Theor Appl Genet (1996) 93:1–8

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Genetic variation in natural populations of the cereal cyst nematode (*Heterodera avenae* Woll.) submitted to resistant and susceptible cultivars of cereals

Received: 4 February 1996 / Accepted: 8 March 1996

Abstract The purpose of the present work was to study the genetic characteristics of cereal cyst nematode (*Heterodera avenae*) populations re-established after the long-term use of resistant oat cultivars in field conditions. Population features were analyzed through fitness components and variation in enzymatic polymorphism (esterase and malate dehydrogenase loci). The longest (6 year) use of the same resistance genes (oat cv Panema) at high frequency (Rotation IB) led to the selection of a resistance-breaking pathotype and to a decrease in viability which suggested either a founder effect or a lower reproductive potential for the new pathotype. Analysis of esterase allelic frequencies led to the conclusions that: (1) the genetic constitution of this pathotype was different from the reference population maintained on the susceptible host (oat cv Peniarth), and (2) that the esterase locus may develop a disequilibrium linkage with loci involved in virulence[1]. Random mating was recorded at the whole-field level but not always at the single-plant level, suggesting that cultivation practices such as annual ploughing could play a major role in homogenizing subpopulations developed in the vicinity of a plant. These phenomena showed that the long-term use of highly effective resistance could provok marked genetic modifications in populations. These risks should be taken into account when deciding strategies for optimal use of resistance genes in nematode management programs.

Key words Oat · *Heterodera avenae* · Resistance-breaking pathotype · Enzymatic polymorphism · Population genetics

Communicated by J.MacKey

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[1] Nematodes overcoming the resistance of cv Panema did not differ from *H. avenae* species following RFLPs in ribosomal DNA

Introduction

The cereal cyst nematode, *Heterodera avenae* Woll., is one of the most damaging species to cereals and is widely distributed through the world. It shows an important polyphenism which is expressed through differences in virulence (pathotypes) and in hatching temperature requirements (ecotypes) (Rivoal and Cook 1993). Its biological cycle begins by the emergence of second stage juveniles preserved in cysts which represent the brown chitinized tegument of dead females (Fig. 1). These juveniles penetrate the roots and establish themselves close to the vascular cylinder where they induce the formation of nutrient cells. The nematodes develop into white lemon-shape females or filiform males after three moults. Their reproduction is strictly amphimictic. The free males are attracted by the gelatinous matrix of the adult females attached to the host root where they intimately intrude before fertilization (Person and Rivoal 1979). After fertilization the females die and become cysts. These nematodes complete only one generation in a year. Attempts at recognizing differences between pathotypes using biochemical markers (isoenzymes) has demonstrated a high genetic polymorphism in this species, especially for esterase and malate dehydrogenase loci (Bergé et al. 1981; Dalmasso et al. 1982). Further analysis on the esterase polymorphism has shown significant differences in genotype and allelic frequencies both between nearby cropping fields and within the same field (Bossis and Rivoal 1989).

The use of resistant cultivars is the most efficient way to control the pathogen in cereals. Several resistance genes are indeed available in oats, barley and wheat.

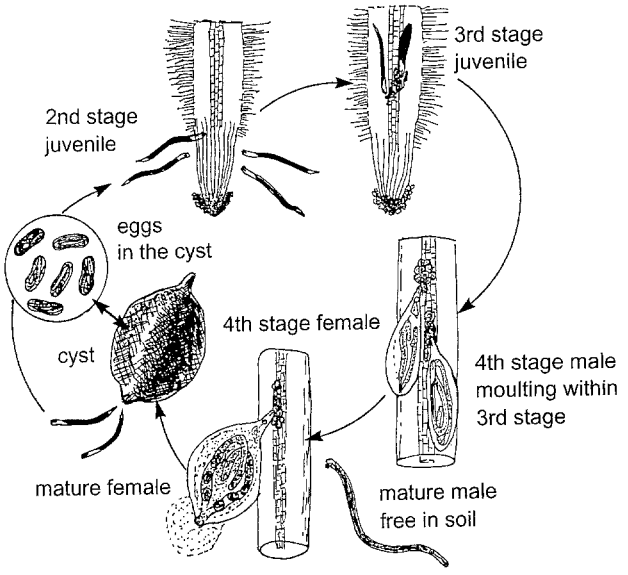

Fig. 1 Life cycle of the cereal cyst nematode *Heterodera avenae*

Mostly based on oligogenic systems, they almost completely inhibit the development of females (Rivoal and Cook 1993). The frequent use of monospecific resistance often contributes to the selection of new virulent pathotypes (Turner et al. 1983). In long-term cropping (1982–1993) with resistant cereal cultivars (oat cv Panema, predominantly cultivated), we have studied the consequences on the population dynamics of the target species *H. avenae*. These cultivars caused dramatic decreases in nematode populations to almost undetectable densities by biological assessment methods routinely applied in diagnostic laboratories. Nevertheless these populations may increase rapidly following the re-introduction of susceptible cultivars into the rotation. The population of *H. avenae* re-established in this way included some nematodes able to overcome the resistance of the oat cv Panema (Rivoal et al. 1995).

Dramatic distrubances in demography and high selection pressure (60% frequency of the same resistant cultivar on a 12-year cropping period) could have caused modifications in the population genetic structure of *H. avenae*, as already shown in several nematode species (Turner 1990; Young et al. 1986). The purpose of the present work was to study the genetic characteristics of the re-established populations after the frequent use of resistant cultivars compared to populations which experimentally had never been exposed to plant resistance genes. The population features were analyzed through fitness components and variation in enzymatic allele frequencies. The polymorphism in restriction fragments length in genes and internal transcribed spacers (ITS) of ribosomal DNA was furtherly studied to evaluate the species status of nematodes able or unable to overcome the resistance of the oat cv Panema.

Materials and methods

Biological materials

The populations of *H. avenae* employed were located in the Argentan plain in Normandy (France) where an experimental design to assess the tolerance capacity of new resistant advanced lines of wheat was based on three pairs of blocks, each 360 m^2 (6 × 60 m). These were cropped with different resistant and susceptible cultivation frequencies to establish nematode densities above (rotations A) or below (rotations B) the damage thresholds (Rivoal et al. 1991, 1995). Only blocks I and III were involved in the present study. Soil nematode densities were estimated from samples of ten soil cores (5 × 18 cm) taken from each of 4–6 equidistant 10 m^2 (2 × 5 m) areas, along the middle of each block in October of each year. Techniques for extracting, counting nematodes and identifying antagonistic fungi have been published previously (Rivoal et al. 1995). Figure 2 summarizes the population dynamics of nematodes in each rotation according to the cereal species and cultivars cropped.

The experimental field was predominantly infested by pathotype Ha11 of *H. avenae* (Rivoal, unpublished). It has been previously demonstrated that the resistance of *Avena sterilis* I.376, which cv Panema is derived from, to this pathotype is based on three dominant genes (Cook et al. 1978; Clamot and Rivoal 1984). The breaking of cv Panema resistance had been consistently recorded only in rotation IB from soil sampled both in January and in June (Rivoal et al. 1995). IA and IB, IIIA and IIIB will be considered as field subpopulations.

Fitness components

The reproductive potential of each population was characterized both by viability (the ability of second stage juveniles to develop into females or cysts) and fecundity (the mean number of the progeny produced by these females or cysts). Both parameters had been assessed on nematodes developing on the susceptible host oat cv Peniarth.

Infective second-stage juveniles were obtained from cysts sampled in October and stored at 7 ± 1°C until emergence. Cysts were sampled in 1991 for rotations IIIA and IIIB, and in 1992 for rotations IA and IB (see Tables 1, 2). Strict viability had been assessed by inoculating juveniles into oat plantlets cultivated on 2% agar medium in Petri dishes, at 16 ± 1°C and a 16 h photoperiod; 48 (3 × 16) juveniles were inoculated on each plantlet with more than 30 replicates per population.

A second assessment of viability, with a concomitant measure of fecundity, was based on the production of white females or/and cysts on oat plants cultivated in plastic tubes (2.5 × 8 cm) filled with either naturally infested soil for populations IIIA and IIIB in 1992, or a mixture of sable de Fontainebleau (85%) and kaolin (15%) infested by two full cysts contained in 250-µm nylon mesh bags for populations IA and IB in 1993. White females and cysts were counted 3–4 months after inoculation by washing the cereal roots above a 250-µm mesh sieve. All these newly formed females and cysts were squashed to evaluate the juvenile content as a measure of the mean fecundity. These experiments were conducted under the same temperature and photoperiod conditions as previously mentioned, with more than 15 replicates per population.

Enzymatic polymorphism

The enzymatic polymorphism for esterase and malate dehydrogenase loci was studied in 1993 on the field subpopulations of nematodes from rotations IA, IB, IIIA and IIIB cropped with the susceptible oat cv Peniarth (see Fig. 2) Three types of sampling were carried out. *Sampling 1*. At the post-flowering stage of the cereal (15 June 1993), 40 plants were uniformly sampled in each experimental strip, according to a grid of four 54-m lines separated by 1 m in width and located at 1.5 m from the outer and inner borders of each parcel. Along each line the plants were sampled at 6-m intervals. The plant roots were immediately placed in an insulated picnic bag for transport to the laboratory for further storage at 3 °C. A single female from each plant

was used for each enzymatic locus giving 39 replicates in subpopulations IA and IB, and 40 replicates in subpopulations IIIA and IIIB.
Sampling 2. Enzymatic polymorphism was analyzed on a large sample of females developed on two individual plants of cv Peniarth from the rotation IIIB, generating two replicates each of 65 and 69 single females, and called IIIB-Plant1 and IIIB-Plant2, respectively.
Sampling 3. A third analysis compared the genetic polymorphism of nematodes collected from rotation IB and reared in a room at the same temperature and photoperiod conditions as given previously, either on five plants of the resistant oat cv Panema (IB-Panema) or on two plants of the susceptible cv Peniarth (IB-Peniarth). The number of single females analyzed was respectively 38 and 51 for esterase, 44 and 50 for malate dehydrogenase.

All the females were extracted from the oat roots by washing under tap water. They were then handpicked under a stereomicroscope and placed separately on humid filter paper in a Petri dish. They were immediately stored at −70 °C. An aliquot collection of 200 females was taken from each of the four field subpopulations, crushed in 250 µl of extraction buffer and directly stored at −70 °C.

Allozyme analysis was carried out on single white females using cellulose acetate gels and standard methods (Hebert and Beaton 1989). Esterase b (EST) and malate dehydrogenase (MDH) were assayed for all subpopulations. Electrophoretic mobilities were judged by visual comparisons of electromorphs against a reference 200-female sample and the relevant R_f values, i.e., the ratio between the migration distance of the enzyme and the migration distance of the front (Hoelzel 1992).

Ribosomal DNA polymorphism

Partial sequences from internal transcribed spacer ribsomal DNA (ITS rDNA) obtained by the polymerase chain reaction (PCR) were compared on isolates of 40 single white females from subpopulations IA, IB-Panema, and pathotype Ha41 of *H. avenae* and *Heterodera schachtii*, another cyst nematode pathogenic to beet crop. Conditions for DNA extraction and amplification were according to the technique modified from De Jong et al. (1989) and Caswell-Chen et al. (1992).

DNA extraction was carried out on single females crushed in 90 µl of lysis buffer (0.1 M Tris HCL, 50 mM EDTA, 1% SDS, 0.17 NaCl, pH 8) and digested with 10 µl of Proteinase K (5 µg/µl) over night at 37 °C. The DNA was then extracted using 25 Phenol/24 Chloroform/1 isoamyl buffer and precipated with isopropanol. Following this 2.5 ng of DNA was re-suspended in 5.3 µl of 5T-E buffer (5 mM Tris, 0.1 mM EDTA pH 8) and stored at 4 °C.

Two 21-nucleotide sequences, 5′-TTG-ATT-ACG-TCC-CTG-CCC-TTT-3′ and 5′-TTT-CAC-TCG-CCG-TTA-CTA-AGG-3′ positioned on the 18*s* and 26*s* ribosomal genes and isolated from a *Xiphinema* library by Vrain et al. (1992), were used to amplify the ITS region. These primers were manufactured by the Eurogentec Society (CHRU, Laboratoire de Biologie Moléculaire, Angers-F). The reaction mixture contained 10x *Taq* buffer, 0.44 mM $MgCl_2$, 0.1 mM each of dATP, dCTP, dGTP and dTTP, 0.25 µM of both primers, 0.5 units of *Taq* DNA polymerase, 2.5 ng of single female DNA and de-ionized water to a volume of 50 µl. For each isolate amplification was carried out in four phases in the thermocycler (Perkin Elmer Cetus DNA Thermal Cycler 480). The first phase consisted of one cycle of 30 s at 94 °C. The second phase consisted of 40 cycles with denaturation at 94 °C for 1 min, annealing at 72 °C for 1 min, and extension at 72 °C for 1 min. A last cycle at 72 °C for 5 min ended the amplification. The amplified DNA bands were monitored by electrophoresis through 1.5% agarose gels buffered in 1 × TAE + ethidium bromide, in a Hoefer HE 99 apparatus.

The product of amplification was digested with 14 enzymes (*Hin*dIII, *Hpa*II, *Sau*3A, *Mae*II, *Mae*III, *Nde*II, *Dde*I, *Hin*fI, *Pst*I, *Alu*I, *Bam*Hl, *Rsa*I, *Hae*III, *Ita*I) at 37 °C for 12 h in the recommended buffer (Boehringer). The DNA fragments thus generated were separated by electrophoresis in the same conditions as described previously. For each population and each restriction enzyme, the bands were recorded on a matrix with 0 or 1 corresponding to the absence or presence of individual bands, respectively.

Statistical analysis

For fitness components, one-way analyses of variance were performed wherever necessary on $\log_{10}$- or arcsine-transformed data with the SAS program (SAS Institute Inc. 1988). Means were classified as different according to the Newman-Keuls at $P \leq 0.05$.

Allelic and genotypic frequencies between nematode populations were compared with a χ^2 test, after pooling the lower frequency alleles A, D, E, for esterase b, and A and B for malate dehydrogenase, in order to minimize problems due to a very small numbers in genotypic classes. Hardy-Weinberg equilibrium conditions, *F*-statistic estimators (Wright), and Nei and Rogers genetic distances were computed with Biosys-1 (Swofford and Selander 1981).

The RFLP matrix was computed with a PHYLIP package to calculate the dissimilarity coefficients between population isolates. A cluster was established from Nei genetic distances (Nei and Li 1979).

Results

Fitness components

Differences were observed for viability and fecundity between subpopulations according to the duration of susceptible oat (cv Peniarth) and/or wheat (cvs Arminda and Fidel) cropping after use of the resistant oat cv Panema (Fig. 2; Tables 1 and 2). Five years after the re-introduction of the susceptible host, the re-established subpopulation IIIB showed a significantly higher viability than IIIA, measured either in Petri dishes or in tubes (sampling in October 1991; Table 1). In contrast, only 2 years after the re-introduction of susceptible cultivars the viability is significantly lower in the re-established subpopulation IB compared to IA where resistant cultivars had never been cropped (sampling in October 1992; Table 2). This difference is significant only in Petri-dish experiments. In tube experiments, the viability of subpopulation IA could have been underestimated, due to the antagonistic effect of the identified fungus, *Cylindrocarpon destructans*, known to be destructive to *H. avenae* females. This could also have caused a delay in the development of the nematode, accounting for the significantly higher number of white females, and could be responsible for the concomitant decrease in fecundity. This fungus had not been recorded in females and eggs of subpopulation IB. The fecundity of females was always higher in re-established subpopulations, at whatever date the susceptible cultivars had been re-introduced. Monocultures of susceptible hosts (rotations A, blocks I and III) caused an increased percentage of inviable progeny, partly explaining the density dependant limiting population dynamics of *H. avenae* under such farming practices (Fig. 2). The lower viability in the earlier re-established subpopulation IB was unexpected. It could have depended on a combination of two independent factors: an inbreeding effect from survivors acting as founders and/or a lower reproductive potential in nematodes virulent to cv Panema. The absence of *C. destructans* in subpopulation IB revealed the indirect decreasing effect of resistance cropping on the development of nematode endoparasitic fungi.

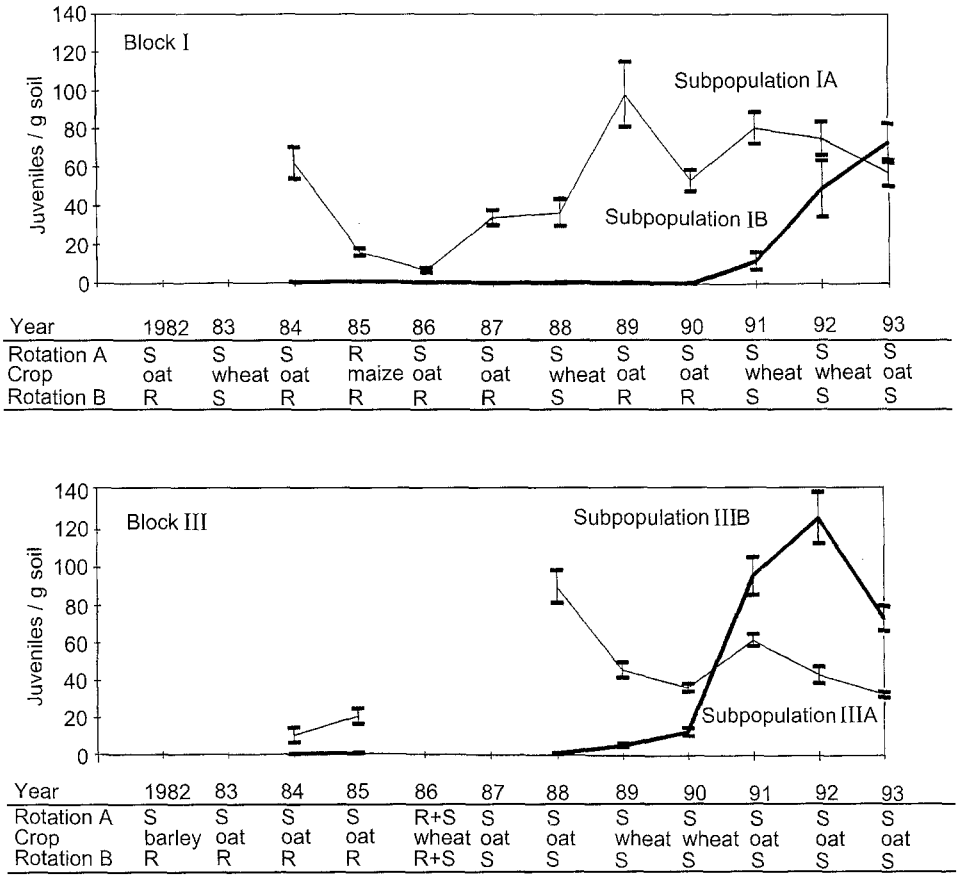

Fig. 2 Population dynamics of *H. avenae* in field strips cropped with resistant (*R*) and suscepetible (*S*) cereals in long-term rotations (1982–1993). Each point represents the mean of 4–5 replicates. *Vertical bars* show the standard error of the mean. The resistant and susceptible cultivars were cv Panema and cv Peniarth for oats, cv Welam and cv Aramir for barley, respectively. Susceptible wheats were cv Arminda except in 1983, Block I (cv Talent) and in 1990, Block III (cv Fidel). The tolerance capacity of new resistant advanced lines of wheat was carried out in 1986 (Block III)

Table 1 Fitness components of subpopulations of *H. avenae* from experimental rotations IIIA and IIIB (year 1992)

Item	Tests	Replicates		IIIA	IIIB
Viability (number of respective life stage found)	Petri plates	> 30	White females	8.3a	10.2b
	Tubes	> 15	Cyst	17.3a	35.3b
			White females	7.4a	16.1b
			Total	24.7a	52.3b
Fecundity (juveniles/cyst)	Tubes	> 15	Viable	99.2a	154.5b
			Total (viable + unviable)	144.7a	175.5b
			%Viable/total	70.0a	85.4b
Mean initial density (juveniles/g of soil)				62.2	93.3
Soil sampling (October)					1991

Enzymatic polymorphism

As previously recorded (Bergé et al. 1981; Dalmasso et al. 1982, Bossis and Rivoal 1989), the monomeric esterase and the dimeric malate dehydrogenese revealed an important polymorphism, with five and four alleles, respectively (Table 3, Fig. 3). All allelic frequencies were below the 95% limit. Except for allele A, rare alleles were not observed either for esterase or for MDH. MDH allele A was also absent in the IB-Peniarth subpopulation.

A Hardy-Weinberg equilibrium has been observed in the four subpopulations, IA, IB, IIIA and IIIB, assuming a random mating in the field populations. The different cropping rotations did not seem to have disturbed panmictic reproduction, as shown by the insignificant low values of *F* statistics for the IA, IB, IIIA and IIIB subpopulations, indicating a non-excess of heterozygosity (Table 3).

In contrast, subpopulations sampled on single oat plants showed greater positive or negative *F* values,

Table 2 Fitness components of subpopulations of *H. avenae* from experimental rotations IA and IB (year 1993)

Item	Tests	Replicates		IA	IB
Viability (number of respective life stage found)	Petri plates	> 30	White females	13.7a	7.9b
	Tubes	> 15	Cyst	63.5	66.6a
			White females	6.5a	0.5b
			Total	70.8a	67.9a
Fecundity (juveniles/cyst)	Tubes	> 15	Viable	248.3a	313.7b
			Total (viable + unviable)	319.1a	365.3a
			%Viable/total	78.0a	85.6b
Mean initial density (juveniles/g of soil)				75.0	48.8
Soil sampling (October)					1992

Table 3 Allele frequencies at esterase and malate dehydrogenase loci for different subpopulations of *H. avenae* and for three sampling methods (see text for explanation). The formula $F^2N(K-1)$ follows a χ^2 law with $K(K-1)/2$ degrees of freedom; K is the number of alleles at the locus of interest and N the number of individuals in the subpopulation. Negative F values indicate an excess in heterozygotes, positive values a deficit. The test is significant at $P \leq 0.05$

Method of sampling		1				2		3	
Locus	Alleles	IA	IB	IIIA	IIIB	IIIB-Plant1	IIIB-Plant2	IB-Panema	Ib-Peniarth
Estérase b	A	0.077	0.013	0.013	0.038	0.054	0.044	0.013	0.020
	B	0.269	0.423	0.412	0.375	0.323	0.397	0.539	0.304
	C	0.359	0.410	0.363	0.313	0.415	0.390	0.276	0.412
	D	0.077	0.064	0.138	0.138	0.092	0.044	0.026	0.118
	E	0.218	0.090	0.075	0.138	0.115	0.125	0.145	0.147
Sample size		39	39	40	40	65	69	38	51
F static estimator (Wright)		−0.204	0.097	−0.047	−0.054	0.013	0.156*	0.077	0.314*
Hardy-Weinberg equilibrium (P)		0.171	0.197	0.749	1.000	0.316	0.041*	1.000	0.019*
Malate dehydrogenase	A	0.013	0.064	0.050	0.025	0.040	0.008	0.057	0.000
	B	0.269	0.359	0.225	0.287	0.290	0.383	0.352	0.490
	C	0.449	0.359	0.463	0.387	0.310	0.367	0.295	0.300
	D	0.269	0.218	0.262	0.250	0.360	0.242	0.295	0.210
Sample size		39	39	40	40	65	69	44	50
F statistic estimator (Wright)		−0.150	0.125	0.077	−0.095	−0.202*	−0.004	0.033	0.201
Hardy-Weinberg equilibrium (P)		0.336	0.052	0.523	0.739	0.012*	0.599	0.537	0.094

expressing an excess of heterozygosity for IIIB-Plant2 ($F = +0.156$; $P = 0.041$) at the esterase locus or a deficit for IIIB-Plant1 ($F = -0.202$; $P = 0.012$) at the MDH locus. These results indicate that matings do not occur randomly in the vicinity of a single plant. A critical aspect of the sampling conditions is demonstrated by the highest and significant deficit of heterozygosity observed for the IB-Peniarth subpopulation at the esterase locus ($F = +0.314$, $P = 0.019$) which may have resulted from the collection of females from only two plants.

A pairwise comparison of samples from subpopulations IB and IA gave significant differences in allelic frequencies (Table 4). The highest χ^2 value involved the comparison of samples from subpopulations IB-Panema and IB-Peniarth, demonstrating that even subpopulations originating from the same field strip may differ genetically. The differences could be due to a greater density of resistance-breaking individuals in the IB-Panema sample than in IB-Peniarth where multiplication of both avirulent and virulent pathotypes had been allowed.

The occurrence of the resistance-breaking pathotype to cv Panema seems responsible for the genetic differentiation. This is confirmed by the lack of significant differences in allelic frequency comparisons between IIIA and IIIB where resistance-breaking phenomena had not been recorded at a consistent level (Rivoal et al. 1995). Multiplication of nematodes on single plants of the susceptible cv Peniarth (compare IIIB-Plant1/IIIB-Plant2) did not introduce any genetic dissimilarity.

The differences between these subpopulations are confirmed through Nei (N) and Rogers (R) genetic distances. The higher divergence ($N = 0.031$; $R = 0.197$) was again expressed between nematodes on IB-Panema

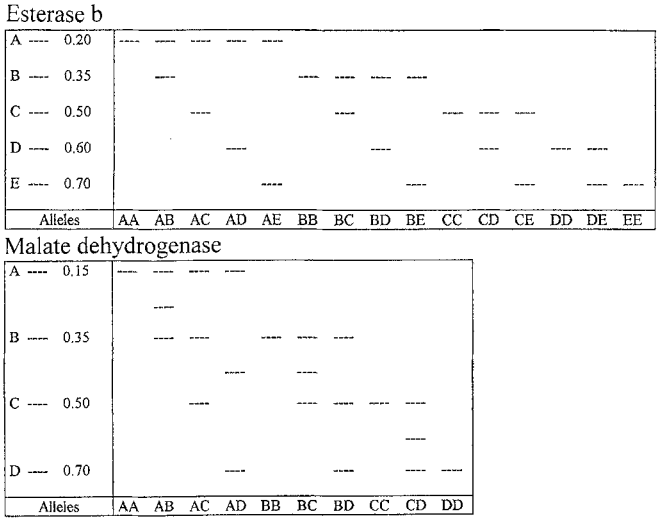

Fig. 3 Allelic patterns and genotypes of monomeric esterase b and dimeric malate dehydrogenase revealed on single females in populations of *Heterodera avenae*, using cellulose acetate gels

Table 4 Selected paired comparisons of allelic frequencies in different subpopulations of *H. avenae* for the esterase b and malate dehydrogenase loci. Differences are significant at $P \leq 0.05$ (*), $P \leq 0.01$ (**)

Comparison of subpopulations	Esterase b		Malate dehydrogenase	
	ddl	χ^2	ddl	χ^2
IB/IA	2	9.03*	2	3.40
IB-Panema/IB-Peniarth	2	10.94**	2	2.05
IIIB/IIIA	2	1.58	2	0.92
IIIB-Plant1/IIIB-Plant2	2	1.77	2	3.75

and the field subpopulation IA. IB-Panema nematodes were not distinct from the field subpopulation in IB but lay at an intermediate genetic distance with the IB-Peniarth subpopulation ($N = 0.017$; $R = 0.156$). The IIIB-Plant1 and IIIB-Plant2 subpopulations did not differ at all ($N = 0.002$; $R = 0.086$).

The ribosomal DNA primers allowed amplification of ITS1, ITS2 and the 5.8*s* gene (Vrain et al. 1992). The four populations studied yielded only one fragment, approximately 1.2 kb long. Digestion of the amplification product occurred with all enzymes except *Hin*dIII and *Bam*H1. In every case the RFLPs were completely similar between the IA, IB-Panema and the pathotype Ha41 of *H. avenae*. According to the stringency level of this technique we may consider that these three populations belong to the same species. In contrast, *H. schachtii* presented similar bands to those of the *H. avenae* population cluster only with respect to the enzymes *Nde*II and *Pst*I. The genetic divergence coefficient between the two species was 1.4604 (Fig. 4).

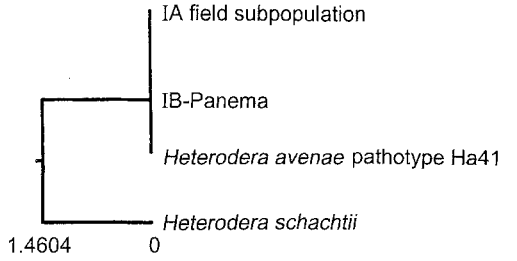

Fig. 4 Genetic dissimilarity between different populations of *Heterodera* nematodes, in respect of RFLPs of ribosomal DNA, calculated using Nei distance

Discussion

The primary objective of nematode management programmes is to reduce the population densities to below damage thresholds. Studies on population dynamics (Pf/Pi ratio) and their impact on crop production have been carried out on plant parasitic nematodes, especially in the genus *Heterodera* (McSorley and Phillips 1993). Studies on the consequences of crop rotation on the genetic structure of nematode populations are, however, infrequent and to-date have rarely involved enzyme polymorphism (Caswell and Roberts 1987). Nematodes such as *H. avenae* are very small animals (an average 500 μm width for white females or cysts) and sufficient quantities of enzymes are difficult to extract from single individuals. It would have been preferable to use more polymorphic loci but, unfortunately, attempts to find other informative enzymatic systems on single females have been unsuccessful.

The panmictic reproduction of the nematode population is consistent at the field level and confirmed the previous results of Bossis and Rivoal (1989). Tremendous decreases in nematode densities and re-colonization resulting from resistant and susceptible plant cropping has not significantly disturbed the random mating of populations. We cannot exclude the possibility that non-random mating at the field level occurred during the phase of extremely low population density but it would have disappeared after 2–3 years of susceptible host cropping. In contrast, subpopulations sampled from individual plants reared in pots showed non-random mating tendencies.

We should have expected the same pattern both at plant and field levels as an excess of homozogosity at the plant level should be found, or even amplified with a Wahlund effect, for the whole field population (Hart and Clark 1989). Such as paradoxical observation could be explained by such cultivation practices as ploughing. This could serve to disrupt regular inbreeding that occurs at the plant level when infective juveniles originating from the same cyst could interbreed in the vicinity of the plant root system (Caswell and Roberts 1987). Annual ploughing certainly caused a regular mixing of alleles at each generation for the whole field population.

Our long-cropping experiment attained several goals of experimental population genetic studies, which are able to detect, characterize and measure the intensity of natural selection where it is occurring (Lewontin 1985).

The main and unexpected result of the present study was to observe the selection of a new resistance-breaking pathotype showing a fitness and an enzymatic polymorphism different from the original field population. Selection for nematode virulence to specific resistance genes through repeated exposure to resistant plants has rarely been experimentally demonstrated under agricultural field conditions (Roberts 1993).

The major disturbance factor for allelic frequency is probably the appearance of nematodes breaking the resistance of cv Panema, which causes a structuring in the field subpopulation IB. Sharper differentiation of populations for the esterase locus could be explained by a linkage disequilibrium between this locus and those associated with virulence to cv Panema. Turner (1990) had earlier observed a contrasting increase and decrease in allelic frequencies for the enzymes phosphoglucosisomerase, phosphoglucomutase and hexokinase between virulent populations of the potato cyst nematode *Globodera pallida* and their unselected avirulent counterparts from geographical isolates. The same polymorphisms in enzymatic allele range or in ribosomal DNA RFLPs between the population virulent to cv Panema and the field population, which had never experimentally subjected to the resistance genes, indicate specific similarities between both nematode isolates. Ferris et al. (1994) had also observed that the rDNA ITS sequences were highly conserved among all the *H. avenae* stricto sensu isolates analyzed. The breaking of resistance genes from *A. sterilis* I.376 by *H. avenae* pathotype has never been observed previously. It occurs in Sweden with several Gotland-strain populations belonging to the *H. avenae* complex, particularly with the Etelhem isolate (Ireholm 1994). Nevertheless, this Swedish population is easily differentiated to *H. avenae* stricto sensu by using RFLPs in ribosomal DNA (Bekal and Rivoal, unpublished).

The re-establishment of populations from undetectable densities also contributed to modifications in population genetic structure. Unfortunately our experimental design could not indicate whether the lower viability of the re-established subpopulation IB resulted from a fitness cost in the wider virulence pathotype, an inbreeding effect in founding survivors, or both phenomena together. The significant difference in allelic frequencies between subpopulations IB and IA suggest that the new virulent pathotype to cv Panema occurs at a consistent frequency. The appearance of this virulent pathotype in only one experimental subpopulation poses questions about the heterogeneous distribution of virulence genes in the field and the mechanisms of selection involved. Rivoal et al. (1995) did not observe any acquired virulence in the geographically closed subpopulation IIB when submitted to the highest selection pressure (oat cv Panema cultivated at 64% frequency from 1982 to 1993) indicating that the virulence genes are either infrequent in this location or else could not be progressively selected, as in the case of mitotically parthenogenetic *Meloidogyne incognita* in tomato (Jarquin-Barberena et al. 1991). In contrast, 3 years of successive host cropping (wheat cv Arminda in 1991 and 1992, oat cv Peniarth 1993) might have contributed to the strong increase in the frequency of virulence genes in subpopulation IB, in spite of a lower genotypic fitness. Nevertheless Turner (1990), and more recently Beniers et al. (1995), have not found a decrease in fitness of *G. pallida* pathotype with increasing virulence to patato cv Darwina bred from different sources of resistance in *Solanum vernei.*

These studies in population genetics complete our investigations on the population dynamics of *H. avenae* exposed to the repeated use of resistance genes. Undoubtedly new in nematology, they must be further developed to evaluate the effects of different agronomical practices such as rotations, the use of resistant cultivars, and fallowing, on the genetic evolution of pathogenic populations.

Acknowledgements The results presented in this paper are part of a research programme with the integrated management of nematode densities supported by BAYER, grant P 00931 and by INRA, AIP 92/9505.

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