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A COMPARATIVE STUDY OF THE LIFE STRATEGIES OF
TWO BACTERIAL-FEEDING NEMATODES UNDER
LABORATORY CONDITIONS. III.
INFLUENCE OF THE INITIAL NEMATODE DENSITY ON THE
INTERACTIONS OF *AROBELOIDES NANUS* (DE MAN 1880)
ANDERSON AND *DOLICHORHABDITIS DOLICHURA*
(SCHNEIDER 1866) ANDRÁSSY 1983 IN MIXED CULTURES

ABSTRACT: Population development and interactions between two bacterial-feeding nematodes *Acrobeloides nanus* and *Dolichorhabditis dolichura* were studied in mixed cultures at different initial density of nematodes.

Population dynamics of the two studied species differed significantly in both competition experiments, with the initial numerical advantage of *A. nanus* as well as with the initial numerical advantage of *D. dolichura*. In all experimental variants population peak of *D. dolichura* occurred always earlier than that of *A. nanus*.

Population dynamics of *A. nanus* and *D. dolichura* depended significantly on the initial density of the second species in the mixed cultures. The higher initial density of *D. dolichura* the less abundant populations of *A. nanus* were noticed. However, no consistent trend in the influence of the *A. nanus* initial density on the abundance of *D. dolichura* was found.

Numerical advantage of *D. dolichura* never led to the extinction of *A. nanus* and in the opposite, numerical prevalence of *A. nanus* in two cases resulted in a complete elimination of *D. dolichura*.

Population dynamics of *A. nanus* and *D. dolichura* in mixed cultures showed a distinct difference in the way of food utilisation by these two species. As food resources become scarce with time, *D. dolichura* density decreased but *A. nanus* density

started to increase. It appears therefore that, under the present laboratory conditions, *A. nanus* was the better competitor. That species maintained a positive growth rate even at low food concentrations at which its competitor could no longer be sustained.

KEY WORDS: life strategy, bacterial-feeding nematodes, inter-species interaction, competitive abilities in a changing environment

1. INTRODUCTION

Populations of bacterial-feeding nematodes cultured under laboratory conditions provide interesting model systems for studying inter-specific interactions (Nicholas 1984, Poinar and Hansen 1986, Sohlenius 1988). Nevertheless, until now relatively little is known about competition among the bacterial-feeding nematodes. *Rhabditis* spp. are known to have a strong depressing influence on other bacterial-feeding nematodes like *A. nanus*, *Cephalobus* sp., *Plectus* sp. (Sohlenius 1973, Sohlenius and Sandor 1989). In microcosm experiments as the density of *A. nanus* increased, the abundance of *Aphelenchoides* decreased

till its total extinction (Roswall *et al.* 1977, Bååth *et al.* 1981, Sohlenius 1985). A considerable decrease in numbers of *Panagrolaimus rigidus* in the presence of *A. nanus* was observed also in pot experiments by Kozłowska and Domurat (1971). In other experiments the abundance of *A. nanus* decreased when it was cultured together with *Mesodiplogaster lheritieri* (Anderson and Coleman 1981) or *Aphelenchus aveanae* (Ingham *et al.* 1985).

Ilieva-Makulec (2001a, b) found pronounced differences in some life-history and population parameters of the two bacterial-feeding nematodes *A. nanus* and *D. dolichura* under laboratory conditions. The consequences of these differences between the two nematodes probably will result in an interesting type of mixed species system when they will compete with each other. That is why the aim of this work was to investigate the effect of varying initial density of *A. nanus* and *D. dolichura* on the outcome of their interaction in mixed species cultures.

2. MATERIALS AND METHODS

The experiments were conducted in Petri dishes with a diameter of 10 cm. 25 ml of 0.6% sterile bactoagar (without any nutrients) to create a situation in which the two species would be limited by the abundance of available food with time) with a known density of bacteria *Sporosarcina* sp. – 8×10^8 colony forming units (cfu ml⁻¹) was poured into sterile Petri dishes. Each plate was inoculated

with varying number of young females of each species (Table 1). The animals used to start the cultures were selected randomly from monoxenic cultures of the two nematode species with the density of a food organism – *Sporosarcina* sp. – 8×10^8 cfu ml⁻¹. The experiments were conducted in the darkness at $20 \pm 1^\circ\text{C}$. All experimental animals were inoculated at the central part of the plates.

Twice per week all experimental plates were uncovered for 30 minutes to be aerated. The experiments lasted 56 days. Every two weeks population numbers of each species were determined in 6 replicate plates. As the methods used were destructive, enough cultures (in total 72 in each experiment) were set up to provide material for successive samplings.

An analysis of variance (ANOVA) was applied to the density of the two nematode species on the effects of initial nematode ratio (three initial values of that ration in each experiment), nematode species (two species) and time of the study (4 sampling dates during the study period). Analyses of variance were performed on all experimental results using the Statgraf statistical package. The significantly different means ($P < 0.05$) were separated using Tukey's Honestly Significant Difference.

3. RESULTS

3.1. INITIAL NUMERICAL ADVANTAGE OF *A. NANUS*

3.1.1. Population dynamics of *A. nanus* and *D. dolichura*

Population dynamics of the two studied species differed significantly (ANOVA, $P < 0.05$) in all three cases when *D. dolichura* with a constant initial density of 5 females was cultured together with *A. nanus* with a density of 10, 25 or 50 individuals (Fig. 1A, B, C). *A. nanus* populations were significantly more dense than populations of *D. dolichura* (Tukey test, $P < 0.05$).

Both nematode species reached their population peaks at different time. At the initial density of 10 individuals, *A. nanus* reached maximum after 42 days of culturing (Fig. 1A). After the peak, population numbers of that species started to decrease. In the

Table 1. Experimental design

I experiment – initial numerical advantage of <i>A. nanus</i>		
Variant	Initial nematode density (ind. plate ⁻¹)	
	<i>A. nanus</i>	<i>D. dolichura</i>
1	10	5
2	25	5
3	50	5
II experiment – initial numerical advantage of <i>D. dolichura</i>		
Variant	Initial nematode density (ind. plate ⁻¹)	
	<i>A. nanus</i>	<i>D. dolichura</i>
1	5	10
2	5	25
3	5	50

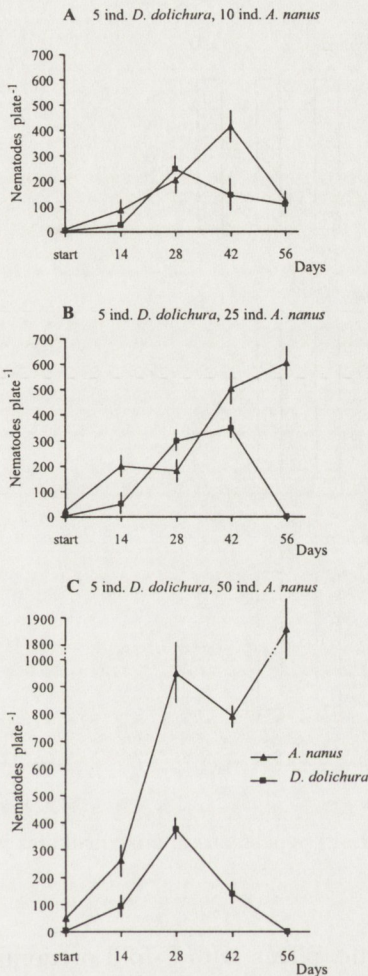


Fig. 1. Population dynamics of *A. nanus* and *D. dolichura* in mixed cultures with the initial numerical advantage of *A. nanus* (mean densities \pm S.D.)

other two variants, apart from some fluctuations, the density of *A. nanus* tended to increase continuously and reached maximal values at the end of the experiment i.e. in 56 days (Fig. 1B, C).

Population peak of *D. dolichura* in the same cultures was observed earlier – after 28 days when the initial predominance of *A. nanus* was two and 10 times higher and after 42 days when its advantage was 5-fold higher. After the peak *D. dolichura* density decreased continuously till to the end of the experiments (Fig. 1A, B, C).

The obtained results showed that, 56 days was the time *A. nanus* (with the initial density of 5 or 50 ind.) needed to reach an absolute dominance and to eliminate *D. dolichura* (with the initial density of 5 ind.) from the mixed cultures.

D. dolichura competed successfully to some extent only with the two-fold initial prevalence of *A. nanus* which resulted in a similar abundance of both species at the end of the experiment (Fig. 1A).

Population development of *A. nanus* depended significantly on its initial density (ANOVA, $P < 0.05$) (Fig. 1A, B, C). The higher initial density the more dense populations of that species were found in the course of the experiment (Tukey test, $P < 0.05$).

Population dynamics of *D. dolichura* also differed significantly in dependence on the initial density of *A. nanus* ($P < 0.05$) (Fig. 1A, B, C). However, no consistent trend in the influence of the *A. nanus* initial density on the abundance of *D. dolichura* was found. For example the most dense populations of *D. dolichura* were observed in the plates with 5-fold initial predominance and the lowest populations at 2-fold advantage of *A. nanus* (Tukey test, $P < 0.05$).

3.1.2. Age structure of *A. nanus* and *D. dolichura* populations

Juvenile forms predominated in the populations of both studied species. At the initial density of 10 ind. plate⁻¹ age structure of *A. nanus* was stable during the study period. (Fig. 2A). More evident fluctuations in the age structure of that species were observed in the cultures with the other two initial densities (Fig. 2B, C).

Percent share of *D. dolichura* juveniles increased with time in all three variants (Fig. 2D, E and F). However, 14 days after the beginning of the experiment very high abundance (40% of the whole population) of *D. dolichura* adults was found in the plates with 5-fold prevalence of *A. nanus* (Fig. 2E).

3.2. INITIAL NUMERICAL ADVANTAGE OF *D. DOLICHURA*

3.2.1. Population dynamics of *A. nanus* and *D. dolichura*

Population dynamics of the two studied species differed significantly (ANOVA, $P < 0.05$) in each variant where *A. nanus* with a constant initial density of 5 individuals was cultured together with *D. dolichura* at a density of 10, 25 or 50 individuals (Fig. 3A, B, C). Over the whole experiment in the cultures where the initial density of *D. dolichura* was

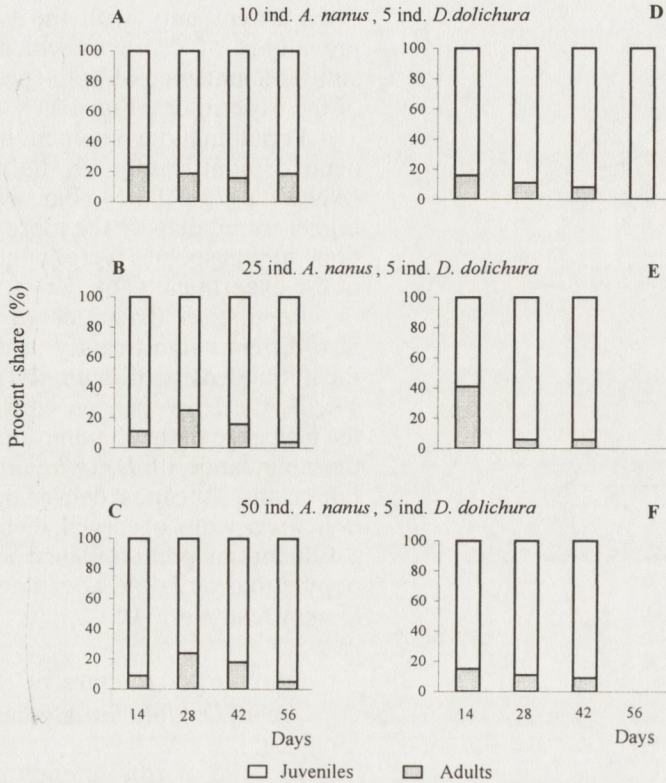


Fig. 2. Age structure of *A. nanus* (A, B, C) and *D. dolichura* (D, E, F) populations in mixed cultures with the initial numerical advantage of *A. nanus*

5- or 10-fold higher its populations were significantly higher than the *A. nanus* populations (Tukey test, $P < 0.05$) (Fig. 3B, C). Only in the cultures with 2-fold initial advantage of *D. dolichura*, much denser were the populations of *A. nanus* ($P < 0.05$) (Fig. 3A).

Population peaks of the studied species in that experiment similarly to the previous one were observed at different time. The abundance of *A. nanus* increased slowly in the cultures with 2- and 5-fold prevalence of *D. dolichura* and reached the highest values after 56 days (Fig. 3A, B). In the cultures with 10-fold prevalence of *D. dolichura*, population development of *A. nanus* was negligible and the low abundance of that species was observed over the whole experiment (Fig. 3C).

Population peak of *D. dolichura* was observed after 14 days in the cultures with initial density of 25 or 50 ind. plate⁻¹ (Fig. 3B, C) and after 28 days in the cultures with initial density of 10 ind. plate⁻¹, thus in all cases earlier than that in *A. nanus*. After the maximum the abundance of *D. dolichura* started to decrease in all experimental combinations.

In the plates with 2-fold predominance of *D. dolichura* it was found that after 42 days of culturing *A. nanus* won the competition and its populations were two times denser than the populations of *D. dolichura*. During the next 14 days the abundance of *A. nanus* increased evidently and at the end (after 56 days) it was even 20 times more abundant than *D. dolichura* (Fig. 3A).

In the cultures with 5-fold prevalence of *D. dolichura*, *A. nanus* needed 56 days to reach the same density as *D. dolichura* (Fig. 3B).

In the third variant, *D. dolichura* maintained its numerical advantage over *A. nanus* till the end of the experiment (Fig. 3C).

Population dynamics of *A. nanus* and *D. dolichura* depended significantly on *D. dolichura* initial density (ANOVA, $P < 0.05$). The higher initial density of *D. dolichura* the less abundant populations of *A. nanus* but more abundant populations of *D. dolichura* were found (Tukey test, $P < 0.05$).

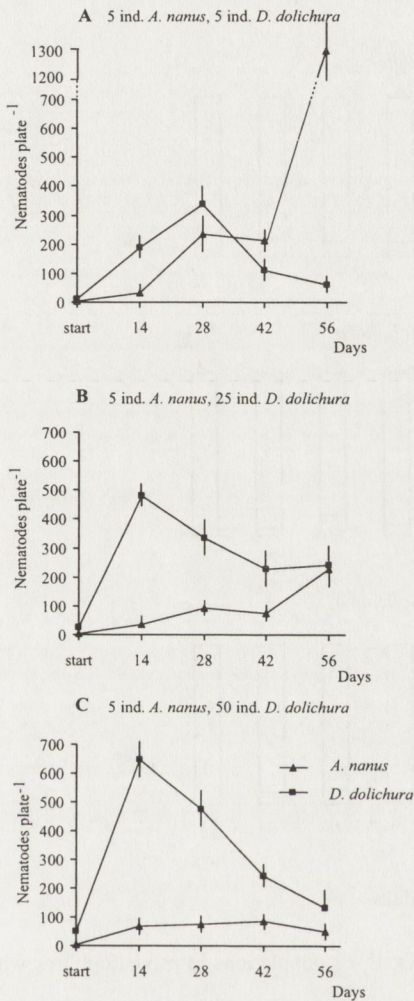


Fig. 3. Population dynamics of *A. nanus* and *D. dolichura* in mixed cultures with the initial numerical advantage of *D. dolichura* (mean densities \pm S.D.)

3.2.2. Age structure of *A. nanus* and *D. dolichura* populations

Juvenile forms predominated in populations of both studied species but much sharper was the dominance observed in the case of *D. dolichura* (Fig. 4). Changes in the age structure of *A. nanus* populations in the course of the experiment were similar in the cultures with 5- and 10-fold numerical advantage of *D. dolichura*. Percent share of *A. nanus* adults in these two variants increased evidently after 28 and 42 days of culturing (Fig. 4B, C). In the cultures with 2-fold prevalence of *D. dolichura* the highest proportion of adults in *A. nanus* populations was noticed after 14 days of the experiment. After

that, the percent share of that age group started to decrease slowly till the end of observations (Fig. 4A).

The proportion of juveniles in the *D. dolichura* populations was maintained at a very high level over the whole experiment. After 56 days of culturing juveniles accounted for 100% of the *D. dolichura* populations in all three variants of that experiment (Fig. 4D, E, F).

4. DISCUSSION

Population dynamics of *A. nanus* and *D. dolichura* in the present competition experiments differed significantly. Population peak of *D. dolichura* occurred always earlier than that of *A. nanus*. This confirms Yeates' (1984) finding that genera with similar feeding habits may show a time sequence in their population peaks.

Population dynamics of *A. nanus* and *D. dolichura* in mixed cultures show a distinct difference in the way of food utilisation by these two species. *D. dolichura* specialised in using initially high food supply. Present results concerning population dynamics of *A. nanus* and *D. dolichura* are similar to the results of Bouwman *et al.* (1994). In the microcosm studies, the above authors observed that populations of *Rhabditis* sp. reached their maximum faster, already in 18 days, and after that the populations abundance continuously decreased till the end of the experiment (180 days). At the same time *Acrobeloides bütschlii* from the same microcosms showed totally different population dynamics. Its density increased continuously and the highest populations of that species were found at the end of the experiment. Bouwman and Zwart (1994) also observed that species from Rhabditidae to a higher degree than the species from Cephalobidae needed high density of bacteria and showed more evident fluctuations in the populations densities.

The results concerning the age structure of *A. nanus* and *D. dolichura* populations in present experiments were similar with the previous observations of Ilieva-Makulec (2001b) in a study of the population dynamics of the same species in relation to initial food level. The observed in present experiment higher fluctuations of the ration between adults and juveniles in the case of *D. dolichura* in comparison to *A. nanus* as in

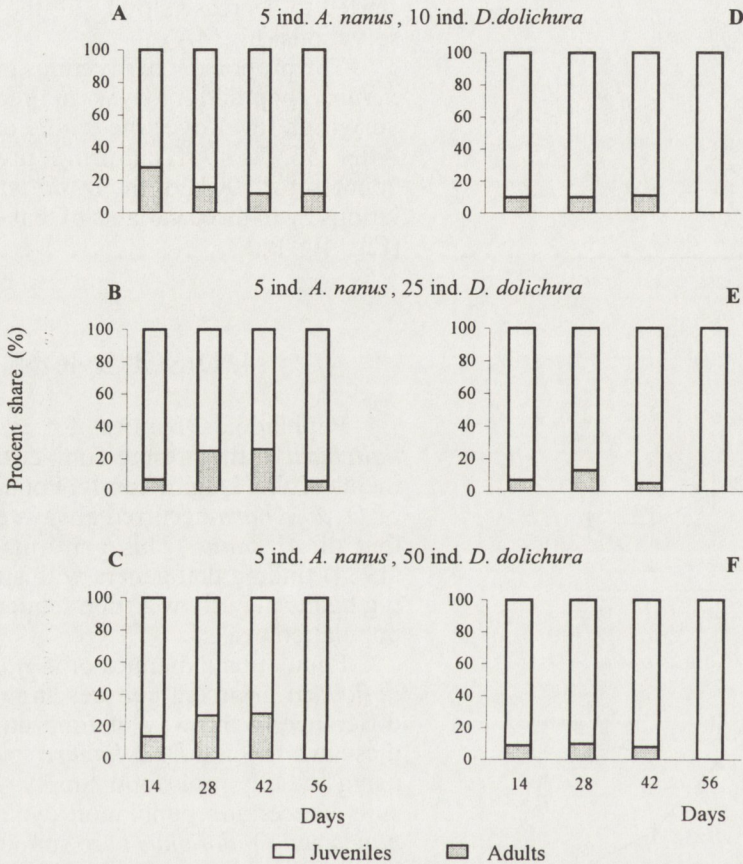


Fig. 4. Age structure of *A. nanus* (A, B, C) and *D. dolichura* (D, E, F) populations in mixed cultures with the initial numerical advantage of *D. dolichura*

Ilieva-Makulec (2001b) could be explained by low growth and reproduction rates of the first species in such, probably unfavourable, food conditions.

It is known that the outcome of species interaction is not explicitly fixed and may change with time and in the space (Wiens 1977, Connell 1983, Shoener 1983). In the fundamental works on inter-specific competition under laboratory conditions it was found that the cultures started with numerical predominance of one of the species always lead to the extinction of the second species (Gause 1934, Park 1962, Leslie *et al.* 1968). The obtained results showed that the outcome of competitive interactions between the two studied species depends on the ratio between their initial numbers. Simply manipulating the initial abundance of the two nematode species resulted in two different competitive outcomes: I – one species dominates from the start; II – the dominant species

changes with time. In all cultures where *A. nanus* had the initial numerical prevalence type I outcome was observed, while in the cultures where *D. dolichura* had numerical advantage, types I and II were demonstrated. Numerical advantage of *D. dolichura* never lead to the extinction of *A. nanus* and in the opposite situation numerical prevalence of *A. nanus* in two cases resulted in complete elimination of *D. dolichura*. Of course, changes of the species competitive abilities in this study should be connected with changes in culture conditions in respect to food (the two species competed for a single limiting resource). As food resources become scarce with time, *D. dolichura* density decreased but *A. nanus* density started to increase. This shows a higher efficiency of resource utilisation of *A. nanus* in comparison to *D. dolichura*. It appears therefore that, under the present laboratory conditions *A. nanus* is a better competitor. *A. nanus* can maintain a

positive growth rate even at low food concentrations at which its competitor can no longer be sustained. These results confirm Ilieva-Makulec's (2001b) findings about higher food requirements of *D. dolichura* in comparison to *A. nanus*.

Present results show that observed inter-specific differences in the life-history strategy, in combination with the temporal heterogeneity of the environment could explain patterns of species composition and resource utilisation.

5. SUMMARY

Pronounced differences in some life-history and population parameters of the bacterial feeding nematodes *Acrobeloides nanus* and *Dolichorhabditis dolichura* under laboratory conditions (Ilieva-Makulec 2001a, b) suggest that the consequences of these differences between the two nematodes probably will result in an interesting type of mixed species system when they will compete with each other. That is why in the present study population development and interactions between two bacterial-feeding nematodes *A. nanus* and *D. dolichura* were investigated in mixed cultures at different initial density of nematodes (Table 1). To create a situation in which the two nematode species would be limited by the abundance of available food (bacteria *Sporosarcina* sp.) with time, the food organism with known density (8×10^8 cfu ml⁻¹) was provided only once, at the beginning of the experiments.

Population dynamics and age structure of the two studied species differed significantly in both competition experiments, with the initial numerical advantage of *A. nanus* (Figs 1 and 2) as well as with the initial numerical advantage of *D. dolichura* (Figs 3 and 4). In all experimental variants population peak of *D. dolichura* occurred always earlier than that of *A. nanus*.

Population development of *A. nanus* and *D. dolichura* depended significantly on their initial density. The higher initial density the more abundant populations of those species were found in the course of the experiment (Figs 1 and 3).

Population dynamics of *A. nanus* and *D. dolichura* depended significantly also on the initial density of the second species in the mixed cultures. The higher initial density of *D. dolichura* the less abundant populations of *A. nanus* were noticed. However, no consistent trend in the influence of the *A. nanus* initial density on the abundance of *D. dolichura* was found. Numerical advantage of *D. dolichura* never led to the extinction of *A. nanus* and in the opposite, numerical prevalence of *A. nanus* in two cases resulted in a complete elimination of *D. dolichura* (Figs 1 and 3).

Population dynamics of *A. nanus* and *D. dolichura* in mixed cultures showed a distinct difference in the way of food utilisation by these two species. As food resources become scarce with time, *D. dolichura* density decreased but *A. nanus* density started to increase. Thus, changes in culture conditions in respect to food (the two species competed for a single limiting resource) was connected with changes of the species competitive abilities. It appears therefore that, under the present laboratory conditions, *A. nanus* was the better competitor. That species maintained a positive growth rate even at low food concentrations at which its competitor could no longer be sustained.

Present results show that observed inter-specific differences in the life-history strategy, in combination with the temporal heterogeneity of the environment could explain patterns of species composition and resource utilisation.

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