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Basic Demography of *Caenorhabditis remanei* Cultured under Standard Laboratory Conditions

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Abstract: Species of the *Caenorhabditis* genus have been used as model systems in genetics and molecular research for more than 30 years. Despite this, basic information about their demography, in the wild and in the lab, has remained unknown until very recently. Here, we provide for the first time a closely quantified life-cycle of the gonochoristic nematode *C. remanei*. Using C. *elegans* protocols, modified for an outcrossing nematode, we estimated the basic demography for individuals of two strains (JU724 and MY12-G) which were recently isolated from the wild. We used a half-sib breeding design to estimate the phenotypic variance of traits of related (within line) and unrelated individuals (between lines) of the two strains cultured in a common environment in the lab. Comparisons between these strains showed that JU724 was characterized by significantly lower overall lifetime fecundity and by differences in age-specific fecundity relative to MY12-G, but there were no differences in their life expectancy and reproductive lifespan. We found high phenotypic variance among all traits. The variance within lines was relatively high compared to the low variation between lines. We suggest this could be the result of high gene flow in these wild-type strains. Finally, comparisons between species suggest that, despite the differences in reproductive strategies (i.e., sex ratios, lifetime fecundity), *C. remanei* has developmental time similar to the hermaphroditic N2 strain *of C. elegans*.
Key words: Caenorhabditis remanei, ecology, lifecycle, JU724, MY12-G, phenotypic variance.

The *Caenorhabditis* genus comprises a group of bacteriophagous free-living nematodes commonly found in soil associated with invertebrates or in rotting fruits (Baird, 1999; Barriare and Felix, 2006; Chen et al., 2006; Kiontke and Sudhaus, 2006). The genus has 19 described species, some of which are morphologically indistinguishable but diverse in their natural habitats and reproductive modes (Kiontke and Sudhaus, 2006). Their use has had a huge impact on increasing our understanding of the mechanisms affecting gene expression, neurotransmitter function in the nervous system, pathways in development and the ageing process (Fitch, 2005). Despite this, the importance of environmental and ecological factors that control their demography in the wild or in the lab has been ignored until very recently (but see Chen et al., 2006).

Recent ecological studies on *C. elegans* have suggested the presence of high genetic variance within populations in the wild (Barriere and Felix, 2005; Haber et al., 2005; Sivasundar and Hey, 2005), among natural populations from different geographical origins (Cutter et al., 2006) and between lab stocks (Stewart et al., 2004). Moreover, there is a good body of evidence that life-history traits exhibit variance within isolates and differ between lab strains cultured in common environments. For example, studies have reported differences in body size, lifetime fecundity, sex ratio, reproductive length, plug formation, lifespan and dauer formation (Hodgkin and Doniach, 1997; Gems and Riddle, 2000; McCulloch and Gems, 2003; Viney et al., 2003; Chen et al., 2006; Harvey and Viney, 2007).

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In contrast, the ecology of other *Caenorhabditis* species has received much less attention. Fifteen of the 19 described species are known to reproduce strictly by outcrossing (gonochoristic/dioecious reproduction, Sudhaus and Kiontke, 1996; Baird, 2002; Kiontke and Sudhaus, 2006). However, only three of these species have been subject to any systematic studies: *C. japonica*, *C. remanei* and *C. brenneri* (referred to henceforth as outcrossing species). *Caenorhabditis remanei* (Sudhaus, 1974) has received most attention from an ecological perspective. Although it has been isolated from only a few places around the world, China, France, Germany, Hungary, Japan, Switzerland and the US, (Sudhaus, 1974; Baird, 1999; Barriere and Felix, 2005; Sudhaus and Kiontke, 2007), it is likely to be as widespread as its relative *C. elegans* (Fitch, 2005). Based on samples of *C. remanei* collected around the world, recent studies suggest that *C. remanei* could be particularly restricted to temperate latitudes (Sudhaus and Kiontke, 2007). Genetic studies have found high variability within and between *C. remanei* populations (Cutter et al., 2006), which is likely to translate to phenotypic variance. In the field, it has been mainly found as a dauer stage associated with terrestrial invertebrates such as isopods, snails and beetles and collected from rotting fruits (Baird, 1999; Kiontke and Sudhaus, 2006). Compared to *C. elegans*, the outcrossing species are known to have higher genetic variance (Jovelin et al., 2003; Cutter et al., 2006; Phillips, 2006; Dolgin et al., 2007). Detailed information on the demography of the outcrossing species is generally anecdotal and is assumed to be similar to *C. elegans*. Although these species are morphologically indistinguishable, they differ in their reproductive biology in that *C. remanei* females need male sperm to reproduce, whereas *C. elegans* hermaphrodites are able to produce and store their own sperm.

This study describes for the first time the life cycle and demographic parameters of *C. remanei* under

standard laboratory conditions using protocols developed for *C. elegans*, but modified for a gonochoristic species. We conducted laboratory experiments to quantify two vital rates: age-specific fecundity and survivorship. Based on these, we then derived seven additional life-history parameters: lifetime fecundity, life expectancy, reproductive lifespan, generation time, population growth rate, stable age distribution and reproductive value. We compared these traits across two different strains recently isolated from the wild. Moreover, we used a half-sib breeding design to explore the phenotypic variance within a group of relatives compared to the offspring of unrelated individuals.

Materials and Methods

General maintenance and procedures: Two wild-type strains of *C. remanei*, JU724 (from China) and MY21-G (from Germany), were used in this study. Both strains were obtained from frozen stocks provided by M. A. Felix from the Nematode Biological Resource Centre in France and N. Timmermeyer from the Animal Ecological Centre in Germany, respectively. Briefly, the Chinese strain was isolated from soil in Zhouzhuang, Jiangsu, China, in May 2005. The German strain was isolated from rotten apples in Tübingen, Germany, in September 2006. Both strains were recovered from the field following standard techniques as described by Barriere and Felix (2006). Once samples were obtained, the original source population was maintained as a large outbred population (assorted mating) and recultured by "chunking" four random pieces of agar (approx. 1 cm^2) for approximately two generations. Then it was sub-divided into five lines and finally stored in several eppendorf tubes and maintained at -80°C, following lab protocols described by Hope (1999). Individuals recovered from these stocks were used for the assays. All individuals were cultured in a constant temperature incubator, maintained in NGM petri dishes and fed on a lawn of *Escherichia coli* (OP50 strain).

Prior to each assay, a sample from a specific line was thawed at room temperature for a few minutes, poured into a NGM petri dish and stored at 20°C. Approximately 2 d later, five gravid females were randomly selected from each line and transferred into individual petri dishes. The L4 offspring from these females were used to initialize all assays. Petri dishes of 30 mm diam. were used to carry out all the assays, and all work was done at 20°C.

Life-history assays: The life history assays were divided into two sections. First, we standardized the lab protocols and described the basic demography of the species using the JU724 strain. We quantified egg hatching, development time, fecundity and survival rates (referred to henceforth as vital rates) of different individual female nematodes from a particular line given continuous access to males. Second, using the developed lab protocols on both strains, we compared the vital rates of JU724 and MY12-G. The objective here was to estimate the variance among individuals, between lines and across strains. We followed 25 individuals from each strain (five per line).

Egg hatching: Five pregnant females at early stage (1 d after pairing) and five more at a later stage (2 d after pairing) were taken from the initializing stock and isolated individually in petri dishes. These females were monitored and transferred every hour into a new petri dish until eggs were found (time 0). Subsequently, females were removed and petri dishes monitored at 2-hr intervals until all eggs hatched. JU724 strain was used for this assay.

Development time: Ten virgin females randomly chosen from the L4 initializing stock were individually isolated with one male (time 0). Mating and egg laying took place ad lib. Individuals were monitored at 12-hr intervals for a period of 4.5 d to estimate numbers at each particular life stage and adult sex ratio. Simultaneously, mature females and males were removed to avoid overlapping generations. This assay was used to describe changes in egg, larvae and adult frequency over time. Larval counts were divided into two ages: larvae between first and third stage (L1-L3) and female larvae with distinguishable L4 features (undeveloped vulva; Sternberg, 2005). Adult counts were divided into females (spiky tail and vulva) and males (fan-like tail; Hodgkin, 1987). The JU724 strain was used for this assay.

Vital rates: Initially, a virgin female was paired with four young males for 48 hr (referred from here to henceforth as age 2 or 2-d old adults). To avoid any possibility that female lifetime fecundity may be sperm-limited, females were subsequently transferred into a new petri dish with four new young males on alternate days (Baird et al., 1994). Transfers were continued until the female stopped laying eggs (max. six transfers). A female was recorded as dead if no movement was observed or it failed to respond to a gentle touch with a platinum wire. Age-specific fecundity was estimated by counting the number of juvenile larvae present in each plate. Plates were monitored 2 d after the female was previously transferred to account for the number of larvae observed. Five virgin females (one from each of the original five lines described above) were randomly selected for this assay and paired with unrelated males from the four alternate lines. In total, 25 females from each of the strains, JU724 and MY12-G, were assessed.

Demographic and statistical analysis: Seven additional demographic parameters were calculated for *C. remanei* using the data collected from the vital rates assays. We applied well-known methods in demography (Caswell, 2001) to calculate the lifetime fecundity, life expectancy, reproductive lifespan, generation time, population growth rate, stable age distribution and reproductive value. The definition and calculation of these demographic parameters used here are summarized in Table

TABLE 1. Description and calculations of demographic parameters used in this study. Caswell, 2001 was used as a reference.

Estimate	Description	Acronyms and calculation
Age-specific survival or survivorship function	Proportion of individuals surviving from birth ($x0$) to age x	l_i
Age-specific fecundity or maternity function	Offspring per individual aged x per unit time i	M_i
Lifetime fecundity	Number of offspring produced per individual in their lifetime	$LF = \sum_{i=0}^{\infty} M_i$
Reproductive lifespan	Number of reproductive days from start of reproduction	RL
Life expectancy	Number of days to live from age x_0	$E = \sum_{i=0}^{\infty} l_i$
Population growth rate	Rate at which population grows in discrete time	λ = dominant eigenvalue of projection matrix **A**
Generation time	Expected mean time between a female having offspring and when her daughters have their offspring	$T = \frac{\sum l_x m_x x}{\sum l_x m_x}$
Stable age distribution	The age distribution at which the whole population as well as all the age classes grow at a rate λ	**A w** = λ **w**; right eigenvector of **A**
Reproductive value	Relative reproductive contribution to the population growth rate by an individual at age x	**v A** = λ **v**; left eigenvector of **A**
Elasticity	The effects of proportional changes in the entries of matrix **A** on the population growth rate λ	$e_{ij} = \frac{a_{ij}}{\lambda}\frac{\partial\lambda}{\partial a_{ij}}$

1. Briefly, a projection matrix **A** was constructed, containing the age-specific reproductive estimates (F_i) on the first row and survival probabilities (P_i) on the subdiagonal, calculated from the age-specific data (Caswell, 2001). Matrix methodologies were used to estimate population growth rate (λ), the stable age distribution (**w**) and reproductive value (**v**).

In addition, we calculated the elasticity of the population growth rate with respect to age-specific parameters for the two strains (Table 1). The elasticities quantify the proportional change in λ given a small proportional change in a vital rate (either F_i or P_i) (Benton and Grant, 1999; Caswell, 2001). Since λ can be used as a measure of fitness (Benton and Grant, 1999), elasticities can be used to anticipate the intensity and direction of selection on different life-history parameters (Lande, 1982; Benton and Grant, 1999).

Model construction and comparison: Using mixed-effects models, we analyzed the pattern of variation of the estimated traits among individuals (within lines), between lines and across strains. Model syntax used here denotes fixed variables with upper case letters and random variables with lower case letters. We used subscripts to denote different levels of the data as follows: l for individual observations (1,2,…,50), k for the line (1,2…,10), j for the strain (1,2) and i for the age (0,2,4,…,16 days) of the lth individual. In some cases, we used $\hat{\beta}$ to describe the average of a trait across observations followed by a superscript denoting which trait we referred to (e.g., $\hat{\beta}^E$ refers to the average life expectancy of all the individuals used in the experiment – see Table 1 for trait acronyms). We presented the variance components in terms of percentages of the total variance attributable to each effect (e.g., percentage of the variance within lines $= \sigma_{line}^2 / [\sigma_{line}^2 + \sigma_{\varepsilon}^2]$, and the percentage of the error variance is presented similarly). We assumed that the variances of random effects were normally distributed with mean zero.

All statistical analyses were done using R 2.7.1 software (R project for statistical computing: http://www.r-project.org). Data were analysed by fitting mixed-effects models using the "lmer" function ("lme4" package). We estimated the relative effects of different sources of variance on phenotypic traits. We compared the variance among individuals (within lines) and between lines (within strain), here treated as random factors, and differences across strains (here treated as a fixed effect). In addition, survivorship was analyzed by fitting survival models using the "Surv" function ("survival" package) and testing whether the probability of dying was constant across time or whether it changed across ages (by fitting Exponential and Weibull models, see Ricklefs and Scheuerlein, 2002; Crawley, 2007).

Model comparison was done using Likelihood Ratio Tests (LRT) for nested models. For unnested models, the model with the lowest AIC value was chosen. See Table 2 for the LRT and AIC values for each model. In addition, we provide a summary of the descriptive statistics for the preferred models (Table 3 and Table 4).

RESULTS

Basic demography of C. remanei (strain JU274): We did not detect significant differences in egg hatching patterns between pregnant females at the early and late stage (χ^2 = 2.96, 4df, P = 0.57). Therefore, all 30 eggs were analyzed together to estimate average hatching

TABLE 2. AIC and log likelihood (logLik) values for vital rates models. Bold letters correspond to the preferred model for each trait according to the AIC. Model syntax as in the text (upper case letters denote fixed variables and lower case letters denote random variables). Random variables are included within brackets (similar to R syntax for 'lmer' function). The symbol ":" denotes an interaction.

Models	Model syntax	AIC	logLik	DF
Lifetime fecundity				
Model 1	$LF_{jkl} \sim \text{Strain}_j + (1\|\text{line}_k)$	659.62	−326.81	3
Model 2	$LF_{jkl} \sim \hat{\beta}^{Ro} + (1\|\text{line}_k)$	665.49	−330.74	2
Model 3	$\boldsymbol{LF_{jkl} \sim \text{Strain}_j}$	**659.65**	**−326.83**	**2**
Life Expectancy				
Model 4	$E_{jkl} \sim \text{Strain}_j + (1\|\text{line}_k)$	334.96	−164.48	3
Model 5	$E_{jkl} \sim \hat{\beta}^{E} + (1\|\text{line}_k)$	335.64	−164.82	2
Model 6	$\boldsymbol{E_{jkl} \sim \hat{\beta}^{E}}$	**333.64**	**−164.82**	**1**
Reproductive lifespan				
Model 7	$RL_{jkl} \sim \hat{\beta}^{RL} + (1\|\text{line}_k)$	231.97	−113.99	2
Model 8	$RL_{jkl} \sim \text{Strain}_j + (1\|\text{line}_k)$	233.46	−113.73	3
Model 9	$\boldsymbol{RL_{jkl} \sim \hat{\beta}^{RL}}$	**231.97**	**−113.99**	**1**
Age-specific fecundity				
Model 10	$\boldsymbol{M_{ijkl} \sim \text{Age}_i + \text{Strain}_j + \text{Age}_i\text{:Strain}_j + (\text{age}_i\|\text{ind}_l)}$	**4026.3**	**−1949.2**	**64**
Model 11	$M_{ijkl} \sim \text{Age}_i + \text{Strain}_j + \text{Age}_i\text{:Strain}_j + (\text{age}_i\|\text{ind}_l) + (\text{age}_i\|\text{line}_k)$	4097.4	−1939.7	109
Model 12	$M_{ijkl} \sim \text{Age}_i + \text{Strain}_j + \text{Age}_i\text{:Strain}_j + (1\|\text{ind}_l)$	4110.9	−2035.4	20
Model 13	$M_{ijkl} \sim \text{Age}_i + \text{Strain}_j + (\text{age}_i\|\text{ind}_l)$	4026.2	−1957.1	56

time. At 20°C, eggs hatched between 12 and 20 hr after being laid (13.8 ± 2.4 SD, n = 30). The rate of nematode development was measured by following the offspring of 10 females on a NGM petri dish. After pairing (time 0), egg peak number on the surface occurred at 1.21 ± 0.46 SD d (Fig. 1a). Subsequently, juvenile larvae (L1-L3) were most abundant at 1.58 ± 0.54 SD d (Fig. 1a). After this time, larvae exhibited sex-specific features; peak numbers of female L4 larvae were recorded at 2.50 ± 0.55 SD d (Fig. 1b). Male L4 larvae were difficult to distinguish from adult males, therefore, the adult male counts include both L4 and adult stages; they peaked at 2.87 ± 0.70 SD d. Adult females and males exhibited similar dynamics; highest numbers were recorded at 2.59 ± 0.60 SD d (Fig. 1c). Sex ratio of females to males did not differ from unity (χ^2 = 2.20, 1df, P = 0.86).

Females of *C. remanei* produced 328.24 ± 39.00 SE (59.41% CV –coefficient of variation) offspring during their lifetime. They can live up to 16.08 ± 1.55 SE (44.19% CV) d, while their reproductive lifespan can last up to 9.84 ± 0.48 (27.47% CV) d. Moreover, they produced most of their offspring early during their lives; on average, 90% of the offspring were produced by day 6 (Fig. 2a). The survival analysis suggested that females' mortality rate was not constant during their lives but increased towards the ends of their lives (Weibull model: intercept = 2.85 ± 0.07 SE, log(scale) = -1.83 ± 0.36 SE; LRT compared to exponential model: χ^2 = 12.58, 1 df, P < 0.01; Fig. 2c).

Using these age-specific fecundity and survival values, we estimated four demographic parameters to describe the life cycle of the worm in more detail. We found that the population growth rate measured over discrete time (λ) was 11.39 ± 30 SE/d. The time to increase by a factor of λ (generation time) was 2.81 ± 0.26 SE d. The stable age distribution at a given time can be seen in Figure 3a, suggesting that approx. 90% of the

TABLE 3. Descriptive statistics to describe *C. remanei* demographic parameters: lifetime fecundity, life expectancy and reproductive lifespan. The models included here are the most parsimonious models to describe the phenotypic variance across strains, between lines and between individuals assayed in this study. (Note that, since the line effect was not significant, it is not included in these models). Model syntax and AIC values can be seen in Table 2. $\hat{\beta}$ represents the intercept of the regression model. Standard residual error is represented by ε. Fixed and random variables are denoted by the letters F and R, respectively.

Model	Parameter	Type of variable	Estimate	SE	t-value	*P*
3	Lifetime fecundity					
	$\hat{\beta}^{LF}$	F	328.24	34.07	9.63	<0.01
	Strain MY12-G	F	169.32	48.19	3.51	<0.01
	ε	R		170.40		
6	Life expectancy					
	$\hat{\beta}^{E}$	F	16.84	0.93	18.03	<0.01
	ε	R		6.60		
9	Reproductive lifespan					
	$\hat{\beta}^{RL}$	F	10.08	0.34	29.83	<0.01
	ε	R		2.39		

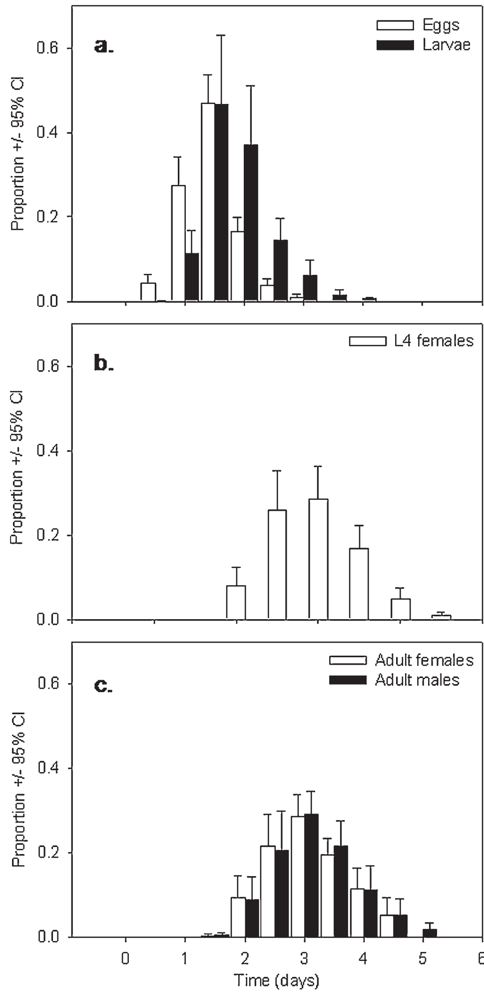

FIG. 1. *C. remanei* development time at 20°C in the lab. Bars represent the proportion of: (a) eggs and larvae, (b) pre-adult females (L4) and (c) adult females and males found on 10 NGM-petri dishes over time.

population in the lab is comprised of < 1-d-old larvae, while the older age classes are rare. The reproductive value distribution suggests that the 2-d-old adults contribute most to the next generation and the contribution of older females decreases rapidly as they age (Fig. 3b).

The elasticity estimates to a change of a vital rate on λ decreased exponentially with age (Fig 3c), indicating that a change in the survival of worms up to the first stage (e.g L1-L3), before reproduction, would have the highest potential impact on λ. Production of offspring by young adults (2-d-old) had the second highest elasticity value. In general, the production of offspring at a given age has a higher elasticity value compared to the survival estimate of the same age.

Vital rates: comparison between strains: We compared the estimates of vital rates between strains. Given the nested breeding design (individuals within lines and lines within strains), we were interested in quantifying the effect of the variation between and within lines on the overall phenotypic trait. We used mixed effects models to describe such variation and to compare strains.

We analyzed lifetime fecundity (*LF*) by fitting a model to describe the observations in relation to the mean lifetime fecundity of all individuals sampled from the *j*th strain ($Strain_j$ fixed effect), plus a random effect representing the deviation for the *k*th line, and the error term (ε_{jkl}) representing the deviation in lifetime fecundity for the *l*th individual from the *k*th line. The model was:

$$LF_{jkl} = Strain_j + line_k + \varepsilon_{jkl}; \text{(Model 1, Table 2)}$$

This model suggested that females from the JU724 strain produced significantly lower numbers of offspring (lifetime fecundity: 328.24 ± 39.00 SE) compared to females from the MY12-G strain (497.60 ± 27.72 SE; $\chi^2 = 7.87$, 1 df, $P < 0.05$; Model 1 vs. Model 2, Table 2). However, the variance between lines was low compared to the variances within lines (percentage of variance components: $\sigma_{line}^2 < \sigma_\varepsilon^2$: 1.86 and 98.14%, respectively). Therefore, the model could be written without adding the variance term to describe the effect of the *k*th line, and the final model becomes: $LF_{jkl} = Strain_j + \varepsilon_{jkl}$; (Model 3, Table 2, Table 3). Model comparison using the AIC values made no clear distinction between models (Model 1 vs. Model 3, Table 2). Therefore, the simplest model was preferred.

We used the same approach to analyze the life expectancy, (*E*), of the *l*th worm from the *k*th line and the *j*th strain. The starting model was:

$$E_{jkl} = Strain_j + line_k + \varepsilon_{jkl}; \text{(Model 4, Table 2)}$$

where the $Strain_j$ describes the mean lifetime fecundity of JU724 and MY12-G. However, we did not detect statistical differences between strains (number of days lived: 16.08 ± 1.55 and 17.60 ± 0.92 SE, JU724 and MY12-G, respectively, $\chi^2 = 0.68$, 1 df, $P = 0.41$; Model 4 vs. Model 5, Table 2, Fig. 2c,d). Therefore, the model could be better formulated as:

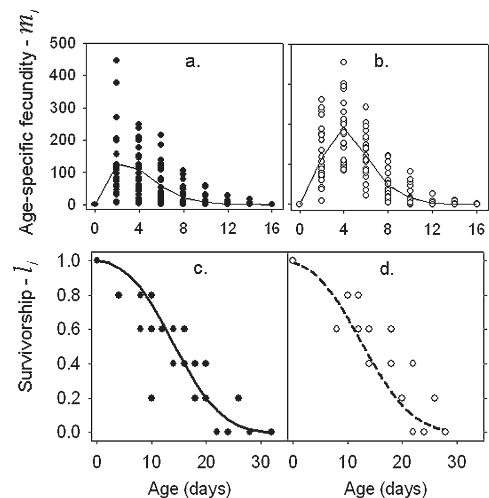

FIG. 2. Age-specific fecundity (a and b) and survivorship (c and d) of females at 20°C. JU724 and MY21 are represented by filled and open symbols, respectively.

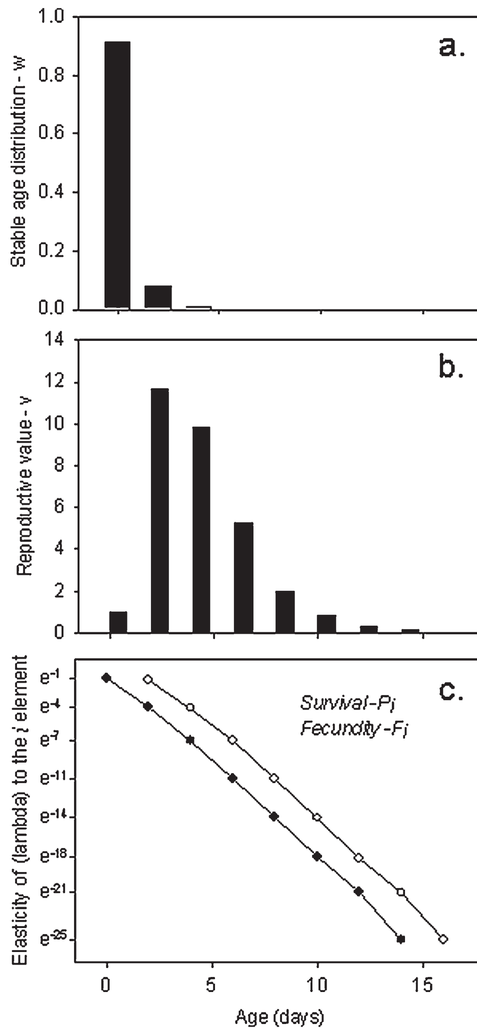

FIG. 3. (a) Stable age distribution; (b) age-specific reproductive value; and (c) the elasticity (log scale) of λ to changes in age-specific survival probability (*Pi,* filled symbols) and age-specific reproductive estimate (*Fi,* open symbols) for JU724.

$E_{jkl} = \hat{\beta}^E + line_k + \varepsilon_{jkl}$ (Model 5, Table 2), where $\hat{\beta}^E$ represents the average life expectancy of all the individuals used in the experiment. However, there was a low variance between lines compared to the variance within lines ($\sigma_{line}^2 < \sigma_\varepsilon^2$: ~0.01and 99.99%, respectively), thus, a model with only the average population life expectancy, $\hat{\beta}^E$, provided a more parsimonious model than one including the variance term to describe the effect of the *l*th individual from the *k*th line (Model 5 vs. Model 6, Table 2). The final model was: $E_{jkl} = \hat{\beta}^E + \varepsilon_{jkl}$; (Model 6, Table 2, Table 3).

Similar to the previous analysis, we did not detect statistical differences between strains (number of reproductive days: 9.84 ± 0.48 SE and 10.32±0.46 SE, JU724 and MY12-G, respectively, $\chi^2 = 0.52$, 1 df, $P = 0.47$; Model 7 vs. Model 8, Table 2). The starting model for Reproductive Lifespan (*RL*) was:

$$RL_{jkl} = \hat{\beta}^{RL} + line_k + \varepsilon_{jkl}; \text{(Model 7, Table 2)}$$

Again, we found a low variance between lines compared to the variance within lines ($\sigma_{line}^2 < \sigma_\varepsilon^2$: ~0.01 and 99.99%, respectively). Adding a variance term to describe the effect of the *l*th individual coming from the *k*th line did not improve the fit of the model (Model 8 vs. Model 9, Table 2). The final model was: $RL_{jkl} = \hat{\beta}^{RL} + \varepsilon_{jkl}$; (Model 9; Table 2, Table 3).

Observations of the number of offspring the *l*th female produced at each stage of its life (*M*) were analyzed following similar steps. Our previous results (see *Basic demography of C. remanei*) showed how fecundity varied in relation to the age of the females. Therefore, we used age as a fixed variable and the subscript *i* to denote the age of the *l*th individual. The best model was:

$M_{ijkl} = Age_i + Strain_j + Age_i \times Strain_j + age_i|ind_l + \varepsilon_{ijkl}$ (Model 10, Table 2, Table 4), where the bar | denotes the age-specific variance between individuals (ind_l).

We found that females from the MY12-G strain not only produced on average more offspring, but there was a significant interaction between strain and age ($\chi^2 = 15.80$, 8 df, $P < 0.5$; Model 10 vs. Model 13, Table

TABLE 4. Descriptive statistics of (a) fixed and (b) random variables to describe the age-specific fecundity of *C. remanei* (Model 10, Table 2). This represents the preferred model to describe the phenotypic variance across strains, between individuals and over time. $\hat{\beta}$ represents the fixed intercept of the mixed-effect model. Residual variance is represented by ε. Model syntax and AIC values can be seen in Table 3. These results are the analysis of a total of 362 observations of 50 individuals. Note that *P* values are not included in this analysis; for further information see Bates and Sarkar (2005).

(a) *Fixed variables*	Estimate	SE	t-value
$\hat{\beta}$	126.76	20.25	6.26
Age 4	−17.20	26.66	−0.65
Age 6	−69.52	25.91	−2.68
Age 8	−105.15	21.12	−4.98
Age 10	−118.17	18.26	−6.47
Age 12	−124.73	19.70	−6.33
Age 14	−124.73	21.28	−5.86
Age 16	−128.64	21.27	−6.05
strain MY12-G	−8.76	28.64	−0.31
Age 4:strain MY12-G	89.12	37.71	2.36
Age 6:strain MY12-G	74.64	36.64	2.04
Age 8:strain MY12-G	32.99	29.86	1.11
Age 10:strain MY12-G	16.93	25.82	0.66
Age 12:strain MY12-G	10.15	27.84	0.36
Age 14:strain MY12-G	7.67	30.07	0.26
Age 16:strain MY12-G	11.95	30.02	0.40
(b) *Random variables*	Variance	SD	Percentage of the total variance
Age 2	10,117.29	100.59	22.56
Age 4	25,294.34	159.04	56.41
Age 6	5,474.03	73.99	12.21
Age 8	1,582.73	39.78	3.53
Age 10	1,649.41	40.61	3.68
Age 12	237.09	15.40	0.53
Age 14	346.60	18.62	0.77
Age 16	3.91	20.10	0.01
ε	132.94	11.53	0.30

2, Fig. 2a,b). In particular, MY12-G females had higher fecundity at ages 4 and 6 compared to females from JU724. Other age-specific fecundities were similar (Table 4).

Concerning the correlation among fixed effects, which describes the relationship between ages and interactions with the strains, we found that the fecundities at adjacent ages were always positively correlated, high fecundity at age 2 is negatively correlated with fecundity from age 6 and onwards (Correlation of Fixed Effects: Table 5), high fecundity at age 6 is positively correlated with the subsequent ages, and that both strains had the same patterns.

Regarding the random effect, which describes the variation among individuals (within line) and between lines, we found a significant difference in the age-specific variance between individuals (χ^2 = 172.57, 44 df, $P < 0.001$; Model 10 vs. Model 12, Table 2). The partition of variance components into age-specific variance between individuals suggested high variation at ages 2, 4, 6, 8 and 10 (22.36, 55.91, 12.10, 3.50 and 3.65%, respectively, Table 4), but these decreased as individuals age. Moreover, age-specific correlations among random effects (Variance Correlation of Random effects, Table 5), which describes the pattern of variation over time, suggested that the random effects of adjacent ages are always positively correlated. Few negative correlations were noticed, for instance, only age 16 seems to have negative correlations with the random effects for all other ages.

Finally, the variance among lines was low compared to the variance within lines (Model 10 vs. Model 11, Table 2). Therefore, the additional term to describe the effect of the age-specific variance of lth individuals from kth lines did not improve the fit of the model and consequently was not included.

Trade-offs between the vital rates: We described the relationship between traits using correlation analysis and similar model constructions as before. We were interested in the nature of the relationship between traits (positively or negatively related) and the response between strains (either additively or within an interaction).

We described the relationship between the number of days lived (E) and lifetime fecundity (LF) for individuals from the kth line within the jth strain. The model was:

$$E_{jkl} = LF_{jkl} + Strain_j + line_k + \varepsilon_{jkl}; \text{(Model 1, Table 6)}$$

We found very little evidence for a fecundity-survival trade-off; worms producing more offspring during their lifetime did not have a shorter lifespan on average ($LF_{jkl[\text{slope}]} = -0.01 \pm 0.01$ SE, t-value = -1.61). There was neither an effect of the strain (χ^2 = 0.16, 1df, P = 0.68; Model 2 vs. Model 1, Table 6) nor an interaction between the strain and the slope of LF_{jkl} (χ^2 = 2.32, 2 df, P = 0.31; Model 3 vs. Model 2, Table 6). Therefore, the model could be better written as: $E_{jkl} = LF_{jkl} + line_k + \varepsilon_{jkl}$ (Model 2, Table 6). Moreover, we found very low variance between lines compared to the variance within lines (~0.01 and 99.99%). A model excluding the line variance effect was therefore more parsimonious (Model 2 vs. Model 4, Table 6). The final model was: $E_{jkl} = LF_{jkl} + \varepsilon_{jkl}$; (Model 4, Table 3, Table 7)

We described the life expectancy (E) in relation to the number of reproductive days (RL) for the lth worm from the kth line within the jth strain. The model was:

$$E_{jkl} = RL_{jkl} + Strain_j + line_k + \varepsilon_{jkl}; \text{(Model 5, Table 6)}$$

We found very low evidence of a relationship between life expectancy and the number of reproductive days ($RL_{jkl[\text{slope}]} = 0.09 \pm 0.40$ SE, t-value = 0.14, Model 5). Moreover, there is no evidence of either an effect of the strain (χ^2 = 0.70, 1 df, P = 0.70; Model 5 vs. Model 6, Table 6) nor an interaction between the strain and RL (χ^2 = 0.67, 2 df, P = 0.72; Model 7 vs. Model 6, Table 5). Therefore, the model could be written without the Strain effect: $E_{jkl} = RL + line_k + \varepsilon_{jkl}$; (Model 6, Table 6). Moreover, we found very low variance between lines compared to the variance within lines (~0.01 and 99.99%). A model excluding the line variance effect was therefore more parsimonious (Model 6 vs. Model 8, Table 6). Therefore, the final model was: $E_{jkl} = RL_{jkl} + \varepsilon_{jkl}$ (Model 8; Table 5, Table 7).

We described the number of reproductive days (RL) in relation to lifetime fecundity (LF) for individuals from the kth line within the jth strain. The model was:

$$RL_{jkl} = LF_{jkl} + Strain_j + line_k + \varepsilon_{jkl}; \text{(Model 9, Table 6)}$$

We found low evidence that reproductive lifespan of worms was correlated with their lifetime fecundity ($LF_{jkl[\text{slope}]} = 0.01 \pm 0.01$ SE, t-value = 5.44). There was neither a significant effect of the strain (χ^2 = 1.53, 1 df, P = 0.22, Model 9 vs. Model 10, Table 5) nor an interaction between the strain and the slope (χ^2 = 3.83, 2 df, P = 0.15, Model 11 vs. Model 10, Table 6). Therefore, the model could be written as: $RL_{jkl} = LF_{jkl} + line_k + \varepsilon_{jkl}$ (Model 10, Table 7). Moreover, although the variance between lines was low compared to the variance within lines, (21.70 and 78.30%, respectively, Table 6), we found evidence that individuals from one line were more similar to each other with respect to lifetime fecundity than individuals from other lines (Model 10 vs. Model 12, Table 6).

Discussion

Caenorhabditis remanei lifecycle: In this study we describe for the first time the lifecycle and demographic parameters of *C. remanei* grown under standard laboratory conditions (Fig. 4). Using the assays developed for this species, we re-constructed its life cycle and found that *C. remanei* has a short generation time when

TABLE 5. Summary of Model 10 (Table 2) to describe the age-specific fecundity. This includes the correlations for the fixed (A) and random effects (B) of the mixed-effect model. See Methods for details about the model syntax (which is similar to R) and Results for more details. Linear mixed-effects model (fit by maximum likelihood): age-specific fecundity ~ (Age) * (Strain) + (Age + 0 | ind)

A. Correlation of fixed effects:

Name	$\hat{\beta}$	Age 4	Age 6	Age 8	Age 10	Age 12	Age 14	Age 16	strainMY12-G	Age 4:strain MY12-G	Age 6:strain MY12-G	Age 8:strain MY12-G	Age 10:strain MY12-G	Age 12:strain MY12-G	Age 14:strain MY12-G
Age 4	−0.10														
Age 6	−0.82	0.45													
Age 8	−0.92	0.32	0.96												
Age 10	−0.91	0.45	0.86	0.90											
Age 12	−0.98	0.22	0.85	0.93	0.94										
Age 14	−0.98	0.03	0.84	0.93	0.85	0.95									
Age 16	−0.97	0.05	0.72	0.85	0.86	0.94	0.93								
strainMY12-G	−0.71	0.07	0.58	0.65	0.64	0.69	0.69	0.68							
Age 4:strain MY12-G	0.07	−0.71	−0.32	−0.22	−0.32	−0.16	−0.02	−0.04	−0.10						
Age 6:strain MY12-G	0.58	−0.32	−0.71	−0.68	−0.61	−0.60	−0.59	−0.51	−0.82	0.45					
Age 8:strain MY12-G	0.65	−0.22	−0.68	−0.71	−0.63	−0.66	−0.66	−0.60	−0.92	0.32	0.96				
Age 10:strain MY12-G	0.64	−0.32	−0.61	−0.63	−0.71	−0.67	−0.60	−0.61	−0.91	0.45	0.86	0.90			
Age 12:strainMY12-G	0.69	−0.16	−0.60	−0.66	−0.67	−0.71	−0.67	−0.67	−0.98	0.22	0.85	0.93	0.94		
Age 14:strainMY12-G	0.69	−0.02	−0.59	−0.66	−0.60	−0.67	−0.71	−0.66	−0.98	0.03	0.84	0.93	0.85	0.95	
Age 16:strainMY12-G	0.68	−0.04	−0.51	−0.60	−0.61	−0.67	−0.66	−0.71	−0.97	0.05	0.72	0.85	0.86	0.95	0.93

B. Correlation of random effects:

	Correlation						
Name	Age 2	Age 4	Age 6	Age 8	Age 10	Age 12	Age 14
Age 4	0.56						
Age 6	−0.06	0.51					
Age 8	0.10	0.57	0.95				
Age 10	0.45	0.94	0.43	0.41			
Age 12	0.31	0.87	0.56	0.54	0.86		
Age 14	−0.14	−0.39	0.43	0.45	−0.51	−0.34	
Age 16	−0.08	−0.24	−0.62	−0.62	−0.25	−0.13	−0.47

TABLE 6. AIC and Log Likelihood values for correlations across vital rates. Bold letters correspond to the most parsimonious according to the AIC (see Methods). Model syntax as in the text (upper case letters denote fixed variables and lower case letters denote random variables). Random variables are included within brackets (similar to R syntax for 'lmer' function). The symbol ":" denotes an interaction.

Model	Model syntax	AIC	logLik	DF
Life expectancy vs. Lifetime fecundity				
Model 1	$E_{jkl} \sim LF_{jkl} + \text{Strain}_j + (1\|\text{line}_k)$	334.45	−163.22	4
Model 2	$E_{jkl} \sim LF_{jkl} + (1\|\text{line}_k)$	334.60	−164.30	3
Model 3	$E_{jkl} \sim LF_{jkl} + \text{Strain}_j + LF_{jkl}{:}\text{Strain}_j + (1\|\text{line}_k)$	336.28	−163.14	5
Model 4	$\mathbf{E_{jkl} \sim LF_{jkl}}$	**334.60**	**−164.30**	**2**
Life expectancy vs. Reproductive lifespan				
Model 5	$E_{jkl} \sim RL_{jkl} + \text{Strain}_j + (1\|\text{line}_k)$	335.59	−164.80	4
Model 6	$E_{jkl} \sim RL_{jkl} + (1\|\text{line}_k)$	336.94	−164.47	3
Model 7	$E_{jkl} \sim RL_{jkl} + \text{Strain}_j + RL_{jkl}{:}\text{Strain}_j + (1\|\text{line}_k)$	338.93	−164.46	5
Model 8	$\mathbf{E_{jkl} \sim RL_{jkl}}$	**335.59**	**−164.79**	**2**
Lifetime fecundity vs. Reproductive lifespan				
Model 9	$RL_{jk} \sim LF_{jk} + \text{Strain}_j + (1\|\text{line}_k)$	215.48	−103.74	4
Model 10	$\mathbf{RL_{jk} \sim LF_{jk} + (1\|line_k)}$	**215.01**	**−104.50**	**3**
Model 11	$RL_{jk} \sim LF_{jkl} + \text{Strain}_j + RL_{jkl}{:}\text{Strain}_j + (1\|\text{line}_k)$	215.18	−104.50	5
Model 12	$RL_{jk} \sim LF_{jk}$	217.60	−105.80	2

cultured at 20°C; maturation takes an average of 1.25 days after hatching. A mature female completes its lifecycle from maturation to death in an average of 16.08 days. Therefore, the complete life cycle from birth to death required approx. 17.33 days; about 7% of the total lifespan of a worm is allocated to maturation into adult, 57% is spent in a reproductive mode and 36% in a post-reproductive mode. These life history characteristics, in addition to the high lifetime fecundity, are typical of species at the fast end of the "slow-fast" continuum or life history variation (Saether et al., 1996).

In our study, we were interested in quantifying the maximum lifetime fecundity of *C. remanei* females. Therefore, we maintained males continuously with each female to ensure that sperm supply was not limited. Our estimate of *LF* is comparable to that quantified for *C. vulgaris* females mated multiple times (mean ± SE: $LF_{C.r.}$=328.24 ± 34.37 and $LF_{C.v.}$ = 401.00 ± 70.00, our study and Baird et al. (1994) respectively; note that *C. vulgaris* is a junior synonym for *C. remanei,* Sudhaus and Kiontke, 1996). Not surprisingly, these *LF* estimates are higher than those for females singly mated ($LF_{C.V.}$ = 169.00 ± 34.00, Baird et al., 1994). This suggests that *C. remanei* demography may be affected by sperm limitation at times, as the continuous supply of sperm significantly increases *LF*.

Regarding the maximum lifetime fecundity, it is arguable whether the optimal growth temperature for *C. remanei* is 20°C. Comparisons of growth curves at 15, 20 and 25°C for *C. elegans* showed that the best temperature among these was 20°C (Byerly et al., 1976). However, as *C. remanei* has a higher thermal tolerance than *C. elegans* (Baird et al., 1994), it is possible that the optimum temperature for *C. remanei* is also higher, and therefore the demography presented here may not be the one producing the highest possible population growth rate. The reaction norm of *C. remanei* life-history parameters across a range of temperatures remains to be studied.

Life history comparisons between C. remanei and C. elegans: Compared to *C. elegans*, *C. remanei* is fundamentally different in that it strictly reproduces by outcrossing.

TABLE 7. Descriptive statistics to describe the trade-offs between demographic parameters of *C. remanei*. The representation of the variables in here is the same as in Table 3 (Note that the variance between lines has significant effects only in the relationship between reproductive lifespan and lifetime fecundity). Model syntax and AIC values can be seen in Table 5. Fixed and random variables are denoted by the letters F and R, respectively.

Model	Parameter	Type of variable	Estimate	SE	t-value	P
4	*Life expectancy vs.:*					
	Lifetime fecundity$_{[slope]}$	F	-0.01	0.00	−1.01	0.32
	$\hat{\beta}^{E}$	F	18.91	2.26	8.36	<0.01
	ε	R		6.60		
8	Reproductive lifespan$_{[slope]}$	F	0.09	0.40	0.22	0.83
	$\hat{\beta}^{E}$	F	15.95	4.13	3.86	<0.01
	ε	R		6.67		
10	*Reproductive lifespan vs.:*					
	Lifetime fecundity$_{[slope]}$	F	0.00	0.00	5.32	
	$\hat{\beta}^{RL}$	F	6.71	0.74	9.02	
			Variance	SD	Percentage of the Variance	
	line (Intercept)	R	0.89	0.94	21.70	
	ε	R	3.22	1.79	78.30	

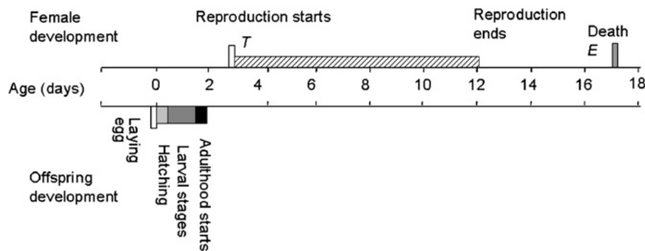

FIG. 4. Reconstruction of the *C. remanei* lifecycle at 20°C. Parameters were estimated using the development time and vital rates assays. *T* is generation time and *E* is lifespan (see Table 1). Note: Absolute estimates are used to illustrate the overall lifecycle.

Therefore, differences in their demography would not be surprising. Indeed, comparisons between our results and the information available for *C. elegans* suggest differences in lifetime fecundity, population growth rate, reproductive lifespan and population sex ratio, but not in generation time. We found that *C. remanei* has higher lifetime fecundity, and consequently a higher population growth rate, compared to *C. elegans* ($\lambda_{C.r}$ = 11.39 and $\lambda_{C.e}$ = 3.49, our study and Chen et al., 2006, respectively). *Caenorhabditis elegans*'s population growth rate is 1.6 times lower, even after accounting for its two-fold advantage resulting from its production of almost solely hermaphrodites. Also, the reproductive lifespan of *C. elegans* is only about a half of that of *C. remanei* (approx. five days, Chen et al., 2006 compared to apporx. 10 days, this study). These results are not surprising, since the mode of reproduction of the former species limits its reproductive potential. *Caenorhabditis elegans* hermaphrodites produce up to 300 sperm that are used to fertilize its eggs (Byerly et al., 1976). Experimental studies have shown that lifetime fecundity can be higher if hermaphrodites are mated (up to 695 progeny, LaMunyon and Ward, 1995; Hodgkin and Doniach, 1997). However, this behavior is not common in the lab, although it remains controversial whether outcrossing happens in the wild or not (Barriere and Felix, 2005; Sivasundar and Hey, 2005). We presume that, other factors being equal (e.g., male abundance), *C. remanei* could potentially outcompete the hermaphroditic *C. elegans* under favorable conditions. However, the fact that, among *Caenorhabditis* species, *C. elegans* is more widely spread compared to the outcrossing species (Fitch, 2005) suggests that there are other important factors too, such as the ability to resist harsh environments (e.g., dauer formation) and the ability of a single hermaphrodite to disperse and colonize new habitats, that are likely to affect fitness.

Our results show that the average generation time of a *C. remanei* female is approx. 2.81 days. Our observed value is similar to those estimates obtained for both wild-caught individuals (mean 3.13 days 95% CI: 2.83 – 3.47, Chen et al., 2006) and the commonly used strain N2 of *C. elegans* (3.83 95% CI: 3.83 - 3.87, Chen et al., 2006). Although the only methodological distinction between this study and Chen et al. (2006) is that the latter is based on experiments conducted on cohorts, the differences are small and therefore somewhat surprising, considering the differences in reproductive mode in these species. Unlike *C. elegans, C. remanei* females do not allocate time to the production of sperm or rely on the transfer of sperm by males. In contrast, hermaphrodites first allocate time to sperm production before switching to the onset of oogenesis (Hodgkin and Barnes, 1991; Ellis and Schedl, 2006). Therefore, gonochoristic females might be expected to have a shorter generation time compared to hermaphrodites of this genus. However, to our knowledge, there is no more detailed information available about the development time of males and females of gonochoristic nematodes.

Comparison across strains and between individuals: We compared the response of four life-history traits of two geographically distant strains of *C. remanei* in a common environment. The results suggest no differences across traits, with the exception of lifetime fecundity. Females of the MY12-G strain produced 1.5 times more progeny compared to females of the JU724 strain. We have no information of the mechanistic reason for the lower lifetime fecundity found in JU724. Since we do not know about *C. remanei's* ecology in the wild, or about the environmental conditions and their associated selection pressures in the natal areas of these strains, it is difficult to assess the biological significance of these differences. In *C. elegans*, previous studies on a large number of strains and wild-caught isolates have reported differences between a variety of life-history traits (see Introduction: Hodgkin and Doniach, 1997; Gems and Riddle, 2000; McCulloch and Gems, 2003; Chen et al., 2006; Harvey and Viney, 2007). We presume that the strains used in this study only represent a small sample of the wide spectrum of genotypes found in the wild. Additional work on other available strains could help to describe the diversity of life-history traits of *C. remanei*.

At the individual level, we found high phenotypic variance between individuals. Reproductive span was the least variable vital rate, followed by life expectancy and finally lifetime fecundity. The existence of high phenotypic variance among individuals is consistent with studies on *C. elegans*. Significant variance has been found in a range of traits in both genetically homogeneous and heterogeneous populations (e.g., Hodgkin and Doniach, 1997; Gems and Riddle, 2000; McCulloch and Gems, 2003; Chen et al., 2006; Harvey and Viney, 2007). For *C. remanei*, there is limited information about the underlying genetic components responsible for the phenotypic variance (e.g., Dolgin et al., 2007). A recent study showed that inbred and outcrossed populations of *C. remanei* exhibit similar levels of phenotypic variance for brood size (Dolgin et al., 2007). However, to our knowledge, the amount of variance attributed to the resemblance between groups has

never been quantified before. In this study, we used a half-sib breeding design to explore the variance components attributed to the within-group (i.e., *k*th line effect) and between-group effect (i.e., *l*th individual). Interestingly, we found low line effects in all the measured traits.

We only detected a significant line effect in the relationship between reproductive lifespan and lifetime fecundity. Related individuals shared a similar relationship between these two traits. It can be presumed that the distribution of important fitness traits such as the observed fecundity and life expectancy can result from previous selection, but other traits and behaviours can be differently linked to these vital rates and therefore result in trade-offs. However, since phenotypic variance has also been found in inbred lines (Dolgin et al., 2007), it is possible that other genetic factors can affect the genetic value, e.g., dominance deviation, interaction deviation and/or sensitivity of some genotypes to particular environments (Falconer and Mackay, 1996; Mrode, 2005).

We know little about the genetic variance of these strains; therefore, the inferences from the data should be made with caution. We propose two possible (but not unique or exclusive) explanations that could have contributed to the small phenotypic variation between lines compared to the variation within lines. First, it could be that the females from which the offspring were generated for the fitness assays were highly genetically related to each other. Therefore, the random mating could be the source of extra added variance. Second, it might be that there is little genetic variation for these traits as a result of the same evolutionary pressures across *C. remanei's* populations. Previous research has found high genetic variance but little population structure (Cutter et al., 2006), suggesting random mating and high rate of gene flow across populations of *C. remanei* (Sudhaus and Kionte, 2007). To date, we do not know much about the proximate mechanisms of gene flow in this species. The association of nematodes with soil invertebrates is considered to be responsible for the movement and dispersion of individuals across microhabitats (Baird et al., 1994; Baird, 1999; Sudhaus and Kionte, 2007).

Although we lack information on the source of phenotypic variance observed, it can have important evolutionary implications. For instance, it has been suggested that a populations' persistence and response to stressful conditions can be linked to the level of phenotypic variation present in the population when the variance is due to genetic components (Crow, 1989). Our populations of *C. remanei* present high levels of phenotypic variance, and, if this variation reflects underlying genetic variance, then these populations will have a correspondingly high evolutionary potential (Houle, 1992). Together with the elasticity estimates, we found that, as expected in a rapidly growing population, changes in survival and fecundity at early stages can have the most effect on fitness (measured here as λ). Therefore, other factors being equal (e.g., mutation rates, pleiotropic effects, heritability, trade-offs), we could expect that early life traits would be easily shaped in response to selection pressures.

Conclusions and some future directions: The demographic parameters estimated in this study provide a useful description of *C. remanei* demography under standard laboratory conditions. We found evidence of high phenotypic variance among individuals compared to the low variance between selected lines and strains from two different geographic locations. The next challenge will be to understand what components of this variance are attributable to additive genetic effects or other sources of variation inherent to the genetics of this species. Moreover, from a more general point of view, it would be interesting to assess if populations' persistence is linked to the genetic and phenotypic background of a population.

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