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REPRODUCTION AND INTER-BREEDING WITHIN AND BETWEEN POPULATIONS OF *XIPHINEMA DIVERSICAUDATUM* (NEMATODA: DORYLAIMOIDEA)

by
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The reproductive biology of longidorid nematodes has been studied mainly in the field (Taylor, 1967; Taylor and Murant, 1968; Taylor and Thomas, 1968; Thomas, 1969; Cotten, 1976) because they are usually difficult to culture in the laboratory (Griffin and Darling, 1964; Flegg, 1968; Cohn and Mordechai, 1969). The few laboratory studies have been done by using either naturally infested field soils or suspensions of the nematode added to sterilised field soil (Yassin, 1969; Cotten, *et al.*, 1970; Flegg *et al.*, 1970: Cotten, 1973).

Populations of *Xiphinema index* Thorne *et* Allen, a thelytokous species, have been raised from single females (Dalmasso and Younes, 1969; Wyss, 1978; Dalmasso, 1979) also Brown and Coiro (1985) have examined the longevity, reproductive span and total reproductive capacity of individual females. Brown and Coiro (1983) found that female *X. diversicaudatum* (Micoletzky) Thorne from a Scottish population had a reproductive span of 36 wk on strawberry host plants and produced 180-200 progeny, equivalent to one egg every 21 day° above a minimum daily threshold of 5°C. However, using the same host plant species Flegg *et al.* (1970) had earlier reported that during a seven month period pairs of *X. diversicaudatum* from an English population produced a mean of only 6.3 eggs.

This difference in the rate of reproduction of populations of *X. diversicaudatum* was further investigated using females from ten populations mated with males from their own and from the Scottish population. The reproduction of a morphometrically dissimilar population of *X. diversicaudatum* from Spain was also examined and the results are reported here.

Materials and Methods

The populations of *X. diversicaudatum* used came from (1) Dundee, Scotland; (2) Aylesford, England; (3) Saint-Katherina-Lombeek, Belgium; (4) Lombardia region, Italy; (5) the Var region, France; (6) San Diego, California, USA; (7) Kostinbrod, Bulgaria; (8) Alexandra, New Zealand; (9) Sandefjord, Norway; (10) Holziken, Switzerland and (11) Cazalegas, Spain. The French population came from a glasshouse whereas the others were from natural biotopes. All populations were kept as breeding colonies at the Scottish Crop Research Institute (Brown and Topham, 1985).

Reproduction within populations

Experiments were done using strawberry (*Fragaria* x *ananassa* Duch. cv. Cambridge Favourite) produced by plant tissue culture techniques (Boxus, 1984). Plantlets were grown singly in 25 cc plastic-pots, without drainage holes, containing a 1:2 steam-sterilised soil/air-dried sand mixture with an aggregate and particle size $< 1500\ \mu m$ and $> 250\ \mu m$. Nematodes were extracted from their soils by the method of McElroy *et al.* (1977) and twenty treatment combinations were established. With each of ten populations (1 to 10 above) five replicates each with a pre-adult female (= 4th stage juvenile with discernible female genital primordium) and three males and ten replicates each only with a pre-adult female were established. The nematodes were hand-picked into the plastic-pots containing the host plants. All replicates were placed in a temperature controlled cabinet (Taylor and Brown, 1974) for 12 wk at $18 \pm 1°C$, RH 90% and with supplementary lighting to provide a 16 hr day-length. To prevent the plants from becoming too large they were trimmed after 6 wk leaving only the two youngest leaf stalks.

The nematodes were extracted from the pots (McElroy *et al.*, 1977) after 12 wk and counted. Those initially added (now all adults) were discarded and all juveniles (first generation progeny) transferred to clean pots. New plantlets were added and the pots returned to the temperature controlled cabinet for 12 wk to allow further development of the juveniles. The nematodes were then re-extracted from the pots and three males and one pre-adult female hand-picked into clean pots containing a new strawberry plantlet. After a further 12 wk the nematodes were extracted from the pots and all juveniles (second generation progeny) counted.

Interbreeding with the Scottish population

In a concurrent experiment the same techniques were used to determine the fecundity of females from the *X. diversicaudatum* populations mated with males from the Scottish population. Initially, five replicates were set up with three males from the Scottish population and one pre-adult female from each of the other populations. In a subsequent experiment the reproduction of the progeny from these matings was determined as described above.

Reproduction by X. diversicaudatum *from Spain*

X. diversicaudatum from Spain are anatomically similar to but morphometrically dissimilar from those of the other populations (Brown and Topham, 1985). An experiment as described above was therefore done to examine the fecundity of Spanish females mated with Spanish and with Scottish males. In addition to strawberry, *Petunia hybrida* Vilm. and *Lolium perenne* L. were used as hosts.

Data analysis

Numbers of progeny were converted to natural logarithms for statistical analysis and detransformed values are given in Table I. A non-orthogonal analysis of the log progeny means from Table I is presented as Table II. Due to differences in the numbers of females producing progeny between populations, between generations and between crosses it is impractical to list least significant ratios (LSR's) for all combinations. For guidance, therefore, LSR's for 2 means of 4 and 2 means of 12 are given in Table I.

Results

Reproduction within populations

Of the pre-adult females initially added > 50% were recovered alive 12 wk later and when males were absent no progeny were produced. When males were present all populations produced progeny, the numbers of

Table I - *Detransformed log mean numbers of progeny produced by individual females from ten populations of* Xiphinema diversicaudatum *(selfs), and by females from nine populations when crossed with males from a Scottish population (crosses), during 12 weeks on strawberry (*Fragaria x ananassa *cv. Cambridge Favourite) host plants.*

Origin of Population	Selfs				Crosses				Grand mean
	generations[1]				generations[1]				
	first (P1)		second (P2)		first (F1)		second (F2)		
	females[2]	progeny[3]	females[2]	progeny[3]	females[2]	progeny[3]	females[2]	progeny[3]	
Scotland	4	22.87	4	29.67	—	—	—	—	
England	2	7.77	2	10.38	2	8.41	3	26.84	11.25
Belgium	2	6.49	4	9.49	3	14.15	3	21.98	12.18
Bulgaria	4	31.50	2	58.56	4	60.34	3	32.79	42.52
France	2	10.38	2	13.20	4	30.57	4	19.30	19.11
Italy	4	30.57	4	39.25	4	51.42	5	35.16	38.09
New Zealand	4	20.49	5	51.94	2	44.70	5	50.40	40.04
Norway	4	24.29	5	26.84	3	30.88	3	47.47	29.26
Switzerland	4	22.20	3	21.76	5	17.46	3	32.79	22.20
USA	3	50.91	2	19.69	2	16.95	2	50.40	28.50

[1] Males and pre-adult females which developed from the first generation were used in a test similar to the first. Progeny produced in the second test were considered the second generation.

[2] Number of females producing progeny from five replicates per generation per population, each replicate initially containing one pre-adult female and three males.

[3] Mean number per female.

NOTE: It is impractical to give a comprehensive listing of Least Significant Ratios (LSR's), therefore, for guidance, the LSR's at the 5% level of probability for 2 means each of 4 values and 2 means each of 12 values are 11.82 and 5.42 respectively.

Table II - *Analysis of variance for Table I ___ non-orthogonal analysis of log progeny means.*

Source of variation	Degrees of freedom	F ratio	P[1]
Populations allowing for crosses and generations	8	7.48	***
Generations allowing for populations and crosses	1	2.04	NS
Crosses allowing for populations and generations	1	7.03	*
Two factor interactions	17	0.67	NS
Three factor interactions	8	1.83	NS

[1] NS, not significant; * and ***, significant at 0.05 and 0.001 respectively.

which varied between individual females. The means for the populations did not differ significantly between the first (P1) and second (P2) generations. Between populations, there were significant (<0.001) differences in the mean numbers of progeny produced in both P1 and P2 (Tabs. I and II).

As there were no significant differences between generations (Tab. II) the mean numbers of progeny per female (P1+P2) were used to provide a better assessment of reproductive rates over a 12 wk period. Females from the English, Belgian and French populations produced relatively few progeny (8-11), Swiss and Norwegian females produced an intermediate number (27-30) whereas those from the other populations produced a relatively large number of progeny (38-47).

Interbreeding with the Scottish population

As with the previous experiment > 50% of the females initially added survived and produced progeny when mated with Scottish males. The first generation progeny (F1) were reproductively viable and produced a second generation (F2). As previously, the females produced different numbers of progeny, the mean numbers of which also differed significantly between populations. There were no significant differences between the F1 and F2 generations. Combining the results from the F1 and F2 generations showed that the English and Belgian females and their progeny again had

substantially smaller reproductive rates than the other populations (Tabs. I and II).

The mean reproductive rates of the selfed (P1 + P2) and the interbred (F1 + F2) populations were similar. However, the English, and the Belgian and French females produced substantially fewer progeny when selfed (P1 + P2) than when crossed with Scottish males (F1 + F2).

Reproduction by females from a Spanish population and ability to interbreed with males from a Scottish population

Of five replicates containing one female and three males from the Spanish population, juveniles were recovered from two with *F. x ananassa* plantlets, from one with *P. hybrida* and from one with *L. perenne*. In pots containing Scottish females mated with Scottish males more juveniles were recovered although the numbers under *L. perenne* were less than with *P. hybrida* or *F. x ananassa* as hosts. When Spanish pre-females were placed with Scottish males, juveniles were not recovered from replicates with *P. hybrida* or *L. perenne* but 4 juveniles were present in one replicate with *F. x ananassa* (Tab. III).

Discussion

The similarity of the results for the different generations suggests that significant differences occur in the reproductive rate between populations of *X. diversicaudatum*. Brown and Coiro (1985) and Coiro and Brown (1984) reported the reproductive span and rate of *X. index* to be affected by host plant, species and cultivar. In the present study only *F. x ananassa* plants were used as it reputedly is a good host for *X. diversicaudatum* (Pitcher *et al.*, 1974). Differences in reproduction by populations of *X. diversicaudatum* therefore might be related to populations being adapted to different host plants. Furthermore, as the reproductive rates obtained here with the English and Scottish populations agree with those obtained earlier by Flegg *et al.* (1970) and Brown and Coiro (1983) it is likely that some populations of *X. diversicaudatum* have reproductive rates inherently less than that of others.

Significant morphometric differences have been reported between the populations of *X. diversicaudatum* used in this study, the Spanish nematodes being least like those of the other populations (Brown and Topham,

Table III - *Numbers of progeny produced by individual females from Scottish and Spanish populations of* Xiphinema diversicaudatum *(selfs), and by females from the Spanish population when crossed with males from the Scottish population (crosses), during 12 weeks on three plant species.*

Population	Host					
	Fragaria x ananassa		*Petunia hybrida*		*Lolium perenne*	
	females[1]	progeny[2]	females[1]	progeny[2]	females[1]	progeny[2]
Scotland (selfs)	4	39[3] (24-56)	3	40 (37-43)	5	10 (6-15)
Spain (selfs)	2	2.5 (2-3)	1	13	1	5
Spain / Scotland (crosses)	1	4	0	0	0	0

[1] Number of females producing progeny from five replicates per host per population, each replicate initially containing one pre-adult female and three males.

[2] Mean number per female.

[3] Mean (minimum-maximum).

1985). Also, only the Spanish nematodes did not readily breed with themselves or interbreed with the Scottish population, thus, this population would seem to be atypical. Despite morphometric differences the anatomical similarity and successful inter-breeding between the other populations is evidence that these populations probably belong to the same classical biological species, *X. diversicaudatum* (inter-breeding and gene interchange between individuals; Mayr, 1970). However, successful interbreeding between populations is not conclusive evidence that they belong to one species as disparate species of animals, including helminths, can produce fertile hybrids in the laboratory (Mayr, 1970; Poinar and Hansen, 1983).

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SUMMARY

Reproduction by individual female *Xiphinema diversicaudatum* from eleven populations and by females which developed from the progeny of these original females was examined in a laboratory study. Unmated females did not produce progeny thus reproduction by *X. diversicaudatum* is unlikely to be thelytokus. Females from all populations produced progeny when mated and numbers of progeny produced by individual females differed. Similarly, mean numbers of progeny produced by females from the populations varied significantly ($P < 0.001$) in the first and the second generations of progeny. Females from nine of the populations when inter-bred with males from a Scottish population produced reproductively viable progeny. Few females from a Spanish population produced progeny when mated with Spanish or Scottish males and the use of three species of host plants little affected reproduction by these nematodes. It is concluded that despite morphometric differences the anatomical similarity and successful inter-breeding between populations is evidence that probably they all belong to one classical biological species, *X. diversicaudatum*.

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