

# Mating-induced Male Death and Pheromone Toxin-regulated Androstasis

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## Abstract

How mating affects male lifespan is poorly understood. Using single worm lifespan assays, we discovered that males live significantly shorter after mating in both androdioecious (male and hermaphroditic) and gonochoristic (male and female) *Caenorhabditis*. Germline-dependent shrinking, glycogen loss, and ectopic expression of vitellogenins contribute to male post-mating lifespan reduction, which is conserved between the sexes. In addition to mating-induced lifespan decrease, worms are subject to killing by male pheromone-dependent toxicity. *C. elegans* males are the most sensitive, whereas *C. remanei* are immune, suggesting that males in androdioecious and gonochoristic species utilize male pheromone differently as a toxin or a chemical messenger. Our study reveals two mechanisms involved in male lifespan regulation: germline-dependent shrinking and death is the result of an unavoidable cost of reproduction and is evolutionarily conserved, whereas male pheromone-mediated killing provides a novel mechanism to cull the male population and ensure a return to the self-reproduction mode in androdioecious species. Our work highlights the importance of understanding the shared vs. sex- and species-specific mechanisms that regulate lifespan.

**Keywords:** *Caenorhabditis*, mating, pheromone, death

## Introduction

The interplay between the sexes influences an individual's longevity<sup>1-3</sup>. *Caenorhabditis* female lifespan is shortened after mating through receipt of male sperm and seminal fluid<sup>4</sup>, and separately by exposure to male pheromone<sup>5</sup>. However, previous studies reported contradictory results on how mating influences male lifespan<sup>3,6</sup>. Therefore, whether and how male lifespan is affected by prolonged exposure and interactions with females is largely unknown.

The *Caenorhabditis* genus consists of both androdioecious (male and hermaphroditic) and gonochoristic (male and female) species. In androdioecious species such as *C. elegans*, the population is dominated by hermaphrodites, which reproduce by self-fertilization. Males are usually very rare (less than 0.2% for the standard lab strain N2) and are produced due to spontaneous X chromosome nondisjunction<sup>7,8</sup>. Under stressful conditions, more oocytes experience chromosome non-disjunction, thus androdioecious species periodically undergo explosions of male populations. The existence of males in androdioecious species may reduce inbreeding and facilitate adaptation to changing environments<sup>9</sup>. By contrast, in gonochoristic species such as

*C. remanei*, 50% of the population is male, and females and males must mate to reproduce. The mating efficiency of *C. elegans* males is very low compared to *C. remanei* males<sup>8</sup>. Gonochoristic species females secrete pheromones that attract males<sup>10</sup>, and have distinct behaviors during mating compared to hermaphrodites<sup>11,12</sup>. How males in androdioecious and gonochoristic species cope with these different mating situations remains poorly understood. Moreover, the utility of killing females by exposure to male pheromone in gonochoristic populations<sup>5</sup> is unclear.

Here we report that after mating, *Caenorhabditis* males suffer from germline-dependent shrinking and death, just as in the case of mated *C. elegans* hermaphrodites and *C. remanei* females<sup>4</sup>. However, *C. elegans* males and hermaphrodites have differential sensitivity to male pheromone-dependent toxicity, while *C. remanei* seem immune to this toxicity, and instead use sex-specific pheromones to identify mates. Thus, androdioecious and gonochoristic species differentially utilize pheromone for mating vs hermaphroditic maintenance, while both species suffer the cost of mating through germline-dependent shrinking and death.

## Results

### *C. elegans* males live shorter after mating

*C. elegans* hermaphrodites shrink up to 30% and live 40% shorter after mating<sup>4</sup>. We wondered if males also experience such extreme post-mating changes. Traditional lifespan assays are performed using grouped worms; however, grouped males live shorter than solitary males<sup>13</sup>, which could mask the lifespan shortening effect of mating in males. Therefore, we measured the lifespans of solitary males and single males paired with a single hermaphrodite for 6 days from Day 1 to Day 6 of adulthood. (We used *fog-2(q71)* worms in our assay; *fog-2* males are equivalent to wild-type (N2) males, while *fog-2* hermaphrodites are self-spermless<sup>4</sup>, enabling identification of successful mating.) Mated male lifespan was decreased

~35% compared with the unmated solitary males (Fig. 1A, Table S1), similar to the lifespan decrease of mated hermaphrodites<sup>4</sup>. Also like females, males shrank after 6 days' mating; by Day 7, the mated males were 10% smaller than the unmated solitary males control (Fig. 1B,C, Table S2).

Males die faster when paired with a hermaphrodite for a longer period: mating with a hermaphrodite for one day did not affect the lifespan of the male, while 2-3 days' mating shortened male lifespan by 15%, 4-5 days' mating reduced their lifespan by 25%, and 6 days' mating increased the reduction to over 35% (Fig. 1D). By contrast, the number of hermaphrodites paired with the single male during mating had much less effect compared to mating duration (Fig. 1E, Fig. S1A,B). The time at which mating occurs within the reproductive period is also not critical for males' post-mating

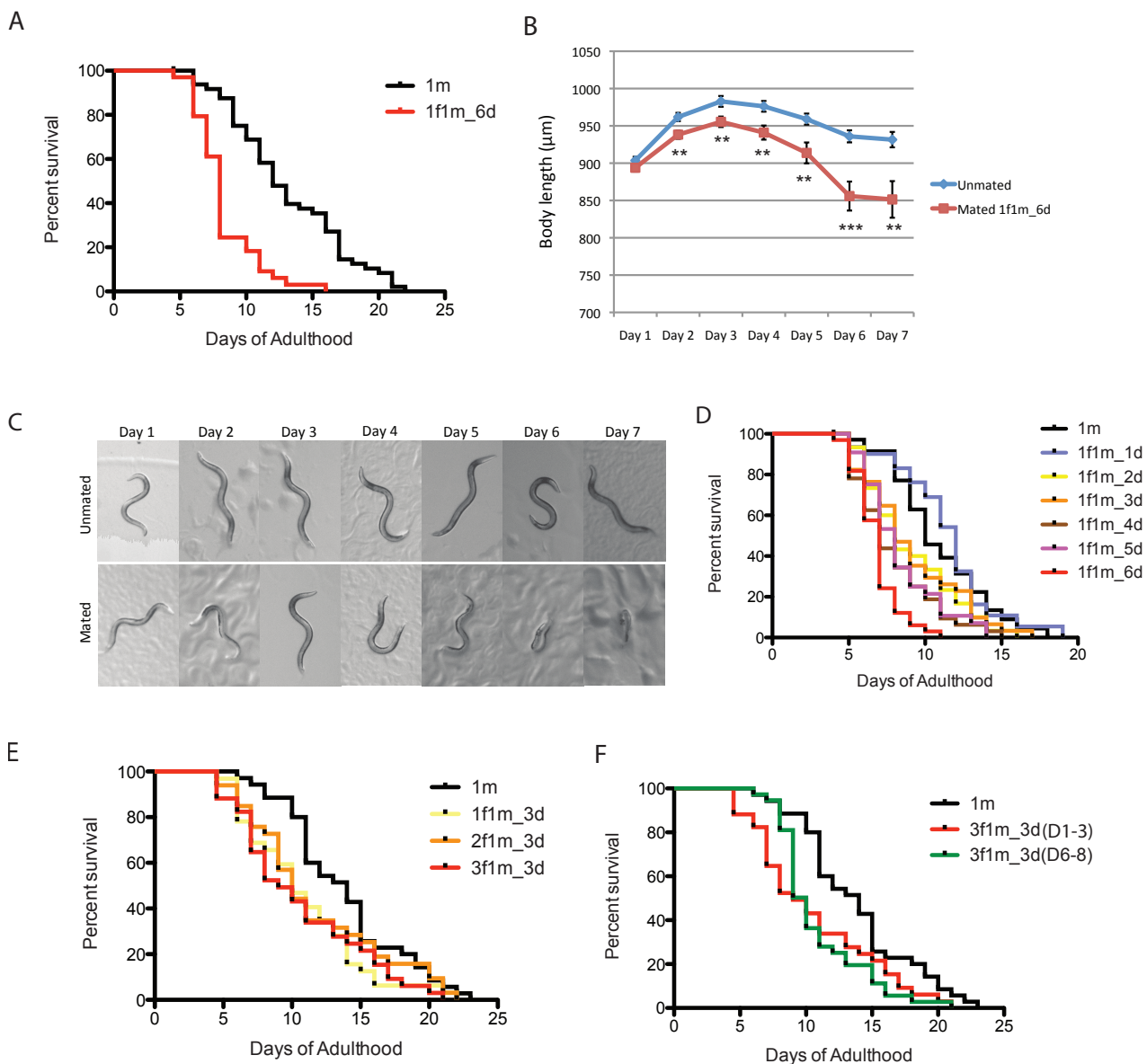


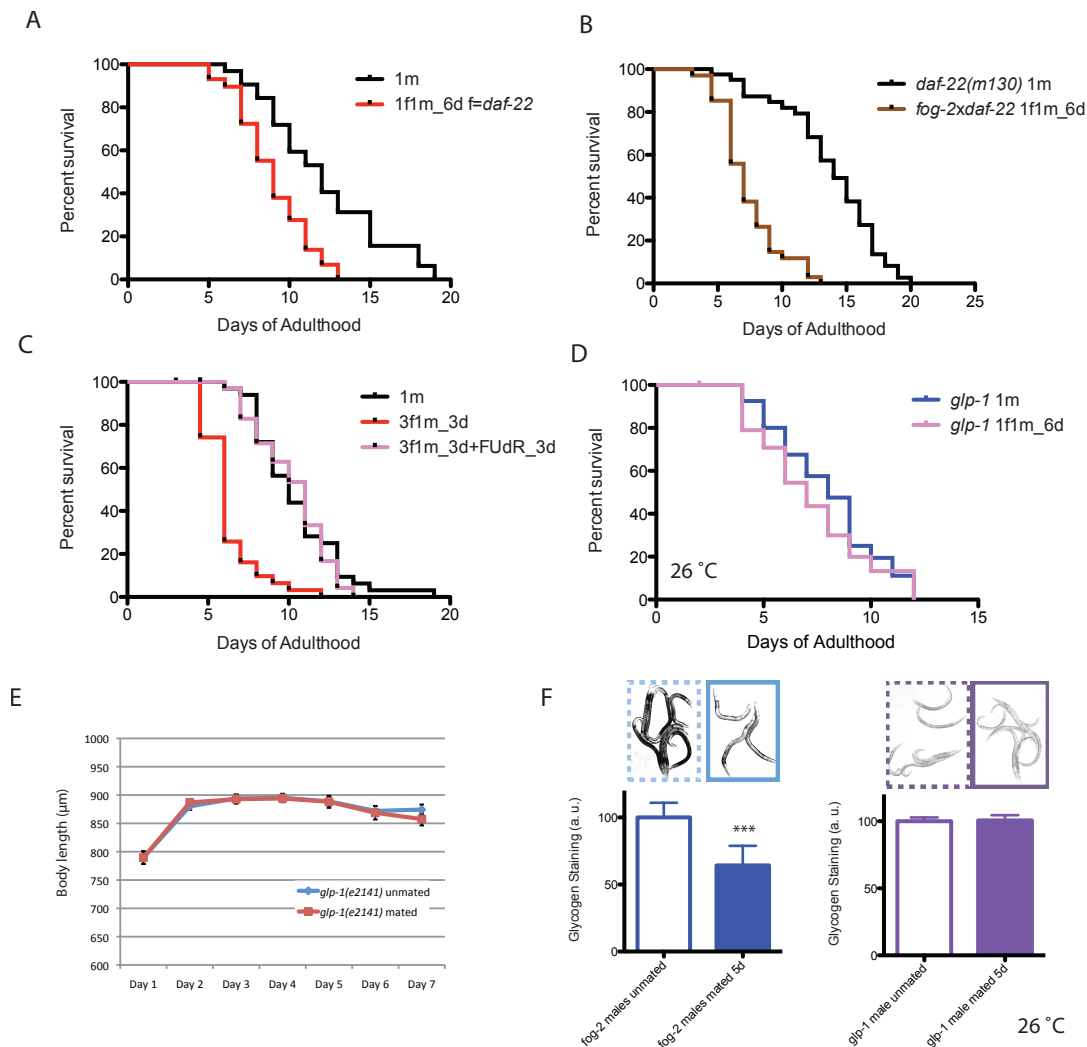
Figure 1. *C. elegans* males shrink and die early after mating.

lifespan decrease; given the same mating duration, males mated with hermaphrodites for the first three days of adulthood had a similar lifespan decrease as those mated with hermaphrodites during Days 6-8 of adulthood (Fig. 1F).

### *C. elegans* males' post-mating shrinking and death are germline-dependent

We wondered whether pheromone is required for mating-induced death in males. To distinguish pheromone from a direct mating effect, we tested *daf-22(m130)* mutants, which are deficient in ascaroside pheromone biogenesis<sup>15</sup>. Wild-type males still died early post-mating when paired with a *daf-22* hermaphrodite for 6 days (Fig. 2A). Likewise, *daf-22* mutant males lived shorter after 6 days' mating (Fig. 2B), indicating that the post-mating lifespan decrease in our single-worm pair lifespan assay is due to mating itself rather than pheromone from either sex.

Elevated germline proliferation is one of the major causes of hermaphrodites' early death after mating<sup>4</sup>. We wondered whether this killing mechanism is conserved in males. Adult treatment with the DNA replication inhibitor 5-fluorodeoxyuridine (FUdR) has little effect on lifespan and meiosis at low dosage (50  $\mu$ M)<sup>16</sup>, but rapidly blocks germline proliferation in mated hermaphrodites<sup>4</sup>. When treated with 50 $\mu$ M FUdR during the three-day mating period, male lifespan was unchanged (Fig. 2C). FUdR treatment also eliminated male post-mating lifespan decrease in our 6 days' mating regime (Fig. S1C,D). Additionally, lacking the germline prevented both shrinking and death: mating caused neither shrinking nor lifespan decrease in germline-less *glp-1(e2141)* males (Fig. 2D,E, Fig. S1E). These results suggest that germline-mediated post-mating lifespan regulation is conserved between sexes to a large extent.

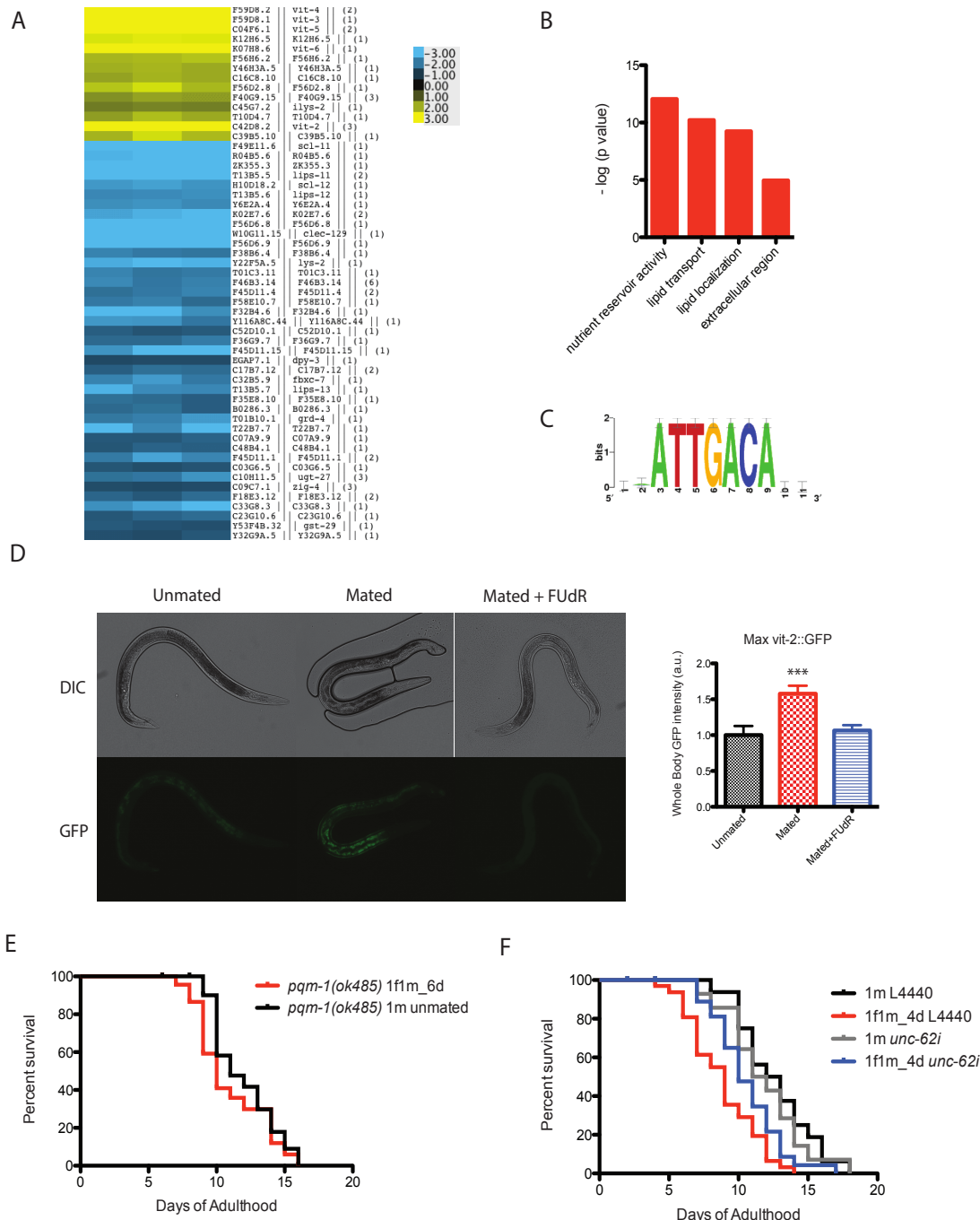


**Figure 2 Male post-mating shrinking death is germline-dependent.**

We have shown previously that osmotic stress resistance correlates well with shrinking in mated hermaphrodites, whereas fat loss does not account for such shrinking<sup>4</sup>. Changes of glycogen levels *in vivo* accurately reflect the osmotic perturbation in the environment<sup>17</sup>; therefore, we measured the glycogen level using iodine staining, and found that mated wild-type worms lost about 30% of the glycogen storage post-mating in a germline-dependent manner (Fig 2F). The mating-induced glycogen storage decrease and subsequent shrinking is conserved between sexes (Fig. S2).

### Vitellogenin dysregulation contributes to male post-mating death

To further characterize male post-mating death, we performed genome-wide transcriptional analysis of mated and unmated males: we paired a single male with a hermaphrodite for 3.5 days of mating, then picked the males individually from the hermaphrodites on Day 4 for microarray analysis (Fig. S3A). As a control, we collected the same number of age-matched solitary males. 14 genes were significantly up-regulated and 41 were significantly down-regulated (FDR=0%; SAM<sup>18</sup>;



4 **Figure 3** Microarray analysis reveals vitellogenin's role in male post-mating death.

Table S3, Fig. 3A). Genes whose expression decreased in mated males include extracellular proteins (*scl-11*, *scl-12*, *zig-4*) and predicted lipase-related hydrolases (*lips-11*, *lips-12*, *lips-13*). The most enriched gene ontology (GO) categories were ribonucleoside monophosphate biosynthetic/metabolic process and extracellular region for the down-regulated genes, and nutrient reservoir activity and lipid transport for the up-regulated genes (Fig. 3B, Fig. S3B).

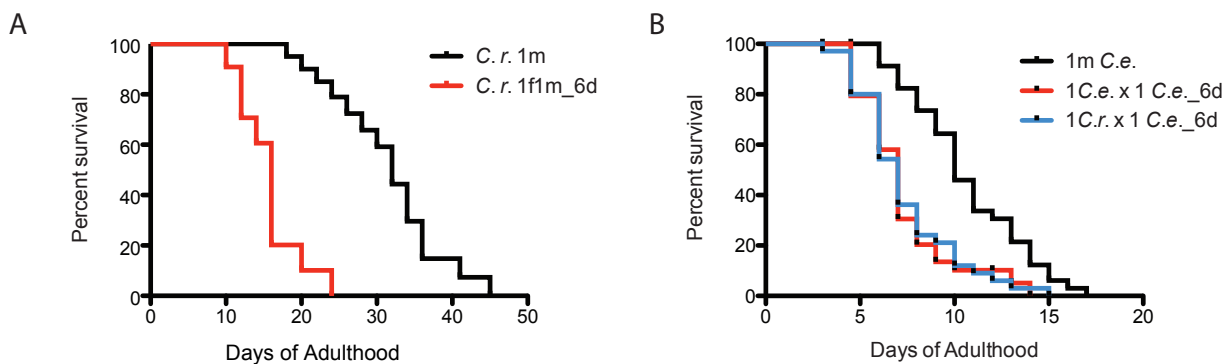
Surprisingly, vitellogenins (*vit-4*, *vit-3*, *vit-5*, *vit-6*, *vit-2*), which encode yolk protein precursors made in the female/hermaphrodite intestine for transport into developing oocytes<sup>19</sup>, were the top up-regulated genes in mated males. They were expressed on average 19 times higher in mated males than in solitary unmated males (Table S3). Males normally do not express *vit* genes, as they produce no oocytes. We confirmed our microarray finding using VIT-2::GFP males: mating induced ectopic expression of VIT-2::GFP, especially in the anterior intestine in males. Such overexpression was germline-dependent (Fig. 3D, S3D). Overproduction of vitellogenins is deleterious for hermaphrodites: vitellogenins accumulate in the head and body of older hermaphrodites<sup>20</sup>; long-lived insulin signaling mutants repress *vit* gene expression<sup>21</sup>; and knockdown of the *vit* genes in wild-type hermaphrodites extends lifespan<sup>21</sup>. The DAE (DAF-16 Associated Element) motif is present in most *vit* genes, which are also Class 2 DAF-16 genes<sup>21</sup>. Thus, we tested the function of PQM-1, the DAE-dependent transcription factor<sup>22</sup>, in male post-mating death. Mated *pqm-1(ok485)* knockout males lived as long as the unmated control (Fig. 3E), suggesting it is important for post-mating death. The binding motif for UNC-62, a master transcription regulator of *vit* genes in hermaphrodites<sup>23</sup>, also emerged in unbiased motif analysis (Fig. 3C). Using RNAi, we found that knocking down *unc-62* was sufficient to rescue the lifespan decrease in mated males (Fig. 3F). Thus, the mis-expression of vitellogenins upon mating contributes to post-mating death in males.

### Mating-induced early death in males is evolutionarily conserved within *Caenorhabditis*

Previously, we showed that *C. remanei* females, like *C. elegans* hermaphrodites, also shrink and die faster after mating<sup>4</sup>, suggesting that the mechanisms are evolutionarily conserved in females. Likewise, we found that male *C. remanei* also lived significantly shorter after mating with a female *C. remanei* for 6 days (Fig. 4A). However, while female death requires successful cross-progeny production, as *C. remanei* males do not induce post-mating death of *C. elegans* hermaphrodites<sup>4</sup>, *C. elegans* males died early when mated with a *C. remanei* female for 6 days (Fig. 4B), suggesting that a component of mating specific and autonomous to the male, rather than a transferred substance or pheromone, is responsible for male death in both species.

### Grouped males also have reduced lifespans in *C. elegans* and *C. remanei*

When male *C. elegans* are housed together, they live shorter compared with solitary males<sup>13</sup>, and the death rate increases with the number of males in a dose-dependent manner<sup>13</sup> (Fig. 5A). (This might be the reason a previous report failed to report shortened lifespan of males after mating, because grouped males were used as the control<sup>3</sup>.) *C. elegans* male lifespan is very sensitive to male density: just two males together significantly reduced each individual's lifespan. In a group of eight males, the individual lifespan had a more dramatic 36% decrease compared with the solitary control (Fig. 5A). *C. remanei* male lifespan was also influenced by male density, although to a lesser degree than *C. elegans* males (Fig. 5C). *C. elegans* males tend to form clumps and attempt to mate with each other. By contrast, *C. remanei* males rarely form clumps, having much reduced male-male interaction<sup>13</sup> (Fig. 5A,C insets). We thought such male-male mating attempts might also lead to post-mating lifespan decrease in a germline-dependent manner as we observed in males mated with females. To test this hypothesis, we placed



**Figure 4 Mating-induced early death in males is conserved.**

the grouped males and solitary controls on FUdR plates to inhibit germline proliferation. In the presence of FUdR, grouped *C. remanei* males had no lifespan decrease (Fig. 5D). However, grouped *C. elegans* males still lived significantly shorter (11% decrease compared with solitary control,  $p=0.0032$ , Fig. 5B), indicating that a germline-independent factor also contributes to *C. elegans* male lifespan reduction when other males are present.

### Male pheromone-dependent toxicity leads to reduced lifespan in grouped *C. elegans*

It was shown previously that *C. elegans* hermaphrodites can be killed by male pheromone secreted by grouped males<sup>5</sup>. We wondered whether male pheromone also affects male lifespan. We held 8 *daf-22(m130)* (pheromone-less) males together, and found that they lived as long as the solitary wild-type males, suggesting that male pheromone kills males (Fig. 5E). Grouped *daf-22* males lived just slightly shorter than solitary

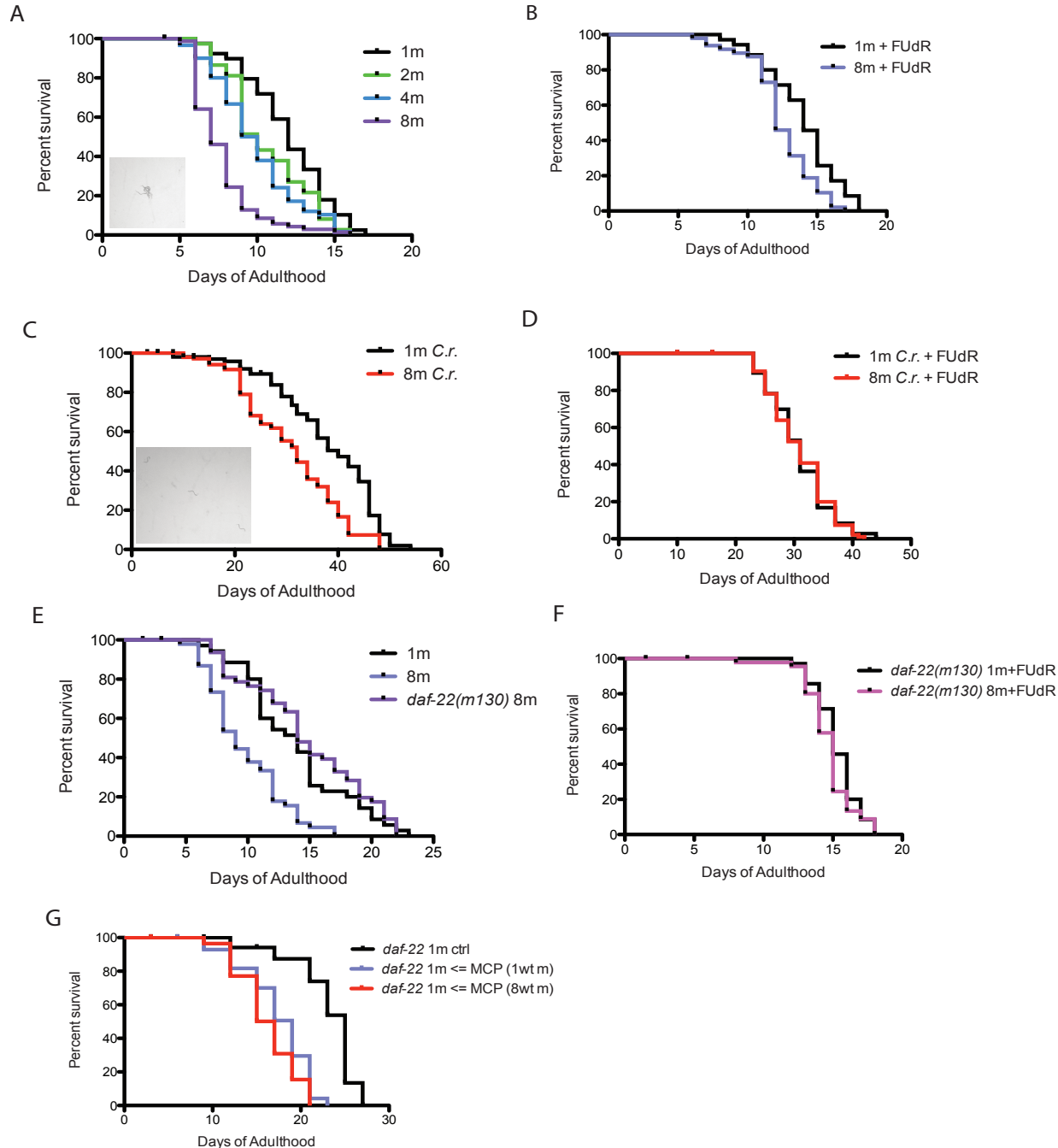


Figure 5 Grouped *C. elegans* males live shorter due to male pheromone.

*daf-22* males (Fig. S4A). The remaining lifespan difference can be explained by germline up-regulation due to mating attempts, since *daf-22* males also formed clumps (Fig. S4A inset), and this lifespan difference was completely eliminated when the experiment was performed in the presence of FUdR (Fig. 5F). Therefore, in grouped *C. elegans* males, early death is due to a combination of germline up-regulation and male pheromone. In fact, males are the victims of their own pheromone: the lifespan of *daf-22* males was significantly reduced when they were maintained on plates conditioned by only one wild-type male (Fig. 5G, S4B), suggesting that *C. elegans* males are extremely sensitive to male pheromone-dependent toxicity.

### *C. elegans* and *C. remanei* have different sensitivity to male pheromone's toxicity

We wondered whether in a true male/female species, male pheromone-mediated death is also present, and if there are cross-species effects. We confirmed that *C. elegans* hermaphrodites die early when grown on plates conditioned with a large number of *C. elegans* males, as shown previously<sup>5</sup> (Fig. 6A, 30 males per plate for conditioning). *C. elegans* hermaphrodites also died early when exposed to *C. remanei* male pheromone (Fig. 6A). By contrast, multiple trials of *C.*

*remanei* females on male-conditioned plates failed to reveal any sensitivity to either *remanei* or *elegans* male pheromone (Fig. 6B). We then tested the sensitivity of both hermaphrodites and males to low levels of pheromone (8 males per plate for conditioning), and found that *C. elegans* hermaphrodites were not as sensitive to male pheromone as males were (Fig. 6C). By contrast, both *C. remanei* males and females were insensitive to low or high amounts of pheromone (Fig. 6D). Thus, *C. elegans* males are most sensitive to male pheromone-dependent toxicity, *C. elegans* hermaphrodites have intermediate sensitivity, and *C. remanei* appear to be immune to male pheromone toxicity (Fig. S5B).

## Discussion

### Germline activation induces deleterious changes that cause males to die

*C. elegans* males and hermaphrodites share many post-mating changes. As we found previously for mated females and hermaphrodites<sup>4</sup>, *Caenorhabditis* males also experience germline-dependent shrinking, glycogen loss, and death after mating. Germline up-regulation also leads to ectopic expression of vitellogenins, which contributes to the post-

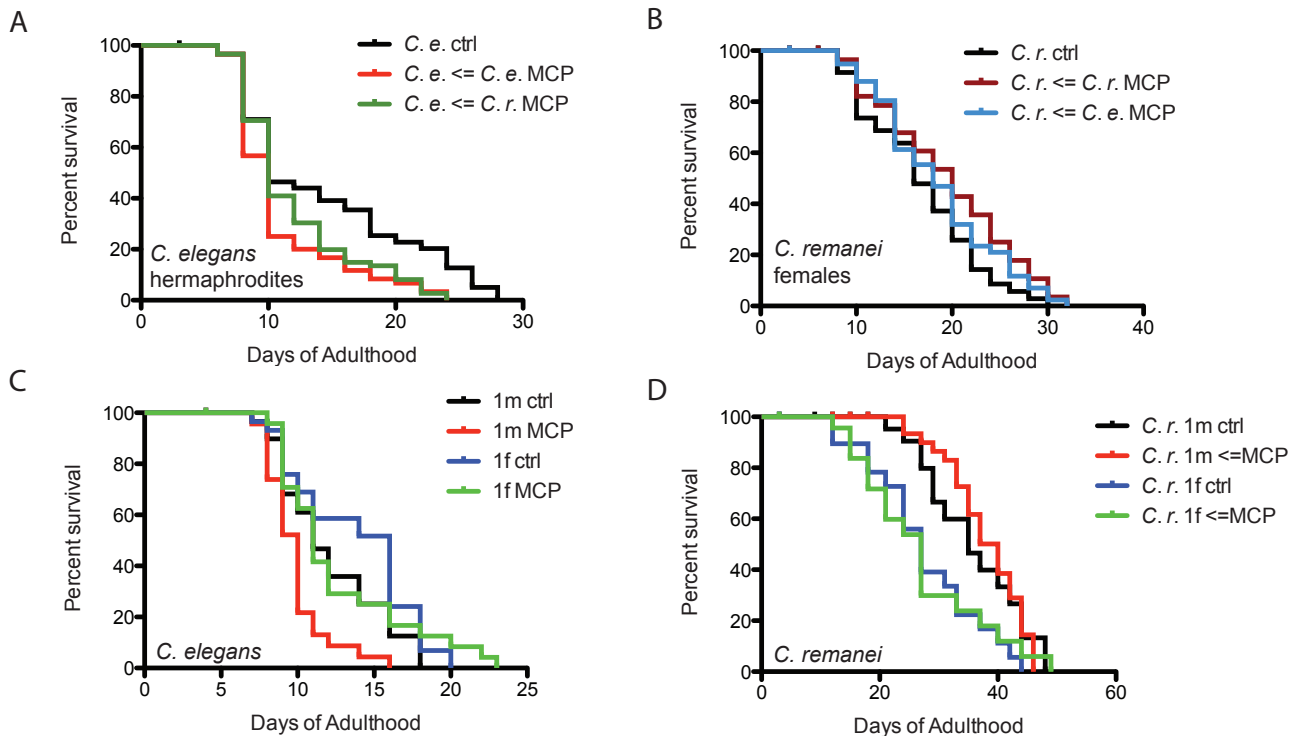
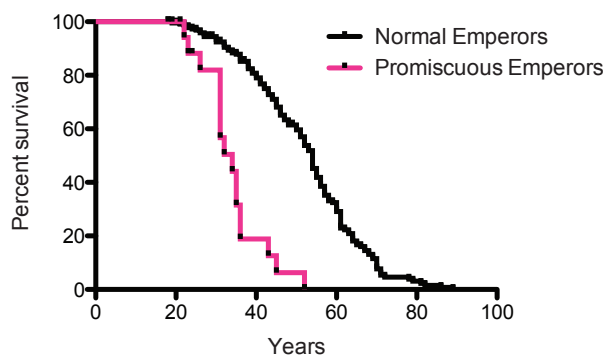


Figure 6 Only *C. elegans* is sensitive to male pheromone's toxicity.

mating lifespan decrease in males. Previously, these yolk protein precursors were only noted to be expressed in hermaphrodites, since males do not produce oocytes, which normally take up vitellogenins in females. Mating also induces significant overexpression of *vit* genes in hermaphrodites<sup>24</sup>, indicating that vitellogenin expression is closely coupled with mating-induced germline up-regulation in both sexes. Such coupling may be strong enough to overcome the repression of male vitellogenin expression. The striking similarity of germline-dependent post-mating changes in *Caenorhabditis* males and females suggests that this mechanism is largely conserved between sexes, and may represent an unavoidable cost of reproduction as a result of mating.



**Figure 7 Average lifespan of Chinese emperors**

Such germline-dependent lifespan shortening appears to be conserved between sexes and across species over large evolutionary distances. Male post-mating death is conserved beyond the *Caenorhabditis* genus, as *Drosophila* males also die earlier after mating<sup>2</sup>. Interestingly, this phenomenon may also be present in human males: in ancient imperial China, those emperors notorious for their lifelong extreme promiscuous sexual behavior lived 35% shorter than their counterparts ( $34 \pm 2$  yrs compared with  $52 \pm 1$  yrs, Fig. 7, Table S4). Limited by the style of historical records in ancient China, other death-contributing factors such as sexually transmitted diseases cannot be ruled out. However, a previous study showed that the lifespan of Korean eunuchs was significantly longer than the lifespan of non-castrated men with similar socio-economic status<sup>25</sup>. Together, these results suggest that some aspects of germline-dependent male post-mating death may be evolutionarily conserved.

### Male pheromone-induced killing as a strategy to selectively reduce the male population

In addition to the mating-induced lifespan decrease, *C. elegans* are subject to killing by male pheromone-dependent toxicity, while *C. remanei* are not. Our study shows that androdioecious and gonochoristic species have different

sensitivities to male pheromone. The androdioecious species (*C. elegans*) males do not appear to use pheromones as efficiently as chemical messengers to facilitate mating, since they are less able to distinguish hermaphrodites' pheromone from other species' female or male pheromone; in fact, *C. elegans* males are even slightly attracted to their own male pheromone, in part explaining their clumping<sup>10</sup> (Fig. S5A). On the other hand, male pheromone is very toxic to *C. elegans* males. Thus, to *C. elegans* males, pheromones serve primarily as toxins to kill males. By contrast, *C. remanei* (gonochoristic species) males are extremely attracted by pheromone produced by *C. remanei* females, even at a low concentration, and are slightly repelled by male pheromone<sup>10</sup> (Fig. S5A), but *C. remanei* are immune to both *elegans* and *remanei* male pheromone toxicity (Fig. 6B,D). Thus, the gonochoristic species *C. remanei* uses pheromones primarily as chemical messengers to locate mates. It is also worth noting that such female pheromone-mediated attraction is completely abolished in the presence of male sperm<sup>10</sup>. In *C. elegans*, males are attracted to old, self-spermless hermaphrodites<sup>26,27</sup>, suggesting that pheromone retains the function as a chemical messenger under some circumstances in *C. elegans*. However, due to the presence of self-sperm in the hermaphrodites, *C. elegans* males do not use pheromone as a primary tool to seek young and middle-aged hermaphrodites.

*Caenorhabditis* species might utilize pheromones in such different ways due to their different modes of reproduction. In the androdioecious species *C. elegans*, males are normally rare (0.2%), so the chance that any worm he encounters will be a hermaphrodite is very high; thus, there may be less selection pressure to evolve pheromones as chemical messengers to seek out mates. However, periodically there are explosions of male populations in androdioecious species (e.g., under stressful conditions) to allow outcrossing and ensure genetic diversity<sup>9</sup>. After this period, however, males are more costly to maintain, and there is pressure to return to a primarily hermaphroditic population. It is notable that because *C. elegans* males are XO, rather than XY, males may have no selfish drive to maintain their own chromosomes. From the perspective of species, using male pheromone as a dose-dependent toxin may be an effective way to cull the male population and ensure the species returns to the self-reproduction mode when the stressful condition has passed. Use of the pheromone as a toxin to kill males may have arisen to aid the return to hermaphroditism, which can also be promoted by increased hermaphroditic progeny production and decreased mating rates<sup>28</sup>; these factors could also act in tandem with the selected pheromone-dependent killing of males. Hermaphrodite death at high male pheromone concentration (which would happen extremely rarely in nature) might simply be a rather infrequent result of collateral damage, as the hermaphrodites are less sensitive than males to the toxin. Male-specific culling also occurs in species such as *Drosophila bifasciata*, in which *Wolbachia* infection leads to the killing of male embryos, suggesting that sex ratio can be



controlled through male-killing<sup>29</sup>. Mathematical modeling shows that selection in *C. elegans* favors low populations of males<sup>30</sup>, and our model provides a mechanism for how this may be achieved.

By contrast, the preponderance of males in a 50:50 population, as in the case of *C. remanei*, makes the use of pheromone as a toxin less likely, as it would cause too much off-target death to be useful for sperm competition. Our cross-species results suggest that *remanei* male pheromone is toxic to *C. elegans*, but both *C. remanei* males and females are immune to both *elegans* and *remanei* pheromone (Fig. 6A,B). These results also suggest that the toxic effect of pheromone may not be due to the pheromone itself, but rather to a receptor-mediated sensitivity to pheromone that is specific to *C. elegans*, with a greater effect in males than in hermaphrodites. Instead, *C. remanei* pheromone is used to distinguish males from females, an important distinction in 50:50 mixed populations. Like *C.*

*elegans*, the primary mode of sperm competition in *C. remanei* appears to involve seminal fluid transfer of factors that cause the mother to die after producing the father's progeny, before she has a chance to re-mate<sup>4</sup>, rather than through a pheromone-based mechanism (Fig. 8).

In summary, germline-dependent early death after mating is conserved between sexes and perhaps even across great evolutionary distances, and is likely due to an unavoidable cost of mating, the result of mated animals ramping up germline proliferation and subsequently exhausting using their own resources as fast as possible to produce the next generation of progeny. The differential use of pheromones as toxins or chemical messengers by males in androdioecious and gonochoristic species demonstrates that they adopt different strategies to compete, mate, and maintain optimal population ratios.

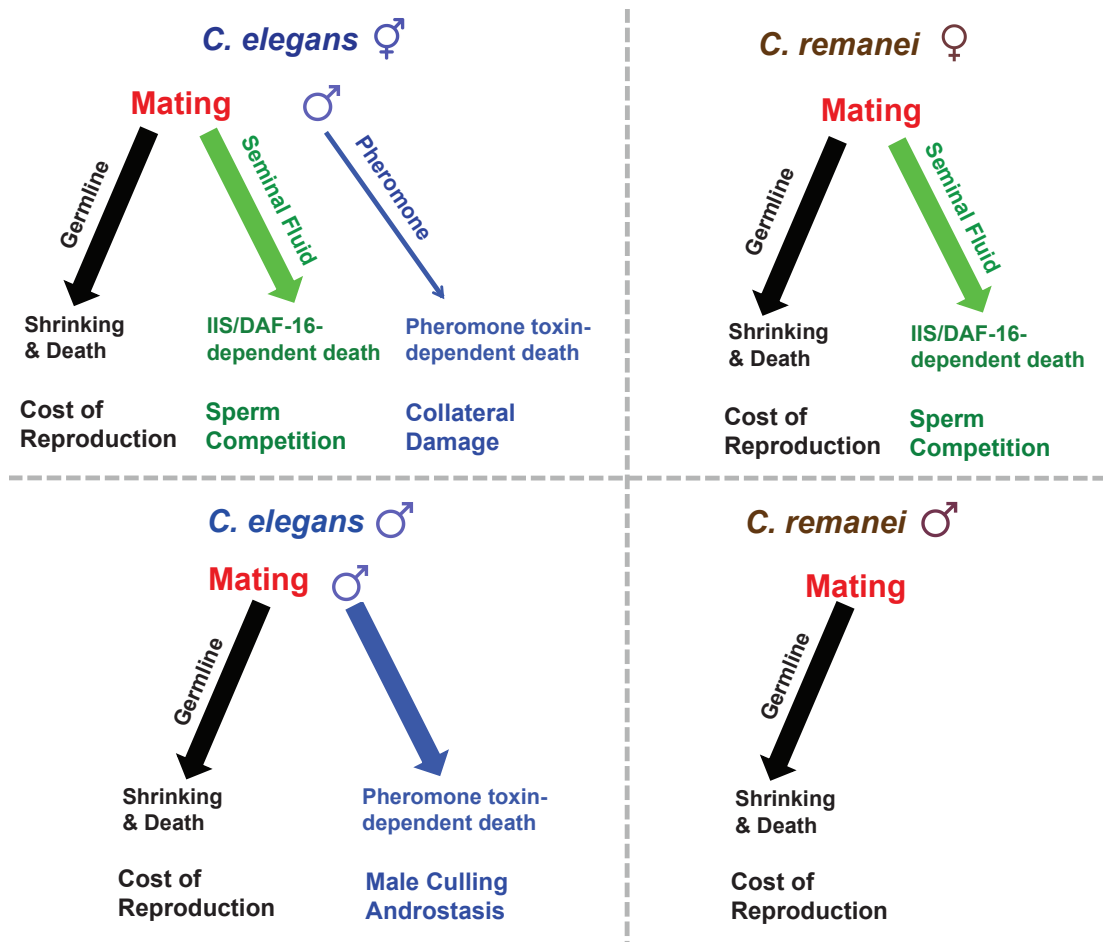


Figure 8 Simplified model of how mating and male pheromone affect lifespan in *C. elegans* hermaphrodites (upper left); *C. remanei* females (upper right); *C. elegans* males (lower left); *C. remanei* males (lower right).

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## **Author Contributions**

C.S., A.M.R., and C.T.M. designed experiments. C.S. and A.M.R. performed experiments. C.S. and C.T.M. wrote the paper.

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## Materials and Methods

### Strains:

CB4108: *fog-2(q71) V*

CB4037: *glp-1(e2141) III*

DR476: *daf-22(m130) II*

RT130: pwIs23 [vit-2::GFP] (translational fusion)

PB4641: *Caenorhabditis remanei*

### Individual male mating lifespan assays:

All the lifespan assays were performed at room temperature (about 20-21°C); except for *glp-1* males' lifespan assays (performed at 25-26°C). 35mm NGM plates were used for all the experiments in this study. 20  $\mu$ l of OP50 was dropped onto each plate to make a bacterial lawn of ~10 mm diameter. The next day, one synchronized late L4 male and one late L4 hermaphrodite/female were transferred onto each 35 mm NGM plate. For experiments in Fig. 1E, 1F, S1A-B,D, 2C, multiple L4 hermaphrodites were transferred together with one male. One late L4 male of the same age and genotype was transferred onto the control plates. Except for Fig. 2A, *fog-2(q71)* hermaphrodites were used as the hermaphrodites in the mating assay, because *fog-2* hermaphrodites do not have self sperm, thus allowing us to easily detect successful mating (i.e. eggs and progeny on the plates). We only included males that were able to produce progeny in our analysis. However, for the experiments regarding *glp-1* males, mating on FUDR, and inter-species cross between *C. elegans* males and *C. remanei* females, we included all the males in the analysis. Worms were transferred onto new plates every other day. If the hermaphrodites were lost or bagged, new unmated Day 1 *fog-2* hermaphrodites were added as replacement. Males and hermaphrodites/females were kept together for 6 days (unless

noted otherwise in the text); afterwards only males were transferred on to newly seeded plates every 2-3 days. For RNAi experiments in Fig. 3F, synchronized eggs were transferred onto NGM plates with RNAi bacteria, late L4 males were transferred and paired with *fog-2* L4 hermaphrodites onto NGM plates seeded with OP50 (to eliminate the possible effect on mating efficiency for different RNAi treatments). Two days later, males and hermaphrodites were transferred onto fresh plates seeded with corresponding RNAi bacteria and males were maintained on RNAi bacteria thereafter. When lifespan assays were completed, Kaplan-Meier analysis with log-rank (Mantel-Cox) method was performed to compare the lifespans of different groups.

### Grouped males:

35mm NGM plates were used for all the experiments in this study. 20  $\mu$ l of OP50 was dropped onto each plate to make a bacterial lawn of ~10 mm diameter. The next day, eight synchronized late L4 males were transferred onto each plate. (Two or four males per plate for experiment in Fig. 5A.) One late L4 male of the same age and genotype was transferred onto the control plates. Males were transferred onto fresh plates every two days, when the males were lost or dead, males from other plates were transferred together to make the size of the group stable.

### Male-conditioned plates (MCP) setup:

Male-conditioned plates for lifespan assays were prepared as previously described<sup>5</sup>. Briefly, 60  $\mu$ l of OP50 was dropped onto each 35mm NGM plate to make a bacterial lawn of ~25 mm diameter. Young Day 1 wild-type males (*fog-2* males) were transferred onto each plate. Two days later, they were removed and worms for lifespan assays were immediately transferred onto these male-conditioned plates. These male-conditioned plates were being prepared throughout the course of the lifespan assays (Fig. S4B). For the experiments in Fig. 6A,B, 30 males were used for each conditioning plate. For experiments in Fig. 6C,D, 8 males were used for conditioning and for the experiment in Fig. 5G, only 1 male was used for conditioning for each plate.

### Body size measurement:

Images of live males on 35mm plates were taken daily for the first week of adulthood with a Nikon SMZ1500 microscope. Image J was used to analyze the body size of the worms. The middle line of each worm was delineated using the segmented line tool and the total length was documented as the body length of the worm. T-test was performed to compare the body size differences between groups of males in the same day.

### **FUDR experiment:**

FUDR was added to the NGM media to the final concentration of 50  $\mu$ M. Late L4 males and hermaphrodites were transferred onto NGM+FUDR plates seeded with OP50. Worms were transferred every two days, and were kept on FUDR plates for different period of time (3 days, 6 days or lifetime as indicated by text).

### **Glycogen staining:**

Glycogen staining was performed according to a well-described protocol<sup>17</sup>. Mating of males was set up as previously described. Right before staining, live males of the same group were picked into a M9 droplet with 1M sodium azide on a 3% agarose pad. Immediately after the liquid was dry, the pad was inverted over the opening of a 50g bottle of iodine crystal chips (Sigma) for 1 minute. After the color stained by iodine vapor on the pad disappear (non-specific staining), the worms were immediately imaged by a Nikon microscope. Due to uncontrollable differences, it is hard to compare the staining performed at different times. Thus, worms from the groups of comparison were mounted onto the same pad (separate M9 droplet if there is no visible difference). Image J was used to compare the mean intensity of iodine staining after the background was subtracted. T-test was performed to compare the staining between different groups (on the same pad).

### **GFP intensity quantification:**

10-20 worms of each group were imaged by Nikon Ti. Image J was used to measure the mean and the maximum GFP intensity of the whole body area. T-test analysis was performed to compare the GFP intensity of different groups of worms.

### **Mated males microarrays:**

We paired a single male with a *fog-2* hermaphrodite for about 3.5 days of mating, then picked the males individually from the hermaphrodites on Day 4 for microarray analysis. As a control, solitary males were collected at the same time. About 150 males (on 150 individual 35mm plates) were collected for each condition and replicate. Three biological replicates were performed. RNA was extracted by heat-vortexing method. Two-color Agilent microarrays were used. The detailed steps and analysis were performed according to a previous report<sup>31</sup>.

### **Pheromone chemotaxis assay:**

This assay was modified from a previous assay<sup>10</sup>. 10 Day 1 virgin *C. remanei* or *C. elegans* hermaphrodites were put in 100  $\mu$ l of M9 buffer at room temperature overnight with

shaking. 100 males of either *C. elegans* or *C. remanei* were put in 100  $\mu$ l of M9. The supernatant solutions were then taken for pheromone chemotaxis assay. 60 mm NGM plates (no food) were used for the chemotaxis assay. Two destination spots (supernatant and M9 control) were separated by about 45 mm, the distance from the origin spot to either destination spot is 30mm. Two 1 $\mu$ l drops of 1M sodium azide were first applied to the destination spots. When dry, a drop of 1  $\mu$ l M9 or supernatant was separately added onto the destination spots. Then, over 10 young adult (Day 2) males were placed at the origin spot (try to transfer as little bacteria as possible). After 60 minutes, the paralyzed male worms were scored based on their location. The chemotaxis index was calculated as:  $(\#worms \text{ at supernatant destination} - \#worms \text{ at control destination}) / (\#total \text{ worms} - \#worms \text{ at origin})$ .

### **Analysis of Lifespans of Emperors in Imperial China:**

In ancient China, agriculture was the main source of the country's wealth. The development of agriculture began in the Neolithic Era (10,000 BC), followed by improvements in the Bronze Age (1000 BC). Late in the Warring states eras (771-221 BC), new iron tools were widely adopted, which revolutionized agriculture in China. Ancient China's economy depended heavily if not solely on agriculture.

Qin Shi Huang (#1 on the list below) was the first emperor to unify China. By that time, agriculture had already been well developed and the basic structure and the level of civilization did not change much until the late 1800s. Also, emperors had the best standard of living and medicine care at the time. Therefore, we think the living conditions of emperors in Imperial China (220 BC -1911) should be relatively similar (i.e., the best of agricultural civilization). Thus, although their living time spans over 2000 years, we grouped them together to perform lifespan analysis.

On our list (Table S4), we only included emperors who were over 18 years old when they died and those who reigned over 1 year, in order to exclude the cases of puppet emperors. Those rows marked by grey on the list indicate that the emperor's death is unnatural (killed in a war, rebellion, etc). We treated them in the same way as censored worms in the lifespan assays, by censoring at the time of death. Those highlighted in yellow are emperors with extreme promiscuous sexual behaviors, as documented by official historical records. Those labeled by shaded yellow means they were considered promiscuous and died unnaturally.

The average lifespan of promiscuous emperors was 34 years old, which is 35% shorter than the normal emperors' lifespan (52 years) (Fig. 7, Table S4). It should be noted that these promiscuous emperors were also noted to indulge themselves with alcohol. Thus, excessive alcohol intake could also be the

cause of their shorter lifespans. However, other emperors who were well-known for their lifelong alcohol indulgence were not short-lived (Examples are Yuan Tai Zong #216 on the list, died at 56; Yuan Shi Zu #219 died at 79). Another case worth noting is Song Gao Zong (#178), who was originally fertile but became infertile when he fled south after defeat by his enemies. By the time he re-established his dynasty in southern China, he was only 24, but was reportedly no longer capable of reproduction; he died at the age of 81. His case again suggests the link between germline signal and lifespan, perhaps in the same manner as the suggested lifespan extension of Korean eunuchs documented by Min, *et al.* (2012)<sup>25</sup>.

## Main Figure Legends

### Abbreviations and nomenclature in the paper:

*C. e.*: *C. elegans*

*C. r.*: *C. remanei*

**f1m\_6d**: “f” stands for hermaphrodite/female, “m” stands for male, the number before f/m suggests the amount of worms on the same 35mm plate. “6d” means mating for 6 days.

**AAA x BBB**: hermaphrodites/females of genotype AAA are mated with males of genotype BBB. (male is always listed after the “x”)

**MCP**: male-conditioned plates

### Figure 1. *C. elegans* males shrink and die early after mating.

- (A) Lifespans of unmated solitary and mated *fog-2(q71)* males. Solitary males:  $13.1 \pm 0.6$  days,  $n=50$ ; mated males:  $8.3 \pm 0.4$  days,  $n=34$ ,  $p<0.0001$ . Each male was paired with a *fog-2(q71)* hermaphrodite on a single 35mm plate during Day 1-6 of adulthood. Unless noted, all the hermaphrodites used are *fog-2(q71)*. For all the lifespan assays performed in this study, Kaplan-Meier analysis with log-rank (Mantel-Cox) test was used to determine statistical significance. All the lifespan results are included in Table S1.
- (B) Length of unmated and mated *fog-2* males: t-test,  $**p<0.01$ ,  $***p<0.001$ .
- (C) Representative pictures of the same unmated solitary male and male paired with one hermaphrodite from Day 1-Day 6 of adulthood.
- (D) Male post-mating lifespan decrease is mating duration-dependent: Unmated solitary males:  $10.9 \pm 0.6$  days,  $n=35$ ; one male and one hermaphrodite mating on Day 1 of adulthood:  $11.4 \pm 0.6$  days,  $n=31$ ,  $p=0.3697$ ; mating from Day 1-2:  $9.0 \pm 0.6$  days,  $n=30$ ,  $p=0.0325$ ; mating from Day 1-3:  $9.1 \pm 0.6$

days,  $n=34$ ,  $p=0.0452$ ; mating from Day 1-4:  $7.9 \pm 0.5$  days,  $n=32$ ,  $p=0.0002$ ; mating from Day 1-5:  $8.3 \pm 0.4$  days,  $n=34$ ,  $p=0.0006$ ; mating from Day 1-6:  $6.8 \pm 0.3$  days,  $n=33$ ,  $p<0.0001$ .

- (E) Lifespans of one male paired with different number of hermaphrodites during Day 1-3 of adulthood: solitary unmated males:  $13.8 \pm 0.7$  days,  $n=35$ ; one male with one hermaphrodite:  $10.8 \pm 0.6$  days,  $n=32$ ,  $p=0.0175$ ; one male with two hermaphrodites:  $11.6 \pm 0.9$  days,  $n=33$ ,  $p=0.1435$ ; one male with three hermaphrodites:  $10.6 \pm 0.8$  days,  $n=34$ ,  $p=0.0147$ .
- (F) Lifespans of one male paired with three hermaphrodites for 3 days but at different time of adulthood. Solitary unmated males:  $13.8 \pm 0.7$  days,  $n=35$ ; mating during Day 1-3 of adulthood:  $10.6 \pm 0.8$  days,  $n=34$ ,  $p=0.0147$ ; mating during Day 6-8 of adulthood:  $10.8 \pm 0.6$  days,  $n=37$ ,  $p=0.0022$ .

### Figure 2. Male post-mating shrinking death is germline-dependent.

- (A) Lifespans of *fog-2* males mated with *daf-22(m130)* hermaphrodites. Unmated solitary *fog-2* males:  $12.1 \pm 0.6$  days,  $n=32$ ; mated males:  $9.0 \pm 0.4$  days,  $n=29$ ,  $p=0.0001$ . In the mated group, one *fog-2(q71)* male was paired with one *daf-22(m130)* hermaphrodite from Day 1- Day 6 of adulthood.
- (B) Lifespans of unmated and mated *daf-22(m130)* males. Unmated solitary *daf-22(m130)* males:  $13.8 \pm 0.6$  days,  $n=40$ ; mated *daf-22(m130)* males:  $7.4 \pm 0.4$  days,  $n=34$ ,  $p<0.0001$ . In the mated group, one *daf-22(m130)* male was paired with one *fog-2(q71)* hermaphrodite from Day 1- Day 6 of adulthood.
- (C) FUDR can rescue male post-mating early death. Unmated solitary males:  $10.5 \pm 0.5$  days,  $n=35$ ; one male with three hermaphrodites for three days:  $6.4 \pm 0.3$  days,  $n=31$ ,  $p<0.0001$ ; one male with three hermaphrodites for three days but in the presence of  $50 \mu\text{M}$  FUDR during the three days' mating:  $10.2 \pm 0.4$  days,  $n=36$ ,  $p=0.7086$  (compared with unmated solitary group).
- (D) Lifespans of unmated and mated *glp-1(e2141)* males: unmated solitary *glp-1* males:  $8.0 \pm 0.4$  days,  $n=40$ ; mated *glp-1* males:  $7.2 \pm 0.4$  days,  $n=40$ ,  $p=0.3178$ . The assay was performed at  $26^\circ\text{C}$ , in mated group, one *glp-1* male was paired with one *fog-2* hermaphrodite from Day 1-6.
- (E) Length of mated and unmated *glp-1(e2141)* males. (The same population as in Fig. 2D)
- (F) Glycogen staining of mated and unmated males. Left: mated *fog-2* (wt) males lost over 30% glycogen after 5 days' mating.  $***p<0.001$ . Right: mated *glp-1* males had a similar amount of glycogen as the unmated *glp-1* males. The staining intensity was

normalized to unmated males of each genotype. Representative pictures are shown above the quantitation. Unmated males are framed by dashed lines, and mated males are framed by solid lines.

**Figure 3. Microarray analysis reveals vitellogenin's role in male post-mating death.**

- (A) Expression heatmap of genes whose expression is significantly changed in mated males based on SAM analysis.
- (B) Enriched GO terms for significantly up-regulated genes in mated males.
- (C) Enriched motif associated with significantly up-regulated genes predicted by RSAT (Regulatory Sequence Analysis Tools).
- (D) Ectopic expression of VIT-2::GFP in mated males is germline- dependent. 5 days' mating, pictures were taken on Day 6 of adulthood. Left: images; right: quantification of VIT-2::GFP expression [maximum  $\pm$  SE (error bars)], a.u., arbitrary units. \*\*\*,  $p < 0.001$ , t-test.
- (E) *pqm-1(ok485)* mated males have similar lifespans as unmated controls. Unmated solitary *pqm-1(ok485)* males:  $11.9 \pm 0.5$  days,  $n=25$ ; mated *pqm-1(ok485)* males:  $11.0 \pm 0.6$  days,  $n=29$ ,  $p=0.2782$ . In the mated group, one *pqm-1(ok485)* male was paired with one *fog-2(q71)* hermaphrodite from Day 1- Day 6 of adulthood.
- (F) *unc-62* RNAi suppresses male post-mating early death. Unmated solitary male on L4440:  $12.6 \pm 0.7$  days,  $n=25$ ; mated males on L4440:  $8.8 \pm 0.5$  days,  $n=33$ ,  $p=0.0001$ . Unmated males on *unc-62* RNAi:  $11.9 \pm 0.8$  days,  $n=25$ ; mated males on *unc-62* RNAi:  $10.6 \pm 0.5$  days,  $n=34$ ,  $p=0.1249$  (compared to unmated males on *unc-62* RNAi).

**Figure 4. Mating-induced early death in males is conserved.**

- (A) Mated *C. remanei* males also live shorter. Unmated solitary *C. remanei* males:  $31.4 \pm 1.7$  days,  $n=72$ ; mated *C. remanei* males:  $15.7 \pm 1.2$  days,  $n=28$ ,  $p < 0.0001$ . In mated group: one *C. remanei* male was paired with one *C. remanei* female from Day 1-Day 6 of adulthood.
- (B) Lifespans of *C. elegans* males mated with *C. elegans* hermaphrodites and *C. remanei* females. Unmated solitary *C. elegans* males:  $10.2 \pm 0.6$  days,  $n=35$ ; *C. elegans* males mated with *C. elegans* hermaphrodites:  $7.4 \pm 0.4$  days,  $n=35$ ,  $p=0.0001$ ; *C.*

*elegans* males mated with *C. remanei* females:  $7.4 \pm 0.4$  days,  $n=35$ ,  $p=0.0003$ . In mated groups, one *C. elegans* male was paired with either one *C. elegans* hermaphrodite or one *C. remanei* female from Day 1-6 of adulthood.

- (C) Average lifespan of promiscuous Chinese emperors ( $34 \pm 2$  yrs) is shorter than that of non-promiscuous emperors ( $52 \pm 1$  yr). See Methods and Table S4 for detailed rationale, description, and data.

**Figure 5. Grouped *C. elegans* males live shorter due to male pheromone.**

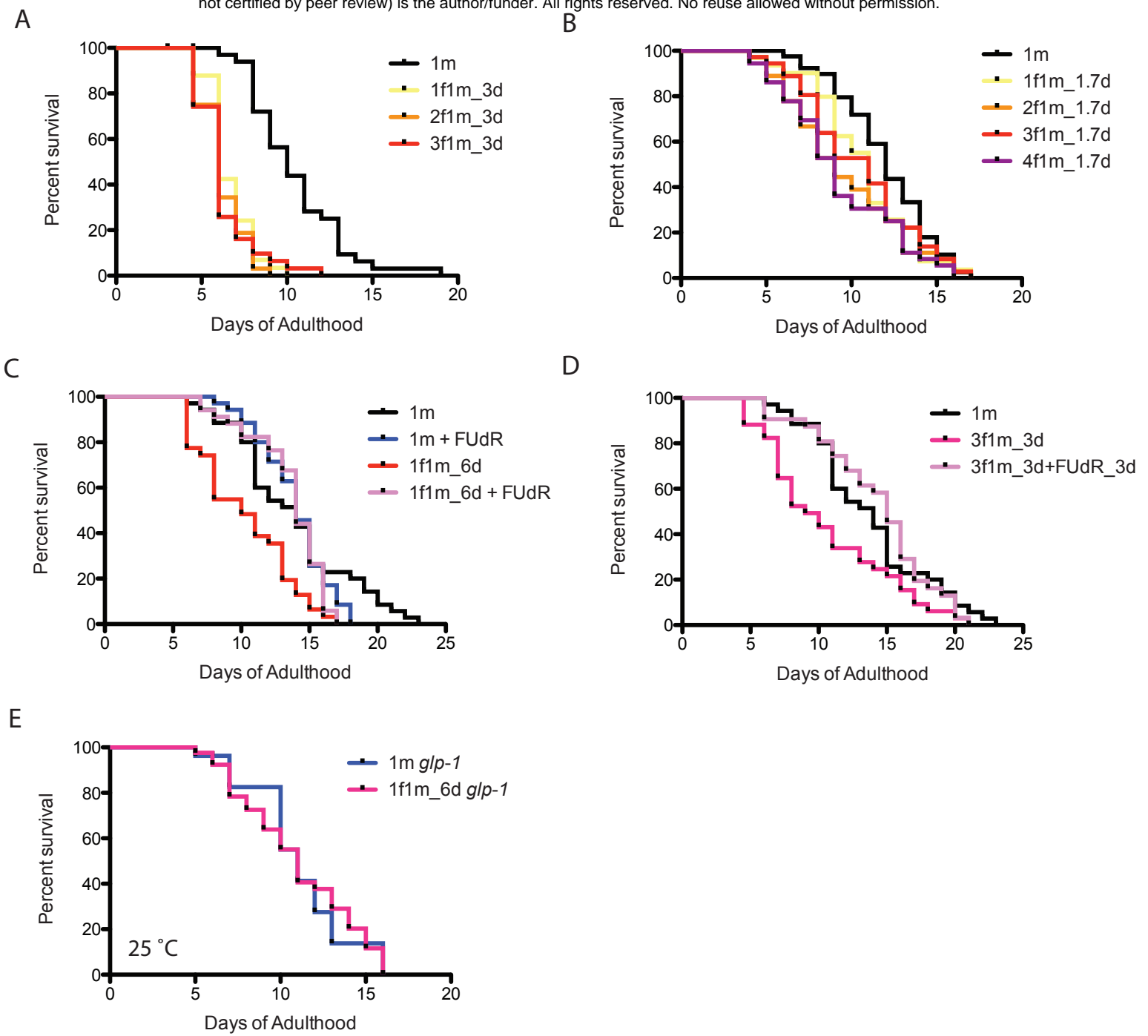
- (A) Lifespans of grouped *fog-2(q71)* males. Solitary males:  $12.0 \pm 0.4$  days,  $n=40$ ; two males:  $10.6 \pm 0.4$  days,  $n=40$ ,  $p=0.0397$ ; four males:  $9.9 \pm 0.4$  days,  $n=60$ ,  $p=0.0012$ ; eight males:  $7.7 \pm 0.2$  days,  $n=80$ ,  $p < 0.0001$ . Inset: clumping of *fog-2* males.
- (B) Lifespans of grouped *fog-2(q71)* males in the presence of  $50\mu\text{M}$  FUDR. Solitary males:  $13.9 \pm 0.4$  days,  $n=35$ ; eight males:  $12.4 \pm 0.3$  days,  $n=48$ ,  $p=0.0032$ .
- (C) Lifespans of grouped *C. remanei* males. Solitary males:  $37.9 \pm 1.1$  days,  $n=120$ ; eight males:  $31.0 \pm 0.9$  days,  $n=160$ ,  $p < 0.0001$ . Inset: *C. remanei* males rarely form clumps.
- (D) Lifespans of grouped *C. remanei* males in the presence of  $50\mu\text{M}$  FUDR. Solitary males:  $30.8 \pm 0.9$  days,  $n=45$ ; eight males:  $30.8 \pm 0.5$  days,  $n=112$ ,  $p=0.9217$ .
- (E) Grouped *daf-22(m130)* males have similar lifespan to solitary wild-type *fog-2* males. Solitary *fog-2* males:  $13.8 \pm 0.7$  days,  $n=35$ ; eight *fog-2* males:  $9.8 \pm 0.5$  days,  $n=48$ ,  $p < 0.0001$ ; eight *daf-22(m130)* males:  $14.7 \pm 0.7$  days,  $n=48$ ,  $p=0.4039$  (compared to solitary males).
- (F) Lifespans are not different between solitary and grouped *daf-22(m130)* in presence of FUDR. Solitary *daf-22(m130)*:  $15.3 \pm 0.3$  days,  $n=35$ ; eight *daf-22(m130)*:  $14.7 \pm 0.3$  days,  $n=48$ ,  $p=0.2117$ .
- (G) *daf-22(m130)* male lifespans on plates conditioned by wild-type *fog-2* males. MCP: male-conditioned plates. Solitary *daf-22(m130)*:  $23.0 \pm 0.9$  days,  $n=30$ ; *daf-22(m130)* on plates conditioned by one *fog-2* male:  $17.3 \pm 0.7$  days,  $n=29$ ,  $p < 0.0001$ ; *daf-22(m130)* on plates conditioned by eight *fog-2* male:  $16.1 \pm 0.6$  days,  $n=30$ ,  $p < 0.0001$ . Details about male-conditioned plates lifespan assays are included in Methods and Fig. S4B.

- fog-2* hermaphrodites control:  $14.4 \pm 0.8$  days,  $n=90$ .  
*fog-2* hermaphrodites on plates conditioned by *fog-2* males:  $10.9 \pm 0.6$  days,  $n=60$ ,  $p=0.0004$ ; *fog-2* hermaphrodites on plates conditioned by *C. remanei* males:  $11.9 \pm 0.5$  days,  $n=90$ ,  $p=0.0042$ .
- (B) Lifespans of grouped *C. remanei* females on plates conditioned by 30 males. *C. remanei* females on control plates:  $15.8 \pm 0.9$  days,  $n=60$ ; *C. remanei* females on plates conditioned by *C. remanei* males:  $19.5 \pm 1.3$  days,  $n=30$ ,  $p=0.0636$ ; *C. remanei* females on plates conditioned by *C. elegans fog-2* males:  $18.5 \pm 10.9$  days,  $n=60$ ,  $p=0.1770$ .
- (C) Lifespans of solitary *C. elegans fog-2* males and hermaphrodites on plates conditioned by eight *fog-2* males. Solitary *fog-2* males on control plates:  $12.1 \pm 0.6$  days,  $n=30$ ; solitary *fog-2* males on male-conditioned plates:  $9.8 \pm 0.4$  days,  $n=28$ ,  $p=0.0046$ . Solitary *fog-2* hermaphrodites on control plates:  $13.8 \pm 0.7$  days,  $n=30$ ; solitary *fog-2* hermaphrodites on male-conditioned plates:  $12.6 \pm 0.9$  days,  $n=29$ ,  $p=0.5965$ .
- (D) Lifespans of solitary *C. remanei* males and females on plates conditioned by eight *C. remanei* males. Solitary *C. remanei* males on control plates:  $35.8 \pm 2.0$  days,  $n=34$ ; solitary *C. remanei* males on male-conditioned plates:  $37.8 \pm 1.2$  days,  $n=34$ ,  $p=0.8501$ . Solitary *C. remanei* females on control plates:  $27.6 \pm 2.2$  days,  $n=24$ ; solitary *C. remanei* females on male-conditioned plates:  $27.0 \pm 2.5$  days,  $n=30$ ,  $p=0.8306$ .

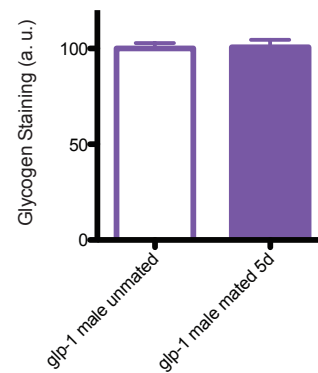
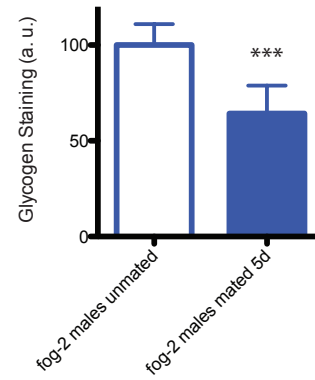
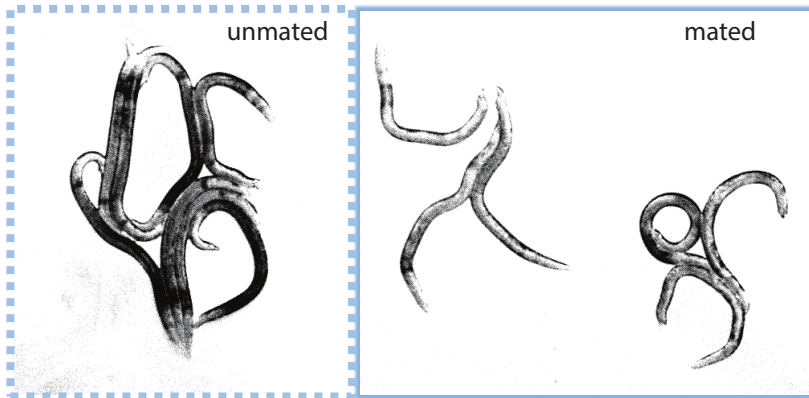
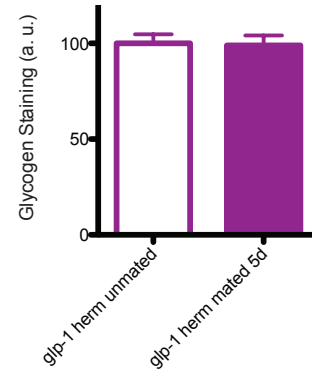
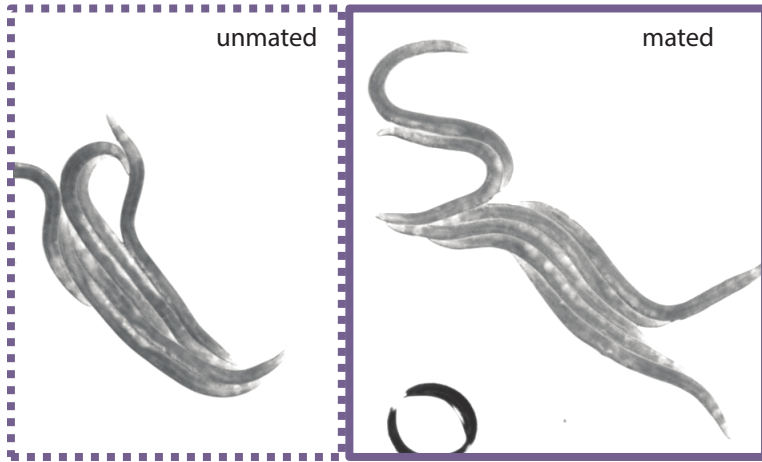
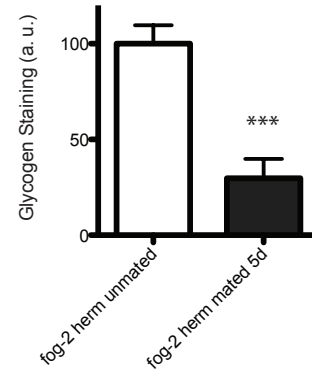
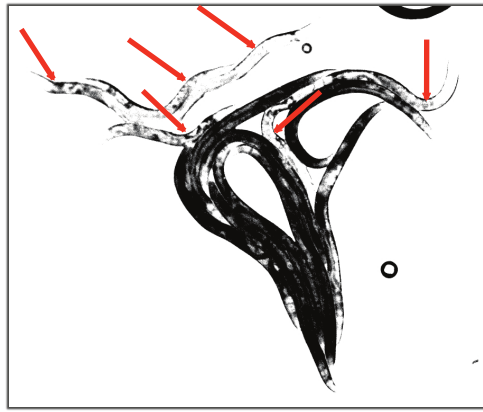
### Figure 7. Average lifespan of Chinese emperors

Average lifespan of promiscuous Chinese emperors ( $34 \pm 2$  yrs) is shorter than that of non-promiscuous emperors ( $52 \pm 1$  yr). See Methods and Table S4 for detailed rationale, description, and data.

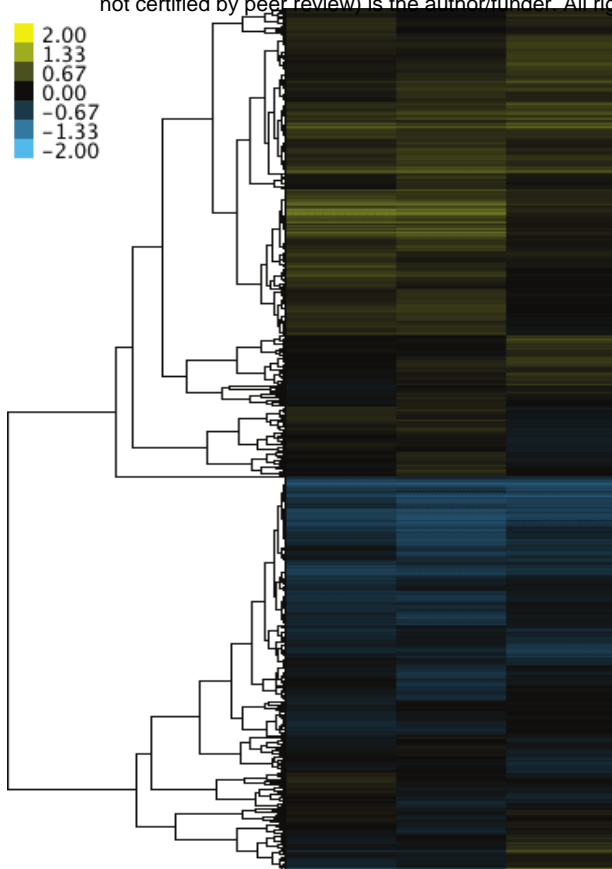
**Figure 8. Simplified model of how mating and male pheromone affect lifespan** in *C. elegans* hermaphrodites (upper left); *C. remanei* females (upper right); *C. elegans* males (lower left); *C. remanei* males (lower right).



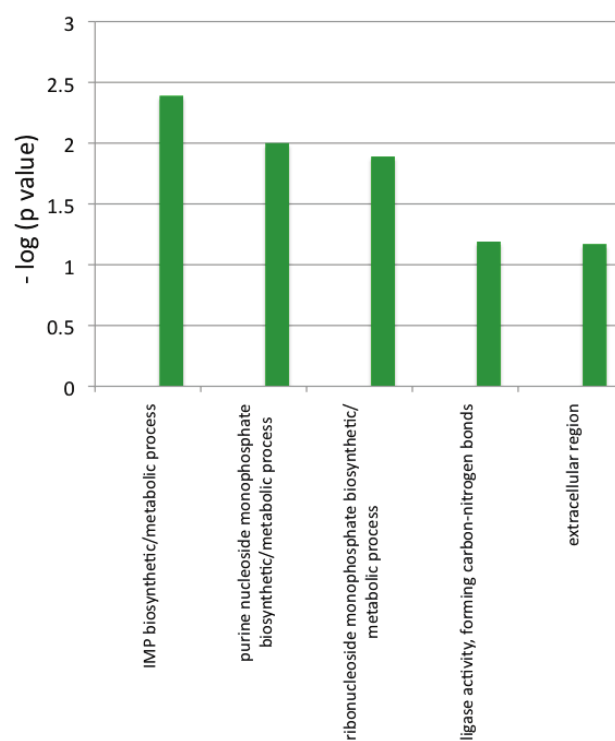




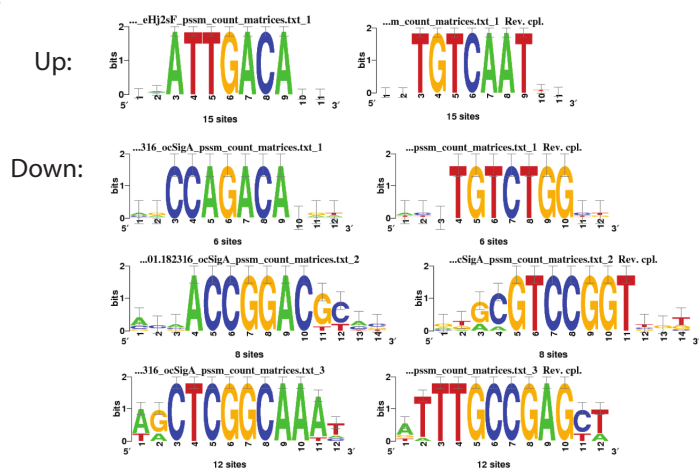
A



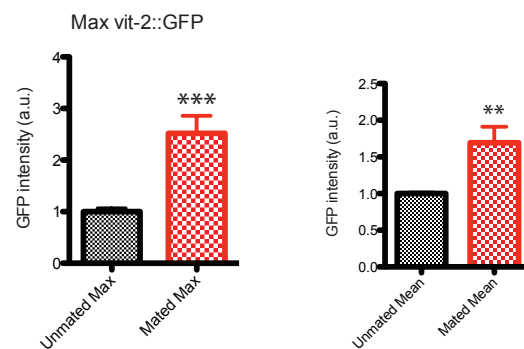
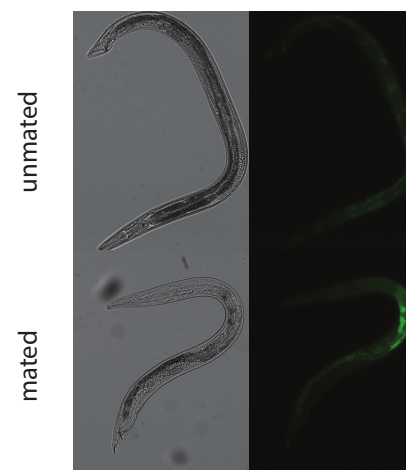
B



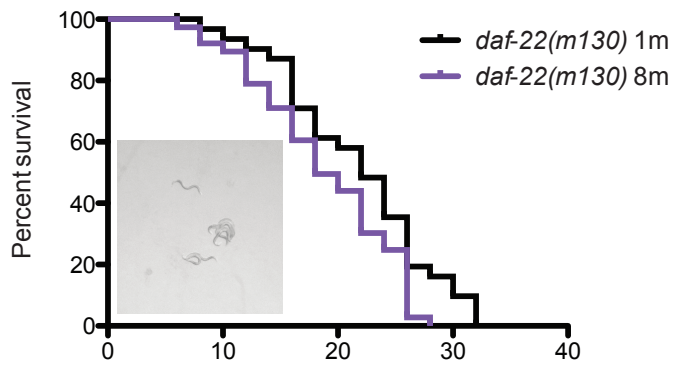
C



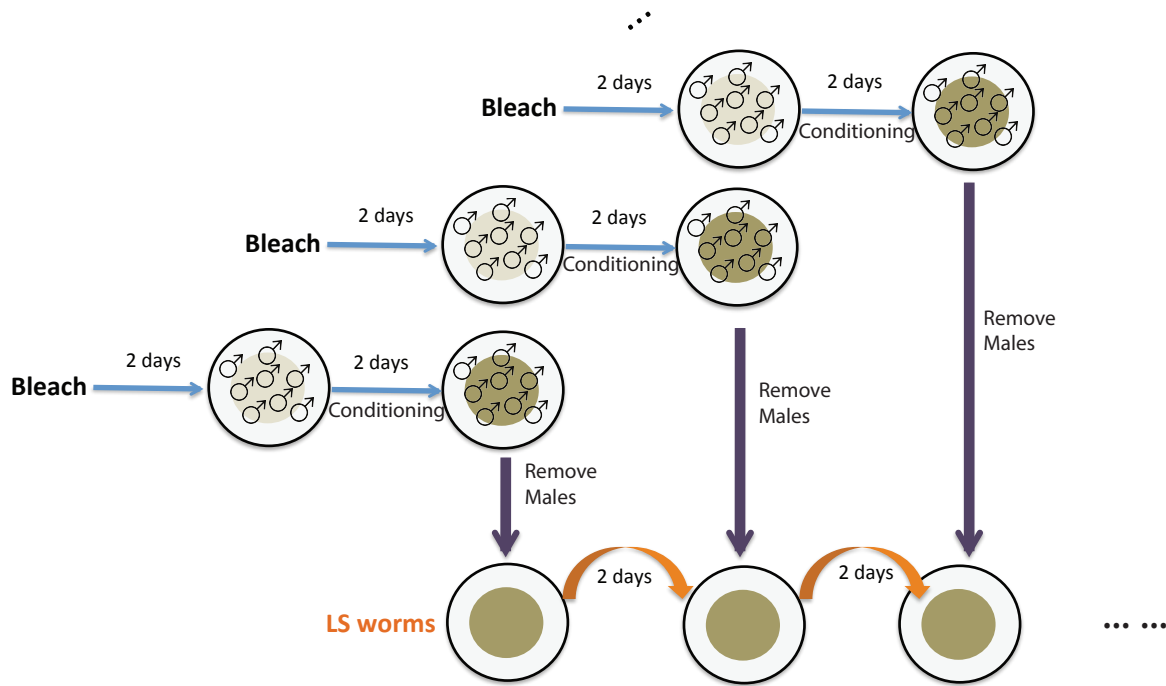
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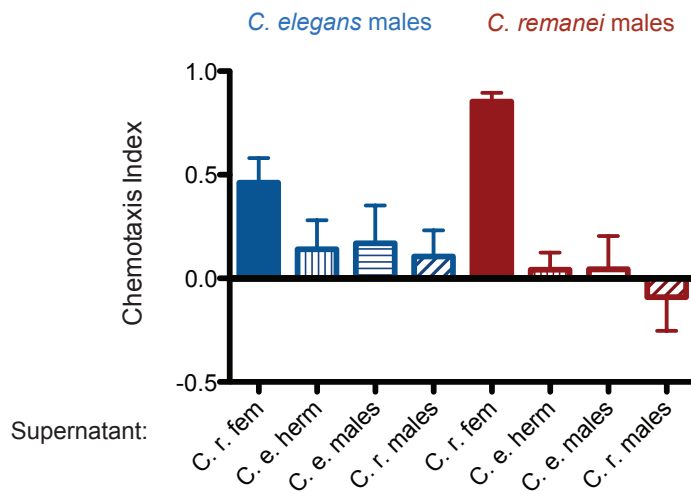
A



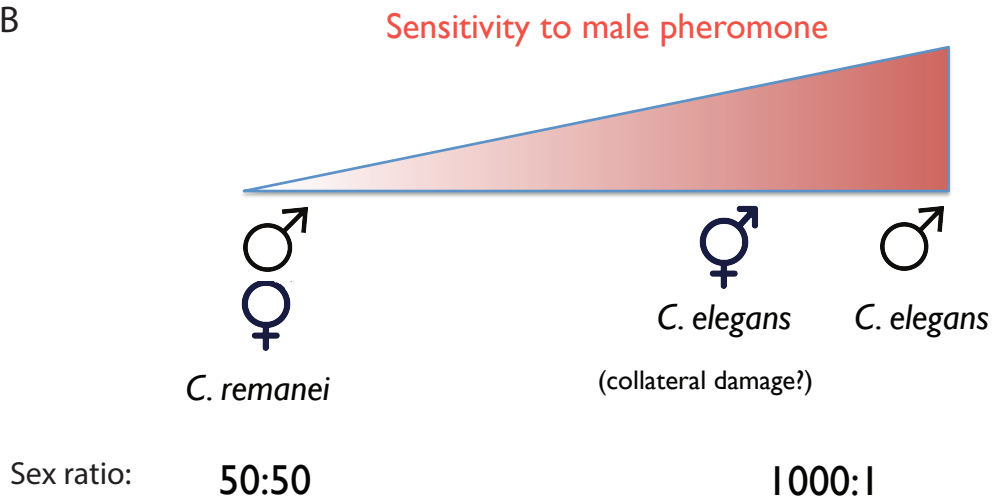
B



A



B



## Supplementary Figures:

### Fig. S1 How mating affects male lifespan.

- (A) Lifespans of one male paired with different number of hermaphrodites during Day 1-3 of adulthood: solitary unmated males:  $10.5 \pm 0.5$  days,  $n=35$ ; one male with one hermaphrodite:  $6.6 \pm 0.2$  days,  $n=33$ ,  $p<0.0001$ ; one male with two hermaphrodites:  $6.3 \pm 0.2$  days,  $n=32$ ,  $p<0.0001$ ; one male with three hermaphrodites:  $6.4 \pm 0.3$  days,  $n=31$ ,  $p<0.0001$ .
- (B) Lifespans of one male paired with different number of hermaphrodites for the first 1.7 days of adulthood: solitary unmated males:  $12.0 \pm 0.4$  days,  $n=40$ ; one male with one hermaphrodite:  $10.6 \pm 0.5$  days,  $n=32$ ,  $p=0.0824$ ; one male with two hermaphrodites:  $9.7 \pm 0.6$  days,  $n=37$ ,  $p=0.0435$ ; one male with three hermaphrodites:  $10.4 \pm 0.6$  days,  $n=36$ ,  $p=0.1575$ ; one male with four hermaphrodites:  $9.3 \pm 0.6$  days,  $n=36$ ,  $p=0.0041$ .
- (C) FUDR can rescue male post-mating early death. Unmated solitary males:  $13.8 \pm 0.7$  days,  $n=35$ ; one male with one hermaphrodite for six days:  $10.3 \pm 0.6$  days,  $n=31$ ,  $p=0.0006$ ; solitary male in the presence of  $50 \mu\text{M}$  FUDR:  $13.9 \pm 0.4$  days,  $n=35$ ,  $p=0.4079$  (compared to unmated solitary group). One male mating with one hermaphrodites for 6 days in the presence of FUDR:  $13.6 \pm 0.5$  days,  $n=34$ ,  $p=0.3992$  (compared to unmated solitary group).
- (D) Lifespans of males paired with three hermaphrodites for 3 days with FUDR: unmated solitary males:  $13.8 \pm 0.7$  days,  $n=35$ ; one male with three hermaphrodites for three days:  $10.6 \pm 0.8$  days,  $n=34$ ,  $p=0.0147$ ; one male with three hermaphrodites for three days but in the presence of  $50 \mu\text{M}$  FUDR during the three days' mating:  $14.3 \pm 0.7$  days,  $n=32$ ,  $p=0.8740$  compared with unmated solitary group.
- (E) Lifespans of unmated and mated *glp-1(e2141)* males: unmated solitary *glp-1* males:  $11.1 \pm 1.0$  days,  $n=27$ ; mated *glp-1* males:  $11.1 \pm 0.5$  days,  $n=43$ ,  $p=0.9149$ . The assay was performed at  $25^\circ\text{C}$ , in mated group, one *glp-1* male was paired with one *fog-2* hermaphrodite from Day 1-6.

### Fig. S2 Glycogen staining of mated vs unmated hermaphrodites and males.

Left: representative pictures of iodine staining of worms. Unmated worms are framed by dashed lines,

whereas mated worms are framed by solid lines. In the first picture, mated and unmated *fog-2* hermaphrodites were mixed together, with red arrows pointing to mated *fog-2* hermaphrodites. Worms were mated from Day 1 – Day 5 and were imaged on Day 5.

Right: quantitation of iodine staining. The intensity of mated worms was normalized to unmated control of the same genotype. Mated *fog-2* hermaphrodites have 30% glycogen compared to unmated *fog-2* hermaphrodites of the same age ( $p<0.0001$ ). Mated *glp-1* hermaphrodites have 99% glycogen compared to unmated *glp-1* hermaphrodites control ( $p=0.6070$ ). Mated *fog-2* males have 64% glycogen compared to unmated *fog-2* males of the same age ( $p<0.0001$ ). Mated *glp-1* males have 101% glycogen compared to unmated *glp-1* males control ( $p=0.7107$ ). Error bars represent SD. \*\*\*,  $p<0.0001$ , t-test.

### Fig. S3 Microarray analysis of mated males.

- (A) Expression heat map of clustered mated males vs unmated males. Individual males were paired with one hermaphrodite for 3.5 days and collected on Day 4 for microarrays.
- (B) Enriched GO terms for significantly down-regulated genes in mated males.
- (C) Enriched motifs in promoter region (1kb upstream of TSS) of significantly up- and down-regulated genes using (RSAT) Regulatory Sequence Analysis Tools ([www.rsat.eu](http://www.rsat.eu)).
- (D) VIT-2::GFP expression in males increases significantly after mating. Upper: DIC and GFP images; Lower: GFP intensity quantitation, left:  $\text{max} \pm \text{SE}$  (error bars); right:  $\text{mean} \pm \text{SE}$  (error bars), a.u., arbitrary units. \*\*,  $p<0.01$ , t-test.

### Fig. S4 Male pheromone and male-conditioned plates (MCP).

- (A) Lifespans of grouped *daf-22(m130)* males. Solitary males:  $21.7 \pm 1.2$  days,  $n=32$ ; eight males:  $18.8 \pm 1.0$

days, n=38, p=0.0394. Inset: *daf-22(m130)* males also form clumps.

- (B) Schematic illustration of how lifespan assays on male-conditioned plates were performed. Detailed description is included in Methods.

**Fig. S5. Male chemotaxis to different pheromones.**

- (A) Supernatant solutions from *C. elegans* males, *C. remanei* males, *C. elegans* N2 hermaphrodites, and *C. remanei* females are used to do the chemotaxis assay. See Methods for detailed description. *C. e.* males to supernatant of *C. r.* females: Chemotaxis Index (CI) is  $0.46 \pm 0.11$  ( mean  $\pm$  SEM, n=12 [plates]); *C. e.* males to supernatant of *C. e.* hermaphrodites: CI =  $0.14 \pm 0.13$  (n=10); *C. e.* males to supernatant of *C. e.* males: CI =  $0.17 \pm 0.17$  (n=12); *C. e.* males to supernatant of *C. r.* males: CI =  $0.11 \pm 0.12$  (n=11); *C. r.* males to supernatant of *C. r.* females: CI =  $0.85 \pm 0.04$  (n=12); *C. r.* males to supernatant of *C. e.* hermaphrodites: CI =  $0.04 \pm 0.08$  (n=12); *C. r.* males to supernatant of *C. e.* males: CI =  $0.04 \pm 0.15$  (n=12); *C. r.* males to supernatant of *C. r.* males: CI =  $-0.09 \pm 0.16$  (n=12).
- (B) Toxicity scale of sensitivity to male pheromone.

## Supplementary Tables:

### Table S1. Lifespan assays summary

Genotype/condition	Mean LS $\pm$ std. error	% change	p value	N	Related Figure
<b>Experiment 1</b>					
1m <i>fog-2</i>	13.1 $\pm$ 0.6	--	--	50	Fig. 1A
1f1m_6d <i>fog-2</i>	8.3 $\pm$ 0.4	-37%	<0.0001	34	Fig. 1A
1f1m_1.5d <i>fog-2</i>	11.9 $\pm$ 0.6	-9%	0.0988	38	
2m <i>fog-2</i>	11.4 $\pm$ 0.5	-13%	0.0149	48	
4m <i>fog-2</i>	9.3 $\pm$ 0.6	-29%	<0.0001	48	
<b>Experiment 2</b>					
1m <i>fog-2</i>	10.9 $\pm$ 0.6	--	--	35	Fig. 1D
1f1m_1d <i>fog-2</i>	11.4 $\pm$ 0.6	+5%	0.3697	31	Fig. 1D
1f1m_2d <i>fog-2</i>	9.0 $\pm$ 0.6	-17%	0.0325	30	Fig. 1D
1f1m_3d <i>fog-2</i>	9.1 $\pm$ 0.6	-17%	0.0452	34	Fig. 1D
1f1m_4d <i>fog-2</i>	7.9 $\pm$ 0.5	-28%	0.0002	32	Fig. 1D
1f1m_5d <i>fog-2</i>	8.3 $\pm$ 0.4	-24%	0.0006	34	Fig. 1D
1f1m_6d <i>fog-2</i>	6.8 $\pm$ 0.3	-38%	<0.0001	33	Fig. 1D
<b>Experiment 3</b>					
1m <i>fog-2</i>	13.8 $\pm$ 0.7	--	--	35	Fig. 1E
1f1m_3d <i>fog-2</i>	10.8 $\pm$ 0.6	-22%	0.0175	32	Fig. 1E
2f1m_3d <i>fog-2</i>	11.6 $\pm$ 0.9	-16%	0.1435	33	Fig. 1E
3f1m_3d <i>fog-2</i>	10.6 $\pm$ 0.8	-23%	0.0147	34	Fig. 1E
<b>Experiment 4</b>					
1m <i>fog-2</i>	10.5 $\pm$ 0.5	--	--	35	Fig. S1A
1f1m_3d <i>fog-2</i>	6.6 $\pm$ 0.2	-37%	<0.0001	33	Fig. S1A
2f1m_3d <i>fog-2</i>	6.3 $\pm$ 0.2	-40%	<0.0001	32	Fig. S1A
3f1m_3d <i>fog-2</i>	6.4 $\pm$ 0.3	-39%	<0.0001	31	Fig. S1A
<b>Experiment 5</b>					
1m <i>fog-2</i>	12.0 $\pm$ 0.4	--	--	40	Fig. S1B
1f1m_1.7d <i>fog-2</i>	10.6 $\pm$ 0.5	-12%	0.0824	32	Fig. S1B
2f1m_1.7d <i>fog-2</i>	9.7 $\pm$ 0.6	-19%	0.0435	37	Fig. S1B
3f1m_1.7d <i>fog-2</i>	10.4 $\pm$ 0.6	-13%	0.1575	36	Fig. S1B
4f1m_1.7d <i>fog-2</i>	9.3 $\pm$ 0.6	-23%	0.0041	36	Fig. S1B
<b>Experiment 6</b>					
1m <i>fog-2</i>	13.8 $\pm$ 0.7	--	--	35	Fig. 1F
3f1m_3d(D1-3) <i>fog-2</i>	10.6 $\pm$ 0.8	-23%	0.0147	34	Fig. 1F
3f1m_3d(D6-8) <i>fog-2</i>	10.8 $\pm$ 0.6	-22%	0.0022	37	Fig. 1F
<b>Experiment 7</b>					
1m <i>fog-2</i>	12.1 $\pm$ 0.6	--	--	32	Fig. 2A
1f1m_6d <i>daf-22</i> x <i>fog-2</i>	9.0 $\pm$ 0.4	-26%	0.0001	29	Fig. 2A
<b>Experiment 8</b>					
1m <i>daf-22</i>	13.8 $\pm$ 0.6	--	--	40	Fig. 2B
1f1m_6d <i>fog-2</i> x <i>daf-22</i>	7.4 $\pm$ 0.4	-46%	<0.0001	34	Fig. 2B
<b>Experiment 9</b>					
1m <i>fog-2</i>	10.5 $\pm$ 0.5	--	--	35	Fig. 2C
3f1m_3d <i>fog-2</i>	6.4 $\pm$ 0.3	-39%	<0.0001	31	Fig. 2C

3f1m_3d+FUdR <i>fog-2</i>	10.2 ± 0.4	-3%	0.7086	36	Fig. 2C
<b>Experiment 10</b>					
1m <i>fog-2</i>	13.8 ± 0.7	--	--	35	Fig. S1C
1f1m_6d <i>fog-2</i>	10.3 ± 0.6	-25%	0.0006	31	Fig. S1C
1m+FUdR <i>fog-2</i>	13.9 ± 0.4	+1%	0.4079	35	Fig. S1C
1f1m_6d+FUdR <i>fog-2</i>	13.6 ± 0.5	-1%	0.3992	34	Fig. S1C
3f1m_3d <i>fog-2</i>	10.6 ± 0.8	-23%	0.0147	34	Fig. S1D
3f1m_3d+FUdR <i>fog-2</i>	14.3 ± 0.7	+4%	0.8740	32	Fig. S1D
<b>Experiment 11 @26°C</b>					
1m <i>glp-1</i>	8.0 ± 0.4	--	--	40	Fig. 2D
1f1m_6d <i>fog-2</i> x <i>glp-1</i>	7.2 ± 0.4	-10%	0.3178	40	Fig. 2D
<b>Experiment 12 @25°C</b>					
1m <i>glp-1</i>	11.1 ± 1.0	--	--	27	Fig. S1E
1f1m_6d <i>fog-2</i> x <i>glp-1</i>	11.1 ± 0.5	0%	0.9149	43	Fig. S1E
<b>Experiment 13 @26°C</b>					
1m <i>glp-1</i>	9.6 ± 0.4	--	--	40	
1f1m_6d <i>fog-2</i> x <i>glp-1</i>	8.8 ± 0.5	-8%	0.238	40	
<b>Experiment 14</b>					
1m <i>pqm-1</i>	11.9 ± 0.5	--	--	25	Fig. 3E
1f1m_6d <i>fog-2</i> x <i>pqm-1</i>	11.0 ± 0.6	-8%	0.2782	29	Fig. 3E
<b>Experiment 15</b>					
1m <i>fog-2</i> (L4440)	12.6 ± 0.7	--	--	25	Fig. 3F
1f1m_4d <i>fog-2</i> (L4440)	8.8 ± 0.5	-30%	0.0001	33	Fig. 3F
1m <i>fog-2</i> ( <i>unc-62i</i> )	11.9 ± 0.8	--	--	25	Fig. 3F
1f1m_4d <i>fog-2</i> ( <i>unc-62i</i> )	10.6 ± 0.5	-11%	0.1249	34	Fig. 3F
<b>Experiment 16</b>					
1m <i>C. r.</i>	31.4 ± 1.7	--	--	72	Fig. 4A
1f1m_6d <i>C. r.</i>	15.7 ± 1.2	-50%	<0.0001	28	Fig. 4A
<b>Experiment 17</b>					
1m <i>C. e.</i>	10.2 ± 0.6	--	--	35	Fig. 4B
1m1f_6d <i>C. e.</i> x <i>C. e.</i>	7.4 ± 0.4	-27%	0.0001	35	Fig. 4B
1m1f_6d <i>C. r.</i> x <i>C. e.</i>	7.4 ± 0.4	-27%	0.0003	35	Fig. 4B
<b>Chinese Emperors LS</b>					
Normal	52.3 ± 1.0	--	--	234	Fig. 7
Promiscuous	34.0 ± 1.9	-35%	<0.0001	21	Fig. 7
<b>Experiment 18</b>					
1m <i>fog-2</i>	12.0 ± 0.4	--	--	40	Fig. 5A
2m <i>fog-2</i>	10.6 ± 0.4	-12%	0.0397	40	Fig. 5A
4m <i>fog-2</i>	9.9 ± 0.4	-18%	0.0012	60	Fig. 5A
8m <i>fog-2</i>	7.7 ± 0.2	-36%	<0.0001	80	Fig. 5A
<b>Experiment 19</b>					
1m+FUdR <i>fog-2</i>	13.9 ± 0.4	--	--	35	Fig. 5B
8m+FUdR <i>fog-2</i>	12.4 ± 0.3	-11%	0.0032	48	Fig. 5B
1m <i>fog-2</i>	13.8 ± 0.7	--	--	35	Fig. 5E
8m <i>fog-2</i>	9.8 ± 0.5	-29%	<0.0001	48	Fig. 5E
8m <i>daf-22</i>	14.7 ± 0.7	+7%	0.4039	48	Fig. 5E



1m+FUdR <i>daf-22</i>	15.3 ± 0.3	--	--	35	Fig. 5F
8m+FUdR <i>daf-22</i>	14.7 ± 0.3	-4%	0.2117	48	Fig. 5F
1m <i>daf-22</i>	17.2 ± 0.6	--	--	35	
8m <i>daf-22</i>	14.7 ± 0.7	-15%	0.0660	48	
<b>Experiment 20</b>					
1m <i>daf-22</i>	21.7 ± 1.2	--	--	32	Fig. S4A
8m <i>daf-22</i>	18.8 ± 1.0	-13%	0.0394	38	Fig. S4A
<b>Experiment 21</b>					
1m <i>C.r.</i>	37.9 ± 1.1	--	--	120	Fig. 5C
8m <i>C.r.</i>	31.0 ± 0.9	-18%	<0.0001	160	Fig. 5C
<b>Experiment 22</b>					
1m <i>C.r.</i> + FUdR	30.8 ± 0.9	--	--	45	Fig. 5D
8m <i>C.r.</i> + FUdR	30.8 ± 0.5	0%	0.9217	112	Fig. 5D
<b>Experiment 23</b>					
1m <i>daf-22</i> ctrl	23.0 ± 0.9	--	--	30	Fig. 5G
1m <i>daf-22</i> ≤1mMCP	17.3 ± 0.7	-25%	<0.0001	29	Fig. 5G
1m <i>daf-22</i> ≤8mMCP	16.1 ± 0.6	-30%	<0.0001	30	Fig. 5G
<b>Experiment 24</b>					
<i>C.e. herm</i> ctrl	14.4 ± 0.8	--	--	90	Fig. 6A
<i>C.e. herm</i> ≤ <i>C.e.</i> MCP	10.9 ± 0.6	-24%	0.0004	60	Fig. 6A
<i>C.e. herm</i> ≤ <i>C.r.</i> MCP	11.9 ± 0.5	-17%	0.0042	90	Fig. 6A
<i>C.r. fem</i> ctrl	15.8 ± 0.9	--	--	60	Fig. 6B
<i>C.r. fem</i> ≤ <i>C.r.</i> MCP	19.5 ± 1.3	+23%	0.0636	30	Fig. 6B
<i>C.r. fem</i> ≤ <i>C.e.</i> MCP	18.5 ± 0.9	+17%	0.1770	60	Fig. 6B
<b>Experiment 25</b>					
1m <i>C. e.</i>	12.1 ± 0.6	--	--	30	Fig. 6C
1m ≤8mMCP <i>C. e.</i>	9.8 ± 0.4	-19%	0.0046	28	Fig. 6C
1f <i>C. e.</i>	13.8 ± 0.7	--	--	30	Fig. 6C
1f ≤8mMCP <i>C. e.</i>	12.6 ± 0.9	-9%	0.5965	29	Fig. 6C
<b>Experiment 26</b>					
1m <i>C. r.</i>	35.8 ± 2.0	--	--	34	Fig. 6D
1m ≤8mMCP <i>C. r.</i>	37.8 ± 1.2	+6%	0.8501	34	Fig. 6D
1f <i>C. r.</i>	27.6 ± 2.2	--	--	24	Fig. 6D
1f ≤8mMCP <i>C. r.</i>	27.0 ± 2.5	-2%	0.8306	30	Fig. 6D

**Table S2. Body size measurements**

<b>Genotype/ condition</b>	<b>N</b>		<b>Day 1</b>	<b>Day 2</b>	<b>Day 3</b>	<b>Day 4</b>	<b>Day 5</b>	<b>Day 6</b>	<b>Day 7</b>
<i>fog-2(q71)</i> unmated	31	<b>Body length ± SE (µm)</b>	903.6 ± 5.0	961.9 ± 5.6	982.8 ± 7.2	976.2 ± 7.2	959.0 ± 7.4	935.9 ± 8.1	931.5 ± 10.3
<i>fog-2(q71)</i> mated	30		893.8 ± 5.1	938.2 ± 5.6	955.4 ± 7.1	941.0 ± 9.5	913.7 ± 14.0	855.9 ± 19.4	851.4 ± 24.5
@20°C		<b>p value</b>	0.1813	0.004	0.0088	0.0044	0.0055	0.0004	0.0015
		<b>% change</b>	-1.1%	-2.5%	-2.8%	-3.6%	-4.7%	-8.6%	-8.6%
<i>glp-1(e2141)</i> unmated	40	<b>Body length ± SE (µm)</b>	789.6 ± 11.3	880.1 ± 6.4	894.0 ± 7.1	895.1 ± 7.3	888.8 ± 7.9	872.0 ± 8.8	874.3 ± 9.0
<i>glp-1(e2141)</i> mated	40		789.6 ± 11.3	887.0 ± 5.9	892.7 ± 8.1	894.0 ± 6.7	888.1 ± 10.8	868.6 ± 12.0	857.5 ± 11.4
@26°C		<b>p value</b>	-	0.4282	0.9083	0.9093	0.9567	0.8173	0.2510
		<b>% change</b>	-	0.8%	-0.1%	-0.1%	-0.1%	-0.4%	-1.9%

**Table S3. Mated males microarray SAM rank table**

A. up-regulated genes (compared to unmated control)

Rank	Sequence Name	Gene Name	Ave. fold change	SAM score	Gene description
1	F59D8.2	vit-4	18.13	13.72	vit-4 is predicted to have lipid transporter activity, based on protein domain information.
2	F59D8.1	vit-3	35.02	13.09	vit-3 encodes a vitellogenin, a precursor of the lipid-binding protein related to vertebrate vitellogenins and mammalian ApoB-100, a core LDL particle constituent (OMIM:107730); VIT-3 is a major yolk component, but as loss of VIT-3 activity via RNA-mediated interference (RNAi) does not result in any abnormalities, VIT-3 likely functions redundantly with other vitellogenins to provide essential nutrients to the developing embryo; VIT-3 is expressed exclusively in the adult hermaphrodite intestine, from which it is secreted into the pseudocoelomic space and finally taken up by oocytes; in males, vit-3 expression may be negatively regulated by MAB-3, a DM binding domain-containing transcription factor required for male sexual development.
3	C04F6.1	vit-5	13.99	11.49	vit-5 encodes a vitellogenin, a lipid-binding protein precursor related to vertebrate vitellogenins and mammalian ApoB-100, a core LDL particle constituent; by homology, VIT-5 is predicted to function as a lipid transport protein; loss of vit-5 activity via large-scale RNA-mediated interference (RNAi) screens indicates that VIT-5 is required for embryogenesis and normal rates of postembryonic growth; VIT-5 is a major yolk component and is expressed exclusively in the adult hermaphrodite intestine from which it is secreted into the pseudocoelomic space and taken up by oocytes.
4	K12H6.5	K12H6.5	6.62	10.59	
5	K07H8.6	vit-6	21.26	10.35	vit-6 encodes a vitellogenin

					precursor protein that is cleaved in the body cavity into two smaller yolk proteins, YP115 and YP88; in <i>C. elegans</i> , vitellogenin genes exhibit stage-, sex-, and tissue-specific expression being expressed exclusively in the adult hermaphrodite intestine.
6	F56H6.2	F56H6.2	4.52	8.25	
7	Y46H3A.5	Y46H3A.5	4.08	8.06	
8	C16C8.10	C16C8.10	3.94	7.29	
9	F56D2.8	F56D2.8	5.03	6.73	
10	F40G9.15	F40G9.15	3.48	6.55	
11	C45G7.2	ilys-2	2.85	6.28	ilys-2 is involved in defense response to Gram-positive bacterium; ilys-2 is predicted to have lysozyme activity, based on protein domain information.
12	T10D4.7	T10D4.7	4.01	6.27	
13	C42D8.2	vit-2	13.96	6.22	vit-2 encodes the vitellogenin homolog YP170; vit-2 is expressed in the adult hermaphrodite intestine and VIT-2 is secreted into the pseudocoelomic space before being taken up by developing oocytes; vit-2 expression is regulated in a sex-, stage-, and tissue-specific manner by the ELT-2/GATA and MAB-3 transcription factors.
14	C39B5.10	C39B5.10	4.81	6.18	

#### B. down-regulated genes (compared to unmated control)

Rank	Sequence Name	Gene Name	Ave. fold change	SAM score	Gene description
1	F49E11.6	scl-11	-23.70	-15.20	scl-11 encodes a predicted extracellular protein that is a member of the <i>C. elegans</i> family of SCP/TAPS domain-containing proteins.
2	R04B5.6	R04B5.6	-8.11	-10.76	R04B5.6 encodes one of two <i>C. elegans</i> sorbitol dehydrogenase orthologs; by homology the product of R04B5.6 is predicted to catalyze the reversible oxidation of sorbitol to fructose in the presence of NAD <sup>+</sup> ; in the embryo, an R04B5.6::gfp fusion is expressed in pharyngeal cells and head neurons.
3	ZK355.3	ZK355.3	-15.78	-10.63	
4	T13B5.5	lips-11	-9.76	-9.88	lips-11 is predicted to have

					hydrolase activity, based on protein domain information.
5	H10D18.2	scl-12	-5.82	-9.38	scl-12 encodes a predicted extracellular protein that is a member of the C. elegans family of SCP/TAPS domain-containing proteins.
6	T13B5.6	lips-12	-5.04	-9.36	lips-12 is predicted to have hydrolase activity, based on protein domain information.
7	Y6E2A.4	Y6E2A.4	-5.22	-9.26	
8	K02E7.6	K02E7.6	-7.29	-9.18	
9	F56D6.8	F56D6.8	-10.08	-8.80	
10	W10G11.15	clec-129	-10.03	-8.00	
11	F56D6.9	F56D6.9	-22.94	-7.93	
12	F38B6.4	F38B6.4	-4.20	-7.49	F38B6.4 is an ortholog of human GART (phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase); F38B6.4 is predicted to have phosphoribosylamine-glycine ligase activity, phosphoribosylformylglycinamide cyclo-ligase activity, phosphoribosylglycinamide formyltransferase activity, and ATP binding activity, based on protein domain information.
13	Y22F5A.5	lys-2	-8.30	-7.46	lys-2 is one of ten C. elegans lysozyme genes; as such, lys-2 can be predicted to have a role in lysozymal function including immune function.
14	T01C3.11	T01C3.11	-4.25	-7.33	
15	F46B3.14	F46B3.14	-4.45	-7.16	
16	F45D11.4	F45D11.4	-4.15	-6.70	F45D11.4, with F45D11.2 and F45D11.3, encodes a nematode-specific protein that entirely consists of one large (~300-residue) 'domain of unknown function' (DUF684) that is found in several other C. elegans proteins; a transcription unit of either F45D11.4, F45D11.2, or F45D11.3 (genes of essentially identical sequence) has a natural nonsense transcript that is up-regulated in vivo by smg[-] mutations, indicating that at least one of these three genes is a natural substrate for SMG-mediated nonsense suppression; since several other natural mRNA substrates of SMG

					suppression (e.g., rpl-3, rpl-8, rpl-10a, rpl-12) have protein products that are involved in translation, F45D11.4 protein may function in translation as well.
17	F58E10.7	F58E10.7	-4.01	-6.33	
18	F32B4.6	F32B4.6	-7.31	-6.19	F32B4.6 is an ortholog of human ABHD11 (abhydrolase domain containing 11).
19	Y116A8C.44	Y116A8C.44	-5.04	-6.12	
20	C52D10.1	C52D10.1	-2.97	-6.09	
21	F36G9.7	F36G9.7	-3.71	-6.06	
22	F45D11.15	F45D11.15	-7.75	-6.04	
23	EGAP7.1	dpy-3	-2.52	-6.04	dpy-3 encodes a cuticular collagen; along with dpy-2, dpy-7, dpy-8, and dpy-10, dpy-3 is required postembryonically for annular furrow formation and/or maintenance; specifically, DPY-3 activity is required for proper assembly of the DPY-7 collagen into the mature extracellular matrix; during each cuticle synthetic period, dpy-3 mRNA is expressed approximately four hours prior to the secretion of new cuticle.
24	C17B7.12	C17B7.12	-3.47	-6.04	
25	C32B5.9	fbxc-7	-4.85	-6.03	
26	T13B5.7	lips-13	-5.67	-5.99	lips-13 is predicted to have hydrolase activity, based on protein domain information.
27	F35E8.10	F35E8.10	-3.41	-5.95	
28	B0286.3	B0286.3	-3.43	-5.95	B0286.3 is an ortholog of human PAICS (phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase); B0286.3 is predicted to have ATP binding activity, based on protein domain information.
29	T01B10.1	grd-4	-4.72	-5.91	grd-4 encodes a hedgehog-like protein, with an N-terminal signal sequence and a C-terminal Ground domain; the Ground domain is predicted to form a cysteine-crosslinked protein involved in intercellular signalling, and it has subtle similarity to the N-terminal Hedge domain of HEDGEHOG proteins; GRD-4 is weakly required for normal molting; GRD-4 is also required for normal adult alae formation, growth to full size, and

					locomotion; all of these requirements may reflect common defects in cholesterol-dependent hedgehog-like signalling or in vesicle trafficking.
30	T22B7.7	T22B7.7	-7.06	-5.90	T22B7.7 is an ortholog of human ACOT9 (acyl-CoA thioesterase 9).
31	C07A9.9	C07A9.9	-3.02	-5.89	
32	C48B4.1	C48B4.1	-3.08	-5.82	C48B4.1 is an ortholog of human ACOX2 (acyl-CoA oxidase 2, branched chain) and ACOX1 (acyl-CoA oxidase 1, palmitoyl); C48B4.1 is predicted to have acyl-CoA dehydrogenase activity, acyl-CoA oxidase activity, and flavin adenine dinucleotide binding activity, based on protein domain information.
33	F45D11.1	F45D11.1	-5.05	-5.73	
34	C03G6.5	C03G6.5	-2.73	-5.70	
35	C10H11.5	ugt-27	-4.36	-5.65	ugt-27 is an ortholog of human UGT3A2 (UDP glycosyltransferase 3 family, polypeptide A2) and UGT3A1 (UDP glycosyltransferase 3 family, polypeptide A1); ugt-27 is predicted to have transferase activity, transferring hexosyl groups, based on protein domain information.
36	C09C7.1	zig-4	-2.52	-5.65	zig-4 encodes a predicted secreted protein that is a member of the immunoglobulin superfamily of proteins; ZIG-4 activity is required for maintenance of ventral nerve cord organization: the AVKL/R and PVQL/R axons of the left and right ventral nerve cords do not maintain their proper spatial positions and drift into the opposite cord; a zig-4::gfp reporter fusion is expressed in the PVT, ASK, BAG, and M2 neurons, with expression also seen during the L1 stage in pharyngeal mesoderm and ectoderm.
37	F18E3.12	F18E3.12	-3.26	-5.65	
38	C33G8.3	C33G8.3	-7.08	-5.62	
39	C23G10.6	C23G10.6	-3.23	-5.57	C23G10.6 is an ortholog of human UGT3A2 (UDP glycosyltransferase 3 family, polypeptide A2) and UGT3A1 (UDP glycosyltransferase 3 family, polypeptide A1); C23G10.6 is predicted to have

					transferase activity, transferring hexosyl groups, based on protein domain information.
40	Y53F4B.32	gst-29	-2.79	-5.54	gst-29 is an ortholog of human HPGDS (hematopoietic prostaglandin D synthase).
41	Y32G9A.5	Y32G9A.5	-2.69	-5.38	



**Table S4. List of Chinese Emperors**

	Non-promiscuous Emperors, natural death
	Non-promiscuous Emperors, unnatural causes
	Promiscuous Emperors, natural death
	Promiscuous Emperors, unnatural causes

	Name	Age at death	Years of reign	Year of birth - Year of death
1	秦始皇 嬴政 Qin Shi Huang Ying Zheng	50	37	259-210 BC
2	秦二世 嬴胡亥 Qin Er Shi Ying Huhai	24	3	230-207 BC
3	汉高祖 刘邦 Han Gao Zu Liu Bang	53	8	247-195 BC
4	汉惠帝 刘盈 Han Hui Di Liu Ying	23	7	210-188 BC
5	汉文帝 刘恒 Han Wen Di Liu Heng	46	23	202-157 BC
6	汉景帝 刘启 Han Jing Di Liu Qi	48	16	188-141 BC
7	汉武帝 刘彻 Han Wu Di Liu Che	70	54	156-87 BC
8	汉昭帝 刘弗陵 Han Zhao Di Liu Fuling	21	13	94-74 BC
9	汉宣帝 刘询 Han Xuan Di Liu Xun	45	25	91-49 BC
10	汉元帝 刘奭 Han Yuan Di Liu Shi	42	16	74-33 BC
11	汉成帝 刘骜 Han Cheng Di Liu Ao	45	26	51-7 BC
12	汉哀帝 刘欣 Han Ai Di Liu Xin	26	7	26-1 BC
13	新朝 王莽 Xin Chao Wang Mang	68	15	45 BC-23
14	汉光武帝 刘秀 Han Guang Wu Di Liu Xiu	63	32	6 BC-57
15	汉明帝 刘庄 Han Ming Di Liu Zhuang	48	18	28-75
16	汉章帝 刘炆 Han Zhang Di Liu Da	32	13	57-88
17	汉和帝 刘肇 Han He Di Liu Zhao	27	17	79-105
18	汉安帝 刘祜 Han An Di Liu Hu	32	19	94-125
19	汉顺帝 刘保 Han Shun Di Liu Bao	30	19	115-144
20	汉桓帝 刘志 Han Huan Di Liu Zhi	36	21	132-167
21	汉灵帝 刘宏 Han Ling Di Liu Hong	34	22	156-189
22	汉献帝 刘协 Han Xian Di Liu Xie	54	31	181-234
23	汉昭烈帝 刘备 Han Zhao Lie Di Liu Bei	63	3	161-223
24	蜀汉后主 刘禅 Shu Han Hou Zhu Liu Shan	65	40	207-271
25	魏文帝 曹丕 Wei Wen Di Cao Pi	40	7	187-226
26	魏明帝 曹叡 Wei Ming Di Cao Rui	34	13	205-239
27	魏齐王 曹芳 Wei Qi Wang Cao Fang	43	15	232-274
28	魏高贵乡公 曹髦 Wei Gao Gui Xiang Gong Cao Mao	20	6	241-260
29	魏元帝 曹奂 Wei Yuan Di Cao Huan	58	5	245-302
30	吴大帝 孙权 Wu Da Di Sun Quan	71	24	182-252
31	吴废帝 孙亮 Wu Fei Di Sun Liang	18	6	243-260
32	吴景帝 孙休 Wu Jing Di Sun Xiu	30	6	235-264
33	吴末帝 孙皓 Wu Mo Di Sun Hao	43	16	242-284
34	晋武帝 司马炎 Jin Wu Di Sima Yan	55	25	236-290
35	晋惠帝 司马衷 Jin Hui Di Sima Zhong	48	16	259-307
36	晋怀帝 司马炽 Jin Huai Di Sima Chi	30	5	284-313
37	晋愍帝 司马邺 Jin Min Di Sima Ye	18	4	300-317
38	晋元帝 司马睿 Jin Yuan Di Sima Yuan	47	5	276-323
39	晋明帝 司马绍 Jin Ming Di Sima Shao	27	3	299-325
40	晋成帝 司马衍 Jin Cheng Di Sima Yan	22	17	321-342
41	晋康帝 司马岳 Jin Kang Di Sima Yue	23	2	322-344
42	晋穆帝 司马聃 Jin Mu Di Sima Dan	19	17	343-361

43	晋哀帝 司马丕 Jin Ai Di Sima Pi	25	4	341-365
44	晋废帝 司马奕 Jin Fei Di Sima Yi	45	6	342-286
45	晋简文帝 司马昱 Jin Jian Wen Di Sima Yu	52	1	321-372
46	晋孝武帝 司马曜 Jin Xiao Wu Di Sima Yao	35	24	362-396
47	晋安帝 司马德宗 Jin An Di Sima Dezhong	37	22	382-418
48	晋恭帝 司马德文 Jin Gong Di Sima Dewen	37	2	385-421
49	楚武悼帝 桓玄 Chu Wu Dao Di Huan Xuan	36	1	369-404
50	成武帝 李雄 Cheng Wu Di Li Xiong	61	30	274-334
51	成幽公 李期 Cheng You Gong Li Qi	26	4	314-338
52	汉昭文帝 李寿 Han Zhao Wen Di Li Shou	44	7	300-343
53	后赵明帝 石勒 Hou Zhao Ming Di Shi Le	60	15	274-333
54	后赵海阳王 石弘 Hou Zhao Hai Yang Wang Shi Hong	22	1	314-335
55	后赵武帝 石虎 Hou Zhao Wu Di Shi Hu	55	15	295-349
56	前燕文明帝 慕容皝 Qian Yan Wen Ming Di Murong Huang	52	12	297-348
57	前燕景昭帝 慕容儁 Qian Yan Jing Zhao Di Murong Jun	42	12	319-360
58	前燕幽帝 慕容暉 Qian Yan You Di Murong Wei	35	10	350-384
59	西燕威帝 慕容冲 Xi Yan Wei Di Murong Chong	28	1	359-386
60	后燕成武帝 慕容垂 Hou Yan Cheng Wu Di Murong Chui	71	12	326-396
61	后燕惠愍帝 慕容宝 Hou Yan Hui Min Di Murong Bao	44	2	355-398
62	后燕昭武帝 慕容盛 Hou Yan Zhao Wu Di Murong Sheng	29	3	373-401
63	后燕昭文帝 慕容熙 Hou Yan Zhao Wen Di Murong Xi	23	6	385-407
64	南燕献武帝 慕容德 Nan Yan Xian Wu Di Murong De	70	7	336-405
65	南燕末主 慕容超 Nan Yan Hou Zhu Murong Chao	26	5	385-410
66	后凉懿武帝 吕光 Hou Liang Yi Wu Wang Lv Guang	62	13	338-399
67	前秦惠武帝 苻洪 Qian Qin Hui Wu Di Fu Hong	66	1	285-350
68	前秦明帝 苻健 Qian Qin Ming Di Fu Jian	39	5	317-355
69	前秦厉王 苻生 Qian Qin Li Wang Fu Sheng	23	2	335-357
70	前秦宣昭帝 苻坚 Qian Qin Xuan Zhao Di Fu Jian	48	28	338-385
71	前秦高帝 苻登 Qian Qin Gao Di Fu Deng	52	8	343-394
72	后秦武昭帝 姚苻 Hou Qin Wu Zhao Di Yao Chang	64	9	330-393
73	后秦文桓帝 姚兴 Hou Qin Wen Huan Di Yao Xing	51	23	366-416
74	后秦末主 姚泓 Hou Qin Mo Zhu Yao Hong	30	1	388-417
75	夏武烈帝 赫连勃勃 Xia Wu Lie Di Helian Bobo	45	18	381-425
76	宋武帝 刘裕 Song Wu Di Liu Yu	60	2	363-422
77	宋少帝 刘义符 Song Shao Di Liu Yifu	19	2	406-424
78	宋文帝 刘义隆 Song Wen Di Liu Yilong	47	29	407-453
79	宋孝武帝 刘骏 Song Xiao Wu Di Liu Jun	35	11	430-464
80	宋明帝 刘彧 Song Ming Di Liu Yu	34	7	439-472
81	齐高帝 萧道成 Qi Gao Di Xiao Daocheng	56	4	427-482
82	齐武帝 萧赜 Qi Wu Di Xiao Ze	54	11	440-493
83	齐郁林王 萧昭业 Qi Yu Lin Wang Xiao Zhaoye	22	1	473-494
84	齐明帝 萧鸾 Qi Ming Di Xiao Luan	47	4	452-498
85	齐东昏侯 萧宝卷 Qi Dong Hun Gou Xiao Baojuan	19	3	483-501
86	梁武帝 萧衍 Liang Wu Di Xiao Yan	86	48	464-549
87	梁简文帝 萧纲 Liang Jian Wen Di Xiao Gang	49	2	503-551
88	梁元帝 萧绎 Liang Yuan Di Xiao Yi	47	3	508-554
89	梁宣帝 萧誉 Liang Xuan Di Xiao Cha	44	8	519-562
90	梁明帝 萧岿 Liang Ming Di Xiao Kui	44	23	542-585
91	陈武帝 陈霸先 Chen Wu Di Chen Baxian	57	3	503-559
92	陈文帝 陈蒨 Chen Wen Di Chen Qian	45	7	522-566
93	陈宣帝 陈顼 Chen Xuan Di Chen Xu	53	14	530-582
94	陈后主 陈叔宝 Chen Hou Zhu Chen Shubao	52	7	553-604

95	北魏道武帝 拓跋珪 Bei Wei Dao Wu Di Tuoba Gui	39	24	371-409
96	北魏明元帝 拓跋嗣 Bei Wei Ming Yuan Di Tuoba Si	32	14	392-423
97	北魏太武帝 拓跋焘 Bei Wei Tai Wu Di Tuoba Tao	45	29	408-452
98	北魏文成帝 拓跋濬 Bei Wei Wen Cheng Di Tuoba Jun	26	13	440-465
99	北魏献文帝 拓跋弘 Bei Wei Xian Wen Di Tuoba Hong	23	11	454-476
100	北魏孝文帝 元宏 Bei Wei Xiao Wen Di Yuan Hong	33	23	467-499
101	北魏宣武帝 元恪 Bei Wei Xiao Wu Di Yuan Ke	33	16	463-515
102	北魏孝明帝 元诩 Bei Wei Xiao Ming Di Yuan Xu	19	13	510-528
103	北魏孝庄帝 元子攸 Bei Wei Xiao Zhuang Di Yuan Ziyou	24	2	507-530
104	北魏节闵帝 元恭 Bei Wei Jie Min Di Yuan Gong	35	1	498-532
105	北魏安定王 元朗 Bei Wei An Ding Wang Yuan Lang	20	1	513-532
106	北魏孝武帝 元修 Bei Wei Xiao Wu Di Yuan Xiu	25	2	510-534
107	东魏孝静帝 元善见 Dong Wei Xiao Jing Di Yuan Jianshan	28	17	524-551
108	西魏文帝 元宝炬 Xi Wei Wen Di Yuan Baoju	45	17	507-551
109	西魏恭帝 拓跋廓 Xi Wei Gong Di Tuoba Kuo	21	3	537-557
110	北齐文宣帝 高洋 Bei Qi Wen Xuan Di Gao Yang	31	10	529-559
111	北齐孝昭帝 高演 Bei Qi Xiao Zhao Di Gao Yan	27	1	535-561
112	北齐武成帝 高湛 Bei Qi Wu Cheng Di Gao Dan	32	4	537-568
113	北齐后主 高纬 Bei Qi Hou Zhu Gao Wei	21	12	556-577
114	北周明帝 宇文毓 Bei Zhou Ming Di Yuwen Yu	27	3	534-560
115	北周武帝 宇文邕 Bei Zhou Wu Di Yuwen Yong	36	18	543-578
116	北周宣帝 宇文赟 Bei Zhou Xuan Di Yuwen Yun	22	1	559-580
117	隋文帝 杨坚 Sui Wen Di Yang Jian	64	24	541-604
118	隋炀帝 杨广 Sui Yang Di Yang Guang	50	14	569-618
119	唐高祖 李渊 Tang Gao Zu Li Yuan	70	9	566-635
120	唐太宗 李世民 Tang Tai Zong Li Shiming	53	23	597-649
121	唐高宗 李治 Tang Gao Zong Li Zhi	56	34	628-683
122	武则天 武曌 Wu Ze Tian Wu Zhao	82	15	624-705
123	唐中宗 李显 Tang Zhong Zong Li Xian	56	6	656-710
124	唐睿宗 李旦 Tang Rui Zong Li Dan	55	8	662-716
125	唐玄宗 李隆基 Tang Xuan Zong Li Longji	78	44	685-762
126	唐肃宗 李亨 Tang Su Zong Li Heng	52	6	711-762
127	唐代宗 李豫 Tang Dai Zong Li Yu	54	17	726-779
128	唐德宗 李适 Tang De Zong Li Shi	64	26	742-805
129	唐顺宗 李诵 Tang Shun Zong Li Song	46	1	761-806
130	唐宪宗 李纯 Tang Xian Zong Li Chun	43	15	778-820
131	唐穆宗 李恒 Tang Mu Zong Li Heng	30	4	795-824
132	唐敬宗 李湛 Tang Jing Zong Li Zhan	18	2	809-826
133	唐文宗 李昂 Tang Wen Zong Li Ang	32	14	809-840
134	唐武宗 李炎 Tang Wu Zong Li Yan	33	6	814-846
135	唐宣宗 李忱 Tang Xuan Zong Li Chen	50	13	810-859
136	唐懿宗 李漼 Tang Yi Zong Li Cui	41	14	833-873
137	唐僖宗 李儇 Tang Xi Zong Li Xuan	27	15	862-888
138	唐昭宗 李晔 Tang Zhao Zong Li Ye	38	16	867-904
139	后梁太祖 朱温 Hou Liang Tai Zu Zhu Wen	61	6	852-912
140	后梁郢王 朱友珪 Hou Liang Ying Wang Zhu Yougui	30	1	884-913
141	后梁末帝 朱友贞 Hou Liang Mo Di Zhu Youzhen	36	10	888-923
142	后唐庄宗 李存勖 Hou Tang Zhuang Zong Li Cunxu	42	4	885-926
143	后唐明宗 李嗣源 Hou Tang Ming Zong Li Siyuan	67	7	867-933
144	后唐闵帝 李从厚 Hou Tang Min Di Li Conghou	21	1	914-934
145	后唐末帝 李从珂 Hou Tang Mo Di Li Congke	52	2	885-936
146	后晋高祖 石敬瑭 Hou Jin Gao Zu Shi Jingtang	51	6	892-942

147	后晋出帝 石重贵 Hou Jin Chu Di Shi Chonggui	61	4	914-974
148	后汉高祖 刘知远 Hou Han Gao Zu Liu Zhiyuan	54	1	895-948
149	后汉隐帝 刘承祐 Hou Han Yin Di Liu Chengyou	20	2	931-950
150	北汉世祖 刘崇 Bei Han Shi Zu Liu Chong	60	4	895-954
151	北汉睿宗 刘钧 Bei Han Rui Zong Liu Jun	43	14	926-968
152	后周太祖 郭威 Hou Zhou Tai Zu Guo Wei	51	4	904-954
153	后周世宗 柴荣 Hou Zhou Shi Zong Chai Rong	39	5	921-959
154	吴太祖 杨行密 Wu Tai Zu Yang Xingmi	54	4	852-905
155	吴烈祖 杨渥 Wu Lie Zu Yang Wo	23	3	886-908
156	吴高祖 杨隆演 Wu Gao Zu Yang Longyan	24	12	897-920
157	吴睿帝 杨溥 Wu Rui Di Yang Pu	38	17	900-937
158	南唐烈祖 李昇 Nan Tang Lie Zu Li Bian	56	6	888=943
159	南唐元宗 李璟 Nan Tang Yuan Zong Li Jing	46	8	916-961
160	南唐后主 李煜 Nan Tang Hou Zhu Li Yu	42	17	937-978
161	南汉高祖 刘岩 Nan Han Gao Zu Liu Yan	54	31	889-942
162	南汉殇帝 刘玢 Nan Han Shang Di Liu Bin	24	1	920-943
163	南汉中宗 刘晟 Nan Han Zhong Zong Liu Sheng	39	15	920-958
164	南汉后主 刘鋹 Nan Han Hou Zhu Liu Chang	38	13	943-980
165	前蜀高祖 王建 Qian Shu Gao Zu Wang Jian	72	18	847-918
166	前蜀后主 王衍 Qian Shu Hou Zhu Wang Yan	28	8	899-926
167	后蜀高祖 孟知祥 Hou Shu Gao Zu Meng Zhixiang	61	1	874-934
168	后蜀后主 孟昶 Hou Shu Hou Zhu Meng Chang	47	31	919-965
169	宋太祖 赵匡胤 Song Tai Zu Zhao Kuangyin	50	17	927-976
170	宋太宗 赵光义 Song Tai Zong Zhao Guangyi	59	21	939-997
171	宋真宗 赵恒 Song Zhen Zong Zhao Heng	55	25	968-1022
172	宋仁宗 赵祯 Song Ren Zong Zhao Zhen	54	41	1010-1063
173	宋英宗 赵曙 Song Ying Zong Zhao Shu	36	4	1032-1067
174	宋神宗 赵顼 Song Shen Zong Zhao Xu	38	18	1048-1085
175	宋哲宗 赵煦 Song Zhe Zong Zhao Xu	25	15	1076-1100
176	宋徽宗 赵佶 Song Hui Zong Zhao Ji	54	25	1082-1135
177	宋钦宗 赵桓 Song Qin Zong Zhao Huan	57	2	1100-1156
178	宋高宗 赵构 Song Gao Zong Zhao Gou	81	35	1107-1187
179	宋孝宗 赵昚 Song Xiao Zong Zhao Shen	68	27	1127-1194
180	宋光宗 赵惇 Song Guang Zong Zhao Dun	54	5	1147-1200
181	宋宁宗 赵扩 Song Ning Zong Zhao Kuo	57	30	1168-1224
182	宋理宗 赵昀 Song Li Zong Zhao Yun	60	40	1205-1264
183	宋度宗 赵禔 Song Du Zong Zhao Qi	35	10	1240-1274
184	宋恭帝 赵昀 Song Gong Zong Zhao Xian	53	2	1271-1323
185	辽太祖 耶律阿保机 Liao Tai Zu Yelv Abaoji	55	11	872-926
186	辽太宗 耶律德光 Liao Tai Zong Yelv Deguang	46	21	902-947
187	辽世宗 耶律阮 Liao Shi Zong Yelv Ruan	34	4	918-951
188	辽穆宗 耶律璟 Liao Mu Zong Yelv Jing	39	18	931-969
189	辽景宗 耶律贤 Liao Jing Zong Yelv Xian	35	13	948-982
190	辽圣宗 耶律隆绪 Liao Sheng Zong Yelv Longxu	61	49	971-1031
191	辽兴宗 耶律宗真 Liao Xing Zong Yelv Zongzhen	40	24	1016-1055
192	辽道宗 耶律洪基 Liao Dao Zong Yelv Hongji	70	46	1032-1101
193	辽天祚帝 耶律延禧 Liao Tian Zuo Di Yelv Yanxi	54	24	1075-1128
194	辽宣宗 耶律淳 Liao Xuan Zong Yelv Chun	61	1	1062-1122
195	辽德宗 耶律大石 Liao De Zong Yelv Dashi	57	12	1087-1143
196	金太祖 完颜阿骨打 Jin Tai Zu Wanyan Aguda	56	9	1068-1123
197	金太宗 完颜晟 Jin Tai Zong Wanyan Sheng	61	12	1075-1135
198	金熙宗 完颜亶 Jin Xi Zong Wanyan Dan	31	14	1119-1149

199	金海陵王 完颜亮 Jin Hai Ling Wang Wanyan Liang	40	12	1122-1161
200	金世宗 完颜雍 Jin Shi Zong Wanyan Yong	67	28	1123-1189
201	金章宗 完颜璟 Jin Zhang Zong Wanyan Jing	41	19	1168-1208
202	金卫绍王 完颜永济 Jin Wei Shao Wang Wanyan Yongji	61	5	1153-1213
203	金宣宗 完颜珣 Jin Xuan Zong Wanyan Xun	61	10	1163-1223
204	金哀宗 完颜守绪 Jin Ai Zong Wanyan Shouxu	37	11	1198-1234
205	夏景宗 李元昊 Xia Jing Zong Li Yuanhao	46	17	1003-1048
206	夏毅宗 李谅祚 Xia Yi Zong Li Liangzuo	21	19	1047-1067
207	夏惠宗 李秉常 Xia Hui Zong Li Bingchang	26	19	1061-1086
208	夏崇宗 李乾顺 Xia Chong Zong Li Qianshun	57	53	1083-1139
209	夏仁宗 李仁孝 Xia Ren Zong Li Renxiao	70	54	1124-1193
210	夏桓宗 李纯祐 Xia Huan Zong Li Chunyou	30	13	1177-1206
211	夏襄宗 李安全 Xia Xiang Zong Li Anquan	42	5	1170-1211
212	夏神宗 李遵頊 Xia Shen Zong Li Zunxu	64	12	1163-1226
213	夏献宗 李德旺 Xia Xian Zong Li Dewang	46	3	1181-1226
214	元太祖 铁木真 Yuan Taizu Tie Mu Zhen	66	22	1162-1227
215	元睿宗 拖雷 Yuan Rui Zong Tuo Lei	41	2	1192-1232
216	元太宗 窝阔台 Yuan Tai Zong Wo Kuo Tai	56	13	1186-1241
217	元定宗 贵由 Yuan Ding Zong Gui You	43	3	1206-1248
218	元宪宗 蒙哥 Yuan Xian Zong Meng Ge	52	9	1208-1259
219	元世祖 忽必烈 Yuan Shi Zu Hu Bi Lie	79	35	1215-1294
220	元成宗 铁穆耳 Yuan Cheng Zong Tie Mu Er	43	13	1265-1307
221	元武宗 海山 Yuan Wu Zong Hai Shan	31	4	1281-1311
222	元仁宗 爱育黎拔力八达 Yuan Ren Zong Aiyulibalibada	36	9	1285-1320
223	元英宗 硕德八剌 Yuan Ying Zong Shuo De Ba La	21	3	1303-1323
224	元泰定帝 也孙铁木儿 Yuan Tai Ding Di Ye Sun Tie Mu Er	36	5	1293-1328
225	元文宗 图帖睦尔 Yuan Wen Zong Tu Tie Mu Er	29	4	1304-1332
226	元明宗 和世㻋 Yuan Ming Zong He Shi La	30	1	1300-1329
227	元惠宗 妥懽帖睦尔 Yuan Hui Zong Tuo Huan Tie Mu Er	51	38	1320-1370
228	元昭宗 爱猷识理达腊 Yuan Zhao Zong Ai Yu Shi Li Da La	40	8	1339-1378
229	元天元帝 脱古思帖木儿 Yuan Tian Yuan Di Tuogusi Tie MuEr	47	10	1342-1388
230	明太祖 朱元璋 Ming Tai Zu Zhu Yuanzhang	71	31	1328-1398
231	明惠宗 朱允炆 Ming Hui Zong Zhu Yunwen	26	4	1377-1402
232	明成祖 朱棣 Ming Cheng Zu Zhu Di	65	22	1360-1424
233	明仁宗 朱高炽 Ming Ren Zong Zhu Gao Chi	48	1	1378-1425
234	明宣宗 朱瞻基 Ming Xuan Zong Zhu Zhan Ji	38	10	1398-1435
235	明英宗 朱祁镇 Ming Ying Zong Zhu Qizhen	38	22	1427-1464
236	明代宗 朱祁钰 Ming Dai Zong Zhu Qiyu	30	7	1428-1457
237	明宪宗 朱见深 Ming Xian Zong Zhu Jianshen	41	23	1447-1487
238	明孝宗 朱祐樞 Ming Xiao Zong Zhu Youtang	36	18	1470-1505
239	明武宗 朱厚照 Ming Wu Zong Zhu Houzhao	31	16	1491-1521
240	明世宗 朱厚熜 Ming Shi Zong Zhu Houcong	60	45	1507-1567
241	明穆宗 朱载坫 Ming Mu Zong Zhu Zaihou	36	6	1537-1572
242	明神宗 朱翊钧 Ming Shen Zong Zhu Yijun	58	48	1563-1620
243	明熹宗 朱由校 Ming Xi Zong Zhu Youjiao	23	7	1605-1627
244	明思宗 朱由检 Ming Si Zong Zhu Youjian	35	17	1610-1644
245	清太祖 努尔哈赤 Qing Tai Zu Nu Er Ha Chi	68	11	1559-1626
246	清太宗 皇太极 Qing Tai Zong Huang Tai Ji	52	17	1592-1643
247	清世祖 福临 Qing Shi Zu Fu Lin	24	18	1638-1661
248	清圣祖 玄烨 Qing Sheng Zu Xuan Ye	69	61	1654-1722
249	清世宗 胤禛 Qing Shi Zong Yin Zhen	58	13	1678-1735
250	清高宗 弘历 Qing Gao Zong Hong Li	89	60	1711-1799

251	清仁宗 颙琰 Qing Ren Zong Yong Yan	61	25	1760-1820
252	清宣宗 旻宁 Qing Xuan Zong Min Ning	69	30	1782-1850
253	清文宗 奕訢 Qing Wen Zong Yi Zhu	31	11	1831-1861
254	清穆宗 载淳 Qing Mu Zong Zai Chun	19	13	1856-1874
255	清德宗 载湉 Qing De Zong Zai Tian	38	34	1871-1908