Rapid adaptive divergence of life-history traits in response to abiotic stress within a natural population of a parthenogenetic nematode

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Sexual reproduction is acknowledged to facilitate adaptation to novel environments while asexual eukaryotes are often regarded as having low adaptive potential. This view has been challenged in a number of studies, but the adaptive potential of asexual populations in the field is poorly documented. We investigated the response of natural populations of the parthenogenetic nematode *Acrobeloides nanus* to imposed divergent selective pressures. For this purpose, we employed a replicated evolution experiment in the field. After 20 years of evolution under abiotic stress and control conditions, life-history traits were assessed in reaction norm- and reciprocal transplant experiments. Both these experiments indicated adaptive divergence within the population of *A. nanus*. Namely, the transplant experiment demonstrated that in the stressed soil environment, body growth rate was more reduced in the nematodes originating from the control treatment. In the reaction norm experiment, survival and reproduction were higher under test conditions corresponding to the native environment of the nematodes. The differences in the analysed traits are discussed in the context of life-history theory. Overall, our results strongly support high adaptive potential of *A. nanus* and suggest that population structure and distribution of asexual species is shaped by local adaptation events.

Keywords: population differentiation; adaptation; life history; asexual reproduction; stress

1. INTRODUCTION

Asexual populations are expected to have lower adaptive potential than the corresponding sexual populations. This prediction follows the reasoning that the absence of recombination in asexuals results in a lower standing genetic variation available for selection (Goddard *et al.* 2005). In addition, without recombination, new beneficial mutations arising in different individuals cannot be combined to create more successful genotypes. Nevertheless, evidence from microevolutionary studies on bacteria and viruses shows that asexual populations are able to increase their fitness in novel environments without population depreciation or extinction (Travisano & Lenski 1996). Less rapid responses can be expected in eukaryotic populations reproducing asexually because of their lower mutation rates and smaller population sizes. The empirical studies appear to confirm this view by reporting very few cases of rapid adaptive evolution in natural populations of asexual eukaryotes (but see Dickson 1962). On the other hand, the absence of recombination restrains erosion of beneficial associations between loci. Therefore, populations are more likely to diverge and local adaptation, once arisen, can be easily maintained.

The first step in studying adaptive divergence involves the detection of phenotypic differences between local

populations and testing the adaptive value of the observed changes. Usually, the local populations are tested in two habitats in a reciprocal transplant experiment and the relative fitness or fitness-related traits are compared. When the fitness of both populations is higher in their native habitats, it can be concluded that the populations are locally adapted (Kawecki & Ebert 2004). The other type of experiment to detect local adaptation involves tests under controlled laboratory conditions, where only certain properties of the field habitat are recreated. Different local populations can, for instance, be reared under the same environmental conditions or be subjected to different levels of a specific environmental factor (reaction norm experiments). The advantage of the latter is that particular environmental factors can be tested as source of divergent selection.

Environmental stress, either of human or non-human origin, causes a sharp reduction in the fitness and fitness-related traits and is therefore an important driver of evolutionary processes (Bijlsma & Loeschcke 2005). Numerous cases are documented where localized stress conditions led to adaptive divergence within populations (e.g. McNeilly 1968; Berglund *et al.* 2004). According to life-history theory, there are two possible scenarios of evolution of life-history traits in a stressful environment. Adverse effects of a stress agent often accumulate over lifetime (e.g. concentration of heavy metals within the body), which can cause an increase in adult mortality (Posthuma & Van Straalen 1993). One of the possible evolutionary responses to this situation involves earlier onset of reproduction and higher reproduction at a younger age. In this way, the fitness effects of increased

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mortality later in life can be avoided (Stanton *et al.* 2000). Indeed, several studies indicated rapid development and early reproduction as evolutionary response to long-term stress (Rice & Mack 1991; Aronson *et al.* 1992). The other possible scenario would involve investment in defence mechanisms like detoxification or excretion. As a result, an individual could enjoy higher adult survival. Because the energy invested in the defence cannot be used for body growth or reproduction, the onset of reproduction is delayed and the rate of offspring production is reduced. This scenario, compared to the previous one, has a broader empirical documentation (Antonovics *et al.* 1971; Grime & Hunt 1975; Campbell & Grime 1992; Donker 1992).

In our study, we focused on a soil-dwelling, bacterivorous nematode *Acrobeloides nanus* de Man, 1880 (Nematoda, Cephalobidae) that reproduces by means of parthenogenesis (e.g. Bird *et al.* 1993; Goldstein *et al.* 1998; Lahl *et al.* 2006). Egg development in *A. nanus* was described as essentially mitotic with rare cases of second meiotic division, where the restoration of diploidy occurs by the fusion of oocyte nucleus with second polar body (Lahl *et al.* 2006). Although few male individuals of *A. nanus* have been reported in two taxonomical studies (Nesterov 1979; Bongers 1988), validity of this record can be questioned not only because of the lack of a mating test, but also because of uncertainty in correspondence of the morphological characteristics of the males with species description of *A. nanus* (T. Bongers, S. Boström 2006, personal communication). Moreover, none of numerous laboratory and field studies of *A. nanus* recorded any males even after application of severe abiotic stresses which are known to induce emergence of males in non-obligate parthenogenetic species (Bird 1971; Triantaphyllou 1973).

Individuals of *A. nanus* can be found in soils of various physical and chemical properties (Bird *et al.* 1993; Korthals 1997; De Goede & Bongers 1998) and constitute a large part of soil nematode communities in extremely different habitats like the deserts of Australia (Bird *et al.* 1993) and Swedish tundra (Sohlenius & Bostrom 1999). Such a broad species niche could be attributed to the possible existence of general purpose genotypes (Lynch 1984) or it could indicate multiple events of adaptation to the local environment (Futuyma & Moreno 1988). Therefore, *A. nanus* is a valuable biological system to investigate the potential of adaptive divergence in asexual species that successfully inhabit broad range of habitats. Showing that a population of *A. nanus* can undergo multiple events of local adaptation within a short time would indicate large adaptive potential that could be responsible for its broad distribution.

To address this question, we used an experimental field where combined treatments of two stress factors (pH level and copper) were applied approximately 20 years ago in a replicated and randomized block design and maintained thereafter. In this way, the population of *A. nanus* that inhabited the field before the treatment application was divided into a number of subpopulations exposed to different treatments of the stress factors. In order to investigate adaptation to the local environments and test the predictions of life-history theory concerning adaptation to a stressful environment, we analysed life-history traits of the populations evolving for 20 years in control

and stress conditions (low pH and high copper load) using reciprocal transplant and reaction norm experiments.

2. MATERIAL AND METHODS

(a) *Experimental field and experimental populations*

(i) *Study species*

Acrobeloides nanus is a free-living, bacterial-feeding nematode that reproduces by parthenogenesis (Wiegner & Schierenberg 1998; Laugsch & Schierenberg 2004). It can be easily cultured on plates with agar media seeded with bacteria. Under laboratory conditions at 20 °C, the generation time is approximately 14 days and 250 eggs are laid during the reproductive period of 35 days. On the basis of previous experiments (A. Doroszuk 2006, unpublished data) and the analysis of body growth rate in the transplant experiment, the number of generations per year under field conditions was estimated as 0.7 (14 generations in 20 years) for the 'stress' plots and 6.6 (132 generations in 20 years) for the control plots.

(ii) *Sampling and field description*

Nematodes were isolated, with standard techniques (Oostenbrink 1960), from soil samples collected from the experimental field at Bovenbuurt, ca 3 km NNE of Wageningen, The Netherlands (Korthals *et al.* 1996). The experimental field was created in 1982 when an agricultural field was divided into 128 plots of 6 × 11 m each and four copper and pH levels were introduced by applying once CuSO₄·5H₂O and sulphur powder or ground calcitic limestone, respectively, in a full factorial design. The plots were arranged into eight blocks, each with a random distribution of all combinations of copper and pH treatments. Only two extreme treatments were used (each represented by four replicate plots): one treatment imposing stress by low pH (4.0) and the copper concentration of 750 kg ha⁻¹ (habitat S) and another control treatment with pH 6.1 without copper addition (habitat C; figure 1). Maize, potatoes and oats have been grown on the field since 1983 in 3-year crop rotation regime. The pH was readjusted every 5 years on average.

About 30 cores (diameter 30 mm) from the top 10 cm of soil mineral layer were taken from each of the plots. Nematodes classified as *A. nanus* were isolated and placed individually on a Petri dish with proteose peptone agar (PPA; 2% technical agar, 0.5% proteose peptone) medium and *Acinetobacter johnsonii*, a soil bacterium, as a food source in order to establish approximately 20 parthenogenetically derived laboratory populations per plot.

About 16–20 laboratory populations per plot were mixed to establish the representative samples of field populations (8 populations = 2 habitats × 4 replicate plots). The individuals of the fourth generation reared in the laboratory were used in all experiments.

(b) *Testing for adaptation*

(i) *Reciprocal transplant experiment*

Eight experimental populations (2 habitats × 4 replicate plots) were reciprocally transplanted between the soils of both habitats collected from the experimental field of Bovenbuurt. For each population, body growth rate expressed as mean relative growth rate (MRGR) was

Figure 1. Distribution of treatments at the experimental field. Roman numbers indicate randomized blocks. habitat C represents control conditions (pH 6.1; Cu=0 kg ha⁻¹); habitat S represents stressful conditions (pH 4.0; Cu=750 kg ha⁻¹).

assessed. The equation for the MRGR is

$$\text{MRGR} = \frac{(\ln w_2 - \ln w_1)}{(t_2 - t_1)},$$

where w_2 refers to the bodyweight (μg) at the end of the experiment (t_2) and w_1 , the bodyweight (μg) at the start of experiment (t_1 ; Radford 1967).

The experiment was started by placing synchronized eggs (Emmons *et al.* 1979) of each experimental population into minicontainers (Lenz & Eisenbeis 1998; Arts *et al.* 2004) with defaunated soil mixed with a suspension of soil bacteria *A. johnsonii* (2×10^8 cells ml⁻¹ yeast extract). The soil was obtained from the plots of the populations' origin and was defaunated by exposure to 60 °C for 3 h. The experiment was performed in the laboratory at 20 °C in dark. Every 3–5 days, one minicontainer of each population was collected. The nematodes were extracted from the minicontainers (Van Bezooijen 1997), heat killed and fixed in 4% formalin. Body length and largest body width were measured for approximately 20 individuals per minicontainer using a microscope at 40 $\times$ magnification with an ocular equipped with a scale. The body length and width measurements were used to calculate body fresh weight with the use of Andrassy's formula (Freckman 1982). These measurements were used to calculate the MRGR per day.

After approximately 12 days from the start of the experiment, 40 μl of concentrated *A. johnsonii* suspension was applied to each minicontainer to prevent food shortage. In order to obtain standardized MRGR values, the following body mass measurements were used for MRGR calculations: the measurements from the last minicontainer before food

addition as initial body mass and the measurements taken first after food addition as the final measurement. The MRGR data were analysed with a mixed-model nested ANOVA using PROC GLM procedure in SAS v. 8.00 (SAS Institute 2000). Field treatment and soil environment were considered fixed factors and replicate plot was nested within the field treatment.

(ii) Reaction norm experiment: copper

For this experiment, 24-well dishes with PPA of six nominal copper concentrations: 0, 60, 100, 120, 150 and 180 mg CuCl₂ l⁻¹ were used. The metal was added to the agar as CuCl₂ solution and 2 μl of *A. johnsonii* suspension was applied. The dishes were incubated overnight at 28 °C prior to use. Eggs of all eight experimental populations were synchronized according to Emmons *et al.* (1979) and transferred individually to separate wells. The experiment started with 12 eggs per population in each copper concentration (total $N=576=12 \text{ eggs} \times 8 \text{ populations} \times 6 \text{ concentrations}$). After hatching, the nematodes were transferred every other day to new wells. On the basis of daily observation, for each individual the duration of juvenile and reproductive periods, total number of offspring, daily reproduction and lifespan were determined. The dishes were incubated at 20 °C in dark.

Comparison of two field treatments in total- and daily reproduction was done by comparing the values of EC₅₀, which were determined using the following logistic model:

$$E = \frac{\alpha}{1 + (x/\text{EC}_{50})^\beta},$$

where E is trait value; α is the trait value at $x=0$; x is copper concentration; EC₅₀ is the concentration of copper that causes 50% effect; and β is the shape parameter of the curve. The curve was fitted using nonlinear regression procedure. Reproductive period was analysed using two-way ANOVA with field treatments and copper concentration as fixed factors. For the analysis of survival curves as well as juvenile period, we implemented log-rank and Wilcoxon tests (SPSS v. 11.0). The data for the replicate populations within habitats were pooled.

(iii) Reaction norm experiment: pH

In order to obtain agar medium with pH values 4.0, 4.5, 5.0, 5.5 and 6.0, citric buffer system in modified nematode growth medium (Wood 1988) was used. The osmolarity of the agar medium was kept constant by adjusting the concentration of NaCl. The experimental set-up, the order of consecutive actions and the life-history traits determined for each individual were the same as in the copper response experiment. The response of the life-history traits was analysed using two-way ANOVA with field treatment and pH level as fixed factors. For the analysis of survival curves as well as juvenile period, we implemented log-rank and Wilcoxon tests. All the analyses were performed using SPSS v. 11.0.

3. RESULTS

(a) Testing adaptation

(i) Reciprocal transplant experiment

The MRGR of the nematodes originating from the plots of both treatments was significantly lower in the soil of habitat S ($F=39.45$, $p<0.001$). However, the adverse effect was more severe for the populations from the plots of

Figure 2. Mean relative growth rate (MRGR) of the experimental populations from habitat C (solid lines) and habitat S (dashed lines) tested under two environmental conditions in a reciprocal transplant experiment. Each line connects MRGR values for one replicate population in two test environments.

habitat C (figure 2). This difference in populations' response to the soil environment was confirmed by significant interaction origin habitat $\times$ test environment ($F=4.74$, $p=0.05$).

(ii) Reaction norms experiment: copper

The populations derived from the plots of habitat S were less affected by copper in total reproduction (figure 3), which is reflected by the significantly higher value of EC_{50} for this trait ($EC_{50}=169.0 \text{ mg l}^{-1}$, Confidence Interval (CI): $162.5\text{--}175.5 \text{ mg l}^{-1}$) compared with the EC_{50} value obtained for the populations from habitat C ($EC_{50}=137.2 \text{ mg l}^{-1}$, CI: $132.8\text{--}141.5 \text{ mg l}^{-1}$). A similar pattern was obtained for daily reproduction where $EC_{50}=201.1 \text{ mg l}^{-1}$ (CI: $191.0\text{--}211.1 \text{ mg l}^{-1}$) for the population from habitat S and $EC_{50}=156.8 \text{ mg l}^{-1}$ (CI: $153.4\text{--}160.1 \text{ mg l}^{-1}$) for the populations from habitat C. Reproductive period was on average shorter in the populations from the plots of habitat C ($F=17.87$, $p<0.001$) and both populations reacted to the higher copper concentration with reduced reproductive period ($F=122.00$, $p<0.001$). Although the significant interaction habitat $\times$ copper concentration ($F=5.423$, $p<0.001$) indicates differences in the response patterns between the populations from different habitats, no conclusion regarding the difference in general tolerance to copper could be made (for the figures see electronic supplementary material). In general, increase in copper concentration in the medium caused decrease in survival probability in populations of both treatments (for the populations from habitat S: log-rank test, $\chi^2=94.32$, $p<0.001$; for the populations from the habitat C: log-rank test, $\chi^2=192.34$, $p<0.001$). The only deviation from this pattern was observed for the survival in $60 \text{ mg CuCl}_2 \text{ l}^{-1}$, where the nematodes survived on average longer than in $0 \text{ mg CuCl}_2 \text{ l}^{-1}$ (for the figures see electronic supplementary material). The differences between the populations originating from different habitats were significant only in the highest concentration of copper tested. In this environment ($180 \text{ mg CuCl}_2 \text{ l}^{-1}$), the nematodes originating from plots of habitat S lived on average longer (Wilcoxon test, $p=0.02$; log-rank test, $p=0.058$)

Figure 3. Response of total reproduction of *A. nanus* originating from the plots of habitat C (closed symbols, solid line, replicate populations pooled) and habitat S (open symbols, dashed line, replicate populations pooled) to different concentrations of copper in the agar medium. Mean $\pm$ s.d. is shown. Values of EC_{50} for both pooled populations are indicated by the triangles ($\pm$ CI).

indicating greater resistance to copper. There was no significant difference between the populations in the response of juvenile period to copper. For both populations, juvenile period was on average longer in higher copper concentrations (log-rank test, $\chi^2=352$, $p<0.001$).

Higher tolerance to copper observed in populations from the habitat S suggests that the nematodes became capable of dealing with higher copper concentrations. Since we used the fourth generation after introducing the nematodes to laboratory culture for this experiment, the observed differences are most likely genetically determined.

(iii) pH response experiment

Populations from the different field habitats showed divergent responses to pH in a number of life-history traits, namely, pH affected reproduction of populations of both origins ($F=12.29$, $p<0.001$). Neither population showed overall advantage in total reproduction across all pH levels ($F=0.196$, $p=0.65$). However, the nematodes from the populations originating from plots of habitat S laid on average more eggs on the medium of lower pH (4.0 and 4.5) compared with the number of eggs laid on the medium of pH 5.0–6.0 (figure 4a). At the same time, the nematodes originating from habitat C laid more eggs on the medium of higher pH (5.0–6.0) in comparison with the nematodes from habitat S. The significance of interaction term habitat $\times$ pH of medium ($F=5.54$, $p<0.001$) indicated differences in the pattern of pH response between populations from different habitats. The analysis of reproductive period revealed similar pattern of the response to pH like the one obtained for reproduction (figure 4b): the effect of pH was significant ($F=11.063$, $p<0.001$) and there was a significant effect of interaction habitat $\times$ pH of medium ($F=4.653$, $p=0.001$). Similar to total reproduction no habitat effect was detected ($F=0.25$, $p=0.62$). Daily reproduction was affected only by the interaction habitat $\times$ pH of medium ($F=3.39$, $p=0.01$) while the responses to pH and habitat were not significant ($F=2.35$, $p=0.054$ and $F=3.45$, $p=0.064$, respectively; figure 4c). The analysis of survival

Figure 4. Reaction norms of the populations originating from habitat C (closed symbols, solid line, replicate populations pooled) and habitat S (open symbols, dashed line, replicate populations pooled) to different levels of pH of the agar medium for (a) total reproduction, (b) reproductive period and (c) daily reproduction. Mean $\pm$ s.e. is shown.

revealed substantial differences between the responses of the populations of different origin to pH (figure 5a,b). On agar medium with pH 4.0, the nematodes originating from habitat S survived longer (log-rank test, $\chi^2=9.397$, $p=0.002$; Wilcoxon test, $\chi^2=7.148$, $p=0.008$), whereas the situation reversed on the medium of pH 6.0. In this case, the nematodes derived from habitat C survived longer (log-rank test, $\chi^2=2.302$, $p=0.129$; Wilcoxon test, $\chi^2=8.390$, $p=0.004$; for figures for all tested pH levels see electronic supplementary material). Length of juvenile period was unaffected by pH (habitat S: log-rank test, $\chi^2=5.696$, $p=0.223$; habitat C: log-rank test, $\chi^2=3.963$, $p=0.411$). In addition, the origin of the populations had no influence on the response to pH (log-rank tests, $p>0.05$ for all tests).

The results strongly suggest that the environment to which the nematodes were exposed in the field determined their response to pH in the laboratory in a number of life-history traits. Since the populations displayed higher values of the traits like survival and total reproduction in the pH conditions that corresponded to the field

Figure 5. Survival probability of the nematodes originating from habitat C (dotted line, replicate populations pooled) and habitat S (solid line, replicate populations pooled) on agar medium of (a) pH 4.0 and (b) pH 6.0.

conditions they evolved in for 20 years, it can be concluded that the differences between these values represent adaptive changes as response to selective pressure that was imposed by pH level in the field.

4. DISCUSSION

While asexual reproduction is supposed to assure the maintenance of a superior genotype in a stable environment, sexual reproduction is considered to provide the basis for rapid evolution. Our results indicated rapid adaptive divergence within the asexual population of *A. nanus* in response to locally imposed selection. Selective agents and time-scale were defined by application of an evolution experiment with manipulated environmental variables in a randomized block design. To our knowledge, this kind of approach has not been used in the studies of adaptive divergence of natural populations until today.

The evidence collected over the last four decades indicated a considerable intraclonal variation in many asexually reproducing species (reviewed in Loxdale & Lushai 2003; Lushai *et al.* 2003). Moreover, artificial selection studies under laboratory conditions showed that populations reproducing by obligate parthenogenesis were able to respond rapidly to strong selection. For example, Wilhoit & Mittler (1991) demonstrated phenotypic response to selection on the body size within 14–16 generations in the greenbug *Schizaphis graminum*. In another study, divergent selection on weight-specific fecundity (WSF) imposed on asexual lineages of *Daphnia pulex* resulted in the differentiation of WSF within two generations (Gorokhova *et al.* 2002). The most rapid response that could be attributed to selection was reported for the pea aphid, *Acyrtosiphon pisum*, selected for different predator defence behaviour. The differences resulting from the disruptive selection were apparent

after a single generation (Andrade & Roitberg 1995). However, the evidence for rapid adaptive response for asexual populations in the field is poor. The rare exceptions include the case study of the spotted alfalfa aphid, *Therioaphis maculata*, where a small introduced asexual population has evolved into numerous variants within a few generations (Dickson 1962).

Although asexual reproduction is expected to maintain local adaptation, only a few cases of adaptive divergence within asexual natural populations have been reported. Prati & Schmid (2000) and Lenssen *et al.* (2004) showed that the clonal plant *Ranunculus reptans* locally adapted to flooding regime. Another group of studies showed adaptation of various asexual species to their hosts. Such adaptation was shown for *Sitobion* aphids (Sunnucks *et al.* 1998), the spider mite *Brevipalpus phoenicis* (Groot *et al.* 2005) and the wheat yellow rust *Puccinia striiformis* f. sp. *tritici* (Enjalbert *et al.* 2005). Some molecular studies on aphid species have provided evidence for local selection (reviewed in Lushai *et al.* 2003).

Testing local adaptation requires the incorporation of reaction norm or reciprocal transplant experiments (Kawecki & Ebert 2004). In order to interpret the observed life-history patterns as resulting from adaptation, in both reciprocal transplant and reaction norm experiments, the assumption of positive correlation of the analysed trait with fitness must be fulfilled. Our reaction norm experiment demonstrated clear differences between populations of both habitats in a number of fitness-related traits. The traits analysed in reaction norm experiments, like total reproduction and survival, influence fitness directly and therefore deliver a straightforward interpretation of the results. In the reciprocal transplant experiment we analysed the MRGR of the nematodes. The relationship of body growth rate with fitness is expected to be less direct than the one with reproduction or survival. Nevertheless, the results of the transplant experiment are consistent with the findings of the reaction norm experiments.

In the reciprocal transplant experiment, we used defaunated soil from the experimental field from where the nematodes originated. Since, in the field, nematodes are likely to experience negative interactions with competing predacious and pathogenic species, use of defaunated soil could result in releasing the pressure caused by these interactions. As a consequence, MRGR values measured in the experiment are expected to be, on an average, higher than in the field. Additionally, since the strength of interspecific interactions can differ between stressed and unstressed habitats (Morin 1999), we cannot exclude the possibility that the pattern of MRGR response in defaunated soil does not entirely overlap with the MRGR response under field conditions. Regardless of these limitations, the results of the implemented transplant experiment can be regarded as an indication for adaptation to local abiotic soil conditions as the observed response could be attributed to the differences in soil properties defined by the experimental manipulation (copper load and soil pH).

There are several aspects that should be taken into consideration while interpreting local adaptation events. Dispersal rate is one of them, since it has profound influence on the capability of local populations to diverge. It is known that individuals of *A. nanus* can passively

disperse by wind (Lee 2002) and through human activities, such as ploughing. The active dispersal in soil is not likely to play a major role in gene flow of nematodes, since the distance travelled per year for most examined nematode species is estimated to range from several centimetres to several metres (Hunt *et al.* 2001). In general, the migration of nematodes among the experimental plots can be regarded as limited. The second factor that is relevant for the adaptive potential of the populations under selection is mutation rate. There are no estimates of genome-wide mutation rate for *A. nanus*, but the recent studies on the spontaneous mutation rate in inbred lines of *Caenorhabditis elegans* using DNA sequencing imply that the mutation rate is high, approximately 2.1 mutations per genome per generation (Denver *et al.* 2004). Based on the comparative analysis of SSU rDNA between different nematode clades, it can be concluded that the mutation rate of *A. nanus* is likely to be of comparable magnitude as the one of *C. elegans* (A. W. G. Van der Wurff, unpublished data). Such high mutation rate would facilitate adaptation by increasing genetic variation (De Visser & Rozen 2005). Other factors that operate together with mutation rate are population size and generation time. We found that the populations inhabiting a single plot (6 × 11 m), regardless of habitat type, consisted of approximately 2×10^5 – 5×10^5 individuals. This is a relatively low number compared to those of prokaryotic organisms or of some eukaryotic species that were found to respond fast to adaptation to a change in the environment (Lushai *et al.* 2003). In addition, generation time of *A. nanus* under field conditions is relatively long (number of generations per year was estimated as 0.7 and 6.6 for the nematodes from habitat S and habitat C, respectively). Such long generation times together with relatively low population size suggest that high mutation rate could be considered the main factor to facilitate rapid adaptive divergence within the population of *A. nanus*.

Since the level of genetic variation in parthenogenetic species does not depend on the mutation rate alone, the knowledge about other genetic mechanisms that operate in *A. nanus* would contribute to a better understanding of its adaptation. Polyploidy, which is common in mitotic parthenogenetic nematodes, is expected to result in higher levels of genetic variation through increased levels of heterozygosity (Lokki 1976). Additionally, certain aspects of chromosome nature and egg development have the potential to result in higher levels of genetic variation in parthenogenetic species (Asher 1970). The studies on egg development in *A. nanus* indicated maturation division equivalent to the first meiotic division in sexual species, which results in the formation of a single large polar body. Therefore, parthenogenesis in *A. nanus* can be regarded as essentially mitotic. In rare cases, a second meiotic division was observed with subsequent fusion of oocyte nucleus and second polar body which was concluded to restore diploidy (Lahl *et al.* 2006). These observations suggest that neither higher ploidy level nor the fusion of the second polar body with the oocyte nucleus is likely to play significant role in generating higher heterozygosity levels and, consequently, higher genetic variation. On the other hand, in the taxon Nematoda (with few exceptions), chromosomes have a holokinetic nature (Maddox *et al.* 2004), which is expected to increase the frequency of chromosomal rearrangements. Overall, because

mechanisms responsible for high adaptability of this species remain unresolved, there is a need for analysis of the levels and sources of genetic variation in populations of *A. nanus*. Genetic analysis could also help to answer the question about cryptic sex. Since cryptic sex can influence the adaptive potential of species that reproduce predominantly by parthenogenesis; discovering such events in *A. nanus* would require revision of the conclusions of the presented work.

The copper response experiment indicated higher survival and reproduction of the nematodes from habitat S in higher copper concentrations. These results suggest that some kind of detoxification or repair mechanisms (probably costly in terms of energy) may be involved. It has been demonstrated that some metal-tolerant populations exhibit lower metabolic rate and slow growth to conserve energy or other resources (Antonovics *et al.* 1971; Donker 1992). Contrary to the results of these studies, our results suggest that the nematodes from habitat S could be even selected for an increase in metabolic rate. This could be concluded from earlier onset of reproduction of the nematodes from habitat S in the control test medium (Cu=0) and their higher daily reproductive output in higher copper concentrations (data not shown). The results of the pH response experiment also support this scenario. The nematodes performed better on an average in the pH levels corresponding to the pH of the environment from where they originated. This type of response was observed not only for survival, but also for total reproduction and reproductive period, which suggests that the improvement in survival was not coupled with the decrease in metabolic rate.

In conclusion, the presented evidence for rapid local adaptation provides strong support for high adaptive potential of *A. nanus*. The evolution of specialized phenotypes in this species indicates that local adaptation may actually contribute more to the determination of population structure and distribution of asexual species than previously acknowledged. Studies on phenotypic evolution in asexual populations in replicated evolution experiments provide the opportunity to test hypotheses concerning the direction and the magnitude of phenotypic changes in the context of known selective agent, time- and geographical scale. Combination of this approach with population genetic analysis of population structure in future studies would provide a powerful tool to reveal mechanisms behind evolutionary dynamics of asexual populations.

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