Evolution of male longevity bias in nematodes

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Summary

Many animal species exhibit sex differences in aging. In the nematode *Caenorhabditis elegans*, under conditions that minimize mortality, males are the longer-lived sex. In a survey of 12 independent *C. elegans* isolates, we find that this is a species-typical character. To test the hypothesis that the *C. elegans* male longevity bias evolved as a consequence of androdioecy (having males and hermaphrodites), we compared sex-specific survival in four androdioecious and four dioecious (males and females) nematode species. Contrary to expectation, in all but *C. briggsae* (androdioecious), males were the longer-lived sex, and this difference was greatest among dioecious species. Moreover, male lifespan was reduced in androdioecious species relative to dioecious species. The evolutionary theory of aging predicts the evolution of a shorter lifespan in the sex with the greater rate of extrinsic mortality. We demonstrate that in each of eight species early adult mortality is elevated in females/hermaphrodites in the absence of food as the consequence of internal hatching of larvae (matricide). This age-independent mortality risk can favour the evolution of rapid aging in females and hermaphrodites relative to males.
Key words: aging; *Caenorhabditis briggsae*; *Caenorhabditis elegans*; evolution; nematode.

Introduction

Sex differences in lifespan occur in many animal species. For example, in the USA in the year 2000, life expectancy from birth of women was 79.5 years, compared with 74.1 years in men (National Center for Health Statistics, www.cdc.gov.nchs). Where sex differences in lifespan exist in mammals, it is typically males that exhibit reduced longevity relative to females (Smith, 1989). Sex differences in lifespan have also been recorded in many invertebrate species, such as the fruitflies *Drosophila melanogaster* (Lints *et al.*, 1983) and *Ceratitis capitata* (Carey *et al.*, 1995), the housefly *Musca domestica* (Rockstein & Lieberman, 1959) and the nematode *Caenorhabditis elegans* (Johnson & Hutchinson, 1993; Gems & Riddle, 2000b). Understanding the

evolutionary and physiological determinants of such gender gaps in lifespan is one goal of biogerontology.

Measured sex differences in lifespan may be understood in terms of an underlying constitutional longevity that is reduced by the deleterious effects of reproductive biology (progeny production, reproductive hormones) and sex-related behaviour (seeking and competing for mates, territorial defense) (Carey *et al.*, 1995). The relative contributions of extrinsic (environmental, behavioural) and intrinsic determinants of lifespan may be gauged by analysis of age-specific mortality patterns. Throughout the animal kingdom, population aging is characterized by an exponential increase in mortality rate with increasing age (reviewed in Wachter & Finch, 1997). Mortality due to extrinsic causes may be distinguished as that component of overall mortality that is age-independent, and distinct from the age-dependent mortality rate acceleration (Finch, 1990). Potentially, sex differences in lifespan may result from a difference either in age-independent mortality, associated more with extrinsic factors, or in the rate of acceleration of the mortality rate with increasing age, i.e. in aging itself. In the present study we document sexual dimorphism in lifespan in populations of free-living nematode species in order to test hypotheses about the evolution of sex differences in aging. Although the limited sample sizes used here preclude biodemographic analysis, survival was measured under conditions designed to minimize mortality due to environmental factors and reproductive behaviour, thereby maximizing the contribution of aging to measured lifespan.

The evolutionary theory of aging provides a powerful explanatory framework for understanding why aging occurs. Aging is not a directly selected trait, but rather evolves as the result of a decreased force of natural selection at older ages (Medawar, 1952; Williams, 1957; Rose, 1991). The latter occurs because mortality resulting from extrinsic hazards leads to progressive rarity of members of increasingly older age groups. For this reason, it has been proposed, late-acting deleterious mutations accumulate in populations, causing aging (Medawar, 1952). An elaboration of this model is that natural selection may favour pleiotropic mutations which are beneficial early in life, but deleterious later in life (antagonistic pleiotropy) (Williams, 1957). The evolutionary theory also provides a potential explanation for sex differences in the rate of aging. As proposed by G. C. Williams: 'Where there is a sex difference, the sex with the higher mortality rate and lesser rate of increase in fecundity should undergo the more rapid senescence' (Williams, 1957).

C. elegans occur as protandrous hermaphrodites (XX) that can reproduce either by self-fertilization or by mating with males (XO). In this species, observed sex differences in survival are culture condition-dependent, as documented in the medfly (Carey & Liedo, 1995; Carey *et al.*, 1995). When cultured individually, male lifespan in the standard laboratory strain N2 is 10–20% longer than that of hermaphrodites (Gems & Riddle, 2000b).

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When cultured in groups, mating with hermaphrodites or attempted mating with other males in single-sex groups greatly reduces male survival (Gems & Riddle, 2000b), such that there is no sex difference in lifespan in mated populations (Gems & Riddle, 1996). Single culture therefore provides a more accurate estimate of the potential physiological lifespan and best reflects life expectancy due to senescence.

The *C. elegans* N2 strain, in which the underlying (constitutive) male longevity bias was documented, is derived from an isolate collected some time before 1956 from near Bristol, UK (Hodgkin & Doniach, 1997). It has since been maintained through cryopreservation and subculture independently in many laboratories, including the *Caenorhabditis* Genetics Center (biosci/UMN/edu/CGC). When lifespan of various N2 stocks was measured under common culture conditions, fertility and survival varied among the isolates (Gems & Riddle, 2000a). Presumably genetic variation arises among N2 lines as novel mutations fix under the force of genetic drift. We have investigated whether the male longevity bias seen in N2 is species-typical for *C. elegans*, by surveying sex differences in survival in other wild isolates of *C. elegans*.

Why might such a male longevity bias have evolved? As described, the evolutionary theory of aging suggests that under natural conditions males experience a lower rate of extrinsic mortality, and/or a higher probability of reproduction at later ages (Williams, 1957). Although wild *C. elegans* populations have not been studied, it is probable that reproduction in males is skewed towards older ages relative to hermaphrodites. This is because hermaphrodites rapidly commence reproduction due to their ability to self-fertilize, and cease reproduction after depletion of ~300 self-sperm (in the absence of mating). Males in such populations must find a mate and copulate successfully in order to reproduce. Moreover, they do not have a deterministic reproductive endpoint, unlike unmated hermaphrodites. A male longevity bias may therefore have evolved as a consequence of protandrous hermaphroditism. This hypothesis can be tested because it predicts that male longevity bias will occur in androdioecious but not dioecious species.

By similar reasoning, protandrous hermaphrodites are likely both to begin and to end reproduction sooner than females because they do not have to encounter and mate successfully with a male to commence reproduction and, in the absence of mating, experience an early endpoint to reproduction due to depletion of self-sperm. From this, a further prediction is that dioecious females will evolve longer lifespans than hermaphrodites. To test these predictions, we have measured lifespan in both sexes of four androdioecious and four dioecious nematodes, two of each belonging to the two genera *Caenorhabditis* and *Oscheius*.

The force of natural selection is predicted to be greater on the effects of genes exerted prior to the onset of reproduction rather than once reproduction has commenced. The *C. elegans* dauer larva is a facultative diapausal third stage larva that forms in response to crowding, reduced food and high temperature (Cassada & Russell, 1975; Riddle & Albert, 1997). *C. elegans*

males form dauer larvae more readily than do hermaphrodites (Ailion & Thomas, 2000). Given that the FOXO transcription factor DAF-16 controls dauer larva formation (Lin *et al.*, 1997; Ogg *et al.*, 1997), and plays a determining role in the male longevity bias (Gems & Riddle, 2000b), it is possible that the latter is a pleiotropic consequence of selection for increased dauer larva formation in males. Thus, if there is pleiotropy between propensity to form dauer larvae and longevity, then in different species we expect the relative propensity of a given sex to form dauer larvae to correlate with a similar longevity bias. We therefore tested for a correlation between the sex bias in dauer larva formation and longevity.

Male longevity bias may arise from direct selection upon late life reproductive value relative to females and hermaphrodites. Females and hermaphrodites can internally hatch larvae independently of aging. In wild-type *C. elegans*, when food is scarce, gravid hermaphrodites desist from laying their eggs (Trent *et al.*, 1983). This effect is mediated by reduced serotonin levels (Horvitz *et al.*, 1982), and may be an adaptation to ensure that eggs are laid where food is available. If no food is encountered, eggs eventually hatch within the uterus, and the emerging larvae devour the mother (matricide). Ultimately, all that remains is the transparent cuticle of the mother, with the writhing larvae trapped within (a 'bag of worms', illustrated for *O. myriophila* in Fig. 3). Under natural conditions of variable food, females may often experience mortality risks at young ages, which in turn can favour the evolution of rapid senescence.

Here we document that the *C. elegans* male longevity bias is a species-typical trait, and not an oddity of the standard laboratory strain, N2. We establish that male longevity bias is not unique to androdioecy, and in fact occurs with greater magnitude in dioecious species. Finally, we document that early mortality in young, starved females and hermaphrodites is elevated by internal hatching of larvae. These observations suggest that increased mortality due to internal hatching of larvae may contribute to the evolution of reduced female/hermaphrodite longevity in terrestrial nematodes.

Results

C. elegans male longevity bias is species typical

To establish whether male longevity bias is a trait of *C. elegans* as a species, rather than a novelty of the N2 strain, we measured lifespans of males and hermaphrodites from 12 independent wild isolates of *C. elegans*. Collections and characteristics of these strains were described by Hodgkin & Doniach (1997). Strains varied in copulatory plug formation (Hodgkin & Doniach, 1997), social behaviour (de Bono & Bargmann, 1998), lifespan (Johnson & Hutchinson, 1993), body size (Knight *et al.*, 2001) and dauer larva formation (M. E. Viney, M. P. Gardner and J. A. Jackson, personal communication).

We also found significant variation in survival among these isolates when data from the sexes are combined ($P < 0.0001$, log rank test). Male longevity exceeded hermaphrodite longevity
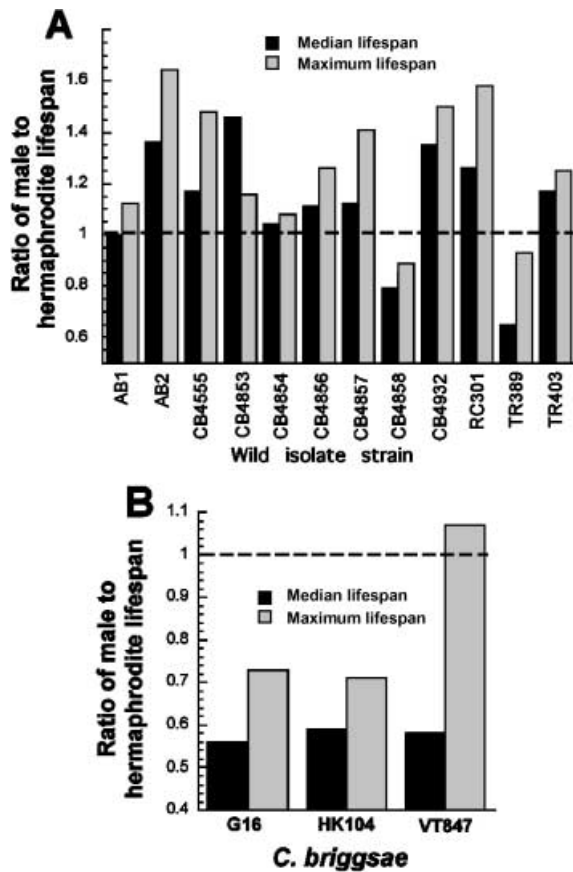
Fig. 1 Ratio of male to hermaphrodite lifespan in two species of *Caenorhabditis*: (A) 12 *C. elegans* wild isolates; (B) three *C. briggsae* isolates.

in nine out of 12 isolates (Fig. 1A, Table 1); this is more than expected by chance according to Fisher's Exact Test (*P* = 0.04), although not according to the more stringent Test for Equal or Given Proportions (*P* = 0.15). In two isolates with relatively

short-lived males, the absolute male longevity was uncharacteristically low and males appeared sickly. Excluding these strains (CB4858 and TR389), the observation of nine male-biased isolates from a sample of 10 is significant as judged by either test (Test for Equal or Given Proportions, *P* = 0.027). We conclude that male longevity bias is a species-typical characteristic of *C. elegans*.

Male longevity bias is not unique to androdioecy

To test whether male longevity bias is unique to androdioecious nematodes, we measured sex-specific longevity in two hermaphroditic and two dioecious species in each of two genera, *Caenorhabditis* and *Oscheius*. Note that *R. remanei* (formerly *C. vulgaris*) is, like *C. elegans*, *C. briggsae* and *C. remanei* spp. *vulgaris*, a species of *Caenorhabditis* (Sudhaus & Kiontke, 1996). When using comparative methods to study covariation in life history traits, it is important to take into account the possible confounding effect of phylogeny (Felsenstein, 1985; Harvey & Keymer, 1991). However, the phylogenetic relationship between the four *Caenorhabditis* species studied remains unclear (Fitch *et al.*, 1995; Delattre & Felix, 2001). In the case of *Oscheius, O. dolichura* (dioecious) and *O. species* (androdioecious) are more closely related to each other than to *O. myriophila* (androdioecious) (Sudhaus & Fitch, 2001), suggesting that the latter two species represent evolutionarily independent occurrences of androdioecy. The phylogenetic relationship between these three species and *O. dolichuroides* has not been reported. On the assumption that androdioecy is derived from dioecy, we can assume that within each genus androdioecy has evolved at least once.

Since we do not know the exact phylogenetic relationship between the eight species studied, we have treated the phylogenetic effects within each genus as negligible, but adjusted for the effect of genus in our analysis using a two-way ANOVA.

Table 1 Lifespans of *C. elegans* wild isolate males and hermaphrodites

| Isolate | Median lifespan (days) ± 95% CI | | Maximum lifespan (days) | | N* | | |
	M[1]	H[2]	M	H	M	H	P†
AB1	28.0 (28.0, 26.0)	28.0 (29.0, 26.0)	37.0	33.0	57 (60)	21 (39)	0.64
AB2	34.0 (37.0, 32.0)	25.0 (27.0, 24.0)	46.0	28.0	60 (61)	26 (36)	< 0.0001
CB4555	28.0 (30.0, 26.0)	24.0 (27.0, 22.0)	43.0	29.0	53 (60)	31 (36)	< 0.0001
CB4853	25.5 (26.5, 24.0)	17.5 (21.5, 15.5)	36.0	31.0	95 (120)	48 (66)	< 0.0001; 0.0002
CB4854	29.0 (32.5, 25.0)	28.0 (31.0, 25.0)	39.0	36.0	30 (54)	19 (36)	0.014
CB4856	25.0 (27.0, 24.0)	22.5 (25.0, 19.75)	36.5	29.0	105 (120)	48 (66)	0.21; 0.0051
CB4857	28.0 (30.0, 26.0)	25.0 (26.0, 23.0)	41.0	29.0	47 (60)	28 (36)	< 0.0001
CB4858	17.0 (19.0, 15.5)	21.5 (23.0, 20.0)	23.5	26.5	76 (90)	48 (66)	< 0.0001; 0.03
CB4932	31.0 (31.0, 27.0)	23.0 (26.0, 21.0)	42.0	28.0	50 (60)	21 (35)	< 0.0001
RC301	26.5 (28.5, 25.0)	21.0 (23.0, 19.0)	42.0	26.5	100 (120)	49 (61)	< 0.0001; < 0.0001
TR389	15.0 (15.0, 12.0)	23.0 (26.0, 20.0)	26.0	28.0	26 (30)	16 (20)	0.0026
TR403	30.5 (32.5, 29.0)	26.0 (29.5, 21.5)	40.7	32.5	95 (131)	42 (66)	0.014; < 0.0001

[1]M, males; ^{2}H, hermaphrodites.
*Number of senescent deaths (starting population).
†Probability that male and hermaphrodite survival differ by random chance (log rank test). Two significance values correspond to results from two separate replicates.
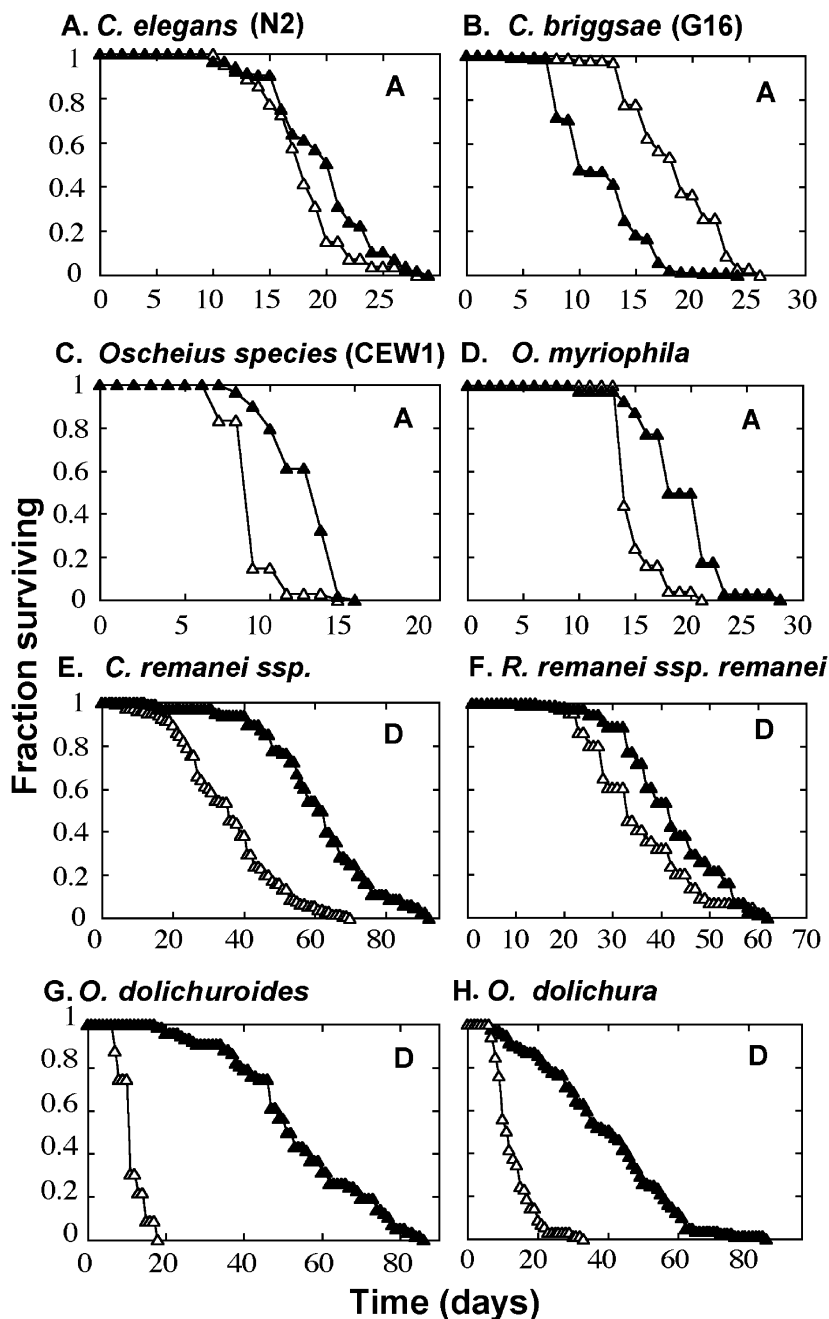
Fig. 2 Survival of males (filled symbols) and hermaphrodites/females (open symbols) of terrestrial nematodes in monoxenic liquid culture (22.5 °C): A, androdioecious; D, dioecious. (A) *C. elegans* N2; (B) *C. briggsae* G16; (C) *Oscheius* sp. CEW1; (D) *O. myriophila*; (E) *C. remanei* ssp.; (F) *Rhabditis remanei* ssp. *remanei*; (G) *O. dolichuroides*; (H) *O. dolichura*.

The use of an ANOVA here also assumes that within each species, reproductive mode has impacted independently upon the pattern of aging. Differences were observed between the two genera: median lifespan in *Oscheius* was shorter than in *Caenorhabditis* in females/hermaphrodites ($P = 0.0004$, ANOVA) but not in males ($P = 0.63$).

Contrary to prediction, males were the longer-lived sex in all species, except for *Caenorhabditis briggsae* (androdioecious), in which hermaphrodites were much longer lived than males in three independent isolates tested (Figs 1B, 2 and Table 2). Furthermore, the ratio of male-to-female median lifespan in dioecious species was significantly greater than for male-to-hermaphrodite in androdioecious species ($P = 0.018$, two-way ANOVA on normalized data). There was no interaction between genus and reproductive mode affecting lifespan sex ratio ($P = 0.09$). The largest sex difference measured was in the dioecious species *O. dolichuroides* in which males were ~4.6-fold longer-lived than females.

To test the hypothesis that androdioecy leads to the evolution of shorter lifespan in hermaphrodites, we compared median lifespans in androdioecious and dioecious species, controlling for the effect of genus. Males were included in this analysis, although we had no expectation of the impact of the reproductive mode on the pattern of male aging. For *C. elegans* we

Table 2 Lifespans of eight terrestrial nematode species

Species (hermaphroditic/dioecious)	Median lifespan ± 95% CI (days)		M : H/F median lifespan	Maximum lifespan (days)		M : H/F max. lifespan	N*	
	H/F	M		H/F	M		H/F	M
C. elegans N2 (H)	17.7 (19.0, 16.5)	19.8 (21.3, 18.3)	1.12	23.0	29.0	1.26	165 (3)	135 (3)
C. briggsae G16 (H)	21.0 (22.3, 20.0)	11.7 (13.0, 10.7)	0.56	26.0	19.0	0.73	153 (3)	140 (3)
C. briggsae VT847 (H)	17.0 (17.0, 15.0)	10.0 (10.0, 8.0)	0.59	17.0	12.0	0.71	46 (1)	39 (1)
C. briggsae HK104 (H)	24.0 (26.0, 20.5)	14.0 (15.0, 13.0)	0.58	30.0	32.0	1.07	30 (1)	36 (1)
Oscheius sp. (H)	9.0 (9.0, 9.0)	13.5 (13.5, 12.0)	1.50	11.0	12.0	1.09	34 (1)	59 (2)
O. myriophila (H)	14.0 (15.0, 14.0)	19.5 (21.0, 18.0)	1.39	18.0	25.0	1.39	27 (1)	40 (1)
C. remanei ssp. (D)	35.8 (40.0, 57.0)	60.5 (64.0, 57.0)	1.69	67.0	89.5	1.34	105 (2)	90 (2)
Rhabditis remanei ssp. *remanei* (D)	33.0 (35.0, 30.0)	40.0 (43.0, 38.0)	1.21	54.5	56.5	1.04	101 (2)	108 (2)
O. dolichura (D)	11.5 (13.5, 10.0)	40.0 (45.0, 33.0)	3.48	35.0	76.5	2.19	70 (2)	95 (2)
O. dolichuroides (D)	11.0 (12.0, 9.5)	50.5 (56.0, 45.5)	4.59	15.0	83.0	5.53	24 (1)	64 (2)

H/F, hermaphrodites/females; M, males.
*Number of senescent deaths (number of trials). Survival curves of males and hermaphrodites/females were significantly different ($P < 0.05$) for all replicates of all species except for one replicate of *R. remanei* ($P = 0.17$, log rank test).

Table 3 Sex bias to dauer formation in different nematode species

Species (hermaphroditic/dioecious)	% dauer formation ± SE		M : H/F dauer formation ratio*	N	P†
	M	H/F			
C. elegans N2 (H)	15.1 ± 9.6	7.5 ± 5.5	2.0	1920	> 0.1
C. briggsae G16 (H)	28.9 ± 1.7	36.2 ± 3.6	0.8	531	> 0.1
C. briggsae VT847 (H)	0.4 ± 0.4	35.4 ± 7.3	0.01	439	< 0.05
R. remanei ssp. *remanei* (D)‡	12.5	41.7	0.3, 0.6§	608, 53	N/A
O. myriophila (H)	8.1 ± 1.0	49.8 ± 10.6	0.2	558	> 0.05

*Values larger than one represent an increased tendency by males to form dauers.
†Probability that mean per cent male and hermaphrodite/female dauer formation differ by random chance (Student's *t*-test, normalized data).
‡Standard error not applicable as only one replicate performed where number of adults was scored.
§Male : hermaphrodite dauer formation ratio based on scoring dauers only and assuming 50 : 50 sex ratio.

took the mean of the observed lifespans of the 12 wild isolates (males, 25.6 days; hermaphrodites, 23.3 days), and likewise for the three *C. briggsae* isolates (males, 11.9 days; hermaphrodites, 20.7 days).

Contrary to our prediction, female lifespan across species was not consistently greater than that of hermaphrodites. Although females appeared longer lived overall ($P = 0.018$, two-way ANOVA), there was an interaction between reproductive mode and genus ($P = 0.016$), reflecting the fact that females were longer lived than hermaphrodites in *Caenorhabditis* but not in *Oscheius*. Unexpectedly, dioecious males were longer lived than androdioecious males ($P = 0.011$) (Table 2). This finding implies that androdioecy leads to the evolution of reduced lifespan in males. A possible explanation for this finding is presented in the discussion.

Sex bias in dauer larva formation in terrestrial nematodes

We tested the possibility that evolution of male longevity bias was due to pleiotropy between dauer larva formation and longevity.

Dauer larva formation was induced with crowding and starvation in four species of nematode. Contrary to prediction, the proportion of males forming dauer larvae was not consistently associated with relative male longevity (Table 3). In *C. briggsae* there was a hermaphrodite bias to dauer larva formation in one of two isolates. In *R. remanei* spp. *remanei* and *O. myriophila*, dauer larva formation occurred more frequently in hermaphrodites and females, despite the fact that males were the longer-lived sex.

Internal hatching of larvae increases mortality in females and hermaphrodites

Matricide provides a potential explanation for the evolution of male longevity bias in the nematode species studied here. We therefore tested its occurrence in the eight species of our study. Matricide was observed in all cases, with maximum levels in different species ranging from 8.3% to 100% after 4 days without food (Fig. 3). However, there was no correlation between the level of matricide and the magnitude of the male longevity bias.
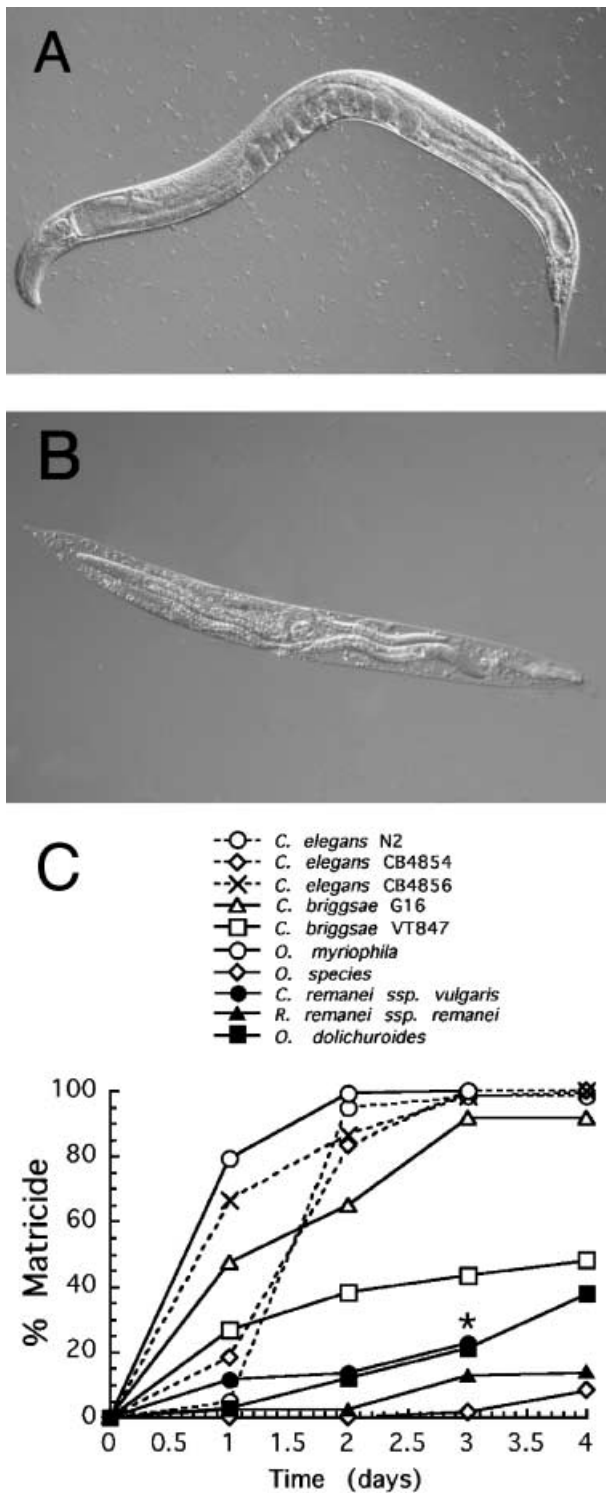
Fig. 3 Matricide (bagging) in terrestrial nematodes. (A) *O. myriophila* adult female. (B) *O. myriophila* 'bag of worms'. (C) Deaths due to matricide over a 4-day period for several nematode species.

Discussion

In this study we show that under conditions that reduce extrinsic mortality, a male longevity bias frequently characterizes both androdioecious and dioecious nematode species. We propose that internal hatching of larvae increases age-independent hermaphrodite and female mortality, and this favours the evolution of rapid senescence relative to males. We also find that androdioecy is associated with short male lifespan relative to dioecy, suggesting that androdioecy favours the evolution of reduced male longevity.

Using 12 *C. elegans* wild isolates, we have shown that the male longevity bias previously observed in the N2 laboratory strain is a species-typical trait. In the exceptional wild isolates where hermaphrodites are longer lived, absolute male lifespan is short, perhaps because the isolate contains sex-specific deleterious alleles. The wild isolate KR314 may be a further instance of this: here males are impotent because they carry the *mab-23(e2518)* mutation (Hodgkin & Doniach, 1997). A previous study of *C. elegans* wild-type strains reported a male longevity bias in two of three wild isolates tested, but not in N2 (Johnson & Hutchinson, 1993). However, in that study lifespan was measured in grouped populations in liquid culture, and we have found that in liquid culture, grouping slightly reduces male lifespan (data not shown).

To explore how nematode sexual dimorphism in aging may evolve, we compared sex lifespan differentials of androdioecious and dioecious species under common culture conditions. Although species may vary in how suitable the culture is for the longevity of each sex, these differences are likely to be random with respect to sex in a sufficiently large sample of species. Furthermore, observations suggest that sex longevity differentials are robust across culture methods. In *C. elegans* male longevity bias was observed in populations maintained on agar plates (Gems & Riddle, 2000b). In *O. dolichuroides* and *C. remanei* ssp. (B. Fletcher and D. Gems, unpublished observations), and in *C. briggsae*, *O. myriophila* and *O. species* (Patel *et al*., 2002), female lifespan is invariant when measured with agar plate culture and liquid culture. How adults survive under conditions similar to their natural habitat is an open and important question.

Our comparison of hermaphroditic and dioecious species does not support the hypothesis that the male longevity bias is a consequence of androdioecy because overall the male longevity bias was even greater in dioecious species. However, it cannot be entirely ruled out that increased male longevity has evolved for different reasons in hermaphroditic and dioecious nematodes. We suggest that the widespread occurrence of male longevity bias among nematode species may be at least in part the evolutionary consequence of increased mortality in females/hermaphrodites due to internal hatching of larvae.

An unexpected finding was that males of hermaphroditic species are shorter-lived overall than those of dioecious species (Table 2). We propose a novel hypothesis to account for the evolution of reduced male lifespan in androdioecious species: that it is a consequence of their rarity relative to hermaphrodites.

In wild populations, the incidence of males in hermaphroditic species generally is likely to be low. In *C. elegans*, among progeny of self-fertilizing hermaphrodites, spontaneous males occur at a frequency of ~0.2% owing to non-disjunction of the X chromosome during meiosis (Hodgkin, 1983); the rate of spontaneous male generation in *C. briggsae* is comparable to that in *C. elegans* (Nigon & Dougherty, 1949). The significance of males in wild populations of androdioecious nematodes remains unclear (Chasnov & Chow, 2002; Stewart & Phillips, 2002).

The rarity of androdioecious males, like the rarity of older age groups, would be expected to lead to a reduction in the force of selection against mutations with male-specific deleterious effects. This is because genes with male-specific effects may be present for long periods only in hermaphrodites, where they may have little or no effect on fitness, thus existing in a selection shadow. According to this hypothesis, the degree of shortevity of male nematodes of hermaphroditic species is likely to reflect their rarity in natural populations relative to the opposite sex. That hermaphrodites are nonetheless the shorter-lived sex in most androdioecious species may reflect the greater evolutionary impact on aging of factors such as matricide.

This hypothesis could explain why, in *C. briggsae*, males are the shorter-lived sex. One unusual characteristic of *C. briggsae* is that when males mate with hermaphrodites, the resulting progeny are initially largely hermaphrodites, rather than equal proportions of hermaphrodites and males as in *C. elegans*. This is due to precedence in fertilization by X-bearing out-cross sperm in this species, which does not occur in *C. elegans* (LaMunyon & Ward, 1997). Although wild populations of *C. briggsae* have not been studied, it has to be assumed that this further reduces the overall incidence of males in this species. Potentially, the short lifespan of *C. briggsae* males could have evolved as the result of their extreme rarity in the wild.

Our findings with eight terrestrial nematode species raise the question of whether male longevity bias is a characteristic of nematodes as a whole, including plant and animal parasitic nematodes. Apart from studies of *C. elegans*, there are few published reports in which sex differences in aging in nematode species are described (reviewed in Gems, 2001). In the case of the free-living nematodes *Panagrellus redivivus* (Abdulrahman & Samoiloff, 1975) and *Rhabditis tokai* (Suzuki *et al.*, 1978), females appear longer lived; in the plant parasitic nematode *Ditylenchus triformis*, males appear longer lived (Hirschmann, 1962). However, a problem with interpreting these findings is that culture conditions may not have been optimal for survival. Specifically, unmated males were not typically maintained in isolation.

Although little is known about aging in animal parasitic nematodes, survival has been studied in the filarial nematode parasite *Onchocerca volvulus*, which causes river blindness in humans. *O. volvulus* adults were excised from subdermal nodules (onchocercomata) from populations at varying intervals after eradication of the blackfly secondary host. Using this approach, the maximum lifespan for *O. volvulus* adults was estimated at 13–14 years (Plaisier *et al.*, 1991). In nodules from endemic areas, females outnumber males by approximately 1.5 : 1 (Albiez

et al., 1984; Karam *et al.*, 1987). However, in aging populations of *O. volvulus*, this ratio decreased, suggesting that male life expectancy may exceed that of females (Karam *et al.*, 1987).

Another question raised by our results is whether there are constraints on the evolution of different longevities in different sexes given that natural selection is acting upon the same genome (apart from the presence/absence of one X chromosome). Potentially, female or hermaphrodite longevity could be greater than it would otherwise be due to selection for greater longevity in males, since gene effects on male aging may also influence the other sex. Alternatively, increased mortality in females or hermaphrodites resulting in the evolution of reduced longevity could reduce male longevity. One extreme example of a nematode species with different adult forms and different adult longevities is *Strongyloides ratti* (reviewed in Gems, 2001). Parthenogenetic females of this species exist in the gut of rodents, and exhibit a maximum lifespan of at least a year (Gemmill *et al.*, 1997), yet the genotypically identical free-living adult *S. ratti* females age and die after little more than a week. In this nematode, at least, there appears to be little constraint upon the degree of difference in the rate of aging that may evolve in genotypically identical forms. The effect of such constraints in the evolution of sex-specific traits is discussed elsewhere (Partridge & Endler, 1987).

How do these findings relate to sex differences in mammalian lifespan? In many mammals, including humans, females are the longer-lived sex. Such differences are at least in part attributable to hormonal differences between the sexes (Diamond, 1982; Holden, 1987). It has also been suggested that the homogametic sex may be the longer-lived because sex chromosome diploidy protects against recessive X-linked mutations (Smith & Warner, 1989). However, this explanation is clearly not applicable to *C. elegans*, where hermaphrodites are the homogametic sex and are shorter-lived. The results presented here are more consistent with the view that sex differences in lifespan are the consequence of sex differences in the evolutionary forces moulding longevity and aging.

The results of this study raise the possibility that the short lifespan of *C. elegans* may result from sex-specific evolutionary forces leading to reduced lifespan: matricide in hermaphrodites and rarity in males. A future challenge will be to integrate the evolutionary theory of aging with the new classical genetics of aging (Partridge & Gems, 2002). In the case of *C. elegans*, sex differences in aging are dependent on the FOXO transcription factor DAF-16 (Gems & Riddle, 2000b). It will be interesting to discover how far the genetic determinants of aging identified in *C. elegans* can account for the evolution of different longevities among diverse nematode species in an evo-devo (or evo-gero) approach.

Experimental procedures

Culture conditions and nematode strains

Nematodes of all species were raised on NGM agar plates spread with the bacterium *Escherichia coli* OP50 as a food

source (Sulston & Hodgkin, 1988). Because male–male interactions reduce lifespan in *C. elegans*, males of all species were kept in solitary culture throughout lifespan measurement trials (Gems & Riddle, 2000b). Due to the leaving behaviour of solitary males, it was not possible to maintain them on agar plates. Therefore, for lifespan measurements animals of both sexes were cultured one per well in 96-well microtitre plates, in a suspension of *E. coli* in S medium (Sulston & Hodgkin, 1988) (concentration 1×10^8–1×10^9 cells mL^{-1}). Lifespan measurements were performed at 22.5 °C.

Wild isolates of *C. elegans* used were: AB1 (Adelaide), AB2 (Adelaide), CB4555 (Pasadena), CB4853 (Altadena), CB4854 (Altadena), CB4856 (Hawaii), CB4857 (Claremont), CB4858 (Pasadena), CB4932 (Taunton), RC301 (Freiburg), TR389 (Madison) and TR403 (Madison) (Hodgkin & Doniach, 1997). The N2 (Bristol) isolate used was the male stock supplied by the *Caenorhabditis* Genetics Center. *Caenorhabditis briggsae* isolates studied were: G16 (Gujarat), HK104 (Japan) and VT847 (Hawaii). Other nematode species employed were: CEW1 *Oscheius* sp., DF5018 *O. dolichuroides*, DF5033 *O. dolichura*, EM435 *O. myriophila*, EM464 *C. remanei* ssp. and SB146 *Rhabditis (Caenorhabditis) remanei* ssp. *remanei*.

Survival analysis

Animals were raised on agar plates and transferred to liquid culture at the fourth larval stage (L4). Thus, all lifespan measurements were performed on unmated animals. For initial analysis of *C. elegans* wild isolates, hermaphrodites were transferred daily during the egg-laying period (approximately 1 week) and then weekly thereafter, while males were transferred at weekly intervals throughout. However, subsequent tests showed that lower male transfer rates slightly reduced male lifespan in some instances (data not shown). In all subsequent survival trials therefore both males and hermaphrodites were transferred daily during the egg-laying period, and then approximately weekly thereafter. In the case of dioecious species, both sexes were transferred at approximately weekly intervals from the outset.

Animals were scored as dead when they failed to move when touched with a platinum wire. Individuals dying from non-senescent causes such as internal hatching of eggs (matricide) and animals in wells with microbial contaminants were censored.

Dauer larva formation

The sex bias in dauer formation was measured as follows. L4 animals were picked and left to mate overnight (2 males : 1 hermaphrodite or 1 male : 1 female). In hermaphroditic species, in order to obtain mixed-sex cohorts for analysis, eggs laid during the first 24 h were discarded, to reduce the presence of self-progeny. Mixed sex nematode stocks were allowed to reach starvation point at 25 °C, at which time dauers were picked and transferred to plates at 15 °C with plentiful food, allowed to recover, and sexed. All animals on the starved plate at the L3 stage or beyond, which would be of the same generation as

the dauer larvae, were sexed. All the remaining animals on the plate were newly hatched larvae, indicating that the F1 generation alone was scored, in its entirety. The percentage of males and hermaphrodites forming dauers was then calculated, and the ratio of male : hermaphrodite/female dauer formation obtained. Thus, a ratio of 1 signifies an equal tendency to form dauers by the two sexes.

Analysis of matricide

Hermaphrodites, or males and females, were picked as fourth-stage larvae, and left on plates for 24 h. Gravid 1-day-old females/hermaphrodites were then placed singly in M9 buffer in 96-well plates, and animals scored daily over 4 days for death due to matricide. For dioecious species, control mated females were also placed onto plates streaked with *E. coli* in order to check that enough sperm had been transferred during mating to allow fertilization of eggs over the 4 days.

Statistical methods

Survival analyses were performed using the Kaplan Meier method on censored data, and the significance of differences between survival curves calculated using the log rank test. Median rather than mean lifespans were calculated since typical age-specific death frequencies do not show a normal distribution. Survival data were re-sampled 50 000 times using R software (www.r-project.org) to obtain a mean of median values across replicates, and the 95% confidence limits around them. The effect of reproductive mode on lifespan was estimated using a two-way ANOVA. Mean percentage dauer formation values were compared using a Student's *t*-test, having first normalized the percentages by arcsine transformation. Results were considered significant at $P = 0.05$. The statistical software used was JMP v.3 (SAS Institute Inc. Cary, NC, USA).

Acknowledgments

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