

Responses to stress of *Caenorhabditis elegans* populations with different reproductive strategies

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Summary

1. Hermaphroditic and gonochoric reproduction are essentially different reproductive strategies that may lead to diverging population responses to adverse environmental conditions. Each strategy implies different physiological mechanisms, which affect life-history traits and represent different ways of dealing with stress.

2. We studied the performance of hermaphroditic vs gonochoric strains in the nematode *Caenorhabditis elegans* exposed to cadmium stress at the individual and population level.

3. Under control conditions, the gonochoric strain started reproduction earlier than the hermaphroditic strain at a smaller size. This was due to an earlier switch from sperm to oocyte production triggered by male sperm availability. Under cadmium stress hermaphrodites showed a decrease in the size at onset of reproduction, presumably as a strategy to maintain a high population growth rate. In contrast the body size of gonochoric nematodes was not affected.

4. A process-based model (DEBtox) was used as a tool for analysing life-history data and calculating population growth rates. The model fitted the data well using physiologically relevant parameters such as ageing, survival or reproduction related parameters. The simultaneous fit of all life-history traits was used to obtain populations growth rate estimates.

5. The differences between the two *C. elegans* strains were reflected at the population level. Lower population growth rates, as calculated by DEBtox, were found in the gonochoric strain, largely determined by the proportion of males in the offspring.

6. From the overall results we suggest that the differences found between both populations are due to the reproductive strategy. Under control conditions, CB strain (with gonochoric reproduction) does not favour population growth rates in the short term due to faster ageing and copulation costs on survival. Furthermore, in response to stress this strain also showed lower performance than the N2 hermaphroditic strain, mainly due to a higher sensitivity of survival to the stressor.

Key-words: Cadmium, gonochoric, hermaphroditic, life history, population level effects

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Introduction

The reproductive strategy of an organism is of great importance in determining its performance under different environmental conditions. For instance, Colegrave, Kaltz & Bell (2002) investigated whether sexual recombination in *Chlamydomonas* generates more variation necessary to adapt to changing environments compared to asexual

populations. Fitness of sexually reproducing populations exceeded that of asexual ones, and they concluded that sex accelerates adaptation to novel food environments. In the case of changing ambient temperatures, Grieg, Borts & Louis (1998) reported that sexual populations of yeast adapted more rapidly to higher temperatures than did asexual ones. The significance of sexual reproduction in the studies of population recovery following toxic stress (Forbes & Depledge 1992) shows the importance of considering different reproductive strategies, since significant differences can be found in the way and efficiency of their response to stress.

The advantages of sexual reproduction and more specifically gonochorism (separation of sexes in different individuals) over asexual reproduction have been sought primarily in relation to the production of variable offspring, with mutation clearance and partial escape from coevolving parasites as the most accepted prevailing theories (West, Lively & Read 1999). These theories suggest that gonochoric populations can respond more efficiently to changes in environmental conditions since they have higher genetic variance. An increased genetic variation provides a larger opportunity to respond to selection than populations where there is no genetic exchange, such as those that have asexual or hermaphroditic (both sexes in one individual) reproduction (Maynard-Smith 1978). Gonochoric populations would therefore have the long-term advantage of a higher rate of adaptation although the short-term benefits are not so clear.

Gonochoric reproduction is a complicated and costly way to reproduce in comparison to asexual or hermaphroditic strategies. Extra costs for sex involve finding a mate and inducing it to cooperate (takes time and energy), competition for mates, possible increase of predation, and the extra investment of producing males in the offspring among others.

Gonochoric reproduction implies investing energy in different ways than in asexual reproduction. The patterns of allocation among the different traits can be understood within the framework of life-history theory. One of the principles is that organisms are constrained by trade-offs in energy allocation, and thus life-history strategies have evolved (Van Straalen & Hoffmann 2000). Different groups of animals vary in their physiological allocation of energy depending on their life-history strategy. The energy allocation rules can be related to life-history traits such as body size and reproductive output, and can be expressed in terms of energy budgets (Kooijman 2000). After exposure to toxicants the allocation patterns depend on the mode of action of the toxicant and the toxicokinetics (Van Straalen & Hoffmann 2000). Yet, the advantages of each of the two reproductive modes in their responses to adverse environmental conditions are unclear.

We studied the responses to stress in two populations that differ in reproductive strategy in the nematode *Caenorhabditis elegans* (Nematoda, Rhabditidae). *C. elegans* usually reproduces hermaphroditically but can switch to gonochoric reproduction in the presence of males. It has an average life span of about 15–20 days at 15 °C, and produces 250–300 eggs during its lifetime. The hermaphroditic mode of reproduction implies that one individual produces both male and female gametes to self-fertilize. At the end of the third larval stage of hermaphrodites, the gametes begin to mature as sperm in the proximal end of the hermaphroditic gonad (Kimble & Ward 1988), and as maturation proceeds the type of gamete matured in the distal end switches to oocytes. Because almost all sperm are matured before the first oocytes mature, the individual possesses its full complement of sperm when fertilization starts (Hirsh, Oppenheim

& Klass 1976; Kimble & Ward 1988). There is also a limit to the number of oocytes that can be produced by the hermaphrodite since there are a fixed number of germ cells available to mature into sperm and eggs. On maturity, a hermaphrodite releases a series of self-fertilized eggs until sperm is depleted and a few remaining unfertilized oocytes are laid. After this, egg release ceases (Ward & Carrel 1979). Males are able to fertilize hermaphrodites, and in this case the number of offspring produced can double that of a non-mated hermaphrodite owing to the higher availability of sperm. This mode of reproduction does not seem to be the predominant one since males are usually infrequent, but when gonochoric reproduction does occur male sperm out-competes hermaphroditic sperm almost completely. This is due to the competitive superiority of male sperm, which is larger and has faster motility (Singson, Hill & L'Hernault 1999). The higher reproductive output produced by gonochoric individuals can be expected to have an effect on other traits. Previous studies with *C. elegans* already showed that mated hermaphrodites had a shorter life span than unmated ones. Although this was initially thought to be an effect of the larger brood size that they produced, it was found to be an effect of copulation. The stress of copulation reduces hermaphrodite life span either directly or by increasing susceptibility to infection by the bacteria used as food (Gems & Riddle 1996).

To be able to fully understand the underlying physiological mechanisms that lead to the observed effects at the population level, a description of the organism's response (survival, growth, reproduction) throughout its life is necessary. The dynamic energy budget (DEB) theory describes the functioning of individual organisms based on a set of simple rules for metabolic organization (Kooijman 2000, 2001), where exposure to toxicants is regarded as a change in energetic parameters. This theory makes use of the formalism of individual-based population modelling to make the link from individual to population level. This insight inspired the development of DEBtox (Kooijman & Bedaux 1996), a suite of models to analyse toxicity experiments in a process-based manner. The DEBtox method has been extended recently to model effects at the population level by means of simultaneously fitting data from the different life-history traits (Jager *et al.* 2004a). This approach was used in this paper to model the effect at the population level of hermaphroditic and gonochoric *C. elegans* individuals under toxic stress. Cadmium was chosen as a known chemical stressor expected to have effects on the life-history traits of this nematode (Ura *et al.* 2002; Swain *et al.* 2004).

Materials and methods

The N2 strain of *C. elegans* was selected as the most suitable for life-cycle testing involving hermaphroditic reproduction, because of the low occurrence of males in this strain. These populations were started with only non-mated hermaphrodites and screened regularly to remove any males. The closely related CB (CB4856)

strain was selected for the gonochoric reproduction experiments, owing to the greater activity and occurrence of males in this strain. In this case populations were started with gravid hermaphrodites that had been fertilized by males, by means of identifying a copulatory plug that remains on the hermaphrodites after copulation (Barker 1995; Hodgkin & Doniach 1997). The ratio of males to hermaphrodites in the offspring of these gravid hermaphrodites was therefore close to 1:1. In these experiments each hermaphrodite was kept with one male at all times, and checked for a copulatory plug to ensure that gonochoric reproduction had taken place. The N2 and CB strains were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota).

Cadmium chloride (CdCl_2) was purchased from Merck, Schuchardt, Germany (>99% pure). The nominal concentrations used were 0, 2, 6, 8, 10 and 12 mg CdCl_2/l agar (0, 10.9, 32.7, 43.6, 54.5 and 65.5 μM). Chemical analysis of the soluble fraction of the agar was performed with inductively coupled plasma atomic emission spectrometry (ICP-AESO; Centraal Laboratorium, Wageningen University Researchcentrum) after 3, 6, 10, 13 and 19 days of preparation of the dishes to monitor any changes in the available fraction of CdCl_2 throughout the experiments (due to binding of compound to the plastic walls or matrix of the agar) (Fig. 1). Precaution was taken against any changes in the cadmium concentrations by using the dishes no longer than 5 days after preparation, since a decrease in cadmium availability was observed after day 6. The relationship between nominal and measured-back concentrations is linear, allowing us to refer to the nominal concentrations for better clarity throughout this study without interfering with the observed patterns in the results.

All nematodes were reared on NGM agar plates seeded with strain OP50 of *Escherichia coli* as a food source (Brenner 1974; Riddle 1988). Stock cultures of OP50 were stored at -80°C and the bacterial cultures were grown in autoclaved LB medium (10 g peptone, 10 g yeast

extract, 5 g NaCl/l water) for 16 h at 37°C and 150 r.p.m. Populations of these nematodes were maintained at 15°C .

All experiments were started by synchronizing populations. Gravid adults were transferred to Petri dishes (6 cm diameter) and allowed to lay eggs for a period of 4 h, after which they were removed. After hatching the juveniles were allowed to develop into gravid adults after which a second synchronization was performed, this time on agar containing the corresponding concentration of toxicant. In this way we obtained the individuals for study, which were exposed throughout their whole life span (since egg stage) to the respective concentrations. Two separate experiments were carried out: one to record the reproductive output and survival of the individuals and a second one to obtain the body growth measurements throughout their life span.

REPRODUCTION AND SURVIVAL

The newly hatched nematodes were transferred to individual wells (12 well plates from Greiner Bio-one, Kremsmuenster, Austria) for observation. Each well contained 2 ml agar with the corresponding concentration of CdCl_2 . The nematodes were daily transferred to a new well to count the number of offspring per individual. Reproduction was recorded for 7–12 individuals at each concentration. Survival was recorded when food pumping rate had ceased and the nematodes showed no movement after gently touching them with a small needle. Survival data were recorded in 20–40 individuals per treatment.

GROWTH

Separate growth experiments were performed for hermaphrodites and gonochoric individuals. In both cases, the newly hatched synchronized individuals were kept on Petri dishes (6 cm diameter) with 10 ml agar containing the different concentrations of toxicant. A maximum of 15 individuals were kept on each dish (in the case of gonochorism 15 hermaphrodites and 15 males) to avoid competition. Pictures were taken of 8–10 individuals per treatment with a Cool Snap camera (Photometrics, USA) at intervals of 6 h at the beginning of the growth curve, and longer intervals towards the end. Additionally another experiment was performed to measure the size at onset of reproduction in 0, 6, 8 and 10 mg Cd l^{-1} for both reproductive strategies. Six individuals were synchronized (within 1 h) for each concentration and kept in individual wells. They were observed every 3 h and pictures were taken when the first egg was laid.

The pictures were digitalized with the Image Pro Express 4.0.1 software package (Media Cybernetics, USA) to obtain length and area measurements of the nematodes at the different time points.

MODELLING

The DEBtox method to analyse the life-history traits and the effects of toxicants on these traits is described

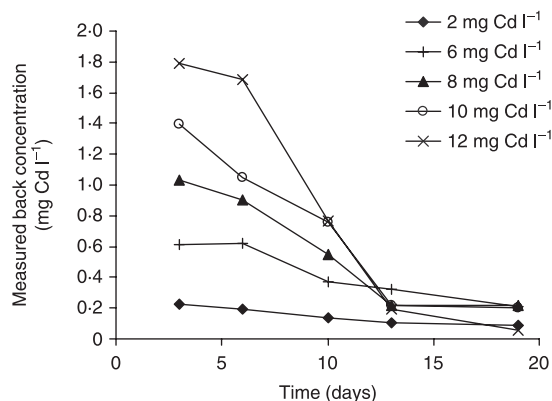


Fig. 1. Chemical analysis of cadmium in soluble fraction of agar at different time points. ◆ nominal 2 mg Cd l^{-1} , + nominal 6 mg Cd l^{-1} , ▲; nominal 8 mg Cd l^{-1} , ○ nominal 10 mg Cd l^{-1} , × nominal 12 mg Cd l^{-1} . Analysis was performed with ICP-AES. A decrease in the measured concentrations is visible after the sixth day.

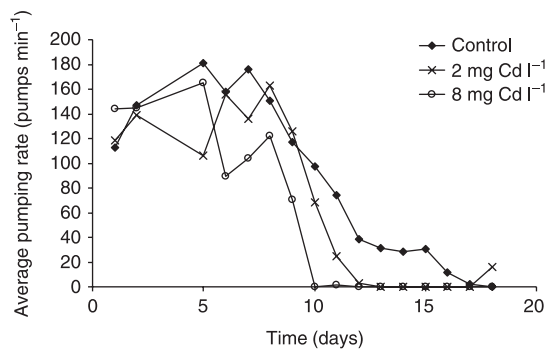


Fig. 2. Average pumping rate throughout time in different concentrations of cadmium. Low pumping rates are observed in newly hatched larvae, which gradually increase until the adult stage is reached supporting observations made by Knight *et al.* (2002) where size of the buccal cavity limits the feeding rate at early time points and thereby growth of the larval worms. This leads to deviations in the expected Von Bertalanffy growth curve of nematodes.

in more detail by Jager *et al.* (2004, 2005). These models are based on the DEB theory with some adaptations. A consequence of the DEB theory is that isomorphic animals grow according to a Von Bertalanffy curve as long as the environment remains constant. The rationale underlying this specific curve is that food uptake is generally proportional to a surface area, whereas maintenance costs are proportional to body volume (Kooijman 2000). However, nematode growth curves are generally sigmoid when length is plotted against time (Byerly, Cassada & Russell 1976), showing a slower initial growth than predicted by the DEB model. An explanation for this phenomenon seems to be that the size of the buccal cavity (i.e. the tube-like mouth cavity, opening up into the oesophagus or pharynx) limits the feeding rate, and thereby growth of the larval worms (Knight *et al.* 2002). Modifications of the growth curves are explained in more detail by Jager *et al.* (2005). These observations are also supported by the observed low pumping rates of newly hatched larvae, which gradually increased until the adult stage was reached. This shows that the buccal cavity was indeed limiting feeding (and therefore growth) in these early time points, whereas at a later stage, the nematodes were able to feed freely and the growth curves showed the expected pattern at this point (Fig. 2).

Apart from the deviations due to the details of growth in the nematodes, some changes were also made to the model describing the effects of old age (senescence). Many aspects of nematode physiology will change with age, but in life-cycle toxicity studies, the primary concerns are the effects on survival and reproduction. Basically, the model by Van Leeuwen, Kelpin & Kooijman (2002) was followed. This model is based on the oxidative stress hypothesis: aerobic respiration produces free radicals that cause damage to cell components. This damage accumulates in the cell, leading to the ageing phenotype. This approach was successfully applied to life-cycle studies with the springtail *Folsomia candida* (Jager 2004).

We applied the same model to nematodes with a few modifications. Firstly, the maintenance rate was not taken as a free model parameter, but was linked directly to the rate constant for growth (Kooijman & Bedaux 1996). Further, old-age effects were not related to damage density but to the absolute amount of oxidative damage in the organism's body, and no additional feedback mechanisms were assumed.

A different mechanism for the old-age effects on reproduction was assumed for each strategy. In hermaphrodites, egg production ceases when the storage of sperm cells is depleted (Ward & Carrel 1979), leading to a sudden halt of reproduction. In contrast, gonochoristic *C. elegans* show a more gradual decline in egg production, presumably due to ageing effects (oxidative damage) on the developing egg.

Finally, the observed toxic effects at the highest concentration were less than expected from the model. To accommodate this behaviour, we assumed that the uptake of cadmium is saturating at higher external concentrations according to Michaelis–Menten kinetics. Similar saturation was for example shown for cadmium in aquatic oligochaetes (Steen Redeker & Blust 2004) and in fish (Van Ginneken, Bervoets & Blust 2001).

The model fits for all traits and both reproductive modes were done simultaneously, which led to a total of eight data sets. These include survival, cumulative reproduction and two growth data sets (one of which incorporates old age sizes) for each strategy (Fig. 3). Simultaneous fitting was required because the data sets include common parameters. It was assumed that most of the parameters do not differ between the hermaphroditic and gonochoric strains, such as the growth parameters and the intrinsic sensitivity to cadmium. However, the ageing parameters needed to be given different values since gonochoric reproduction had a negative effect (copulation damage) on survival, which was accounted for as a higher damage killing rate, reflecting faster ageing in these individuals than in the hermaphrodites. The ageing parameters also differ because of the different mechanisms that govern the reproduction patterns at old ages (sperm shortage in hermaphrodites *vs* oxidative damage in gonochoric individuals).

Difficulties were encountered when matching the data sets in the model fitting procedure because the experiments for growth were done separately from the determination of survival and reproduction. This led to slight variations in the initial part of the growth curve (i.e. the reproduction data may have resulted from animals with a slightly shifted growth pattern, compared with the body size data). This problem was solved by determining the body size at the onset of reproduction independently. In this way, the length at the start of reproduction became a fixed parameter.

The model was implemented in Matlab® Version 7-0 (Release 14, Mathworks, MA, USA), and model fitting was based on maximum likelihood estimation. Confidence intervals were generated using profile likelihoods

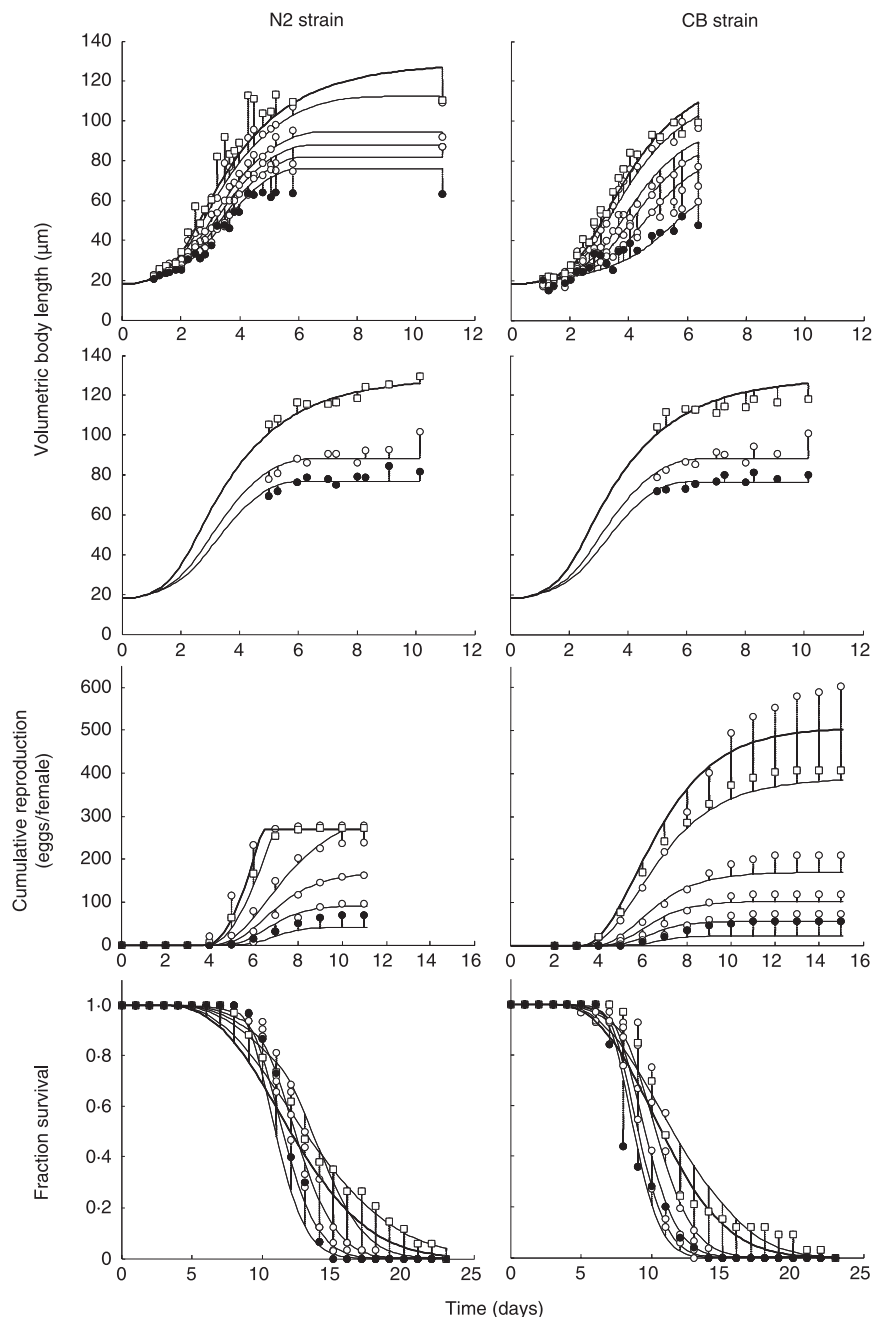


Fig. 3. Data and simultaneous model fits for all end-points of the life-cycle study with *C. elegans* exposed to cadmium, for both reproductive strategies. $\square$ control, $\circ$ concentrations 2, 6, 8 and 10 mg Cd l⁻¹, $\bullet$ 12 mg Cd l⁻¹ (highest concentration). These include survival, cumulative reproduction, and two growth data sets for each strain. The top graphs show the data points for all concentrations until adult stage (day 6), whereas the bottom graphs include the data points for three concentrations until the old-age stage (day 10) to calculate the point at which growth stops completely. Simultaneous fitting was required because the data sets include common parameters. The effects of cadmium on the different end-points are visible as concentrations increase, as well as differences in reproduction and survival between the two strategies.

(Meeker & Escobar 1995). The intrinsic rate of population increase was calculated according to the Euler–Lotka equation, as a function of exposure concentration (Jager *et al.* 2004). Further, the population effects at limiting food densities were predicted. This was possible as food limitation has predictable consequences in DEB theory (Jager *et al.* 2005); although care must be taken, as this calculation is an extrapolation beyond the information provided by the experimental data (e.g. it was assumed that the intrinsic sensitivity to cadmium is not affected by food level).

Results

Good model fits were obtained for the data sets, with few differences between the parameters for N2 and CB populations (Table 1). Fertilization by males was not affected by cadmium exposure (*t*-test, $n_1 = 7$, $n_2 = 6$, $n_3 = 7$, $t_1 = 0.096$, $t_2 = 2.205$, $t_3 = 1.842$, $P > 0.05$); the male: hermaphrodite ratio in the F1 of the gonochoric (CB) experiments ranged from 0.45 to 0.51 in control conditions, 8 mg Cd l⁻¹ and 12 mg Cd l⁻¹.

Table 1. Parameters used in the model

	N2 strain	CB strain
Physiological parameters		
Von Bertalanffy growth rate (day ⁻¹)	0.488 (0.476–0.520)	
Initial length (µm)	18.1 ^a	
Length at first reproduction (µm)	83.4 ^a	69.9 ^a
Maximum length (µm)	130 (127–132)	
Maximum reproduction rate (eggs day ⁻¹)	336 (297–370)	
Energy investment ratio (–)	10 ^a	
Time before hatching (day)	0.375 (9 h) ^a	
Length where ingestion is half of maximum (µm)	30.0 (29.8–30.4) (growth 1) 29.8 (28.9–30.6) (growth 2) 31.0 (30.8–31.3) (repro/surv)	31.1 (30.9–31.4) (growth 1) 29.8 (29.1–30.6) (growth 2) 30.9 (30.7–31.2) (repro/surv)
Ageing parameters		
Damage killing rate (day ⁻¹)	0.0185 (0.0146–0.0229)	0.0268 (0.0207–0.0338)
Damage tolerance on reproduction (–)	8 ^a	3.04 (2.67–3.80)
Maximum total number of eggs	269 (248–288)	8 ^a
Toxicological parameters		
Elimination rate (day ⁻¹)	0.0138 (0.0125–0.0161)	
Half-saturation conc. (mg l ⁻¹)	22.9 (14.5–28.8)	
NEC for survival (mg l ⁻¹)	0.559 (0.488–0.619)	0.265 (0.219–0.311)
Killing rate (mg l ⁻¹ day ⁻¹)	1.00 (0.674–1.51)	1.30 (0.935–1.67)
NEC for effects on assimilation (mg l ⁻¹)	4.20e-6 (0–0.00637)	
Tolerance concentration (mg l ⁻¹)	1.00 (0.751–1.08)	
Decrease in length at first repro due to Cd stress (–)	0.931 (0.665–1.24)	0 ^a

^aIndicates parameter is fixed, not estimated from the data. Confidence intervals obtained from DEB model in brackets. NEC, no effect concentration.

The decrease in reproduction at increasing cadmium levels was clearly visible in both strains. Both in control conditions and in the lowest concentration (2 mg Cd l⁻¹) CB yielded a higher reproductive output than N2, and in both strains the individuals exposed to 2 mg Cd l⁻¹ reproduced more than the controls. However, apart from these two cases all other concentrations showed similar reproduction for both strains (Fig. 3).

Differences were found in the onset of reproduction between the two strains which was probably governed by the reproductive strategy. N2 started reproduction after 100 h with an average volumetric length (cubic root of the volume) of 83.4 µm, whereas CB started reproduction earlier at 84 h with an average volumetric length of 69.9 µm. The differences between the size at onset of reproduction were found to be significant at control conditions (*t*-test, $n_1 = 6$, $n_2 = 6$, $t = 6.780$, $P < 0.05$). At increasing cadmium concentrations, size at onset of reproduction decreased in N2 whereas CB individuals showed little reduction in size (Fig. 4). This suggests a difference between toxicokinetics of cadmium between the two strains.

Cadmium affected survival with visible differences between the curves of the two strains. Lower survival was observed in CB under all conditions. This negative effect on survival was accounted for in the model as a higher damage killing rate (Table 1). This ageing parameter reflected faster ageing in CB individuals than in N2 individuals.

The traits were best described by the model when a decrease in assimilation was assumed as the mode

of action for cadmium, implying that either cadmium affects the feeding rate of the organisms directly, or by decreasing the efficiency with which energy from food is assimilated (Kooijman & Bedaux 1996). The last assumption was difficult to test experimentally but the first assumption was supported by the observation of pharyngeal pumping rates which were found to be significantly lower at increasing concentrations of cadmium (Fig. 2). These observations were also reported in earlier studies on feeding inhibition in *C. elegans* exposed to sub-lethal concentrations of cadmium (Jones & Candido 1999). Body size as well as reproduction were decreased by cadmium, presumably due to the effect on assimilation, without any differences in the physiological parameters of CB and N2 individuals apart from onset of reproduction (Table 1).

Population growth rate was negatively affected by cadmium. Compared with N2, lower values were recorded for CB individuals in all treatments (Fig. 5). Furthermore, CB individuals seemed more affected by the cadmium than N2 individuals. In control conditions, CB presented only a slightly lower population growth rate than N2, but as the concentration of cadmium increased this difference grew visibly. At limiting food levels, the model predicts that the population growth rate collapses at a certain concentration of toxicant, as individuals either do not reach the size threshold for reproduction or do not grow past the food-limited larval stage. The model also showed the increasing effects of cadmium for both strains when food level is reduced as could be seen from the decrease in the concentration at which growth rate collapses.

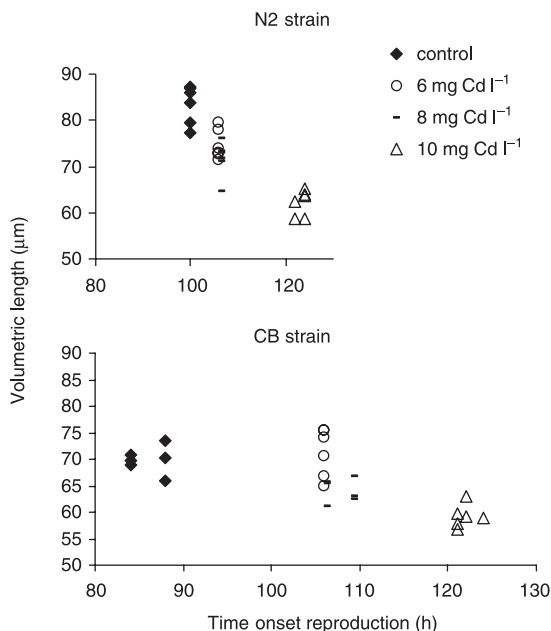


Fig. 4. Size and time of reproduction onset in N2 and CB individuals with increasing concentrations of cadmium. Differences in the onset of reproduction sizes were found to be significant between the two strategies (*t*-test, $P < 0.05$). With increasing concentrations of cadmium, N2 individuals gradually decrease their size at onset of reproduction whereas CB individuals show little reduction in size. This suggests a difference in how cadmium stress was dealt with for onset of reproduction. N2 starts reproduction later and at a larger size than CB. This difference in timing is caused by the larger threshold size for reproduction of N2. This variation in threshold size is probably related to reproductive strategy, where the populations differ in the timing of the switch from sperm production to oocyte production during maturation. Concentrations: ◆ control, ○ 6 mg l⁻¹, – 8 mg l⁻¹, △ 10 mg l⁻¹.

Discussion

ONSET OF REPRODUCTION

N2 started reproduction later and at a larger size than CB (Fig. 4). The difference in timing is caused by the larger threshold size for reproduction of the N2 individuals. The variations in the threshold size are probably related to reproductive strategy, where the two populations differ in the timing of the switch from sperm production to oocyte production during maturation. This switch to oocyte maturation and ovulation is dependent on several gamete signalling events (McCarter *et al.* 1999), in which sperm play an important role. Their presence is necessary for triggering oocyte maturation and basal gonadal sheath activity (Singson 2001). A reduction in sperm production allows an earlier switch and therefore onset of reproduction can take place at a smaller size. Mating probably leads to this decrease in hermaphroditic sperm production and earlier switch to oocyte production. It provides an earlier source of mature sperm that can trigger the maturation of oocytes sooner than in non-mated hermaphrodites, where this does not occur until all sperm have matured.

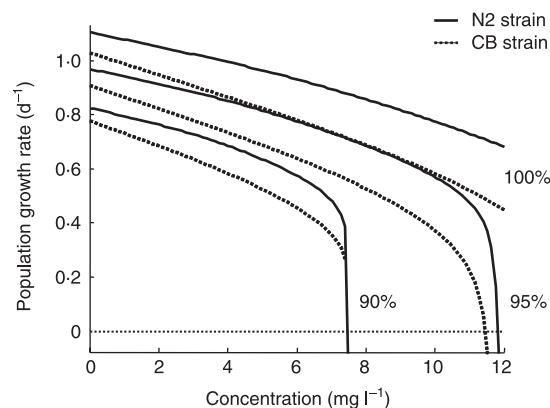


Fig. 5. Modelled intrinsic rate of population increase for *C. elegans*, exposed to cadmium at different food levels (shown as percentage of the maximum ingestion rate). Solid line, N2 strain; dotted line, CB strain. A decrease in the modelled population growth rates is observed with increasing concentrations of cadmium, also showing lower values for CB individuals in control conditions as well as in all of the cadmium concentrations. Furthermore, individuals from CB strain seem more affected by the cadmium stress than individuals from the N2 strain. At limiting food levels, the model predicts that the population growth rate collapses at a certain concentration of toxicant, as individuals either do not reach the size threshold for reproduction or do not grow past the food-limited larval stage. The model also shows the increasing effects of cadmium for both strategies when food level is reduced.

In contrast to CB, the onset of reproduction in N2 occurs at a smaller size as cadmium concentration increases. This may reflect a strategy of N2 in response to lower growth rates (and not to the toxicant *per se*). It is conceivable that the number of sperm cells is such that it maximizes fitness (Cutter 2004). The production of more sperm cells does not necessarily increase the population growth rate because it simultaneously delays reproduction. In a stressed situation (such as Cd exposure), the outcome of this trade-off may be different, and less sperm production may maximize fitness. Thus, the threshold size in N2 is probably being reduced by a decrease in sperm production, triggering an earlier switch to oocyte production. CB individuals, however, already start reproduction at a small size in control conditions, with reduced sperm production in comparison to non-mated N2 individuals, and earlier maturation of oocytes. In CB individuals, the presence of male sperm allows hermaphroditic sperm production to stop early. This happens both in control conditions and in the different concentrations, giving way to similar sizes of reproduction onset. A further reduction in size might not be possible for reproduction to take place.

REPRODUCTION

Cadmium also affected reproduction (Fig. 3). In the control and at the lowest concentration (2 mg Cd l⁻¹) the decline in reproduction is governed by sperm limitation (sudden halt in reproduction due to lack of sperm) in hermaphroditic N2, and ageing/oxidative

damage (gradual decrease of reproduction) in gonochoric CB. In this case, gonochorism produces higher lifetime reproductive output than hermaphroditism although it must be taken into account that half the offspring will be males. However, at higher cadmium concentrations the decline in reproduction is governed by toxicity rather than by the aforementioned mechanisms. Cadmium limits the resources available to the organisms for egg production and therefore comparable reproductive outputs in these cadmium concentrations (6, 8, 10 and 12 mg Cd l⁻¹) can be seen for both strains.

The reproductive output in the lowest concentration of cadmium (2 mg Cd l⁻¹) is higher than that of the control organisms. This apparent hormesis occurs for both N2 and CB individuals, and is visible only in the reproduction data. Hormesis has been defined as an adaptive response to low levels of stress that causes a disruption in homeostasis, resulting in improved fitness or modest overcompensation, for some physiological systems for a finite period (Calabrese & Baldwin 2001). The model does not incorporate this effect but responds to this data set by slightly increasing the curve for the control response.

GROWTH, AGEING AND SURVIVAL

With increasing concentrations of cadmium, a reduction in the growth rate was also observed. The ageing model assumes that a reduction in growth implies lower respiration rates and therefore less oxidative damage, which could lead to an increased longevity. This is one possible explanation for the effect, which is visible between days 5 and 10, where the survival curves of the exposed individuals rise above the control (Fig. 3). After these early time points the toxic effects of cadmium could be counteracting any increase in longevity caused by a reduced respiration, showing a decreasing percentage of survival in time with increasing concentrations.

In all cases, it can be seen that the survival of CB individuals is lower than that of N2 individuals due to the difference in the rate of ageing (Table 1). This faster ageing (higher killing rate) is partly caused by mating costs and the model of oxidative damage accumulation may not be entirely explanatory in this case. Survival in gonochoric CB individuals is more sensitive to cadmium than in N2 (lower no effect concentration, higher killing rate in CB) probably because the mating costs increase their sensitivity. They are, however, not more sensitive to cadmium for growth and reproduction through a decrease in assimilation, which indicates that effects on assimilation and effects on the hazard rate (for survival) may not be directly related.

THE SIGNIFICANCE OF GONOCHORIC REPRODUCTION IN *C. ELEGANS*

The major differences found between the two strains were onset of reproduction (and how cadmium affects it), ageing and sensitivity of survival to cadmium. When

all end-points are considered simultaneously, the analyses suggest that gonochoric reproduction in this species does not seem to favour population growth rates in the short term. It is a complicated and costly way to reproduce, with copulation costs affecting the survival curves and a progeny composed of 50% of males, which also lowers the population growth rates. As performance of the gonochoric strain is expected to be lower compared with the hermaphroditic strain in the short term, it might not be surprising that there are lower frequencies of *C. elegans* males found in nature than of hermaphrodites.

For a long time, the evolution and significance of sex has been a major puzzle in evolutionary biology because of its two-fold costs (Maynard-Smith 1978) and to the challenge of unravelling the advantages that might outweigh the disadvantages of this mode of reproduction. One of the most widely accepted theories, known as the 'Red Queen' hypothesis, suggests that continuous development is needed for an evolutionary system to maintain its fitness relative to the systems with which it is coevolving (Van Valen 1973). Another theory states that sex could act to encourage the removal of deleterious genes (Kondrashov 1988). The fact is that sex is an almost universal phenomenon and few groups of higher eukaryotes have persisted by asexual reproduction for a long period of time (Barton & Charlesworth 1998). However, it can be considered that gonochorism in *C. elegans* is probably not cost-effective under stable conditions, making asexual reproduction more effective in this case. In wild-type *C. elegans* males are found at about 0.05% of the population, but this seems to increase under extreme conditions. Heat shocks of 30 °C for 6 h are commonly used in laboratory cultures to increase the rate of non-disjunction of the X chromosome in gametogenesis and therefore obtain a greater number of males (Sulston & Hodgkin 1988). A male pool could be providing this species with an emergency mechanism to confront the occurrence of adverse conditions, justifying what seems to be a less efficient and more costly reproductive strategy otherwise.

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