Extraordinary plasticity in aging in *Strongyloides ratti* implies a gene-regulatory mechanism of lifespan evolution

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Summary

Aging evolves as the result of weakened selection against late-acting deleterious alleles due, for example, to extrinsic mortality. Comparative studies of aging support this evolutionary theory, but details of the genetic mechanisms by which lifespan evolves remain unclear. We have studied aging in an unusual nematode, *Strongyloides ratti*, to gain insight into the nature of these mechanisms, in this first detailed examination of aging in a parasitic nematode. *S. ratti* has distinct parasitic and free-living adults, living in the rat small intestine and the soil, respectively. We have observed reproductive and demographic aging in parasitic adults, with a maximum lifespan of 403 days. By contrast the maximum lifespan of free-living adults is only 5 days. Thus, the two adults of *S. ratti* have evolved strikingly different rates of aging. Parasitic nematode species are frequently longer-lived than free-living species, presumably reflecting different extrinsic mortality rates in their respective niches. Parasitic and free-living female *S. ratti* are morphologically different, yet genetically identical. Thus, the 80-fold difference in their lifespans, the greatest plasticity in aging yet reported, must largely reflect evolved differences in gene expression. This suggests that interspecific differences in lifespan may evolve via similar mechanisms.
Key words: comparative aging; evolution; nematode; phenotypic plasticity.

Introduction

Aging may evolve as the result of accumulation in populations of fitness-neutral, late-acting, deleterious mutations (Medawar, 1952). Alternatively, aging may reflect action of alleles that increase early life fitness (e.g. by increasing reproductive output)

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but have deleterious, late-life effects (Medawar, 1952; Williams, 1957). Such antagonistic pleiotropy is better supported by experimental investigation (Partridge & Gems, 2002). Yet, the molecular genetic mechanisms, genes and pathways by which lifespan evolves remain obscure.

A prediction of the evolutionary theory of aging is that organisms in environments producing a low rate of extrinsic mortality will age more slowly than those in environments that generate a high rate of extrinsic mortality (Medawar, 1952; Williams, 1957; Edney & Gill, 1968). This has been tested, and largely supported, by comparative studies. For example, bats have longer lifespans than rodents of similar size; this is thought to be due to the lower extrinsic mortality rate of bats, due to their ability to escape predation through flight (Wilkinson & South, 2002). Similarly, island opossums were found to age more slowly compared with their mainland cousins, presumably due to reduced predation, and hence reduced extrinsic mortality, on islands (Austad, 1993). Among social insects there are intraspecific, intercaste differences in lifespans and survival rates (Page & Peng, 2001), that for ants, may be due to different extrinsic mortality rates (Chapuisat & Keller, 2002). It is likely that the same evolutionary, genetic and mechanistic explanations of aging apply in both intra- and interspecific settings (Williams, 1957).

Here we describe a remarkable intraspecific difference in aging in *Strongyloides ratti*, an unusual species of nematode. The life cycle of this organism includes both free-living and parasitic reproductive adults. The parasitic adults are female only (Viney, 1994) and live in the small intestine of their rat host; the free-living adults live in faeces and soil outside of the host (Fig. 1). The parasitic females reproduce by parthenogenesis; thus, parasitic and free-living female morphs are genetically identical (Viney, 1994; Harvey *et al.*, 2000).

These two adult forms are morphologically different, principally in the overall body size and the relative size and morphological arrangement of the pharynx (Fig. 1). The parasitic morph is larger than the free-living morph (*c.* 2.4 and 1 mm, respectively). The relative length of the pharynx is also greater in the parasitic morph than in the free-living morph (*c.* 25% and 10–15% of body length, respectively). The pharynx of the free-living morph is of a typical 'rhabditid' form, with distinct sections; in contrast the parasitic morph has a so-called 'filariform' pharynx without distinct sections. This difference in pharynx morphology is likely to reflect the different diets of the two morphs. However, beyond these differences, there are no obvious morphological adaptations of the parasitic form to its lifestyle though, undoubtedly, various biochemical and physiological adaptations to a parasitic lifestyle must exist. An expressed sequence tag
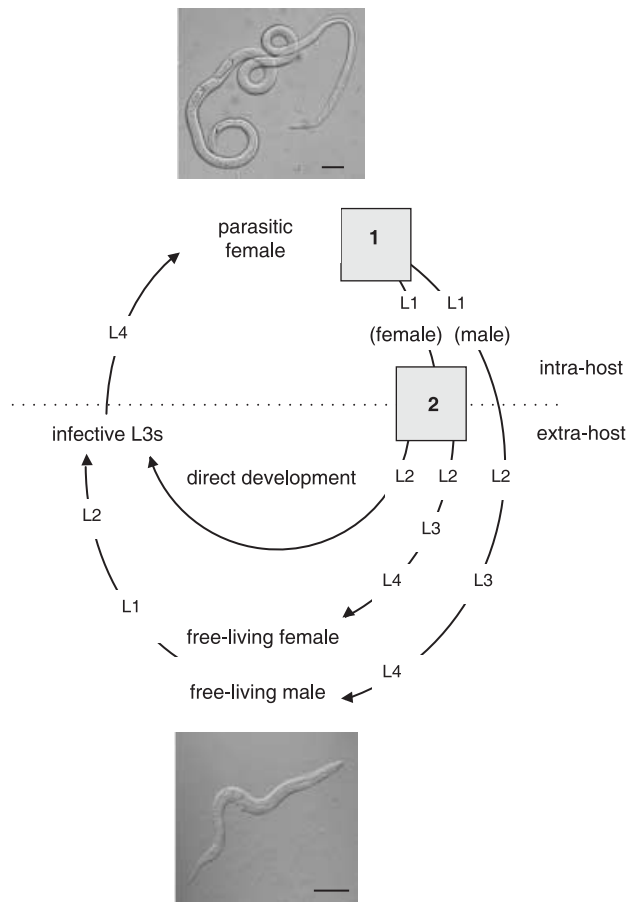
Fig. 1 Life cycle of *Strongyloides ratti*. The parasitic stage is female only and lives in the gut of its rat host and reproduces by mitotic parthenogenesis (Viney, 1994), passing eggs and larvae in faeces into the external environment. The eggs can develop either directly into infective third stage larvae (iL3s) or into free-living adult females and males (Harvey *et al.*, 2000). These adults mate by conventional sexual reproduction and produce progeny which develop into iL3s and infect new hosts (Viney *et al.*, 1993). The two developmental switches are shown as grey boxes: (1) the sex determination event; (2) the female-only developmental switch, after (Harvey *et al.*, 2000). Numbered larval stages are shown as L. The life cycle contains both parasitic and free-living generations; an adult parasitic female (top) and free-living adult female (bottom). Bars = 100 μM.

(EST) analysis of different stages of the *S. ratti* life cycle has shown that there are extensive differences in gene expression between parasitic and free-living stages (Thompson *et al.*, 2005). This EST analysis showed that approximately 40% of some 4000 likely genes of *S. ratti* are expressed in the parasitic female morph but not in the free-living stages of the life cycle (Thompson *et al.*, 2005). Given that the parasitic and free-living adult females are genetically identical, the differences in their biology (morphology, physiology, biochemistry, aging) must be due largely to differences in gene expression.

The life history of *S. ratti* raises questions about the evolution of lifespan. Free-living nematode species are short-lived, with maximum lifespans of a few weeks or months; by contrast, some parasitic nematodes can survive for many years. For example, while the soil-dwelling nematode *Caenorhabditis elegans*, much used as an experimental model, lives for 2–3 weeks, adults of the human filarial parasite *Onchocerca volvulus* can live for up to 12 years (reviewed in Gems, 2000). However, there is a paucity of information on aging in parasitic nematodes, which to some extent reflects the difficulty of directly observing this type of organism. The longer lifespans of parasitic nematodes seems likely to be an evolutionary consequence of lower extrinsic mortality rates within the relatively protected environment of the host.

In the laboratory, *S. ratti* infections of immunocompetent hosts are limited by the host immune response. Most parasitic females are lost after approximately 4 weeks and all after approximately 10 weeks, after which the hosts are strongly immune to reinfection (Gemmill *et al.*, 1997; Kimura *et al.*, 1999; Wilkes *et al.*, 2004). However, natural infections of *S. ratti* appear to be longer lived than laboratory infections, as judged by the observed prevalence of infection in wild rats (Fisher & Viney, 1998). This is likely to be due to a combination of both lower immune function and lower levels of infection in wild rats compared with laboratory rats (Viney *et al.*, 2005). Thus, wild *S. ratti* parasitic adults seem likely to experience a relatively low rate of extrinsic mortality compared with the free-living adults of *S. ratti*. Evolutionary theory would therefore seem to predict the evolution of long and short lifespans in parasitic and free-living *S. ratti* adults, respectively. Yet can this happen, given that these two forms of reproductive adult are products of the very same genome? Does the presence of a single genome from which two adult forms are generated act as a constraint to the evolution of longevity and aging? What can this tell us about the genetic mechanisms by which aging evolves?

Here we describe a careful comparative analysis of the biology of aging in *S. ratti*, with emphasis on the parasitic female. We describe the effects of age on parasitic female mortality rate, fecundity, autofluorescence (age-pigment, or lipofuscin) and, in both adult forms, on ultrastructure. Taking these findings together with those of an earlier study of aging in free-living *S. ratti* adults (Gardner *et al.*, 2004), this gives the first full picture of aging in this species. *S. ratti* parasitic females are long-lived (maximum lifespan, ~1.1 years), and senesce as judged by age-associated changes in their reproduction and mortality rate, although not ultrastructure or autofluorescence. Compared with parasitic females, free-living adults show massively accelerated aging, both in terms of their mortality rate and reproduction, as well as age-associated degenerative changes in their gross morphology, ultrastructure and autofluorescence (Gardner *et al.*, 2004; this study). The extraordinary plasticity in adult longevity that *S. ratti* exhibits implies a significant role for gene-regulatory mechanisms in the evolution of longevity.

Results

Strongyloides ratti parasitic adults are long-lived and exhibit demographic senescence

To ascertain whether the lifespan of *S. ratti* parasitic females is limited by intrinsic aging (senescence) or by extrinsic causes, we
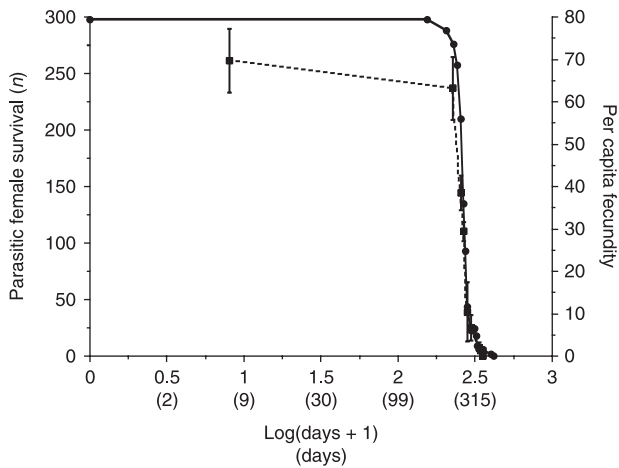
Fig. 2 Aging and fecundity of the parasitic and free-living females of *Strongyloides ratti*. (A) The estimated number (● —) and the mean (±1 SE) daily *per capita* fecundity (■ -------) of parasitic females, shown on a log scale, with the approximate number of actual days given in parentheses beneath.

Table 1 Mean and maximum lifespan, initial mortality rate (IMR), mortality rate doubling time (MRDT) and fecundity for the parasitic and free-living adult female morphs of *Strongyloides ratti*

| Morph	Lifespan (days) Mean (± SE) Maximum	IMR^2 (10^{-4} days^{-1}) (95% CI)	MRDT† (days) (95% CI)	Lifetime fecundity
Parasitic	271.3 (± 1.6) 403	5.6 (4.0–7.9)	22.7 (20.8–24.8)	~16 000
Free-living*	3.0 ± (0.1) 5	16.8 (9.7–29.0)	0.79 (0.65–0.96)	~40
Ratio	80	0.23	29	400

*Aging of 100 free-living females was determined at 25 °C, with a food source of streptomycin-resistant OP50 on agar plates containing 400 μg mL^{-1} streptomycin (Gardner *et al.*, 2004). These data were used to calculate the IMR and MRDT using a maximum likelihood approach (Pletcher, 1999) to obtain parameter estimates for the initial mortality rate and the Gompertz function (and hence IMR and MRDT). The actual observed maximum lifespan is reported here. Note, in Gardner *et al.* (2004), intercept and slope parameters (and hence IMR and MRDT, respectively) of the Gompertz model were determined by regression of ln(mortality rate) values against time.
†The IMR and the MRDT of the parasitic and free-living female morphs are significantly different ($\chi^2 = 2388.2$, d.f. = 1, $P < 0.0001$; $\chi^2 = 1763.5$, d.f. = 1, $P < 0.0001$, respectively).

first measured age-specific mortality rate and age changes in fertility in this morph. The occurrence of an exponential increase in mortality rate with increasing age is characteristic of limitation of life by biological aging, and we aimed to test for this. To this end, a cohort of nude rats, which are immunologically permissive to *S. ratti* infections (Gemmill *et al.*, 1997; Harvey *et al.*, 2000), were infected with *S. ratti*. The survival of these nematodes was then estimated by periodically culling small numbers of infected rats, and counting the number of parasitic females present. Mortality rates were then estimated from the decline in nematode numbers. However, it should be noted that it is unclear to what extent older parasitic females are passed from the gut as the result of their death, or of aging-associated loss of fitness. Thus, it is possible that our analysis underestimates parasite lifespan and overestimates parasite age-specific mortality rate. It should also be noted that *S. ratti*, in common with most parasitic nematodes, do not multiply within the host. Further, the conditions under which the infected rats were maintained precluded accidental infection (e.g. with their own offspring). Thus, in this study a cohort of parasitic *S. ratti* females was present in each infected rat at the start of each trial from which individual worms were then lost gradually over time.

Population aging was measured in this manner in two separate experiments, each running for approximately 1 year: a preliminary experiment (see Supplementary material) to refine the sampling schedule, and then a second experiment in which the principal measurement of senescence was made, which is reported here. The results of the two experiments are consistent with one another. For parasitic females, population aging (i.e. a significant increase in mortality rate with increasing age) was detectable only after 154 days of adulthood. This corresponded to mean (± SE) and maximum lifespans of 271 ± 1.6 and 403 days, respectively (Fig. 2, Table 1). Parasitic females showed an exponential increase in mortality rate with age, supporting

the view that lifespan is limited by senescence, rather than other, extrinsic causes of mortality. The Gompertz model for age-dependent mortality rate provided the best fit to these data, taking into account the number of parameters for the parasitic female morphs ($P = 0.024$). The mortality rate doubling time (MRDT) (Finch *et al.*, 1990) was 22.7 (95% CI 20.8–24.8) days. The initial mortality rate (IMR) was 5.6 (95% CI 4.0–7.9) × 10^{-4} day^{-1}. This is the first experimental demonstration of population (demographic) aging in a parasitic nematode. The longevity of parasitic *S. ratti* females contrasts strikingly with genetically identical, free-living adult females, which have mean (± SE) and maximum lifespans of only 3.0 ± 0.1 and 5 days, respectively, even under optimized culture conditions (Fig. 2, Table 1) (Gardner *et al.*, 2004).

Strongyloides ratti parasitic adults exhibit reproductive senescence

As a second approach to assess aging in the parasitic morph, we measured age changes in the rate of reproduction. The estimated mean (± SE) daily per capita fecundity was 57.1 ± 9.5 offspring for the first ~250 days of adulthood, after which it rapidly declined (Fig. 2). Like the Gompertzian acceleration of age-specific mortality, this change in reproduction in late life is consistent with aging in parasitic females. The estimated lifetime production of progeny was ~16 000 per *S. ratti* parasitic female, compared with maximum lifetime fecundity of less than 40 in the free-living females (Gardner *et al.*, 2004). This difference in lifetime fecundity might account for the evolution of the peculiarly short lifespan of free-living females (see Discussion).
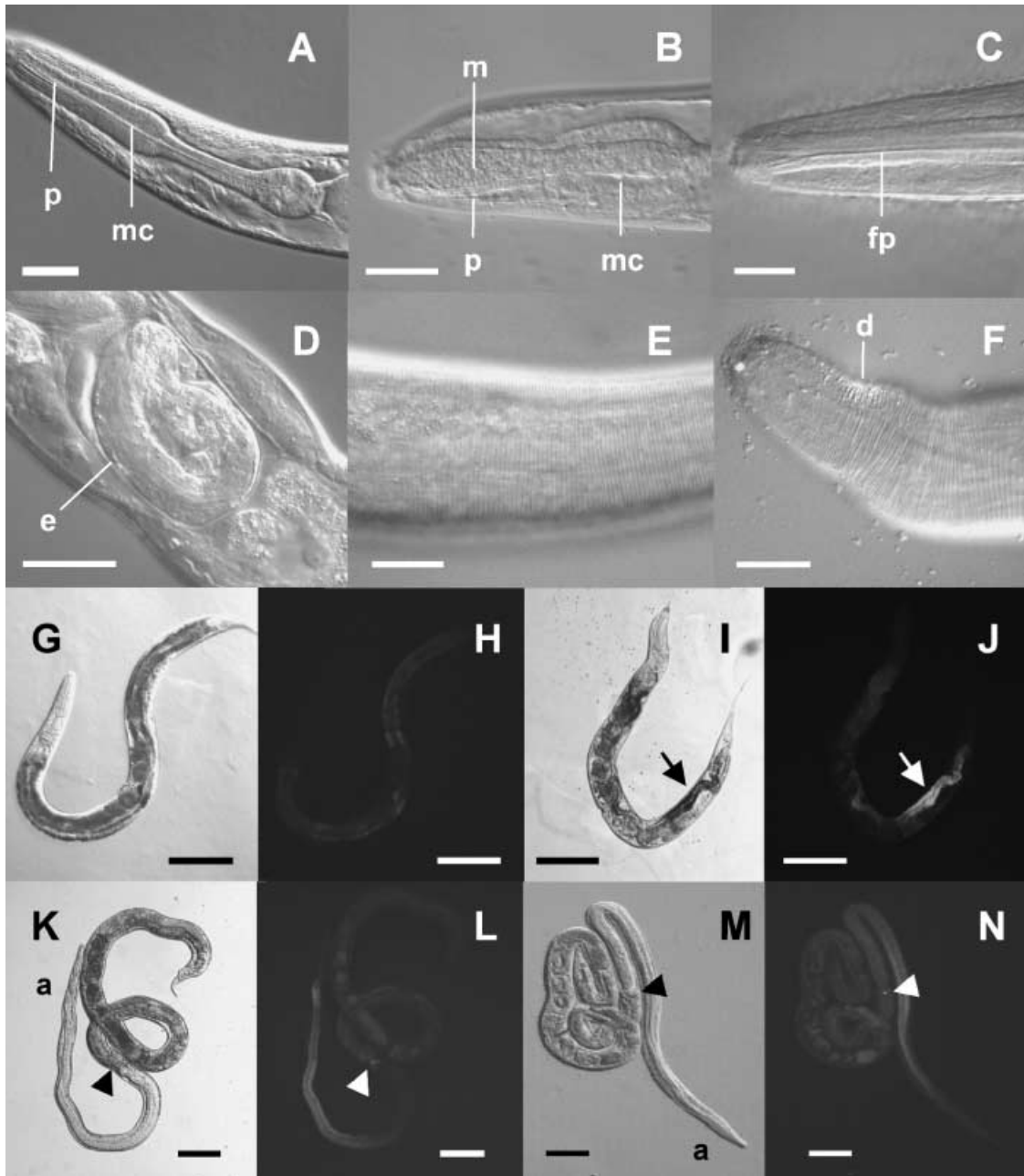
Fig. 3 Age-associated changes in the morphology and autofluorescence during aging in *Strongyloides ratti*, viewed using differential interference contrast (DIC) microscopy and epifluorescence microscopy, respectively. (A) Head region of 1-day-old free-living *S. ratti* female. (B) Head region of 4-day-old free-living female at slightly higher magnification. Here the lumen of the procorpus and metacorpus is packed with microbes. (C) Anterior end of 11-month-old parasitic female, showing no obvious age-associated changes. (D) Apparently normal embryo in uterus of 11-month-old parasitic female. (E) Cuticle (near head) of 6-day-old parasitic female, showing radial annulae (vertical stripes) that are well formed. (F) Cuticle of 11-month-old parasitic female, showing annulae that are slightly disorganized in places, and some minor damage, d, Bars = 20 μM. (G–N) Bright field and epifluorescence images of free-living and parasitic females, showing effects of aging on autofluorescence. (G, H) One-day-old free-living female. (I, J) Four-day-old free-living female with increased autofluorescence in the intestine (arrows). (K, L) Seven-month-old parasitic female. (M, N) Eleven-month-old parasitic female. Arrowhead, junction between pharynx and intestine. Note the absence of autofluorescence, particularly from the intestine (posterior to the pharynx), and the lone punctum of autofluorescence at the junction of the pharynx and the intestine in (N). Bars = 100 μM. a, anterior end of animal; e, embryo; fp, filariform pharynx; p, procorpus; mc, metacorpus of pharynx; m, microbial digesta.

Age changes in morphology and autofluorescence in *S. ratti* adults

We examined the age-associated changes in the morphology of *S. ratti* adults, first using differential interference contrast (DIC or Nomarski) microscopy. Free-living adult females of *S. ratti* show striking age-associated degenerative changes, including intestinal cell atrophy and blockage of the pharynx and intestinal lumen with packed microbial digestate (Fig. 3A,B; Gardner *et al.* 2004). By contrast, even 11-month-old parasitic females of
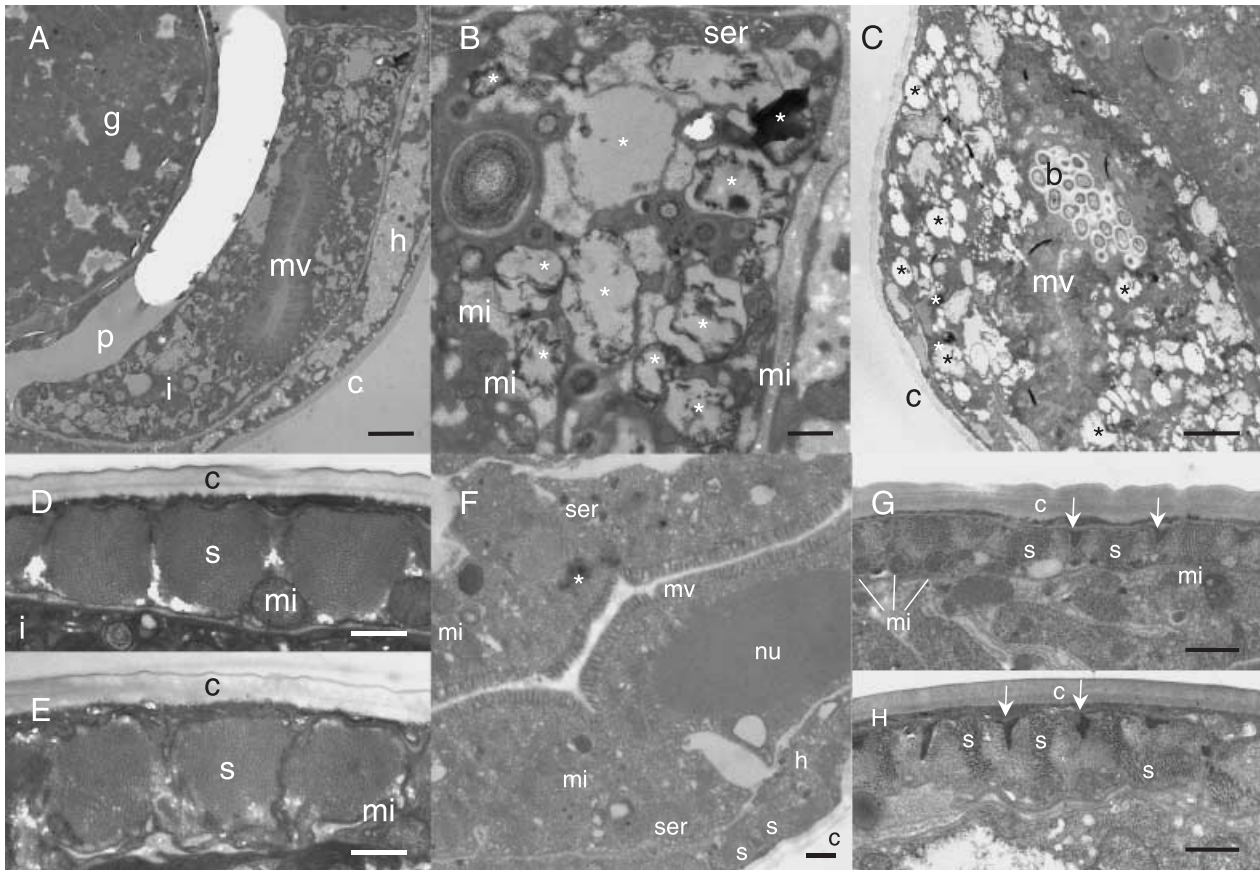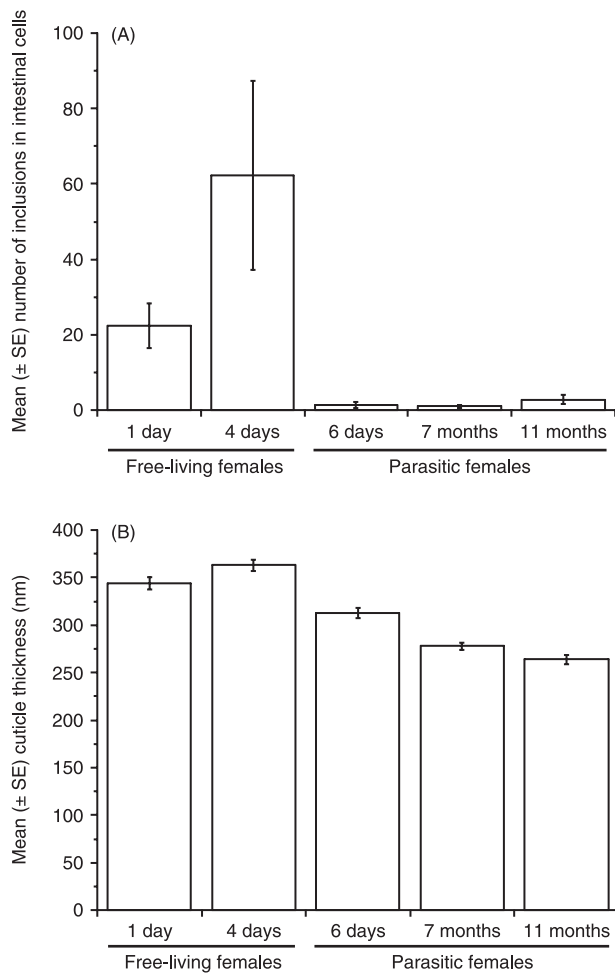
Fig. 4 Age-associated changes in ultrastructure in free-living and parasitic females of *Strongyloides ratti*. All figures show transverse sections of the mid-body region. (A–E) Free-living females. (A, B) One-day-old adult; (B) shows detail of top right corner of intestinal cross section in (A). Note the necrotic appearance of the cytoplasm and presence of dark, electron opaque material, even in this young adult. Bars = 2 μm and 0.5 μm, respectively. (C) Four-day-old adult, view of intestine in advanced state of necrosis. Bar = 2 μm (D, E) Aging of the cuticle and underlying sarcomeres; (D) 1-day-old adult, (E) 4-day-old adult. Note the good preservation of cuticle and sarcomeres. Bar = 0.5 μm. (F–H) Parasitic females; (F) 11-month-old parasitic female, view of intestine. Bar = 0.5 μm. Note the well-preserved ultrastructure even at this advanced age. Cytoplasm appears rich with mitochondria and smooth endoplasmic reticulum. (G, H) Cuticle and underlying sarcomeres; (G) 6-day-old female, (H) 11-month-old female. Note the slight loss of organization of the sarcomeres in (H). Bars = 0.5 μm. Arrows, dense bodies that interface with the hypodermis and cuticle. b, clump of bacteria; c, cuticle; g, gonad; h, hypodermis; i, intestine; mi, mitochondria; mv, microvilli; nu, nucleus; p, pseudocoelomic space; s, sarcomere; ser, smooth endoplasmic reticulum; *regions of cytoplasmic breakdown or electron dense material.

S. ratti showed no marked degenerative changes. Major organs (pharynx, intestine, gonad) appeared unchanged (Fig. 3C), and apparently normal embryos were seen in the gonad (Fig. 3D); however, in some individuals there was slight damage and wrinkling in the cuticle, particularly in the head region (Fig. 3E,F).

In free-living adult females, there is a striking age-associated increase in gut autofluorescence (Fig. 3G–J, Gardner *et al.*, 2004). This autofluorescent material is likely to be the molecular waste material lipofuscin, as seen in aging *C. elegans* (Epstein *et al.*, 1972; Davis *et al.*, 1982). Accumulation of molecular waste of this nature is ubiquitous in animal aging, and reflects age increases in molecular damage, and impairment of cellular turnover mechanisms. We examined autofluorescence in parasitic females of *S. ratti*, but little was seen in 7- or even 11-month-old animals, and there was no apparent increase in this with age (Fig. 3K–N). This suggests that these long-lived parasitic females are highly efficient at avoiding, repairing or turning over molecular damage.

Age changes in ultrastructure of *S. ratti* adults

Next, we used transmission electron microscopy (TEM) to study and compare age changes in ultrastructure of free-living and parasitic females of *S. ratti*. In free-living females striking deteriorative age changes were evident in several tissues. Most salient was a marked deterioration in the ultrastructure of the intestinal cells, which developed a damaged and disorganized appearance in most animals (Fig. 4A–C). In particular, much of the cross-sectional area of intestinal cells consisted of a variety of inclusions whose appearance suggests pathology. Some of these inclusions were ovate, and resembled lipid droplets but often containing electron opaque material with a sooty appearance; others were more irregular and more electron-opaque. These intestinal cell inclusions increased in number with age (Fig. 5A) but, surprisingly, were already abundant in 1-day-old adults (Fig. 4A,B). This implies that by the time they reach maturity, free-living females of *S. ratti* are already in an advanced

Fig. 5 Quantitative analysis of age changes in ultrastructure in free-living and parasitic females of *Strongyloides ratti*. (A) Mean number (± 1 SE) of lipid-like and electron-dense inclusions (probable lipofuscin) visible in cross sections of intestinal cells. Number of sections examined: Free-living adults, six each for 1- and 4-day-old females; parasitic females, five, nine and six for 6-day-, 7-month- and 11-month-old females, respectively. (B) The mean (± 1 SE) cuticle thickness (nm) of 1- and 4-day-old free-living females are 344 ± 6 ($n = 9$) and 363 ± 6 nm ($n = 9$), respectively, which are not significantly different ($\chi^2 = 1.03$, d.f. = 1, $P = 0.31$). The mean (± 1 SE) cuticle thickness of 6-day-, 7-month- and 11-month-old parasitic females are 313 ± 5.0 ($n = 6$), 278 ± 3.67 ($n = 9$) and 264 ± 4.67 nm ($n = 9$), respectively, which are also not significantly different ($\chi^2 = 5.56$, d.f. = 2, $P = 0.062$).

state of senescence. In *Caenorhabditis*, similar degenerative changes in the intestine of older animals have been observed using TEM, where electron-opaque inclusions were taken to be lipofuscin (Epstein *et al.*, 1972). By contrast, in parasitic adults there were very few such inclusions (Fig. 4F), and their number changed little with age (Fig. 5B). These results are consistent with the age-associated increases in autofluorescence seen in free-living but not parasitic adults. In some old free-living adults bacteria from the intestinal lumen were present in the intestinal cytoplasm, and in others in the pseudocoelom (data not shown). This suggests that invasion by bacteria may hasten the demise of these rapidly aging nematodes.

We also studied the effect of aging on the cuticle, which in *C. elegans* has been reported to grow thicker with increasing age (Herndon *et al.*, 2002). In *S. ratti* there were no age-associated changes in cuticle thickness in either adult morph (Figs 4 and 5B). Another age-associated change observed in *C. elegans* is structural damage to muscle, i.e. sarcopenia (Herndon *et al.*, 2002). In free-living *S. ratti* females slight age-associated changes in muscle structure were sometimes seen, and more so in parasitic females where the sarcomeres appeared somewhat less tightly packed in older worms than in younger worms (Fig. 4).

In summary, in parasitic females of *S. ratti* we saw few age-associated changes in gross morphology, ultrastructure, or intestinal autofluorescence. This contrasts with free-living females where there are striking age-associated changes in morphology and ultrastructure and increases in intestinal autofluorescence (Gardner *et al.*, 2004; this study).

Discussion

In this study we examined and compared aging in free-living and parasitic adults of the unusual nematode, *S. ratti*. Our results demonstrate a very high degree of phenotypic plasticity in aging in this organism. However, it is possible that extrinsic factors have, to some extent, contributed to mortality of both adult morphs, although we have tried to minimize this. In *C. elegans*, lifespan is shortened by their bacterial food source (Garigan *et al.*, 2002), such that treatment with antibiotics increases lifespan. Here, although free-living *S. ratti* were cultured in the presence of streptomycin, electron microscopy revealed bacteria within the bodies of some older worms, suggesting that lifespan may still be reduced by bacterial invasion. However, necrotic changes in ultrastructure are evident in all individuals, particularly in the intestine, even in some 1-day-old females. This suggests that bacterial invasion is a consequence of endogenous aging, rather than a primary cause of age increases in mortality rate. It is likely, though, that we have slightly underestimated the potential longevity of free-living *S. ratti* adults.

The longevity of parasitic adults may also have been underestimated. Our estimates of survival and mortality rate are based on loss of parasitic adults from the intestine, which seems likely to be a consequence of aging. However, this loss could reflect either mortality or reduced fitness. The passing of senescent, but live, worms from the host could explain the near absence of pathology observed in older parasitic adults, as implied by DIC, autofluorescence and TEM observations (Figs 3–5). Thus, our results suggest that we may have slightly underestimated longevity in both free-living adults and, perhaps to a greater extent, in parasitic adults. Crucially, though, this implies that we have not overestimated the phenotypic plasticity of aging in this organism.

Our results provide strong evidence that aging limits the lifespan of parasitic females of *S. ratti*, which have a maximum lifespan of over a year. The free-living morph of *S. ratti* also senesces, but only attains a maximum lifespan of less than a week. Direct comparison of the parameters that describe the aging of these two morphs (Table 1) show that they have an

80-fold difference in their lifespan; the IMR of the parasitic morph is approximately threefold less and the MRDT approximately 30 times greater in the parasitic morph, compared with the free-living female morph. Thus, both morphs appear to senesce but, comparatively, this senescence is massively accelerated in the free-living females. These differences exist despite the fact that long- and short-lived adult females are genetically identical. There is some evidence that this pattern of plasticity in aging may be common among related nematodes in the family Strongyloididae (Gems, 2000). For example, in *Parastrongyloides trichosuri*, a parasite of Australian possums, free-living adults have a maximum lifespan of only 11 days, and parasitic infections (in immunocompetent hosts) can last for up to 274 days (Grant *et al.*, 2006).

Strongyloides ratti is an organism subject to a complex pattern of evolutionary selective pressure, since it has both free-living and parasitic adults (Viney, 1996). Entirely free-living nematode species are typically shorter-lived than parasitic species (Gems, 2000), which is likely to be an evolutionary consequence of lower extrinsic mortality rates in the latter. As shown here, free-living and parasitic *S. ratti* females have, like purely free-living and parasitic nematode species, evolved short and long lifespans, respectively. What is intriguing in *S. ratti* is the way that the moulding of adult longevity by the extrinsic mortality rate apparently occurs independently in the two morphs, despite their sharing the same genome. One might have expected that selection for longevity in the parasitic female would purge the genome of late-acting deleterious mutations, resulting in long lifespan in the free-living adults. In fact, the latter are actually shorter lived than any known free-living nematode species. One possibility is that the shortevity of the free-living adults has evolved as a consequence of the greater fecundity of the parasitic females. This condition could favour pleiotropic alleles that increase parasitic fecundity but reduce lifespan of free-living adults. Alternatively, the shortevity of free-living adults could reflect the fact that this is a facultative phase (Viney, 1996). Thus, given multiple, successive parasitic generations, selection on free-living adult-specific fitness traits may be weakened, much as they are by elevated extrinsic mortality.

That the two morphs of *S. ratti* are genetically identical implies that their different lifespans result largely from differences in gene expression (although environmental factors may, to some extent, have influenced our mortality rate data; see above). These results suggest that in molecular genetic terms, 'late-acting mutations' or 'antagonistic pleiotropy' reflect differences in the expression of genes promoting longevity or aging, at least in *S. ratti*. Potentially, interspecific differences in lifespan are enacted by similar evolved differences. This implied view of lifespan as a highly plastic, regulated trait is consistent with the rapid evolution of interspecific differences in lifespan. For example, humans and chimpanzees have evolved a difference in maximum lifespan of some 50 years since divergence from a common ancestor only 5–6 million years ago; recent evidence suggests that altered gene expression contributes to such differences (Khaitovich *et al.*, 2005).

One established regulator of animal aging is the insulin/IGF-1 signalling pathway (Tatar *et al.*, 2003; Kenyon, 2005), which may determine aging by regulating the level of somatic maintenance (Kirkwood, 1977; McElwee *et al.*, 2004). It is possible that different lifespans evolve via the action of this and other such pathways and, or the processes they regulate. Given the remarkable plasticity of aging of *S. ratti*, as described in this study, this species represents a useful model organism for studies of regulated mechanisms that are potent determinants of aging and longevity, and for understanding the mechanisms contributing to evolved differences in lifespan.

Experimental procedures

Parasitic and free-living morphs

The *S. ratti* isofemale line, ED321 Heterogonic (Viney, 1996) was used throughout. To measure aging in the parasitic morph, 80 female nude rats (RNU-*rnu*) (Harlan, UK) were inoculated subcutaneously with 1000 infective third-stage larvae. Nude rats were used because these are immunologically fully permissive to *S. ratti* infections (Viney, 1996). The infections were monitored by a combination of (i) culling groups of infected rats and directly determining the number of parasitic females present and (ii) measurement of the number of eggs passed in the faeces (Wilkes *et al.*, 2004) with measure (i) concentrated from day 250 postinfection (see Supplementary material) such that sufficient data (measure i) were available to accurately measure the rate of senescence at great age. The age of the parasitic females is measured as days postinfection; the prepatent period for *S. ratti* is 4 days postinfection. A preliminary experiment was undertaken (see Supplementary material) to refine the sampling schedule of the experiment that is reported here. These 80 nude rats were sacrificed according to the following schedule as the number of days postinfection (number of rats): 7 (10), 226 (7), 255 (6), 266 (7), 281 (6), 298 (7), 337 (7), 352 (6), 355 (6), 367 (6), 388 (5), 403 (4) and 415 (3). The lifetime fecundity of the parasitic females is the summation of the number of eggs and larvae passed in faeces per day throughout this second infection, with values interpolated between sample days. To determine aging of the free-living females of *S. ratti*, they were cultured at 25 °C with a food source of streptomycin-resistant OP50 on agar plates containing 400 μg mL^{-1} streptomycin (Gardner *et al.*, 2004).

Mortality analysis

Mortality data for both the parasitic and free-living morphs was fitted to the Gompertz, Gompertz-Makeham, logistic and logistic-Makeham data models (Pletcher, 1999) using maximum likelihood methods in Winmodest version 1.0. Because these four models are nested models, we used the log-likelihood ratio test to determine which model gave the best fit: the Gompertz model provided the best fit to the data, taking into account the number of parameters. This model assumes that mortality increases exponentially with age as, $m(t) = Ae^{\alpha t}$, where $m(t)$ is

the mortality rate at time *t*, *A* is the initial mortality rate (IMR) and α is the Gompertz exponential function (Finch, 1990). Testing hypotheses concerning mortality parameter values can be developed by introducing an extended model, resulting in a modified log-likelihood function (Pletcher, 1999). To test hypotheses concerning such parameter values, we constrained certain parameters (e.g. α) to be equal, under the null hypothesis. We compared *A* and α between the parasitic and free-living morphs, with twice the difference between the maximum log-likelihood estimates being distributed as a χ^2 random variable with 1 degree of freedom.

Morphological analysis

Parasitic females were obtained from infected rats as previously described (Wilkes *et al.*, 2004) and DIC microscopy and autofluorescence observations were made as previously described (Gardner *et al.*, 2004). For both DIC microscopy and autofluorescence a total of 6-day- (*n* = 17), 7-month- (*n* = 15) and 11-month-old (*n* = 6) animals were examined. For the TEM studies free-living females 1 and 4 days old (maintained at 25 °C) (Gardner *et al.*, 2004) and parasitic females 6 days, 7 months, and 11 months old were fixed (Ashton *et al.*, 1995), treated with microwave radiation and embedded using standard methods. Serial sections (70–100 nm) were mounted on slotted grids and examined with a JEOL (Garden City, Herts, UK) 1200 EX TEM operated at 80 KV. For each of these five samples, sections were obtained from three worms each at the anterior, middle and posterior portion, giving a total of 45 sections. The mean number of lipid-like and electron-opaque inclusions within the intestinal cells was determined from the sections in which this could be viewed. Cuticle thickness was measured every 45° around the circumference of each section and the mean value per section was used to compare cuticle thickness between worms of different ages.

Acknowledgments

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Supplementary material

The following supplementary materials available for this article:

Appendix S1 Measuring the survival of parasitic females of *Strongyloides ratti*: preliminary experiment.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1474-9726.2006.00226.x (This link will take you to the article abstract).

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