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Slow development as an evolutionary cost of long life

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Summary

1. Life-history theory predicts a trade-off between early-life fitness and life span. While the focus traditionally has been on the fecundity-life span trade-off, there are strong reasons to expect trade-offs with growth rate and/or development time.
2. We investigated the roles of growth rate and development time in the evolution of life span in two independent selection experiments in the outcrossing nematode *Caenorhabditis remanei*.
3. First, we found that selection under heat-shock leads to the evolution of increased life span without fecundity costs, but at the cost of slower development.
4. Thereafter, the putative evolutionary links between development time, growth rate, fecundity, heat-shock resistance and life span were independently assessed in the second experiment by directly selecting for fast or slow development. This experiment confirmed our initial findings, since selection for slow development resulted in the evolution of long life span and increased heat-shock resistance.
5. Because there were no consistent trade-offs with growth rate or fecundity, our results highlight the key role of development rate – differentiation of the somatic cells per unit of time – in the evolution of life span.
6. Since development time is under strong selection in nature, reduced somatic maintenance resulting in shorter life span may be a widespread cost of rapid development.

Key-words: antagonistic pleiotropy, development time, growth, life span, stress resistance, trade-off

Introduction

Ageing, or senescence, is a multifaceted physiological deterioration of organismal function, which increases the probability of death with age and ultimately limits life span (Finch 1994; Gems & Partridge 2013). Ageing has long been at the forefront of fundamental and applied research, but despite tremendous progress in recent years we are still far from fully understanding why and how ageing evolves (Williams *et al.* 2006; Gems & Partridge 2013; Jones *et al.* 2014). Because of extrinsic mortality caused by a variety of biotic and abiotic factors, mutations that cause the loss of vitality in late-life are shadowed from natural selection and can accumulate in the population, which forms the basis of the mutation accumulation theory of ageing (Medawar 1952; Charlesworth 1994). Importantly, since ageing is considered maladaptive under mutation accumulation, this theory does not predict a trade-off between early and late function (reviewed in Maklakov, Rowe & Friberg 2015). In 1957, George Williams (Williams 1957) developed the antagonistic pleiotropy theory by reasoning that an allele that contributes to increased performance in early-life can spread in the population even at the cost of late-life performance. Thus, ageing can be seen as an adaptation under antagonistic pleiotropy, and a physiological account of this theory, the disposable soma theory of ageing (Kirkwood 1977), suggests that such early-life benefits can include increased fecundity, increased growth rate and rapid development (Kirkwood & Austad 2000; Stearns *et al.* 2000), traded-off against somatic maintenance and, therefore, life span (Kirkwood 1977; Labbadia & Morimoto 2015a).

The relationship between fecundity and several proxies of somatic maintenance is well studied, and increased reproductive effort is often associated with decreased life span (Flatt 2011; Boonekamp *et al.* 2014) and reduced stress resistance (Labbadia & Morimoto 2015b). However, in recent decades, a number of studies have failed to support the trade-off between fecundity and life span (reviewed in: Edward & Chapman 2011; Flatt 2011; Maklakov & Immler 2016). When life span extension does not reduce fecundity, it is likely that the cost of long-life is offset by other life-history traits that affect fitness, such as

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growth and development (Ricklefs 2006). The connection between growth and proxies for somatic maintenance has been recognized for quite some time and increased growth is often associated with reduced longevity (Olsson & Shine 2002; Lee, Monaghan & Metcalfe 2013) and reduced stress tolerance (Sørensen, Kristensen & Loeschcke 2003). However, the causal relationship between growth and life span is more difficult to establish because of the confounding differences in body size. Nevertheless, recent experimental work suggests that increased growth rate results in reduced life span when body size is controlled for (Lee, Monaghan & Metcalfe 2013).

It is possible, however, that some of the effects associated with growth rate may result from differences in development time. Since development is the differentiation of the soma, while growth is the increase in mass over time, these processes are fundamentally different on the cellular level (van der Have & de Jong 1996). Theory (Stearns 1992; Blanckenhorn & Demont 2004) and experimental work (Johansson & Rowe 1999; Lind *et al*. 2011) show that short development time is beneficial in time-constrained environments. Furthermore, development time is often under sexual selection during scramble competition (Andersson 1994). Despite development time being a component of the calculation of growth rate, these traits can be adjusted independently (Ball & Baker 1996; Lind & Johansson 2011). However, the relationship between development time and life span is rarely investigated. In a landmark study, Stearns *et al*. (2000) selected *Drosophila melanogaster* flies for different random mortality rates and found that high mortality was associated with short life span, high early fecundity, small size and fast development (growth was not calculated), suggesting that both reproduction, development and possibly growth can all be involved in trade-offs with life span. However, since most organisms continue to develop (have cell divisions) throughout the growth phase, the effects of growth and development are notoriously difficult to separate.

We set out to investigate the effects of reproduction, growth rate and development time on the evolution of life span in the dioecious nematode *Caenorhabditis remanei*. Because its reliance on temporary food sources such as rotten fruit, which is the natural habitat of *C. remanei* (Fitch 2005; Félix & Braendle 2010), development time is expected to be under strong selection in this species. Moreover, *Caenorhabditis* nematodes have a fixed number of cells and do not continue cell division after maturation, making it possible to partly separate the effects of development time and growth rate, since all post-maturation growth (which is the majority of growth) is caused by the increase in cell volume (Lambie 2002). Before maturation, development and growth cannot be fully separated.

Here we use two independent selection experiments to show a trade-off between adult survival and juvenile development. First, using previously established selection lines, we tested the hypothesis that life span extension can be offset by trade-offs with development time or growth rate. Previously published work on these lines has demonstrated that while selection under random increased mortality results in lowered life span in favour of increased fecundity, selection under increased mortality due to heat-shock results in increases in both fecundity and life span (Chen & Maklakov 2012; replotted in Fig. 1d–e). We examined development time and juvenile growth, and found that longer adult life span in the heat-shock lines does come at a cost of longer juvenile development. To explore the link between these traits, we established a second selection experiment where we directly selected on fast and slow development time, and investigated the correlated responses in growth, fecundity, heat-shock stress resistance and life span of both sexes. We found that lines selected for slow development invested in somatic maintenance because they were more stress resistant and longer lived than their fast-developing counterparts, while fecundity was not affected and no consistent pattern was found for growth rate. We discuss these results in the light of the disposable soma theory of ageing and suggest that development time emerges as an important trait involved in trade-off with stress resistance and life span.

Materials and methods

EXPERIMENTAL PROCEDURES

Larval development of mortality-selected lines

We used experimental lines, created by artificial selection from the wild-type SP8 strain of *C. remanei*. For a full description of the selection procedure, see Chen & Maklakov (2012). Briefly, the lines were subjected to two crossed selection regimes: mortality source [random (R) or condition-dependent (C-d)] and mortality rate [high (H) or low (L)], resulting in four selection regimes (HR, LR, HC-d, LC-d). Condition-dependent mortality was imposed using heat-shock at 40 °C for either 110 (HC-d) or 70 (LC-d) minutes, while random mortality was imposed by random removal of an equal number of worms as were killed by heat-shock in the condition-dependent mortality treatment. Four replicate lines were subjected to each selection regime, resulting in a total of 16 lines. The lines evolved under these conditions for 12 generations, and were subsequently kept for two generations under relaxed selection and frozen for future use.

In this experiment, we assayed development time to and size at sexual maturity of females from these lines. Sexual maturity was determined by the presence of a mating plug; already as L4 larvae females were constantly subjected to mating attempts by males and we therefore considered successful mating as a good indicator of sexual maturity. Females are mated shortly after sexual maturity.

Before each assay, worms were recovered from freezing and cultivated for two generations under standard laboratory conditions (Stiernagle 2006). Worms fed on standard *Escherichia coli* OP50 during the first two blocks, while the antibiotics streptomycin and nystatin were added to agar for the last two blocks to combat bacterial infections, and these worms were therefore feeding on the streptomycin-resistant strain OP50-1. The assays were conducted in four blocks, each containing one replicate line of each selection treatment, with the exception of the lines LR2 and HR2, which were replicated in two blocks for logistical reasons. For each line, 10 mated females (second day of adulthood) were allowed to lay eggs on a seeded plate for 1 h. After 54 h, when the offspring were

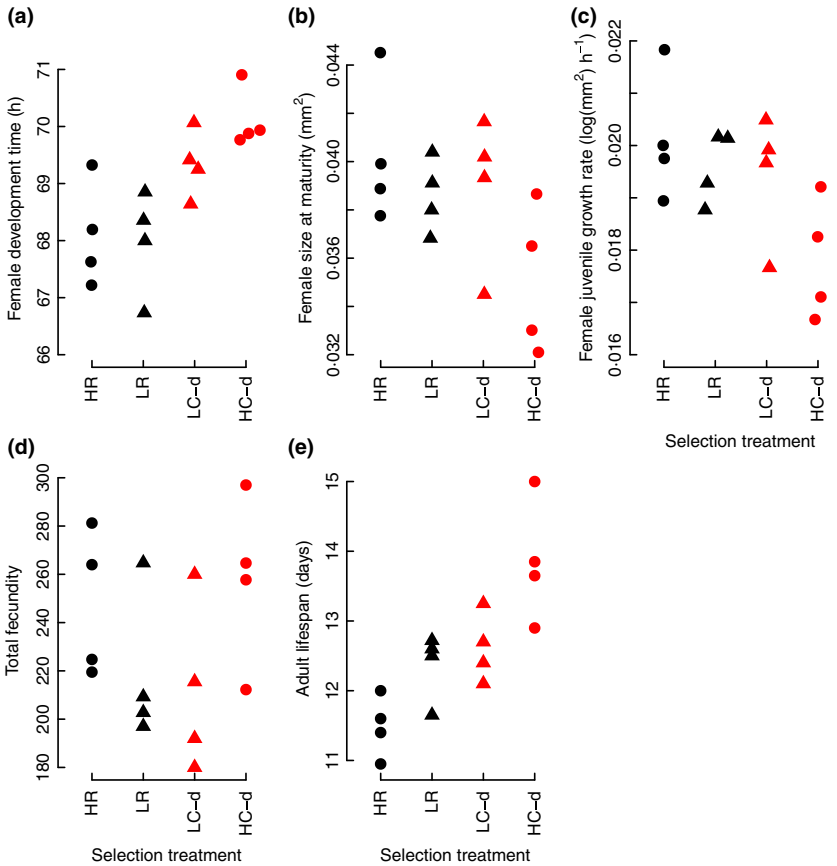

Fig. 1. Juvenile development time, size at sexual maturity, growth rate, total fecundity and life span of females from random (white) or condition dependent mortality regime (red). High mortality rate is designated by circles and low rate by triangles. Symbols represent line means, controlled for block effects. d–e are reproduced from Chen & Maklakov (2012).

in the L4 larval stage, male larvae were removed and replaced by the same number of sexually mature males (second day of adulthood) from the wild-type SP8 strain. From 59 h after egg laying, plates were monitored hourly, and mated females were scored for development time from egg to sexual maturity and photographed using a Lumenera Infinity2-5C digital microscope camera mounted on a Leica M165C stereo microscope (Heerbrugg, Switzerland). Size was measured as the cross-sectional area using ImageJ 1.46r (http://imagej.nih.gov/ij/).

Selection for fast and slow development time

As for mortality selection, the wild-type SP8 population was used as the founder population for the selection experiment on development time. We created 12 replicate lines split equally between fast-development and low-development selection. For each replicate line, 25 females (day two of adulthood) were allowed to lay eggs for 2 h on a seeded plate, which results in 200–300 eggs laid. A short egg-laying period was necessary to synchronize egg-laying to increase the precision in estimating the development time. 61 h later, the plates were monitored hourly for the presence of mated females. In fast-development lines, the first 25 mated females (identified by the presence of a mating plug) from each line were transferred to the next generation, together with 25 randomly selected males from the same line. In slow-development lines, mated females present on the plate were continuously discarded until 25 females were left unmated; these late-maturing females were transferred to the next generation, together with 25 randomly selected males from the same line. Synchronous egg laying for 2 h on a new plate was performed the following day (day two of adulthood), which marks the beginning of the next generation of selection. The selection procedure was repeated for six generations, after which the selected lines were expanded under relaxed selection for two generations and frozen at −80 °C for future assays (Stiernagle 2006).

Larval development and body size in development-selected lines

To investigate the response to selection on development time, we assayed larval development and size at maturity in females and males from all development-selected lines. The female assay was performed in the same way as described above for the mortality-selected lines, with a few minor changes. To combat bacterial contamination, the antibiotics streptomycin, kanamycin and nystatin were added to agar and bacterial LB. Moreover, the females were kept with similar aged males from the same line. The assay of female development time corresponds exactly to the selection procedure and was run in two blocks, each block containing three replicate lines of each of the two treatments. Males can be accurately scored as adults by the presence of the cuticularized fan (Emmons & Sternberg 1997), and were photographed and removed from the plates once they became adult. The male assay was run in three blocks, each block containing two replicate lines of each of the two treatments.

Life span assays in development-selected lines

Life span assays were established using 10 age-synchronized worms of the same sex in the L4 stage (54 h old) as focal worms with 10 worms of the opposite sex from the ancestral SP8 population as standard background worms. Worms were transferred daily to new plates and sex ratio was adjusted to the focal sex throughout the assay. The life span assay was run using five replicate plates for

each line and sex combination, resulting in 120 plates and 1200 focal worms. Worms were considered dead if they did not respond to gentle prodding by the picker. Missing worms were censored, as were females dying from matricide (bagging, caused by internal hatching of eggs).

Adult size and growth rate assays in development-selected lines

Body size measurements were conducted using age-synchronized worms in the L4 stage. To estimate growth, individual worms were kept with two worms of opposite sex, and photographs were taken on days 2, 4 and 6 (corresponding to adult age 0, 2 and 4); adult age 4 being the day of the maximal size of females and nearly maximal size of males (Lind *et al.* 2016). Size was measured as the cross-sectional area. Individuals of the non-target sex were replaced when lost. The experiment was run in a separate block for each sex, consisting of seven replicate individuals per line and block for females, and 10 replicate individuals for males (because of male-biased dispersal from plates, the male replicate number was higher).

Reproduction of development-selected lines

The same individuals were used for the reproduction and growth assays. Individual females with access to two males were allowed to lay eggs on a plate for c. 24 h; the hatched offspring were killed using chloroform 2 days later and counted. The number of offspring on a plate was divided by the exact time each female had been present on the plate, and multiplied by 24 h, to get a daily reproduction estimate. Reproduction was scored daily until the death of each female.

As for females, the same males were used in the growth and reproduction assays. However, because male reproduction is limited by access to females, male age-specific reproduction was assayed by placing a single male together with eight virgin females (second day of adulthood) for 3 h; afterwards, the male was removed and the females were allowed to lay eggs for another 3 h. The developing offspring were killed and counted 2 days later. This assay was performed every third day, starting at day one, until the death of each male.

Heat-shock resistance of development-selected lines

The heat-shock resistance assay was performed in the same way as for the mortality-selected lines (Chen & Maklakov 2012). Mixed-sex populations consisting of 25 females and 25 males were set up as L4 larvae and assayed for heat-shock resistance at day 3 of adulthood. Heat-shock was induced by exposing the worms to 40 °C for 110 min. Vigour was inspected the next day, and highly mobile individuals, *class A* following Herndon *et al.* (2002), were considered resistant to heat-shock. The assay was run in three blocks, the first set up with two replicate plates per line, the second and third block with six replicate plates per line.

STATISTICAL ANALYSES

We analysed development time, size at maturity and juvenile growth rate of mortality- and development-selected lines in separate linear mixed effect models, with block and line as random effects. For mortality-selected lines, mortality source (R or C-d) and mortality rate (H or L) were modelled as crossed fixed factors. For growth rate, the size at maturity was multiplied by a constant (100) to avoid negative numbers, and growth rate calculated as log(size at maturity)/development time ($mm^2\ h^{-1}$). For development-selected lines, selection regime was the only fixed effect. Since males and females were scored in different experiments run at different times, they were analysed separately. Response variables were log-transformed before analyses. The models were fitted using the LME4 package (Bates *et al.* 2015) in the statistical software R 3.2.2 (R Core Team 2015), and chi-square-tests of fixed effects were performed using the CAR package.

Heat-shock resistance of the development-selected lines was scored as the number of resistant and non-resistant worms on each replicate plate, and this response variable was analysed in a generalized linear mixed model with a binomial error structure, with selection regime and sex as fixed factors and line and block as a random factors using the LME4 package.

Survival of development-selected lines was analysed in Cox proportional hazard models with Gaussian random effects using the COXME package for R. Selection regime and sex were fitted as fixed factors, and line and plate as nested random factors.

Adult size of development-selected lines was analysed in linear mixed models with age and age^2 as covariates to estimate the direction and curvature of the growth curve. Selection treatment was fitted as a fixed effect, crossed with age and age^2, while line was treated as a random effect. Males and females were analysed in different models, as they were scored in different experiments. Adult growth rate was analysed both in a nonlinear model and in the linear models of growth between adult age 0 and 2, and adult age 0 and 4. The nonlinear model was a three-parameter asymptotic exponential function of the form: Size $(t) = a - be^{-kt}$, which describes nematode adult growth (Lind *et al.* 2016), where the coefficient a denotes the asymptote, b the initial size and k the growth rate. Because of only three data-points per individual, it was not possible to fit the three-parameter model on the individual level; therefore the model was fitted on the selection line level and the coefficients extracted per line. The significances of the coefficients were thereafter tested in separate linear models (ANOVAs), with selection regime fitted as a fixed factor. Only individuals that survived until day 4 were used for adult growth rate analyses.

Reproduction was analysed as rate-sensitive individual fitness λ_{ind} (Brommer, Merilä & Kokko 2002) as well as total reproduction. λ_{ind} encompasses the timing and number of offspring and is thus analogous to the intrinsic growth rate of a population (Stearns 1992). It is estimated by solving the Euler-Lotka equation for each individual. Selection treatment was treated as a fixed factor, and line as a random factor. The sexes were analysed separately, since they were scored in different experiments.

Results

MORTALITY-SELECTED LINES

We found that females from lines evolving under heat shock (C-d lines) had longer development time than lines evolving under random mortality (mortality source: $\chi^2 = 11{\cdot}93$, d.f. = 1, $P = 0{\cdot}001$; mortality rate: $\chi^2 = 1{\cdot}38$, d.f. = 1, $P = 0{\cdot}241$; mortality source × rate: $\chi^2 = 0{\cdot}14$, d.f. = 1, $P = 0{\cdot}705$). HC-d lines had highest absolute values for development time (Fig. 1a), but there was no interaction between mortality rate and source.

In contrast, we found no difference in size at maturity (mortality source: $\chi^2 = 1{\cdot}42$, d.f. = 1, $P = 0{\cdot}234$; mortality rate: $\chi^2 = 0{\cdot}80$, d.f. = 1, $P = 0{\cdot}373$; mortality source × rate: $\chi^2 = 2{\cdot}32$, d.f. = 1, $P = 0{\cdot}128$), despite the smallest absolute

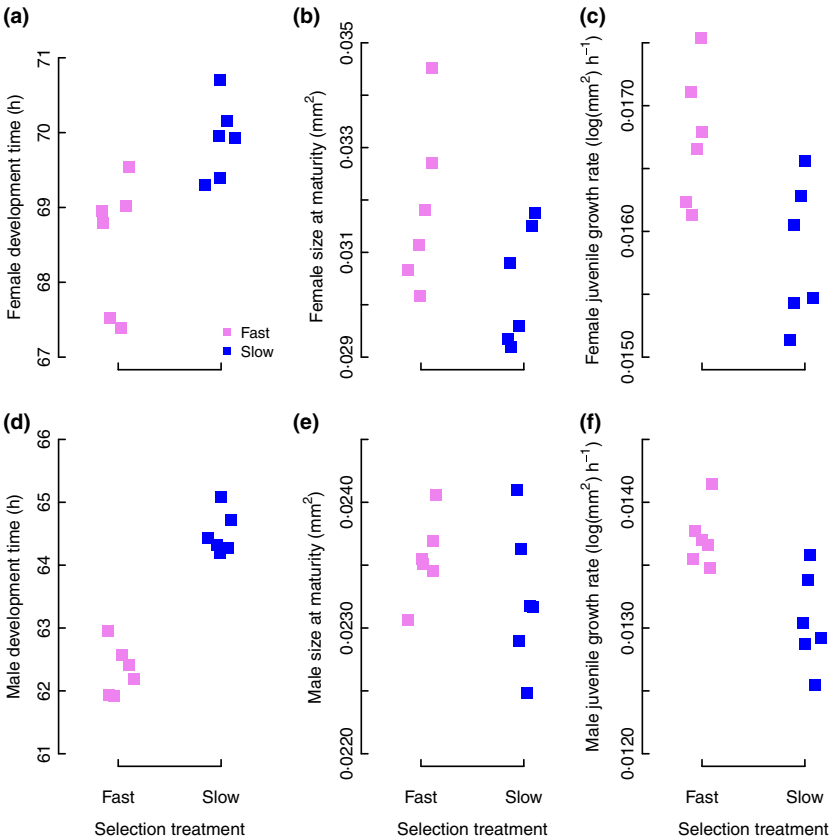

Fig. 2. Juvenile development time (a, d), size at maturity (b, e) and growth rate (c, f) of females (a–c) and males (d–f) from development-selected lines. Symbols represent line means, controlled for block effects.

size of individuals from the HC-d lines (Fig. 1b). The same was true for growth rate (mortality source: $\chi^2 = 2{\cdot}75$, d.f. = 1, $P = 0{\cdot}098$; mortality rate: $\chi^2 = 1{\cdot}16$, d.f. = 1, $P = 0{\cdot}281$; mortality source × rate: $\chi^2 = 1{\cdot}98$, d.f. = 1, $P = 0{\cdot}159$, Fig. 1c).

DEVELOPMENT-SELECTED LINES

The lines had responded to selection, since females from fast-development lines matured earlier than their counterparts from slow-development lines ($\chi^2 = 9{\cdot}77$, d.f. = 1, $P = 0{\cdot}002$, Fig. 2a). The same was also true for the correlated response to selection in males ($\chi^2 = 81{\cdot}30$, d.f. = 1, $P < 0{\cdot}001$, Fig. 2d). No difference in size at maturity was found for females ($\chi^2 = 3{\cdot}35$, d.f. = 1, $P = 0{\cdot}067$, Fig. 2b) or males ($\chi^2 = 1{\cdot}80$, d.f. = 1, $P = 0{\cdot}180$, Fig. 2e). Nevertheless, for both sexes, the fast-development lines had higher growth rate until maturity, driven by their faster development time (females: $\chi^2 = 7{\cdot}31$, d.f. = 1, $P = 0{\cdot}007$, Fig. 2c; males: $\chi^2 = 14{:}34$, d.f. = 1, $P < 0{\cdot}001$, Fig. 2f). Females took longer to develop than males, but were larger at maturity and had higher growth rate.

Size was also measured at days 0, 2 and 4 of adulthood in an independent experiment using different individuals. Because size was measured at fixed times, it directly corresponds to overall growth rate (juvenile and adult) but cannot be compared to size at maturation, which was measured at different times (when they matured). For females, the size (and, therefore, overall growth rate) did not differ between the selection regimes (Selection: $\chi^2 = 0{\cdot}21$, d.f. = 1, $P = 0{\cdot}650$; Age: $\chi^2 = 749{\cdot}65$, d.f. = 1, $P < 0{\cdot}001$; Age^2: $\chi^2 = 297{\cdot}03$, d.f. = 1, $P < 0{\cdot}001$; Selection × Age: $\chi^2 = 0{\cdot}008$, d.f. = 1, $P = 0{\cdot}929$; Selection × Age^2: $\chi^2 = 0{\cdot}009$, d.f. = 1, $P = 0{\cdot}924$, Figs 3 and S1, Supporting Information), which contrasts with the difference in pre-adult growth rate. However, the pre-adult difference in growth rate in males was still present, as fast-development males were larger (and, therefore, had higher overall growth rate) (Selection: $\chi^2 = 8{\cdot}46$, d.f. = 1, $P = 0{\cdot}004$; Age: $\chi^2 = 371{\cdot}46$, d.f. = 1, $P < 0{\cdot}001$; Age^2: $\chi^2 = 465{\cdot}47$, d.f. = 1, $P < 0{\cdot}001$; Selection × Age: $\chi^2 = 0{\cdot}49$, d.f. = 1, $P = 0{\cdot}484$; Selection × Age^2: $\chi^2 = 0{\cdot}0002$, d.f. = 1, $P = 0{\cdot}989$, Figs 3 and S1).

We analysed adult growth rate as the linear growth between adult days 0 and 2, adult days 0 and 4 and as nonlinear growth using a three-parameter asymptotic model. For days 0 to 2, we found no difference in adult growth neither in females (Selection: $\chi^2 = 0{\cdot}007$, d.f. = 1, $P = 0{\cdot}934$) nor in males (Selection: $\chi^2 = 1{\cdot}202$, d.f. = 1, $P = 0{\cdot}273$). For days 0 and 4, we also found no difference in adult growth neither in females (Selection: $\chi^2 = 0{\cdot}031$, d.f. = 1, $P = 0{\cdot}861$) nor in males (Selection: $\chi^2 = 1{\cdot}494$, d.f. = 1, $P = 0{\cdot}222$). The trend was, however, stronger in males. When analysing nonlinear adult growth on pooled line-data, we again found no difference in females (*parameter a*: $F = 0{\cdot}032$, d.f. = 1, 10, $P =$

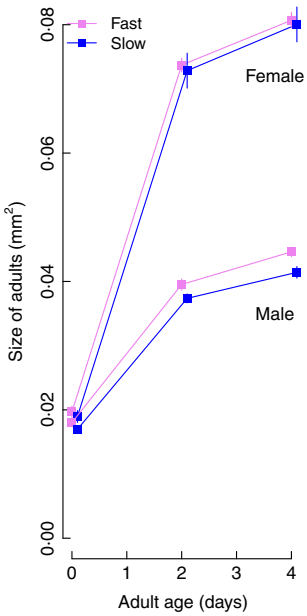

Fig. 3. Adult size at age 0, 2 and 4 days of adulthood for females and males from development-selected lines. Cell divisions (development) stops after maturation, all post-mitotic growth is therefore caused by increases in cell size. Error bars represent ±SE.

0·862; *parameter b*: $F < 0{\cdot}001$, d.f. = 1, 10, $P = 0{\cdot}990$; *parameter k*: $F = 0{\cdot}065$, d.f. = 1, 10, $P = 0{\cdot}805$) nor in males (*parameter a*: $F = 4{\cdot}098$, d.f. = 1, 10, $P = 0{\cdot}051$; *parameter b*: $F = 1{\cdot}456$, d.f. = 1, 10, $P = 0{\cdot}225$; *parameter k*: $F = 0{\cdot}475$, d.f. = 1, 10, $P = 0{\cdot}506$) and although the growth rate (parameter *k*) differed more between selection treatments for males than for females, it was again far from significance.

Reproduction was measured daily, starting at adult age 0. We found no difference in individual fitness (measured as λ_{ind}) in any sex (females: $\chi^2 = 0{\cdot}12$, d.f. = 1, $P = 0{\cdot}722$; males: $\chi^2 = 1{\cdot}72$, d.f. = 1, $P = 0{\cdot}190$); the same was also true for lifetime reproductive success (females: $\chi^2 = 0{\cdot}03$, d.f. = 1, $P = 0{\cdot}872$; males: $\chi^2 = 0{\cdot}26$, d.f. = 1, $P = 0{\cdot}612$) (for daily reproduction, see Figs 4, S2 and S3).

In both sexes, fast-development lines were less resistant to heat-shock ($\chi^2 = 6{\cdot}46$, d.f. = 1, $P = 0{\cdot}011$, Fig. 5). Females were less resistant to heat-shock than males

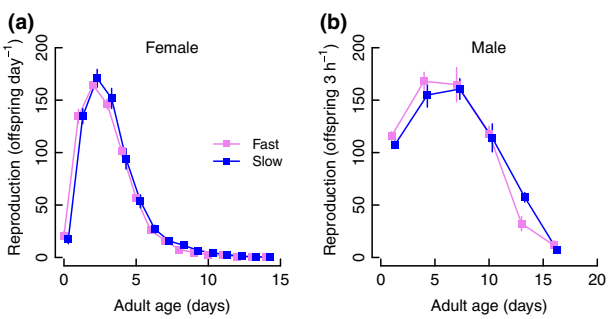

Fig. 4. Reproduction of females (a) and males (b) from the development-selected lines.

($\chi^2 = 449{\cdot}90$, d.f. = 1, $P < 0{\cdot}001$), and the difference between fast-development and slow-development lines was larger for males, as shown by the interaction between selection regime and sex ($\chi^2 = 10{\cdot}59$, d.f. = 1, $P = 0{\cdot}001$). No overdispersion was present in the model ($\chi^2 = 969{\cdot}58$, ratio = 0·973, $\text{d.f.}_{\text{residual}} = 997$, $P = 0{\cdot}727$).

Fast-development lines had lower survival under standard laboratory conditions than slow-development lines ($z = -2{\cdot}76$, $P = 0{\cdot}0058$) (Fig. 6). Males lived longer than females ($z = -6{\cdot}70$, $P < 0{\cdot}001$), but the effect of selection regime on life span was the same in both sexes, as no interaction was found between selection regime and sex ($\chi^2 = 0{\cdot}02$, d.f. = 1, $P = 0{\cdot}880$). Similar results were found when females dying of matricide were scored as dead instead of censored (selection regime: $z = -3{\cdot}19$, $P = 0{\cdot}001$; sex: $z = -11{\cdot}50$, $P < 0{\cdot}001$; selection regime × sex: $\chi^2 = 0{\cdot}33$, d.f. = 1, $P = 0{\cdot}565$).

Discussion

The antagonistic pleiotropy and disposable soma theories suggest that ageing evolves because of the trade-offs between early-life and late-life function (Williams 1957), where reduced investment in somatic maintenance results in shorter life span (Kirkwood 1977). The reproduction-life span and growth-life span trade-offs are commonly investigated in this regard. Increased reproductive effort is often associated with decreased life span (Flatt 2011; Boonekamp *et al.* 2014), but a number of recent studies have failed to support this view (reviewed in Flatt 2011; Edward & Chapman 2011; Maklakov & Immler 2016). When no cost of reproduction is observed, the trade-offs with growth and development are likely (Metcalfe & Monaghan 2003; Ricklefs 2006; Gems & Partridge 2013; Maklakov & Immler 2016). In line with this approach, the life span cost of fast growth has been repeatedly observed (Eklund & Bradford 1977; Lints & Soliman 1977; Olsson & Shine 2002), also when controlling for body size (Lee, Monaghan & Metcalfe 2013). However, the potential cost of fast development has not received much attention (but see Stearns *et al.* 2000). Since development time is an integrated part of the calculation of growth rate this is hardly surprising, but development (the differentiation of soma) is fundamentally different from growth (the accumulation of mass) (van der Have & de Jong 1996). Moreover, growth and development can be subject to different selection pressures and can be adjusted independently (Ball & Baker 1996; Lind & Johansson 2011). The experimental difficulties in separating growth from development can be partly resolved in organisms showing post-mitotic growth, i.e. where cellular development stops at maturation when the majority of growth remains to be completed. *Caenorhabditis* nematodes offer such a possibility because most of the growth in these organisms occurs after the completion of development and sexual maturation (Lambie 2002). We capitalized on this to investigate the life span cost of growth and development in *C. remanei* in two independent

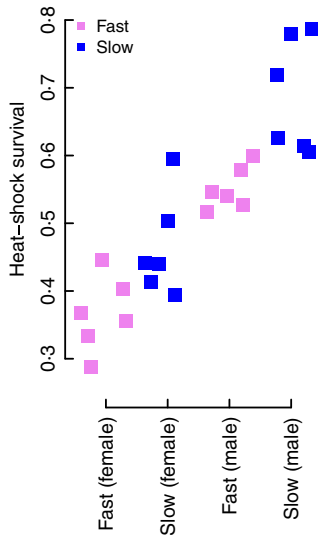

Fig. 5. Heat-shock resistance of females and males from development-selected lines. Symbols represent line means.

selection experiments, focusing on both juvenile (pre-maturation) and adult (post-maturation) growth; the latter can be totally separated from development and constitutes the majority of mass increase, especially for females (Fig. 3).

We found that females from high heat-shock mortality (HC-d) lines of *C. remanei* that exhibit high fecundity and long life (Chen & Maklakov 2012) had evolved increased development time, while juvenile growth was not significantly affected (Fig. 1a,c). We then confirmed that slow development is an evolutionary cost of long life and high stress resistance in another selection experiment targeting female development time. Worms from lines selected for long female development time had increased tolerance to heat stress (Fig. 5) and longer life span than worms selected for short female development time (Fig. 6), and this effect was present in both sexes. Thus, across two independent selection experiments focusing on different traits, we found that high heat-shock resistance and long life span are traded-off against development time. The reciprocal effect resulting in both selection experiments strongly argues for a realized genetic correlation between development time and somatic maintenance according to quantitative genetic theory (Roff 1997). This result is broadly in line with antagonistic pleiotropy and disposable soma theories of ageing that emphasize the trade-off between early-life function and investment in somatic maintenance (Kirkwood 1977).

The co-evolution of long life and resistance to heat-stress in both experiments, combined with the finding that males are both longer lived and more stress resistant than females, suggests that investment in somatic maintenance prolongs life span (Kirkwood 1977). Heat-shock resistance is associated with long life and immunity in nematodes (Amrit, Boehnisch & May 2010) and up-regulation of heat-shock proteins have a protective effect against a wide variety of environmental and genetic stressors (Sørensen, Kristensen & Loeschcke 2003). Moreover, there is generally a positive relationship between stress resistance and long life (Rose *et al.* 1992; Holzenberger *et al.* 2003), which confirms both traits usefulness as proxies for somatic maintenance (Labbadia & Morimoto 2015a, b). We show that these traits are strongly genetically correlated in *C. remanei*, in both selection experiments.

In contrast to the clear relationship between development time, heat-shock tolerance and life span in both selection experiments, the results for body size and growth rate were mixed. Size at maturity did not significantly differ between any treatments (Figs 1b and 2b,e). Because of the pronounced differences in development time, both males and females from fast-developing lines from the development-selection experiment showed higher growth rate until maturity, which was, however, not found in the mortality-selection experiment. The effect of juvenile growth could perhaps be shown with greater statistical power in some comparisons but not in others, and the overall picture is that we had stronger and clearer response in development time, even though these traits covary prior to sexual maturation.

After sexual maturation, where the major part of growth takes place, growth and development are no longer confounded, because of the postmitotic growth in *C. remanei* (i.e. cell size growth without additional cell divisions). No difference in adult growth rate between fast-development and slow-development lines was found for females or males, in any growth rate calculation (linear or nonlinear adult growth). Males from fast-developing lines were,

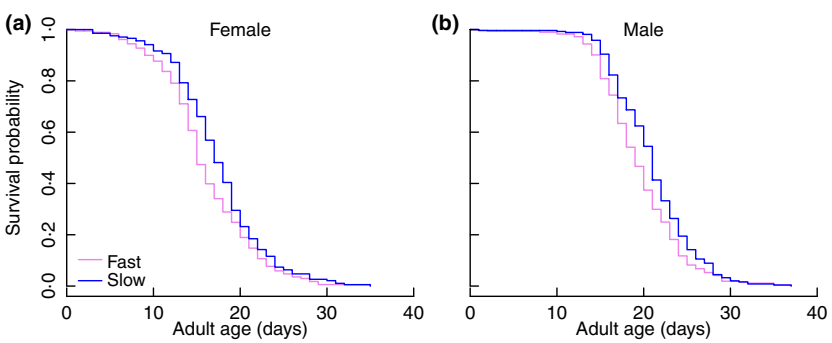

Fig. 6. Survival probabilities under normal laboratory conditions of females (a) and males (b) from development-selected lines.

however, larger, but this difference in total growth (juvenile and adult) was attributed to differences in juvenile growth. Total growth did not differ in females, despite a difference in juvenile growth, and the females had equal adult size. The mortality-selected lines were not scored for adult growth rate, but did not significantly differ in juvenile growth. Thus, we found a trade-off between development time and life span in all experiments and sexes, but despite the fact that most growth (especially for females) occurs after maturation, adult growth did not contribute to the differences in life span. Therefore, development and juvenile growth are responsible for the differences in life span. While development and growth naturally covary prior to maturation, development showed the strongest response to selection. We cannot rule out that growth before maturation is fundamentally different from growth after maturation. However, because long development time, rather than slow growth rate, was consistently associated with increased life span and stress resistance across both experiments, we suggest it is more parsimonious to assume that development plays an important role in longevity in this system.

It could be argued that our selection criterion in the development-selection experiment, the presence of a mating plug, reflects female resistance to mating rather than development time. However, mating takes place shortly after sexual maturation. The symmetrical response in the mortality-selected lines and the correlated evolution of development time of males, the non-selected sex in the development-selection experiment, confirm that our criterion of choice reflects development time in both sexes rather than a female-specific behavioural trait. Direct selection on age at reproduction does not result in a response in development time in *D. melanogaster*, further demonstrating the independence of these traits (Partridge, Prowse & Pignatelli 1999).

Development time has seldom been investigated in relation to ageing, but when scored in selection lines of *D. melanogaster*, some stress resistant lines had slower development (Bubliy & Loeschcke 2005). Moreover, a trade-off was also found between fast development, small size, high early fecundity and short life span in *D. melanogaster* evolving under high random mortality (Stearns *et al.* 2000). Since development is the differentiation of cells and tissues by cell division and DNA replication, while growth is the increase in mass over time by protein synthesis (van der Have & de Jong 1996), they can result in fundamentally different costs. The cost of fast growth is normally explained in terms of reduced maintenance (Kirkwood 1977; Metcalfe & Monaghan 2003) and increased damage, such as increased oxidative stress (Rollo 2002), while costs of fast development may also lie in compromised DNA synthesis and cell/tissue differentiation. In the life-history literature, it has long been recognized that development rate is under direct selection in time-constrained environments (Johansson & Rowe 1999; Lind *et al.* 2011), that selection for protandry is taxonomically widespread (Andersson 1994) and that organisms are able to adjust development independent of growth (Ball & Baker 1996; Lind & Johansson 2011). Given the boom-and-bust reproductive biology of *Caenorhabditis* nematodes that often inhabit such ephemeral habitats as rotten fruits (Fitch 2005; Félix & Braendle 2010), slow development time is likely to be costly. Although food shortage can speed up development in some organisms (Blanckenhorn 1999), *Caenorhabditis* nematodes respond to reduced food by longer development time (Klass 1977). Future work should focus on disentangling the roles of these different processes in the evolution of ageing.

We found mixed evidence for the trade-off between fecundity and life span. In the mortality-selected lines, previously published work has shown that increased random mortality did result in increased fecundity and reduced life span, but when mortality was increased by heat-shock, fecundity was also increased along with life span and heat-shock resistance (Chen & Maklakov 2012), but at the cost of slow development. The lines selected for fast or slow development time did not differ in fecundity, implying that the increased fecundity observed in the previous mortality-selection experiment was genetically correlated with other traits than development. Importantly, because selection for development time affected neither total fecundity nor rate-sensitive individual fitness, the reciprocal effect on development and somatic maintenance in both experiments argues for a genetic correlation between these traits. While the existence of life span-reproduction trade-offs is well supported (Flatt 2011; Boonekamp *et al.* 2014) and also present in our random mortality lines (Chen & Maklakov 2012), an increased number of studies have failed to find this trade-off (see Edward & Chapman 2011; Maklakov & Immler 2016), and this study adds to that number. Our results do not imply that fecundity-longevity trade-offs are absent in nematodes, since (i) it is present in the random mortality lines; and (ii) down-regulation of the nutrient-sensing TOR pathway, which plays a key role as a regulator of life span across different taxa (Johnson, Rabinovitch & Kaeberlein 2013), results in increased life span at the cost of reduced growth and female fecundity in *C. remanei* (Lind *et al.* 2016). Large females often produce more eggs and the fact that female adult size was not affected by selection on development time may explain the lack of a fecundity cost. Nevertheless, these results, together with previously published work, suggest that the lack of the fecundity cost of life span extension can be explained by trade-offs with other key life-history traits, such as development time.

Our main finding is that development time emerges as a key trait in the evolution of life span. This result was obtained in two independent selection experiments. In both experiments, we found evidence for genetic trade-off between fast development and somatic maintenance, measured as heat-shock resistance and life span. In contrast, we found mixed evidence for fecundity-life span and growth-life span trade-offs. Because development time is

often under strong selection in natural time-constrained environments (Johansson & Rowe 1999; Lind *et al.* 2011), our results suggest that development-life span trade-off may play an important role in the evolution of life span in natural populations, in addition to trade-offs with reproduction and growth. We suggest that facultative life-history shifts, where development is adjusted independent of growth rate (Ball & Baker 1996; Lind & Johansson 2011) offers an experimental approach to decouple these factors and their role for investment in somatic maintenance in organisms with continuous cell divisions.

Authors' contributions

M.I.L. and A.A.M. conceived the ideas and designed methodology; M.I.L., H-.y.C., S.M., A.C.G.G., H.C., M.K.Z., J.A. and T.L. collected the data; M.I.L. analysed the data; M.I.L. and A.A.M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.45dn8 (Lind *et al.* 2017).

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Supporting Information

Details of electronic Supporting Information are provided below.

Fig. S1. Adult size at age 0, 2 and 4 days of adulthood for females and males from fast (violet) or slow (blue) selected lines.
Fig. S2. Daily reproduction of females from development-selected fast (violet) and slow (blue) lines.
Fig. S3. Three hours reproduction of males from development-selected fast (violet) and slow (blue) lines.