Environmental variation and life-history evolution: experiments on *Caenorhabditis remanei*

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This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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May, 2009

Abstract

Organisms are constantly altering their phenotypes in response to changing environments. Many of these differences are known to be due to genetic changes. However, some of the differences between individuals will be due to phenotypic plasticity. Phenotypic plasticity is the property of a given genotype to produce different phenotypes in response to distinct environments (Pigliucci 2001). Phenotypic plasticity can be adaptive and may provide with the means to thrive across a wide range of environments. Thus it represents one solution to surviving in a variable environment. Maintaining high population genetic variance is also recognized as enabling a population to respond to a changing environment. Both constitute phenotypic responses to changing environments, but rely on quite different mechanisms. The purpose of my project is to examine by what means, population history can influence the responsiveness of populations to environmental change. In order to approach this question I used a model species (*Caenorhabditis remanei*) and selection experiments in the laboratory.

Caenorhabditis species are widely used in research, for instance, to study mechanisms affecting gene expression and their effects on individual's phenotype. Despite this, we have a limited understanding of the importance of environmental factors that control their demography in the laboratory or in nature. Particularly, the demography of other nematode species other than *C. elegans* has until very recently been ignored. Thus, I described the basic demography of *C. remanei* cultured under standard laboratory conditions. I compared the life history of two geographically distant populations of *C. remanei* under standard laboratory conditions. Differences between populations were expected to be present as a consequence of local adaptation to environmental conditions. My results show that *C. remanei* cultured in the laboratory has a short generation time, but it is surprisingly similar to the generation time of *C. elegans*. Moreover, I found that there was little difference in the life history across populations. Between individuals, I found high phenotypic variance, which would be partially the result of high genetic diversity within the population.

C. elegans and *C. remanei* are morphologically indistinguishable. However, they differ in their reproductive biology; the former facutatively reproduces by selfing, whereas the latter can only produce progeny by crossing (hermaphroditism and gonochorism, respectively). Sexual conflict, different reproductive strategies between males and females, has

previously been identified in the soil nematode of *C. elegans*. However, evidence of sexual conflict is lacking in gonochoristic species of nematode. Thus, I conducted an experiment to examine the effect of the number of males present on females' fecundity and survival rate. My results show that increasing the number of males increases female fecundity. Thus, suggesting that *C. remanei* females are sperm limited. However, there is a threshold, a further increase in the number of males reduced survival rate. These results are in agreement with the theory of sexual conflict.

Environmentally-dependent traits are universally common across species. For *C. remanei*, life-history traits such as fecundity and survival are expected to be genetic and environmentally dependent, but these dependencies remain very poorly understood. Thus, in order to improve our understanding of the response of *C. remanei*'s life history traits to changing environments; I exposed three populations of worms (two wild type isolates and a half-diall cross between them) to six temperatures and assessed their response. I used a half-sib breeding design as a means to estimate gene-environment interaction for all traits. Differences between populations were expected to be due to differences in genetic composition. I found that *C. remanei* fecundity is optimal at 17 °C, a higher growth temperature than that established for *C. elegans*. Although worms cultured at 5 and 30 °C significantly reduced their fecundity, it was still permissive for some individuals.

Not all plastic traits are expected to be adaptive. It is recognised that heterogeneous environments select for plasticity. Thus, in order to manipulate the plasticity levels, I maintained populations for 50 generations in two different environments: constant temperature and predictably fluctuating temperature. Life-history components were quantified at three times during the course of the experiment (generation 1, 20 and 50). If plasticity is adaptive, it could be under strong selection in the fluctuating environment. After the selection experiment, comparisons between populations evolved in these different environments allowed me to quantify how two different evolutionary pressures shaped strains' life history, and how this response depended on likely levels of genetic diversity (i.e. between the pure strains and the hybrid). In both environments, I found changes in the reproductive schedules. Although I did not detect significant changes in the lifetime fecundity after the selection experiment, females showed an increase in their early fecundity. This shift in reproductive parameters shows adaptation as a consequence of the environmental pressures. These results are in agreement with the theory of life-history evolution.

In theory, a plastic genotype has a wider ecological breath compared with one with reduced or no plasticity. After 50 generations in each environment, populations were assayed at three temperatures to assess whether population history can influence the responsiveness of populations (e.g. tolerance to temperature). Higher levels of plasticity (i.e. tolerance) were expected in populations maintained in a fluctuating environment compared to the more stable environment. I found that worms from a fluctuating environment showed an increase in their tolerance to stressful conditions, while worms cultured in a constant environment showed no change. Thus, I successfully selected for populations with high and low levels of plasticity.

Adaptive plasticity is expected to increase individual's fitness across a range of environments because it expresses the "matching" phenotype according to environmental cues. However, a plastic genotype with the machinery to match the environment could be at disadvantage compared to a less plastic genotype when the environment is not changing. This disadvantage is expected to be linked to the reallocation of resources in the maintenance of genetic and cellular machinery that enables it to detect changes in the environment and in the production of the matching phenotype. Thus, to test this hypothesis, I translocated populations between the two environments. After the translocation, plastic worms moved back into the constant environment reproduced very poorly compared to worms before the selection took place and compared to the less plastic worms (reared in a constant environment). This strongly supports the idea that plastic strategies can turn an individual into "The Jack of all trades, but Master of none".

Candidate's declaration

I declare that the work recorded in this thesis is entirely my own, except where otherwise stated, and that it is also of my own composition. Much of the material included in this thesis has been produced in co-authorship with my supervisors, and my personal contribution to each chapter is as follows:

Chapter 2. In press as: Diaz, S.A., Lindström J., and Haydon, D.T. Basic Demography of *Caenorhabditis remanei* Cultured under Standard Laboratory Conditions. Journal of Nematology. The idea was developed by SAD, JL and DH. SAD carried out all the data collection and the majority of the statistical analysis and was senior author in the manuscript.

Chapter 3. *In preparation for submission as*: Diaz, S.A., Haydon, D.T. and Lindström J. Between a rock and a hard place: sperm limited fecundity and polyandry induced mortality in female nematodes *Caenorhabditis remanei*. The idea was developed by SAD, JL and DH. SAD carried out all the data collection and the majority of the statistical analysis and was senior author in the manuscript.

Chapter 4. *In preparation for submission as*: Diaz, S.A., Lindström J., and Haydon, D.T. Tolerance of *Caenorhabditis remanei* to stressful conditions: comparison across populations. The idea was developed by SAD, JL and DH. SAD carried out all the data collection and the majority of the statistical analysis and was senior author in the manuscript.

Chapter 5. *In preparation for submission as*: Diaz, S.A., Haydon, D.T. and Lindström J. Life-history evolution in fluctuating environments: a long-term selection experiment on *Caenorhabditis remanei*. The idea was developed by SAD, JL and DH. SAD carried out all the data collection and the majority of the statistical analysis and was senior author in the manuscript.

Chapter 6. *In preparation for submission as*: Diaz, S.A., Haydon, D.T. and Lindström J. Fluctuating environmental conditions select for increased phenotypic plasticity, but with a cost: an experimental evaluation using *Caenorhabditis remanei*. The idea was developed by SAD, JL and DH. SAD carried out all the data

collection and the majority of the statistical analysis and was senior author in the manuscript.

I further declare that no part of this work has been submitted as part of any other degree.

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January 2009

Acknowledgements

First I would like to thank my supervisors Jan Lindström and Dan Haydon. From my first day in the PhD, until the last moments of this thesis, they have always offered advice and listened to my thoughts. Starting a nematode laboratory in the department was not the easiest thing, nevertheless, their constant support made it possible. I have enjoyed every single step during the PhD; numerous meetings talking about experimental design and data analysis were always coupled with great laughs. I feel in debt to them after all their constant encouragement; not only because the incubators, but because their joy to celebrate small or big moments during my time here. I would like to thank Jan for taking into consideration the idea of having me as his student and then Dan to join in this boat trip.

Using *Caenorhabditis* remanei as a model species has been "interesting". *C. remanei* worked very well as a model system in my research, but also provided the means of meeting people interested in many areas of Evolution. I want to thank Marie-Anne Félix, Hinrich Schulenburg and the people in Tubingen, Glyn Ball, Scott Baird, Simon Harvey, among others who were always happy to transfer their knowledge about worms. Although the work with the nematodes had its ups and downs, they were a fantastic species to work with; I would like to thank them as well (at least 1,800 females and around 6,300 males were used in all the assays, plus many more were kept in culture during the experiment).

I would like to thank as well many people who were happy in reading my drafts manuscripts on top of their own work: Douglas (almost my third supervisor), Sunny, Liz, Andy H., Margaret and Dave – they all were very helpful in improving my vocabulary and grammar. Being part of the GK Building has been an amazing and life changing experience. I got the chance to coincide with many people; many who are gone (David, Veronica, Pep, Yeonita, Alison, Pierre, Mi Ran, Seb, Caro and Adriana –who were there with me during the ups and downs); many who are still around (Maria, Andy H, Hawthorne, Peter, Karim, Davina, Liz, Marco, Flavie, Andy T., Gus, Vic, Clare, among others); my running mate Britt (who was always up for a run and I will miss!); and many more. Thanks as well to Malcolm, Barbara, Richard, Kate A., Graeme, Roman, Aileen, Kate G., Karen, Bill, Pat, Neil and many other who were always happy to have a chat in the corridor or in the social room (plus also offered their advice when I needed!). Thanks as well to Nick lobster and the skipping crew (Sunny and Annabelle - my most recent flat

mate who has been trying her best in improving my style and wine-tasting skills!). Thanks to M&S for making “Simply Food”.

Finally I would like to thank my family. They have been very supportive while I have been away from Mexico. Gracias Silvia, Pedro and Tony, for all your love and positive thinking.

I was supported by a CONACyT (Mexico) studentship during this PhD.

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1 General introduction

1.1 Primer

'It is not the strongest of species that survive or the most intelligent but the ones most responsive to change'

Charles R. Darwin, 1859

In order to conserve species or populations, it is important not only to understand the reasons why they become extinct but also to understand the mechanisms that keep them extant. Extinction is likely due to the combined effects of deterministic and stochastic processes that affect individuals within a population, and environmental change is likely to be an important driver of extinction. Particularly, environmental variability linked to climate change, such as rising temperatures and extreme-erratic changes (increased incidence of floods, storms, cyclones and hurricanes), can act as stressors that reduce the performance of individuals in their habitat, and ultimately their numbers in a population (Begon et al., 1996; Walther et al., 2002).

Darwin, in 1859, without an understanding of genetics, proposed that in a changing environment *responsive* species have an advantage. Our current understanding about the genetic basis behind morphological and physiological responses highlights the importance of having plastic strategies to cope with a changing environment (Pigliucci, 2005). This is covered in evolutionary ecology by the concepts of phenotypic plasticity and adaptive plasticity (Via and Lande, 1985). However, these broad concepts also bring up numerous more detailed questions that are at the heart of evolutionary ecology but not fully understood: What evolutionary pressures select for highly responsive (i.e. plastic) organisms? Can we artificially manipulate the responsiveness of an organism? Are there limits to the response? Does being responsive incur costs? Do these costs depend on environmental variability? These questions, among others, have been recently highlighted in numerous reviews (e.g. Via et al., 1995; Pigliucci, 1996; DeWitt et al., 1998; Agrawal, 2001; Callahan et al., 2008). However, empirical evidence about them has accumulated very slowly (Pigliucci, 2005). Moreover, there seem to be contradictory findings across taxa (e.g. Scheiner and Berrigan, 1998; and Bell and Galloway, 2008). The lack of detailed knowledge and the contradictions in the existing results thus highlight the importance of further research. In this introduction, I review the general theory and empirical evidence regarding how organisms cope with environmental variation, research on phenotypic plasticity, experimental evolution of plasticity and the potential costs and limits of plasticity.

1.2 Environmental variability

Natural environments are constantly changing, and environmental fluctuations may change in both their frequency and magnitude (Boyce et al., 2006). For instance, changes in frequency might result in longer favourable or unfavourable seasons for individual species (Boyce et al., 2006). Rhythmically repetitive changes, such as seasons or tidal movement are ubiquitous across the planet (Begon et al., 1996). However, infrequent extreme environmental states may have greater influence on population dynamics, increasing extinction risk, for instance, than either more occasional or moderate changes (Pike et al., 2004).

It is possible that organisms, populations and ecological communities do not respond to averaged environmental conditions (Walther et al., 2002) but are more likely to be responsive to spatial heterogeneity (Walther et al., 2002) and the frequency of extreme temporal events, such as extreme ocean atmosphere dynamics and temperatures (Parmesan, 2006). The study of the consequences of environmental conditions outside the natural range of species is important, for example, for understanding the likely consequences of changing environmental patterns due to climate change.

1.3 The mechanisms underlying organisms' response to changing environment

Species and populations' response to climate change, or to any other source of selection, can be the consequence of at least two separate mechanisms: 1) populations that maintain high genetic variation are likely to include pre-adapted phenotypes that can increase in frequency in response to environmental change (Via et al., 1995), or alternatively, 2) a single genotype can thrive by adjusting its response to different environmental conditions through phenotypic plasticity (Via et al., 1995). Possession of a phenotype that can match environmental conditions is likely to be a critical asset in reducing the risk of extinction induced by environmental change. Both processes can result in populations persisting through time in changing environments. However, the underlying mechanisms and consequently their costs and limitations are potentially very different.

1.3.1 Genetic variation

Maintenance of genetic variation within a population has been associated with various population level factors, including population size and its variation, and migration rate (which in turn increases outbreeding opportunities) (Frankham, 2005). The genetic phenomena of mutation, epistasis, and pleiotropy are also associated with levels of genetic variation (Roff, 2002; Frankhman, 2005). In nature, many studies have found high amounts of genetic variation within a population (see Davis and Shaw 2001 and Gienapp et al., 2008 for recent reviews). Although there is still some controversy surrounding the generality of empirical studies linking enhanced genetic variance and a population's survival probability (Frankham, 2005), this concept is generally accepted.

1.3.2 Phenotypic plasticity

Phenotypic plasticity is considered as a beneficial solution to living in heterogeneous environments (Via et al., 1995). Phenotypic plasticity can be defined as the characteristic of a particular genotype to produce different phenotypes in response to environmental conditions (Schlichting and Pigliucci, 1998; West-Eberhard 2003). It can involve a single or numerous physiological, morphological and or behavioural reponses of an organism to an environmental stimulus (Silvertown, 1998). Recently, plasticity has been proposed as an adaptation for organisms to cope with varying environments (Bell and Galloway 2008). Thus, organism with higher levels of plasticity could be expected to perform better over a range of environments in comparison with a less plastic organism (e.g. higher environmental tolerace). However, at least in theory, it is expected that the "optimal" fit of organisms to environmental variability must involve some compromise between a matching response to environmental variation and tolerating it (Begon et al., 1996). Phenotypic plasticity is usually described by the *reaction norm*; where a trait of a genotype is described as a function of an environmental gradient (Via et al., 1995; Schlichting and Pigliucci, 1998). Thus, reaction norms can describe both non-responsive or responsive traits (Figure 1.1.A and Figure 1.1.B-C, respectively; Schlichting and Pigliucci, 1998), and the non-response or response across genotypes (Figure 1.1.A-B and Figure 1.1.C; Pigliucci, 2005). Authors use one or the other depending whether they refer to plasticity of a trait as the property of a genotype or across a population of genotypes (i.e. gene by environment interaction or GEI; Pigliucci, 2005). In this thesis, I use phenotypic plasticity to describe the responsiveness of a trait to an environmental gradient.

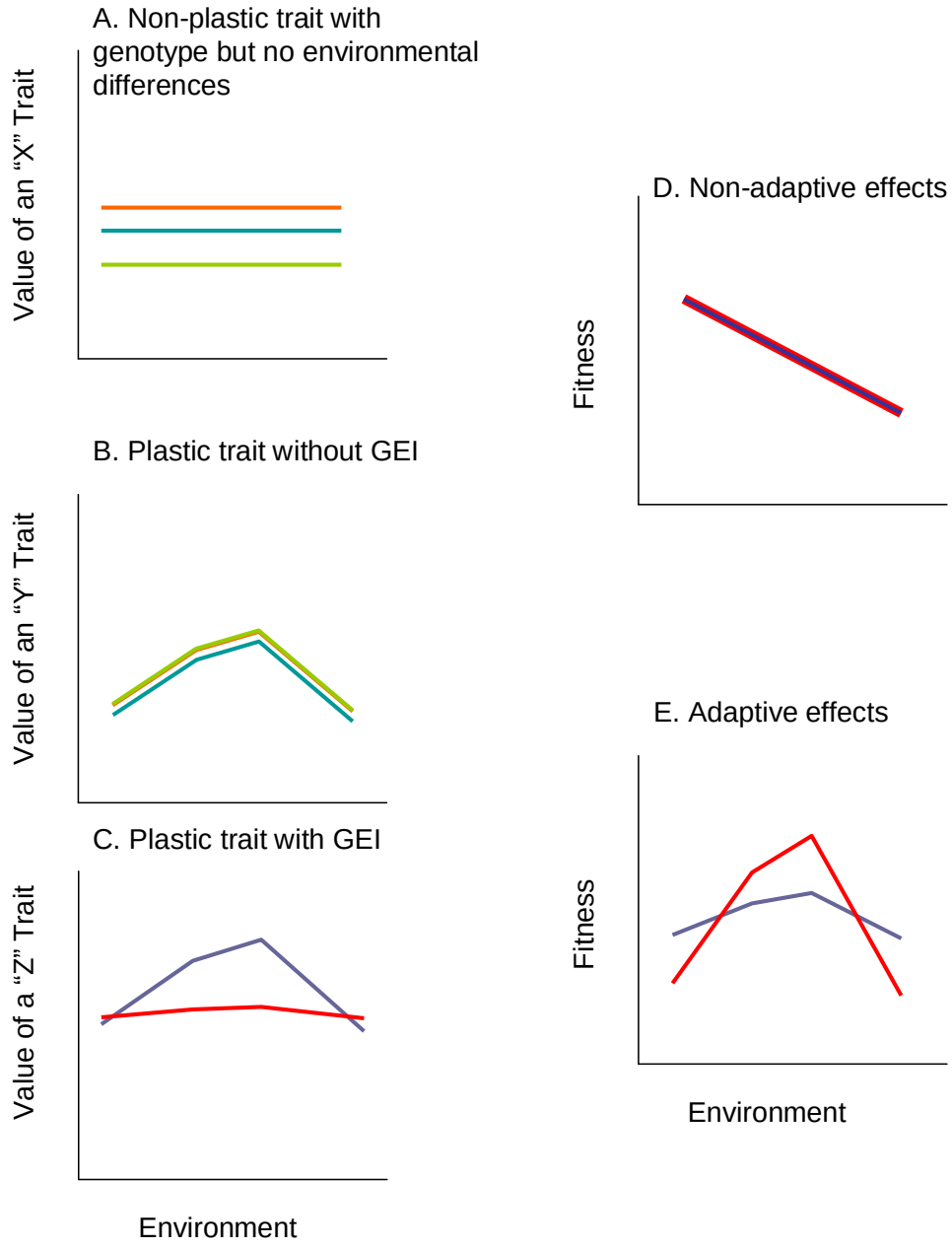

Figure 1.1. Panels to illustrate different types of reaction norms. A-C are illustrative figures to describe the response of traits to the environment. A. Represents a trait that is not responsive to the environment (e.g. *Rana arvalis* body size in relation to predation, Lardner, 1998), but there are quantitative differences across genotypes. B. Represents a trait that is responsive to the environment, but there are no differences across genotypes (e.g. *Drosophila melanogaster* development time in relation to latitude, James et al., 1997); and C) represents a trait that is responsive to the environment and in addition genotypes respond differently (*Caenorhabditis elegans*, Gutteling et al. 2007). *GEI= Gene by environment interaction. D-E are hypothetical examples of the effects of two levels of plasticity (from Figure C; e.g. blue genotype with higher level of plasticity compared to the red genotype) on fitness, D) differences in the level of plasticity have no effect on fitness, whereas E) shows that the level of plasticity on fitness (i.e. the blue genotype has a wider niche breath compared to the red genotype).

Phenotypic plasticity has been documented in nature in a range of species along altitudinal or latitudinal gradients (e.g. Ackerly et al., 2000). Empirical manipulations have documented phenotypic plasticity arising under certain conditions (e.g. Reznick et al., 2001, Hautekeete et al., 2002), for instance, changes in phenology and clutch size between morphs in relation to temperature (*Tetrix undulata*, Forsman, 2001) and predator presence (*Daphnia hyaline*, Stibor, 1992; *Poecilia reticulata*, Reznick et al., 2001 review).

Phenotypic plasticity is not always expected to be adaptive (West-Eberhard, 2003). However, a plastic response to an environmental gradient can be seen as adaptive if the genotypes with phenotypic plasticity can cope with a changing environment better than less-plastic ones and when there are genes regulating such traits (e.g. GEI). In such cases, phenotypic plasticity is expected to be under strong selection (West-Eberhard, 2003). For instance, we could imagine plastic trait that has no direct effect on fitness (Figure 1.1.D). Conversely, a trait could be environmentally dependent and with direct effects on fitness (Figure 1.1.E).

It is considered that environmental variability selects for phenotypic plasticity (West-Eberhard, 2003). However, there is a lack of understanding on wheather all varying environments facour the evolution of plasticity. Reseach on microorganisms suggests that the scale of environmental heterogeneity can influence the evolution of phenotypic plasticity. Reboud and Bell (1977) conducted an evolutionary experiment in which populations of an unicellular alga (*Chlamydomonas*) were exposed to spatially or temporally varying environments (alteration between light and dark phases). Populations exposed to spatial heterogeneity evolved into dark- and light-adapted specialist, whereas populations cultured in the temporally varying environment evolved genotypes with phenotypic plasticity (referred by Reboud and Bell (1997) as phenotypically plastic generalist). These results highlight the importance of the scale of environmental variability in the evolution of phenotypic plasticity and the maintenance of genetic diversity (Silvertown 1998).

To this point in this introduction, phenotypic plasticity has been considered as a beneficial characteristic: individuals can persist in a varying environment. However, having plasticity might as well have evolutionary consequences that are not always beneficial (DeWitt 1998). For instance, the reduction in genetic diversity could reduce the evolutionary potential in a population (DeWitt 1998). Moreover, although plasticity may enhance fitness, organisms are not expected to be infinitely plastic as there are numerous possible factors limiting phenotypic changes (Via and Lande, 1985; Van Tienderen, 1991;

West-Eberhard, 2003). For instance, there are many examples in which an increase in reproduction reduces an individual's survival, and female fecundity is often limited by age at maturation (Roff, 2002). Phenotypic plasticity constraints can also be due to maintenance or production of certain traits (DeWitt et al., 1998). Although the plasticity cost has been commonly measured in terms of fitness decline, the reduction of fitness is thought to be mainly due to the production and maintenance of genetic and cellular machinery necessary to be plastic (Scheiner, 1993, DeWitt et al., 1998). For example, to be able to detect changes in environmental conditions, individuals must allocate energy during development to producing and maintaining a specific machinery. This allocation will reduce energy available for other activities and can also affect fitness traits such as fecundity (DeWitt et al., 1998). In theory, it is therefore expected that organisms with relatively plastic traits, compared to less plastic organisms, will pay a fitness cost, and that this should be particularly marked in the absence of environmental fluctuation. Although these concepts are well established, empirical research into the costs of phenotypic plasticity are accumulating only slowly (DeWitt et al., 1998). One of the reasons why the progress in answering questions about phenotypic plasticity has not been faster is that our understanding about evolutionary pressures that select for plasticity is still limited.

It is not clear either what the exact costs involved in phenotypic plasticity are. For instance, Hughes et al. (2007) cultured populations of *E. coli* in cycling pH, randomly fluctuating pH, constant acid, and constant base environments. Their results suggest that individuals can increase their tolerance to extreme acid and alkaline environments (i.e. change their reaction norm) if they have been reared in a fluctuating environment previously. However, contrary to these predictions, populations with higher plasticity show no apparent fitness cost when moved back to a constant environment. In fact, many studies have not found any apparent cost associated with phenotypic plasticity (e.g. DeWitt, 1998; Agrawal et al., 2002; Relyea, 2002; Van Kleunen and Fischer, 2005; Caruso et al., 2006; Callahan et al., 2008). It seems plausible, therefore, that organisms often pay no price for having a trait in an environment where that trait is not advantageous.

However, it is important to note that the absence of apparent costs of having plasticity does not necessarily mean that there are no costs. This is because our ability to determine which traits contribute to fitness is limited and the same trait is not necessarily costly in all environments (e.g. DeWitt et al., 1998; Steinger et al., 2003; Pigliucci, 2005). Thus, advancing the field requires experimental studies that incorporate manipulations of environmental variability and detailed monitoring of the resulting fitness components of organisms living in these environments.

1.4 Model species for evolutionary experiments

Selection experiments, as exemplified by Hughes et al. (2007)'s study, are important tools for addressing evolutionary questions. In addition, it is important to choose an appropriate model species. For obvious reasons (short generation time and easy maintenance), studies on adaptation to environmental conditions have mainly been conducted on small organisms (e.g. bacteria, Bennett et al., 1992; algae, Reboud and Bell, 1997; Kassen and Bell, 1998; viruses, Weaver et al., 1999; Daphnia, Scheiner and Yampolsky, 1998; free-living nematodes, Brun, 1965; Drosophila, Dobzhansky, 1947).

Over the years, the use of metazoans such as flies, free-living nematodes and water fleas in evolutionary experiments has significantly increased (Daphnia spp. Scheiner and Yampolsky, 1998; Drosophila spp., Dobzhansky, 1947; Caenorhabditis spp.; e.g. Brun, 1965). Compared to uni-cellular organisms, studying metazoans provides an opportunity to describe processes of birth and death with more detail. This allows, for instance, breaking down fitness into life-history components such as reproductive and survival schedules. In addition, another advantage of using these species has been the bulk of information already available concerning their physiology, development and genetics (for a recent review, see Hedges, 2002). Their increasing use is also potentially linked to the availability of completed genome sequences of several model species (Ponting, 2008). *C. elegans* was the first multi-cellular organism to have its genome sequenced in 1998 (The *C. elegans* sequencing consortium, 1998). *C. elegans* is part of the Caenorhabditis species-complex which comprises one of the most widely studied metazoans. The use of Caenorhabditis spp. as model species has had a huge impact in increasing the understanding of genetics, neurobiology, embryonic development and the ageing process over the last 30 years (Brener, 1974; Fitch, 2005). However, despite the availability of its genome, we understand little about their ecology in the wild. Particularly, we know little about the ecological and evolutionary pressures that have shaped their life histories (Fitch, 2005).

Using Caenorhabditis species as a model system in evolutionary ecology has numerous advantages; its basic biology, physiology and genetics are well known compared to many other animals (Epstein and Shakes, 1995). Moreover, compared to other invertebrates (e.g. Drosophila), Caenorhabditis individuals can be cultured under similar environmental conditions throughout their life. Thus, the possibility of inverted selection, due to the use of different growing conditions between juveniles and adults, is diminished.

The *Caenorhabditis* genus comprises a group of bacteriophagous nematodes with small body sizes, and short generation time (Kiontke and Sudhaus, 2006). They are 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). Only four of these 19 species have been studied in any detail: *C. briggsae*, *C. remanei*, *C. brenneri* (or *C. sp. PS1010*) and *C. elegans* (Elegans group, Kiontke et al., 2004). Although these species are morphologically very similar, *C. elegans'* reproductive biology differs from that of the other three. *C. elegans* females have a facultative reproductive biology, thus they can produce progeny by mating with males or/and by self-fertilisation. In contrast, the other species are known to reproduce strictly by outcrossing (referred by others as gonochoristic/dioecious reproduction, Sudhaus and Kiontke, 1996; Baird, 2002; Kiontke and Sudhaus, 2006).

Both self-fertilisation and outcrossing strategies can be potentially advantageous in some circumstances: outcrossing species are known to display higher genetic diversity within populations, while self-fertilizing species are not constrained by having to find a mate (Jovelin et al., 2003; Cutter et al., 2006; Phillips, 2006; Dolgin et al., 2007). Since populations with low levels of genetic variability might have a limited rate of evolution (Fisher, 1930), the use of outcrossing species might provide researchers with the means for avoiding inbreeding depression and increasing the evolutionary potential in the laboratory.

The ecology of *C. remanei* is better known than that of other outcrossing nematodes (e.g. Baird, 1999; Berriere and Felix, 2005). *C. remanei*'s basic reproductive biology is assumed to be similar to its relative *C. elegans* but it does not have hermaphroditic reproduction (Baird, 2002). A hermaphrodite can produce up to 300 eggs and males up to 1000 sperm. Its life cycle (i.e. egg to egg cycle) takes approximately 60 hrs at 20 °C to complete but this is sensitive to temperature: 45hrs at 16 °C and 95hrs at 25 °C, respectively (Epstein and Shakes, 1995). After hatching (incubation ~18 hrs) the larva goes through 4 larval stages (L1-L4) before reaching maturity (Hope, 1999). An important stage during a Caenorhabditis sp. life cycle is the so-called “dauer” stage, which is a developmentally arrested larval form that does not feed. Studies indicate that dauer formation is caused by a pheromone produced by the adults under unfavourable conditions such as crowding, food scarcity and high temperatures (Riddle and Albert, 1997; Ailion and Thomas, 2000; Viney et al., 2003; Harvey and Viney, 2008). The variability of dauer

stage development represents a reaction norm example of phenotypic plasticity induced by environment conditions (Viney et al., 2003; Harvey and Viney, 2008).

Another important characteristic of the Caenorhabditis species-complex is that individuals grown under laboratory conditions produce all their eggs in a short time (50-60 hrs at 25 °C) once they reach maturity. After this period, adults usually can live up to 3 weeks. Males reared individually under laboratory conditions have a lifespan 10-20% longer than females (McCulloch and Gems, 2003), but this difference does not exist when individuals are reared in groups (Gems and Riddle, 1996). This result reflects the trade-off between survival and reproduction in males. Van Voorhies et al. (2005) simulated conditions in natural soil habitats and found a greatly reduced longevity (ca. 10%) of *C. elegans* compared to standard laboratory conditions, and it is likely that *C. remanei* in the wild will have a correspondingly shorter lifespan.

C. elegans has a widespread global distribution (Fitch, 2005) and although *C. remanei* has been isolated in only a few countries around the North Hemisphere (Sudhaus, 1974 unpublished data; Barriere and Felix, 2005; Baird, 1999), it is likely to be equally widespread. Genetic studies have found high variability within and between *C. remanei* populations, which could suggest it is also more widely spread than documented in the literature (A. Cutter pers. comm.).

1.5 Aims and objectives of the current study

The main objective of my PhD is to quantify whether individuals with plastic phenotypes pay a cost when living in a constant environment. I addressed this question by conducting selection experiments in the lab and using a nematode species, *Caenorhabditis remanei*, as a model system. The research involved several aims to accomplish the main question (see Figure 1.2; section A, B, C, D): to describe *C. remanei* demography in the laboratory (Chapter 2); to study the effect of the number of males on *C. remanei* females' life history (Chapter 3); to describe levels of plasticity of *C. remanei* females measured as the level of tolerance over a range of temperatures (Chapter 4); to describe changes in life-history traits and tolerance to temperature of populations of *C. remanei* cultured in two environmental regimes for 50 generations (Chapter 5); and to investigate the evolution of plasticity after the selection experiment and whether having plasticity incurred any cost (Chapter 6).

As information on the demography of the outcrossing species in the Caenorhabditis genus is generally anecdotal and merely assumed to be similar to *C. elegans*, I quantified

the reproductive and survival schedules of *C. remanei* cultured in standard laboratory conditions (Chapter 2, section A.1 in Figure 1.2).

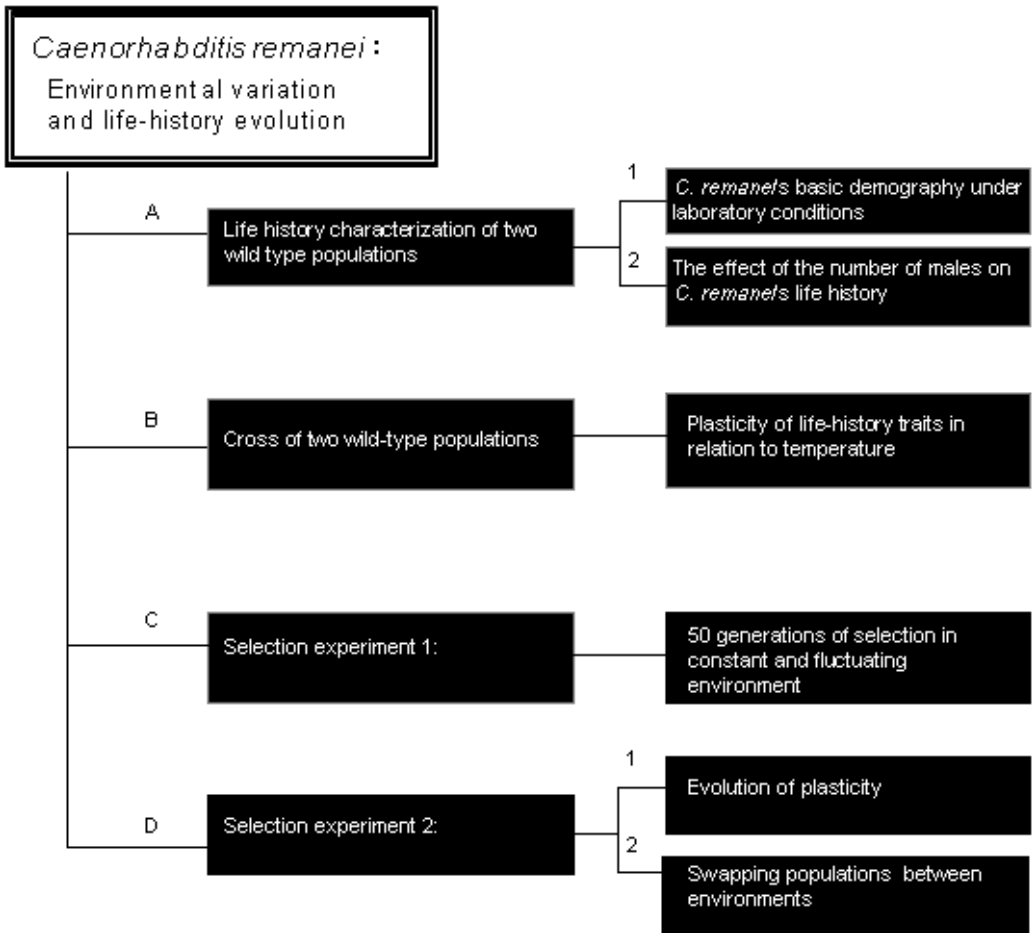

Figure 1.2. Thesis layout. See the text for more details.

Using C. *elegan's* protocols, modified for an outcrossing nematode I characterised the basic demography for individuals of two strains (JU724 and MY12-G). These strains were recently isolated from the wild (2005 and 2006, respectively). They were acquired from frozen samples that were kept frozen after their isolation from the wild. Therefore, I considered that they represent natural populations, not yet adapted to laboratory conditions. I used a half-sib breeding design throughout the work to estimate the phenotypic variance of traits of related (within replicate) and unrelated individuals (between replicates) of the strains cultured in a common environment in the lab. The purpose of this was to quantify the variance between related and unrelated individuals as a proxy to quantify the genetic structure across the population (Maynard Smith, 1989).

Although *C. remanei* and *C. elegans* 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 (Byerly

et al., 1976, Kimble and Ward, 1988). Recent research on *C. elegans* has shown sperm limitation and evidence of a potential sexual conflict in this hermaphroditic species (Kleeman and Basolo, 2007). Similar processes could be expected in other free-living nematodes of the *Caenorhabditis* genus too, however, evidence of either in gonochoristic species is lacking. Therefore, as part of my research, I investigated in Chapter 3 (section A.2 in Figure 1.2) to what extent female reproductive and survival schedules depend on the number of males present.

Phenotypic plasticity is a widespread phenomenon (Via et al., 1995). In Chapter 4 (section B in Figure 1.2), I quantified the response of life-history traits to temperature. This was required as a baseline to describe thermal tolerance of the two geographically distinct strains and a half-diallel cross between them, which allowed a comparison not only between different strains, but what might be a more genetically diverse hybrid population. Differences in the thermal tolerance of these populations could be caused by local adaptations or by changes in gene frequencies due to hybridisation. The objective was to test whether local adaptations and hybridisation, had different effects on levels of phenotypic plasticity.

In the rest of the thesis (Chapter 5-6, sections C and D in Figure 1.2), I focused on the effects of constant and predictably-fluctuating environments on the evolution of phenotypic plasticity. Despite numerous theoretical studies describing the evolution of life-history traits in fluctuating and uncertain environments (e.g. Tuljapurkar, 1989; Tuljapurkar, 1990; Orzack and Tuljapurkar, 2001), there is little empirical evidence relating to it. Thus, in Chapter 4 (section C in Figure 1.2), the evolutionary consequences of environmental conditions on fitness were assessed. I cultured populations of *C. remanei* under two thermal regimes, constant and predictably-fluctuating, for 50 generations. I compared the response of life-history traits in these environments at generation 1, 20 and 50.

In theory, the existence of predictability in the environment can select for individuals that vary their life histories according to environmental cues (Roff, 2002). Thus, in Chapter 6 (section D.1 and D.2 in Figure 2), I investigated the evolution of phenotypic plasticity in populations exposed to two environmental regimes (constant and predictably fluctuating temperature). I expected that individuals that have been selected for a fluctuating environment would have more plastic responses. Moreover, after the translocation between environmental regimes, I expected that individuals with plasticity phenotypes would pay a fitness cost when moved to a constant environment.

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

2.1 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 a developmental time similar to the hermaphroditic N2 strain *of C. elegans*.

2.2 Introduction

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 laboratory 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).

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.

2.3 Material and methods

2.3.1 General maintenance and procedures

Two wild-type strains of *C. remanei*, JU724 (from China) and MY12-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 re-cultured 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.

2.3.2 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).

2.3.3 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. The JU724 strain was used for this assay.

2.3.4 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.

2.3.5 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.

2.3.6 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 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**).

Estimate	Description	Acronyms and calculation
Age-specific survival or survivorship function	Proportion of individuals surviving from birth (x_0) to age x	l_x
Age-specific fecundity or maternity function	Offspring per individual aged x per unit time	M_x
Lifetime fecundity	Number of offspring produced per individual in their lifetime	$LF = \sum_{x=0}^{\infty} M_x$
Reproductive lifespan	Number of reproductive days from start of reproduction	RL
Life expectancy	Number of days to live from age x_0	$E = \sum_{x=0}^{\infty} l_x$
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	$e_{ij} = \frac{a_{ij}}{\lambda} \frac{\partial \lambda}{\partial a_{ij}}$

Table 2.1. Description and calculations of demographic parameters used in this study. Caswell, 2001 was used as a reference.

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).

2.3.7 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 *l*th 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).

2.4 Results

2.4.1 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 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. 2.1a). Subsequently, juvenile larvae (L1-L3) were most abundant at 1.58 ± 0.54 SD d (Fig. 2.1a). After this time, larvae exhibited sex-specific features; peak numbers of female L4 larvae were recorded at 2.50 ± 0.55 SD d (Fig. 2.1b). 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. 2.1c). Sex ratio of females to males did not differ from unity (χ^2= 2.20, 1df, *P* = 0.86).

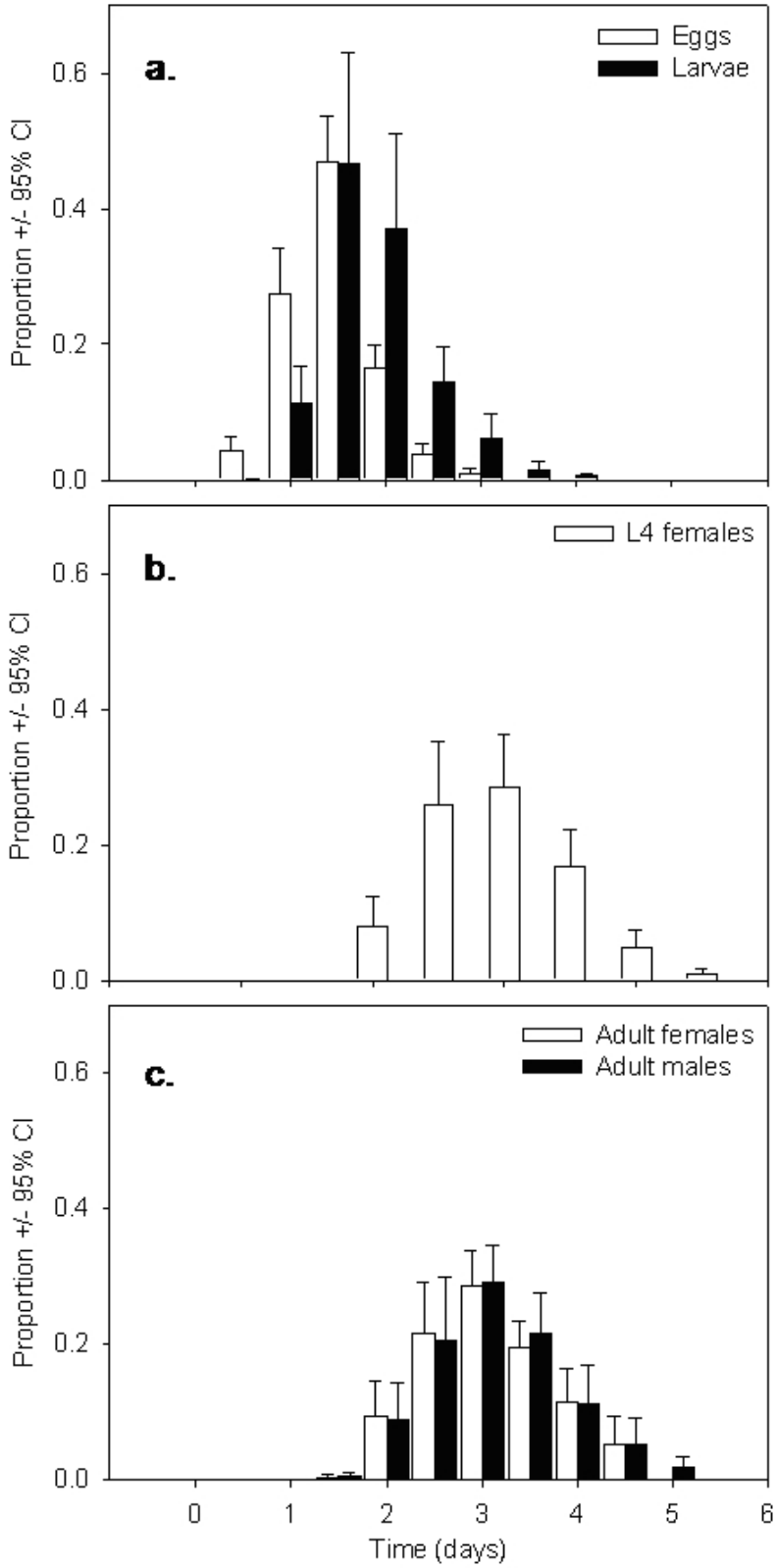

Figure 2.1. *C. remanei*'s development time at 20 °C in the lab. Bars represent the proportion of: (a) eggs and larvae, (b) pre-adult females (L4) and (c) adult females and males found on 10 NGM-petri dishes over time.

Females of *C. remanei* cultured under laboratory conditions at 20 °C 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. 2.2a). 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: $\chi^2 = 12.58$, 1 df, $P < 0.01$; Fig. 2.2c).

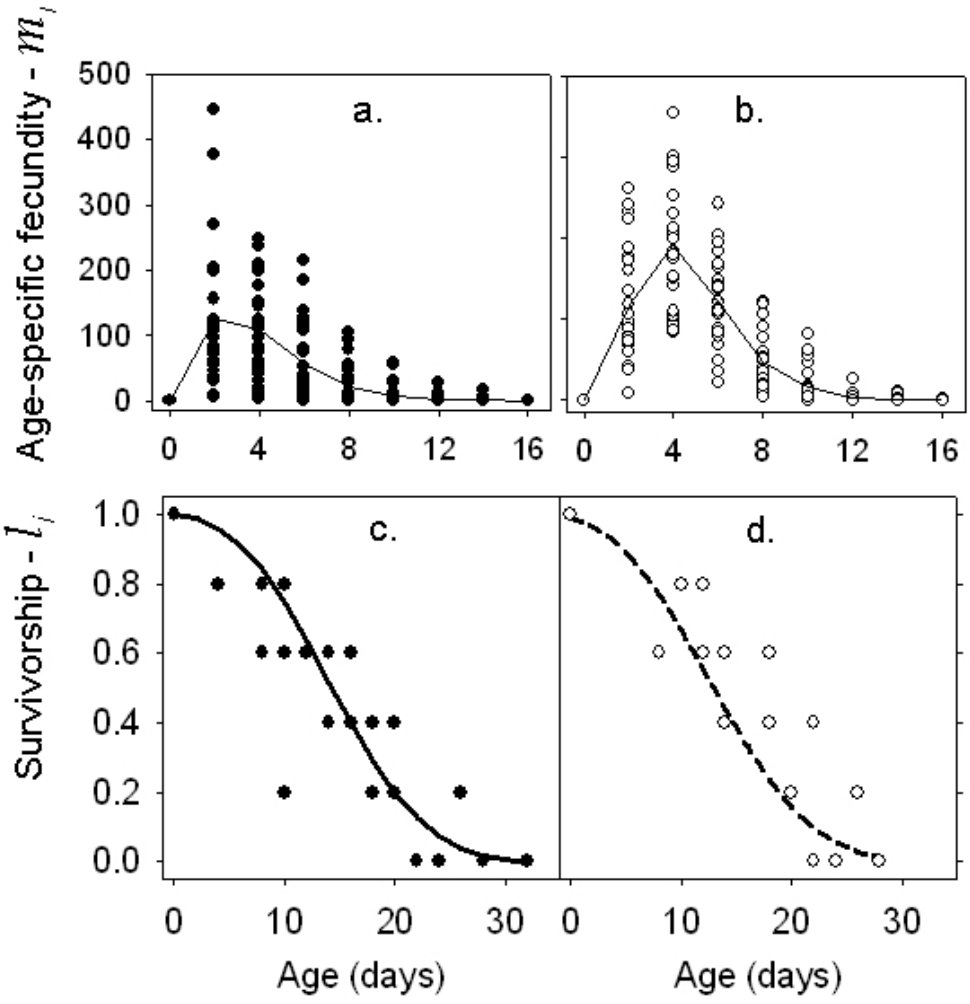

Figure 2.2. Age-specific fecundity (a and b) and survivorship (c and d) of females at 20 °C. JU724 and MY21-G are represented by filled and open symbols, respectively.

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 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. 2.3b).

The elasticity estimates to a change of a vital rate on λ decreased exponentially with age (Fig. 2.3c), 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.

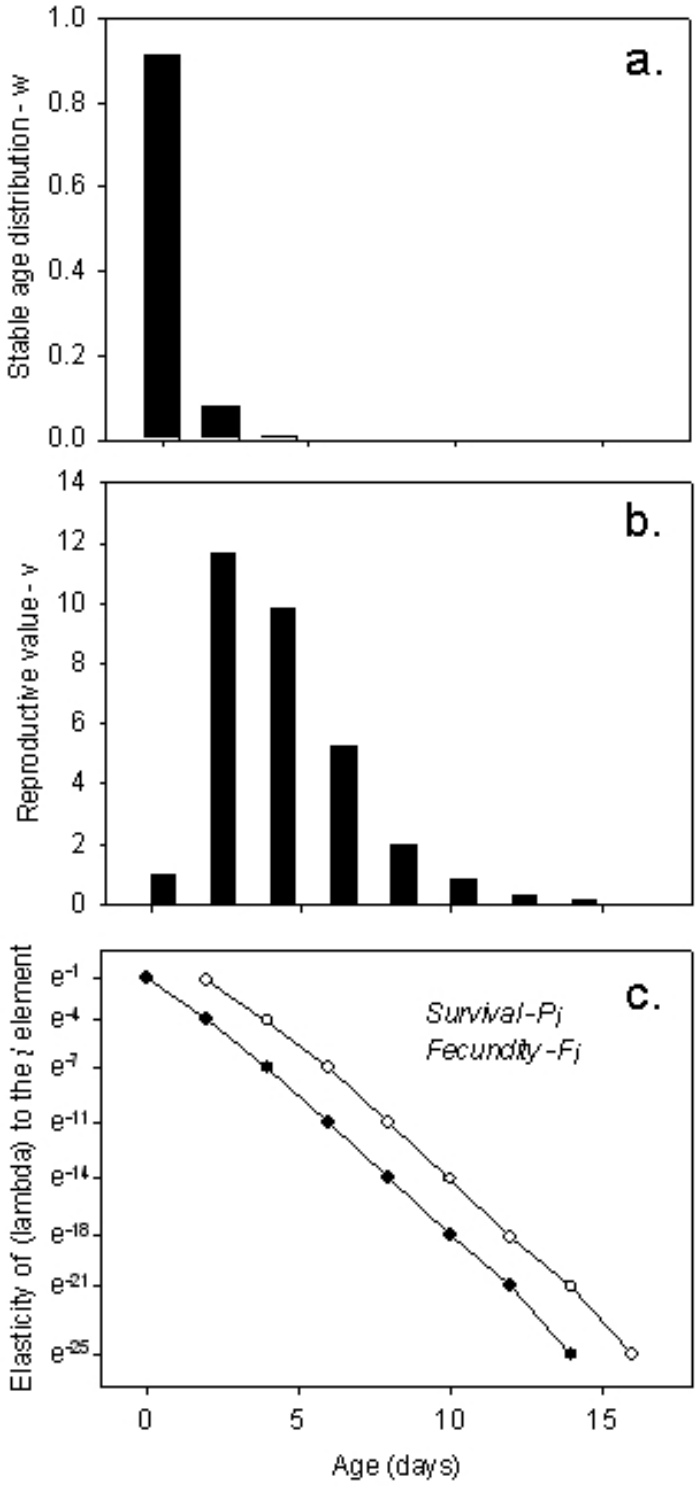

Figure 2.3. (a) Stable age distribution; (b) age-specific reproductive value; and (c) the elasticity (log transformed) of λ to changes in age-specific survival probability (Pi, filled symbols) and age-specific reproductive estimate (Fi, open symbols) for JU724.

2.4.2 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:

$LF_{jkl} = Strain_j + line_k + \varepsilon_{jkl}$; (Model 1, Table 2.2)

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 2.2). However, the variance between lines was low compared to the variances within lines (percentage of variance components: $\sigma_{line}^2 < \sigma_{\square}^2$: 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 2.2, Table 2.3). Model comparison using the AIC values made no clear distinction between models (Model 1 vs. Model 3, Table 2.2). Therefore, the simplest model was preferred.

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

Table 2.2. AIC and log likelihood (logLik) values for vital rates models. Bold letters correspond to the preferred model for each trait according to the AIC (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.

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:

$E_{jkl} = Strain_j + line_k + \varepsilon_{jkl}$; (Model 5, Table 2.2)

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. 2.2c,d). Therefore, the model could be better formulated as: $E_{jkl} = \hat{\beta}^{E} + line_k + \varepsilon_{jkl}$ (Model 4, 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 ($\sigma_{line}^2 < \sigma_{\square}^2$: ~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 2.2). The final model was: $E_{jkl} = \hat{\beta}^{E} + \varepsilon_{jkl}$; (Model 6, Table 2.2, Table 2.3).

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 2.2). The starting model for Reproductive Lifespan (*RL*) was:

$RL_{jkl} = \hat{\beta}^{RL} + line_k + \varepsilon_{jkl}$; (Model 7, Table 2.2)

Again, we found a low variance between lines compared to the variance within lines ($\sigma_{line}^2 < \sigma^2$: ~0.01and 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 2.2). The final model was: $RL_{jkl} = \hat{\beta}^{RL} + \varepsilon_{jkl}$; (Model 9; Table 2.2, Table 2.3).

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 (days)					
	$\hat{\beta}^{E}$	F	16.84	0.93	18.03	<0.01
	ε	R		6.60		
9	Reproductive lifespan (days)					
	$\hat{\beta}^{RL}$	F	10.08	0.34	29.83	<0.01
	ε	R		2.39		

Table 2.3. Descriptive statistics to describe *C. remanei* demographic parameters (lifetime fecundity, life expectancy and reproductive lifespan) of females cultured at 20 °C. The models included here are the preferred 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 2.2. $\hat{\beta}$ 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.

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 2.2, Table 2.4), 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 (χ^2 = 15.80, 8 df, P<0.5; Model 10 vs. Model 13, Table 2.2, Fig. 2.2a,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 2.4).

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 2.5), high fecundity at age 6 is positively correlated with the subsequent ages, and that both strains had the same patterns.

(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

Table 2.4. Descriptive statistics to describe *C. remanei* demographic parameters (lifetime fecundity, life expectancy and reproductive lifespan) of females cultured at 20 °C. The models included here are the preferred 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 2.2. $\hat{\beta}$ 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.

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 2.5. Summary of Model 10 (Table 2) to describe the age-specific fecundity. This includes the correlations of 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).

2.4.3 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 *k*th line within the *j*th strain. The model was:

$E_{jkl} = LF_{jkl} + Strain_j + line_k + \varepsilon_{jkl}$; (Model 1, Table 2.6)

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 ($LF_{\boldsymbol{jkl}[\text{slope}]}$ = -0.01 ± 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 2.6) nor an interaction between the strain and the slope of $LF_{\boldsymbol{jkl}}$ (χ^2 = 2.32, 2 df, *P* = 0.31; Model 3 vs. Model 2, Table 2.6). Therefore, the model could be better written as: $E_{jkl} = LF_{jkl} + line_k + \varepsilon_{jkl}$ (Model 2, Table 2.6). 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 2.6). The final model was: $E_{jkl} = LF_{jkl} + \varepsilon_{jkl}$; (Model 4, Table 3, Table 2.7).

We described the life expectancy (*E*) in relation to the number of reproductive days (*RL*) for the *l*th worm from the *k*th line within the *j*th strain. The model was:

$E_{jkl} = RL_{jkl} + Strain_j + line_k + \varepsilon_{jkl}$; (Model 5, Table 2.6)

We found very low evidence of a relationship between life expectancy and the number of reproductive days ($RL_{jkl[\text{slope}]}$ = 0.09 ± 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 2.6) nor an interaction between the strain and *RL* (χ^2 = 0.67, 2 df, *P* = 0.72; Model 7 vs. Model 6, Table 2.5). Therefore, the model could be written without the Strain effect: $E_{jkl} = RL + line_k + \varepsilon_{jkl}$; (Model 6, Table 2.6). 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 2.6). Therefore, the final model was: $E_{jkl} = RL_{jkl} + \varepsilon_{jkl}$ (Model 8; Table 2.5, Table 2.7).

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	$E_{jkl} \sim \boldsymbol{LF_{jkl}} + (1\|\text{line}_k)$	334.60	-164.30	3
Model 3	$E_{jkl} \sim \boldsymbol{LF_{jkl}} + \text{Strain}_j + LF_{\boldsymbol{jkl}}:\text{Strain}_j + (1\|\text{line}_k)$	336.28	-163.14	5
Model 4	$\boldsymbol{E_{jkl} \sim LF_{jkl}}$	**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	$\boldsymbol{E_{jkl} \sim RL_{jkl}}$	**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	$\boldsymbol{RL_{jk} \sim LF_{jk} + (1\|\text{line}_k)}$	**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

Table 2.6. AIC and Log Likelihood values for correlations across vital rates. Bold letters correspond to the preferred model (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.

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

$RL_{jkl} = LF_{jkl} + Strain_j + line_k + \varepsilon_{jkl}$; (Model 9, Table 2.6)

We found low evidence that reproductive lifespan of worms was correlated with their lifetime fecundity ($LF_{jkl[\text{slope}]}$ = 0.01 ± 0.01 SE, t-value = 0.44). There was neither a significant effect of the strain (χ^2 = 1.53, 1 df, *P* = 0.22, Model 9 vs. Model 10, Table 2.5) nor an interaction between the strain and the slope (χ^2 = 3.83, 2 df, *P* = 0.15, Model 11 vs. Model 10, Table 2.6). Therefore, the model could be written as: $RL_{jkl} = LF_{jkl} + line_k + \varepsilon_{jkl}$ (Model 10, Table 2.7). Moreover, although the variance between lines was low compared to the variance within lines, (21.70 and 78.30%, respectively, Table 2.6), 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 2.6).

Model	Parameter	Type of variable	Estimate	SE	t-value	P
	Life expectancy vs.:					
4	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	<0.01
	$\hat{\beta}^{RL}$	F	6.71	0.74	9.02	<0.01
			Variance	SD	Percentage of the Variance	
	line (Intercept)	R	0.89	0.94	21.70	
	ε	R	3.22	1.79	78.30	

Table 2.7. 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.

2.5 Discussion

2.5.1 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. 2.4). Using the assays developed for this species, we re-constructed its life cycle and found that *C. remanei* has a short generation time when 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).

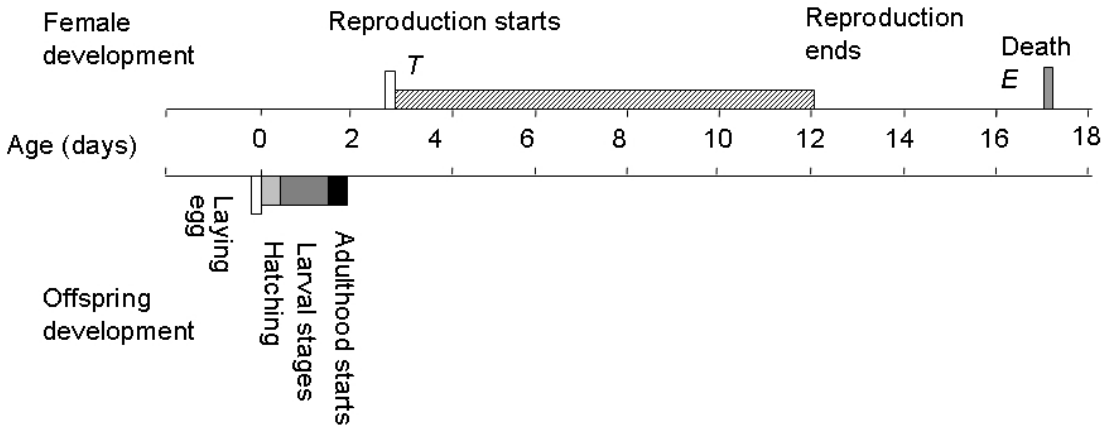

Figure 2.4. Re-construction of *C. remanei*'s lifecycle at 20 °C. Parameters were estimated using the development time and vital rates assays. T is generation time and E is lifespan (see Table 1). Note: Absolute estimates are used to illustrate the overall lifecycle.

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 20ºC. Comparisons of growth curves at 15, 20 and 25°C for *C. elegans* showed that the best temperature among these was 20ºC (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.

2.5.2 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. 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 approx. 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 behaviour 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 favourable 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.

2.5.3 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.

2.5.4 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

3 Between a rock and a hard place: sperm limited fecundity and polyandry induced mortality in female nematodes *Caenorhabditis remanei*

3.1 Abstract

In many sexually reproducing species, females are sperm limited and actively mate more than once which leads to sperm competition between males. If females gain from multiple matings due to increased fecundity, for instance, this can lead to a sexual conflict as the optimal mating frequency for females and males can be different. Thus, males with traits evolved to reduce females' re-mating rates are not uncommon. However, the same traits can also reduce directly or indirectly female survival. Evidence of this sexual conflict is ubiquitous across several taxa. Here, we examine the evidence for this form of conflict in the free-living nematodes of the *Caenorhabditis* genus. Members of this group are extensively used to describe developmental and physiological processes. Despite this, evidence of sexual conflict in gonochoristic species is lacking. In this study, we found evidence of sexual conflict in *C. remanei* cultured under laboratory conditions. In our first experiment, we found that females' fecundity increased with the number of males present which suggests that females' reproduction may be sperm limited. However, increased number of males present also reduced female survival. A second experiment ruled out the possibility of a density dependency effect reducing female survival when more males were present as increasing female density correspondingly did not affect female survival.

3.2 Introduction

In sexually reproducing species, the relative interests between sexes are seldom identical, leading to sexual conflict (Partridge and Hurst, 1998; Arnqvist and Rowe, 2005). For instance, females are often sperm limited and require more than one mating in order to maximise their reproduction (e.g. Pitnick, 1993; Pitnick and Markow, 1994), and the resulting polyandry intensifies sperm competition (Chapman et al., 1995; Birkhead, 2000; Pai et al., 2007). It is commonly thought that antagonistic coevolution of male and female traits (Chapman, 2006) has led to the evolution of a variety of male traits to aid their reproduction; for instance, aggression towards partners, mate guarding (Chapman et al., 1995), copulatory plugs (Hodgkin and Doniach, 1997), stimulants of oocyte production (Chapman et al., 1995), faster sperm (LaMunyo and Ward 1995) and toxic components inside the seminal fluid (Chapman et al., 1995). In many cases, these traits can actually cause harm to the female and may even reduce her overall life expectancy (Chapman et al., 1995; Eberhard, 1996; Arnqvist and Rowe, 2005). In response, females display traits such as male avoidance (Kleemann and Basolo, 2007), mate choice (Arnqvist and Rowe, 2005), expulsion of sperm (Kleemann and Basolo, 2007) and/or maintenance of harsh environmental conditions inside female reproductive tract (Eberhard, 1996).

Evidence for sexual conflict is widespread across the animal kingdom, and therefore free-living nematodes should not be an exception. Caenorhabditis nematodes, particularly *C. elegans*, are among the most studied metazoan organisms. Despite the often-detailed knowledge of their genetic and developmental pathways (Francis et al., 2003), our understanding of the selective forces shaping their life-history is still scarce. One exception is Gems and Riddle's (1996) experiment in the hermaphrodite *C. elegans* demonstrating that female mating reduced hermaphrodite lifespan whereas gamete production per se or reception of the seminal fluids apparently did not, and therefore the trade-off between reproduction and longevity is mediated by cost of copulations in *C. elegans*. This cost indicates a potential for sexual conflict over the timing of mating. Most of the individuals are sequential hermaphrodites and males are very rare, comprising less than 1 % of the population (Ward and Carrel, 1979).

The hermaphrodites are sperm limited (Byerly et al., 1976, Kimble and Ward, 1988) but one would expect them to want to use up all the self-sperm before mating with males in order to avoid the cost of mating. However, the males do not seem to pay a cost for mating and would consequently try to mate whenever they have an opportunity. Indeed,

Kleeman and Basolo (2007) have shown this to be the case: if the hermaphrodites have self-sperm left, they are more likely to move away from males and to eject their sperm after insemination, whereas the self-depleted hermaphrodites do the opposite. It is possible that there is even more scope for sexual conflict in the gonochoristic Caenorhabditis species, such as *C. remanei* as they could also be sperm limited but cannot self-fertilise.

In this study, we experimentally manipulated the mating frequency of *C. remanei* females by varying the number of males present, and quantified its effect on female survival and fecundity rates. More specifically, we addressed the following questions under laboratory conditions: 1) Do mated females show reduced life expectancy in comparison to unmated females, indicating costs of mating? 2) Does increased number of matings increase female fecundity? In addition, to rule out the possibility that a possible reduction in female life expectancy with increasing number of males present is merely due to density dependence in survival, we also manipulated the number of unmated females present.

3.3 Material and methods

3.3.1 General maintenance

A wild-type strain of *C. remanei* (JU724), originally isolated in China, was used in these experiments. The strain was obtained from frozen stocks provided by M. A. Felix in the Nematode Biological Resource Centre in France. Individuals were maintained according to standard laboratory protocols (Hope, 2001): they were cultured in a temperature-controlled incubator at 20°C and fed on a lawn of *Escherichia coli* (OP50 strain). Petri dishes of 35mm diameter, half filled with standard nematode media (NGM), were used throughout the whole experiment. Before the start of each experiment, the progeny from a single pair was used to select twenty-five young females of similar age (approximately 36 hours after hatching). These females were individually raised in isolation for 24 hours prior to the start of the experiment to assure virginity. For males, mature worms were selected from a growing population.

3.3.2 Experimental design

The first part of the experiment was designed to quantify the effect of the number of males on female survival and fecundity. At the start of the experiment, the twenty-five

females were split randomly and evenly into five different treatment groups, with either 1, 2, 4, 8 or 16 males, creating five replicates per treatment (time 0). To provide a continuous food supply, the females were transferred daily into a new petri dish with the same number of males as before. At each transfer, the males previously used were removed and replaced by new males randomly selected from the offspring of five different females; they were approximately 36-hours old at the time of the transfer. After the last transfer, males were removed and individual females were monitored daily until death. Previous experience showed that transferring females after certain age increases the likelihood of their death (In this study 65% of the females survived until the sixth transfer). This may have been due to possible damage caused by the worm picker. To minimise the extra mortality caused by transfers *per se,* females were transferred no more than six times during the duration of the experiment. Death was recorded if the female showed no movement and failed to respond to a gentle touch with the worm picker.

As it is theoretically possible that female mortality increases with the number of males simply due to density dependent factors we also looked at the survival of unmated females in the absence of males at two densities, 1 and 16 females per petri dish, using 5 replicates of each. These females were transferred daily to new petri dishes, as in the first part of the experiment. Similar to all the other treatments, these females were individually transferred for 6 days and then monitored daily until death was recorded.

3.3.3 Statistical and demographic analysis

3.3.3.1 Life expectancy(LE)

The number of days alive, *x*, was used to estimate the life expectancy (*LE*) of females in each treatment. For mated females, the effect of the number of males on female life expectancy was tested using regression analysis. For unmated females, we used ANOVA analysis to compare the mean and variance of each treatment.

3.3.3.2 Fecundity (LF)

The number of offspring produced by a female at each stage interval was used to calculate the age-specific fecundity (m_x) and lifetime fecundity ($LF = \Sigma m_x$). The effect of the number of males on female *LF* was analysed using regression models. The age-specific fecundity (m_x) data were analysed using mixed-effects models to describe the effect of the males on the number of offspring produced at each age interval (lmer function in “lme4

package" from R, Crawley, 2007). The model included *Age* (x =0,1,2,…,6) as a categorical variable and the number of males as fixed factors, and a random factor that referred to the effect of the *i*th individual (since the data contained repeated measures). We presented the variance components in terms of the percentage of the total variance attributable to each age effect, σ_{Agex}^2, and the error deviation, σ_ε^2 (for example, the percentage of variance at Age0: = $\sigma_{Age0}^2 / [\sum_{Age0}^2 + \sigma_\varepsilon^2]$).

3.3.3.3 Trade-offs among fitness components

The relationship between life expectancy and lifetime fecundity was investigated using regression models. Using the sign of the coefficient to establish either a positive, a negative, or absence of a relationship between the two vital traits.

3.3.3.4 Model comparison

The number of males was used as a continuous variable in all the models for each trait and each model was compared to its quadratic form to reveal possible non-linear effects. The linear and quadratic regression models were compared by contrasting their residuals (using 'anova' function, F-test, in R software from R project for statistical computing: http://www.r-project.org). The results are presented with the corresponding *F* statistics, degrees of freedom (d.f.) and P-values (P). For mixed-effect models, we used likelihood-ratio tests (LRT) to choose the most parsimonious model between a linear or a quadratic model. The comparison for nested models included the χ^2 statistic for a given number of d.f. and the P-value. For unnested models, the Akaike information criterion (AIC) was used to assess if the random effect added a significant effect into the model. Data are presented as mean± se.

3.4 Results

3.4.1 Life expectancy (LE)

Females' life expectancy decreased in relation to an increase in the number of males present (Figure 3.1A). However, the reduction in lifespan was not linear with an increase in males (Table 3.1A). This was shown by the comparison between the linear and quadratic models, adding the quadratic term to the model provides a better fit to the observed data (model comparison: *F*= 5.50, 1 d.f., P=0.02). For the unmated females, we

found similar life expectancies between virgin females cultured individually and in a cohort (number of days: 27.20±0.67 and 24.10±0.39, respectively, model comparison: *F*= 3.19, 1 d.f., P=0.11).

3.4.2 Fecundity (LF)

The lifetime fecundity of females differed between the treatments (Figure 3.1B). Lifetime fecundity tended to increase with the number of males up to a peak fecundity with seven males (Table 3.1B). However, increasing their number further resulted in declining lifetime fecundity. The nonlinear relationship between fecundity and the number of males was confirmed by comparing the linear and quadratic models, showing that the nonlinear model provides a better fit to the data (model comparison: F= 4.5, 1 d.f., P=0.04, Table 3.2).

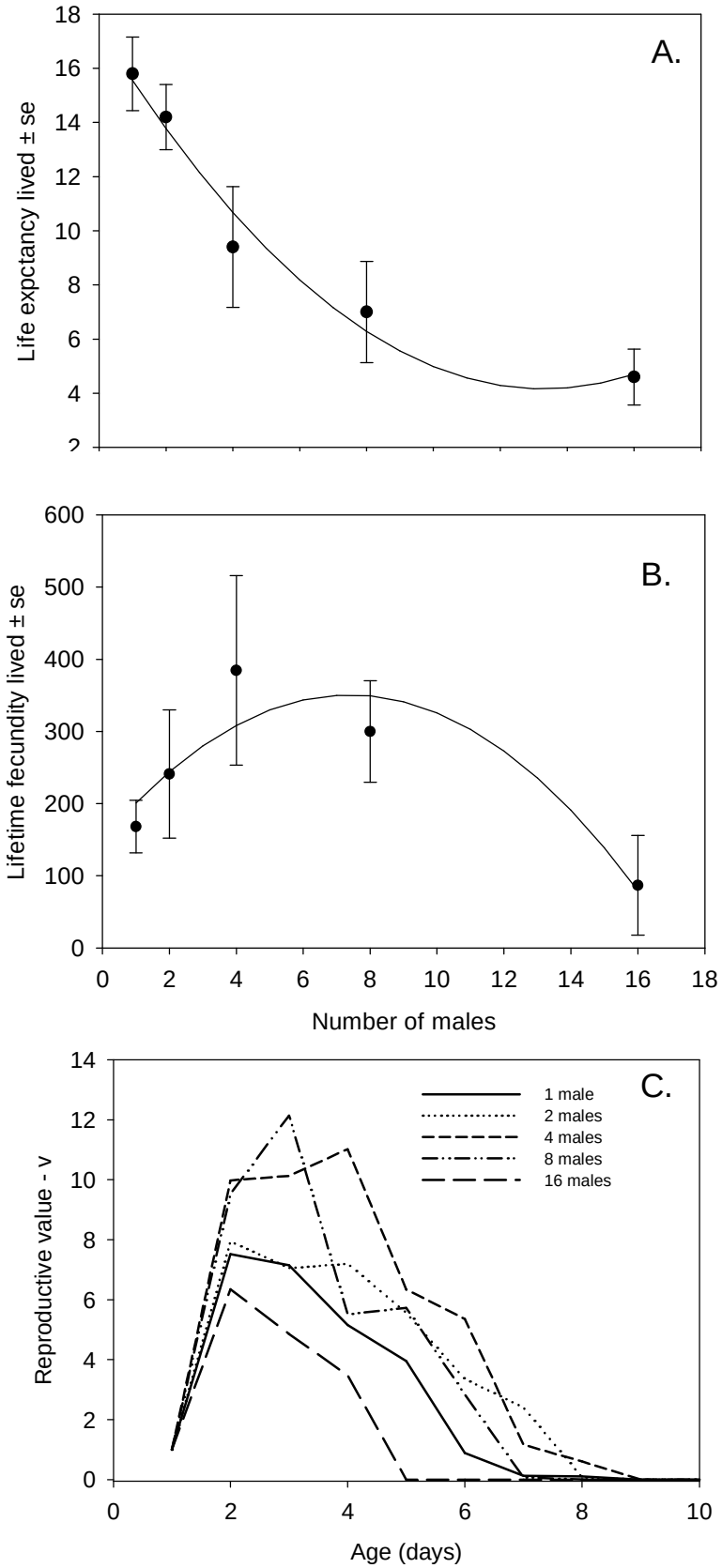

Figure 3.1. The effect of the number of males on females' (A) life expectancy (number of days); (B) lifetime fecundity (number of larvae produced). Dots represent the observations (±se) in each treatment and the continuous lines the predicted values from the quadratic models; and (C) Reproductive value functions of each treatment.

Parameter	Mean±SE	t-value	P	$F_{[df]}$	R^2	P
A) LE						
Linear model						
Intercept	14.57 ± 1.15	12.61	<0.01	$25.34_{[1,23]}$	0.50	<0.01
Males	-0.70 ± 0.14	-5.03	<0.01			
Residual std. error	3.82					
Quadratic model						
Intercept	17.48 ± 1.63	10.72	<0.01	$17.9_{[2,22]}$	0.59	<0.01
Males	-2.00 ± 0.57	-3.53	<0.01			
Males^2	0.08 ± 0.03	2.34	<0.05			
Residual std. error	3.49					
B) LF						
Linear model						
Intercept	292.16±60.82	4.80	<0.01	$1.50_{[1,23]}$	0.02	0.23
Males	-9.01±7.36	-1.22	0.23			
Residual std. error	200.9					
Quadratic model						
Intercept	150.62±87.39	1.72	0.09	$3.12_{[2,22]}$	0.15	0.06
Males	53.94±30.41	1.77	0.09			
Males^2	-3.65±1.72	-2.12	<0.05			
Residual std. error	187					
C) LE~LF						
Linear model						
Intercept	14.38±1.67	8.61	<0.01	$12.15_{[2,22]}$	0.48	<0.01
Lifetime fecundity	0.01 ± 0.01	0.16	0.88			
males	-0.70 ± 0.14	-4.73	<0.01			
Residual std. error	3.90					
Quadratic model						
Intercept	16.72 ± 1.71	9.76	<0.01	$12.82_{[3,21]}$	0.59	<0.01
Lifetime fecundity	0.01 ± 0.01	1.27	0.21			
Males	-2.27 ± 0.59	-3.80	<0.01			
Males^2	0.09 ± 0.03	2.69	<0.05			
Residual std. error	3.41					

Table 3.1. Descriptive statistics of (A) life expectancy, (B) lifetime fecundity, and (C) trade-offs models. (A) Models include the linear relationship between the number of days a female lived and the treatment (number of males present), and the quadratic model also has this in quadratic form. The variables in lifetime fecundity models and trade-offs models in (A). Models are presented with the corresponding statistics to test the significance of the parameters in the model (see Methods).

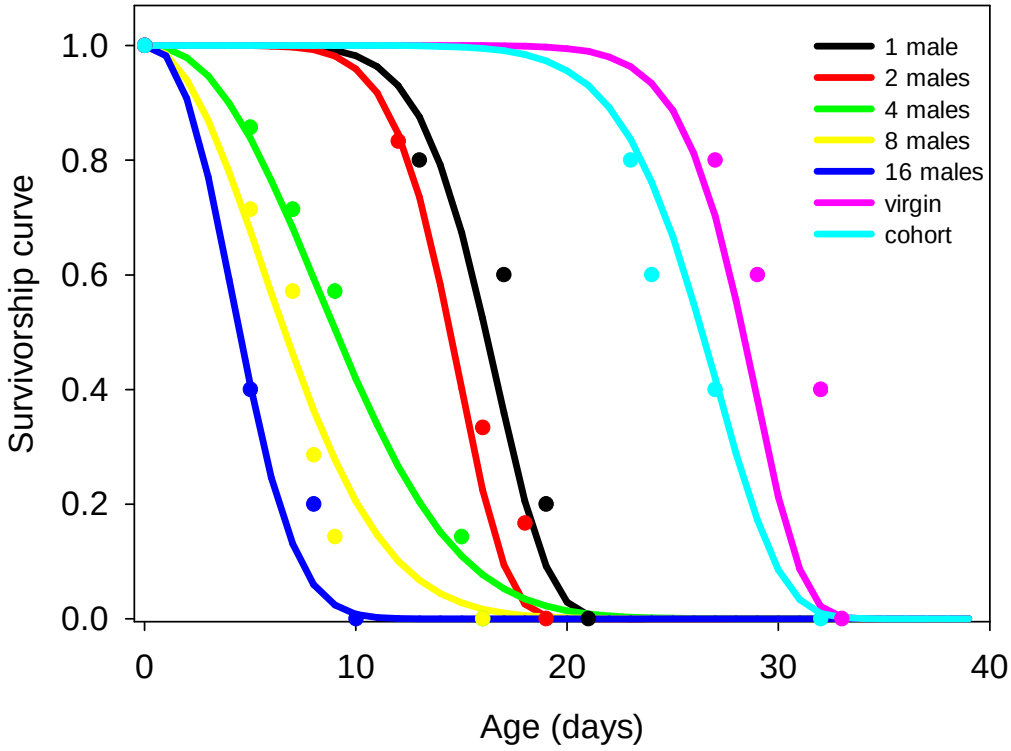

Figure 3.2. Illustration of survivorship (l_x) curves for each treatment. The label for each treatment is at the left of each line; except for unmated females raised individually. Observations are represented by dots and the fitted model by lines. The fit curves represent the Weibull model ($S_x = e^{-(scale*x)*shape}$); for details see, Ricklefs and Scheuerlein 2002, Crawley 2007).

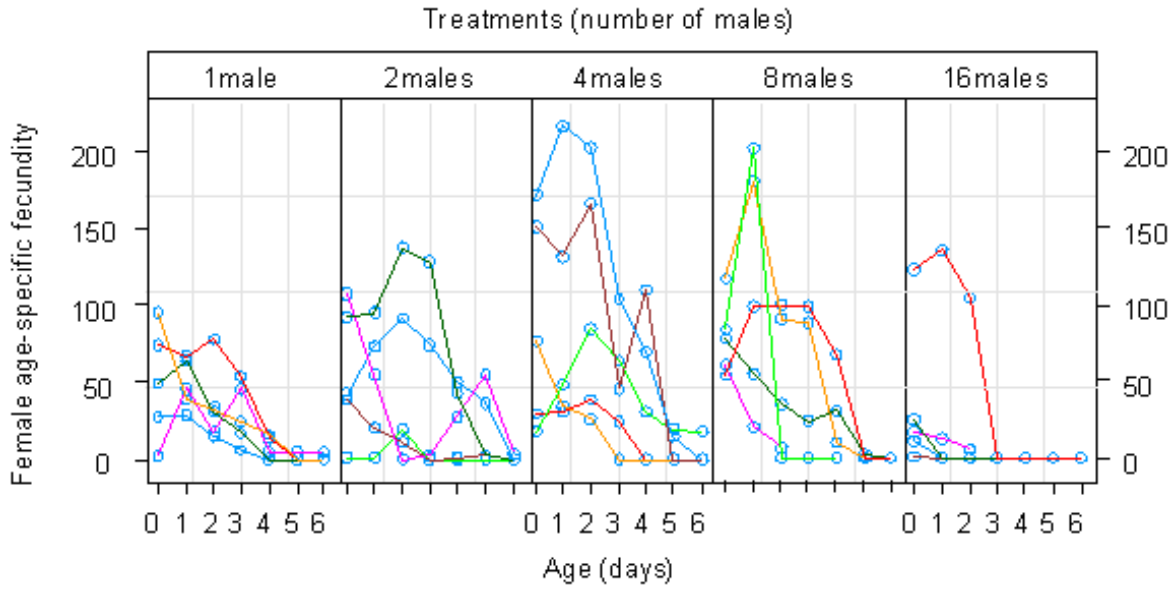

Figure 3.3. Illustration of age-specific fecundity (m_x) across treatments. Individuals' observations are represented by lines.

3.4.3 Age-specific fecundity *(m_x)*

To quantify the production of offspring over time, and to compare the effect of different treatments on it, we used mixed-effects model approach. We were interested in describing the number of offspring produced by the *i*th female at each stage of its life (m_{xi}) and the effect of the number of males (*Males*) using the following model:

$m_{xi} = Age_x + Males + (Age_x \mid ind_i) + \varepsilon_{xi}$; (Model 1)

where the bar | denotes the age-specific variance between individuals ($Age_x|ind_i$).

Since our previous analysis showed that lifetime fecundity was affected by the number of males present, our initial model similarly described how an increase in the number of males would affect the observed data independent of age (Model 1). This model was compared with a set of alternative models to test the following hypotheses:

1. The effect of the number of males on female fecundity depends on her age (Model 2 Table 3.2).
2. The effect of the number of males on females' age-specific fecundity is not linear (Model 3 Table 3.2).
3. The effect of the number of males has a non-linear effect on female fecundity, and this effect is different at different ages of the female (Model 4 Table 3.2).
4. The age-specific individual variation is similar across ages (removal of $Age_x|ind_i$, Model 5, Table 3.2).

We found no support for hypothesis 1 (χ^2=0.42, 6 d.f, P=0.99, Model 1 vs. Model 2), thus, all age-specific fecundities responded similarly to an increase in the number of males. However, as suggested by the *LF* models, we found that the relationship between the number of males and age-specific fecundity was not linear (hypothesis 2, χ^2=8.07, 1 d.f, P<0.01, Model 1 vs. Model 3). This model suggested that all age-specific fecundities increased similarly with an increase in the number of males present up to ca. 7 males, but a further increase resulted in a decline in all age-specific fecundities. Further analysis indicated no support for hypothesis 3 (χ^2=0.67, 6 d.f, P=0.99, Model 3 vs. Model 4), thus,

the non-linear relationship between fecundity and number of males was not age-specific. Finally, the analysis of variance components suggested a similar individual variance across ages (AIC Model 5 < AIC Model 3, Table 3.4). Therefore a simpler model was preferred: $m_{xi} = Age_x + Males + Males^2 + (1 \mid ind_i) + \varepsilon_{xij}$; (Model 5, Table 3.2)

In summary, we found that the number of males had a positive effect on females age-specific fecundity up until *ca*. 7 males and that a further increase in the number of males had a negative effect on fecundity independently of the female age (Figure 3.1B). It is also reflected in the shape of the reproductive value functions when compared between treatments (Figure 3.1C). This model also suggested that the variance between individuals is larger than the individual variance across ages (63.32 and 36.68 %, σ_ε and σ_{ind} respectively).

Regarding the correlations among age-specific fecundities, we found that adjacent fecundities are always positively correlated, except for the fecundity between age0 and age1 (Table 3.3C). Moreover, the fecundity at age0 is negatively correlated with all the subsequent ages.

Models	Model syntax	AIC	logLik	DF
Model 1	M_{xi} ~Age$_x$ + Males + (Age$_x$|ind$_l$)	1644.94	-786.47	36
Model 2	M_{xi} ~Age$_x$ + Males + Age$_x$:Males + (Age$_x$|ind$_l$)	1656.51	-786.25	42
Model 3	M_{xi} ~Age$_x$ + Males + Males^2 + (Age$_x$|ind$_l$)	1638.87	-782.43	37
Model 5	**M_{xi} ~Age$_x$ + Males + Males^2 + (1|ind$_l$)**	**1563.05**	**-771.52**	**10**
Model 4	M_{xi} ~Age$_x$ + Males + Age$_x$:Males + Males^2 + (Age$_x$|ind$_l$)	1650.19	-782.09	43

Table 3.2. AIC and log likelihood (logLik) values for age-specific fecundity models. Bold letters correspond to the preferred model for each trait (see methods). Model syntax used upper case letters to denote fixed variables and lower case letters for random variables. Random variables were included within brackets (similar to R syntax for 'lmer' function). The symbol ":" was used to denote an interaction and the bar | denotes the variance of individual given the named variable.

(A) Fixed variables:	Mean	SE	t value
$\hat{\beta}$	50.17	14.25	3.52
Age1	4.28	9.09	0.47
Age2	-10.04	9.09	-1.11
Age3	-31.18	9.61	-3.24
Age4	-46.61	9.61	-4.85
Age5	-62.64	9.92	-6.32
Age6	-66.63	10.09	-6.60
Males	8.86	4.53	1.95
Males^2	-0.63	0.26	-2.46
(B) Random effects	Variance	SD	Percentage of the total variance
Ind (Intercept)	597.66	24.45	36.68
ε	1031.71	32.12	63.32

C) *Correlation of Fixed Effects:*

	(Intr)	age1	age2	age3	age4	age5	age6	males
age1	-0.32							
age2	-0.32	0.50						
age3	-0.32	0.47	0.47					
age4	-0.32	0.47	0.47	0.47				
age5	-0.32	0.46	0.46	0.45	0.45			
age6	-0.33	0.45	0.45	0.45	0.45	0.44		
males	-0.77	0.00	0.00	0.00	0.00	0.02	0.03	
I(males^2)	0.69	0.00	0.00	0.01	0.01	-0.01	-0.02	-0.97

Table 3.3. Descriptive statistics of (A) fixed and (B) random variables to describe Model 2 (Table 3.2) and (C) correlation of fixed factors. Summary of the correlations for the fixed effects for the same mixed-effects model. The model describes the preferred model to describe the effect of the number of males on the age-specific fecundity. $\hat{\beta}$ represents the fixed intercept of the mixed-effect model, ind represents the random effect of the intercept and ε represents the residual standard error. Model syntax and AIC values can be seen in Table 3.2. These results are the analysis of a total of 154 observations of 25 individuals. Note that P values are not included in these results, for further discussion of the lme4 package see Bates and Sarkar (2005).

3.4.4 Trade-offs between vital rates

Among all the females, there was no clear evidence that the number of days alive was related to the total number of offspring produced by a female (F=0.97, 1 d.f, P=0.33, R^2=0.01). However, between treatments, the analysis suggested that an increase in the number of males accentuates the negative relationship between lifetime expectancy and lifetime fecundity (Males = -2.27, Table 3.1C). Moreover, we found support for a non-linear relationship between the number of males and the correlation between vital rates (Table 3.1C, model comparison of linear vs. quadratic: F=7.25, 1 d.f., P=0.01)

3.5 Discussion

Here, we report for the first time evidence of sexual conflict in *C. remanei* females reared under laboratory conditions. Females' lifetime fecundity was shown to be related to the number of males present; females with access to four males had the highest fecundity, and reducing or increasing the number of males from this resulted in a lower fecundity. This suggests a potential for sexual conflict over the mating frequency of *C. remanei* females. Increasing the number of matings is only beneficial for a female up to a certain point, whereas males are unlikely to suffer from the number of matings as fast (see e.g. Gems and Riddle, 1996). These results also suggest that males may directly or indirectly harm their partner and that this damage becomes apparent after only a relatively low number of matings. In our second experiment, we found that mating *per se* also reduces the life expectancy: the life expectancy of unmated females was 50% longer than that of females mated with one male. This is further supported by the fact that reduced lifespan was not a result of density-dependent reduction of survival, as the presence of equivalent numbers of females did not reduce female survival as much as that of males.

Females of many species mate with more than one male during their fertile period (reviewed in Birkhead and Pizzari 2002). For many species, multiple matings increases female's breeding success, which has been linked to an increase in the availability and queality of sperm and other compounds inclided in the seminal fluid. (Birkhead and Pizzari 2002). Emprircal evidence suggests that sperm limitation in fecundity is not uncommon in nature (Ridley, 1988; Wendell et al., 2002; Preston et al., 2001) and there are many different reasons for this in different species. In invertebrates, the common agreement is that a single insemination is not enough to fertilise all female oocytes (Ridley, 1988). For instance, increasing the number of sperm has a positive effect on fecundity in species such as snails (Chen and Baur, 1993), several species of crabs (Sato & Goshima, 2007, Sato et al., 2006), crayfish (Rubolini et al., 2007), the spiny lobster (MacDiarmid and Butler, 1999), several species of *Drosophila* (Pitnick, 1993; Pitnick and Markow, 1994), and as well in the nematode of *C. elegans* (Ward and Carrel, 1979). In *C. elegans*, experimental studies have shown that increasing the number of sperm can increase the fecundity in hermaphrodites (Hodgkin and Barnes, 1991; LaMunyon and Ward, 1995), and a mutation that increases the number of sperm produced by a hermaphrodite significantly enhances fecundity (*ca.* 499 progeny, Hodgkin and Barnes, 1991). Similarly, hermaphrodites mated with males show enlarged brood size (ca. 695 progeny, LaMunyon and Ward, 1995).

The increase in fecundity in *C. remanei* and *C. elegans* could be not only due an increase in sperm number, but as well due to transferred compounds included in the seminal fluid, therefore increasing females/hermaphrodite fecundity. Studies have found that transferred nutrients are common across invertebrate species (Thornhill and Alcock 1983; Cordero 1996). For instance nuptial gifts provided by the males can influence copulating success with a given male (Birkhead and Pizzari 2002). Moreover, it could be possible that males could perceive the increased risk of sperm competition and decide to increase not only the number of matings but at the same time the allocation of sperm and or nutrients for the female (Birkhead and Pizzari 2002).However, the apparent benefit of increased female fecundity comes with a cost of reduced life expectancy. This trade-off has been at the heart of the study of life-history evolution for over half a century (William, 1966; Reznick et al., 2002; Roff, 2002; Promislow, 2003). The general idea is that the positive contribution of fecundity to fitness may affect directly or indirectly other life-history components, for instance, by reducing time and energy to forage (Daly, 1978), increasing predation rate (Arnqvist, 1989; Rowe, 1994), the risk of physical injury (Parker, 1979), or the risk of disease (Hurst et al., 1995; Knell and Webberley, 2004), which, in turn, can reduce survival (Reznick et al., 2002). Notably, this fecundity survival trade-off can be caused by the number of matings itself, without assuming further energetic costs of reproduction. Manipulative experiments across a range of species have indeed shown reduction in life expectancy linked to the number of mates, for instance, in the tropical butterfly (*Bicyclus anynana*, Fischer, 2007) and in Drosophila (Fowler and Partridge, 1989) and *C. elegans* (Gems and Riddle, 1996). At the mechanistic level, studies suggest that reduced life expectancy is the consequence of physiological changes linked to hormonal regulation, intermediary metabolism and allocation, immune function, reproductive proteins, and defenses against stress and toxicity (for a recent review, see Harshman and Zera, 2007).

In invertebrates, the effect of reproduction on the reduction of average life expectancy in mated females (compared to unmated females or mated once) can vary: 8% reduced life expectancy in the tropical butterfly *Bicyclus anynana* (Fischer, 2007), 16% life expectancy in Drosophila (Fowler and Partridge, 1989). In free living nematodes, studies report 11% reduction for *Panagrellus redivivus* (Abdulrahman and Samoiloff, 1975). We found a 37% reduction in *C. remanei;* whereas for *C. elegans*, this cost has been shown to vary from zero to 42%, (VanVoohies, 1992; Gems and Riddle, 1996). Interestingly, in *C, elegans*, this high cost seems to be directly related to the number of copulations, as neither egg production nor reception of sperm affected female longevity (Gems and Riddle, 1996). It is

possible that this was caused by the seminal fluids, as has been shown to be the case in Drosophila. Their seminal fluid contains chemicals that can stimulate egg production, suppress fertilisation by sperm from previous matings, and decrease female life expectancy (Chapman et al., 1995; Chapman, 2008). Gems and Riddle (1996) addressed this question in another experiment, using mutant males capable of stimulating of oocyte production but incapable of fertilisation, and males that apparently are capable of mating but neither produce nor transfer sperm. The results showed that in both cases hermaphrodite females' life expectancy was reduced. The common conception is that males cause stress or physical damage that ultimately reduces the life expectancy of their partner. During mating, a male scans the body of his partner before inserting the spicules into the female or hermaphrodite's vulva. The spicules' function is linked to the correct attachment to the vulva (Barr and Garcia, 2006). Thus, by attaching himself to the vulva by his spicules a male can aid his own reproductive success but may harm the female. Despite this harm to the female, selection could favour males that cause more harm (Edvarsson and Tregenza, 2005; Parker, 2006).

Increasing understanding of Caenorhabditis biology has made them a suitable model system to study sperm competition and sexual conflict. In *C. elegans* the males form a copulatory plug which has shown to increase the time between copulations and reduce the chance of subsequent matings being successful (Barker, 1994). Although we did not quantify this trait systematically in *C. remanei*, we noticed that the size of these plugs can be considerable and that there is a lot of variation in the plugs deposited; some females had plugs corresponding to 20 – 25% of their body size. We suspect that reduced life expectancy may result from damage due to the insertion of spicules, which may in turn be linked to the increasing time of male scanning before a successful copulation due to the presence of a plug previously deposited. Future work should address whether the plug size has an effect on female survival and whether males pay a cost for increasing the number of their matings.

4 Tolerance of *Caenorhabditis remanei* to stressful conditions: comparison across populations

4.1 Abstract

Climate change threatens the persistence of many species. Phenotypic plasticity has been suggested as one of the mechanisms that mitigate against the risk of extinction. In theory, a plastic genotype has a wider ecological breath and could potentially endow higher fitness in novel environments compared with one with reduced or no plasticity. Local adaptations and additive genetic variance, linked to hybridisation, are important factors with potentially different effects on phenotypic plasticity. In this study, I used three strains (two wild-type isolates and a half-diallel cross) of the free-living nematode *Caenorhabditis remanei* and a half-sib breeding design to quantify the plasticity of life-history traits of three populations of worms cultured under a range of temperatures under laboratory conditions. My results describe for the first time the plasticity of four life-history traits of *C. remanei* with regard to changing temperatures; 17° C (± 0.30 sd) was the optimal temperature for fecundity, while 4° C (±0.2.5 sd) maximised survival. The results suggest that the three populations share similar overall thermal breath as evaluated under laboratory conditions but differ in the precise shape of their reaction norms assessed with respect to temperature, suggesting genetic differences and local adaptation across populations. However, the results from the half-sib breeding design exhibit high between-individual variance, which was particularly expected in the half-diallel cross. Moreover, across temperatures, I found low variance between replicates compared to the variance within replicates, thus suggesting low gene-environment interaction for all traits.

4.2 Introduction

Natural environments are not stable in time or space, and their variability is a challenge for organisms inhabiting them. Currently, climate change and its consequences on ecological systems, threatens the persistence of local populations and even many species and around the world (Thomas et al., 2004). Phenotypic plasticity has been recently highlighted as a potentially important factor for mitigating extinction risk (Nussey et al., 2007; Charmantier et al., 2008). Phenotypic plasticity can be defined as the ability of a given genotype to produce distinct phenotypes in response to a changing environment (e.g. Pigliucci, 2001, 2005). Phenotypic plasticity has been typically represented using the *reaction norm*, where the value of a phenotypic trait expressed by a particular genotype is described as a function of an environmental gradient (Via et al., 1995). The significance of phenotypic plasticity is that more plastic individuals (i.e. genotypes) can "match" their phenotype to current environmental conditions; when plastic individuals can cope with a range of environments better than less plastic individuals, phenotypic plasticity can be evolutionarily adaptive.

Due to the ubiquity of temporal fluctuations and spatially heterogeneity in environmental conditions phenotypic plasticity can be expected to be very common in nature, and studies in the wild and in laboratory conditions have indeed reported phenotypic changes in physiological, morphological and life-history traits as a consequence of changing environmental conditions across a wide range of taxa (Byerly et al., 1976; Epstein and Shakes 1995; Visser et al., 1998; Nussey et al., 2005; Charmantier et al., 2008). For instance, the common ground cricket (*Allonemobius socious*) exhibits a cline in the number of broods produced in one season (from single to multiple), varying within its geographical range (Howard and Furth, 1986; Roff, 2002). This has shown to be due to phenotypic plasticity and not due to a simple genetic polymorphism (Roff and Bradford, 2000; Roff, 2002). In *Drosophila melanogaster*, phenotypic plasticity of traits, such as developmental time, in response to temperature has been identified in several geographically different populations reared under laboratory conditions (James et al., 1997). However, despite having phenotypic plasticity, James and collaborators (1997) found that developmental time showed no latitudinal variation between populations in the in the degree of plasticity. Thus, it implies that populations have phenotypic plasticity but there might have not been significant genetic differentiation between populations. In contrast, in the free-living nematodes, *Caenorhabditis elegans*, laboratory studies have found that different strains show both phenotypic plasticity and significant genetic

variation in several life-history traits, such as fecundity, in response to temperature (Gutteling et al., 2007).

In nature, the relationship between genetic variation and phenotypic plasticity is poorly understood. They are commonly viewed as alternative (though not exclusive) means for coping with environmental variability (Schlichting and Pigliucci, 1998). However, when plasticity is a consequence of differences in the reaction norms across genotypes (plasticity at population level, Pigliucci, 2005), it could facilitate the maintenance of genetic variation by acting as a buffer against natural selection (Schlichting and Pigliucci, 1998). Our current understanding of the variation in individual reaction norms (or plasticity) and their response to selection in the wild relies to high degree on quantifying phenotypic variance (Nussey et al., 2007). The use of model species in the laboratory and modern genetic methods of analysis have allowed substantial progress in the description of the effects of genetic and environmental variance (the genotype by environment interaction, or GEI) on the phenotype and between-individual variance (e.g. Sgro and Hoffmann, 1998, Gutteling et al., 2007).

Phenotypic plasticity and GEI may play an important role in the evolution of life histories (Roff, 2002). Populations with sufficient genetic variance for the evolution of plasticity may contain different genotype frequencies as a result of local adaptation. (Pigliucci, 2005). As a consequence, comparisons across different populations can reveal plasticity as well as different GEIs. Populations with low genetic variance, due to low population density for instance, may also show accentuated response to inbreeding as inbreeding depression can negatively affect the response of plasticity to selection (Potvin and Tousingagnt, 1996). One way to avoid such effects can be by artificially mating two inbred populations (Maynard Smith, 1989). In nature, natural hybridisation can produce a relatively fit hybrid, with higher levels of phenotypic plasticity, that may be able to invade novel habitats (Arnold, 1997). However, it is recognised that hybridisation can "sweep" away locally adapted genes (Maynard Smith, 1989). Therefore, local adaptations and hybridisation are important factors with potentially different effects on phenotypic plasticity.

In this study I describe for the first time the plasticity of life-history traits of the free-living nematode *C. remanei* cultured in a wide range of temperatures under laboratory conditions. First, I used a half-sib breeding design and examined the response of two vital rates to six temperatures (5, 10, 15, 20, 25 and 30 º C). Using data derived from these rates, I further derived three important life-history components: life expectancy, lifetime

fecundity and reproductive lifespan. I used two wild-type isolates from geographically-distant populations of *C. remanei* to illustrate the response of different populations, with possible local adaptation, to a temperature gradient. Second, I assessed whether changing additive genetic variance could have an effect on plasticity using a half-diallel cross of the two wild-type populations. Finally, I used mixed-effect statistical analyses to describe the individual variance between and within related individuals from the half-sib breeding design. This approach explores the between-individual variation in reaction norms, and can he helpful in describing the evolutionary potential at population level (Nussey et al., 2007).

Phenotypic plasticity has been documented under laboratory conditions in the free-living nematode *C. elegans* (Byerly et al., 1976; Epstein and Shakes, 1995; Gutteling et al., 2007; Harvey and Viney, 2007), but there is limited information on how related species respond to environmental gradients. *C. remanei* is a gonochoristic nematode closely related to *C. elegans*. Although these species are morphologically indistinguishable, *C. remanei* differs from *C. elegans* in that the females need sperm to reproduce. Recently, *C. remanei's* genome has been sequenced (http://dev.wormbase.org/db/seq/gbrowse/c_remanei/; Haag et al., 2007). Therefore, information pertaining to *C. remanei*'s life cycle and plasticity will be useful as a base line for future studies of its demography in the lab and potential use in evolutionary studies.

4.3 Material and methods

4.3.1 Strains

I used two wild-type strains of *C. remanei*, JU724 and MY12-G, which were recently isolated from the field. Both strains were obtained from frozen stocks provided by M. A. Felix from the Nematode Biological Resource Centre in France (JU724) and N. Timmermeyer from the Animal Ecological Centre in Germany (MY12-G). The Chinese strain was isolated from soil in May 2005 and the German strain was isolated from rotten apples in September 2006. Five replicates (out-bred populations) from each of these strains have been previously used in another study to characterise their life history at 20 °C in the lab (Diaz et al., *in press*). JU724 and MY12-G (referred to henceforth as JU and MY, respectively - see Table 4.1 for acronyms) were used to create a half-diallel cross (hereafter referred to as HYB). This third population consisted of the F1 progeny of a female JU by a male MY, and the reciprocal crosses (ten matings each – the progeny of 20 females in total). All the crosses were conducted at 20 °C. This population was maintained for

approximately 5 generations through randomised mating. It was then sub-divided into five lines (referred to henceforth as replicates) and finally stored in several eppendorf tubes and maintained at -80°C using the same protocols as those used for JU and MY (Diaz et al., in press). Individuals recovered from these frozen stocks were used for all subsequent 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). Petri dishes of 30 mm diameter were used to conduct all assays.

Acronyms	Use
JU	JU724 strain
MY	MY12-G strain
HYB	Half-diallel cross
LE	Life expectancy
LF	Lifetime fecundity
RL	Reproductive lifespan
m	Age-specific fecundity
l	Survival rate
Temp	Temperature
Temp^2	Temperature to the power of two
Age	Age of an individual
ind	Individual

Table 4.1. Symbol used in the text and figures.

4.3.2 Temperature treatments

Under laboratory conditions, I characterised populations' life-history traits in response to a range of temperatures (5, 10, 15, 20, 25 and 30 º C). Prior to each assay, a frozen sample from a specific replicate was thawed at room temperature for a few minutes and then poured onto a NGM-petri dish. The following day, it was moved to the assigned temperature (i.e. 5, 10, 15, 20, 25 or 30 º C). Approximately two days later (except for those individuals raised at 5 º C, which required 4 days), five gravid females were randomly selected from each replicate and transferred into individual petri dishes. The L4 offspring from these females were used to initialize all assays.

4.3.3 Life-history assays

I quantified fecundity and survival rates (referred to henceforth as vital rates) of different individuals from all replicates at each temperature. Using laboratory protocols previously developed to quantify the vital rates of *C. remanei* (Diaz et al., in press), I compared four life-history traits of JU, MY and HYB and their responses across a range of temperatures.

The life-history traits I quantified were life expectancy, lifetime fecundity, reproductive lifespan and age-specific fecundity (see Table 4.1 for acronyms). The objective here was to estimate the average vital rates at each temperature, together with the variance among individuals, between replicates and across strains. I followed 25 individuals from each strain (five per replicate) at each temperature. A virgin female was paired with four unrelated young males for 48 hours. On alternate days after this, the female was subsequently transferred into a new petri dish with four new unrelated young males (Diaz et al., in press; Baird et al., 1994). Transfers were continued until the female stopped laying eggs. Then the female was monitored on alternate days to score the date of death. Age-specific fecundity was estimated by counting the number of juvenile larvae present in each plate. Plates were monitored two days after the female was transferred and the number of larvae counted. In total, 150 females from each population (JU7, MY and HYB) were assessed in this way

4.3.4 Model construction and comparison

Using mixed-effects models, I analysed the average performance of worms of different strains, across temperatures, together with the pattern of variance of the estimated traits across temperatures, among individuals (within replicates) and between replicates.

Model syntax used here denotes fixed variables with upper case letters and random variables with lower case letters. I used subscripts to denote different levels of the data as follows: *m* for individual observations (1,2,…,450), *l* for the replicate (1,2…,15), *k* for the Strain (1,2 and 3), *j* for the Temperature (5, 10,…,30) and *i* for the Age (0,2,…,14 days) of the *m*th individual.

The syntax of the random effects was the following: "(1|replicate$_{lkj}$)" term describing the random effect (intercept) of the deviation from the population mean of the average life-history trait for the *l*th replicate within the *k*th strain at the *j*th temperature (90 levels); the "(1| replicate$_{kl}$)" effect is a random variable (intercept) representing the deviation from the population mean of the average life-history trait for the *l*th replicate within the *k*th strain (15 levels); the "(temp|replicate$_{kl}$)" term is a random variable (slope) representing the deviation of the *l*th replicate within the *k*th strain from the population mean of the average life-history trait across temperatures. The ε_{jklm} is a random variable representing the deviation of the life-history trait for the *m*th worm of the *l*th replicate of the *k*th strain maintained at the *j*th temperature (Faraway, 2006). I presented the variance components in terms of percentages of the total variance attributable to each effect (e.g.

percentage of the variance within replicates = $\sigma_{replicate}^2 / [\sigma_{replicate}^2 + \sigma_{\square}^2]$, and the percentage of the error variance is presented similarly). I assumed that the deviations for each individual random effect were normally distributed with mean zero and fixed variance ($N(0, \sigma^2)$).

The starting model to describe the hierarchical structure of each response variable was M0 (*LE*, *LF* and *RL* –shown in Table 4.2). However, for all traits, the random effect describing the deviance of the *l*th replicate within the *k*th strain was not significant (χ^2= 0.02, 1 d.f., P=0.89), therefore it was not included in the competing models. Moreover, although in some instances there was low evidence of a significant effect of the random effect to describe the deviance of the *l*th replicate within the *k*th strain at the *j*th temperature, such terms describe the non-independent observations of the experimental design. Thus, it was included in all competing models for quantifying accurately the effect of the fixed terms (Faraway, 2006).

In most of the instances (models), I included a quadratic term (fixed effect) to represent a non-linear increase or decrease of a trait in response to temperature. This allowed us to describe how each trait co-varied with respect to temperature. I used random effects for the replicate deviance (i.e. intercept), and the linear and quadratic deviance at the replicate level. This allowed the overall pattern to vary between replicates in terms of the response, and in terms of its curvature (Pinheiro and Bates, 2000). Strain and age was treated as a categorical variable, whereas temperature was treated as continuous.

All statistical analysis was done using R 2.7.1 software (R project for statistical computing: http://www.r-project.org). Mixed-effects models were fitted using the “lmer” function (“lme4” package, version: 0.999375-27). In addition, I analysed survivorship by fitting survival models using the “Surv” function (“survival” package, version: 2.34-1) 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 performed using Likelihood Ratio Tests (LRT) for nested models. For un-nested models, the most parsimonious model with lowest AIC value was chosen. In all instances, pair-wise comparison of competing model are embodied in the text; first the competing vs. the simplest. Unless otherwise stated, the results are presented by a mean effect ± standard error (se).

4.4 Results

4.4.1 Lifetime Fecundity (LF) of the strains used

I found that the strains used in this study varied in the average number of offspring produced (Figure 4.1). Although MY produced on average 36% more offspring compared to JU, I did not detect significant differences between the wild-type isolates (average number of offspring of MY= 468.10±43.04 and JU= 344.20±70.54, t-value= -1.50m d.f. = 14.89, P= 0.15. The crosses between both isolates varied in their average *LF*, for instance, the JU female by MY male cross produced 123.20±20.43 offspring, whereas the reciprocal mating produced 334.50±26.38 (t-value=-6.32, d.f.= 16.94, P<0.001). The F1 (half-diallel cross= HYB) progeny from these two crosses produced significantly more offspring than the JU female by MY male cross and the reciprocal cross (HYB: 481.20±29.04, t-value= -9.86, d.f.= 15.89, P<0.001 and t-value= -3.68, P<0.01, respectively).

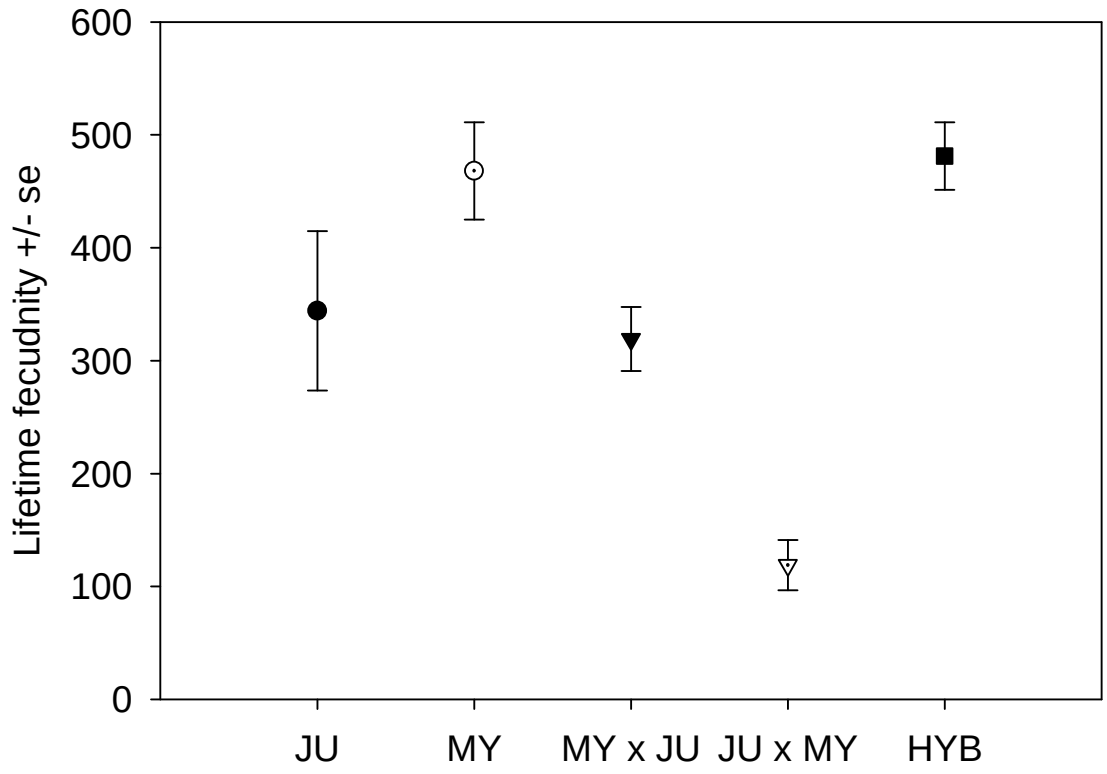

Figure 4.1. Lifetime fecundity of three population of *C. remanei* used in this study. Parental strains (JU and MY, filled and opened circle, respectively); MY female and JU male cross, and the reciprocal (filled and opened triangle, respectively); and the F1 of these crosses (HYB, filled squared).

4.4.2 Plasticity

4.4.2.1 Life Expectancy (LE)

The results suggested that high temperatures negatively affected *LE* (M2 vs. M1: χ^2= 91.24, 1 d.f., P<0.001, Table 4.2A; Figure 4.2A). However, the decrease was not linear, a quadratic term added to the model has a significant effect (M3 vs. M2: χ^2= 14.219, 1 d.f., P<0.001, Table 4.2A). The average *LE* was similar across strains (M4 vs. M3: χ^2= 2.6079, 2 d.f., P=0.27, Table 4.2A), though I found that the average *LE* across temperatures varied between strains (M5 vs. M3: χ^2= 11.064, 4 d.f., P<0.05, Table 4.2A). However, I did not find evidence that strains had different optimum temperatures (there was no significant interaction between quadratic terms and strains (M6 vs. M5: χ^2= 4.0894, 2 d.f., P=0.13, Table 4.2A).

Regarding the variance components, there was not any statistical support for a random effect of replicate on the slope governing the reduction in *LE* with temperature (M7 vs. M5: χ^2= 0.02, 2 d.f., P=0.90, Table 4.2A) or a quadratic deviance at the replicate level with regard temperature (M8 vs. M5: χ^2= 0.01, 3 d.f., P=0.98, Table 4.2A). The most appropriate model contained the fixed effects of temperature (as a quadratic), strain, and its interaction with temperature and a random effect to describe the hierarchical structure of the data (M5; Table 4.3A, Figure 4.2A).

4.4.2.2 Lifetime fecundity (LF)

I found that extreme temperatures decreased *LF* (M3 vs. M1: χ^2= 116.03, 1 d.f., P<0.001; Figure 4.2B; Table 4.2B). The average *LF* was significantly different between strains (M4 vs. M3: χ^2= 15.84, 2 d.f., P<0.001; Table 4.2B); while the interaction between strain and temperature was not significant (M5 vs. M4: χ^2= 1.28, 2 d.f., P=0.53; Table 4.2B), the interaction between the quadratic term and strains was (M6 vs. M4: χ^2= 8.65, 2 d.f., P<0.05, Table 4.2B). This suggested that strains had different optimum temperatures for *LF*.

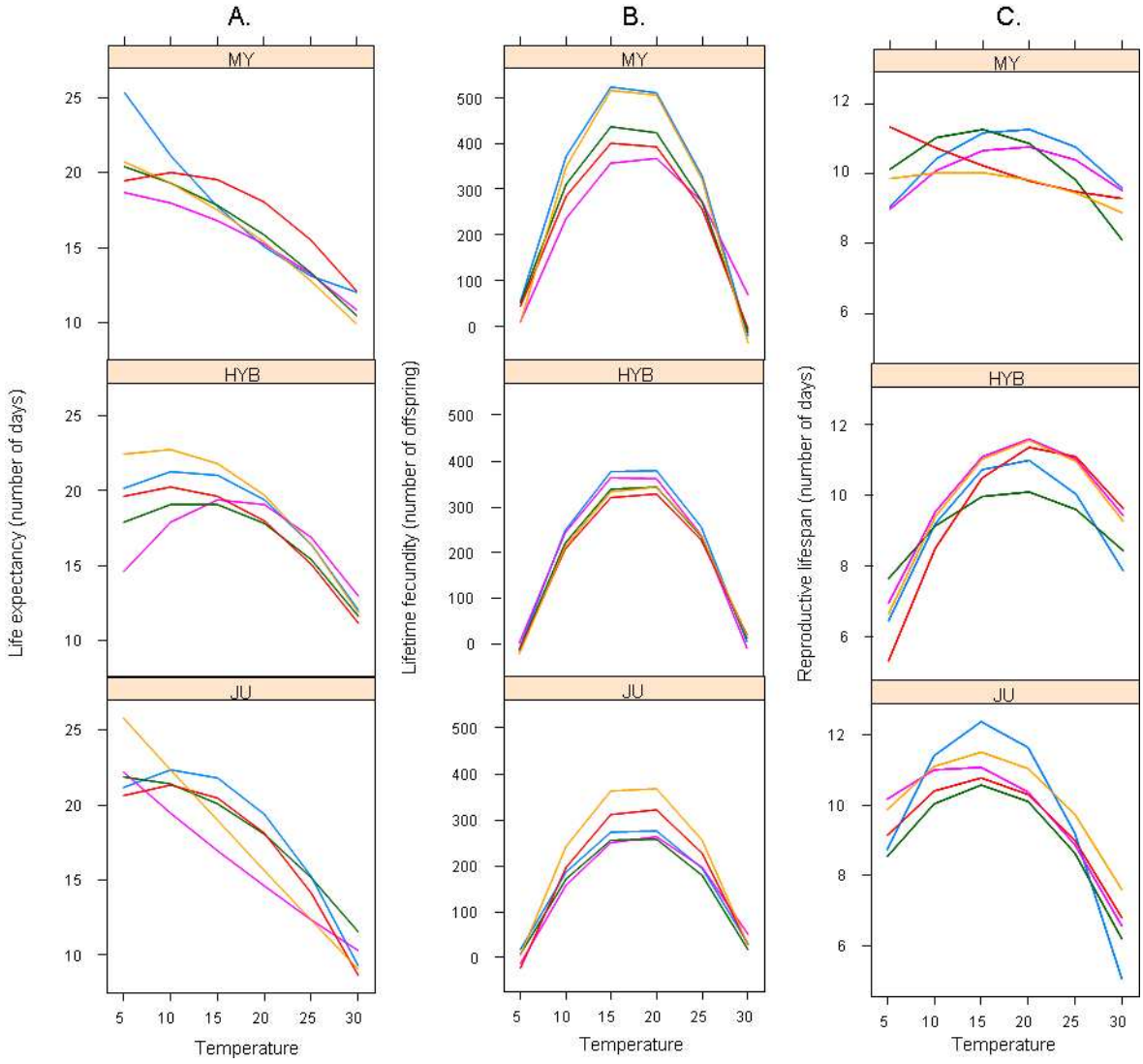

Figure 4.2. Life-history traits of three populations of *C. remanei* cultured in six temperatures. Each plot represents A) *LE*, B) *LF* and C) *RL*, and the subplot (rows) represents the strain MY, HYB and JU. Lines represent the replicate response to temperature (predicted from the preferred model –see Table 4.2).

Regarding the variance components, while I found no support for a random effect of replicate on the temperature slope (M7 vs. M6 χ^2= 0.01, 2 d.f., P=0.99, Table 4.2B), governing the relationship between temperature and *LF*, I found evidence for a random effect of the replicate on the quadratic deviance of the temperature and *LF* (M8 vs. M6: χ^2= 18.72, 5 d.f., P<0.01, Table 4.2B). Therefore, the preferred model contains the fixed terms for temperature (linear and quadratic), strain, and its interaction with temperature in both the linear and quadratic terms; and the random terms for the linear and quadratic deviance at the replicate level. (M8, Table 4.3B, Figure 4.2B).

4.4.2.3 Reproductive lifespan (RL)

I found evidence that the overall *RL* decreased with increasing temperature (M2 vs. M1: χ^2= 0.49, 1 d.f., P=0.48; Table 4.2C, Figure 4.2C). However, the rate of reduction was not linear (M3: vs. M2: χ^2= 27.78, 2 d.f., P<0.001, Table 4.2C). The observations suggested that extreme temperatures reduce the *RL* disproportionately. Moreover, I found evidence to suggest that, although the average *RL* across temperatures was similar (M4 vs. M3: χ^2= 3.35, 2 d.f., P=0.18, Table 4.2C), the relative rate of response varied significantly between strains (M6 vs. M3: χ^2= 22.07, 4 d.f., P<0.001, Table 4.2C). Thus, suggesting that strains had different optimum temperatures for *RL*. For example, the average *RL* of MY did not vary with temperature, whereas the other two strains showed significant variation in *RL* across temperature.

Regarding the variance components, I found evidence of a random effect of replicate on the slope of changing temperature (M7 vs. M6: χ^2= 6.3244, 2 d.f., P<0.05, Table 4.2B). Therefore, the preferred model contained the fixed terms for temperature (as a quadratic), strain, and a strain temperature interaction (with both linear and quadratic terms); and the random terms for the deviance of the replicate with respect to temperature (M7 Table 4.3C, Figure 4.2C).

Sub-	Model	Syntax	AIC	logLik	d.f.
A.	*LE*				
	M0	1 + (1|replicate$_{lk}$) + (1| replicate$_{lkj}$)	2950.3	-1471.2	4
	M1	1 + (1| replicate$_{lkj}$)	2948.3	-1471.2	3
	M2	Temp + (1| replicate$_{lkj}$)	2859.1	-1425.5	4
	M3	Temp + Temp^2 + (1| replicate$_{lkj}$)	2846.8	-1418.4	5
	M4	Temp + Temp^2 + Strain$_k$ + (1| replicate$_{lkj}$)	2848.2	-1417.1	7
	M5	**Temp + Temp^2 + Temp *strain + (1| replicate$_{lkj}$)**	**2843.8**	**-1412.9**	**9**
	M6	Temp * Strain$_k$ + Temp^2* Strain$_k$ + (1| replicate$_{lkj}$)	2843.7	-1410.8	11
	M7	Temp + Temp^2 + Temp * Strain$_k$ + (temp | replicate$_{lkj:k}$)	2847.8	-1412.9	11
	M8	Temp + Temp^2 + Temp * Strain$_k$ + (temp + temp^2 | replicate$_{lkj:k}$)	2853.6	-1412.8	14
B.	*LF*				
	M0	1 + (1|replicate$_{lk}$) + (1|replicate$_{l:k:j}$)	5783.4	-2887.7	4
	M1	1 + (1| replicate$_{lkj}$)	5781.4	-2887.7	3
	M2	Temp + (1| replicate$_{lkj}$)	5785.4	-2887.7	5
	M3	Temp + Temp^2 + (1| replicate$_{lkj}$)	5669.5	-2829.7	5
	M4	Temp + Temp^2 + Strain$_k$ + (1| replicate$_{lkj}$)	5657.7	-2821.8	7
	M5	Temp + Temp^2 + Temp * Strain$_k$ + (1| replicate$_{lkj}$)	5660.5	-2821.3	9
	M6	Temp * Strain$_k$ + Temp^2* Strain$_k$ + (1| replicate$_{lkj}$)	5656.0	-2817.0	11
	M7	Temp* Strain$_k$ + Temp^2* Strain$_k$ + (temp | replicate$_{lkj:k}$)	5659.6	-2816.8	13
	M8	**Temp *** Strain$_k$ **+ Temp^2*** Strain$_k$ **+ (temp +temp ^2 | replicate$_{lkj:k}$)**	**5646.9**	**-2807.5**	**16**
C.	*RL*				
	M0	1 + (1|replicate$_{lk}$) + (1| replicate$_{lkj}$)	2209.9	-1101.0	4
	M1	1 + (1| replicate$_{lkj}$)	2207.9	-1101.0	3
	M2	Temp + (1| replicate$_{lkj}$)	2209.5	-1100.7	4
	M3	Temp + Temp^2 + (1| replicate$_{lkj}$)	2184.2	-1087.1	5
	M4	Temp + Temp^2 + Strain$_k$ + (1| replicate$_{lkj}$)	2184.8	-1085.4	7
	M5	Temp + Temp^2 + Temp * Strain$_k$ + (1| replicate$_{lkj}$)	2170.1	-1076.0	9
	M6	Temp * Strain$_k$ + Temp^2* Strain$_k$ + (1| replicate$_{lkj}$)	2167.8	-1072.9	11
	M7	**Temp *** Strain$_k$ **+ Temp^2 *** Strain$_k$ **+ (temp | replicate$_{lkj}$)**	**2160.6**	**-1067.3**	**13**

	M8	Temp * $Strain_k$ + Temp^2 * $Strain_k$ + (temp + temp^2| $replicate_{lkj}$)	2166.4	-1067.2	16
D.	m_i				
	M1	1 + (1| ind_{mlkj}) + (1| $replicate_{lk}$) + (1| $replicate_{lkj}$)	43138	-21564	5
	M2	1 + (1| ind_{mlkj}) + (1|$replicate_{lkj}$)	43137	-21564	4
	M3	1 + (1| ind_{mlkj})	43135	-21564	3
	M4	Age_i + (1| ind_{mlkj})	41451	-20715	11
	M5	Age_i + Temp + (1| ind_{mlkj})	41453	-20715	12
	M6	Age_i * Temp + (1| ind_{mlkj})	41466	-20713	20
	M7	Age_i + Temp +Temp ^2+(1| ind_{mlkj})	41118	-20546	13
	M8	Age_i * Temp + Temp ^2+ Age_i:Temp^2 + (1| ind_{mlkj})	39741	-19842	29
	M9	Age_i * Temp + Temp ^2+ Age_i:Temp^2 + $Strain_k$ + (1| ind_{mlkj})	39713	-19826	31
	M10	Age_i * Temp + Temp ^2+ Age_i:Temp^2 + $Strain_k$ * Age_i + (1| ind_{mlkj})	39666	-19786	47
	M11	Age_i * Temp + Temp ^2+ Age_i:Temp^2 + $Strain_k$ * Age_i + Temp * $Strain_k$ + (1| ind_{mlkj})	39668	-19785	49
	M12	Age_i * Temp + Temp ^2+ Age_i:Temp^2 + $Strain_k$ * Age_i + Temp * $Strain_k$ + ITemp^2* $Strain_k$ +	39656	-19777	51
	M13	**Age_i * Temp +Temp ^2+ Age_i:Temp^2 + $Strain_k$ * Age_i + Temp * $Strain_k$ +Temp^2* $Strain_k$**	**26040**	**-12925**	**95**
E.	l_i				
	M1	1	-1481.40	2966.80	2
	M2	factor($Temp_j$)	-1379.50	2773.00	7
	M3	factor($Temp_j$) + $Strain_k$	-1376.90	2771.80	9
	M4	**factor($Temp_j$)* $Strain_k$**	**-1366.00**	**2770.00**	**19**

Table 4.2. AIC and log likelihood (logLik) values for vital rates models. Bold letters correspond to the preferred model for each trait (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.

Sub-table	Model			
A.	M5. *LE*			
	Fixed effects:	Estimate	Std. Error	t value
	(Intercept)	22.33	1.43	15.58
	Temp	0.07	0.16	0.43
	Temp^2	-0.02	0.00	-3.85
	StrainHYB	-3.69	1.47	-2.51
	StrainMY	-2.71	1.47	-1.84
	Temp:StrainHYB	0.22	0.08	2.92
	Temp:StrainMY	0.11	0.08	1.43
	Random effects:			
	Groups Name	Variance	Std.Dev.	% of variance
	replicate(Intercept)	0.000	0.00	0
	Residual	31.23	5.59	100
B.	M8. *LF*			
	Fixed effects:	Estimate	Std. Error	t value
	(Intercept)	-265.36	57.08	-4.65
	Temp	64.12	7.85	8.17
	StrainHYB	-48.36	80.72	-0.60
	StrainMY	-71.37	80.72	-0.88
	Temp^2	-1.81	0.22	-8.41
	Temp:StrainHYB	10.02	11.10	0.90
	Temp:StrainMY	25.79	11.10	2.33
	StrainHYB:Temp^2	-0.29	0.30	-0.97
	StrainMY:Temp^2	-0.79	0.30	-2.60
	Random effects:			
	Groups Name	Variance	Std.Dev.	% of variance
	replicate(Intercept)	0.00	0.00	0.00
	temp	118.02	10.86	0.90
	temp^2	0.12	0.35	0.00
	Residual	13025.00	114.12	99.10
C.	M7. *RL*			
	Fixed effects:	Estimate	Std. Error	t value
	(Intercept)	7.20	1.50	4.81
	Temp	0.55	0.17	3.22
	StrainHYB	-5.20	2.12	-2.46
	StrainMY	-0.57	2.12	-0.27
	Temp^2	-0.02	0.00	-4.32
	Temp:StrainHYB	0.38	0.24	1.60
	Temp:StrainMY	-0.01	0.24	-0.03
	StrainHYB:Temp^2	0.01	0.01	-0.77
	StrainMY:Temp^2	0.01	0.01	0.50
	Random effects:			
	Groups Name	Variance	Std.Dev.	% of variance
	replicate(Intercept)	5.22	2.28	43.77
	temp	0.01	0.08	1.53
	Residual	5.98	2.45	46.86

Table 4.3. Descriptive statistics of the response of the life-history traits of *C. remanei* (LE, LF and RL). These are the preferred models to describe the phenotypic variance across temperature gradients, between strains, between replicates and between individuals assayed in this study. (Note that since the replicate effect was not significant, thus, it is not included in these models). Model syntax and AIC values can be seen in Table 4.2.

4.4.2.4 Life Expectancy (LE) and Lifetime Fecundity (LF) correlation

I found evidence that individuals who produced more offspring had shorter life expectancy (M1 vs. M0: χ^2= 6.51, 1 d.f., P<0.05, Table 4.4A; Figure 4.3). Although a rise in temperatures increased the strength of this negative relationship, adding a temperature-*LF* interaction term did not improve the model (M2 vs. M1: χ^2= 3.36, 1, P= 0.06, Table 4.4A). However, I found that the rate of decline of *LE* as *LF* increased varied across temperatures (M3 vs. M1: χ^2= 14.39, 3, P<0.05, Table 4.4A), suggesting that extreme temperatures force a steeper trade-off. Strains did not show differences in the *LE*-*LF* trade-off across temperatures (M4 vs. M3: χ^2=1.37, 2, P=0.50, Table 4.4A). The preferred model contains terms for temperature (both linear and as a quadratic); strain, and a strain temperature interaction (with linear term); *LF*, and a *LF*-temperature interaction (with both linear and quadratic term) and a random term to describing the hierarchical structure of the data (M3, Table 4.5A).

4.4.2.5 Life Expectancy (LE) and Reproductive Lifespan (RL) correlation

I found little evidence of a relationship between *LE* and *RL* (M1 vs. M0: χ^2= 2.71, 1 d.f., P=0.09, Table 4.4B; Figure 4.4). I found neither a linear (M2 vs. M1: χ^2= 0.21, 1 d.f., P=0.65, Table 4.4B) or non-linear (M3 vs. M1: χ^2= 0.21, 2 d.f., P=0.90, Table 4.4B) effect of temperature on this trade-off. Moreover, the trade-off did not vary between strains (M4 vs. M1: χ^2= 0.50, 2 d.f., P=0.78, Table 4.4B). The preferred model simply contained terms for temperature (both linear and as a quadratic); strain, and a strain temperature interaction (with linear term); *RL*, a *RL*-temperature interaction (both linear and quadratic terms) and the random effect to describe the hierarchical structure in the data (M1, Table 4.5B).

4.4.2.6 Reproductive lifespan (RL) and lifetime fecundity (LF) correlation

I found evidence that worms that produced more offspring had longer reproductive lifespan (M1 vs. M0: χ^2= 50.94, 1, P<0.001, Table 4.4C, Figure 4.5). Inclusion of temperature-*LF* improved the model (M2 vs. M1: χ^2= 9.42, 1, P<0.01, Table 4.4C). Moreover, the relationship was not linear, extreme temperatures had a more positive effect on the *RL*-*LF* trade-off (M3 vs. M2: χ^2= 32.52, 1, P<0.001, Table 4.4C). However, I did not detect differences in the response between strains (M4 vs. M3: χ^2= 2.87, 2, P= 0.24, Table 4.4C).

The preferred model contained terms for temperature (both linear and as a quadratic); strain, and a strain temperature interaction (with both linear and quadratic terms); *LF*, and a *LF*-temperature interaction (both linear and quadratic terms) and a random term to describe the deviance of the replicate and its deviance on the relationship between *RL* and changing temperature (M3, Table 4.5C).

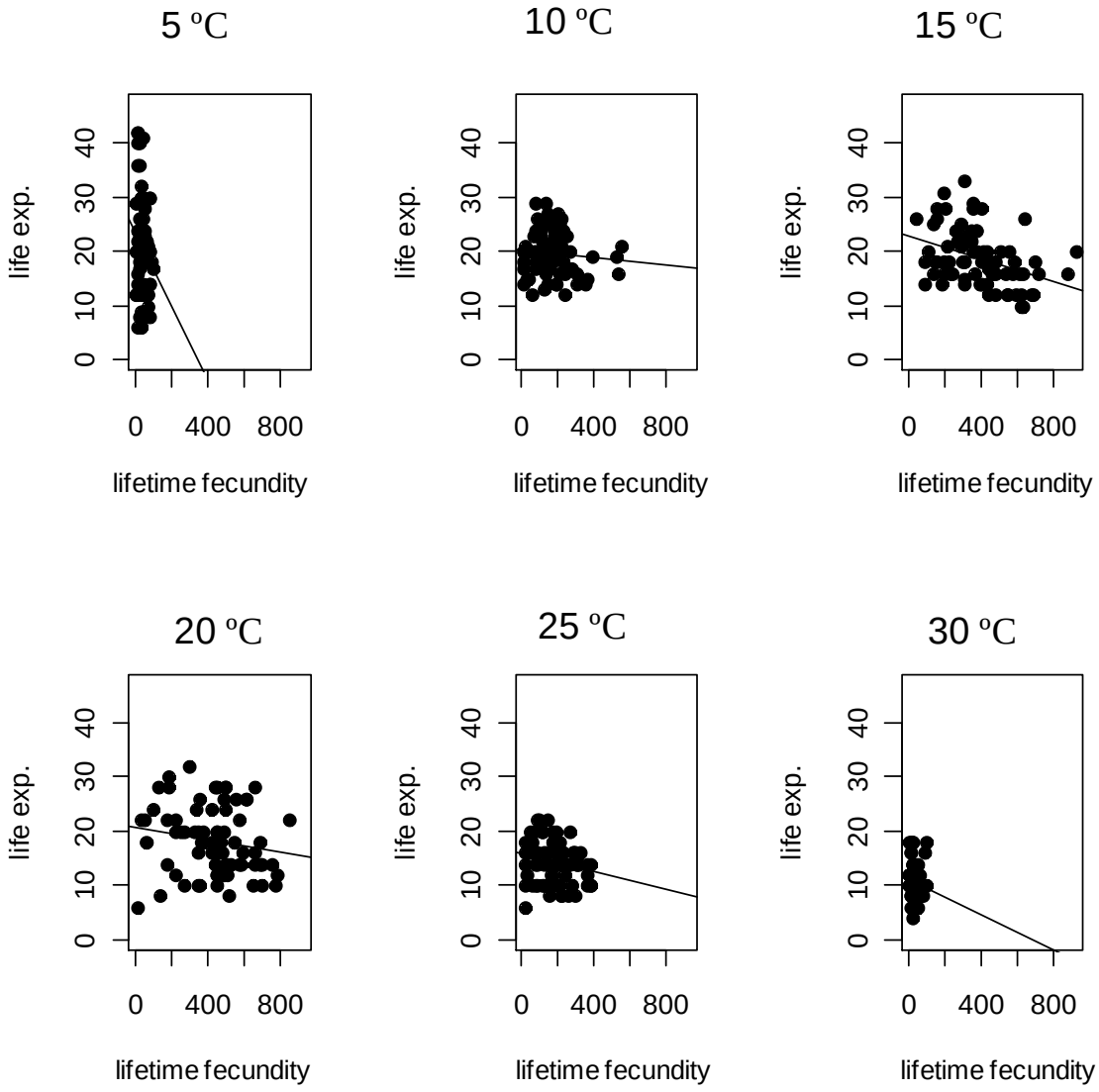

Figure 4.3. LE and LF correlation of *C. remanei* at different temperatures. Filled circles represent observations for each individual. The line represents a regression model for each temperature.

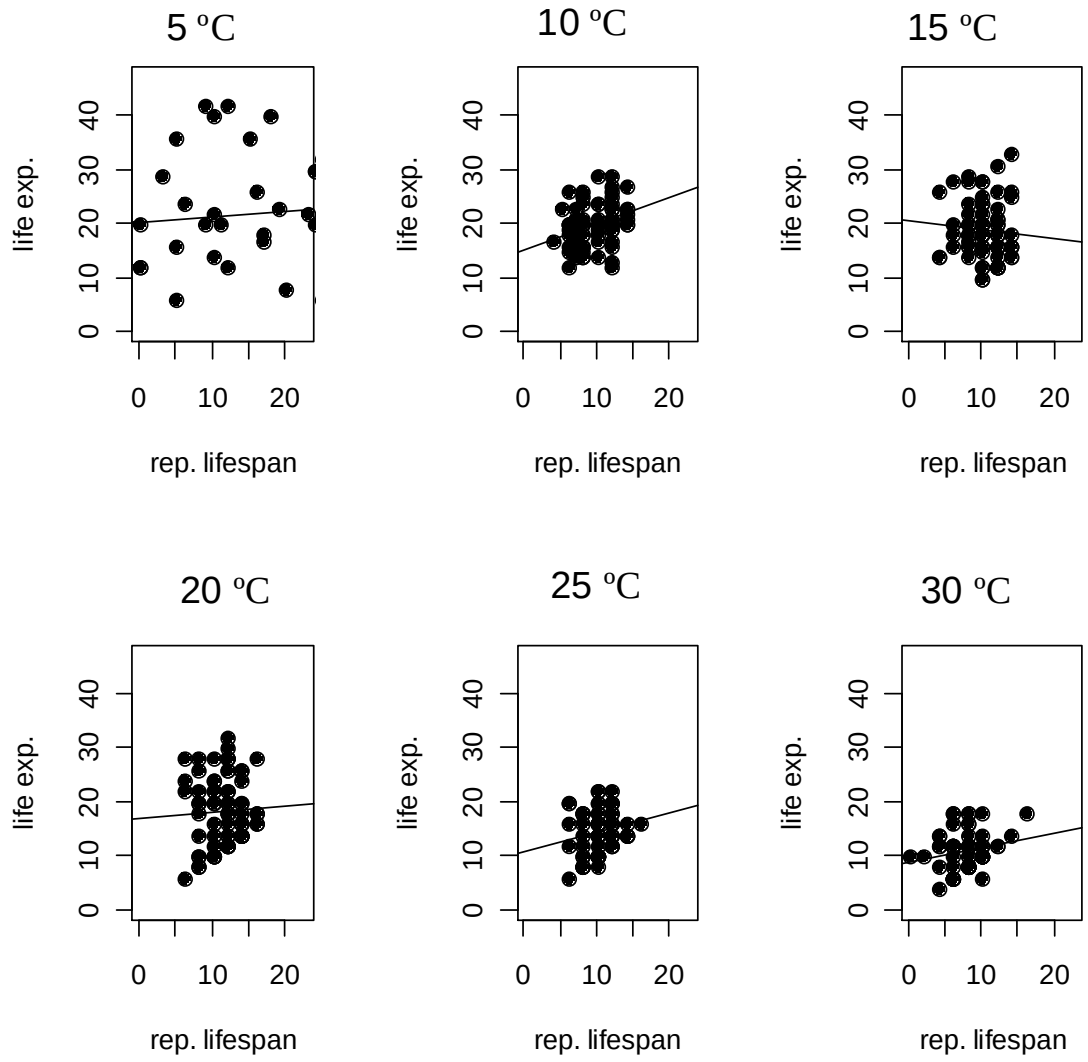

Figure 4.4. As Fig. 4.3, but showing *LE* and *RL* correlation.

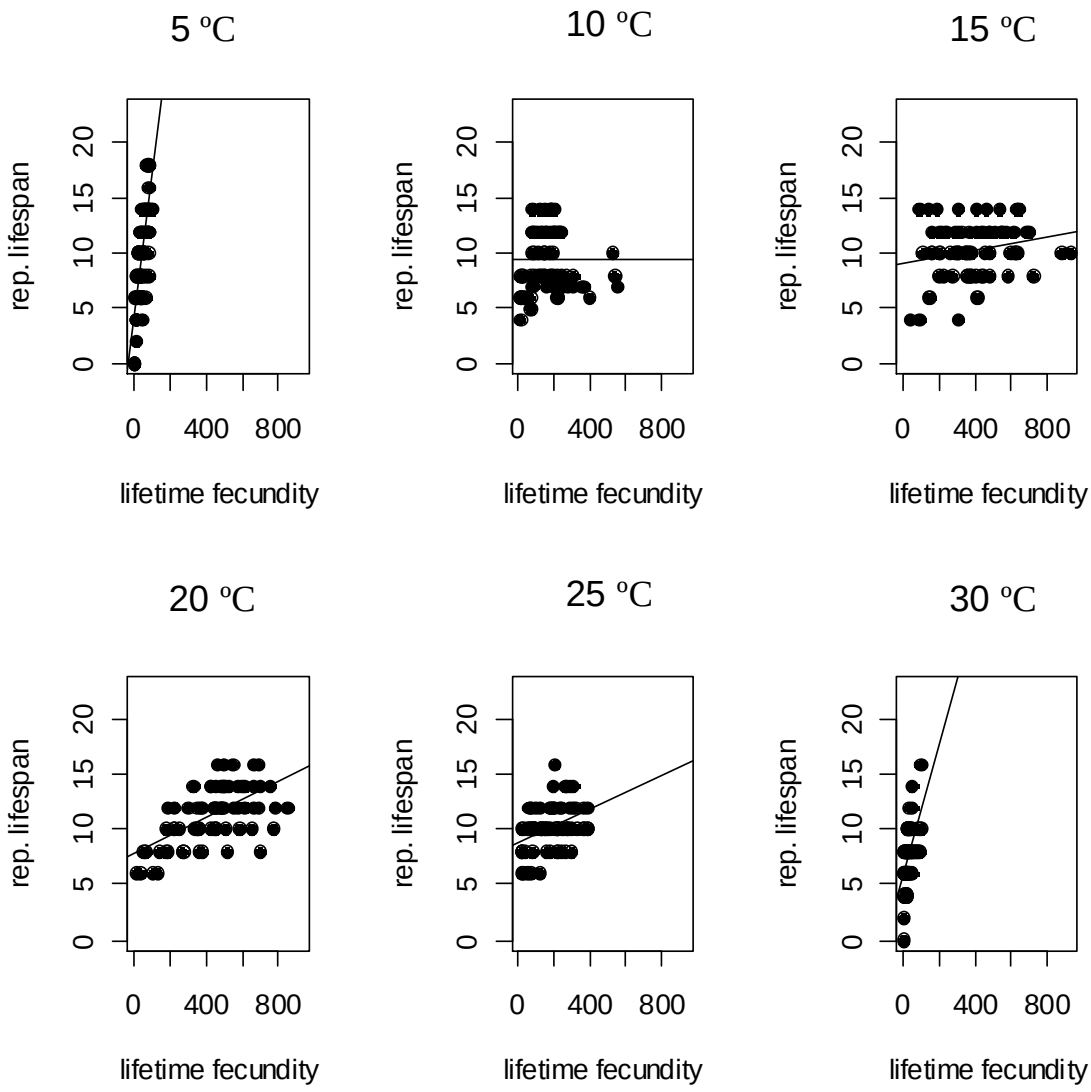

Figure 4.5. As Fig. 4.3, but showing *RL* and *LF* correlation.

Subtable	Model	Syntax	AIC	logLik	Df
A.		*LE~LF*			
	M0	Temp + Temp^2 + Temp* $Strain_k$ + (1|$replicate_{lkj}$)	2843.8	-1412.9	9
	M1	Temp + Temp^2 + Temp* $Strain_k$ + LF + (1|$replicate_{lkj}$)	2839.3	-1409.6	10
	M2	Temp + Temp^2 + Temp* $Strain_k$ + LF + Temp:LF + (1|$replicate_{lkj}$)	2837.9	-1408.0	11
	M3	**Temp+ Temp^2 + Temp* $Strain_k$ + LF + Temp:LF + Temp^2:LF + (1| $replicate_{lkj}$)**	**2835.4**	**-1405.7**	**12**
	M4	Temp + Temp^2 + Temp* $Strain_k$ + LF + Temp:LF + Temp^2:LF + $Strain_k$:LF + (1|$replicate_{lkj}$)	2838.0	-1405.0	14
B.		*LE~RL*			
	M0	Temp +Temp^2 + Temp * $Strain_k$ + (1|$replicate_{lkj}$)	2843.8	-1412.9	9
	M1	**Temp+Temp^2 + Temp* $Strain_k$ + RL + (1|$replicate_{l:k}$)**	**2843.1**	**-1411.5**	**10**
	M2	Temp +Temp^2 + Temp * $Strain_k$ + RL + Temp:RL + (1|$replicate_{lkj}$)	2844.9	-1411.4	11
	M3	Temp +Temp^2 + Temp * $Strain_k$ + RL + Temp :RL +Temp^2:RL + (1|$replicate_{lkj}$)	2846.9	-1411.4	12
	M4	Temp +Temp^2 + Temp * $Strain_k$ + RL + $Strain_k$:RL + (1|$replicate_{lkj}$)	2850.3	-1411.2	14
C.		*RL~LF*			
	M0	Temp * $Strain_k$ + Temp^2* $Strain_k$ + (temp | $replicate_{lkj}$)	2160.8	-1067.4	13
	M1	Temp * $Strain_k$ + Temp^2* $Strain_k$ + LF + (temp | $replicate_{lkj}$)	2111.9	-1042.0	14
	M2	Temp * $Strain_k$ + Temp^2* $Strain_k$ + LF + Temp:LF + (temp| $replicate_{lkj}$)	2104.5	-1037.2	15
	M3	**Temp * $Strain_k$ +Temp 2* $Strain_k$ + LF + Temp:LF + Temp^2:LF + (temp| $replicate_{lki}$)**	**2073.9**	**-1021.0**	**16**
	M4	Temp * $Strain_k$ +Temp^2* $Strain_k$ + LF + Temp:LF + Temp^2:LF + $Strain_k$:LF + (temp | $replicate_{lkj}$)	2075.1	-1019.5	18

Table 4.4. AIC and Log Likelihood values for correlations across vital rates. Bold letters correspond to the preferred model (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.

Sub-table	Model			
A.	M3 *LE~LF*			
	Fixed effects:	Estimate	Std. Error	t value
	(Intercept)	22.00	1.72	12.77
	Temp	0.36	0.23	1.55
	Temp^2	-0.03	0.01	-3.99
	StrainHYB	-3.63	1.45	-2.50
	StrainMY	-1.06	1.51	-0.70
	LF	-0.05	0.02	-2.86
	Temp:StrainHYB	0.22	0.07	2.95
	Temp:StrainMY	0.06	0.08	0.76
	Temp:LF	4.67E-03	1.96E-03	2.39
	Temp^2:LF	-1.17E-04	5.49E-05	-2.13
	Random effects:			
	Groups Name	Variance	Std.Dev.	% of variance
	replicate(Intercept)	0.00	0.00	0.00
	Residual	30.26	5.50	83.49
B.	M1 *LE~RL*			
	Fixed effects:	Estimate	Std. Error	t value
	(Intercept)	21.08	1.62	13.03
	Temp	-0.01	0.16	-0.04
	Temp^2	-0.01	0.00	-3.03
	StrainHYB	-3.06	1.52	-2.02
	StrainMY	-2.56	1.47	-1.74
	RL	0.16	0.10	1.65
	Temp:StrainHYB	0.19	0.08	2.40
	Temp:StrainMY	0.10	0.08	1.26
	Random effects:			
	Groups Name	Variance	Std.Dev.	% of variance
	replicate (Intercept)	0.00	0.00	0.00
	Residual	31.05	5.57	100.00
C.	M3 *RL~LF*			
	Fixed effects:	Estimate	Std. Error	t value
	(Intercept)	9.16	1.61	5.69
	Temp	0.09	0.18	0.47
	StrainHYB	-5.56	2.16	-2.58
	StrainMY	-2.13	2.18	-0.98
	Temp^2	-0.01	4.68E-03	-1.52
	LF	0.05	0.01	5.53
	Temp:StrainHYB	0.41	0.24	1.73
	Temp:StrainMY	0.06	0.24	0.24
	StrainHYB:Temp^2	-0.01	0.01	-0.83
	StrainMY:Temp^2	2.40E-03	0.01	0.39
	Temp:LF	-0.01	9.24E-04	-5.43
	Temp^2:LF	1.51E-04	2.54E-05	5.97
	Random effects			
	Groups Name	Variance	Std.Dev.	% of Variance
	replicate (Intercept)	6.28	2.51	57.16
	temp	0.01	0.09	0.07
	Residual	4.70	2.17	42.77

Table 4.5. 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 4.4. (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 4.4.

4.4.2.7 Age-specific fecundity (m_i)

Given the number of observations of the *m*th worm and the hierarchical structure within the data (*m*th individual nested within the *l*th replicate within the *k*th strain at the *j*th temperature), M3 was the starting model in this analysis (Table 4.2D). I used the random effect (1| ind_{mlkj}), describing 4050 observations of 450 individuals (temp:strain:replicate:ind), to quantify the effects of age, temperature and strain on the age-specific fecundity. The number of offspring declined as females aged (M4 vs. M3: χ^2= 1699.1, 8 d.f., P<0.001, Table 4.2D, Figure 4.6). In common with *LF*, I found that extreme temperatures had a negative effect on the overall age-specific fecundity of individuals (M7 vs. M4: χ^2= 337.88, 2 d.f., P<0.001 Table 4.2D). Moreover, the effect of extreme temperatures varied with individual age (M8 vs. M7: χ^2= 1408.2, 16 d.f., P<0.001, Table 4.2D). For instance, the age-specific fecundity of individuals at age 6 was relatively more sensitive to extreme temperatures. As with *LF*, I found evidence that age-specific fecundity differed between strains (M9 vs. M8: χ^2= 32.18, 2 d.f., P<0.001, Table 4.2D); MY producing relatively more offspring. Moreover, MY produced relatively more offspring at ages 2 and 4 compared to the other two strains (M10 vs. M9: χ^2= 81.25, 18 d.f., P<0.001, Table 4.2D). Although I did not detect a significant interaction between temperature and strain (M11 vs. M10: 2.46, 2 d.f., P=0.30, Table 4.2D), I found a significant interaction between strain and the quadratic term of temperature (M12 vs. M10: χ^2=16.02, 2 d.f., P<0.001, Table 4.2D). For instance, MY produced significantly more offspring at intermediate temperatures compared to the other two strains. In addition, I found significant differences in the age-specific variance between individuals (M13 vs. M12: χ^2= 13704, 44 d.f., P<0.001, Table 4.2D). For instance, the age-specific variance was greater at ages (days) 0, 2 and 6 compared to the other ages (36.12, 33.48 and 22.05 % of the variance). The preferred model contained terms for age, temperature (with both linear quadratic terms); and age-temperature interaction (with both linear quadratic terms); strain, strain-temperature interaction (with both linear and quadratic terms), and strain-age interaction, and a random effect for age (Model 13, Table 4.2D; see Table 4.6).

A) Fixed factors

Parameter	Estimate	.Std. Error.	t value
intercept	-114.70	9.50	-12.08
Age2	-32.99	10.28	-3.21
Age4	34.03	12.84	2.65
Age6	85.25	11.39	7.49
Age8	106.20	10.17	10.44
Age10	113.40	9.67	11.73
Age12	114.30	9.56	11.96
Age14	114.50	9.55	12.00
Age16	114.60	9.50	12.06
Temp	23.15	1.18	19.58
I(Temp^2)	-0.65	0.03	-19.77
StrainHYB	9.98	5.07	1.97
StrainMY	25.10	5.07	4.95
Age2:Temp	8.94	1.28	6.98
Age4:Temp	-5.26	1.60	-3.29
Age6:Temp	-16.44	1.42	-11.59
Age8:Temp	-21.19	1.27	-16.73
Age10:Temp	-22.76	1.20	-18.90
Age12:Temp	-23.05	1.19	-19.36
Age14:Temp	-23.14	1.19	-19.46
Age16:Temp	-23.15	1.18	-19.57
Age2:I(Temp^2)	-0.26	0.04	-7.16
Age4:I(Temp^2)	0.14	0.04	3.18
Age6:I(Temp^2)	0.46	0.04	11.58
Age8:I(Temp^2)	0.60	0.04	16.84
Age10:I(Temp^2)	0.64	0.03	19.06
Age12:I(Temp^2)	0.65	0.03	19.54
Age14:I(Temp^2)	0.65	0.03	19.66
Age16:I(Temp^2)	0.65	0.03	19.76
Age2:StrainHYB	-5.67	5.47	-1.04
Age4:StrainHYB	-8.77	6.83	-1.28
Age6:StrainHYB	-8.83	6.06	-1.46
Age8:StrainHYB	-9.88	5.41	-1.83
Age10:StrainHYB	-10.04	5.15	-1.95
Age12:StrainHYB	-10.23	5.09	-2.01
Age14:StrainHYB	-10.14	5.08	-2.00
Age16:StrainHYB	-10.18	5.05	-2.02
Age2:StrainMY	-0.43	5.47	-0.08
Age4:StrainMY	-4.61	6.83	-0.67
Age6:StrainMY	-14.25	6.06	-2.35
Age8:StrainMY	-20.18	5.41	-3.73
Age10:StrainMY	-23.25	5.15	-4.52
Age12:StrainMY	-23.99	5.09	-4.72
Age14:StrainMY	-24.23	5.08	-4.77
Age16:StrainMY	-24.36	5.05	-4.82
Temp:StrainHYB	0.03	0.06	0.59
Temp:StrainMY	-0.06	0.06	-1.12
I(Temp^2):StrainHYB	0.00	0.00	-0.64
I(Temp^2):StrainMY	0.00	0.00	0.66

B) Random factors

Groups	Name	Variance	% Variance
ind1	age0	1822.70	36.12
	age2	1689.20	33.48
	age4	1112.60	22.05
	age6	339.03	6.72
	age8	70.58	1.40
	age10	8.55	0.17
	age12	1.53	0.03
	age14	0.07	0.00
	age16	0.02	0.00
Residual		1.80	0.04

Table 4.6.Descriptive statistics of the effect of temperature on *C. remanei* age-specific fecundity. The table contains the a) fixed factors and b) random factors of the preferred model (Model syntax and AIC values can be seen in Table 4.2). Number of observations: 3101, number of individuals: 450.

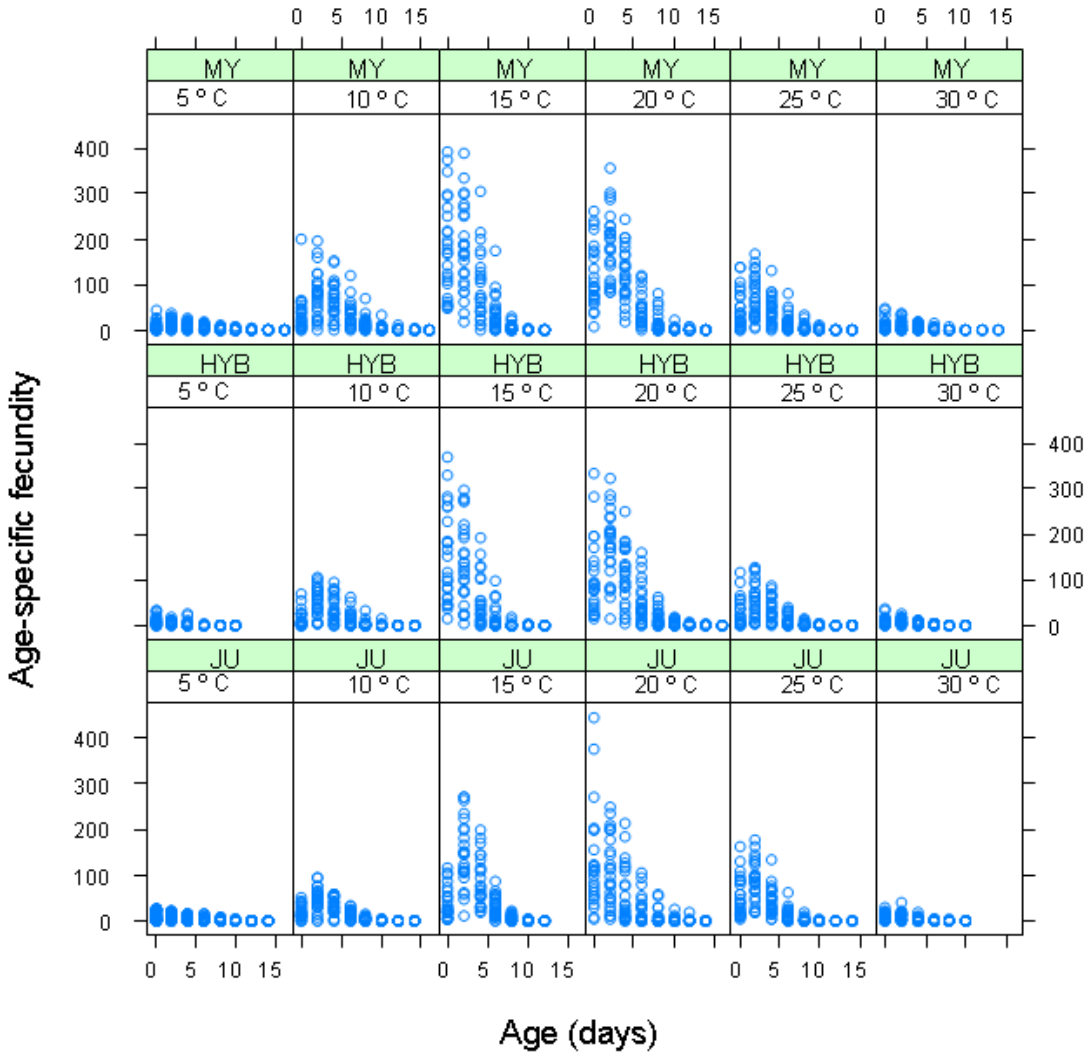

Figure 4.6. Age-specific fecundity of individuals of *C. remanei* in response to temperature. The Figure represents the three strains (MY, HYB and JU –from the top to the bottom) cultured at 5, 10 15 a 20 25 and 30 ° C. Circles represent single observations.

4.4.2.8 Survival (l_i)

I found a significant difference in survival rate among individuals maintained at different temperatures. (M2 vs. M1: χ^2= 203.75, 5 d.f., P<0.001; Table 4.2E; Figure 4.7). I found that strains responded differently to temperature (M4 vs. M2: χ^2= 226.96, 12 d.f., P<0.01; Table 4.2E; Table 4.7). Moreover, the analysis suggested that the probability of dying was not constant across time (log-likelihood of Exponential model = 3431.41), instead, the mortality rate increased with age (Weibull model =2732.02; Exponential vs. Weibull: χ^2= 699.32, d.f. 1, P<0.001). The preferred model included the fixed terms of temperature (as a categorical variable), strain, the interaction term between them (M4; Table 4.2E and Table 4.7).

Parameter	Estimate	Std. Error	z-value	P-value
(Intercept)	3.29	0.06	58.88	<0.001
Temp10	-0.22	0.08	-2.81	<0.01
Temp15	-0.15	0.08	-1.97	<0.05
Temp20	-0.32	0.08	-4.07	<0.001
Temp25	-0.63	0.08	-8.07	<0.001
Temp30	-0.86	0.08	-11.00	<0.001
StrainHYB	-0.13	0.08	-1.62	0.11
StrainMY	-0.03	0.08	-0.32	0.75
Temp10:StrainHYB	0.11	0.11	1.00	0.32
Temp15:StrainHYB	-0.01	0.11	-0.08	0.94
Temp20:StrainHYB	0.25	0.11	2.24	<0.05
Temp25:StrainHYB	0.29	0.11	2.63	<0.001
Temp30:StrainHYB	0.24	0.11	2.18	<0.05
Temp10:StrainMY	-0.11	0.11	-1.00	0.32
Temp15:StrainMY	-0.25	0.11	-2.24	<0.05
Temp20:StrainMY	0.05	0.11	0.45	0.65
Temp25:StrainMY	0.09	0.11	0.78	0.43
Temp30:StrainMY	0.06	0.11	0.57	0.57
Log(scale)	-1.28	0.04	-35.30	<0.001

Table 4.7. Survival analysis (Weibull model). Results of the preferred -survival model to describe the survival rate of individuals from three strains cultured across a range of temperatures. Note that temperature is used as a categorical variable. The model syntax can be seen in Table 4.2E, Model 41).

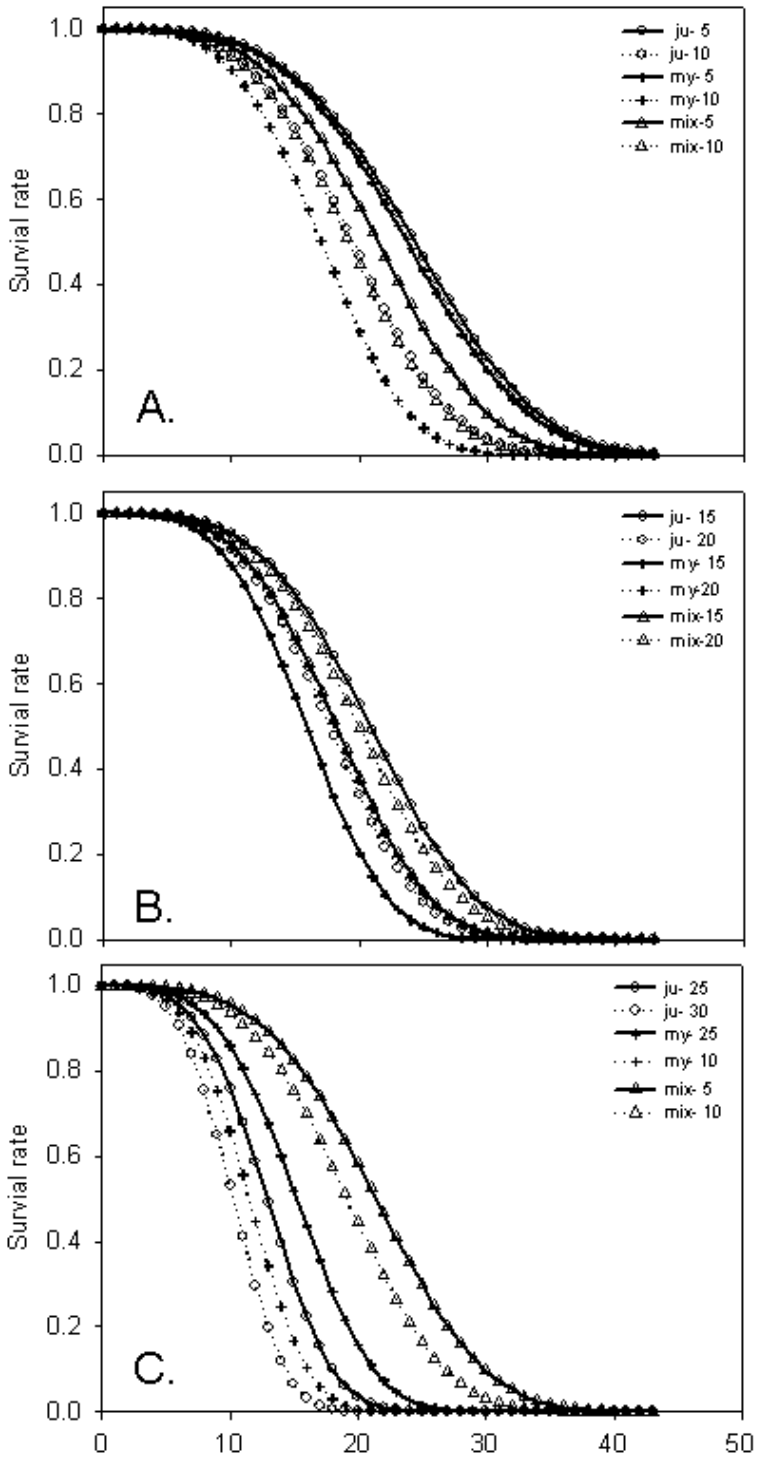

Figure 4.6. Survival rate of *C. remanei* growing at a) 5 and 10, b) 15 and 20 and c) 25 and 30 ° C. Open circles refer to JU, cross to MY and triangle to HYB. The lines represent the predicted values of the model in Table 4.7).

4.5 Discussion

4.5.1 General results

This study illustrates for the first time, the phenotypic plasticity of *C. remanei* life-history traits in response to temperature under laboratory conditions. I used protocols previously developed for *C. remanei* (Diaz et al., *in press*) to describe the relationship between life expectancy, lifetime fecundity and reproductive lifespan in relation to six temperatures (5, 10, 15, 20, 25 and 30 °C). In general, I found a non-linear relationship between all life-history components and temperature. Across strains, the highest fecundity was found at 20 °C, and the lowest at 5 and 30 ° C; the highest life expectancy was recorded at 15 °C and the lowest at 30 °C; and the peak of reproductive lifespan was found at 10 °C and the lowest at 30 °C. According to the quadratic models, maximum life expectancy was expected at 1.75, 4.50 and 7.25 °C; lifetime fecundity at 17.87, 17.30 and 17.73 °C; and reproductive lifespan at 13.75, 13.75 and 23.25 °C (JU, MY and HYB, respectively for each trait). Thus, for all the traits, comparisons across strains suggest a similar optimal temperature of 17 - 18° C (± 0.30 sd) but they differed in the precise shape of the relationship between temperature and respective life-history traits. This is in agreement with several other studies on *C. elegans*. Variation in tolerance to extreme temperatures is known to occur between strains of *C. elegans* (Fatt and Dougherty, 1963) and it is presumably equally likely to be encountered across other Caenorhabditis species as well (Kiontke, 1999; Kiontke and Sudhaus, 2006; Cutter et al., 2006). Compared to *C. elegans*, *C. remanei* exhibits higher thermal breath (Baird et al., 1994; Gutteling et al., 2008). For many invertebrates, the tolerance to stressful temperatures is linked to an increase in heat-shock proteins and changes in the membrane phospholipids (Feder and Hofmann, 1999; Hoffmann et al., 2003; Rea et al., 2005; Murray et al., 2007). In *C. elegans*, the genetic basis underlying differences in heat tolerance between strains is considered rather simple (Cutter et al., 2006): empirical studies have shown that these are linked to actual differences in gene composition (Gutteling et al., 2007; Harvey and Viney, 2007). The same genetic and physiological mechanisms could underlie the increased tolerance of *C. remanei* to stressful temperatures compared to *C. elegans*. However, direct evidence for this is lacking and the ecological significance of temperature tolerance is unclear since we know so little about the ecology of *C. remanei* in the wild.

4.5.2 The hybrid

Inbreeding depression is often associated with a decline in fertility and growth rate (Maynard Smith, 1989; Keller and Waller, 2002). However, when inbred lines are crossed, the F1 is usually as vigorous as the original outbred population (Maynard Smith, 1989).

The results from the two parental crosses of HYB (JU female and MY male cross and the reciprocal) suggest that certain gene combinations have relatively low vigour compared to the wild-type strains. In addition, the half-diallel cross (HYB) shows similar fecundity and optimal growth temperatures compared to JU and MY. Thus, the crosses produced a fit hybrid (HYB) population with no apparent signs of inbreeding depression. In *C. elegans*, studies have documented that crosses between different isolates can lead to outbreeding depression (Dolgin et al., 2007) but there was no evidence of this in *C. remanei*.

4.5.3 Reaction norms and GEI

The main objective of this study was to describe the reaction norms of three important life-history traits in response to temperature. I used a half-sib breeding experiment to describe whether the plasticity of traits had a genetic basis (i.e. GEI). I approached this question by looking at the difference in the offspring traits produced by each replicate (variance between individuals (within replicates) vs. variance between replicates). The analyses suggested that there was high phenotypic variance among individuals (ε_{mlkj}) which was not related to replicate (i.e. "(1|replicate$_{lkj}$)"). This was a surprising result as, in theory, high phenotypic variance might be the consequence of high additive genetic variance (Maynard Smith 1989).

Two potential (but not exclusive) explanations could underlie the suggested low GEI across the populations of worms sampled in this study. First, it might imply that different genotypes (described here as replicates) produced similar phenotypes across a range of temperatures. It is recognised that canalization can reduce the phenotypic variation (Waddington, 1942; Rutherford and Lindquist 1998). Moreover, epigenetic mechanisms that favour canalization are expected to be favoured by natural selection (Siegal and Bergman, 2002). It is also possible that there is really very little inter-continental variation between *C. remanei* populations so that all the individuals assayed were actually highly similar (Diaz et al., in press). Under these circumstances, the reaction norms would reflect the plasticity of similar genotypes. Environmental heterogeneity is

expected to promote local adaptation, thus resulting in the maintenance of polymorphism and genetic diversity at the species level (Roff, 2002). Given the variability of soil characteristics across time and space (Lee, 1994), we might expect a high degree of local adaptation in *C. remanei* populations.

Identifying the number and location of loci that contribute to phenotypic plasticity is not a trivial problem, particularly as phenotypic traits are not always governed by single genes. In addition to the approach of using experimental breeding designs, describing the genetic basis of a phenotypic trait can be conducted by sampling stretches of the DNA that are closely linked to the genes that underlie the trait in question. Using modern quantitative genetic techniques, studies have started to look at the genotype-phenotype relationship by mapping quantitative trait loci (QTL) in different environments. In relation to temperature, recent results suggest that in *C. elegans* fecundity has a significant GEI (Gutteling et al., 2007). Gutteling and collaborators (2007) quantified the phenotypic variance between and among replicates, in addition to several QTLs at each temperature. Their results suggest that there is allelic sensitivity to temperature. For Drosophila, similar studies suggest a significant GEI in life expectancy (Vieira et al., 2000). Thus, a GEI could be as well expected in populations of *C. remanei*.

Another important assumption usually made when interpreting the results from experimental studies of genetic architecture is that the experiments are based on representative, unbiased sampling of wild-type individuals. In the half-sib breeding design I used here, a female was mated with several apparently unrelated males. However, if the strains used in this study were already inbred, this mating design would not be able to detect a replicate effect, making it difficult to disentangle the genetic basis of the plasticity. Another approach would be to use the isofemale line technique (Parsons and Hosgood, 1968; David et al., 2005). This approach has the potential to describe the genetic architecture of quantitative traits of natural populations under laboratory conditions (David et al., 2005). It consists of isolating wild females to initiate a full sib family by allowing the progeny to interbreed (David et al., 2005). As a result, the line will be partially inbred since it will be founded by genes from a single female and from as few as one male (David et al., 2005). In the present [my] study, using unrelated males might have had the opposite effect, hence increasing the genetic differences between the individuals sampled within a replicate.

4.5.4 Correlations between life-history traits

Life-history theory is based on the assumption that selection will act to maximise fitness (Roff, 2002). However, the absence of a "*Darwinian demon*" (Law, 1979), which simultaneously maximises all fitness components, suggests the existence of trade-offs between life-history components (Roff, 2002). For instance, it is commonly thought that a direct or indirect increase in fecundity may reduce energy available to other fitness components such as survival. Despite the simplicity and appeal of the trade-off theory, it is difficult to distinguish causation from correlation (Roff, 2002). Thus, inferences from statistical correlations must be made with caution. I found a negative relationship between lifetime fecundity and life expectancy. Moreover, the analysis suggested that extreme temperatures accentuate the negative relationship between traits. A similar, but positive association, was found between reproductive lifespan and lifetime fecundity. These results could suggest that the genetic correlations (underlying the phenotypic correlation) are environmentally dependent. For instance, for some individuals maximising fecundity can be more costly at certain temperatures (DeWitt et al., 1998). If genetic correlations underlie the observed phenotypic correlations, the evolution of plasticity and life history might be constrained in heterogeneous environments (Pigliucci, 2005). However, similar to the reaction norms of life-history traits, the analysis suggests weak support for a GEI for each correlation.

4.5.5 Conclusions

My results suggest plasticity with regards to changing temperatures for all the life-history traits of *C. remanei* studied here. These results intuitively suggest that, in agreement with results on other ectotherms, *C. remanei*'s performance is limited by temperature (Cossins and Bowler, 1987). Moreover, compared to *C. elegans*, *C. remanei* has higher thermal tolerance.

5 Life-history evolution in fluctuating environments: a long-term selection experiment on *Caenorhabditis remanei*

5.1 Abstract

Environments are spatially and temporally heterogeneous and this variation is considered to be partially responsible for shaping the life-history adaptations of populations and species. In nature, organisms are physiologically limited to a range of conditions that allow their normal functions, and there are many documented examples of adaptations to constant extreme conditions but empirical evidence of adaptations to fluctuating environments is more limited. However, the study of the evolutionary consequences of fluctuating environmental conditions on fitness is important, for example, for understanding the likely consequences of changing environmental patterns due to climate change. In this study, I used the nematode *Caenorhabditis remanei* to study the evolution of life-history traits in two thermal regimes, constant and predictably fluctuating, for 50 generations. I used three strains, wild-type strains JU724 and MY12-G (originally from China and Germany) and a half-diallel cross between them, which allows a comparison not only between different strains but also between likely levels of genetic diversity (i.e. between the pure strains and the hybrid). The results show that thermally fluctuating conditions are generally suboptimal for nematodes reared in laboratory conditions, resulting in up to 60% reduction in performance across different life-history traits compared to nematodes cultures in standard conditions. For worms in the fluctuating environment, I did not detect changes in lifetime fecundity despite the relatively long-term opportunity for adapting to these environments. However, the timing of reproduction shifted towards younger age in the fluctuating environment in the course of selection, resulting in an increase in the generation time from 3.68 (generation 1) to 3.8 (generation 50) – a strong demonstration of adapting to the fluctuating environment as it marks the difference between extinction and possible persistence in such an environment.

5.2 Introduction

The natural environment is constantly changing (Karl et al. 1995; Easterling, 2000). Temporal and spatial fluctuations create a diversity of habitats in which organisms are born, develop, produce progeny and die. Thus, species life histories are actively affected by natural selection acting on individual variation within populations (Charlesworth, 1980). From an evolutionary perspective, temporal fluctuations and spatial complexity are considered to promote species diversity across habitats (Tews et al., 2004), and within a species, populations living in different environments show adaptations to local environmental conditions (Futuyma and Moreno, 1988; Grant and Grant, 1993). While studies have described the importance of environmental effects on populations' demography, the evolutionary consequences are not always obvious. Two main issues still remain unanswered. First, what are the consequences of the fluctuating environments on populations' genetic diversity, and second, what life-history traits are favoured in a fluctuating environment? Here, the focus will be on the life-history traits.

Depending on the frequency of the environmental variation, relative to the lifetime of an individual, natural selection can change the tolerance of individuals to a wider range of temperatures (Via and Lande, 1985; Scheiner, 1993). Thus, environmental variation occurring at a short time scale relative to generation time might promote phenotypic plasticity (Roff, 2002). As empirical evidence regarding the consequences of living in fluctuating environments has received little attention, in this study, I focus on life-history trait evolution in fluctuating environments.

In the absence of genetic constraints (Roff, 2002), organisms should evolve to match the average state of the environment, and the breadth of adaptation should evolve to match the range of environmental variation (Bradshaw, 1965; Southwood, 1977; Futuyma and Moreno, 1988). In theory, selection will favour those genotypes that have the highest growth rate compared to other genotypes in the population, especially if we assume an equilibrium population in a constant environment (Charlesworth, 1980; Benton and Grant, 2000; Roff, 2002). In constant favourable environments, where the probability of dying is close to zero, selection is expected to favour "fast" life cycles (Wilbur and Rudolf, 2006). Thus, in a species with a short

maturation time, early reproduction might be adaptive since there are no potential benefits of delaying reproduction. In contrast, when the environment is fluctuating, either in a predictable or unpredictable manner, changes in the direction of selection may favour individuals with delayed reproductive schedules (Wilbur and Rudolf, 2006). Variation in the age of maturity can be explained when bigger/older individuals produce more or higher quality offspring than smaller/younger parents (Stearns and Crandall, 1981; Stearns and Koella, 1986).

Mathematical models of the effects of temporal variation on life history traits have received considerable attention (e.g. Tuljapurkar 1989; Tuljapurkar, 1990; Orzack and Tuljapurkar, 2001; Altwegg et al., 2007). These models commonly describe the effects on temporal variability on fitness by breaking down the environmental effects that contribute to the fitness of each age-class (Stearns, 2000). We can imagine two scenarios, temporal variation reducing 1) the survival of juveniles or 2) the survival of adults (Murphy, 1958; Roff, 2002). Murphy (1968) was one of the first to theoretically consider these two scenarios and he predicted that long life expectancy and late maturity might be the consequence of evolutionary pressures causing a reduction in the survival of juveniles (i.e. pre-reproductives as referred by Murphy, 1968). In contrast, low or variable adult survival might cause evolutionary pressure toward early reproduction (Murphy, 1698). Empirical evidence shows that different age classes are indeed often affected differently by environmental variation. For instance, in nature, low temperatures during winter affect juvenile but not adult survival of populations of asp viper (*Vipera aspis*) (Altwegg et al., 2005). Thus, from an evolutionary perspective, it is important to determine whether fluctuating environments reduce juvenile or adult survival.

In addition to theoretical studies, empirical studies have also demonstrated the limits of evolutionary change (Cohan and Graf, 1985; Krebs and Loeschcke, 1996; Gibbs et al., 1997). In theory the rate of response to selection should be linked to the additive genetic variance present in a population (Fisher 1930), and empirical studies have corroborated this prediction (e.g. Reznick et al., 1997; Hendry and Kinnison, 1999; Roff, 2002). Thus, inbreeding depression, as a consequence of low population density for instance, can negatively affect the rate of evolutionary change (Arnold, 1993; Potvin and Tousingagnt, 1996). In addition, life-history can be constrained

through the cost of adapting to abiotic conditions (Harshman and Hoffman, 2000); for instance, empirical studies have reported that an increase in adult cold resistance was accompanied by a decrease in early fecundity of two species of Drosophila, suggesting a life-history trade-off. (Watson and Hoffmann, 1996). Another limiting element is that the trait in question, or the mechanisms which increase tolerance to fluctuating environments, must have some genetic basis to be transmitted to future generations. Again, research on Drosophila suggests that although heritabilities for thermal tolerance, for instance, can vary between populations, the heritability of tolerance to stressful conditions is not uncommon (e.g. Watson and Hoffmann, 1996; Jenkins and Hoffmann, 1999).

For obvious reasons (short generation time and easy maintenance), research on the effects of environmental fluctuation on adaptation have mainly been conducted on small organisms (bacteria, Bennett et al., 1992; algae, Reboud and Bell, 1997; Kassen and Bell, 1998; viruses, Weaver et al., 1999; Daphnia, Scheiner and Yampolsky, 1998; Drosophila, Haley and Birley, 1983). Experimental evolutionary studies investigating the adaptation of microorganisms to temperature have been particularly successful (Huey, 1982; Huey et al., 1991; Bennett et al., 1992). Bennett and colleagues (1992) investigated the adaptation of *Escherichia coli* to three constant temperatures (32, 37 and 42 °C) and a thermally fluctuating regime (32/37 °C) for 2,000 generations. They found that all four treatments showed improved fitness compared to an ancestral line, which was previously propagated for a similar length of time at the average temperature (Bennett et al., 1992). For invertebrates, the evolution of thermal tolerance has been mainly documented under laboratory conditions in Drosophila (e.g. Huey et al., 1991; Watson and Hoffman, 1996). For instance, Huey and collaborators found that *Drosophila melanogaster* individuals cultured at low temperatures exhibit faster developmental times after 60 generations of selection (Huey et al., 1991). In an early study, the adaption of the soil nematode *Caenorhabditis elegans* to a gradual increase (0.5°C) in temperature (from 18 to 23 °C) was investigated across generations (Brun, 1965). Although adaptation to gradual change was achieved, any further increase in temperature, even by as little as 0.5 °C, resulted in *ca.* 90% sterile worms in the population (Brun, 1965).

The thermal tolerance of other free-living nematodes, such as *C. remanei*, is considered to be higher compared to *C. elegans* (Chapter 4; Baird et al., 1994). In a previous study, I described the thermal breath of *C. remanei* under laboratory conditions. I found that 17 °C was the optimal growing temperature for fecundity (see Chapter 4). Although extreme temperatures, such as 5 and 25 °C, significantly decreased fecundity, they were still permissive for some individuals (Chapter 4). If tolerance to high/low temperatures has some genetic basis, it would be likely subject to selection.

In this study, the main objectives are to examine the response of fitness components to environmental conditions (constant vs. predictably fluctuating temperature), and the effect of the evolutionary starting point (pure strains vs. hybrid) under laboratory conditions. I used a gonochoristic nematode *C. remanei* as a model species raised under laboratory conditions for the selection experiment. Five replicates of three strains of *C. remanei* were cultured under two environmental regimes, constant (15 °C) and fluctuating (between 5 and 25°C), for 50 generations. I then compared the fecundity and survival rates at generations 1, 20 and 50.

5.3 Material and methods

5.3.1 General protocols

5.3.1.1 Strains

I used three strains of *C. remanei*, two wild-type strains (JU724 and MY12-G; originally from China and Germany, respectively) and a half-diallel cross (HYB). The two wild type isolates were recently isolated from the field. They were provided by M. A. Felix from the Nematode Biological Resource Centre in France and N. Timmermeyer from the Animal Ecological Centre in Germany, respectively. The Chinese strain was isolated from soil in May 2005 and the German strain was isolated from rotten apples in September 2006. The strains were obtained from samples frozen since their isolation in the wild; I assume they represent a natural population that have not been adapted to laboratory conditions. Moreover, I assumed that populations did not adapt to laboratory conditions. The half-diallel cross (HYB) consisted of the F1

progeny of a female JU724 by a male MY12-G , and the reciprocal crosses (Chapter 4). To initiate the selection experiment, I used five replicates from each of these out-bred strains, JU724, MY12-G (referred to henceforth as JU and MY, respectively) and HYB. I used replicates which were stored in eppendorf tubes and maintained at -80 °C prior to the current study. Worms recovered from these stocks were used to initiate the selection experiment. This is a standard procedure (Epstein and Shakes, 1995) and has been shown to have no effect on the life-history characteristics of *C. remanei* (Epstein and Shakes, 1995). All individuals were maintained in NGM petri dishes and fed on a lawn of *Escherichia coli* (OP50 strain) using standard protocols (Hope, 1999).

5.3.2 Selection experiment

5.3.2.1 Temperature regimes

Five replicates from each strain were cultured in two temperature-controlled incubators, one constantly at 15° C, one fluctuating regularly between 5 and 25° C (mean 15° C) , for 50 generations each. In the fluctuating temperature regime, temperature changed from the minimum to maximum every 12 hours. This change took ca. 15 minutes and the cooling from maximum to minimum took ca. 45 minutes.

5.3.2.2 Maintenance and culture

During the experiment, I maintained the population using standard laboratory protocols (Hope 1999). Using aseptic conditions at 20 °C, two random chunks of agar of approximately 1 cm^2 were transferred onto a new NGM plate every 3-4 days for worms in a constant environment and every 4-5 days for worms in a fluctuating environment. These transferring schedules corresponded to *ca.* two generations in each environment. In addition, every 10 generations, I stored 10 samples of each replicate in eppendorf tubes to be maintained at -80 °C (Hope, 1990). Worms recovered from these samples were used to initiate the fitness assays. Petri dishes of 50 mm diameter were used for the maintenance of worms in the experiment.

5.3.3 Fitness assays

Prior to each assay, a frozen sample was thawed at room temperature for a few minutes and then poured into a 50 mm NGM-petri dish. The following day, the worms were moved to the assigned environment (i.e. constant or fluctuating). For worms in a constant environment, approximately two days later, five gravid females were randomly selected from each replicate and transferred into individual petri dishes. For worms in a fluctuating environment, the same procedure was followed approximately 4 days later after thawing. The L4 offspring of these females were used to initialize all assays.

5.3.3.1 Life-history assays

Using lab protocols previously developed to quantify the vital rates of *C. remanei* (Diaz et al. in press), I conducted the life-history assays at generation 20 and 50 (F20 and F50, respectively) in both environments. The life-history traits I quantified were life expectancy, lifetime fecundity, reproductive lifespan and age-specific fecundity. I followed 25 individuals from each strain (five per replicate) from each regime. As I have previously shown that *C. remanei* females maximise their fecundity when given access to ca. four males (Chapter 3), to get an estimate of fecundity which would not be limited by sperm availability, a virgin female was paired with four unrelated young males for 48 hours. On alternate days after this, the female was subsequently transferred into a new petri dish with four new unrelated young males (Diaz et al., in press; Baird et al., 1994). Transfers were continued until the female stopped laying eggs. Then the female was monitored on alternate days to score the date of death (similar to other *C. elegans*' protocols, e.g. Evason et al., 2005; Dolgin et al., 2007). Age-specific fecundity was estimated by counting the number of juvenile larvae present in each plate. Plates were monitored two days after the female was previously transferred to account for the number of larvae observed. In total, 150 females from each population (JU7, MY and HYB) in each regime were assessed.

5.3.3.2 Demographic analysis

I applied well-known methods in demography (Case, 2000) to calculate the generation time and population growth rate. Briefly, I used the age-specific fecundity estimates (m_x) and survival (l_x) at time x to estimate the generation time (T):

$$T = \frac{\sum l_x m_x x}{\sum l_x m_x};$$

5.3.3.3 Model construction and comparison

Using mixed-effects models, I analysed the average performance of worms of different strains, over generations and between regimes, together with the pattern of variance within generations, within regimes, within strains, among individuals (within replicates) and between replicates.

Model syntax used here denotes fixed variables with upper case letters and random variables with lower case letters. I used subscripts to denote different levels of the data as follows: *n* for individual observations (1,2,…,450), *m* for the replicate (1,2…,15), *l* for the Strain (1,2 and 3), *k* for the Generation (F1, F20 and F50), *j* for the Regime (constant and fluctuating) and *i* for the Age (0,2,…,14 days) of the *n*th individual (referred in the models as ind).

The syntax of the random effects was the following: the "(strain| replicate)" term denotes the random variable representing the deviation of the population mean of the average life-history trait for the *m*th replicate (within the *l*th strain; Faraway, 2006), the "(generation| replicate)" term is a random variable representing the deviation of the *m*th replicate from the population mean of the average life-history trait across generations, the "(regime| replicate)" term is a random variable representing the deviation of the *m*th replicate from the population mean of the average life-history trait between regimes, and the "(1| replicate)" effect is a random variable (intercept) representing the deviation from the population mean of the average life-history trait for the *m*th replicate. The ε_{jklmn} is a random variable representing the deviation of the life-history trait for the *n*th worm of the *m*th replicate

(within the *l*th strain) between regimes, and across *k*th generations (Faraway, 2006). I presented the variance components in terms of percentages of the total variance attributable to each effect (e.g. percentage of the variance within replicates = $\sigma_{replicate}^2$ / [$\sigma_{replicate}^2 + \sigma_\varepsilon^2$], and the percentage of the error variance is presented similarly). I assumed that the variances of random effects were normally distributed with mean zero.

5.3.3.4 Statistical analysis

All statistical analysis was 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, version: 0.999375-27). In addition, I analysed survivorship by fitting survival models using the "Surv" function ("survival" package, version: 2.34-1) 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 performed using Likelihood Ratio Tests (LRT) for nested models. For un-nested models, the most parsimonious model with lowest AIC value was chosen. In all instances, pair-wise comparison of competing model are embodied in the text; first the competing vs. the simplest. Unless otherwise stated, the results are presented by a mean effect ± standard error (se).

5.4 Results

5.4.1 Life expectancy (LE)

I found that life expectancy, as measured by the average number of days lived, decreased over generations (Fig. 5.1, M1 vs. M0: χ^2= 60.26, 2, P<0.001; Table 5.1A). The analysis also showed that this reduction in *LE* was different in the two regimes (Fig. 5.1, M2 vs. M1: χ^2= 53.76, 1 d.f., P<0.001; Table 5.1A), so by the end of the experiment worms in the fluctuating regime had the shortest *LE*. In addition, I found a

significant interaction between generation and regime (M3 vs. M2: χ^2= 42.88, 2 d.f., P<0.001; Table 5.1A), indicating that the reduction in *LE* was steepest in the fluctuating regime. While strain was not significant when fitted as a main effect (M4 vs. M3: χ^2= 1.78, 2 d.f., P= 0.40; Table 5.1A), or as an interaction with generation (M5 vs. M3: χ^2= 8.15, 8 d.f., P= 0.41; Table 1.A), the effect of the regime on *LE* varied significantly between strains (M6 vs. M3: χ^2= 10.61, 4 d.f., P<0.05; Table 5.1A); JU exhibited longer *LE* compared to the other strains only in the constant regime. Finally, a model with a three-way interaction term (between strain, generation and regime) was the preferred (M7 vs. M6: χ^2= 66.87, 8 d.f., P<0.001; Table 5.1A), suggesting that the effect of the environmental regime on generation change in *LE* was further dependent on the strain (Figure 5.1). For instance, in a constant environment JU had the largest average *LE* at the beginning of the experiment, but after 50 generations of selection all strains showed similar *LE*; in contrast in the fluctuating environment, strains had similar *LE* before and after the selection experiment. Regarding the variance components, I found little variation across replicates, moreover, models permitting different variances for the replicates of each strain (M8 vs. M7: χ^2= 3.29e-07, 5 d.f., P=0.99; Table 5.1A), generation (M9 vs. M7: χ^2= 3.29e-07, 5 d.f., P=0.99; Table 5.1A) or regime (M10 vs. M7: χ^2= 3.29e-07, 2 d.f., P=0.99; Table 5.1A) were not preferred. Therefore, the final model contained the fixed effects of generation, regime and strain, and the corresponding two- and three-way interaction, and a random effect to describe the hierarchical structure of the data (Model M7 in Table 5.1A; Table 5.2A; Figure 5.1).

	model	Syntax	AIC	LogLik	D.F
A.	*LE*				
	M0	$1 + (1\|\text{replicate}_{jklm})$	2638.4	-1316.2	3
	M1	$\text{Generation}_k + (1\|\ \text{replicate}_{jklm})$	2582.1	-1286.0	5
	M2	$\text{Generation}_k + \text{Regime}_j + (1\|\ \text{replicate}_{jklm})$	2530.3	-1259.2	6
	M3	$\text{Generation}_k + \text{Regime}_j + \text{Generation}_k{:}\text{Regime}_j + (1\|\ \text{replicate}_{jklm})$	2491.5	-1237.7	8
	M4	$\text{Generation}_k + \text{Regime}_j + \text{Generation}_k{:}\text{Regime}_j + \text{Strain}_l + (1\|\ \text{replicate}_{jklm})$	2493.7	-1236.8	10
	M5	$\text{Generation}_k + \text{Regime}_j + \text{Generation}_k{:}\text{Regime}_j + \text{Strain}_l + \text{Regime}_j{:}\text{Strain}_l + (1\|\ \text{replicate}_{jklm})$	2488.9	-1232.4	12
	M6	$\text{Generation}_k + \text{Regime}_j + \text{Generation}_k{:}\text{Regime}_j + \text{Strain}_l + \text{Generation}_k{:}\text{Strain}_l + (1\|\ \text{replicate}_{jklm})$	2495.3	-1233.7	14
	M7	$\mathbf{\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (1\|\ \text{replicate}_{jklm})}$	**2438.0**	**-1199.0**	**20**
	M8	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{strain}_l\|\ \text{replicate}_{jklm})$	2448.0	-1199.0	25
	M9	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{generation}_k\|\ \text{replicate}_{jklm})$	2448.0	-1199.0	25
	M10	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{regime}_j\|\ \text{replicate}_{jklm})$	2442.0	-1199.0	22
B.	*LF*				
	M0	$1 + (1\|\ \text{replicate}_{jklm})$	5814.6	-2904.3	3
	M1	$\text{Regime}_j + (1\|\ \text{replicate}_{jklm})$	5676.2	-2834.1	4
	M2	$\text{Generation}_k + \text{Regime}_j + (1\|\ \text{replicate}_{jklm})$	5680.0	-2834.0	6
	M3	$\text{Generation}_k + \text{Regime}_j + \text{Generation}_k{:}\text{Regime}_j + (1\|\ \text{replicate}_{jklm})$	5655.4	-2821.7	6
	M4	$\text{Regime}_j + \text{Strain}_l + (1\|\ \text{replicate}_{jklm})$	5679.5	-2831.7	8
	M5	$\text{Regime}_j + \text{Strain}_l + \text{Regime}_j{:}\text{Strain}_l + (1\|\ \text{replicate}_{jklm})$	5645.8	-2814.9	8
	M6	$\text{Regime}_j + \text{Strain}_l + \text{Regime}_j{:}\text{Strain}_l + \text{Generation}_k + (1\|\ \text{replicate}_{jklm})$	5649.5	-2814.8	10
	M7	$\text{Regime}_j + \text{Strain}_l + \text{Regime}_j{:}\text{Strain}_l + \text{Generation}_k + \text{Strain}_l{:}\text{Generation}_k + (1\|\ \text{replicate}_{jklm})$	5652.4	-2812.2	14
	M8	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (1\ \|\ \text{replicate}_{jklm})$	5650.1	-2805.0	20
	M9	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{strain}_l\ \|\ \text{replicate}_{jklm})$	5654.4	-2814.2	25
	M10	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{generation}_k\ \|\ \text{replicate}_{jklm})$	5655.6	-2814.8	25
	M11	$\mathbf{\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{regime}_j\ \|\ \text{replicate}_{jklm})}$	**5620.2**	**-2800.1**	**22**
C.	*RL*				
	M0	$1 + (1\|\ \text{replicate}_{jklm})$	2065.99	-1030.00	3
	M1	$\text{Generation}_k + (1\|\ \text{replicate}_{jklm})$	2024.03	-1007.01	5
	M2	$\text{Generation}_k + \text{Regime}_j + (1\|\ \text{replicate}_{jklm})$	1975.05	-981.53	6
	M3	$\text{Generation}_k + \text{Regime}_j + \text{Generation}_k{:}\text{Regime}_j + (1\|\ \text{replicate}_{jklm})$	1957.24	-970.62	8
	M4	$\text{Generation}_k + \text{Regime}_j + \text{Generation}_k{:}\text{Regime}_j + \text{Strain}_l + (1\|\ \text{replicate}_{jklm})$	1952.10	-966.05	10
	M5	$\text{Generation}_k + \text{Regime}_j + \text{Generation}_k{:}\text{Regime}_j + \text{Strain}_l + \text{Generation}_k{:}\text{Strain}_l + (1\|\ \text{replicate}_{jklm})$	1946.13	-959.07	14
	M6	$\text{Generation}_k + \text{Regime}_j + \text{Generation}_k{:}\text{Regime}_j + \text{Strain}_l + \text{Generation}_k{:}\text{Strain}_l + \text{Regime}_j{:}\text{Strain}_l + (1\|\ \text{replicate}_{jklm})$	1942.08	-955.04	16
	M7	$\mathbf{\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (1\|\ \text{replicate}_{jklm})}$	**1927.64**	**-943.82**	**20**
	M8	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{regime}_j\|\ \text{replicate}_{jklm})$	1936.59	-943.29	22
	M9	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{strain}_l\|\ \text{replicate}_{jklm})$	1931.57	-943.79	22
	M10	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{generation}_k\|\ \text{replicate}_{jklm})$	2024.03	-1007.01	25
D.	m_x				
	M0	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (1\ \|\ \text{ind}_{jklmn})$	43562	-21761	20
	M1	$\text{Age}_i + \text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (1\ \|\ \text{ind}_{jklmn})$	41834	-20889	28
	M2	$\text{Age}_i + \text{Generation}_k * \text{Regime}_j * \text{Strain}_l + \text{Age}_i{:}\ \text{Generation}_k + (1\ \|\ \text{ind}_{jklmn})$	41829	-20870	44
	M3	$\text{Age}_i + \text{Generation}_k * \text{Regime}_j * \text{Strain}_l + \text{Age}_i{:}\ \text{Generation}_k + \text{Age}_i{:}\ \text{Regime}_j + (1\ \|\ \text{ind}_{jklmn})$	40068	-19982	52
	M4	$\text{Age}_i + \text{Generation}_k * \text{Regime}_j * \text{Strain}_l + \text{Age}_i{:}\ \text{Generation}_k + \text{Age}_i{:}\ \text{Regime}_j + \text{Age}_i{:}\ \text{Strain}_l + (1\ \|\ \text{ind}_{jklmn})$	39886	-19875	68
	M5	$\text{Age}_i * \text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (1\ \|\ \text{ind}_{jklmn})$	39687	-19680	164
	M6	$\mathbf{\text{Age}_i * \text{Generation}_k * \text{Regime}_j * \text{Strain}_l + (\text{age}\ \|\ \text{ind}_{jklmn})}$	**31480**	**-15532**	**208**
E.	l_x				
	M0	1	2829	-1413	2
	M1	Generation_k	2696	-1344	4
	M2	$\text{Generation}_k + \text{Regime}_j$	2567	-1278	5
	M3	$\text{Generation}_k * \text{Regime}_j$	2389	-1188	7
	M4	$\text{Generation}_k * \text{Regime}_j + \text{Strain}_l$	2386	-1184	9
	M5	$\mathbf{\text{Generation}_k * \text{Regime}_j * \text{Strain}_l}$	**3128**	**-1546**	**18**
	M6	$\text{Generation}_k * \text{Regime}_j * \text{Strain}_l$ -Exponential	2299	-1131	19

Table 5.1. AIC and log likelihood (logLik) values for the life-history traits models, (A) life expectancy, *LE*, (B) lifetime fecundity, *LF*, (C) reproductive lifespan, *RL*, (D) age-specific fecundity, m_x, and (E) survivorship. Bold letters correspond to the preferred model for each trait (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, whereas symbol "*" denotes an interaction plus the main terms.

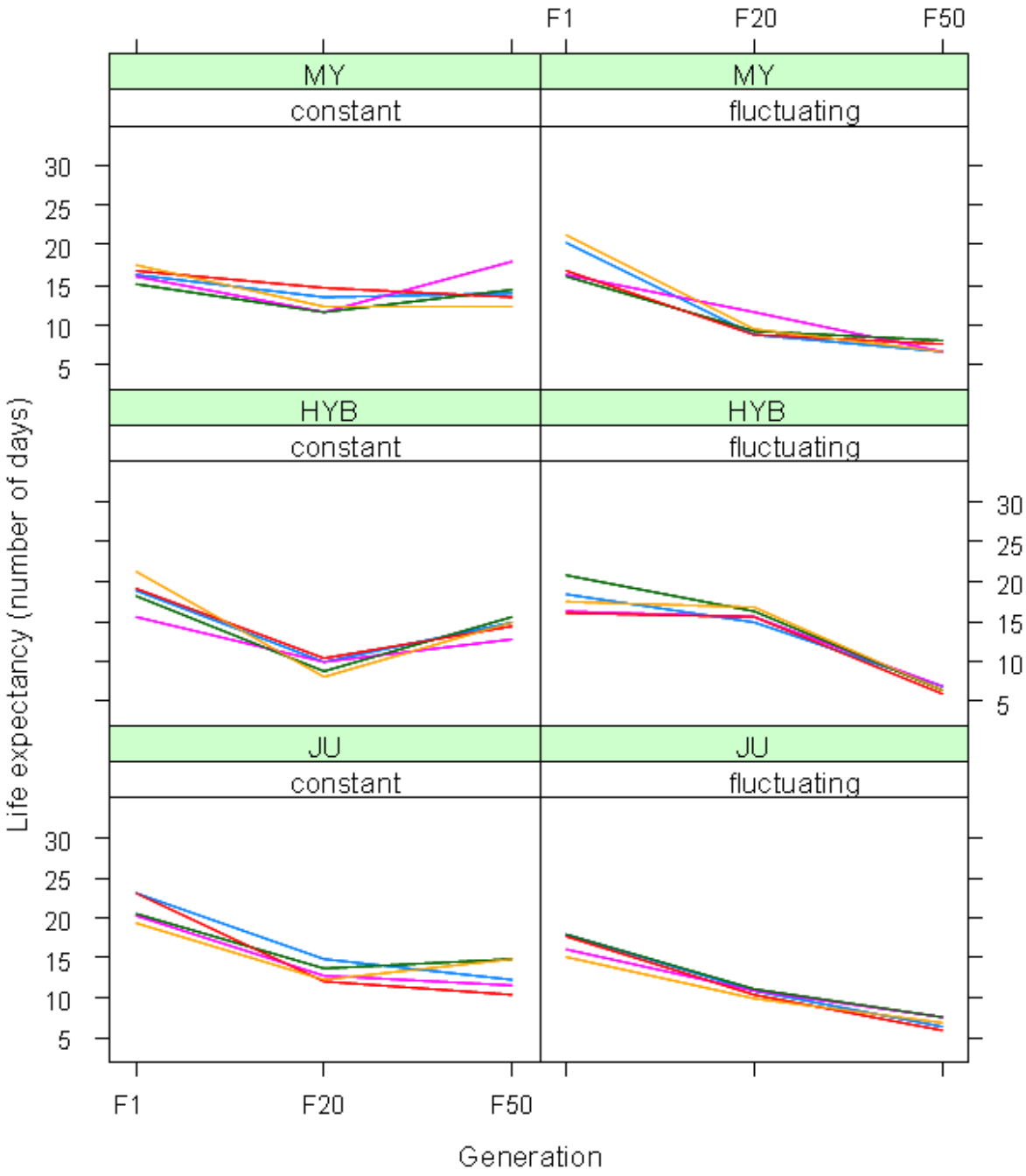

Figure 5.1. Life expectancy (number of days lived) of the three strains of *C. remanei* exposed to two environmental regimes (constant and fluctuating environment) in the beginning of the experiment (F1) and in generations 20 and 50. Each line represents the replicate mean.

5.4.2 Lifetime fecundity (LF)

I followed the same protocols as for *LE* to analyse the lifetime fecundity, *LF*. The average *LF* did not change over generations (Fig. 5.2, M2 vs. M1: χ^2= 0.21, 2 d.f., P= 0.90; Table 5.1B). The regime had a significant effect on *LF* (M1 vs. M0: χ^2= 140.36, 1 d.f., P<0.001; Table 5.1B); the average *LF* was lower in the fluctuating

regime, moreover there was no interaction between the regime and the average number of offspring produced by a female over generations (M3 vs. M1: χ^2= 4.76, 4 d.f., P= 0.31; Table 5.1B; Figure 5.2). In addition, I found that the strains varied in the average number of offspring they produced (M4 vs. M1: χ^2= 24.82, 2 d.f., P<0.001; Table 5.1B); for instance, MY had a higher *LF*. Moreover, the number of offspring produced by a strain varied between environments (M5 vs. M4: χ^2= 13.57, 2 d.f., P<0.01; Table 5.1B); there were differences in the productivity across strains in the constant environment, but not in the fluctuating environment. There was no significant interaction between strain and generation (M7 vs. M5: χ^2= 5.43, 6 d.f., P= 0.49; Table 5.1B), nor did adding a three-way interaction term (between strain, generation and regime) improved the model fit further (M8 vs. M5: χ^2= 19.74, 12 d.f., P= 0.07; Table 5.1B). Regarding the variance components, I found no support for strain- or generation-specific variance terms for the random effect to describe the variance between replicates (M9 vs. M5: χ^2= 1.46, 5 d.f., P= 0.92 and M10 vs. M5: χ^2= 0.22, 5 d.f., P= 0.99, respectively; Table 5.1B. However, the regime-specific variance term was significant (M11 vs. M7: χ^2= 7.04, 2 d.f., P<0.05; Table 5.1B). The variance within the fluctuating regime was smaller compared to the variation in the constant regime (<0.01 and 99.9 %, respectively; Table 5.2B); however, I found a large between individual variance, which was not related to the rest of the terms (69.58%, Table 5.2B). The most parsimonious model contained the fixed effects of regime, strain, regime-strain interaction term and a random effect describing the replicate deviance for each regime (Model M11 in Table 5.2B).

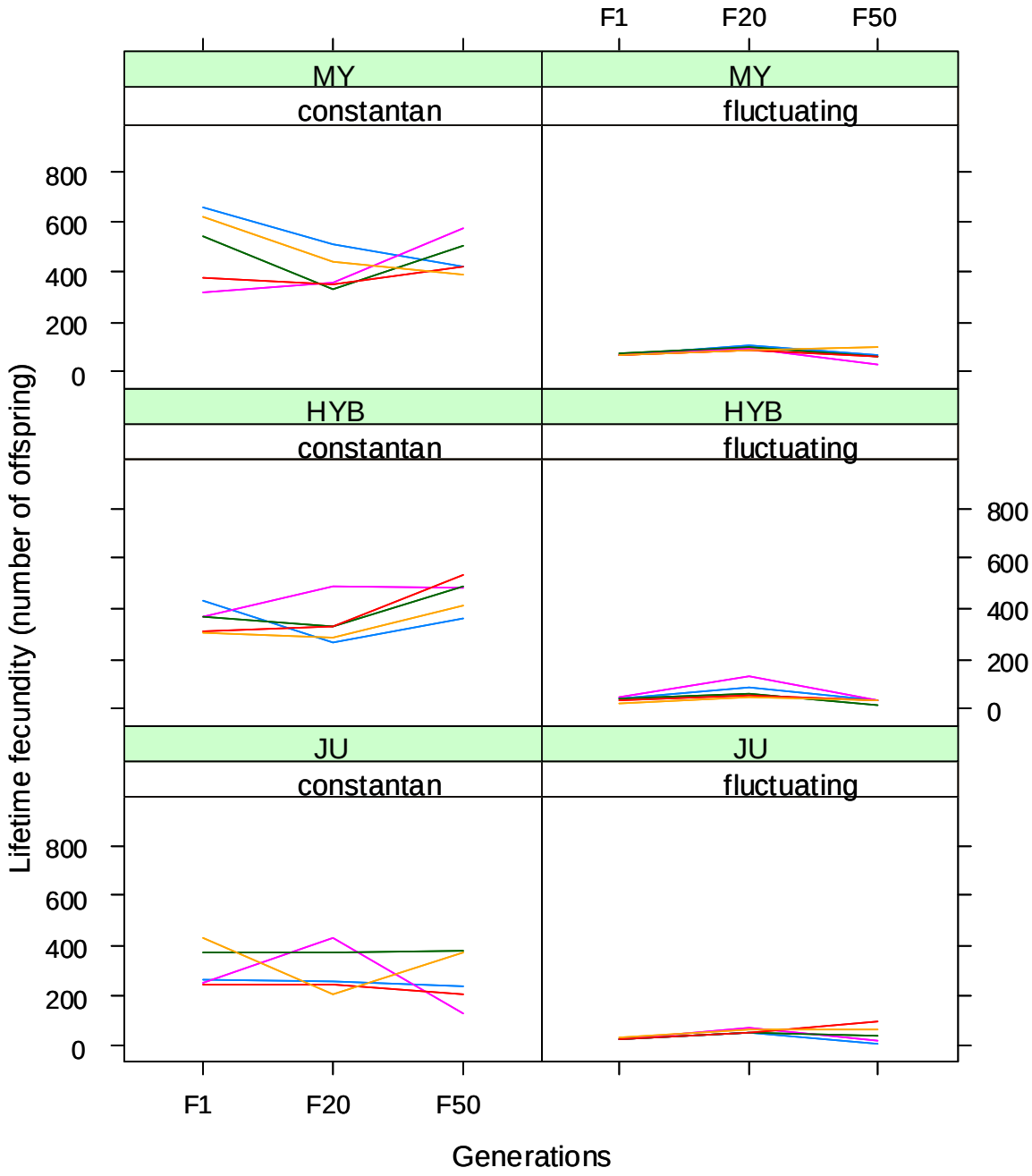

Figure 5.2. As Fig. 5.1, but showing lifetime fecundity (number of offspring).

5.4.3 Reproductive lifespan (RL)

Reproductive lifespan, the number of days taken to produce all the offspring, changed over generations (M1 vs. M0: χ^2= 45.97, 2 d.f., P<0.001; Table 5.1C). In general, the average *RL* shortened over the course of the experiment (Figure 3), and it was also significantly different between the environmental regimes (M2 vs. M1: χ^2= 50.97, 1 d.f., P<0.001; Table 5.1C); worms from the fluctuating regime had shorter *RL* both at the beginning and in the end of the selection experiment. There was also a significant

interaction between regime and generation (M3 vs. M2: χ^2= 21.82, 2 d.f., P<0.001; Table 5.1C). For instance, *RL* shortened in the fluctuating regime from F20 to F50, however, there was no such change in the constant regime (Figure 5.3). In addition, I found that the strains responded differently to the environmental regime (M4 vs. M3: χ^2= 9.14, 2 d.f., P<0.05; Table 5.1C). Moreover, I found a significant interaction between strain and generation (M5 vs. M4: χ^2= 13.97, 4 d.f., P<0.01; Table 5.1C), and strain and regime (M6 vs. M5: χ^2= 8.05, 2 d.f., P<0.05; Table 5.1C). For instance, worms from the HYB strain growing in a constant environment did not show a change in RL but had reduced *RL* in the fluctuating environment (Figure 5.3). Therefore, I found evidence of a significant three-way interaction term between strain, generation and regime (M7 vs. M6: χ^2= 22.44, 4 d.f., P<0.001; Table 5.1C). Regarding the variance components, I found no evidence for strain, generation or regime specific variance terms for the replicate random effect (M8 vs. M7: χ^2= 0.01, 5 d.f., P= 0.99, M9 vs. M7: χ^2= 1.0579, 5 d.f., P= 0.96, and M10 vs. M7: χ^2= 0.0739, 2 d.f., P= 0.96, respectively; Table 5.1C). As a consequence, the final model contained the fixed effects of generation, regime and strain, and the corresponding two- and three-way interaction terms and a random effect to describe the hierarchical structure of the data (Model M7, Table 5.2C).

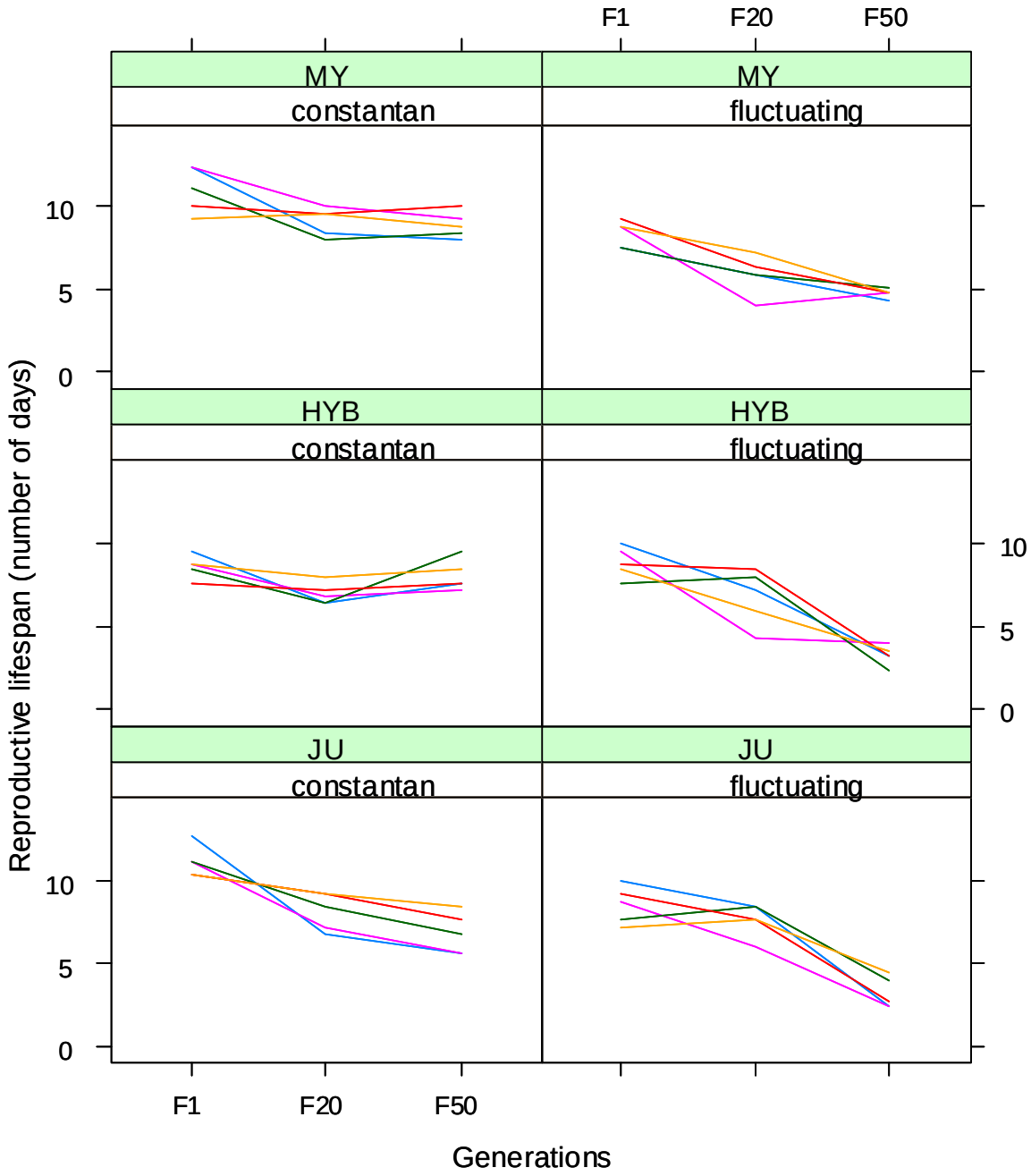

Figure 5.3. As Fig. 5.1 and 5.2, but showing reproductive lifespan (number of days).

Model	Parameter	Estimate	Std. Error	t value
A. M7	*LE*			
	Fixed effects:			
	(Intercept)	21.28	0.69	30.62
	GenerationF20	-8.12	0.98	-8.26
	GenerationF50	-8.48	0.98	-8.63
	RegimeFL	-6.32	0.98	-6.43
	StrainHYB	-2.68	0.98	-2.73
	StrainMY	-4.84	0.98	-4.93
	GenerationF20:RegimeFL	1.80	1.39	1.30
	GenerationF50:RegimeFL	-1.60	1.39	-1.15
	GenerationF20:StrainHYB	-1.04	1.39	-0.75
	GenerationF50:StrainHYB	4.36	1.39	3.14
	GenerationF20:StrainMY	4.48	1.39	3.22
	GenerationF50:StrainMY	6.52	1.39	4.69
	RegimeFL:StrainHYB	3.56	1.39	2.56
	RegimeFL:StrainMY	6.04	1.39	4.35
	GenerationF20:RegimeFL:StrainHYB	5.36	1.97	2.73
	GenerationF50:RegimeFL:StrainHYB	-5.64	1.97	-2.87
	GenerationF20:RegimeFL:StrainMY	-6.72	1.97	-3.42
	GenerationF50:RegimeFL:StrainMY	-7.40	1.97	-3.77
	Random effects:			
	Groups Name	Variance	Std.Dev.	% of Variance
	replicate (Intercept)	0.00	0.00	0.00
	Residual	12.07	3.47	100.00
B. M11	*LF*	Estimate	Std. Error	t value
	Fixed effects:			
	(Intercept)	290.59	23.72	12.25
	RegimeFL	-248.27	27.19	-9.13
	StrainHYB	95.35	33.54	2.84
	StrainMY	164.19	33.54	4.90
	RegimeFL:StrainHYB	-89.49	38.45	-2.33
	RegimeFL:StrainMY	-129.12	38.45	-3.36
	Random effects:			
	Groups Name	Variance	Std.Dev.	% of Variance
	replicate regimeconst	5790.00	76.09	30.42
	regimefluct	4.67E-08	2.16E-04	2.45E-10
	Residual	13242.00	115.08	69.58
C. M7	*RL*	Estimate	Std. Error	t value
	Fixed effects:			
	(Intercept)	11.20	0.39	28.42
	GenerationF20	-3.04	0.56	-5.45
	GenerationF50	-4.40	0.56	-7.89
	RegimeFL	-2.64	0.56	-4.74
	StrainHYB	-2.56	0.56	-4.59
	StrainMY	-0.16	0.56	-0.29
	GenerationF20:RegimeFL	2.08	0.79	2.64
	GenerationF50:RegimeFL	-0.96	0.79	-1.22
	GenerationF20:StrainHYB	1.36	0.79	1.73
	GenerationF50:StrainHYB	3.84	0.79	4.87
	GenerationF20:StrainMY	1.12	0.79	1.42
	GenerationF50:StrainMY	2.24	0.79	2.84
	RegimeFL:StrainHYB	2.88	0.79	3.65
	RegimeFL:StrainMY	-2.62E-15	0.79	-3.32E-15
	GenerationF20:RegimeFL:StrainHYB	-2.48	1.12	-2.22
	GenerationF50:RegimeFL:StrainHYB	-4.08	1.12	-3.66
	GenerationF20:RegimeFL:StrainMY	-2.64	1.12	-2.37
	GenerationF50:RegimeFL:StrainMY	-0.48	1.12	-0.43
	Random effects:			
	Groups Name	Variance	Std.Dev.	% of Variance
	replicate (Intercept)	0.00	0.00	0.00
	Residual	3.88	1.97	100.00

Table 5.2. Summary of the parameters of the preferred models on (A) life expectancy, *LE*, (B). lifetime fecundity, *LF*, and (C). reproductive lifespan, *RL* (see Table 5.1). Model syntax and AIC values can be seen in Table 5.1. In all models, FL and CO refer to fluctuating and constant environments, respectively.

5.4.4 Age-specific fecundity (m_x)

The initial model to analyse the age-specific fecundity (m_x) contained the fixed terms generation, regime and strain and the corresponding two and three-way interaction terms; the previous analysis on *LF* suggested that all these terms significantly affected fecundity (see *Lifetime fecundity* analysis above). I found that fecundity varied with the age of the female (M1 vs. M0: χ^2= 1744.71, 8 d.f., P<0.001; Table 5.1D). In general, individuals produced more offspring per day in the beginning of their reproductive career (between age 0 and 4), after which their reproductive output declined rapidly (Figure 5.4). The analysis suggested that the inclusion of a two-way interaction term between age and generation improved the model (M2 vs. M1: χ^2= 36.86, 16 d.f., P<0.01; Table 5.1D); suggesting an increase in early fecundity over the course of the experiment. Moreover, the interaction between age and regime was significant (M3 vs. M2: χ^2= 1777.07, 8 d.f., P<0.001; Table 5.1D), suggesting that after the selection experiment, the increase in early fecundity for worms in the constant environment was at age 0 and 2, while for worms in the fluctuating environment happened at age 2 and 4. The strains also varied in their age-specific fecundity (M4 vs. M3: χ^2= 213.97, 16 d.f. , P<0.001; Table 5.1D). For instance, MY individuals produced more offspring between age 0 and 4 compared to the other two strains. Moreover, this high fecundity depended of the generation and the regime (M5 vs. M4: χ^2= 390.73, 96 d.f., P<0.001; Table 5.1D). For instance, the high age-specific fecundity in MY worms was mainly significant across generations for worms in the constant environment.

Regarding the variance components, I found a high age-specific variance between individuals between age 0 and 4 (M6 vs. M5: χ^2= 8294.90, 44 d.f., P<0.001; Table 5.1D). The final model to describe the age-specific fecundity contained the fixed effects of age and strain, their interaction term, generation, regime and strain (all with the two and three-way interactions), and the random effect representing the age-specific variance between individuals (Model M6 in Table 5.1.D and *Appendix I*; Figure 5.4).

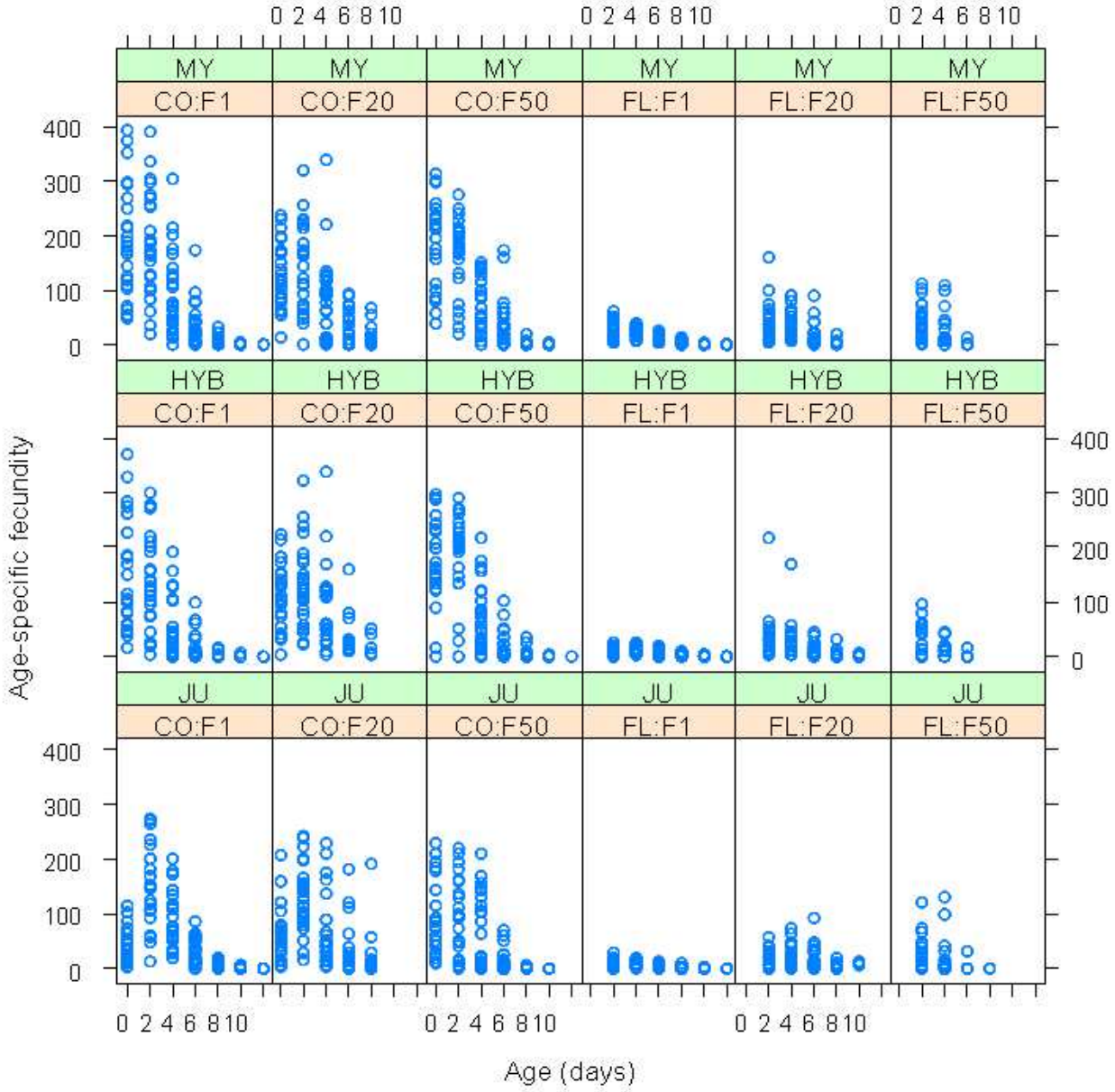

Figure 5.4. Age-specific fecundity of worms of *C. remanei*. The plot represents the observations in the number of offspring produced by MY, HYB and JU (rows) at generation 1, 20 and 50 (F1, F20 and F50, respectively -columns) and cultured in a constant (CO) and fluctuating environment (FL).

5.4.5 Survival (l_x)

I found that the survival rate among individuals declined over the 50 generations (M1 vs. M0: χ^2= 136.86, 2 d.f., P<0.001; Table 5.1E). The analysis suggested that worms in fluctuating environment showed lower survival rate compared to worms in a constant environment (M2 vs. M1: χ^2= 131.66, 1 d.f., P<0.001; Table 5.1E), and after the selection experiment, the decline in survival rate was steepest for worms in the fluctuating regime (M3 vs. M2: χ^2= 181.17, 2 d.f., P<0.001; Table 5.1E). The survival rate varied across strain (M4 vs. M3: χ^2= 7.22, 2 d.f., P<0.05; Table 5.1E); on average, JU had higher survival rte. However, the differences between the survival rate across strains were mainly present at the beginning of the experiment (M5 vs. M4: χ^2= 107.02, 10 d.f., P<0.001; Table 5.1E). Moreover, the analysis suggested that the probability of dying was not constant across time (log-likelihood of Exponential model = 2261.10), instead, the mortality rate increased with age (Weibull model =3092.16; Exponential vs. Weibull: M5 vs. M6: χ^2= 831.05, 1 d.f., P<0.001; Table 5.1E). The preferred model included the fixed terms of generation, regime and strain and the two- and three-way interaction between them (M5; Table 5.1E and Table 5.3; Figure 5.5).

Parameters	Value	Std. Error	z	p
(Intercept)	3.15	0.05	65.14	<0.001
GenerationF20	-0.37	0.07	-5.45	<0.001
GenerationF50	-0.51	0.07	-7.51	<0.001
RegimeFL	-0.28	0.07	-4.12	<0.001
StrainHYB	-0.13	0.07	-1.97	<0.01
StrainMY	-0.27	0.07	-4.02	<0.001
GenerationF20:RegimeFL	-0.28	0.10	-2.92	<0.01
GenerationF50:RegimeFL	-0.61	0.10	-6.33	<0.001
GenerationF20:StrainHYB	-0.22	0.10	-2.31	<0.01
GenerationF50:StrainHYB	0.23	0.10	2.36	<0.01
GenerationF20:StrainMY	0.09	0.10	0.95	0.34
GenerationF50:StrainMY	0.39	0.10	4.07	<0.001
RegimeFL:StrainHYB	0.13	0.10	1.38	0.16
RegimeFL:StrainMY	0.26	0.10	2.66	<0.001
GenerationF20:RegimeFL:StrainHYB	0.66	0.14	4.85	<0.001
GenerationF50:RegimeFL:StrainHYB	-0.40	0.14	-2.92	<0.01
GenerationF20:RegimeFL:StrainMY	-0.20	0.14	-1.49	0.14
GenerationF50:RegimeFL:StrainMY	-0.43	0.14	-3.12	<0.01
Log(scale)	-1.42	0.04	-40.23	<0.001

Table 5.3. Descriptive statistics of the survival model of the evolution of survivorship.

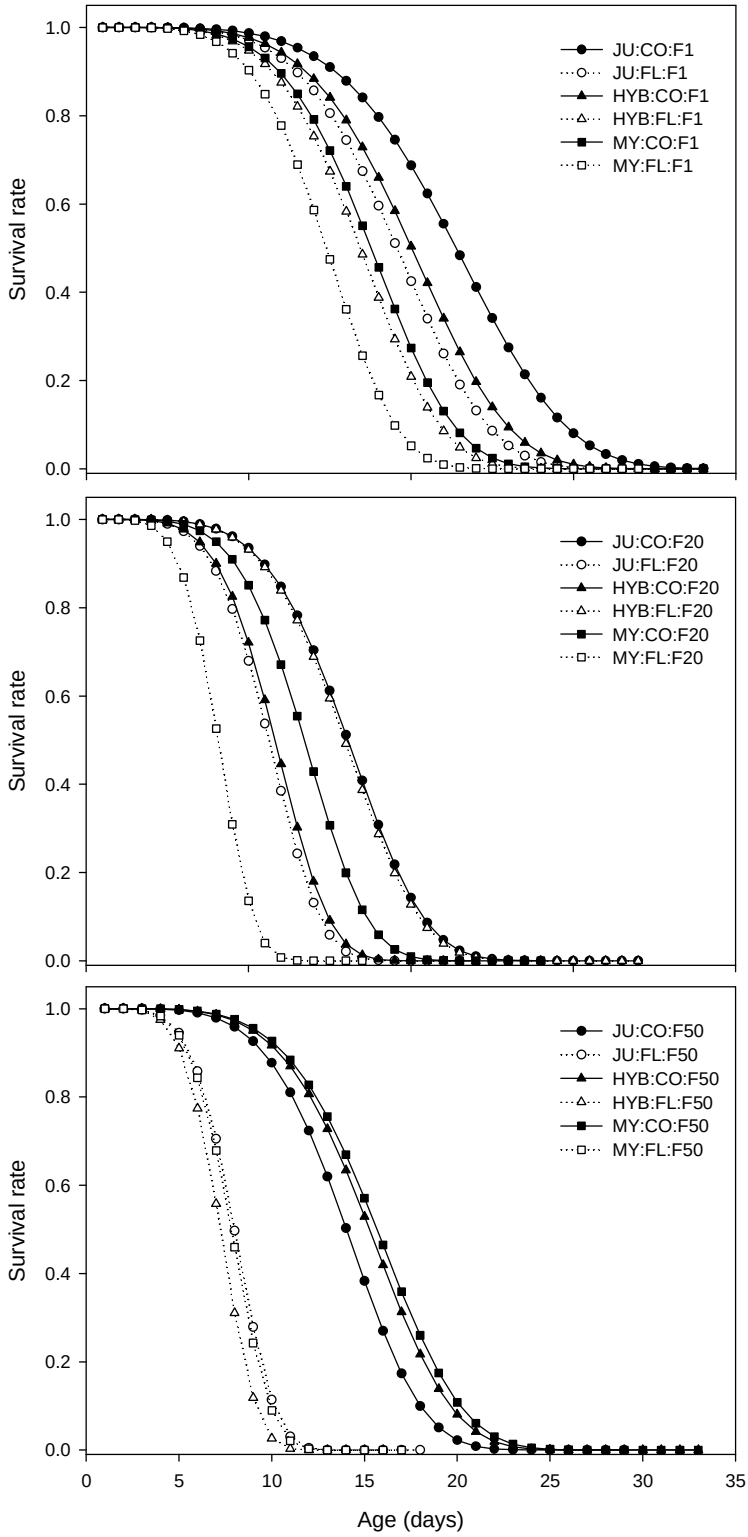

Figure 5.5. Surivival rate of *C. remanei*. The plot represents the proportion of females alive of MY, HYB and JU at generation 1, 20 and 50 (F1, F20 and F50, respectively) and cultured in a constant (CO) and fluctuating environment (FL).

5.4.6 Demography

The generation time (*T*) is shown in Table 5.4. I found that *T* was different between environments and across generations ($F_{3,56}$= 86.91, P<0.001). Worms in a fluctuating environment had longer generation time. In addition, worms from both environments showed a reduction in *T* over the course of the experiment. Although the average *T* was not different between strains ($F_{2,54}$=2.05, P=0.14), I found significant differences between environments and across generations ($F_{11,48}$=7.66, P<0.001).

		Constant		Fluctuating	
		Mean	SE	Mean	SE
F1	JU	2.82	0.09	3.73	0.11
	MY	2.38	0.1	3.54	0.07
	HYB	2.36	0.09	3.77	0.08
F50	JU	2.42	0.09	3.37	0.09
	MY	2.35	0.03	3.68	0.05
	HYB	2.33	0.02	4.36	0.22

Table 5.4. Descriptive statistics of the average generation time (*T*) between families.

5.5 Discussion

Despite considerable theoretical research, the evolution of life-history traits in fluctuating environments has not been extensively investigated at the empirical level (see, Scheiner and Yampolsky, 1998; Hughes et al., 2007). In this study, I conducted an experiment to study the effects of culturing the nematode *C. remanei* under two environmental regimes (constant or predictably-fluctuating temperatures) for a period of 50 generations. I quantified four important life-history components (life expectancy, lifetime fecundity, reproductive lifespan, and age-specific fecundity) at the beginning of the experiment and at generations 20 and 50, and compared the results between two wild-type strains, JU724 and MY12-G, and a half-diallel cross between them. My results show that the fluctuating environment (changing from 5 to 25 °C every 12 hours) reduced all fitness components compared with the constant environment - lifespan was reduced by up to 30%, lifetime fecundity by up to 90%, and reproductive lifespan by up to 24%. Therefore, it is clearly more difficult for the nematodes to perform optimally in a fluctuating temperature regime. In addition, after 50 generations of selection in each environment, life expectancy and reproductive lifespan were also reduced in comparison with the ancestor line in each environment (between 40 and 60 % each). However, the overall lifetime fecundity showed

no such reduction (or improvement) in either of the environmental regimes compared to the starting point before the experiment.

5.5.1 Consequences of living in fluctuating environments on life-history evolution

In accordance with the theory of life-history evolution in unfavourable environments (Murphy, 1968; Roff, 2002), the results from this study show that fluctuating environments reduced the overall female fecundity compared with individuals in a constant environment. This is not surprising since it is clear that low and high temperatures are not optimal for *C. remanei,* or other nematodes (see Chapter 4). However, to my knowledge, there is little empirical research examining the evolutionary consequences of living in fluctuating temperatures. In this study, adaptation to a fluctuating environment potentially occurred through changes in the timing of reproduction (maturation time and age-specific fecundity) and adult survival.

Although early maturation could be beneficial in terms of fitness, it is agreed that delayed maturity can be beneficial if postponed growth increases fertility (Wilbur and Rudolf 2006). In this study, starting the fitness assays in the fluctuating environment took longer compared with the constant treatment (*ca.* 2 days more), mainly due to the lack of pregnant females. Delayed developmental time in a fluctuating environment could be the result of low temperatures delaying physiological processes such as cell division, or the speed of chemical/enzymatic reactions (Hochachka and Somero, 2002). Temperatures could also affect foraging efficiency through reduced locomotion and pharyngeal movement in nematodes (Dusenbery et al., 1978; Dusenbery and Barr, 1980; Raegan et al., 2001).

Once maturation was reached, females selected to grow in a fluctuating environment increased their reproductive effort during their early lifetime (between age 2 and 4) compared with worms at the beginning of the experiment in the same environment. This translates to a change in generation time of 3.80 days at the beginning of the experiment to 3.38 after the selection experiment - an 11.05 % reduction in generation time. Since high early fecundity has important consequences for fitness (Cole, 1954; Caswell, 1989, 2001; Roff 1992; Stearns, 1992), other things being equal, F50 worms would out-compete F1 worms due to the high early fecundity and the resulting faster population growth rate.

In addition, females selected for a fluctuating environment displayed a significant reduction in life expectancy compared with worms at the start of the experiment in the same environment. Moreover, the survival analysis suggested that mortality increased with age. According to life-history theory, either high or variable adult mortality will tend to generate evolutionary pressures towards early fecundity and shorter reproductive lifespan (Murphy, 1968). Therefore, evolutionary pressures might have resulted in changes in resource allocation, by increasing early female reproductive effort and reducing energy availability for adult maintenance (i.e. cost of reproduction).

The cost of reproduction is a pivotal trade-off around which life histories are thought to evolve (Williams, 1966; Stearns, 1992; Roff, 2002; Harshman and Zera, 2006). The traditional theory suggests that the energy available for physiological processes is limited, thus giving rise to trade-offs such as current reproduction versus future reproduction and survival (Stearns, 1992; Roff, 2002). Numerous experimental studies using Drosophila, for instance, have found such a trade-off (e.g. Rose and Charlesworth, 1981; Rose, 1984; Foley and Luckinbill, 2001). Moreover, manipulative experiments suggest that the trade-off is mediated by resource allocation (e.g. Foley and Luckinbill, 2001). For instance, increased early fecundity in Drosophila, as a consequence of direct selection on juvenile feeding rate, results in individuals that accumulate more lipids but display reduced life expectancy as adults (Foley and Luckinbill, 2001). Thus, the reduction in adult survival of *C. remanei* females could be the consequence of an increase in reproductive effort.

5.5.2 Consequences of living in a constant environment on life-history evolution

For worms in a constant regime, the results suggest adaptation to rearing condition by increasing early fecundity. These results are in accordance with life-history theory, suggesting that natural selection will favour “fast” life cycles (Wilbur and Rudolf, 2006), and evolutionary experiments using natural populations reared under constant and favourable laboratory conditions (e.g. *D. melanogaster*, Sgro and Partridge 2000, Rego et al., 2007; *D. subobscura*, Matos et al., 2000, 2002, Rego et al., 2007). For example, Rego and collaborators (2007) found that wild populations of *D. subobscura* showed signs of an increase in early fecundity after being reared under favourable laboratory conditions for 43 generations. Moreover, similar results have been found in laboratory populations of the house mouse *Mus musculus*, a finding thought to be caused by inadvertent selection that

favors early maturation in the laboratory (Bronson, 1984). Thus, individuals that are less likely to escape and more likely to produce progeny in the first few generations of confinement to laboratory housing are potentially selected (Miller et al., 2002). Furthermore, these populations of mouse were more likely to undertake inbreeding (Miller et al., 2002). In the current experiment, the hybrid population was expected to be less likely affected by inbreeding. Moreover, it was expected that the hybrid would show an increased potential for change in traits such as lifetime fecundity, as a consequence of higher additive genetic variance compared with the parental populations. However, the changes in the life-history traits studied here were similar across all the strains. One explanation for the lack response in lifetime fecundity might be that fecundity is already maximised under favourable conditions in the laboratory.

5.5.3 Predictable- vs. unpredictable- fluctuating environments

In theory, predictable environments can select for single genotypes, giving rise to different phenotypes (Roff, 2002). Conversely, if the environment is variable but unpredictable (the environment changes randomly between the time of development of the trait and the time of selection), the population will evolve towards a single phenotype that represents the optimal compromise among environmental states (Scheiner and Yampolsky, 1998). Experimental studies using *Daphnia pluex* have failed to select for plastic genotypes in variable but predictable environments (Scheiner and Yampolsky, 1998). Scheiner and Yampolsky (1998) cultured populations in temporally constant (20 °C), predictable- (i.e. 12 days at 17 °C, 6 days at 20 °C, 12 days at 23 °C, 6 days at 20 °C) and unpredictable-fluctuating temperature (randomly changing every 3 days between 17, 6 and 20 °C). They found that populations exposed to each environment different in the growth rate in the amount of plasticity or adaptation to variable environments (Scheiner and Yampolsky, 1998). Moreover, neither of the fluctuating environments showed maintenance of genetic diversity compared to the constant environment.

In the present study, the temperature was changed every 12 hours in the fluctuating environment. Under favourable conditions, *C. remanei*'s life cycle takes approximately 2.4 days (Diaz et al., in press) while it takes approximately 3.5 days in fluctuating conditions. Hence, in the fluctuating environment, each worm could have experienced each extreme temperature at least three times. Although the transition between temperatures was relatively fast, one temperature might have been more favourable for reproduction. For example, bacteria growing in fluctuating environments show greater preference for high temperatures (Bennett et al., 1992). If there are cues in the environment that provide

information about the present and future conditions, we can expect organisms that perceive such cues to respond rapidly and accurately to environmental change (Pigliucci, 2001). If phenotypic plasticity is heritable, then it would be under strong selection (Pigliucci, 2001). From an evolutionary perspective, it would be important to determine whether populations selected for fluctuating environments are characterised by having phenotypic plasticity. Studies using *E. coli* suggest that fluctuating environments (either temperature or pH) select for populations with phenotypic plasticity (Bennett and colleagues, 1992; Hughes et al., 2007). However, contrary to predictions, having phenotypic plasticity seems to incur no cost: there are no recorded reductions in fitness when these populations are tested in a constant environment, suggesting that the 'jack of all trades' may still be the 'master of all' (Hughes et al., 2007).

5.5.4 Significance of living in a fluctuating environment for nematodes

The results show that thermally-fluctuating conditions affected *C. remanei*'s life-history. To date, little is known about the ecology of free-living nematodes in the wild. Caenorhabditis species have been previously isolated from a wide range of habitats around the world (Sudhaus and Kiontke, 2007). *C. remanei* has been previously collected in several areas of North America, Europe and Asia (Sudhaus, 1974; Baird, 1999; Barriere and Felix, 2005; Sudhaus and Kiontke, 2007) and recent studies suggest that it might be restricted to temperate latitudes (Sudhaus and Kiontke, 2007). Some research has suggested that natural populations of Caenorhabditis potentially experience a wide range of environments in the wild (Sudhaus and Kiontke, 2007). For example, *C. drosophilae* and *C. sonorae* may regularly encounter temperatures of 31°C, much higher than the average desert temperature (Kiontke, 1999). However, the precise thermal niche of wild Caenorhabditis has not yet been confirmed.

5.5.5 Conclusion

Results from this experiment show that changing environments are not optimal for *C. remanei*. Moreover, the evolutionary experiment suggests that there was an evolutionary pressure for increasing early fecundity and reduced adult survival. In addition, due to the stressful temperatures and physiological constraints, worms potentially delayed maturation in the fluctuating compared with the constant environment.

6 Fluctuating environmental conditions select for increased phenotypic plasticity, but with a cost: an experimental evaluation using *Caenorhabditis remanei*

6.1 Abstract

Environment varies temporally and spatially. This makes it very difficult for any genotype to have a perfect match with any environment, as changes in gene frequencies are likely to lag environmental fluctuations as a result of the delayed effects of selection. One solution is phenotypic plasticity, the ability of a single genotype to produce different phenotypes in response to different environments. When phenotypic plasticity is adaptive, it provides organisms with the potential to respond rapidly and effectively to environmental change. Nevertheless, it potently incurs a cost. In this study I use the nematode *Caenorhabditis remanei* to study the evolution of phenotypic plasticity in populations exposed to two environmental regimes (constant vs. predictably fluctuating temperature). The results of the experiment show changes in the reaction norm in response to temperature; at the lowest temperature worms from a fluctuating environment increased their average fecundity and survival by 332 offspring and 51%, respectively, relative to worms maintained in a constant environment, while worms from a fluctuating environment at the highest temperature reduced their average fecundity and survival by 11 offspring and 50%, respectively, relative to worms in a constant environment. Therefore, phenotypically plastic worms showed a wider thermal breath at low temperatures compared to worms selected for a constant environment. Moreover, both the survival and fecundity of worms selected to grow in a fluctuating environment significantly declined when moved back to a constant environment, suggesting that increased phenotypic plasticity has a fitness cost that is manifest in more stable environments.

6.2 Introduction

Species and populations are, to a greater or lesser extent, affected by external environmental factors. Fossil record data (Davis and Shaw, 2002) and natural population studies (Parmesan, 2006) have shown well-observed effects of climate on the distribution and life history of numerous species. However, defining by what mechanisms populations respond to novel environmental conditions – thereby avoiding extinction - is not so straightforward.

From an evolutionary perspective, natural selection has been an important process in shaping species' life histories by favouring strategies that suit local environmental conditions (e.g. Grant and Grant, 1993). Evolution by natural selection is expected to be limited by the genetic diversity present in the population (Fisher, 1930). Moreover, changes in gene frequencies are inevitably constrained by generation time, leading to a potential lag between corresponding phenotypic changes and environmental conditions. (Charlesworth, 1980). Thus, the persistence of an organism exposed to climatic stress or a novel environment may depend on behavioural and physiological changes of a single genotype that can thrive in different environments, rather than evolutionary responses of the genes (Hoffmann, 1995). Phenotypic plasticity is, therefore, considered a potential solution to the challenge of persisting in a changing environment (Schlichting and Pigliucci, 1998; Davis et al., 2005; Charmantier et al., 2008; Gienapp et al. 2008).

Recent research has highlighted the significance of phenotypic plasticity, identifying it as an important property which provides the potential for organisms to respond rapidly and effectively to environmental change (e.g. Charmantier et al., 2008). Phenotypic plasticity can be defined as the characteristic of a particular genotype to produce different phenotypes in response to environmental conditions (Schlichting and Pigliucci 1998). It can be quantified using the *reaction norm*, where a trait of a genotype is described as a function of an environmental gradient (Via et al., 1995).

Examples of phenotypic plasticity are numerous across taxa and show a high diversity across traits, including changes in morphological traits, for example leaf morphology between aerial and aquatic leaves of a plant (e.g. *Proserpinaca spp.*, Bradshaw, 1965), a switch between winged and wingless aphid morphs in response to host plant quality (*Acyrthosiphon pisum*, Dixon and Agarwala, 1999; Muller et al 2001), and life history traits, such as developmental time, for example nematodes of the

Caenorhabditis genus have longer lifecycles at low temperatures compared with high temperatures (*C. elegans*, Byerly et al., 1976; *C. remanei*, see Chapter 4).

However, not all plastic traits are expected to be adaptive (West-Eberhard 2003). Demonstrating adaptiveness in phenotypic plasticity requires showing that plastic individuals can cope with a range of environments better than less plastic ones, and that plasticity is genetically conferred, and can therefore be modified through natural selection (West-Eberhard, 2003). It is generally accepted that there is genetic variation in natural populations for plastic responses (Pigliucci, 2001). However, our understanding about evolutionary pressures that select for, or against, plasticity is still limited; it is not clear if plasticity can be artificially selected, which mechanisms control it, or whether having plasticity incurs any costs.

In theory, predictability in the environmental fluctuations can select for individuals that vary their life histories according to environmental cues (Roff, 2002). The cost of increased plasticity is likely to be expressed in terms of a decline in fitness in some environments, potentially due to the production and maintenance of genetic and cellular machinery necessary for it (Scheiner, 1993, DeWitt et al., 1998). For instance, to be able to detect changes (i.e. cues) in environmental conditions, individuals must allocate energy during development for producing and maintaining specific sensory machinery. This allocation will reduce the energy available for other activities and fitness traits such as fecundity (DeWitt et al., 1998). Although the concepts are well established, empirical research examining the costs of phenotypic plasticity on animals are accumulating only slowly (DeWitt et al., 1998; e.g. Scheiner and Berrigan, 1998; Scheiner and Yampolsky, 1998). Scheiner and Yampolsky (1998) conducted an evolutionary experiment to select for plasticity by raising populations of species under different environmental regimes (constant, predictably fluctuating and randomly fluctuating, Scheiner and Yampolsky, 1998) and their results showed that, contrary to theoretical expectations, populations in fluctuating environments were less plastic.

In this study, populations of the free-living nematode *C. remanei* and a selection experiment were used to test 1) whether environmental variability selects for plasticity and 2) whether having increased plasticity incurred any cost manifest in more constant environments. Previous research showed that wild-type populations of *C. remanei* exhibited plasticity in fitness components in response to temperature under laboratory conditions (Chapter 4). Moreover, populations of this nematode have been maintained under constant and predictably-fluctuating temperatures for 50 generations (Chapter 5).

The analysis of these populations' life history in response to the selection regime suggests adaptation to each environmental regime (Chapter 5). Therefore, changes in the levels of phenotypic plasticity might be expected. Worms selected to grow in a constant environment were expected to show reduced plasticity compared with worms cultured in a fluctuating environment (Experiment 1). To test whether an increase in the plasticity incurred a cost, populations between treatments were transposed at generation 51 and their life-history responses were quantified (Experiment 2). If increased plasticity incurred a cost, individuals with higher plasticity moved back to a moderate and constant environment would be expected to show reduced fitness compared with individuals assayed at the beginning of the experiment.

6.3 Methods

6.3.1 Strains

I used three strains of *C. remanei*, two wild-type strains (JU724 and MY12; originally from China and Germany, respectively) and a half-diallel cross (HYB). The two wild type strains correspond to recently isolated populations from the field (2005 and 2006 each). Samples from these populations were obtained from the Nematode Biological Resource Centre in France and the Animal Ecological Centre in Germany, respectively. The strains were obtained from samples that were kept frozen subsequent to their isolation in the wild so it is reasonable to assume that these populations have not adapted to laboratory conditions. The half-diallel cross (HYB) consisted of the progeny of a female JU724 by a male MY12-G (referred to henceforth as JU and MY, respectively), and reciprocal crosses (Chapter 4). Five replicates (out-bred populations) from each of these three strains have been previously used in another study to characterise the plasticity of their basic life history at a range of temperatures in the laboratory (Chapter 4) and further selected to grow two environmental regimes (Chapter 5).

6.3.2 Experimental procedures

6.3.2.1 Experiment I: Evolution of plasticity

Five replicates, from each of the strains were used to characterise the plasticity of life-history traits of populations in response to a three temperatures (5, 15, and 25 º C).

Lineages from each strain had been previously maintained under two different environmental regimes (CO and FL) for 50 generations. Worms recovered from these stocks were used to assess the changes in plasticity over the course of the selection experiment. Replicates were stored in eppendorf tubes and maintained at -80 °C prior to the current study (Chapter 5). This is a standard procedure (Hope 1999) and has been shown to have no effect on the life-history characteristics of *C. remanei* (Epstein and Shakes, 1995). All individuals were maintained in NGM petri dishes of 50 mm and fed on a lawn of *Escherichia coli* (OP50 strain) using standard protocols (Hope, 1999).

Prior to each assay, a frozen sample from each replicate at generation 50 (F50) was thawed at room temperature for a few minutes and then poured into a 50 mm NGM-petri dish. The following day, it was moved to the assigned temperature (i.e. 5, 15, and 25 º C). Approximately two days later (except for those individuals raised at 5 º C, which required 4 days), five gravid females were randomly selected from each replicate and transferred into individual petri dishes. The L4 offspring from these females were used to initialize all life-history assays.

Life-history assays: Using lab protocols previously developed to quantify the vital rates of *C. remanei* (Diaz et al., in press), life-history assays were conducted on replicates previously frozen at F50. The life-history traits quantified were life expectancy, lifetime fecundity, and age-specific fecundity for individuals cultured at each temperature, from each strain (five individual females per replicate) from each environmental regime. As previously shown, mating *C. remanei* females with four males maximised their fecundity (Chapter 3). Therefore, to get an estimate of fecundity that would not be limited by sperm availability, a virgin female was paired with four unrelated young males for 48 hours. On alternate days after this, the female was subsequently transferred into a new petri dish with four new unrelated young males. Transfers were continued until the female stopped laying eggs. The female was then monitored on alternate days to score the date of death. Age-specific fecundity was estimated by counting the number of juvenile larvae present in each plate. Plates were monitored two days after the female was previously transferred to account for the number of larvae observed. In total, 25 females from each population (JU7, MY and HYB), at each temperature, and from each regime, were assessed.

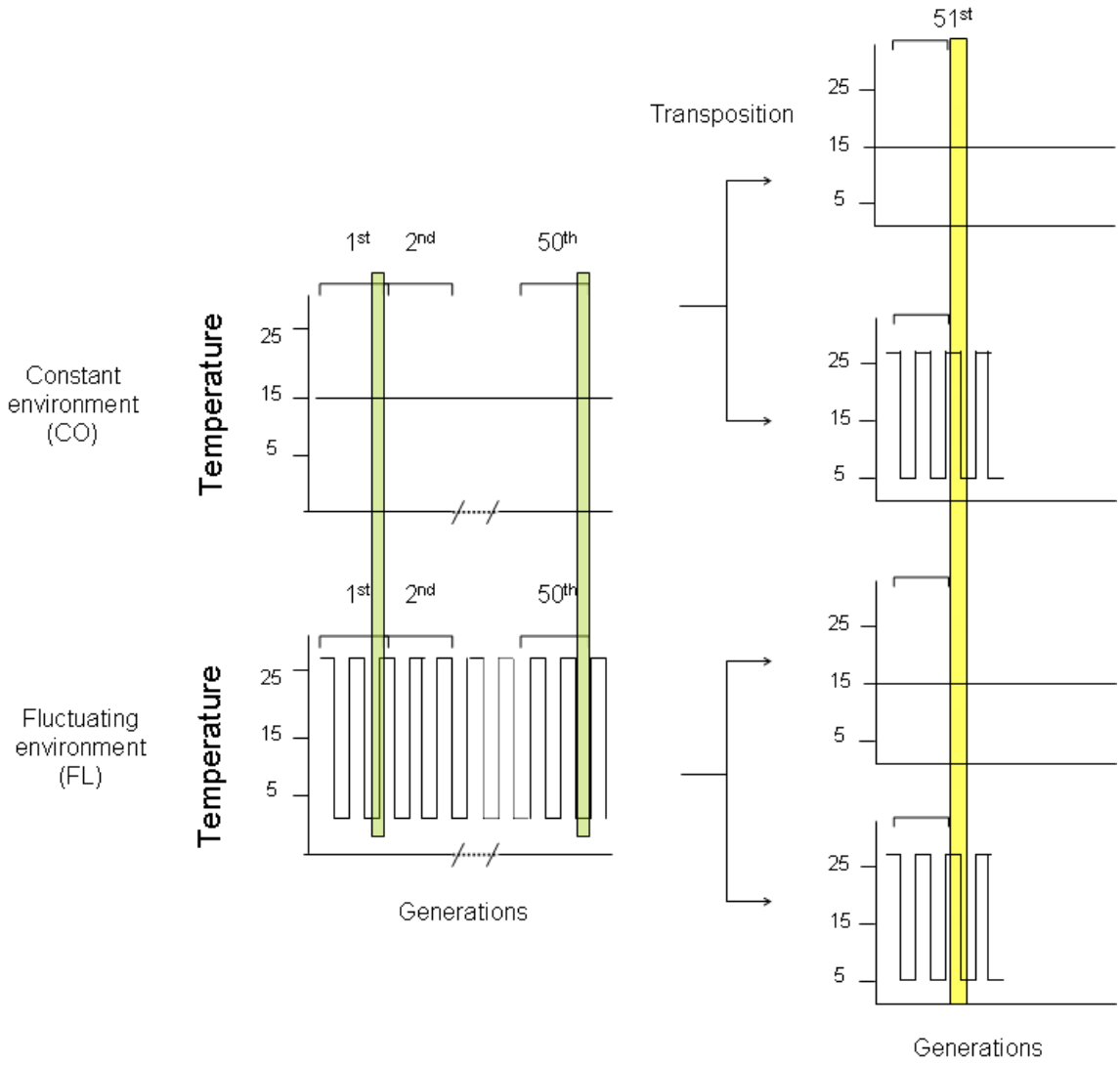

Figure 6.1. Diagram to illustrate the experimental design. Replicates were cultured for 50 generations in each environment (CO and FL). Fitness assays (green bars) were carried out at generation 1 and 50 to quantify changes in plasticity of life-history traits in response to temperature. At generation 50, replicates were subdivided into two populations and transposed between treatments and their life-history traits characterised at generation F51 (yellow bar).

6.3.2.2 Experiment II: Transposition

At generation 50, all replicates were transposed between treatments (i.e. between constant and fluctuating regime) using a fully-crossed design (see Figure 6.1). Since the generation time between regimes was different (Chapter 5), this transposition did not take place on exactly the same dates (see Table 6.1). All replicates were transferred into their assigned environments for one generation, after which fitness assays were conducted in these same environments exactly as described above for the F50 generation.

		Constant	Fluctuating
Start of experiment		04/03	04/03
Frozen sample at	Generation 10	04/25	05/18
"	Generation 20	05/25	07/06
"	Generation 30	06/25	08/24
"	Generation 40	07/24	10/10
"	Generation 50	08/30	11/28
Swapping		08/30	11/28
Frozen sample at	Generation 51	09/03	12/01

Table 6.1. Calendar of dates for the experiment. The selection experiment for both regimes started on the same day (3th of April 2007). Subsequent samples were taken approximately every 50 generations. The translocation between regimes was at different times.

Model construction and comparison: Mixed-effects models were used to analyse the average performance of worms of different strains, over generations, between temperatures and between regimes, together with the pattern of variance within generations, within regimes, within strains, among individuals (within replicates) and between replicates.

Model syntax used here denotes fixed variables with upper case letters and random variables with lower case letters. The subscripts to denote different levels of the data were as follows: *o* for individual observations (1,2,…,450), *n* for the replicate (1,2…,15), *m* for the Strain (1,2 and 3), *l* for the generation (F1, F50CO and F50FL), *k* for the temperature (5, 10 and 15), *j* for the treatment (F1CO, F50CO, F51CO:FL, F1FL, F50FL, and F51FL:CO) and *i* for the Age (0,2,…,14 days) of the *o*th individual (referred in the models as ind).

In Experiment 1, the syntax of the random effects was the following: the "(1| $replicate_{klmn}$)" effect is a random variable (intercept) representing the deviation from the population mean of the average life-history trait for the *n*th replicate , within the *m*th strain, within the *l*th generation at the *k*th temperature (135 levels); and the terms "(strain| $replicate_{klmn}$)", "(generation| $replicate_{klmn}$)" and "(temperature| $replicate_{klmn}$)" denoting the random variable representing the deviation of the *n*th replicate for each strain, generation and temperature, respectively. For the age-specific analysis, the "(age| ind_{klmnoi})" term denotes the random variable representing the deviation of the population mean of the age-specific fecundity for the *o*th individual within the *n*th replicate, within the *m*th strain within the *l*th generation at the *k*th temperature (675 levels). Finally, the ε_{onmlkj} is a random variable representing the deviation of the life-history trait for the *o*th worm of the *n*th replicate within the *m*th strain, at the *l*th generation in the *k*th temperature (Faraway, 2006). For Experiment II, similar syntax was used as for Experiment I, except that treatment (with subscript *j*) was used rather than generation and temperature as factors.

The variance components were presented in terms of percentages of the total variance attributable to each effect (e.g. percentage of the variance within replicates = $\sigma_{replicate}^2 / [\sigma_{replicate}^2 + \sigma_{\varepsilon}^2]$, and the percentage of the error variance is presented similarly). I assumed that the deviations for each individual random effect were normal with mean zero and constant variance ($N(0, \sigma^2)$).

Statistical analysis: All statistical analysis was performed 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, version: 0.999375-27). Model comparison was done using Likelihood Ratio Tests (LRT) for nested models. Unless otherwise stated, the results are presented by a mean ± standard error (se).

6.4 Results

6.4.1 Evolution of plasticity

6.4.1.1 *Life expectancy (LE).*

The average number of days lived by a female was significantly different across temperatures (M1 vs. M0: χ^2= 25.32, 2 d.f, P<0.001; Table 6.2A, Figure 6.2). After the selection experiment, the average LE declined in both regimes (M2 vs. M1: χ^2= 391.36, 2 d.f, P<0.001; Table 6.2A). Moreover, the analysis suggested that the plasticity between regimes was different (M3 vs. M2: χ^2=143.40, 4 d.f, <0.001; Table 6.2A). For instance, at generation 1, the average *LE* declined with increasing temperature. However, after the selection experiment, worms from a constant regime showed the highest *LE* at 15 deg C; while worms from a fluctuating regime had similar *LE* across all temperatures. In addition, the analysis suggested that including a term for strain (M4 vs. M3: χ^2=0.32, 2 d.f, P=0.85; Table 6.2A), a strain:temperature interaction term (M5 vs. M3: χ^2=5.84, 6 d.f., P= 0.44; Table 6.2A) and a strain:generation term (M6 vs. M3: χ^2=7.99, 6 d.f., P= 0.24; Table 6.2A) did not improve the model. However, the inclusion of a three-way interaction term between temperature, generation and strain (in addition to the corresponding main effects) significantly improved the model (M7 vs. M3: χ^2=37.82, 18 d.f , P<0.01; Table 6.2A). This suggested that after the selection experiment, although the three strains showed a similar pattern in response to temperature, the precise changes over the selection experiment and their response to each temperature were relatively different. For instance, MY and HYB

strain cultured in a fluctuating environment exhibited relatively low LE at 25 °C compared to JU; while at other temperatures, the reverse was true. Regarding the variance components, the analysis suggested that neither of the random effects (strain, generation or temperature) had a significant effect in the model (AIC M7 < AIC M8, M9 and M10; Table 6.2A). The final model contained the fixed effects of temperature, generation, strain, and a three-way interaction between them and a random effect to describe the hierarchical structure in the data (Model M7, Table 6.3A, Figure 6.2).

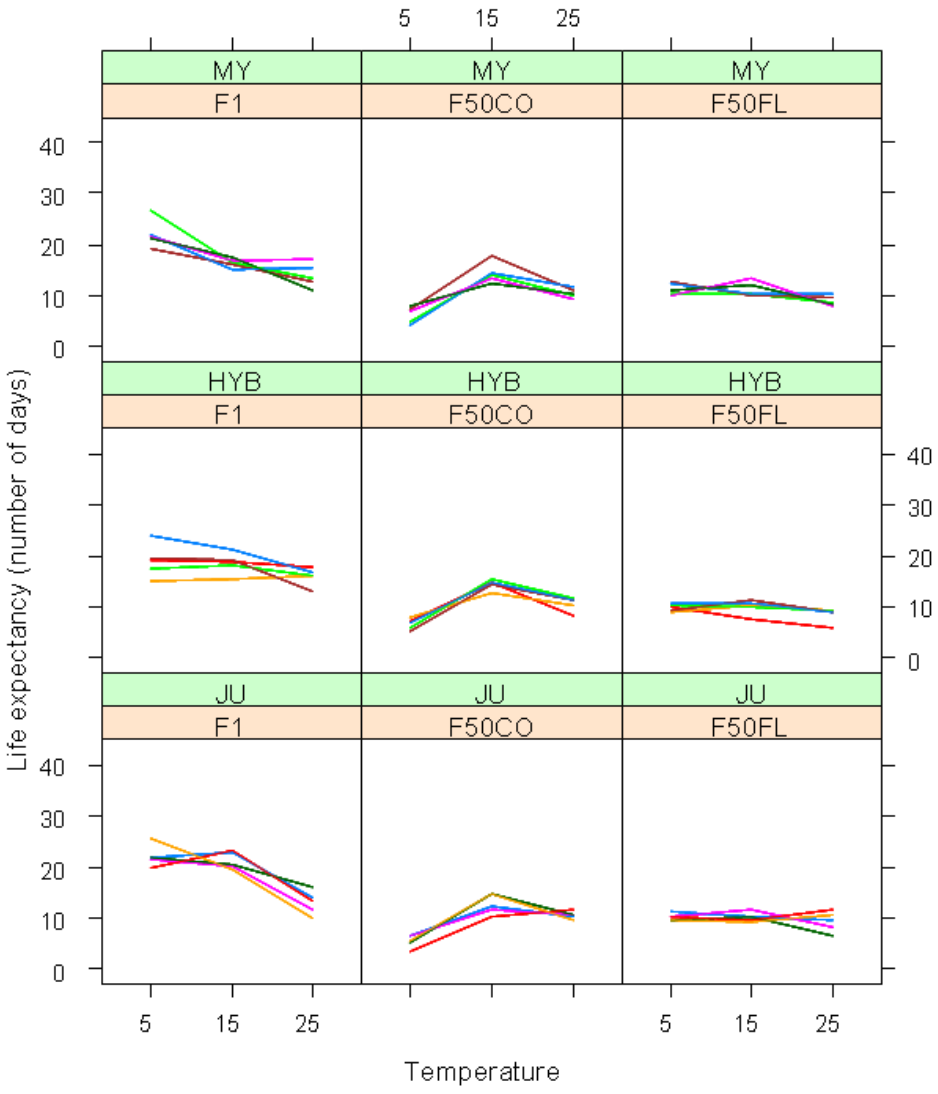

Figure 6.2. Life expectancy of *C. remanei* in response to temperature. Lines represent the plasticity replicates of each strain (MY, HYB and JU) cultured under two environmental regimes (CO and FL) at to generation (F1 and F50).

	Model	Syntax	AIC	logLik	Df
A.	*LE*				
	M0	$1 + (1\|\ \text{replicate}_{klmn})$	4185.6	-2089.8	3
	M1	$\text{Temperature}_k + (1\|\text{replicate}_{klmn})$	4180.0	-2085.0	5
	M2	$\text{Temperature}_k + \text{Generation}_l + (1\|\ \text{replicate}_{klmn})$	4045.2	-2015.6	7
	M3	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{: Generation}_l + (1\|\ \text{replicate}_{klmn})$	3938.2	-1958.1	11
	M4	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{: Generation}_l + \text{Strain}_m + (1\|\ \text{replicate}_{klmn})$	3941.9	-1957.9	13
	M5	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{: Generation}_l + \text{Strain}_m + \text{Temperature}_k\text{: Strain}_m + (1\|\ \text{replicate}_{klmn})$	3944.4	-1955.2	17
	M6	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{: Generation}_l + \text{Strain}_m + \text{Generation: Strain}_m + (1\|\ \text{replicate}_{klmn})$	3942.2	-1954.1	17
	M7	$\mathbf{\text{Temperature}_k * \text{Generation}_l * \text{Strain}_m + (1\|\ \text{replicate}_{klmn})}$	**3936.4**	**-1939.2**	**29**
	M8	$\text{Temperature}_k * \text{Generation}_l * \text{Strain}_m + (\text{strain}\|\ \text{replicate}_{klmn})$	3946.4	-1939.2	34
	M9	$\text{Temperature}_k * \text{Generation}_l * \text{Strain}_m + (\text{generation}\|\ \text{replicate}_{klmn})$	3946.4	-1939.2	34
	M10	$\text{Temperature}_k * \text{Generation}_l * \text{Strain}_m + (\text{temperature}\|\ \text{replicate}_{klmn})$	3946.4	-1939.2	34
B.	*LF*				
	M0	$1 + (1\|\ \text{replicate}_{klmn})$	8408.0	-4200.0	4
	M1	$\text{Temperature}_k + (1\|\ \text{replicate}_{klmn})$	8410.0	-4200.0	5
	M2	$\text{Temperature}_k + \text{Generation}_l + (1\|\ \text{replicate}_{klmn})$	8453.8	-4223.9	3
	M3	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + (1\|\ \text{replicate}_{klmn})$	8329.9	-4159.9	5
	M4	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_m + (1\|\ \text{replicate}_{klmn})$	8323.2	-4154.6	7
	M5	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_n + \text{Temperature}_l\text{: Strain}_m + (1\|\ \text{replicate}_{klmn})$	8273.5	-4125.8	11
	M6	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_m + \text{Temperature}_k\text{: Strain}_m + \text{Generation}_l\text{: Strain}_m + (1\|\ \text{replicate}_{klmn})$	8247.9	-4111.0	13
	M7	$\text{Temperature}_k * \text{Generation}_l * \text{Strain}_m + (1\|\ \text{replicate}_{klmn})$	8218.1	-4092.1	17
	M8	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_m + \text{Temperature}_k\text{: Strain}_m + \text{Generation}_l\text{: Strain}_m + (\text{strain}\|\ \text{replicate}_{klmn})$	8214.1	-4086.0	21
	M9	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_m + \text{Temperature}_l\text{: Strain}_m + \text{Generation}_l\text{:Strain}_m + (\text{generation}\|\ \text{replicate}_{klmn})$	8221.5	-4081.8	29
	M10	$\mathbf{\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_m + \text{Temperature}_k\text{: Strain}_m + \text{Generation}_l\text{: Strain}_m + (\text{temperature}\|\ \text{replicate}_{klmn})}$	**8212.3**	**-4080.2**	**26**
C.	m_x				
	M0	$\text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_m + \text{Temperature}_k\text{:Strain}_m + \text{Generation}_l\text{:Strain}_m + (1\|\text{ind}_{klmnoi})$	63317	-31637	21
	M1	$\text{Age}_i + \text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_m + \text{Temperature}_k\text{:Strain}_m + \text{Generation}_l\text{:Strain}_m + (1\|\text{ind}_{klmnoi})$	61187	-30565	29
	M2	$\text{Age}_i * \text{Temperature}_k + \text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_m + \text{Temperature}_k\text{:Strain}_m + \text{Generation}_l\text{:Strain}_m + (1\|\text{ind}_{klmnoi})$	59474	-29692	45
	M3	$\text{Age}_i * \text{Temperature}_k + \text{Age}_i * \text{Generation}_l + \text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{:Generation}_l + \text{Strain}_m + \text{Temperature}_k\text{:Strain}_m + \text{Generation}_l\text{:Strain}_m + (1\|\text{ind}_{ponml})$	59349	-29614	61
	M4	$\text{Age}_i * \text{Temperature}_k + \text{Age}_i * \text{Generation}_l + \text{Age}_i * \text{Strain}_m + \text{Temperature}_k + \text{Generation}_l + \text{Temperature}_k\text{: Generation}_l + \text{Strain}_m + \text{Temperature}_k\text{: Strain}_m + \text{Generation}_l\text{:Strain}_m + (1\|\text{ind}_{klmnoi})$	59251	-29548	77
	M5	$\text{Age}_i * \text{Temperature}_k + \text{Age}_i * \text{Generation}_l + \text{Age}_i * \text{Strain}_m + \text{Age}_i\text{: Temperature}_k\text{:Generation}_l + \text{Temperature}_k\text{: Strain}_m + \text{Generation}_l\text{: Strain}_m + (1\|\text{ind}_{klmnoi})$	58650	-29216	109
	M6	$\text{Age}_i * \text{Temperature}_k + \text{Age}_i * \text{Generation}_l + \text{Age}_i * \text{Strain}_m + \text{Age}_i\text{: Temperature}_k\text{:Generation}_l + \text{Age}_i\text{:Temperature}_k\text{: Strain}_m + \text{Generation}_l\text{: Strain}_m + (1\|\text{ind}_{klmnoi})$	58499	-29109	141
	M7	$\text{Age}_i * \text{Temperature}_k + \text{Age}_i * \text{Generation}_l + \text{Age}_i * \text{Strain}_m + \text{Age}_i\text{: Temperature}_k\text{: Generation}_l + \text{Age}_i\text{:Temperature}_k\text{: Strain}_m + \text{Age}_i\text{: Generation}_l\text{: Strain}_m + (1\|\text{ind}_{klmnoi})$	58415	-29034	173
	M8	$\mathbf{\text{Age}_i * \text{Temperature}_k + \text{Age}_i * \text{Generation}_l + \text{Age}_i * \text{Strain}_m + \text{Age}_i\text{: Temperature}_k\text{: Generation}_l + \text{Age}_i\text{: Temperature}_k\text{: Strain}_m + \text{Age}_i\text{: Generation}_l\text{: Strain}_m + (\text{age}\|\text{ind}_{klmnoi})}$	**38275**	**-18920**	**217**

Table 6.2. AIC and log likelihood (logLik) values for models to describe the plasticity of life history after the selection experiment. Bold letters represent the preferred model. Random variables are included within brackets. The symbol ":" denotes an interaction and "*" denotes an interaction plus the corresponding main terms.

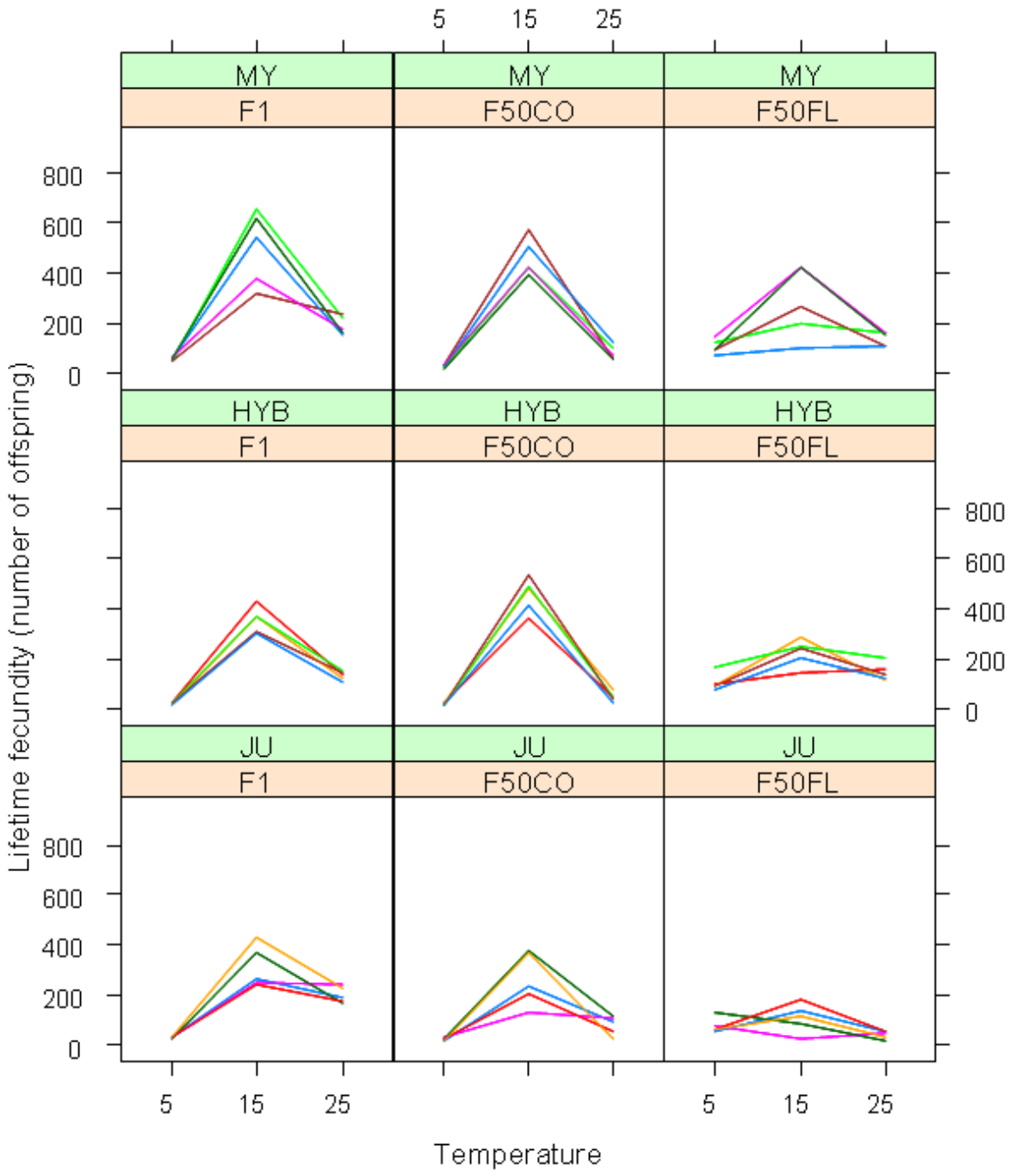

Figure 6.3. As figure 2, but showing the lifetime fecundity of females of *C. remanei*.

6.4.1.2 *Life fecundity (LF)*

The total number of offspring produced by a female varied across temperatures (M1 vs. M0: $\chi 2$= 9.62, 2 d.f., P<0.001; Table 6.2B). For instance, fecundity at 5 and 25 ° C was lower compared to that observed at 15 ° C (Figure 6.3). After the selection experiment, the *LF* of worms from each regime was different (M2 vs. M1: $\chi 2$= 138.80, 2 d.f., P<0.001; Table 6.2B); worms growing in a fluctuating regime exhibited lower LF at the beginning

of the experiment compared to worms in a constant regime. Moreover, the effect of the regime was not the same across temperatures (M3 vs. M2: $\chi2$= 115.01, 4 d.f., P<0.001; Table 6.2B). For instance, worms from a fluctuating regime assayed at 15 ° C exhibited lower *LF* compared to worms in a constant regime. However, worms from the fluctuating regime assayed at either 5 or 25 ° C did not exhibit lower *LF* compared to their counterparts from a constant regime. In addition, the analysis suggested that strains did not vary in their average LF (M4 vs. M3: $\chi2$= 0.32, 2 d.f., P=0.85; Table 6.2B), in their response across temperatures (M5 vs. M4: $\chi2$= 5.84, 6 d.f., P=0.44; Table 6.2B) or between generations (M6 vs. M5: $\chi2$ = 7.99, 6 d.f., P=0.24; Table 6.2B). However, a model including a three-way interaction term between temperature, generation and strain (in addition with their main terms) had a significant effect in the model (M7 vs. M6: $\chi2$ = 37.82, 18 d.f., P<0.01; Table 6.2B). Regarding the variance components, the analysis suggested a model containing a variance term for each strain (M8 vs. M7: $\chi2$= 0.03, 5 d.f., P>0.05; Table 6.2B) or for each generation (M9 vs. M7: $\chi2$= 1.16e-06, 5 d.f., P>0.05; Table 6.2B) was not preferred. However, a model allowing for a variance term for each temperature showed a significant improvement (M10 vs. M7: $\chi2$ = 33.36, 9 d.f., P<0.001; Table 6.2B); the variance at 15 ° C was larger compared to the variance at extreme temperatures. Thus the final model included the fixed terms of temperature, generation, strain, the temperature-generation interaction, strain, temperature-strain interaction, and generation-strain interaction, and a random effect to describe the variance for each temperature (Model M10, Table 6.3B, Figure 6.3).

Model	Parameter	Estimate	Std. Error	t value
A. M7	*LE*			
	Fixed effects:			
	(Intercept)	22.24	0.86	25.98
	Temperature15	-0.96	1.21	-0.79
	Temperature25	-9.28	1.21	-7.67
	GenerationF50_C	-16.80	1.21	-13.88
	GenerationF50_F	-14.00	1.21	-11.57
	StrainHYB	-3.24	1.21	-2.68
	StrainMY	-0.12	1.21	-0.10
	Temperature15:GenerationF50_CO	8.32	1.71	4.86
	Temperature25:GenerationF50_CO	14.48	1.71	8.46
	Temperature15:GenerationF50_FL	0.96	1.71	0.56
	Temperature25:GenerationF50_FL	8.40	1.71	4.91
	Temperature15:StrainHYB	0.56	1.71	0.33
	Temperature25:StrainHYB	6.28	1.71	3.67
	Temperature15:StrainMY	-4.72	1.71	-2.76
	Temperature25:StrainMY	1.24	1.71	0.72
	GenerationF50_CO:StrainHYB	4.44	1.71	2.59
	GenerationF50_FL:StrainHYB	2.80	1.71	1.64
	GenerationF50_CO:StrainMY	0.92	1.71	0.54
	GenerationF50_FL:StrainMY	1.20	1.71	0.70
	Temperature15:GenerationF50_CO:StrainHYB	-0.08	2.42	-0.03
	Temperature25:GenerationF50_CO:StrainHYB	-7.52	2.42	-3.11
	Temperature15:GenerationF50_FL:StrainHYB	-0.36	2.42	-0.15
	Temperature25:GenerationF50_FL:StrainHYB	-6.80	2.42	-2.81

	Temperature15:GenerationF50_CO:StrainMY	5.60	2.42	2.31
	Temperature25:GenerationF50_CO:StrainMY	-2.20	2.42	-0.91
	Temperature15:GenerationF50_FL:StrainMY	4.68	2.42	1.93
	Temperature25:GenerationF50_FL:StrainMY	-2.64	2.42	-1.09
	Random effects:			
	Groups Name	Variance	Std.Dev.	
	replicate (Intercept)	0.000	0.0000	0
	Residual	18.318	4.2799	100
B. M10	*LF*			
	Fixed effects:			
	(Intercept)	46.83	16.31	2.87
	Temperature15	260.87	32.63	8.00
	Temperature25	136.59	19.78	6.91
	GenerationF50_C	-22.97	21.12	-1.09
	GenerationF50_F	10.99	21.12	0.52
	StrainHYB	-37.07	21.12	-1.76
	StrainMY	4.53	21.12	0.22
	Temperature15:GenerationF50_CO	16.61	35.75	0.47
	Temperature25:GenerationF50_CO	-92.33	21.67	-4.26
	Temperature15:GenerationF50_FL	-245.05	35.75	-6.86
	Temperature25:GenerationF50_FL	-125.39	21.67	-5.79
	Temperature15:StrainHYB	111.57	35.75	3.12
	Temperature25:StrainHYB	-4.40	21.67	-0.20
	Temperature15:StrainMY	169.47	35.75	4.74
	Temperature25:StrainMY	9.76	21.67	0.45
	GenerationF50_C:StrainHYB	33.73	25.16	1.34
	GenerationF50_F:StrainHYB	99.53	25.16	3.96
	GenerationF50_C:StrainMY	-1.25	25.16	-0.05
	GenerationF50_F:StrainMY	47.47	25.16	1.89
	Random effects:			
	Groups Name	Variance	Std.Dev.	
	replicate temperature5	0.00	0.00	0.00
	temperature15	6060.20	77.85	40.77
	temperature25	0.00	0.00	0.00
	Residual	8804.90	93.83	59.23

Table 6.3. Summary of models describing changes in plasticity of A) life expectancy and B) lifetime fecundity after the selection experiment. Model syntax and AIC values can be seen in Table 6.2.

6.4.1.3 *Age-specific fecundity (m_x)*

The number of offspring produced by a female declined over time (M1 vs. M0: $\chi 2$ = 2145.30, 8 d.f., P<0.001; Table 6.2C). Females were more fecund between age 0 and 6 days, with a rapid decline thereafter (Figure 6.4). The analysis suggested a significant interaction between age and temperature (M2 vs. M1: $\chi 2$ = 1745.20, 16 d.f., P<0.001; Table 6.2C), suggesting that extreme temperatures decreased early fecundity. Moreover, 5 °C was more detrimental compared to 25 °C. Similar to the lifetime fecundity analysis, worms selected for a fluctuating environment displayed lower age-specific fecundity compared to worms in the constant environment (M3 vs. M2: $\chi 2$ = 157.23, 16 d.f., P<0.001; Table 6.2C), and MY produced relatively more offspring at age 0 and 2 (M4: vs. M3: $\chi 2$ = 130.40, 16 d.f., P<0.001; Table 6.2C). In addition, I found that the inclusion of a three-way interaction between age, temperature and regime further improved the model (M5 vs. M4: $\chi 2$ = 664.74, 32 d.f., P<0.001; Table 6.2C); suggesting that despite worms

from the fluctuating environment having lower age-specific fecundity at 15 °C compared to worms selected in a constant environment, worms from the fluctuating environment exhibited higher fecundity at age 0 and 2 at extreme temperatures than their counterparts from the constant environment (Figure 6.4). Further analysis suggested a significant interaction of strain and both: the age-temperature interaction (M6 vs. M5: $\chi 2 = 214.41$, 32 d.f., $P<0.001$; Table 6.2C) and the age-regime interaction (M7 vs. M6: $\chi 2 = 148.76$, 32 d.f., $P<0.001$; Table 6.2C). These findings suggested that MY exhibited higher early fecundity mainly at 15 °C. However, the differences between strains were small at extreme temperatures. Moreover, MY worms at the beginning of the experiment and selected for the constant environment had higher early fecundity compared to their counterparts. However, these differences were not large in the fluctuating environment. Finally, regarding the variance component, I found a high age-specific variance between individuals (M8 vs. M7: $\chi 2 = 20228.00$, 44 d.f., $P<0.001$; Table 6.2C). The between-individual variance was large during their early life (between age 0 and 4), later the variance between individuals decreases rapidly. The preferred model included the fixed terms of age, temperature, regime, strain, and a two-way interaction term between age and temperature, regime and strain, in addition with three three-way interactions: age-temperature-regime, and age-temperature-strain and age-regime-strain, and a random effect describing the age-specific variance (Model M8 Table 6.2C and *Appendix II*; Figure 6.4).

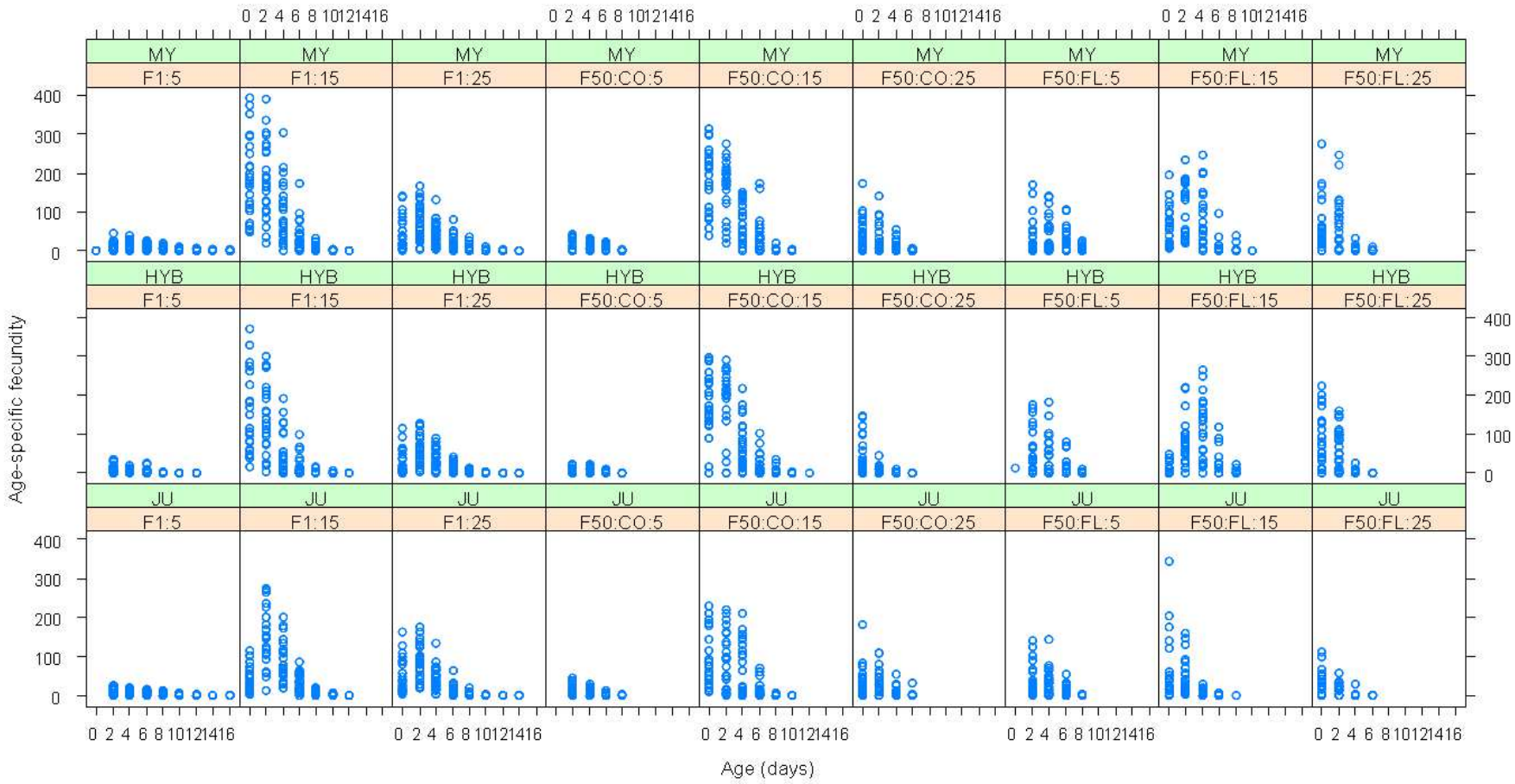

Figure 6.4. Age-specific fecundity (m_x) of females of *C. remanei* in relation to temperature (5, 15 and 25 °C). ASF is represented at generation F1 and at F50 in each environment (CO and FL). Strains (MY, HYB and JU) are represented by rows and the treatments as columns.

6.4.2 Cost of plasticity

I was interested in describing whether populations' past history (selection in a constant or a fluctuating regime) affected population response to the translocation. For the control groups (constant to constant and fluctuating to fluctuating), I did not detect changes in *LE* or *LF* between generation F50 and F51 ($F_{4,145} = 0.29$, P=0.88);. Therefore, for all traits, the analysis was restricted to the transposition between treatments (i.e. constant to fluctuating and fluctuating to constant).

6.4.2.1 Life expectancy (LE)

In both regimes, individuals showed changes in life expectancy after the translocation (Figure 6.5). However, the response to the translocation varied between regimes (M1 vs. M0: χ^2 = 240.51, 5 d.f., P<0.001; Table 6.4A); worms moved from a constant to a fluctuating environment showed a significant decline in *LE*, while worms moved from a fluctuating to a constant environment showed a relative increase in *LE*. Moreover, although the average *LE* was similar across strains (M2 vs. M1: χ^2 = 0.41, 2 d.f., P=0.81; Table 6.4A), the relative response to the translocation was different between strains (M3 vs. M1: χ^2 = 36.82, 12 d.f., P<0.001; Table 6.4A). For instance, HYB worms selected to grow in a constant environment, and transposed to a fluctuating environment had the largest reduction in the number of days lived compared to the other strains; while MY worms selected to grow in a fluctuating environment had the lowest *LE* after the translocation from a fluctuating to a constant environment. Regarding the variance components, including a random term to describe the replicate variance between treatments (M4 vs. M3: χ^2= 4.92, 20 d.f., P>0.5; Table 6.4A), or strains (M5 vs. M3: χ^2= 5.82, 35 d.f., P>0.5; Table 6.4A) did not significantly improve the model. Thus, the final model included the following fixed terms: treatment, strain and the interaction between them; and a random term to describe the hierarchical structure of the data (M3, Table 6.5A, Figure 6.5).

	Model	Syntax	AIC	logLik	Df
A.	*LE*				
	M0	$1 + (1\mid \text{replicate}_{jmn})$	2582.6	-1288.3	3
	M1	$\text{Treatment}_j + (1\mid\text{replicate}_{jmn})$	2352.1	-1168.1	8
	M2	$\text{Treatment}_j + \text{Strain}_m + (1\mid \text{replicate}_{jmn})$	2355.7	-1167.8	10
	M3	$\mathbf{\text{Treatment}_j * \text{Strain}_m + (1\mid \text{replicate}_{jmn})}$	**2339.3**	**-1149.7**	**20**
	M4	$\text{Treatment}_j * \text{Strain}_m + (\text{treatment}\mid \text{replicate}_{jmn})$	2376.9	-1148.5	40
	M5	$\text{Treatment}_j * \text{Strain}_m + (\text{strain}\mid \text{replicate}_{jmn})$	2349.3	-1149.7	25
B.	*LF*				
	M0	$1 + (1\mid \text{replicate}_{jmn})$	5677.3	-2835.6	3
	M1	$\text{Treatment}_j + (1\mid \text{replicate}_{jmn})$	5532.2	-2758.1	8
	M2	$\text{Treatment}_j + \text{Strain}_m + (1\mid \text{replicate}_{jmn})$	5510.7	-2745.4	10
	M3	$\text{Treatment}_j * \text{Strain}_m + (1\mid \text{replicate}_{jmn})$	5493.5	-2726.7	20
	M4	$\mathbf{\text{Treatment}_j * \text{Strain}_m + (\text{treatment} \mid \text{replicate}_{jmn})}$	**5492.2**	**-2706.1**	**40**
	M5	$\text{Treatment}_j * \text{Strain}_m + (\text{strain} \mid \text{replicate}_{jmn})$	5494.0	-2722.0	25
C.	m_x				
	M1	$\text{Treatment}_j + \text{Strain}_m + (1 \mid \text{ind}_{jmnoi})$	42750	-21365	10
	M2	$\text{Age}_i + \text{Treatment}_j + \text{Strain}_m + (1 \mid \text{ind}_{jmnoi})$	41333	-20648	18
	M3	$\text{Age}_i * \text{Treatment}_j + \text{Strain}_m + (1 \mid \text{ind}_{jmnoi})$	39365	-19625	58
	M4	$\text{Age}_i * \text{Treatment}_j + \text{Strain}_m + \text{Age}_i\text{: Strain}_m + (1 \mid \text{ind}_{jmnoi})$	39189	-19520	74
	M5	$\text{Age}_i * \text{Treatment}_j * \text{Strain}_m + (1 \mid \text{ind}_{jmnoi})$	38877	-19274	164
	M6	$\mathbf{\text{Age}_i * \text{Treatment}_j * \text{Strain}_m + (\text{age} \mid \text{ind}_{jmnoi})}$	**28003**	**-13793**	**208**

Table 6.4. As in Table 6.2, but describing the models after the translocation.

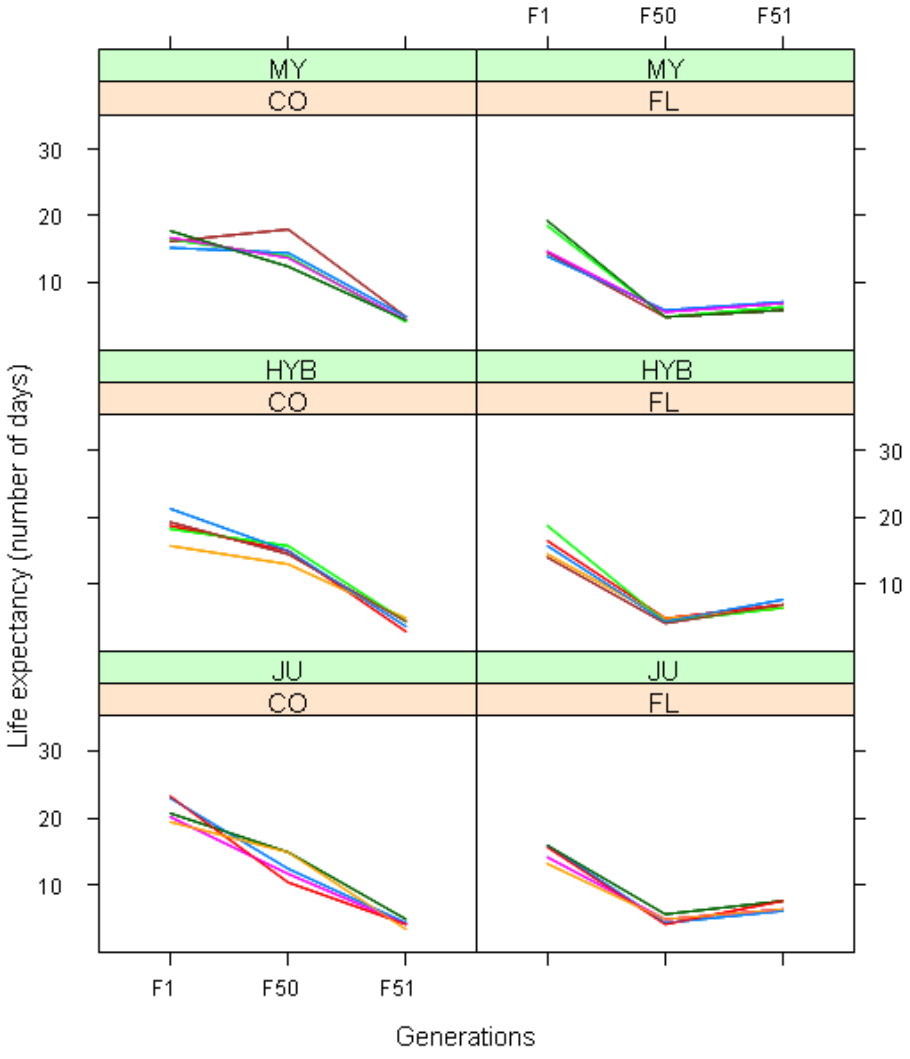

Figure 6.5. Life expectancy of *C. remanei* cultured under two environmental regimes (CO and FL) and after the translocation. Lines represent the plasticity of replicates of each strain (MY, HYB and JU) at three generation (F1, F50 and F51).

6.4.2.2 Lifetime fecundity (LF)

Populations' history affected the response to translocation (M1 vs. M0: χ^2 = 155.10, 5 d.f., P<0.001; Table 6.4B). Worms from the fluctuating regime showed an increase in fecundity after being moved to a constant environment, while worms from a constant regime exhibited a reduction in a fluctuating environment (Figure 6). In addition, and in agreement with previous analysis (see Plasticity section), the average *LF* was different between strains (M2 vs. M1: χ^2 = 25.42, 2 d.f., P<0.001; Table 6.4B) as was their response to the translocation (M3 vs. M2: χ^2 = 37.27, 10 d.f., P<0.001; Table 6.4B). For instance, only MY worms in a constant regime showed a relative increase in fecundity after the selection experiment (Figure 6.6). All the strains in a constant regime showed a reduction in fecundity after the translocation, though JU worms produced lower number of offspring compared to the other strains; and although all strains cultured in a fluctuating regime showed an increase in fecundity after the translocation to a constant environment, worms from MY produced more offspring compared to the other stains (Figure 6.6). Regarding the variance components, the analysis suggested that including a variance term to represent the varying replicate deviations from the mean between treatments had a significant effect in the model (M4 vs. M3: χ^2= 41.23, 20 d.f., P<0.01; Table 6.4B). For instance, the deviation from the population mean was larger in worms coming from a constant regime compared to worms from a fluctuating regime (Table 6.5A). The final model contained a fixed effect for treatment, strain, and a strain:temperature interaction term; and a random effect to describe the deviance for each treatment (Table 6.5A, Figure 6.6).

Model	Parameter	Estimate	Std. Error	t value
A.	*LE*			
	Fixed effects:			
	(Intercept)	21.28	0.62	34.17
	TreatmentCO:F50	-8.48	0.88	-9.63
	TreatmentCO:F51	-17.20	0.88	-19.53
	TreatmentFL:F1	-4.32	0.88	-4.91
	TreatmentFL:F50	-14.56	0.88	-16.53
	TreatmentFL:F51	-12.48	0.88	-14.17
	StrainHYB	-2.68	0.88	-3.04
	StrainMY	-4.84	0.88	-5.50
	TreatmentCO:F50:StrainHYB	4.36	1.25	3.50
	TreatmentCO:F51:StrainHYB	2.60	1.25	2.09
	TreatmentFL:F1:StrainHYB	3.56	1.25	2.86
	TreatmentFL:F50:StrainHYB	2.44	1.25	1.96
	TreatmentFL:F51:StrainHYB	2.68	1.25	2.15
	TreatmentCO:F50:StrainMY	6.52	1.25	5.23
	TreatmentCO:F51:StrainMY	5.24	1.25	4.21
	TreatmentFL:F1:StrainMY	6.04	1.25	4.85
	TreatmentFL:F50:StrainMY	5.32	1.25	4.27
	TreatmentFL:F51:StrainMY	4.52	1.25	3.63
	Random effects:			
	Groups Name	Variance	Std.Dev.	
	replicate (Intercept)	0.00	0.00	0.00

	Residual	9.70	3.11	100.00
B.	*LF*			
	Fixed effects:	Estimate	Std. Error	t value
	(Intercept)	311.32	41.08	7.58
	TreatmentCO:F50	-48.44	53.59	-0.90
	TreatmentCO:F51	-304.56	45.22	-6.74
	TreatmentFL:F1	-284.52	45.22	-6.29
	TreatmentFL:F50	-266.88	45.22	-5.90
	TreatmentFL:F51	-203.96	45.22	-4.51
	StrainHYB	46.32	58.10	0.80
	StrainMY	191.32	58.10	3.29
	TreatmentCO:F50:StrainHYB	147.48	75.78	1.95
	TreatmentCO:F51:StrainHYB	2.52	63.95	0.04
	TreatmentFL:F1:StrainHYB	-36.96	63.95	-0.58
	TreatmentFL:F50:StrainHYB	-58.64	63.95	-0.92
	TreatmentFL:F51:StrainHYB	-41.00	63.95	-0.64
	TreatmentCO:F50:StrainMY	11.24	75.78	0.15
	TreatmentCO:F51:StrainMY	-166.84	63.95	-2.61
	TreatmentFL:F1:StrainMY	-146.48	63.95	-2.29
	TreatmentFL:F50:StrainMY	-171.32	63.95	-2.68
	TreatmentFL:F51:StrainMY	-135.88	63.95	-2.13
	Random effects			
	Groups Name	Variance	Std.Dev.	
	replicate treatmentCO:F1	6650.40	81.55	33.73
	treatmentCO:F50	4133.50	64.29	20.96
	treatmentCO:F51	0.00	0.00	0.00
	treatmentFL:F1	0.00	0.00	0.00
	treatmentFL:F50	0.00	0.00	0.00
	treatmentFL:F51	0.00	0.00	0.00
	Residual	8934.20	94.52	45.31

Table 6.5. . Summary of models describing the effect of the translocation on A) life expectancy and B) lifetime fecundity. Model syntax and AIC values can be seen in Table 6.4.

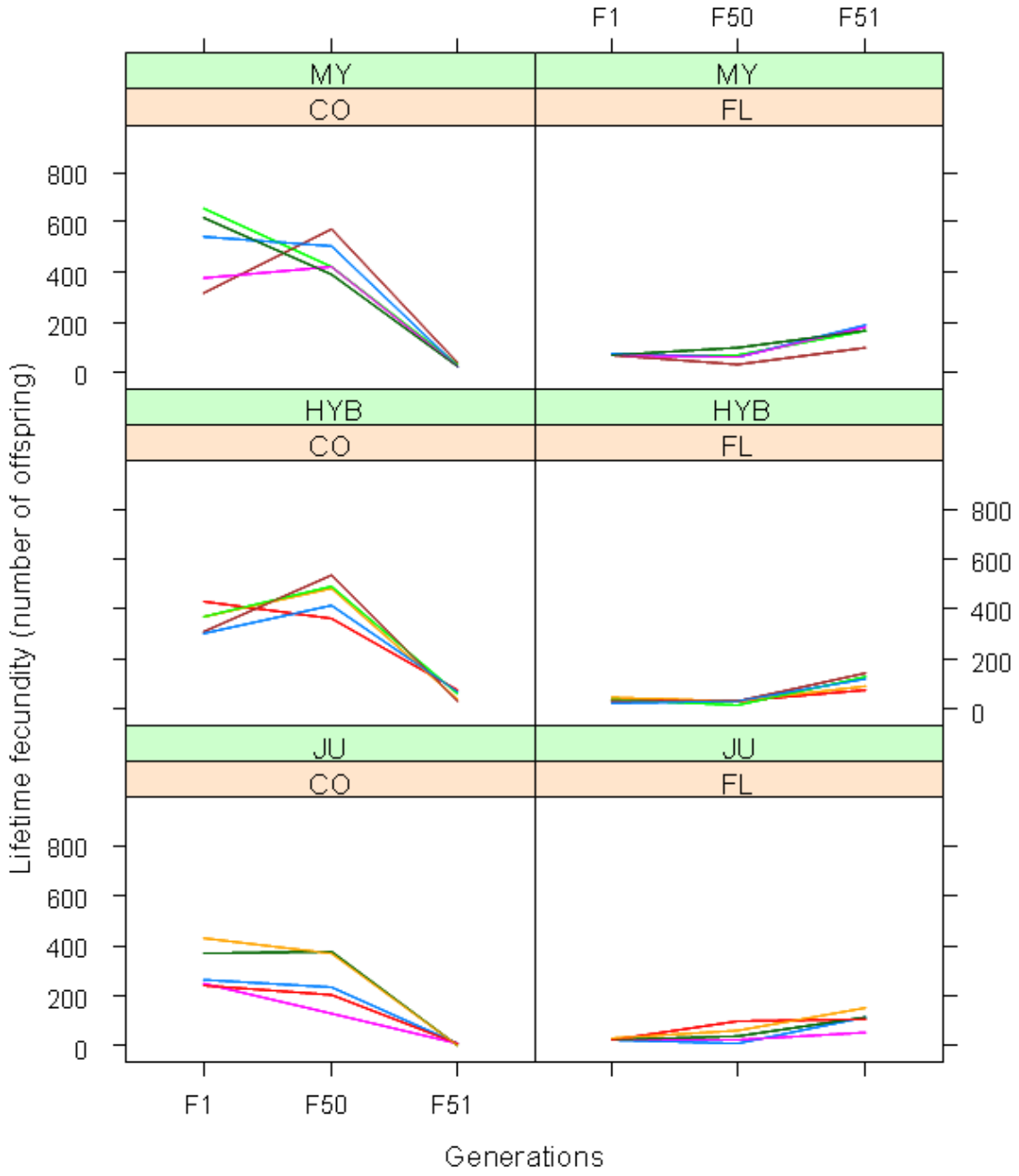

Figure 6.6. As figure 4, but showing the lifetime fecundity of *C. remanei*.

6.4.2.3 *Age-specific fecundity (m_x)*

The starting model was the most parsimonious model describing lifetime fecundity (Table 6.4C). Similar to the previous results of the plasticity of m_x, females are mainly fecund at early ages (M2 vs. M1: χ^2= 1433.0, 8 d.f., P<0.001; Table 6.4C; Figure 7). The inclusion of an interaction between age and treatment improved the model (M3 vs. M2: χ^2= 2047.9, 40 d.f., P<0.001; Table 6.4C), thus suggesting that the transposition had a significant effect on female age-specific fecundity. For instance, worms selected for a constant environment increase their early fecundity from generation F1 to F50, but when these worms were moved to a fluctuating environment their early fecundity was significantly reduced,

moreover, it was lower than the age-specific fecundity in a fluctuating environment at the beginning of the experiment (Figure 6.7). Conversely, worms selected for a fluctuating environment, increased their early fecundity between generation F1 and F50, moreover, when moved to a constant environment, their fecundity was higher than in a fluctuating environment, but still lower compared to worms in a constant environment (either at generation F1 and F50). A further addition of the interaction term between age and strain improved the model (M4 vs. M3: χ^2= 208.08, 16 d.f., P<0.001; Table 6.4C). This suggests that MY exhibited a higher age-specific fecundity compared to the other strains. The inclusion of a three-way interaction between age, treatment and strain further improved the model (M5 vs. M4: χ^2= 492.17, 90 d.f., P<0.001; Table 6.4C), suggesting that increased MY productivity during the early life of a female was most significant at the beginning of the experiment in the constant environment.

Regarding the variance components, the inclusion of the random term to describe the age-specific variance between individuals led to a significant improvement in the model (M6 vs. M5: χ^2= 10962, 44 d.f., P<0.001; Table 6.4C) suggesting higher individual variation at age 0, 2, and 4. Thus, the final model contained the terms of age, strain, treatment, the three-way interaction between them, and an age-specific random term representing the between-individual variation (Model M6 in Table 6.4C and *Appendix III*; Figure 6.7).

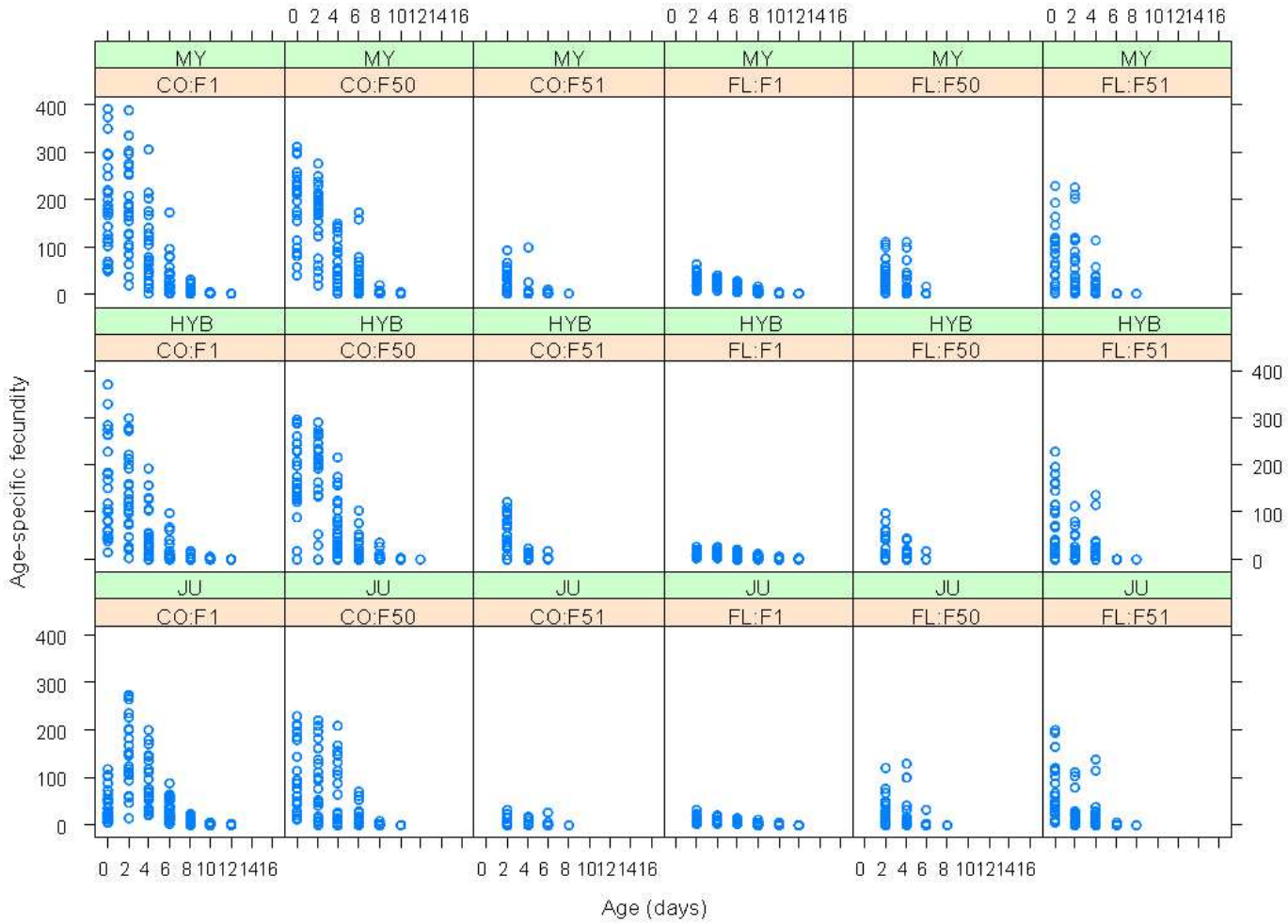

Figure 6.7. Age-specific fecundity (m_x) of females of *C. remanei* before and after the transposition experiment. m_x is represented at generation F1 and F50 in each environment (CO and FL) and after the transposition (F51) into the opposite environment (e.g. F51CO corresponds to worms from a constant environment moved to a fluctuating environment). Strains (MY, HYB and JU) are represented by rows and the treatments as columns.

6.5 Discussion

Despite numerous studies describing the effects of environmental conditions on species and populations' life history, our understanding of the mechanisms enabling adaptations to changeable environments is incomplete (Scheiner and Yampolsky, 1998; Roff, 2002). Phenotypic plasticity is clearly an important attribute in tracking environmental change (Via et al., 1995; Pigliucci, 2000). However, there is little empirical evidence describing 1) the extent to which fluctuating environments result in selection for plasticity and 2) whether increasing plasticity comes with associated costs in the absence of environmental fluctuation. In this study, I addressed the first issue by examining the level of plasticity of populations of *C. remanei* following selection in constant and predictably-fluctuating environment for 50 generations. I found that worms cultured in a fluctuating environment showed an increase in phenotypic plasticity (measured as thermal tolerance) across temperatures compared with worms selected for a constant environment. For instance, at extreme temperatures such as 5 ºC, the fecundity of JU worms selected for the fluctuating environment was 243% greater than the fecundity of worms at the beginning of the experiment at the same temperature. Conversely, the fecundity of JU worms selected for the constant environment was 24% less than the fecundity at the beginning of the experiment at 5 ºC. For survival, JU worms from a fluctuating environment showed an increase of 49% in life expectancy at 5 ºC, while worms from the constant environment displayed a decrease of 76% compared to worms before the selection experiment. Although the relative increase/decrease of each fitness component was different across strains, the pattern was similar when assayed under laboratory conditions. These results suggest that predictably-fluctuating temperatures selected for plasticity. In addition, despite the increased thermal tolerance across temperatures, when worms selected for higher levels of plasticity were moved back to a constant environment, they showed a significant decline in fitness components compared to their counterparts at the beginning of the experiment in a constant environment; JU, MY and HY exhibited a 66, 68 and 68% decline in lifetime fecundity; while the life expectancy reduction was 68, 61 and 63%, respectively. Thus, it is possible that the energy allocated for the production and maintenance of the genetic and physiological machinery for increased plasticity could have resulted in a reduction of energy for other physiological processes such as survival and reproduction. Therefore, suggesting that increased plasticity does incur a fitness cost when living in a constant intermediate environment.

Theoretically, frequent fluctuation in environments occurring within the lifetime of individuals might be expected to select for plasticity (Roff, 2002). If there are cues that give information on the state of the present and future environment, we could expect that genotypes will evolve means of using such cues to develop or display the optimal trait or behaviour (Roff, 2002). In the current study, temperature was changing every 12 hours and individuals were therefore experiencing each 5 and 25 °C temperature at least three times over the course of their lifetime. This predictable environment potentially acted to select for plastic mechanism (including physiological and behavioural changes) and resulted in a change of the reaction norms. Previous studies on multi-cellular organisms have not shown a change in reaction norms as a consequence of prolonged exposure to fluctuating conditions. For instance, Scheiner and Yampolsky (1998) found that populations of Daphnia increased their population growth rate after 10 (parthenogenetic) generations in predictably-fluctuating temperatures. However, despite the adaptation to fluctuating environments, populations did not show significant changes in their reaction norms compared to populations maintained in a constant temperature (Scheiner and Yampolsky, 1998).

The evolution of the reaction norms has been documented under laboratory conditions (Scheiner and Berrigan, 1998; Buckling et al., 2007; Hughes et al., 2007). Previous research has documented the evolution of the reaction norm mainly in unicellular organism as a consequence of manipulating the growing media quality, e.g. absence of particular enzymes (Buckling et al., 2006), or a change in its pH (Hughes et al., 2007). For instance, a recent study cultured populations of *E. coli* under predictable and randomly fluctuating pH conditions (Hughes et al., 2007). Similar to the results in the current study, they found that both populations were characterised by an increase in tolerance to variable pH environments (Hughes et al., 2007). However, contrary to the findings of the current study, none of the populations showed any apparent cost, therefore suggesting that populations became both “the jack and the master of many trades” (Hughes et al., 2007).

Increased plasticity is expected to incur a cost on theoretical grounds (DeWitt et al., 1998). However, studies more commonly report a lack of cost (Scheiner and Berrigan, 1998; Buckling et al 2006) which may reflect our limited ability to determine which traits contribute to fitness and/or in what environments plasticity is costly, rather than a real lack of such cost (e.g. DeWitt et al. 1998; Steinger et al., 2003; Pigliucci, 2005). For several organisms, the tolerance to stressful temperatures is linked to an increase in heat-shock proteins and changes in the membrane phospholipids (Feder and Hofmann, 1999; Hoffmann et al., 2003; Rea et al., 2005; Murray et al., 2007). It has been suggested that an

increase in number, and possible expression and regulation, of heat-shock proteins has potential cost (Krebs and Feder, 1997). Plasticity (measured by increased thermal tolerance) in *C. remanei* could be linked to a similar physiological mechanisms with similar associated costs.

In plants, the cost of plasticity has been recently documented (Bell and Galloway 2008). Plastic populations of an annual plant (*Geranium carolinianum*), which have previously experienced low levels of light, are better at avoiding shaded areas by internode elongation than plants that come from areas with higher levels of light (Bell and Galloway, 2008). However, increased internode elongation ability comes at a cost as it is negatively related to the number of fruits produced (Bell and Galloway, 2008). The demonstrable cost of plasticity, therefore, is likely to vary depending of populations' life history and previous environmental conditions. For C. *remanei*, although the actual mechanism involved in the increased levels of plasticity (i.e. tolerance) is unknown, it the results suggest that populations adapted to the fluctuating environment and this adaptation incurred a cost.

6.5.1 Conclusions

Understanding developmental and physiological mechanisms of the plastic response are important to understanding the evolution of plasticity. In this study the levels of plasticity in *C. remanei* were linked to populations' previous environmental history, demonstrating that fluctuating environments can select for high levels of plasticity compared to constant environments. Moreover, increased levels of plasticity can have a fitness cost if the environment is not fluctuating. Further research should focus on the mechanisms underlying the increased plasticity in *C. remanei*. Although the increased plasticity resulting from selection suggests that there are at least some genes linked with plasticity, we know little about those genes.

7 General Discussion

Biotic and abiotic factors can produce numerous responses in organisms' behaviour and physiology. Exposure to low temperatures in humans, for instance, can trigger behavioural changes such as seeking extra clothing or shelter, reduced motility and physiological changes such as reduction in sweat produced, or increase in transferred blood from the skin towards the core body. Similarly, invertebrates such as nematodes exposed to low temperatures can change their behaviour and migrate to their preferred temperature (Hedgecock and Russell, 1975) and have physiological changes which lead to an increase in body fat (Murray et al., 2007). Conversely, when both humans and nematodes are exposed to warmer conditions, they can produce opposite and reversible changes in their behaviour and physiology. Although the mechanisms between species can differ considerably, environmentally-dependent traits are universally present across nature.

The development and increased availability of molecular techniques to study the mechanisms behind organisms' responses has significantly increased over the second half of the 20th century (Pigliucci 2003). One of the most remarkable findings in this era of genomes, molecular markers, and microsatellites is that organisms' phenotypes are not just a fixed product of their genes, but are also constantly changing and following the natural world. This result has led to a rapid development of ideas about phenotypic plasticity. At least three ideas are generally agreed among the scientific community: plastic strategies are ubiquitous across taxa, numerous phenotypic traits have a genetic basis, and various plastic traits are to some extent adaptive. These topics have been extensively covered — both theoretically and empirically — in recent books and reviews (West-Eberhard 2003, DeWitt and Scheiner, 2004). One question has been frequently identified as the most controversial and puzzling: what limits the evolution of adaptive plasticity (Callahan et al. 2008). In this work, I developed a laboratory experiment to approach this question. For this general discussion, I briefly describe the scope of plasticity before outlining my main results in relation to the current understanding about adaptive phenotypic plasticity and discussing areas for future research.

The concept of phenotypic plasticity embraces the study of environmentally dependent traits of a genotype. Phenotypic plasticity can be studied from at least three perspectives: describing the distribution of traits (e.g. body size, protein expression, paternal care, number of offspring, etc.) of individuals in response to an environmental gradient, understanding the mechanism (e.g. its genetic basis) responsible for observed phenotypic plasticity across individuals, and understanding what evolutionary forces have caused the differential degree of plasticity across individuals. While the first two angles have received considerable theoretical and empirical attention, there are few studies

illustrating the adaptive evolution of phenotypic plasticity, and the consequences and constraints of having different degrees of plasticity.

Adaptive phenotypic plasticity is considered as a solution to a fluctuating environment. For the last 16 years or so, there has been an increasing interest in the study of adaptive phenotypic plasticity. Figure 7.1 shows the results after searching for articles displaying "Adaptive phenotypic plasticity" in the Title and in the Abstract. Although the results can reflect the increase in usage of the concept due to an agreement in semantics across different areas of research, it also indicates the increasing idea of a genotype that performs optimally, compared to others, across a range of natural environmental conditions.

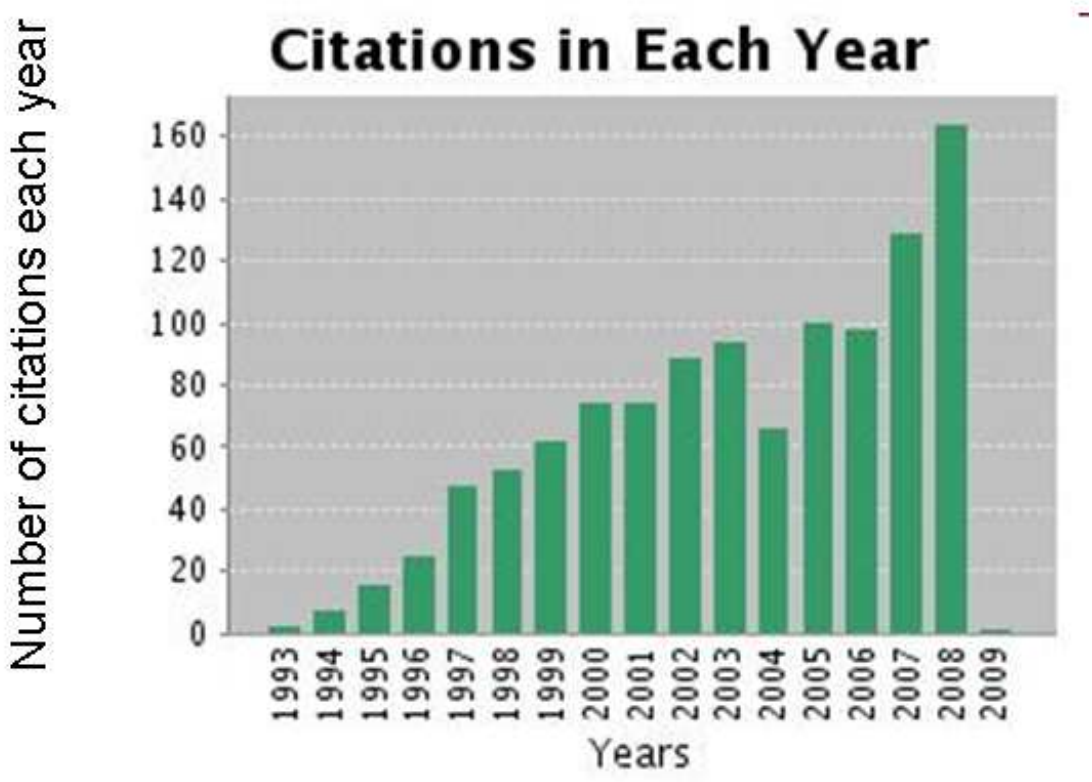

Figure 7.1. Number of papers published from 1900 to 2009 on Adaptive phenotypic plasticity (included in the title, abstract or keywords). Data are from a search using Citation Reports options of the Institute for Scientific Information Science Citation Index. Note that the first year in the x axis is 1993 because no citations were found previous to that date. Sum of the times cited: 1,109.

Adaptive traits may involve increased biomass allocation in plants under favourable temperature conditions compared to low temperatures (e.g. *Arabidopsis thaliana*, Atkin et al., 2006), shade avoidance of plants under low light conditions compared to high light

conditions (e.g. *Geranium carolinianum*, Bell and Galloway, 2008), production of fewer and larger offspring by fish in low predation environments compared to high predation environments (e.g. *Poecilia reticulata*, Reznick and Cardenas, 1996; Bashey, 2006), construction of nests on higher grounds by birds experiencing higher predation risk compared to ground nesting birds (e.g. *Vermivora celata*, Peluc et al., 2008), increased male mate choice in rams due to high levels of female promiscuity (Soay sheep, Preston et al., 2005), production of heat shock proteins to increase thermal tolerance in response to high temperature (e.g. *Undaria pinnatifida*, Henkel and Hofmann, 2008), and arrested growth in free-living nematodes under unfavourable environmental conditions compared to favourable conditions (e.g. *Caenorhabditis elegans*, Viney et al., 2003) among others. Ultimately, these responsive traits are expected to increase individuals' performance (i.e. fitness components) across environments compared to less plastic individuals (DeWitt and Scheiner, 2004).

Although the theoretical framework behind the concept of adaptive phenotypic plasticity, like the adaptive evolution of any other trait, is well established, we understand little about the evolution of plasticity. For instance, to what extent an increase in environmental variability selects for plasticity, and whether having phenotypic plasticity can be costly when the environment is less variable. In this study, I approached these questions using a free-living nematode as a model species and selection experiments to manipulate the level of response between populations. Specific conclusions from each part of my work are discussed in the relevant sections. First, I briefly describe my results in relation to the current understanding about adaptive phenotypic plasticity and then discuss areas for future research.

7.1 Phenotypic plasticity

Part of my work focused on describing how individuals from natural populations responded to biotic and abiotic factors under laboratory conditions (Chapter 3 and 4). This involved quantifying the distribution of several life-history traits. I found that female *C. remanei*'s performance (measured as fecundity rate) in the laboratory changed in response to two factors: the number of males and temperature. Females displayed an optimal performance when paired with *ca*. 7 males and when growing at *ca*. 17 °C (Chapter 3 and 4, respectively). An increase or decrease in both factors would result in a reduction in female performance.

Individual's performance is expected to be the result of having a particular phenotype as a consequence of its genotype and the environmental influence (see Chapter 1Figure 1D). Thus, I had a good estimator of the plasticity (i.e. tolerance or response) of natural populations of *C. remanei* to laboratory conditions.

The results from Chapters 3 and 4 are in agreement with the general view that all biological processes are to some extent environmentally dependent (DeWitt and Scheiner, 2004). A next rational step forward would be to identify what behavioural, morphological, physiological and molecular mechanisms lie behind the variation in performance of *C. remanei* females in response to biotic or abiotic factors.

Within the realm of sexual selection, theory predicts that sexual conflict is partially responsible of the antagonistic coevolution of male and female traits (Chapman, 2006; Chapter 3). A reduction in female mortality rate could be caused by adaptive male traits that increase their reproduction (Chapman, 2006), as well as a consequence of the ability of females in displaying traits, such as male avoidance, to counteract males (e.g. *C. elegans*, Kleemann & Basolo, 2007). From a sexual conflict perspective, it would be interesting to know what mechanisms are responsible for the limited ability (i.e. low plasticity) of *C. remanei* females to increase their reproduction in the presence of high and low number of males.

Regarding temperature, one example of increased plasticity can be the tolerance to stressful temperatures of invertebrates linked to an increase in expression of heat-shock proteins and changes in the number of fatty acids in the membrane (Murray et al., 2007; Rea et al., 2005; Hoffmann et al., 2003; Feder and Hofmann, 1999). Thus, the observed variance in performance between isolates of *C. remanei* (JU and MY12-G) could be the consequence of differential levels of plasticity (e.g. tolerance to stressful conditions) due to local adaptations (Chapter 5). Indeed, a recent study on *C. elegans* showed that there are differences in both the levels of phenotypic plasticity and genotype–environment interactions (GEI) among isolates (e.g. Gutteling et al., 2007). This study is one of the few empirical studies showing the existence of loci displaying environmentally based allelic sensitivity (theory reviewed in Via et al., 1995). Quantitative trait loci (QTL) mapping is a powerful tool for studying the genetic mechanism of plasticity and GEI (Ungerer et al. 2003). Studying the genetic sensitivity among *C. remanei* populations, therefore, should incorporate QTL mapping in the future.

7.2 Significance of phenotypic plasticity

The observed plasticity of natural populations of *C. remanei*, cultured in the lab (Chapter 4), was however not necessarily adaptive. Before the start of my work, *C. remanei* populations were presumably adapted to their local environmental conditions. However, I was interested in comparing individuals with high and low levels of plasticity. Thus, I conducted a selection experiment to manipulate the levels of plasticity among populations of *C. remanei.*

From Chapter 4, it was evident that low and high temperatures in the laboratory were restrictive for many individuals from natural populations of *C. remanei*. Thus, in the selection experiment I cultured populations of *C. remanei* in a fluctuating temperature (predictably changing from low to high temperature) that might select for a high plasticity and at a constant temperature that might select for lower plasticity (Chapter 5).

In both regimes I found a response to selection manifest by increased early fecundity (Chapter 5). Adaptation was mediated by shifting reproductive schedules towards early life since there was no benefit in delaying reproduction. Interestingly, I found that after 50 generations of selection in a fluctuating environment, female lifespan was reduced by nearly 50%. This suggests that increased early fecundity reduced resources available for other biological process, for instance defenses against stress (reviewed by Harshman and Zera, 2007).

These results, however, do not exclude the possibility of a shift due to inadvertent selection as a consequence of the maintenance protocols. In both selection regimes, populations were transferred approximately once every two generations in order to avoid food depletion and diminish negative density-dependant effects. Thus, those worms which had delayed reproduction were potentially less favoured. I tried to diminish this by taking a random sample of individuals of several ages. Although the inadvertent selection is largely unavoidable, populations cultured in both regimes were subjected to identical protocols.

Similar to the previous section, our understanding of the mechanistic basis of these evolutionary adaptations has been limited by a lack of detailed functional information on the underlying biological processes. In addition, regarding the potential inadvertent selection due to maintenance protocols, this could be partially solved by maintaining populations under laboratory conditions designed to be as similar as possible to natural conditions. Other studies have been successful in reproducing semi-field systems in the

laboratory for model organisms such as malaria-infected mosquitoes (Knols et al., 2002). Caenorhabditis' laboratory protocols have not changed significantly over the 30 years that they have been used as a model organism (Brenner, 1974). Thus, it would be beneficial for studies interested in using semi-field systems to direct research into the development of new media cultures for the maintenance of large populations of nematodes under laboratory conditions.

7.3 Cost of high plasticity in a constant environment

Phenotypic plasticity provides individuals with the means to thrive in a heterogeneous environment (DeWitt and Scheiner 2004). However, if the environment is not changing, plastic individuals with the machinery to match the environment can be at a disadvantage compared individuals that are less plastic (DeWitt and Scheiner 2004). In Chapter 6, I presented the results of the main question of this research: Is plasticity costly in a more stable environment? First, I showed that individuals from a fluctuating environment displayed high phenotypic plasticity measured by their higher tolerance to a wider range of temperatures compared to their counterpart in a constant environment. Second, I showed that having high plasticity, which was adaptive in a fluctuating environment, incurred a high cost when the environment was not fluctuating. Individuals from the population in the fluctuating environment moved to a constant temperature after the selection experiment displayed a reduction in fitness of *ca*. 64%. This suggests that having plasticity can be costly in environments in which plasticity is not needed.

Our understanding about the mechanisms of plasticity in life-history traits is limited. Two models have been proposed to describe it: regulatory loci that alter gene expression in different environments, or loci displaying environmentally based allelic sensitivity (Via et al. 1995). Although the genetics of plasticity could lie somewhere in between, and these mechanisms need not be mutually exclusive, tools such as QTL mapping offer the opportunity to further our understanding. Integrating QTL mapping with evolutionary studies could describe not only the genetics of plasticity, but shed light on its evolutionary change.

Research on the evolutionary genetics of plasticity is gathering momentum. Choosing a good model species for the selection experiments is again essential. For obvious reasons, organisms with short generation time are preferred in evolutionary

experiments. Using *C. remanei* was successful in the current work. During the selection experiment populations did not display apparent signs of inbreeding. For instance, *C. remanei*'s reproductive biology (i.e. gonochorism, see Introduction) could have helped to reduce the frequency of homozygotes within the population during the course of the experiment. Homozygotes are more likely to appear in populations where mating between relatives is common, for instance, in the *C. elegans* system. On the other hand, there is evidence of low inbreeding depression in life-history traits of populations of *C. elegans* reared in the laboratory (e.g. Johnson and Wood 1982). However, it is not clear if the same would be true in a long term study using *C. elegans* populations.

The use of *C. elegans* offers several advantages. In addition to the long term history of research on this species (Brenner 1974), *C. elegans'* reproductive biology is advantageous for the use of the isofemale line technique in molecular studies (David et al. 2005; Parsons and Hosgood 1968; see Discussion Chapter 4). *C. elegans* hermaphrodites can self-fertilise to produce progeny (Hodgkin, 1987). This can make the production of iso-hermaphrodite lineages (genetically homogeneous) for quantitative genetics much easier (e.g. Dolgin et al., 2008).

7.4 Final thoughts

The work presented here, along with other research studying how organism respond to environmental variability clearly states that phenotypic plasticity is a ubiquitous trait. Moreover, it is a trait with the potential to evolve, even under laboratory conditions. The evolution of plasticity in the wild can have many ecological consequences, such as the reduction in extinction probability of a population, blooming of invasive species, spreading diseases, antagonistic co-evolution between and within species, among others. Environmental variation caused by natural processes and/or anthropogenic influences can act as selective pressures for plasticity. Thus, the study of the evolution of plasticity in the wild is another open door for future research.

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Appendix I

Summary of the mixed-effects model (Model M6 Table 5.1D) to describe the Age-specific fecundity in relation to the temperature.

The model included 4050 number of observations corresponding to 450 individuals within 15 replicates.

Linear mixed model fit by maximum likelihood
Formula: mx~ Age_i * $Generation_k$ * $Regime_j$ * $Strain_l$ + (age | ind_{jklmn})

A) Fixed effects			
	Estimate	Std. Error	t value
(Intercept)	39.24	7.20	5.45
Age2	107.64	8.86	12.15
Age4	46.64	10.23	4.56
Age6	-8.24	8.51	-0.97
Age8	-32.48	7.62	-4.26
Age10	-37.80	7.21	-5.24
Age12	-39.12	7.21	-5.43
Age14	-39.24	7.20	-5.45
Age16	-39.24	7.21	-5.44
GenerationF20	25.84	10.18	2.54
GenerationF50	57.32	10.18	5.63
RegimeFL	-29.52	10.18	-2.90
StrainHYB	111.72	10.18	10.97
StrainMY	141.16	10.18	13.86
Age2:GenerationF20	-49.20	12.53	-3.93
Age4:GenerationF20	-44.52	14.46	-3.08
Age6:GenerationF20	-28.64	12.04	-2.38
Age8:GenerationF20	-19.04	10.78	-1.77
Age10:GenerationF20	-27.28	10.20	-2.67
Age12:GenerationF20	-25.96	10.19	-2.55
Age14:GenerationF20	-25.84	10.19	-2.54
Age16:GenerationF20	-25.84	10.20	-2.54
Age2:GenerationF50	-115.24	12.53	-9.19
Age4:GenerationF50	-81.36	14.46	-5.63
Age6:GenerationF50	-76.56	12.04	-6.36
Age8:GenerationF50	-63.60	10.78	-5.90
Age10:GenerationF50	-58.76	10.20	-5.76
Age12:GenerationF50	-57.44	10.19	-5.64
Age14:GenerationF50	-57.32	10.19	-5.63
Age16:GenerationF50	-57.32	10.20	-5.62
Age2:RegimeFL	-108.04	12.53	-8.62
Age4:RegimeFL	-51.32	14.46	-3.55
Age6:RegimeFL	0.56	12.04	0.05
Age8:RegimeFL	23.44	10.78	2.18
Age10:RegimeFL	28.08	10.20	2.75
Age12:RegimeFL	29.40	10.19	2.89

Age14:RegimeFL	29.52	10.19	2.90
Age16:RegimeFL	29.52	10.20	2.90
GenerationF20:RegimeFL	-18.72	14.40	-1.30
GenerationF50:RegimeFL	-42.36	14.40	-2.94
Age2:StrainHYB	-120.68	12.53	-9.63
Age4:StrainHYB	-146.56	14.46	-10.13
Age6:StrainHYB	-127.40	12.04	-10.58
Age8:StrainHYB	-116.48	10.78	-10.81
Age10:StrainHYB	-112.80	10.20	-11.06
Age12:StrainHYB	-111.80	10.19	-10.97
Age14:StrainHYB	-111.72	10.19	-10.97
Age16:StrainHYB	-111.72	10.20	-10.96
Age2:StrainMY	-106.04	12.53	-8.46
Age4:StrainMY	-131.24	14.46	-9.07
Age6:StrainMY	-136.52	12.04	-11.34
Age8:StrainMY	-140.32	10.78	-13.02
Age10:StrainMY	-141.60	10.20	-13.88
Age12:StrainMY	-141.08	10.19	-13.84
Age14:StrainMY	-141.16	10.19	-13.86
Age16:StrainMY	-141.16	10.20	-13.85
GenerationF20:StrainHYB	-66.92	14.40	-4.65
GenerationF50:StrainHYB	-29.20	14.40	-2.03
GenerationF20:StrainMY	-79.92	14.40	-5.55
GenerationF50:StrainMY	-55.56	14.40	-3.86
RegimeFL:StrainHYB	-110.44	14.40	-7.67
RegimeFL:StrainMY	-121.48	14.40	-8.44
Age2:GenerationF20:RegimeFL	53.00	17.73	2.99
Age4:GenerationF20:RegimeFL	47.80	20.46	2.34
Age6:GenerationF20:RegimeFL	23.12	17.03	1.36
Age8:GenerationF20:RegimeFL	12.40	15.24	0.81
Age10:GenerationF20:RegimeFL	20.16	14.43	1.40
Age12:GenerationF20:RegimeFL	18.84	14.41	1.31
Age14:GenerationF20:RegimeFL	18.72	14.41	1.30
Age16:GenerationF20:RegimeFL	18.72	14.42	1.30
Age2:GenerationF50:RegimeFL	109.36	17.73	6.17
Age4:GenerationF50:RegimeFL	62.72	20.46	3.07
Age6:GenerationF50:RegimeFL	59.56	17.03	3.50
Age8:GenerationF50:RegimeFL	47.96	15.24	3.15
Age10:GenerationF50:RegimeFL	43.80	14.43	3.04
Age12:GenerationF50:RegimeFL	42.48	14.41	2.95
Age14:GenerationF50:RegimeFL	42.36	14.41	2.94
Age16:GenerationF50:RegimeFL	42.36	14.42	2.94
Age2:GenerationF20:StrainHYB	87.56	17.73	4.94
Age4:GenerationF20:StrainHYB	107.52	20.46	5.26
Age6:GenerationF20:StrainHYB	74.92	17.03	4.40
Age8:GenerationF20:StrainHYB	63.04	15.24	4.14
Age10:GenerationF20:StrainHYB	68.00	14.43	4.71
Age12:GenerationF20:StrainHYB	67.00	14.41	4.65
Age14:GenerationF20:StrainHYB	66.92	14.41	4.64

Age16:GenerationF20:StrainHYB	66.92	14.42	4.64
Age2:GenerationF50:StrainHYB	131.52	17.73	7.42
Age4:GenerationF50:StrainHYB	74.96	20.46	3.67
Age6:GenerationF50:StrainHYB	52.04	17.03	3.06
Age8:GenerationF50:StrainHYB	37.08	15.24	2.43
Age10:GenerationF50:StrainHYB	30.40	14.43	2.11
Age12:GenerationF50:StrainHYB	29.28	14.41	2.03
Age14:GenerationF50:StrainHYB	29.20	14.41	2.03
Age16:GenerationF50:StrainHYB	29.20	14.42	2.03
Age2:GenerationF20:StrainMY	66.20	17.73	3.74
Age4:GenerationF20:StrainMY	90.36	20.46	4.42
Age6:GenerationF20:StrainMY	74.16	17.03	4.36
Age8:GenerationF20:StrainMY	75.88	15.24	4.98
Age10:GenerationF20:StrainMY	80.36	14.43	5.57
Age12:GenerationF20:StrainMY	79.84	14.41	5.54
Age14:GenerationF20:StrainMY	79.92	14.41	5.55
Age16:GenerationF20:StrainMY	79.92	14.42	5.54
Age2:GenerationF50:StrainMY	95.28	17.73	5.38
Age4:GenerationF50:StrainMY	62.40	20.46	3.05
Age6:GenerationF50:StrainMY	77.64	17.03	4.56
Age8:GenerationF50:StrainMY	56.64	15.24	3.72
Age10:GenerationF50:StrainMY	56.40	14.43	3.91
Age12:GenerationF50:StrainMY	55.48	14.41	3.85
Age14:GenerationF50:StrainMY	55.56	14.41	3.86
Age16:GenerationF50:StrainMY	55.56	14.42	3.85
Age2:RegimeFL:StrainHYB	123.44	17.73	6.96
Age4:RegimeFL:StrainHYB	147.88	20.46	7.23
Age6:RegimeFL:StrainHYB	127.08	17.03	7.46
Age8:RegimeFL:StrainHYB	115.52	15.24	7.58
Age10:RegimeFL:StrainHYB	111.68	14.43	7.74
Age12:RegimeFL:StrainHYB	110.52	14.41	7.67
Age14:RegimeFL:StrainHYB	110.44	14.41	7.67
Age16:RegimeFL:StrainHYB	110.44	14.42	7.66
Age2:RegimeFL:StrainMY	102.48	17.73	5.78
Age4:RegimeFL:StrainMY	119.00	20.46	5.82
Age6:RegimeFL:StrainMY	118.64	17.03	6.97
Age8:RegimeFL:StrainMY	120.44	15.24	7.90
Age10:RegimeFL:StrainMY	121.92	14.43	8.45
Age12:RegimeFL:StrainMY	121.40	14.41	8.42
Age14:RegimeFL:StrainMY	121.48	14.41	8.43
Age16:RegimeFL:StrainMY	121.48	14.42	8.43
GenerationF20:RegimeFL:StrainHYB	83.32	20.37	4.09
GenerationF50:RegimeFL:StrainHYB	25.92	20.37	1.27
GenerationF20:RegimeFL:StrainMY	83.36	20.37	4.09
GenerationF50:RegimeFL:StrainMY	52.56	20.37	2.58
Age2:GenerationF20:RegimeFL:StrainHYB	-100.16	25.07	-4.00
Age4:GenerationF20:RegimeFL:StrainHYB	-132.36	28.93	-4.58
Age6:GenerationF20:RegimeFL:StrainHYB	-92.48	24.08	-3.84
Age8:GenerationF20:RegimeFL:StrainHYB	-80.32	21.55	-3.73

Age10:GenerationF20:RegimeFL:StrainHYB	-84.56	20.40	-4.14
Age12:GenerationF20:RegimeFL:StrainHYB	-83.40	20.38	-4.09
Age14:GenerationF20:RegimeFL:StrainHYB	-83.32	20.38	-4.09
Age16:GenerationF20:RegimeFL:StrainHYB	-83.32	20.39	-4.09
Age2:GenerationF50:RegimeFL:StrainHYB	-141.96	25.07	-5.66
Age4:GenerationF50:RegimeFL:StrainHYB	-74.92	28.93	-2.59
Age6:GenerationF50:RegimeFL:StrainHYB	-49.72	24.08	-2.07
Age8:GenerationF50:RegimeFL:StrainHYB	-34.12	21.55	-1.58
Age10:GenerationF50:RegimeFL:StrainHYB	-27.28	20.40	-1.34
Age12:GenerationF50:RegimeFL:StrainHYB	-26.00	20.38	-1.28
Age14:GenerationF50:RegimeFL:StrainHYB	-25.92	20.38	-1.27
Age16:GenerationF50:RegimeFL:StrainHYB	-25.92	20.39	-1.27
Age2:GenerationF20:RegimeFL:StrainMY	-67.52	25.07	-2.69
Age4:GenerationF20:RegimeFL:StrainMY	-100.64	28.93	-3.48
Age6:GenerationF20:RegimeFL:StrainMY	-81.44	24.08	-3.38
Age8:GenerationF20:RegimeFL:StrainMY	-80.28	21.55	-3.73
Age10:GenerationF20:RegimeFL:StrainMY	-83.80	20.40	-4.11
Age12:GenerationF20:RegimeFL:StrainMY	-83.28	20.38	-4.09
Age14:GenerationF20:RegimeFL:StrainMY	-83.36	20.38	-4.09
Age16:GenerationF20:RegimeFL:StrainMY	-83.36	20.39	-4.09
Age2:GenerationF50:RegimeFL:StrainMY	-104.64	25.07	-4.17
Age4:GenerationF50:RegimeFL:StrainMY	-67.28	28.93	-2.33
Age6:GenerationF50:RegimeFL:StrainMY	-76.44	24.08	-3.18
Age8:GenerationF50:RegimeFL:StrainMY	-53.44	21.55	-2.48
Age10:GenerationF50:RegimeFL:StrainMY	-53.40	20.40	-2.62
Age12:GenerationF50:RegimeFL:StrainMY	-52.48	20.38	-2.58
Age14:GenerationF50:RegimeFL:StrainMY	-52.56	20.38	-2.58
Age16:GenerationF50:RegimeFL:StrainMY	-52.56	20.39	-2.58
B) Random effects			
Groups Name	Variance	Std.Dev.	% of var.
ind age0	1294.80	35.98	30.07
age2	1434.60	37.88	33.32
age4	1092.40	33.05	25.37
age6	382.55	19.56	8.88
age8	100.35	10.02	2.33
age10	0.01	0.11	0.00
age12	0.00	0.04	0.00
age14	0.00	0.02	0.00
age16	0.00	0.04	0.00
Residual	1.24	1.11	0.03

Appendix II

Summary of the mixed-effects model (Model M8 Table 6.2.C) to describe the Age-specific fecundity in relation to the temperature.

The model included 6075 number of observations corresponding to 675 individuals within 15 replicates.

Linear mixed model fit by maximum likelihood
Formula: mx~ Age*Temp+Age*Treatment+Age*Strain+Age:Temp:Treatment+
Age:Temp:Strain + Age:Treatment:Strain + (Age - 1| ind1)

Fixed effects:	Estimate	Std. Error	t value
(Intercept)	-7.80	6.26	-1.25
Age2	31.47	7.60	4.14
Age4	27.22	8.85	3.08
Age6	14.87	7.18	2.07
Age8	11.31	6.39	1.77
Age10	9.18	6.29	1.46
Age12	8.36	6.27	1.33
Age14	7.96	6.26	1.27
Age16	7.83	6.26	1.25
Temp15	80.65	7.87	10.25
Temp25	33.89	7.87	4.31
TreatmentF50:CO	4.56	7.87	0.58
TreatmentF50:FL	18.82	7.87	2.39
StrainMIX	9.17	7.87	1.17
StrainMY	14.29	7.87	1.82
Age2:Temp15	33.27	9.55	3.48
Age4:Temp15	-33.52	11.12	-3.01
Age6:Temp15	-62.65	9.02	-6.94
Age8:Temp15	-79.04	8.03	-9.85
Age10:Temp15	-81.11	7.90	-10.27
Age12:Temp15	-81.20	7.88	-10.31
Age14:Temp15	-80.87	7.87	-10.28
Age16:Temp15	-80.71	7.87	-10.26
Age2:Temp25	26.29	9.55	2.75
Age4:Temp25	-0.01	11.12	0.00
Age6:Temp25	-24.05	9.02	-2.67
Age8:Temp25	-33.48	8.03	-4.17
Age10:Temp25	-34.68	7.90	-4.39
Age12:Temp25	-34.48	7.88	-4.38
Age14:Temp25	-34.10	7.87	-4.34
Age16:Temp25	-33.95	7.87	-4.31
Age2:TreatmentF50:CO	-20.63	9.55	-2.16
Age4:TreatmentF50:CO	-12.69	11.12	-1.14
Age6:TreatmentF50:CO	-13.20	9.02	-1.46
Age8:TreatmentF50:CO	-8.53	8.03	-1.06
Age10:TreatmentF50:CO	-6.12	7.90	-0.77
Age12:TreatmentF50:CO	-5.19	7.88	-0.66

Age14:TreatmentF50:CO	-4.77	7.87	-0.61
Age16:TreatmentF50:CO	-4.62	7.87	-0.59
Age2:TreatmentF50:FL	-13.73	9.55	-1.44
Age4:TreatmentF50:FL	-21.56	11.12	-1.94
Age6:TreatmentF50:FL	-15.98	9.02	-1.77
Age8:TreatmentF50:FL	-21.43	8.03	-2.67
Age10:TreatmentF50:FL	-20.28	7.90	-2.57
Age12:TreatmentF50:FL	-19.45	7.88	-2.47
Age14:TreatmentF50:FL	-19.03	7.87	-2.42
Age16:TreatmentF50:FL	-18.88	7.87	-2.40
Age2:StrainMIX	-32.59	9.55	-3.41
Age4:StrainMIX	-30.61	11.12	-2.75
Age6:StrainMIX	-14.46	9.02	-1.60
Age8:StrainMIX	-11.92	8.03	-1.49
Age10:StrainMIX	-9.92	7.90	-1.26
Age12:StrainMIX	-9.41	7.88	-1.19
Age14:StrainMIX	-9.22	7.87	-1.17
Age16:StrainMIX	-9.17	7.87	-1.17
Age2:StrainMY	-30.05	9.55	-3.15
Age4:StrainMY	-16.76	11.12	-1.51
Age6:StrainMY	-8.44	9.02	-0.94
Age8:StrainMY	-10.44	8.03	-1.30
Age10:StrainMY	-12.88	7.90	-1.63
Age12:StrainMY	-13.43	7.88	-1.71
Age14:StrainMY	-13.91	7.87	-1.77
Age16:StrainMY	-14.16	7.87	-1.80
Age0:Temp15:TreatmentF50:CO	29.09	8.62	3.38
Age2:Temp15:TreatmentF50:CO	-11.56	8.05	-1.44
Age4:Temp15:TreatmentF50:CO	-4.61	7.16	-0.64
Age6:Temp15:TreatmentF50:CO	0.52	3.88	0.13
Age8:Temp15:TreatmentF50:CO	0.39	0.98	0.40
Age10:Temp15:TreatmentF50:CO	0.97	0.32	3.03
Age12:Temp15:TreatmentF50:CO	0.65	0.29	2.29
Age14:Temp15:TreatmentF50:CO	0.28	0.28	0.98
Age16:Temp15:TreatmentF50:CO	0.08	0.29	0.28
Age0:Temp25:TreatmentF50:CO	-2.79	8.62	-0.32
Age2:Temp25:TreatmentF50:CO	-49.13	8.05	-6.10
Age4:Temp25:TreatmentF50:CO	-32.93	7.16	-4.60
Age6:Temp25:TreatmentF50:CO	-8.84	3.88	-2.28
Age8:Temp25:TreatmentF50:CO	-0.41	0.98	-0.42
Age10:Temp25:TreatmentF50:CO	0.76	0.32	2.36
Age12:Temp25:TreatmentF50:CO	0.67	0.29	2.34
Age14:Temp25:TreatmentF50:CO	0.27	0.28	0.94
Age16:Temp25:TreatmentF50:CO	0.08	0.29	0.28
Age0:Temp15:TreatmentF50:FL	-77.65	8.62	-9.01
Age2:Temp15:TreatmentF50:FL	-109.70	8.05	-13.63
Age4:Temp15:TreatmentF50:FL	-30.75	7.16	-4.29
Age6:Temp15:TreatmentF50:FL	-26.73	3.88	-6.89
Age8:Temp15:TreatmentF50:FL	-1.95	0.98	-1.99
Age10:Temp15:TreatmentF50:FL	0.68	0.32	2.11
Age12:Temp15:TreatmentF50:FL	0.65	0.29	2.29
Age14:Temp15:TreatmentF50:FL	0.28	0.28	0.98
Age16:Temp15:TreatmentF50:FL	0.08	0.29	0.28

Age0:Temp25:TreatmentF50:FL	18.75	8.62	2.18
Age2:Temp25:TreatmentF50:FL	-58.51	8.05	-7.27
Age4:Temp25:TreatmentF50:FL	-63.56	7.16	-8.88
Age6:Temp25:TreatmentF50:FL	-21.68	3.88	-5.59
Age8:Temp25:TreatmentF50:FL	-2.16	0.98	-2.20
Age10:Temp25:TreatmentF50:FL	0.76	0.32	2.36
Age12:Temp25:TreatmentF50:FL	0.67	0.29	2.34
Age14:Temp25:TreatmentF50:FL	0.27	0.28	0.94
Age16:Temp25:TreatmentF50:FL	0.08	0.29	0.28
Age0:Temp15:StrainMIX	49.05	8.62	5.69
Age2:Temp15:StrainMIX	36.65	8.05	4.55
Age4:Temp15:StrainMIX	24.76	7.16	3.46
Age6:Temp15:StrainMIX	1.27	3.88	0.33
Age8:Temp15:StrainMIX	0.80	0.98	0.82
Age10:Temp15:StrainMIX	0.03	0.32	0.08
Age12:Temp15:StrainMIX	0.12	0.29	0.42
Age14:Temp15:StrainMIX	0.03	0.28	0.09
Age16:Temp15:StrainMIX	0.00	0.29	0.00
Age0:Temp25:StrainMIX	10.99	8.62	1.28
Age2:Temp25:StrainMIX	-8.05	8.05	-1.00
Age4:Temp25:StrainMIX	-7.25	7.16	-1.01
Age6:Temp25:StrainMIX	-1.77	3.88	-0.46
Age8:Temp25:StrainMIX	1.00	0.98	1.02
Age10:Temp25:StrainMIX	0.48	0.32	1.49
Age12:Temp25:StrainMIX	0.19	0.29	0.66
Age14:Temp25:StrainMIX	0.03	0.28	0.09
Age16:Temp25:StrainMIX	0.00	0.29	0.00
Age0:Temp15:StrainMY	79.51	8.62	9.23
Age2:Temp15:StrainMY	56.55	8.05	7.02
Age4:Temp15:StrainMY	32.20	7.16	4.50
Age6:Temp15:StrainMY	4.88	3.88	1.26
Age8:Temp15:StrainMY	-0.92	0.98	-0.94
Age10:Temp15:StrainMY	-0.69	0.32	-2.16
Age12:Temp15:StrainMY	-0.45	0.29	-1.59
Age14:Temp15:StrainMY	-0.23	0.28	-0.80
Age16:Temp15:StrainMY	-0.08	0.29	-0.28
Age0:Temp25:StrainMY	11.53	8.62	1.34
Age2:Temp25:StrainMY	13.37	8.05	1.66
Age4:Temp25:StrainMY	-6.40	7.16	-0.89
Age6:Temp25:StrainMY	-6.03	3.88	-1.55
Age8:Temp25:StrainMY	-1.61	0.98	-1.65
Age10:Temp25:StrainMY	-0.40	0.32	-1.24
Age12:Temp25:StrainMY	-0.41	0.29	-1.45
Age14:Temp25:StrainMY	-0.21	0.28	-0.75
Age16:Temp25:StrainMY	-0.08	0.29	-0.28
Age0:TreatmentF50:CO:StrainMIX	0.08	8.62	0.01
Age2:TreatmentF50:CO:StrainMIX	34.99	8.05	4.35
Age4:TreatmentF50:CO:StrainMIX	17.55	7.16	2.45
Age6:TreatmentF50:CO:StrainMIX	7.63	3.88	1.97
Age8:TreatmentF50:CO:StrainMIX	3.23	0.98	3.29
Age10:TreatmentF50:CO:StrainMIX	0.61	0.32	1.91
Age12:TreatmentF50:CO:StrainMIX	0.13	0.29	0.47
Age14:TreatmentF50:CO:StrainMIX	0.03	0.28	0.09

Age16:TreatmentF50:CO:StrainMIX	0.00	0.29	0.00
Age0:TreatmentF50:FL:StrainMIX	-27.08	8.62	-3.14
Age2:TreatmentF50:FL:StrainMIX	48.61	8.05	6.04
Age4:TreatmentF50:FL:StrainMIX	54.80	7.16	7.65
Age6:TreatmentF50:FL:StrainMIX	11.99	3.88	3.09
Age8:TreatmentF50:FL:StrainMIX	2.93	0.98	2.99
Age10:TreatmentF50:FL:StrainMIX	0.57	0.32	1.78
Age12:TreatmentF50:FL:StrainMIX	0.13	0.29	0.47
Age14:TreatmentF50:FL:StrainMIX	0.03	0.28	0.09
Age16:TreatmentF50:FL:StrainMIX	0.00	0.29	0.00
Age0:TreatmentF50:CO:StrainMY	-13.85	8.62	-1.61
Age2:TreatmentF50:CO:StrainMY	16.16	8.05	2.01
Age4:TreatmentF50:CO:StrainMY	1.15	7.16	0.16
Age6:TreatmentF50:CO:StrainMY	3.93	3.88	1.01
Age8:TreatmentF50:CO:StrainMY	-2.37	0.98	-2.42
Age10:TreatmentF50:CO:StrainMY	-0.80	0.32	-2.49
Age12:TreatmentF50:CO:StrainMY	-0.57	0.29	-2.01
Age14:TreatmentF50:CO:StrainMY	-0.24	0.28	-0.84
Age16:TreatmentF50:CO:StrainMY	-0.08	0.29	-0.28
Age0:TreatmentF50:FL:StrainMY	-28.95	8.62	-3.36
Age2:TreatmentF50:FL:StrainMY	29.08	8.05	3.61
Age4:TreatmentF50:FL:StrainMY	31.73	7.16	4.43
Age6:TreatmentF50:FL:StrainMY	1.76	3.88	0.45
Age8:TreatmentF50:FL:StrainMY	-0.92	0.98	-0.94
Age10:TreatmentF50:FL:StrainMY	-1.05	0.32	-3.28
Age12:TreatmentF50:FL:StrainMY	-0.57	0.29	-2.01
Age14:TreatmentF50:FL:StrainMY	-0.24	0.28	-0.84
Age16:TreatmentF50:FL:StrainMY	-0.08	0.29	-0.28
B) Random effects			
Groups Name	Variance	Std.Dev.	% of Variance
ind1 age0	1391.10	37.30	36.00
age2	1214.20	34.84	31.42
age4	959.97	30.98	24.84
age6	280.43	16.75	7.26
age8	16.49	4.06	0.43
age10	0.42	0.65	0.01
age12	0.00	0.07	0.00
age14	0.00	0.05	0.00
age16	0.02	0.13	0.00
Residual	1.52	1.23	0.04

Appendix III

Summary of the mixed-effects model (Model M6 Table 6.4.C) to describe the age specific fecundity after the translocation.

The model included 4050 number of observations corresponding to 450 individuals within 15 replicates.

Linear mixed model fit by maximum likelihood
Formula: $m_x \sim$ Age*Treatment*Strain+(age-1|ind1)

A) Fixed effects:	Estimate	Std. Error	t value
(Intercept)	39.24	6.95	5.65
Age2	107.64	8.69	12.39
Age4	46.64	9.45	4.94
Age6	-8.24	7.86	-1.05
Age8	-32.48	7.08	-4.59
Age10	-37.8	6.97	-5.43
Age12	-39.12	6.95	-5.63
Age14	-39.24	6.95	-5.65
Age16	-39.24	6.95	-5.65
TreatmentF1FL	-39.24	9.83	-3.99
TreatmentF50CO	57.32	9.83	5.83
TreatmentF50FL	-39.24	9.83	-3.99
TreatmentF51:FL:CO	28.72	9.83	2.92
TreatmentF51CO:FL	-39.24	9.83	-3.99
StrainHYB	111.72	9.83	11.37
StrainMY	141.16	9.83	14.37
Age2:TreatmentF1FL	-97.92	12.29	-7.97
Age4:TreatmentF1FL	-37.32	13.36	-2.79
Age6:TreatmentF1FL	13.28	11.12	1.2
Age8:TreatmentF1FL	34.52	10.01	3.45
Age10:TreatmentF1FL	38.48	9.85	3.91
Age12:TreatmentF1FL	39.12	9.83	3.98
Age14:TreatmentF1FL	39.24	9.83	3.99
Age16:TreatmentF1FL	39.24	9.82	3.99
Age2:TreatmentF50CO	-115.24	12.29	-9.38
Age4:TreatmentF50CO	-81.36	13.36	-6.09
Age6:TreatmentF50CO	-76.56	11.12	-6.89
Age8:TreatmentF50CO	-63.6	10.01	-6.35
Age10:TreatmentF50CO	-58.76	9.85	-5.97
Age12:TreatmentF50CO	-57.44	9.83	-5.85
Age14:TreatmentF50CO	-57.32	9.83	-5.83
Age16:TreatmentF50CO	-57.32	9.82	-5.84
Age2:TreatmentF50FL	-82.96	12.29	-6.75
Age4:TreatmentF50FL	-28.24	13.36	-2.11
Age6:TreatmentF50FL	9.6	11.12	0.86
Age8:TreatmentF50FL	32.48	10.01	3.25
Age10:TreatmentF50FL	37.8	9.85	3.84

Age12:TreatmentF50FL	39.12	9.83	3.98
Age14:TreatmentF50FL	39.24	9.83	3.99
Age16:TreatmentF50FL	39.24	9.82	3.99
Age2:TreatmentF51:FL:CO	-155.16	12.29	-12.63
Age4:TreatmentF51:FL:CO	-95.88	13.36	-7.18
Age6:TreatmentF51:FL:CO	-59.48	11.12	-5.35
Age8:TreatmentF51:FL:CO	-35.48	10.01	-3.54
Age10:TreatmentF51:FL:CO	-30.16	9.85	-3.06
Age12:TreatmentF51:FL:CO	-28.84	9.83	-2.94
Age14:TreatmentF51:FL:CO	-28.72	9.83	-2.92
Age16:TreatmentF51:FL:CO	-28.72	9.82	-2.92
Age2:TreatmentF51CO:FL	-103.72	12.29	-8.44
Age4:TreatmentF51CO:FL	-44.96	13.36	-3.37
Age6:TreatmentF51CO:FL	9.4	11.12	0.85
Age8:TreatmentF51CO:FL	32.48	10.01	3.25
Age10:TreatmentF51CO:FL	37.8	9.85	3.84
Age12:TreatmentF51CO:FL	39.12	9.83	3.98
Age14:TreatmentF51CO:FL	39.24	9.83	3.99
Age16:TreatmentF51CO:FL	39.24	9.82	3.99
Age2:StrainHYB	-120.68	12.29	-9.82
Age4:StrainHYB	-146.56	13.36	-10.97
Age6:StrainHYB	-127.4	11.12	-11.46
Age8:StrainHYB	-116.48	10.01	-11.64
Age10:StrainHYB	-112.8	9.85	-11.45
Age12:StrainHYB	-111.8	9.83	-11.38
Age14:StrainHYB	-111.72	9.83	-11.37
Age16:StrainHYB	-111.72	9.82	-11.37
Age2:StrainMY	-106.04	12.29	-8.63
Age4:StrainMY	-131.24	13.36	-9.82
Age6:StrainMY	-136.52	11.12	-12.28
Age8:StrainMY	-140.32	10.01	-14.02
Age10:StrainMY	-141.6	9.85	-14.38
Age12:StrainMY	-141.08	9.83	-14.36
Age14:StrainMY	-141.16	9.83	-14.36
Age16:StrainMY	-141.16	9.82	-14.37
TreatmentF1FL:StrainHYB	-111.72	13.9	-8.04
TreatmentF50CO:StrainHYB	-29.2	13.9	-2.1
TreatmentF50FL:StrainHYB	-111.72	13.9	-8.04
TreatmentF51:FL:CO:StrainHYB	-105.24	13.9	-7.57
TreatmentF51CO:FL:StrainHYB	-111.72	13.9	-8.04
TreatmentF1FL:StrainMY	-141.16	13.9	-10.16
TreatmentF50CO:StrainMY	-55.56	13.9	-4
TreatmentF50FL:StrainMY	-141.16	13.9	-10.16
TreatmentF51:FL:CO:StrainMY	-129	13.9	-9.28
TreatmentF51CO:FL:StrainMY	-141.16	13.9	-10.16
Age2:TreatmentF1FL:StrainHYB	121.96	17.37	7.02
Age4:TreatmentF1FL:StrainHYB	150.6	18.89	7.97
Age6:TreatmentF1FL:StrainHYB	130	15.72	8.27
Age8:TreatmentF1FL:StrainHYB	117.44	14.16	8.3
Age10:TreatmentF1FL:StrainHYB	113.12	13.93	8.12

Age12:TreatmentF1FL:StrainHYB	111.96	13.9	8.06
Age14:TreatmentF1FL:StrainHYB	111.72	13.9	8.04
Age16:TreatmentF1FL:StrainHYB	111.72	13.89	8.04
Age2:TreatmentF50CO:StrainHYB	131.52	17.37	7.57
Age4:TreatmentF50CO:StrainHYB	74.96	18.89	3.97
Age6:TreatmentF50CO:StrainHYB	52.04	15.72	3.31
Age8:TreatmentF50CO:StrainHYB	37.08	14.16	2.62
Age10:TreatmentF50CO:StrainHYB	30.4	13.93	2.18
Age12:TreatmentF50CO:StrainHYB	29.28	13.9	2.11
Age14:TreatmentF50CO:StrainHYB	29.2	13.9	2.1
Age16:TreatmentF50CO:StrainHYB	29.2	13.89	2.1
Age2:TreatmentF50FL:StrainHYB	118.68	17.37	6.83
Age4:TreatmentF50FL:StrainHYB	136.88	18.89	7.25
Age6:TreatmentF50FL:StrainHYB	126.76	15.72	8.06
Age8:TreatmentF50FL:StrainHYB	116.48	14.16	8.23
Age10:TreatmentF50FL:StrainHYB	112.8	13.93	8.1
Age12:TreatmentF50FL:StrainHYB	111.8	13.9	8.05
Age14:TreatmentF50FL:StrainHYB	111.72	13.9	8.04
Age16:TreatmentF50FL:StrainHYB	111.72	13.89	8.04
Age2:TreatmentF51:FL:CO:StrainHYB	114.6	17.37	6.6
Age4:TreatmentF51:FL:CO:StrainHYB	138.76	18.89	7.34
Age6:TreatmentF51:FL:CO:StrainHYB	120.68	15.72	7.68
Age8:TreatmentF51:FL:CO:StrainHYB	110	14.16	7.77
Age10:TreatmentF51:FL:CO:StrainHYB	106.32	13.93	7.63
Age12:TreatmentF51:FL:CO:StrainHYB	105.32	13.9	7.58
Age14:TreatmentF51:FL:CO:StrainHYB	105.24	13.9	7.57
Age16:TreatmentF51:FL:CO:StrainHYB	105.24	13.89	7.58
Age2:TreatmentF51CO:FL:StrainHYB	168.36	17.37	9.69
Age4:TreatmentF51CO:FL:StrainHYB	147.96	18.89	7.83
Age6:TreatmentF51CO:FL:StrainHYB	127.16	15.72	8.09
Age8:TreatmentF51CO:FL:StrainHYB	116.48	14.16	8.23
Age10:TreatmentF51CO:FL:StrainHYB	112.8	13.93	8.1
Age12:TreatmentF51CO:FL:StrainHYB	111.8	13.9	8.05
Age14:TreatmentF51CO:FL:StrainHYB	111.72	13.9	8.04
Age16:TreatmentF51CO:FL:StrainHYB	111.72	13.89	8.04
Age2:TreatmentF1FL:StrainMY	125.72	17.37	7.24
Age4:TreatmentF1FL:StrainMY	147.36	18.89	7.8
Age6:TreatmentF1FL:StrainMY	143.96	15.72	9.16
Age8:TreatmentF1FL:StrainMY	142.12	14.16	10.04
Age10:TreatmentF1FL:StrainMY	141.4	13.93	10.15
Age12:TreatmentF1FL:StrainMY	141.08	13.9	10.15
Age14:TreatmentF1FL:StrainMY	141.16	13.9	10.16
Age16:TreatmentF1FL:StrainMY	141.16	13.89	10.16
Age2:TreatmentF50CO:StrainMY	95.28	17.37	5.48
Age4:TreatmentF50CO:StrainMY	62.4	18.89	3.3
Age6:TreatmentF50CO:StrainMY	77.64	15.72	4.94
Age8:TreatmentF50CO:StrainMY	56.64	14.16	4
Age10:TreatmentF50CO:StrainMY	56.4	13.93	4.05
Age12:TreatmentF50CO:StrainMY	55.48	13.9	3.99
Age14:TreatmentF50CO:StrainMY	55.56	13.9	4

Age16:TreatmentF50CO:StrainMY	55.56	13.89	4
Age2:TreatmentF50FL:StrainMY	122.72	17.37	7.06
Age4:TreatmentF50FL:StrainMY	135	18.89	7.15
Age6:TreatmentF50FL:StrainMY	136.08	15.72	8.66
Age8:TreatmentF50FL:StrainMY	140.32	14.16	9.91
Age10:TreatmentF50FL:StrainMY	141.6	13.93	10.17
Age12:TreatmentF50FL:StrainMY	141.08	13.9	10.15
Age14:TreatmentF50FL:StrainMY	141.16	13.9	10.16
Age16:TreatmentF50FL:StrainMY	141.16	13.89	10.16
Age2:TreatmentF51:FL:CO:StrainMY	139.76	17.37	8.04
Age4:TreatmentF51:FL:CO:StrainMY	116.68	18.89	6.18
Age6:TreatmentF51:FL:CO:StrainMY	124.16	15.72	7.9
Age8:TreatmentF51:FL:CO:StrainMY	128.16	14.16	9.05
Age10:TreatmentF51:FL:CO:StrainMY	129.44	13.93	9.29
Age12:TreatmentF51:FL:CO:StrainMY	128.92	13.9	9.28
Age14:TreatmentF51:FL:CO:StrainMY	129	13.9	9.28
Age16:TreatmentF51:FL:CO:StrainMY	129	13.89	9.29
Age2:TreatmentF51CO:FL:StrainMY	126.48	17.37	7.28
Age4:TreatmentF51CO:FL:StrainMY	135.84	18.89	7.19
Age6:TreatmentF51CO:FL:StrainMY	135.96	15.72	8.65
Age8:TreatmentF51CO:FL:StrainMY	140.32	14.16	9.91
Age10:TreatmentF51CO:FL:StrainMY	141.6	13.93	10.17
Age12:TreatmentF51CO:FL:StrainMY	141.08	13.9	10.15
Age14:TreatmentF51CO:FL:StrainMY	141.16	13.9	10.16
Age16:TreatmentF51CO:FL:StrainMY	141.16	13.89	10.16
B) Random effects			
Groups Name	Variance	Std.Dev.	% of Variance
ind age0	1206.00	34.73	34.50
age2	1229.00	35.06	35.15
age4	812.37	28.50	23.24
age6	236.65	15.38	6.77
age8	10.81	3.29	0.31
age10	0.22	0.47	0.01
age12	0.01	0.10	0.00
age14	0.00	0.06	0.00
age16	0.01	0.09	0.00
Residual	1.03	1.02	0.03