Longevity in *Caenorhabditis elegans* reduced by mating but not gamete production

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THEORIES of life-history evolution propose that trade-offs occur between fitness components, including longevity and maximal reproduction[1–3]. In *Drosophila*, female lifespan is shortened by increased egg production[4], receipt of male accessory fluid[5] and courting[6]. Male lifespan is also reduced by courting and/or mating[7]. Here we show that in the nematode *Caenorhabditis elegans*, mating with males reduces the lifespan of hermaphrodites by a mechanism independent of egg production or receipt of sperm. Conversely, males appear unaffected by mating. Thus, in *C. elegans* there is no apparent trade-off between longevity and increased egg or sperm production, but there is a substantial cost to hermaphrodites associated with copulation.

The soil-dwelling roundworm *C. elegans* can reproduce either as a self-fertilizing hermaphrodite or by crossing with males. Hermaphrodite reproduction is limited by the number of sperm produced (around 300) because the adult switches to production of oocytes. Oocyte production ceases after depletion of sperm. Cross-fertilization can increase brood size to over 1,000 (ref. 8). Hermaphrodite reproduction *per se* has no detectable effect on longevity since wild-type lifespans have been observed in sterile mutants[9–11] and in animals in which the gonadal precursor cells had been destroyed by laser microsurgery[11]. We tested the effect of exposure to males on lifespan. Using the wild-type (N2) strain and conditions similar to a previous study (ref. 12; Fig. 1), we observed that hermaphrodite lifespan (50% survival) was reduced by 43%, from 16.7 ± 0.5 to 9.6 ± 0.5 days (Fig. 1*a*). Furthermore, hermaphrodite longevity was found to vary with the dosage of males (Fig. 1*c* and Table 1). The increase in mortality reached saturation at a male-to-hermaphrodite ratio of 1.5–2.0 to 1, where a 50% reduction in lifespan was seen. Below this ratio, crossing was less than 100% efficient (that is, there were fewer than 50% male progeny). Exposure to hermaphrodites did not affect male survival, even at low male-to-hermaphrodite ratios (Fig. 1*b* and Table 1).

Our results are the opposite to those reported by Van Voorhies[12], who observed a reduction of lifespan in mated *C.*

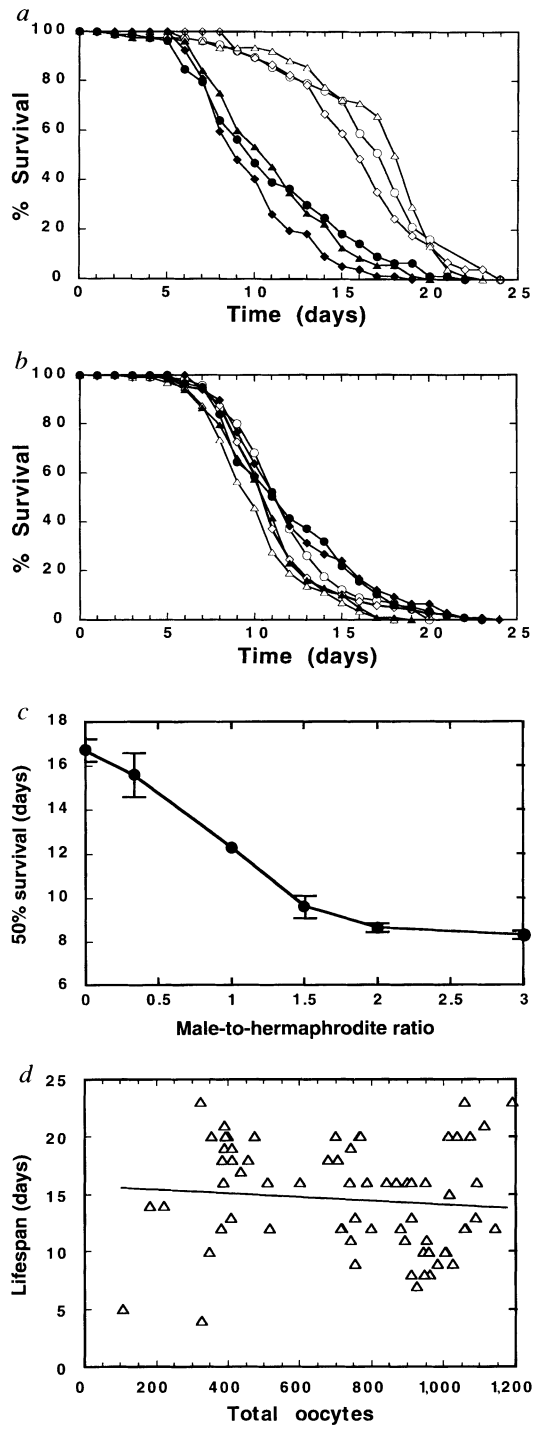

FIG. 1 Effect of mating on *C. elegans* wild-type males and hermaphrodites. *a*, Hermaphrodites; *b*, males. Fifteen L4 males and ten L4 hermaphrodites were placed on each plate as previously described[12]. Total sample size: 229 unmated, 236 mated hermaphrodites; 379 unmated, 358 mated males. Filled symbols, mated; open symbols, unmated. The three different symbols represent three replicate experiments. Hermaphrodite lifespan (50% survival) was significantly decreased by mating (Mann Whitney $U = 0.00$, d.f. = 1, $P < 0.05$). Conversely, male lifespan (50% survival) was unaffected ($U = 7.00$, d.f. = 1, $P > 0.05$). In two replicates, 60-mm Petri plates were used, and in the third, 35-mm plates were used as described[12]. Under these conditions it was also found that incomplete cross-fertilization occurred (60–95%, measured as twice the percentage of male offspring). The small subset of hermaphrodites in the mating experiment that survived to the same maximum lifespan probably experienced partial or no cross-fertilization. The degree of crossing varies with parental sex ratio. *c*, Effects of male dosage on hermaphrodite survival. Error bars represent standard error. *d*, Brood sizes and lifespans of individual briefly mated hermaphrodites.

METHODS. Animals were maintained monoxenically at 20 °C in 60-mm Petri dishes filled with NG agar and seeded with *E. coli* OP50 as the food source[23]. To determine adult lifespans, 15–200 L4 larvae were transferred to fresh plates, then transferred again daily during the egg-laying period, and less frequently thereafter. Death was scored as absence of any movement and failure to move after several light pokes with a platinum wire. The zero point was the time of L4 transfer. To minimize possible costs of copulation and achieve the broad range of brood sizes shown in *d*, each hermaphrodite was mated with three males on a 1-cm spot of bacteria for 5 h at 20 °C, after which the males were removed. Owing to the brevity of the mating period, brood size should, in most cases, be limited by the number of sperm transferred, and not the fitness of the individual hermaphrodites. Animals were transferred daily to fresh plates and each day's progeny counted two days later. Of 62 hermaphrodites incubated with males, 49 proved to have mated on the basis of the presence of male offspring. A further eight unmated hermaphrodites were also scored. A total of 52,086 progeny (48,350 progeny and 3,736 unfertilized oocytes) were scored. The curve was generated by the bestfit function of KaleidaGraph version 2.1.3 (Abelbeck Software). Lifespan was not correlated with brood size ($r = 0.10$, $P \gg 0.05$). Conclusions were the same when fertilized oocytes were excluded.

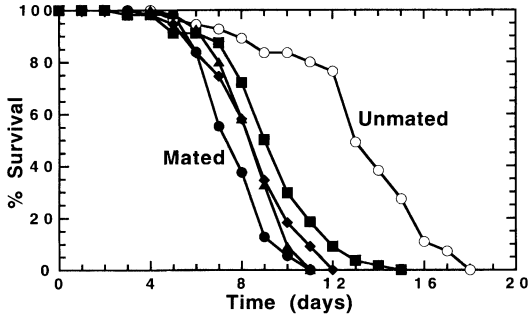

FIG. 2 Lifespan (50% survival) of N2 hermaphrodites at 25 °C was shortened by mating, even with fertilization-defective males (mated versus unmated; $t = 6.70$, $P < 0.05$). Results shown are from one of two duplicate experiments that yielded similar results. The male-to-hermaphrodite ratio was 2:1. The mean sample size per curve was 54.8. As prior tests showed that *fer-1* and *fer-6* males had a reduced lifespan relative to N2 males (50% survival: 5.6, 5.9 and 8.5 days, respectively), males were replaced every 48 h. Survival of hermaphrodites mated with an unreplenished population of N2 males is also shown. Open circles, unmated; diamonds, unreplenished N2 male-mated; filled circles, N2 male-mated; triangles, *fer-1* male-mated; squares, *fer-6* male-mated. Note: the *fer-6*(*hc6*ts) strain used, BA6, also contains a weak *unc* (uncoordinated) mutation[24].

elegans males but not hermaphrodites. Van Voorhies used a short-lived variant of N2 with a hermaphrodite lifespan (50% survival) of 12.0 ± 0.3 days (20 °C). The N2 strain used in our study was the *Caenorhabditis* Genetics Center (CGC) reference N2 male stock. F1 hermaphrodites from crosses between the CGC N2 strain and Van Voorhies' variant had a lifespan indistinguishable from the CGC strain, indicating that the Van Voorhies variant is a recessive, short-lived mutant. However, strain differences fail to explain why our more extensive study differs from the previously reported results, for in our hands the short-lived variant also showed hermaphrodite- but not male-mating costs when grown on the standard food source, *E. coli* strain OP50 (data not shown).

There are several possible causes of reduced lifespan in hermaphrodites exposed to males: (1) metabolic costs of extra egg production; (2) mating-induced physiological changes associated with increased rate of egg production; (3) receipt and/or storage of sperm or seminal fluid; (4) internal hatching of larvae; (5) structural damage sustained during copulation; (6) mating-associated bacterial infection; and (7) non-mating costs associated with the presence of males.

Possible costs of increased egg production were assayed by briefly mating young adult hermaphrodites and comparing brood size with lifespan in individual animals (Fig. 1*d*). Only a small number of unfertilized oocytes are stored by the hermaphrodite, so increased broods require increased investment of resources in oocyte production. No significant correlation between brood size and lifespan was observed, from which we conclude that the decrease in lifespan is not the result of increased egg production. Another possibility is that mating stimulates a physiological response in hermaphrodites associated with resource mobilization to increase the rate of egg production at a cost to somatic maintenance. However, we did not observe an increase in the rate of egg production associated with mating (Table 2).

To investigate whether receipt or storage of sperm affect hermaphrodite lifespan, N2 hermaphrodites were mated with sterile males. Mutant *fer-1*(*hc24*ts) males mate normally at 25 °C and transfer sperm that are capable of stimulating oocyte production but incapable of fertilization[13,14], whereas *fer-6*(*hc6*ts) males appear to copulate normally, but neither stimulate oocyte production nor transfer sperm[13,15]. Figure 2 shows that hermaphrodite lifespan is decreased by mating with either *fer-1* or *fer-6* males. As *fer-6* males do not transfer sperm, this indicates that neither receipt nor storage of sperm is the cause of hermaphrodite lifespan reduction. In *Drosophila*, seminal fluid has been shown to stimulate egg production, suppress fertilization by sperm from previous matings, and decrease female lifespan[5,16–19]. If the cost of mating with *fer-6* males were a side effect of displacement of self-sperm caused by seminal fluid, it might be expected that mating N2 hermaphrodites with *fer-6* males would also result in a reduction of egg laying due to inhibition of self-fertilization, but this was not seen (data not shown). However, it remains possible that *fer-6* males transfer seminal fluid, and that this reduces hermaphrodite lifespan.

We tested whether hatching of larvae inside the uterus caused early hermaphrodite mortality by mating sterile hermaphrodites with sterile males. Mutant *fog-2*(*q71*) hermaphrodites produce no sperm[20], so mating with *fer-6*(*hc6*ts) males at 25 °C produces very few or no fertilized eggs. In two trials using *fer-6* males at a male-to-hermaphrodite ratio of 2:1, a hermaphrodite lifespan of 7.8 ± 0.8 days (25% survival) was seen, compared to 6.5 ± 0.7 days when N2 males were used, and 14.1 ± 0.8 days for unmated hermaphrodites. (Results are expressed as 25% survival because a proportion of unmated *fog-2* hermaphrodites die as young adults.) This result indicates that internal hatching of larvae is not the cause of reduced hermaphrodite lifespan, and it provides further evidence that reduced lifespan is not the result of increased egg production.

To test whether the mere presence of males decreased hermaphrodite longevity, *mab-10(e1248);him-5(e1490)* males, which cannot copulate because of tail defects[21], and *unc-32(e189);him-8(e1489)*

TABLE 1 Effect of sex ratio on longevity (20 °C)

Males/ hermaphrodites	50% Survival of males (days ± s.e.)	50% Survival of hermaphrodites (days ± s.e.)	Maximum lifespan of males (days ± s.e.)	Maximum lifespan of hermaphrodites (days ± s.e.)	*N**	Total hermaphrodites
0	– –	16.7 ± 0.5	– –	23.6 ± 0.6	9	513
0.33	12.2 –	15.6 ± 1.0	24 –	23 ± 1.0	2†	148
1.0	11.8 –	12.3 –	21 –	20 –	1	50
1.5	10.9 ± 0.2	9.6 ± 0.5	21 ± 1.5	19.3 ± 0.9	3	224
2.0	9.5 ± 0.4	8.6 ± 0.2	21 ± 1.5	14.8 ± 1.2	5	251
3.0	11.9 ± 0.4	8.3 ± 0.2	22.3 ± 2.2	13.5 ± 0.4	3	103
–	10.1 ± 0.2	– –	18.1 ± 1.2	– –	9	–

Male: hermaphrodite ratio (*M*:*H*) explains 86.5% of the variation in 50% survival of hermaphrodites (Pearson's $r = 0.93$; all statistics estimated with SYSTAT[22]). The sex ratio effect appeared saturated when $M{:}H \geq 1.5$ ($r = -0.61$, $P = 0.06$). Male 50% survival was not correlated with *M*:*H* ratio ($r = 0.11$, $P \gg 0.05$).

* Number of independent trials. Mean sample size was 64.7 (range: 31–162). Lifespans were found to be independent of population density at the densities used. Animals lost during trials (on average 6.4 and 8.6% of the initial number of hermaphrodites and males, respectively) are excluded from these values. Note that mating causes a slight increase in male maximum lifespan.

† Values for males from two trials were summed to yield an adequate sample size. This was treated as a single trial for males.

TABLE 2 Effect of mating on egg production rate

Treatment	Eggs laid per hour Day 1	Day 2	Day 3
Unmated	6.1 ± 0.2	6.5 ± 1.5	0.6 ± 0.3
Mated once	5.0 ± 0.2	6.9 ± 0.2	5.4 ± 1.9
Mated thrice	4.9 ± 0.4	6.5 ± 0.1	5.1 ± 1.3

N2 hermaphrodites were mated singly with 10 N2 males for five hours (20 °C). The first group was unmated, the second mated only on day one, and the third on three consecutive days. Egg laying was measured over a three-hour period at 24-hour intervals, immediately after mating in the mating test groups. Each group consisted of six animals and the entire experiment was repeated twice. Rates are ±s.e. Non-parametric ANOVA (Kruskal-Wallis) revealed a nearly significant decrease in the rate of egg laying in the two mated groups on day 1 ($P = 0.07$), but not day 2. No increase in egg-laying rate was observed. On day 3, the self sperm of unmated animals were almost depleted.

males, which cannot mate owing to motility defects[21], were used. Maintenance in the presence of such males throughout life, at a male-to-hermaphrodite ratio of 2:1, did not reduce hermaphrodite longevity (data not shown).

These results demonstrate that the cost of mating to hermaphrodite longevity is not associated with egg production or egg laying, the receipt or storage of sperm, internal hatching of eggs, or non-mating effects associated with presence of males. Thus, it would appear that the stress of copulation reduces hermaphrodite lifespan, either directly or by increasing susceptibility to infection by the bacteria used as food. It also remains possible that receipt of seminal fluid is a cause. By contrast with the hermaphrodites, there is no apparent cost to males of increased spermatogenesis or copulation. Sterile hermaphrodites do not have increase lifespan[9–11] and mutations in only one of many genes involved in spermatogenesis, *spe-26*, have been reported to increase lifespan[12], suggesting that lifespan extension in *spe-26* mutants may be a pleiotropic effect unconnected to the defect in spermatogenesis. Taken together, these results do not support the hypothesis that increased gamete production accelerates ageing because of a drain on resources from somatic maintenance functions, or that mating actively stimulates such a diversion of resources. It is possible, however, that such trade-offs might only be observed when resources are limited, that is, in calorically restricted animals. □

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A systematic map of direction preference in primary visual cortex

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NEURONS in the primary visual cortex respond selectively to the orientation of edges and their direction of motion. Orientation preference is mapped in a systematic fashion across the cortical surface, such that neurons in adjacent columns have similar but slightly shifted preferred orientations[1–7]. Microelectrode studies have suggested that direction preference is also arranged in a systematic fashion[8–10], but exactly how this response property is mapped remains unclear. Here we show by optical imaging[4–5] of intrinsic signals[6–7,11–14] in ferret cortical area 17 that there is a mosaic-like map of direction preference. This map consists of numerous regions within which direction preference changes in a slow, continuous fashion. These regions are separated by winding boundaries (fractures) across which direction preference shifts abruptly, often by 180 °. Comparison of direction and orientation preference maps shows that these fractures subdivide iso-orientation domains into regions selective for opposite directions of motion.

Functional maps of direction preference were obtained by collecting separate images of the cortical surface while the animal viewed bar stimuli moving in one of eight directions ($n = 13$ animals). Figure 1*a* shows differential images of direction preference obtained by subtracting the cortical images that were collected while the animal viewed stimuli that moved in opposite directions. These difference images are composed of numerous oval or circular-shaped dark and light patches (iso-direction domains). Iso-direction domains differ in their appearance from iso-orientation domains, which have a more banded or striped appearance (Fig. 1*b*). Neurons located within the centre of iso-direction domains have highly selective directional responses that can be predicted from the optical images ($n = 4$ animals, 10 recordings) (Figs 1*b*, *c* and 2*a*).

The complete organization of direction preferences across a region of visual cortex was determined by vectorially summing the eight images shown in Fig. 1*a*. The result is shown in Fig. 2*a*, where preferred direction is colour coded and indicated by the direction of the arrows overlaid on the map; the length of the arrows represents the magnitude of direction selectivity. The map consists of numerous, distinct regions of high selectivity in which direction preference shifts in a slow, continuous fashion. Figure 2*b* shows that these regions are separated by narrow bands of low selectivity across which direction preference changes abruptly, often by 180 °. In this image, arrows indicating direction preference are overlaid on a magnitude map of direction selectivity where black indicates regions of low selectivity.

Direction and orientation preference maps are similar in some characteristics and strikingly different in others. Both maps are organized at a similar spatial scale (Fig. 3*a*, *d*). Using autocorrelation analysis, we calculated the average repeat distance in the orientation preference map to be 890 ± 29 μm and in the direction preference map to be 867 ± 18 μm ($n = 5$ animals). But the fragmented structure of the direction preference maps, which is especially apparent when the magnitude of the direction signal is plotted (Fig. 3*b*), is very different from that found for orientation preference maps (Fig. 3*e*). Regions of low selectivity in orientation maps are confined to small circular zones located at the centres of orientation pinwheels or, less commonly, short lines that connect adjacent pinwheel centres[5–7]. This difference is also apparent