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Genetic relationships between *Globodera pallida* pathotypes in Europe assessed by using two dimensional gel electrophoresis of proteins

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Summary — The mosaic distribution patterns of the *Globodera pallida* pathotypes in Europe hamper an effective control by means of resistance. These spatial variations in virulence are predominantly determined by three processes : *i)* the genetic structure of the initial populations introduced from South America, *ii)* random genetic drift and *iii)* gene flow. The result of these processes was studied with two dimensional gel electrophoresis (2-DGE) of total protein extracts from young females. 2-DGE of 25 European populations revealed 29 variant proteins encoded by 29 alleles at 11 putative loci. The majority of the populations had widely different allele frequencies at various loci. Only two populations were nearly identical. The similarity dendrogram constructed from the genetic distances of the 25 populations, which had never been exposed to resistance in Europe, showed that current pathotype classification is incapable of reflecting the genetic relationships between the *G. pallida* populations. Populations classified as identical pathotypes were often placed in distinct groups. Since both variation in virulence and proteins are prevalently determined by aforementioned processes, which affect the variation of the entire gene pool, the similarities and dissimilarities revealed by 2-DGE will also be reflected at virulence loci, including those loci not revealed yet by current pathotype schemes. The value of molecular data for breeding for resistance is discussed.

Résumé — ***Relations génétiques entre les pathotypes européens de* Globodera pallida *révélées par l'utilisation de l'électrophorèse des protéines en deux dimensions, sur gel*** — La répartition en mosaïque des pathotypes de *Globodera pallida* en Europe empêche un contrôle efficace au moyen de la résistance. Ces variations spatiales de la virulence proviennent essentiellement de trois processus : *i)* la structure génétique des populations initialement introduites d'Amérique du sud; *ii)* la dérive génétique aléatoire; *iii)* le flux de gènes. Les conséquences de ces processus sont étudiées par électrophorèse sur gel en deux dimensions (2-DGE) d'extraits protéiques totaux de jeunes femelles. La 2-DGE de 25 populations européennes révèle 29 variants protéiques codés par 29 allèles sur 11 loci supposés. La majorité de ces populations montrent des fréquences alléliques largement différentes sur divers loci. Seules, deux populations sont presque identiques. Le dendrogramme de similarité construit à partir des distances génétiques de 25 populations — jamais exposées à la résistance en Europe — montre que la classification usuelle des pathogènes est incapable de refléter les relations génétiques entre populations de *G. pallida*. Des populations attribuées aux mêmes pathotypes sont souvent situées dans des groupes différents. Étant donné que la variabilité concernant les pathotypes et les protéines sont surtout déterminées par les processus cités ci-dessus — lesquels affectent la variabilité du stock génique tout entier — les ressemblances et les dissemblances révélées par la 2-DGE seront également reflétées sur les loci de virulence, en incluant les loci non encore mis en évidence par les schémas relatifs aux pathotypes. La valeur des données moléculaires en vue de croisements pour la résistance est discutée.

Key-words : Potato cyst nematodes, virulence, resistance breeding, protein polymorphism, genealogical relationships.

The growth of resistant potato cultivars is potentially an effective means of controlling of *Globodera rostochiensis* (Woll.) Behrens and *G. pallida* (Stone) Behrens, because selection towards alleles for virulence is rather slow (Jones *et al.*, 1967; Jones & Perry, 1978; Jones *et al.*, 1981; Turner *et al.*, 1983). However, in Europe a wide application of resistance, which is mediated by pathotype specific genes (Kort *et al.*, 1978), is complicated by the presence of populations having already high numbers of virulent individuals for various sources of resistance (Huijsman, 1964). Knowledge of the virulence characteristics of the populations in an area is therefore a prerequisite for an effective introduction of new resistant cultivars. However, testing the various wild *Solanum* species and their progeny on suitable resistant genotypes is laborious and expensive. An additional drawback is that so far no proper monogenic resistance is available against *G. pallida* and breeders try to accumulate an array of genes in commercial cultivars in order to obtain resistance effective against a large part of the European populations. Because the number and nature of the genes involved is unclear (Goffart & Ross,

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1954; Rothacker, 1958; Huijsman, 1974), the progeny of each cross has to be tested against a large number of populations. At present the number of populations tested is arbitrary and depends mainly on the screening capacity. Evidently, knowledge of the genealogical relationships between potato cyst nematode populations, and a better understanding of the nature and extent of the processes conferring interpopulation variation in virulence will improve the efficiency of breeding for resistance.

It is hypothesized that the allele frequencies for virulence in populations, which have never been exposed to corresponding genes for resistance in Europe, are predominantly the result of three processes. First, the mosaic distribution patterns of the eight pathotypes presently known in Europe (Kort *et al.*, 1978), five within *G. rostochiensis* (Ro_1-Ro_5) and three within *G. pallida* (Pa_1-Pa_3), may in part be explained by the genetic structure of the initial populations introduced from the Andean region, their presumed origin, or from elsewhere in South America. Native populations from different localities are often discriminated at various virulence loci (Canto Saenz & de Scurrah, 1977) and the spatial variations observed in Europe are probably in part generated by the introduction of primary founders having distinct provenances. Second, the numerous secondary founding events, e.g. by few cysts adhering to seed tubers, by which Europe has been colonized have offered ample opportunity for random genetic drift to operate. Changes in allele frequencies between generations are also likely to occur when populations are nearly eradicated, e.g. due to the infrequent growth of potatoes and the use of nematicides. Third, despite their poor dispersal abilities, gene flow can probably not be neglected. Indirect evidence that gene flow is not rare comes from the observation that *G. rostochiensis* and *G. pallida*, which have probably been introduced independently (Evans & Stone, 1977), often occur in mixture (Kort & Bakker, 1980).

Although no direct evidence is available, it seems justified to assume that selection caused by adaptation to local environmental conditions is of minor importance in explaining the mosaic distribution patterns of the pathotypes. An argument against a strong selection of any type is, that it is too large a genetic load on its spread and survival. Potato cyst nematodes produce only one generation in a growing season and in a normal crop rotation the time between generations ranges from two to five years. The maximum multiplication factor is on the average 25 (Den Ouden, 1965). These considerations also suggest that the contribution of mutation is irrelevant. Mutation, by itself, is a slow process and time expired since the arrival of the first founders, probably after 1850 (Jones & Jones, 1974), is too short for newly arisen mutants to have changed the allele frequencies noticeably.

In this study we investigated the genetic relationships between 25 *G. pallida* populations from Europe by using two dimensional gel electrophoresis (2-DGE) of proteins (Bakker & Gommers, 1982; Bakker & Bouwman-Smits, 1988*a*, *b*). In view of the numerous molecular data from other organisms (Kimura, 1983), it is reasonable to assume that selection and mutation are not relevant in explaining the protein variations between the descendants of the primary founders. This implies that in the absence of the resistant host, both variation in virulence and proteins in the European potato cyst nematode populations are prevalently determined by the genotypes of the primary founders, random genetic drift and gene flow. Since these processes influence the whole gene pool of a population, electrophoretic data are informative for interpopulation variation at virulence loci, including those not revealed yet by current pathotype schemes. The possibilities and perspectives for using molecular data as a guidance for breeding for resistance are discussed.

Materials and methods

Samples of the *G. pallida* populations listed in Table 1 were obtained from : the Plant Protection Service, Wageningen, the Netherlands (population nos 1, 3, 4, 5, 6, 7, 8, 9, 10, 24, 25); CPRO, Wageningen, the Netherlands (population nos 11, 12, 15, 16, 17, 18, 19, 20, 21); Hilbrands Laboratorium, Assen, the Netherlands (population no. 13); Department of Nematology, Muenster, Federal Republic of Germany (population nos. 22, 23); Department of Nematology, Rothamsted Experimental Station, Harpenden, England (population nos 2, 14). The original samples (more than 100 cysts) were obtained from heavily infested spots in the field. The populations had not or at least not significantly been exposed in Europe to potato cultivars with genes for resistance present in *Solanum vernei* hybrid 62.33.3. Populations were maintained in the greenhouse on cultivars susceptible to all pathotypes.

The virulence characteristics of the populations were estimated by measuring the reproduction on potatoes grown in pots. Multiplication factors of population nos. 1, 3, 4, 5, 6, 7, 8, 9 and 10 were supplied by Ir. C. Miller and Ing. J. Bakker, Plant Protection Service, and of population nos. 11, 12, 15, 16, 17, 18, 19, 20 and 21 by Ing. J. H. Vinke, Foundation for Agricultural Plant Breeding. The ability of population nos. 13, 22, 24 and 25 to overcome resistance was determined by inoculating second stage larvae on roots of sprouts grown on water agar in Petri dishes (Mugniéry & Person, 1976). The numbers of cysts or females produced on *S. vernei* hybrid 62.33.3 were expressed as a percentage of those developed on a general susceptible host (Table 1) and used as an indication for the number of virulent genotypes.

Total protein samples of adult females (Bakker & Bouwman-Smits, 1988*a*), second stage larvae and fourth

Table 1. Ratios between the protein quantities produced by IP-variant encoding alleles at 11 presumptive loci in adult females of 25 *G. pallida* populations [a].

Pathotype, collection location and code [b]	Number of females on *S. vernei* 62.33.3 [c]	Alleles									
		A_1	A_2	A_3	A_4	B_1	B_2	C_1	C_2	D_1	D_2
1. Pa_1, Glarryford, Northern Ireland, 1337	10.4 (2.5)	0	23 (10)	77 (10)	0	100	0	24 (4)	76 (4)	25 (5)	75 (5)
2. Pa_1, Portglenone, Northern Ireland, PORT	-	100	0	0	0	60 (4)	40 (4)	100	0	100	0
3. Pa_2, Smilde, the Netherlands, D-234	2.8 (0.5)	0	0	80 (5)	20 (5)	100	0	0	100	0	100
4. Pa_2, Anlo, the Netherlands, D-236	2.5 (0.4)	0	0	80 (3)	20 (3)	100	0	0	100	0	100
5. Pa_2, Emmen, the Netherlands, D-264	0.8 (0.7)	0	0	100	0	100	0	0	100	0	100
6. Pa_2, Vriezeveen, the Netherlands, D-275	4.3 (1.6)	0	0	100	0	100	0	100	0	100	0
7. Pa_2, Averest, the Netherlands, D-276	1.4 (0.5)	0	25 (5)	75 (5)	0	100	0	75 (6)	25 (6)	35 (4)	65 (4)
8. Pa_2, Hardenberg, the Netherlands, D-286	0.9 (0.3)	0	20 (4)	58 (10)	21 (5)	100	0	0	100	21 (8)	79 (8)
9. Pa_2, Oosterhesselen, the Netherlands, D-287	0.8 (0.4)	0	25 (6)	64 (6)	12 (6)	100	0	0	100	0	100
10. Pa_2, Ommen, the Netherlands, D-301	0.6 (0.4)	0	0	100	0	100	0	100	0	92 (9)	8 (9)
11. Pa_2, ? , the Netherlands, 1095	1.6 (0.5)	0	0	100	0	100	0	0	100	20 (5)	80 (5)
12. Pa_2, Coevorden, the Netherlands, P-2-22	0.7 (0.2)	0	0	100	0	100	0	10 (12)	90 (12)	8 (9)	92 (9)
13. Pa_2, Veendam, the Netherlands, HPL-1	1.5 (0.5)	0	50 (5)	50 (5)	0	100	0	0	100	0	100
14. Pa_2, New Leake, Great Brittain, ST	3.3 (2.1)	0	18 (4)	68 (6)	15 (7)	100	0	10 (12)	90 (12)	18 (8)	82 (8)
15. Pa_3, Valthe, the Netherlands, ROOK	36.7 (23.8)	0	16 (6)	84 (6)	0	100	0	35 (4)	65 (4)	42 (15)	58 (15)
16. Pa_3, Gasselte, the Netherlands, A-75-250-39	31.5 (21.6)	0	0	100	0	100	0	49 (9)	51 (9)	28 (5)	72 (5)
17. Pa_3, Anjum, the Netherlands, 1077	6.5 (1.9)	0	82 (5)	18 (5)	0	100	0	35 (4)	65 (4)	0	100
18. Pa_3, Westerbork, the Netherlands, 1112	11.8 (5.6)	0	21 (6)	79 (6)	0	100	0	100	0	0	100
19. Pa_3, Sleen, the Netherlands, 74-768-20	49.4 (31.8)	0	13 (6)	71 (2)	16 (4)	100	0	25 (5)	75 (5)	62 (11)	38 (11)
20. Pa_3, Vriezeveen, the Netherlands, 75-884-4	10.4 (4.3)	0	0	100	0	100	0	90 (12)	10 (12)	0	100
21. Pa_3, Hardenberg, the Netherlands, 1097	16.3 (6.3)	0	16 (2)	70 (1)	13 (1)	100	0	26 (5)	74 (5)	0	100
22. Pa_3, Frenswegen, Federal Republic of Germany, FR	5.7 (0.6)	0	0	88 (7)	12 (7)	100	0	45 (4)	55 (5)	33 (6)	67 (6)
23. Pa_1, ? , Austria, R 4	47.9 (10.8)	0	0	93 (10)	7 (10)	100	0	10 (12)	90 (12)	35 (5)	65 (5)
24. Pa_3, Far Oer, Denmark, E-1215	9.3 (0.7)	0	64 (5)	36 (5)	0	100	0	30 (9)	70 (9)	76 (5)	24 (5)
25. Pa_3, Cadishead, Great Britain, E-1202	6.7 (0.7)	0	12 (2)	73 (4)	15 (2)	100	0	42 (6)	58 (6)	35 (4)	65 (4)
isoelectric point		5.48	5.52	5.56	5.60	5.50	5.58	5.96	6.20	6.23	6.28
molecular mass [e]		59				56	54	28		31.5	
color [f]		r.b.				r.b.		r.		g.	
detection limit [g]		10-15				< 2.5		15-20		15-20	
13 adult males [g]		0	33 (6)	35 (7)	0	43 (10)	0	0	33 (7)	0	0
13 fourth stage female larvae [g]		0	52 (8)	58 (7)	0	52 (10)	0	0	74 (11)	0	38 (9)
13 second stage larvae [g]		0	81 (8)	88 (10)	0	79 (11)	0	0	78 (9)	0	0

Table 1 (continued)

	Alleles																	
E_1	E_2	F_1	F_2	F_3	G_1	G_2	H_1	H_2	H_3	I_1	I_2	J_1	J_2	J_3	J_4	K_1	K_2	K_3
0	100	21 (4)	13 (4)	66 (5)	0	100	26 (1)	14 (7)	60 (7)	100	0	35 (1)	48 (3)	16 (4)	0	53 (5)	47 (5)	0
0	100	14 (1)	84 (2)	2 (2)	100	0	0	0	100	100	0	0	100	0	0	—[c]	—[d]	—[d]
83 (5)	17 (5)	15 (2)	12 (2)	73 (4)	0	100	51 (2)	0	49 (2)	100	0	0	74 (4)	0	26 (4)	0	8 (9)	92 (9)
82 (7)	18 (7)	20 (5)	8 (2)	72 (2)	0	100	52 (6)	0	48 (6)	100	0	0	77 (5)	0	23 (5)	0	8 (9)	92 (9)
0	100	0	33 (6)	67 (6)	0	100	63 (5)	0	37 (5)	100	0	5 (3)	95 (3)	0	0	0	100	0
0	100	0	35 (5)	65 (5)	0	100	80 (8)	0	20 (8)	100	0	56 (1)	6 (2)	38 (3)	0	80 (5)	20 (5)	0
0	100	0	36 (5)	64 (5)	0	100	31 (3)	31 (3)	38 (6)	100	0	71 (11)	19 (8)	10 (3)	0	0	100	0
0	100	0	63 (4)	37 (4)	0	100	51 (2)	0	49 (2)	100	0	25 (3)	50 (6)	25 (3)	0	0	100	0
0	100	0	92 (5)	8 (5)	0	100	60 (6)	0	40 (6)	100	0	64 (7)	0	0	36 (7)	0	100	0
0	100	0	13 (5)	87 (5)	0	100	44 (8)	0	56 (8)	100	0	71 (10)	29 (10)	0	0	0	100	0
0	100	28 (4)	67 (11)	5 (7)	0	100	40 (5)	0	60 (5)	100	0	11 (1)	43 (2)	0	46 (3)	0	100	0
0	100	21 (5)	71 (5)	7 (3)	0	100	33 (4)	0	67 (4)	100	0	11 (4)	61 (6)	0	28 (2)	0	100	0
0	100	20 (4)	20 (3)	60 (6)	0	100	50 (3)	50 (3)	0	100	0	78 (2)	22 (2)	0	0	48 (10)	52 (10)	0
8 (5)	92 (5)	87 (4)	2 (3)	10 (3)	0	100	40 (5)	0	60 (5)	100	0	44 (5)	26 (4)	30 (1)	0	20 (5)	80 (5)	0
23 (7)	77 (7)	3 (4)	29 (5)	68 (2)	0	100	42 (2)	0	58 (2)	30 (4)	70 (4)	0	59 (2)	41 (2)	0	0	65 (5)	35 (5)
15 (6)	85 (6)	0	28 (6)	72 (6)	0	100	49 (8)	0	51 (8)	18 (6)	82 (6)	0	72 (8)	28 (8)	0	0	76 (4)	24 (4)
0	100	0	26 (3)	74 (3)	0	100	48 (4)	52 (4)	0	100	0	66 (6)	34 (6)	0	0	0	100	0
0	100	0	43 (4)	57 (4)	0	100	52 (2)	48 (2)	0	100	0	23 (2)	77 (2)	0	0	0	100	0
0	100	49 (3)	4 (4)	47 (6)	0	100	61 (12)	0	39 (12)	100	0	95 (3)	0	5 (3)	0	0	65 (4)	35 (4)
0	100	0	100	0	0	100	35 (7)	0	65 (7)	100	0	45 (4)	0	55 (4)	0	74 (9)	0	26 (9)
0	100	14 (5)	53 (5)	33 (4)	0	100	59 (2)	0	41 (2)	100	0	58 (7)	8 (1)	33 (7)	0	65 (5)	35 (5)	0
0	100	0	46 (4)	54 (4)	0	100	59 (11)	22 (5)	19 (6)	100	0	68 (4)	0	32 (4)	0	22 (9)	88 (9)	0
17 (5)	83 (5)	0	9 (5)	91 (5)	0	100	19 (5)	63 (8)	18 (4)	100	0	26 (1)	68 (2)	0	6 (1)	0	100	0
0	100	0	45 (4)	55 (4)	0	100	57 (1)	15 (2)	28 (3)	100	0	0	37 (6)	63 (6)	0	0	100	0
0	100	33 (6)	20 (6)	47 (6)	0	100	27 (2)	27 (2)	46 (6)	100	0	16 (4)	58 (12)	25 (12)	0	17 (3)	83 (3)	0
6.23	6.29	6.32	6.42	6.48	6.44	6.60	6.62	6.63	6.78	6.71	6.84	6.68	6.90	6.97	7.04	6.72	6.80	6.94
60		54			28.5		36	37	36	50	49	56				58		
r.b.		r.b.			r.		b.b.			r.b.		r.b.				r.b.		
< 2.5		2.5-5			10-15		2.5-5			< 2.5		< 2.5				10		
0	34 (9)	0	0	15 (6)	0	66 (14)	0	0	0	104 (13)	0	25 (7)	8 (3)	0	0	23 (5)	20 (7)	0
0	35 (11)	7 (4)	10 (4)	36 (8)	0	91 (13)	31 (8)	28 (8)	0	23 (12)	0	42 (4)	13 (3)	0	0	36 (8)	23 (6)	0
0	68 (10)	0	0	9 (4)	0	68 (9)	0	0	0	104 (19)	0	47 (9)	14 (5)	0	0	26 (5)	20 (7)	0

[a] The ratios and the standard deviations (in parenthesis) were obtained as described in the text. The loci and alleles of the IP-variants are indicated with capitals and numbers, respectively — [b] As designated in the original collections - [c] Number of females expressed as a percentage of those developed on a general susceptible cultivar — [d] No allele product of locus *K* could be traced in population no. 2 — [e] Molecular masses in kilodaltons — [f] Reddish brown (r.b.), red (r.), grey (g.), blackish brown (b.b.) — [g] Relative protein quantities ($x10^2$), estimated from the reference series (see text).

stage female larvae (Bakker & Bouwman-Smits, 1988*b*) were prepared as described. Total protein extracts from adult males were made as follows. Potato cultivar Eigenheimer was inoculated with approximately 200 cysts and after 35 days the adult males were recovered from the soil. Approximately 1200 adult males were handpicked under a dissecting microscope and homogenized in a small mortar in 60 µl 10 mM Tris-HCl, pH 7.4, 5 % (v/v) 2-mercaptoethanol and saturated with 64 mg urea, and stored at - 80 °C until use.

As a standard 35 µg protein was used for electrophoresis. Isoelectric focusing using ampholines pH range 5-7, sodium dodecyl sulfate electrophoresis in 12 % acrylamide and staining (Bakker & Bouwman-Smits, 1988*b*) was done by processing eight samples simultaneously. Reference series for estimating relative protein quantities were prepared by processing sixteen samples at a time. Molecular weight and isoelectric point determinations were as described (Bakker & Bouwman-Smits, 1988*a*).

Population nos. 1, 3, 4, 13, 14, 22, 24 and 25 (Table 1) were studied for two or more subsequent generations. No consistent differences were observed between different generations of the same population.

Protein profiles were evaluated visually by superimposing the original gels on a bench viewer. A total of 224 protein patterns was analysed. The minimum number of replicates per object was two.

The similarity dendrogram was constructed following the UPGMA method (Sneath & Sokal, 1973) with the assistance and computer facilities of Ir. T. Heyerman, Department of Entomology, Agricultural University, Wageningen, the Netherlands.

Results

The genetic differentiation of the 25 *G. pallida* populations (Table 1) was investigated with two dimensional gel electrophoresis (2-DGE) of total protein extracts from adult females. 2-DGE patterns representing the protein composition of 100 individuals are shown in Figure 1.

Comparison of the 25 populations revealed 29 variant protein spots which seem to be the result of amino acid substitutions that alter the net charge (IP-variants). The corresponding IP-variants had the typical characteristics of proteins encoded by alleles at the same locus (Bakker & Bouwman-Smits, 1988*a*). The 29 IP-variants were treated as the products of 29 alleles at 11 loci. The IP-variants are assigned with a capital referring to the locus and a number referring to the allele (Bakker & Bouwman-Smits, 1988*a*). The corresponding IP-variants had a moderate difference in isoelectric point, similar molecular weight, same color and seem to be produced in about equal quantities per haploid set of chromosomes within each individual (Figs 1, 2; Table 1). At loci B, H and I the allele products displayed minor differences in molecular weight. Such dissimilarities in apparent molecular weight have been reported before between corresponding allele products and may be the result of deletions or additions of a stretch of amino acids, but single net charge changing substitutions may be involved as well (Rosenblum *et al.*, 1983). Several more IP-variants, mostly minor protein spots, were detected among the approximate 350 proteins resolved on the original patterns, but only those were included which allowed a proper quantification of their allele frequencies.

The remaining variant protein spots were designated as nonisoelectric point variants (NIP-variants) (Bakker & Bouwman-Smits, 1988*a*). Examples of NIP-variants are shown in Figure 1. Comparison of all populations revealed a total of 44 major NIP-variants. Variants having the characteristics of IP-variants in a number of pairwise comparisons, but of which the corresponding allele products could not be traced in all populations studied were also designated as NIP-variants. Preliminary analysis of the 44 NIP-variants showed that they support the major affinities between the 25 populations based on the IP-variants. For reasons of conciseness the NIP-variants are not included in this report.

The ratios between the protein quantities of the corresponding IP-variants were used as a measure for their allele frequencies (Fig. 2), and were estimated as follows. In case only one of the corresponding IP-variants could be detected in a population after electrophoresing 35 µg total protein, the protein quantity of this IP-variant was given a relative value of 1.0. For each locus one such monomorphic population was chosen and used to make a reference series by electrophoresing protein quantities of 0.875 µg; 1.75 µg; 3.5 µg; 5.25 µg; 7.00 µg and so on in steps of 3.5 µg up to 45.5 µg. Hence these quantities correspond with relative values of 0.025; 0.05; 0.10; 0.15 *et cetera*. In populations with two or more corresponding IP-variants the protein quantities were evaluated visually by comparing the spot size and intensity with those of the reference series. If no clear cut decision could be made, e.g. whether the relative protein quantity was closer to 0.1 or 0.2 the IP-variant was given an intermediate value of 0.15. The relative values so obtained were used to calculate the ratios, which were used as a measure for the allele frequencies. The ratios were estimated by electrophoresing a minimum of two protein samples from each population. Each replicate was evaluated twice. The average ratios and their standard deviations are listed in Table 1. The detection limits of the IP-variants were estimated from the reference series.

Ideally the sums of the relative values of the protein quantities of the corresponding IP-variants should equal 1.0. However, in this study the sums of the relative values usually ranged from 0.8 to 1.2 for loci B, E, F, H, I and J, and from 0.7 to 1.3 for loci A, C, D, G and K. These deviations from 1.0 were mainly due to variations between independent experiments.

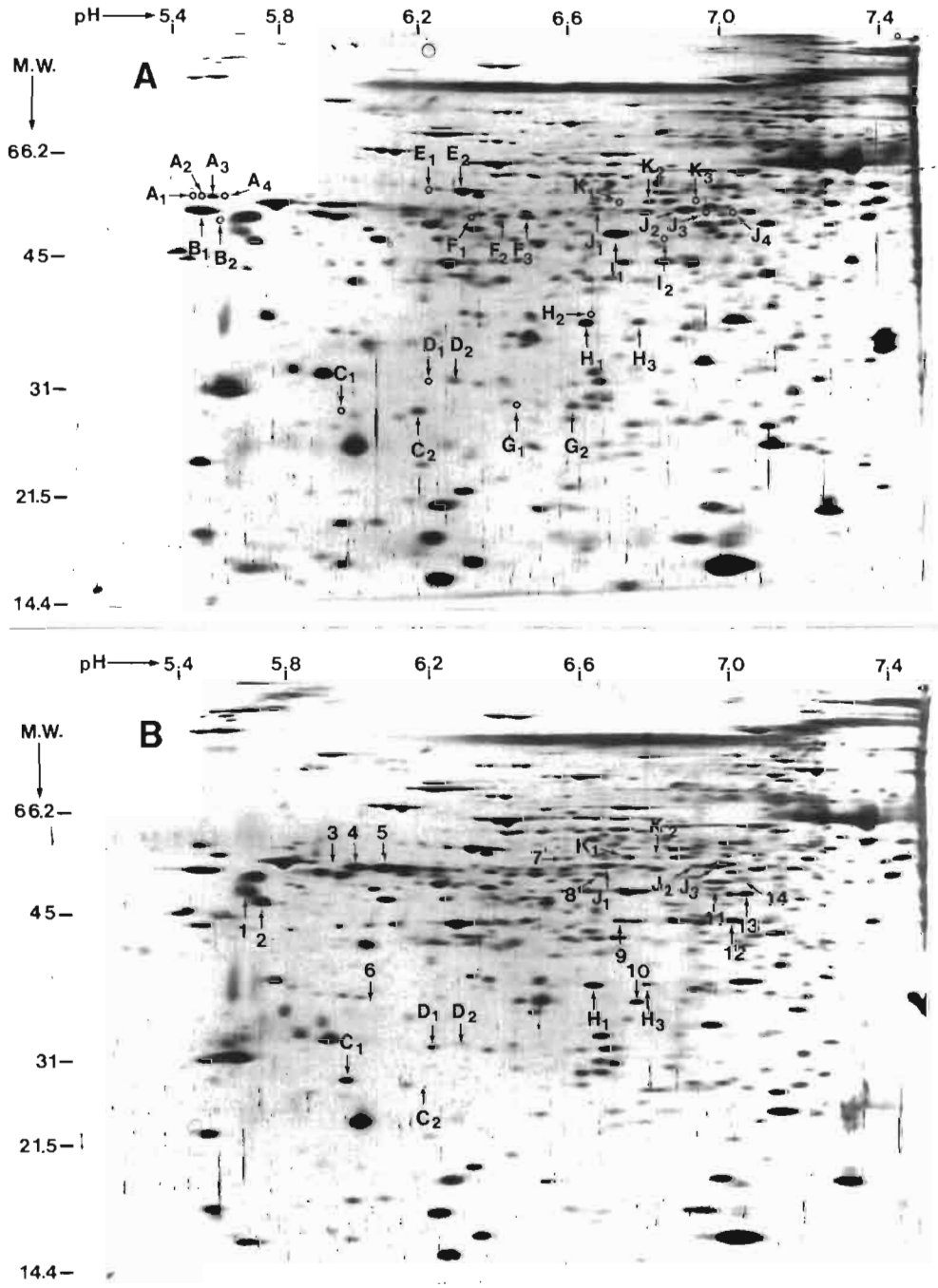

Fig. 1. Protein patterns of a mixture of 100 adult females from *Globodera pallida* population no. 5 (A) and population no. 6 (B). The positions of the isoelectric point variants (IP-variants) detected by the comparison of 25 populations (Table 1) are marked in A. The capitals and numbers refer to the putative loci and alleles, respectively. Qualitative and quantitative differences between the two populations at the IP-variant loci are denoted in B. Dissimilarities between the two populations indicated with arabic numbers in B are nonisoelectric point variants (NIP-variants). Other differences visible on the photographs, e.g. variants with an isoelectric point above pH 7.05, were not consistent. Molecular masses are given in kilodaltons. Further details see text and Table 1.

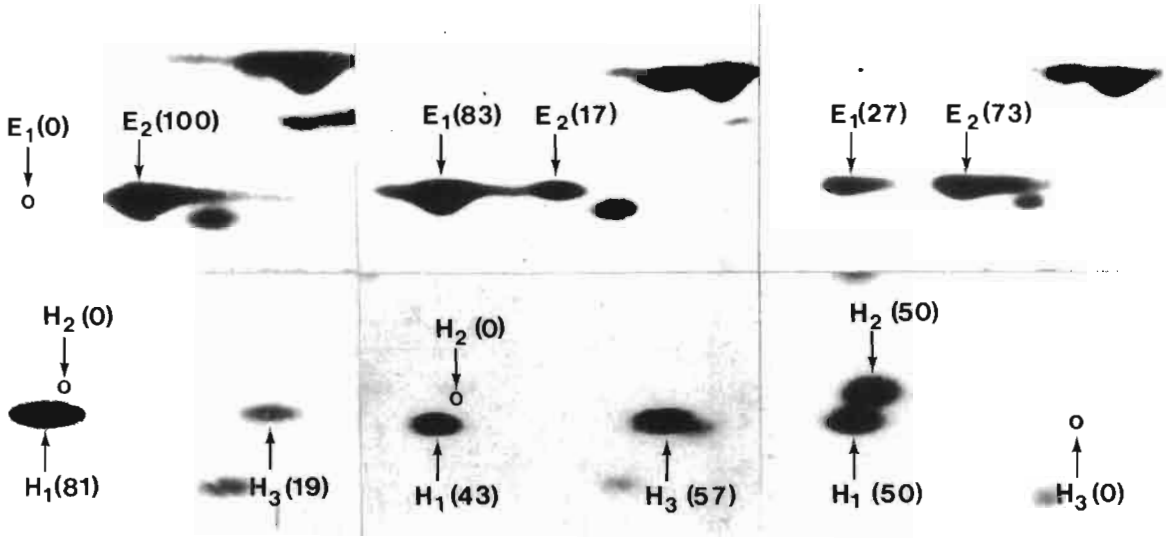

Fig. 2. Details of protein patterns made by electrophoresing a homogenate of 100 adult females. The IP-variants are designated with capitals and numbers referring to the putative loci and alleles, respectively. In parenthesis the estimates of the ratios between the protein quantities of the corresponding IP-variants, which were used as a measure of the allele frequencies. Ratios were estimated as described in the text. Top row. Locus E : population no. 6 (left), no. 4 (middle) and no. 15 (right). Bottom row. Locus H : population no. 6 (left), no. 15 (middle) and no. 13 (right).

Despite these difficulties, measuring the proportion of protein quantities of the corresponding IP-variants seems a valid approach to estimate the allele frequencies. When populations were electrophoresed and stained simultaneously in parallel experiments, deviations between the sums of the relative values for a given locus were small. Deviations between the highest and lowest values of the sums were usually less than 0.2 for loci B, E, F, H, I and J, and less than 0.3 for loci A, C, D, G and K. Moreover, repeated electrophoresis and staining of population nos. 3, 4, 13, 14, 22, 23, 24 and 25 in parallel experiments revealed no consistent differences between the sums of the relative values of any given locus, indicating that we are dealing with codominant alleles of which the products are synthesized in similar amounts.

The similarity dendrogram (Fig. 3) was constructed from a distance matrix following the UPGMA method (Sneath & Sokal, 1973). Genetic distances were obtained by interpreting the ratios between the protein quantities of the eleven IP-variant loci as allele frequencies. Distances were computed according to Rogers (1984). The distances between population no. 2 and the other populations were based on ten loci, because no allele product of locus K could be traced in population no. 2 (Table 1). The distance matrix is available from the senior author upon request.

In order to define the eleven IP-variant loci as well as possible, we investigated also other developmental stages than adult females. A detailed study of population no. 13 showed, that the majority of the IP-variant encoding alleles were also expressed in adult males, fourth stage female larvae and second stage larvae (Table 1). Inspection of Table 1 demonstrates, that at locus F only one allele (F_3) could be traced in adult males and second stage larvae, whereas three alleles (F_1, F_2 and F_3) were present in adult females and fourth stage female larvae. The alleles F_1 and F_2, which both have a frequency of 0.2 in adult females, are probably also expressed in adult males and second stage larvae, but the protein quantities produced by the genes at locus F in those stages are too low to detect such low allele frequencies. In case all corresponding IP-variants were detected, the ratios between the protein quantities in those stages were similar to those in adult females (Table 1).

Discussion

The molecular variation between pathotypes has been studied with various techniques, ranging from disc electrophoresis (Trudgill & Carpenter, 1971; Greet & Firth, 1977; Franco, 1979; Platzer, 1981), isoelectric focusing (Fox & Atkinson, 1985), immunoelectrophoresis (Wharton *et al.*, 1983), two dimensional gel electrophoresis (Stegemann *et al.*, 1982; Ohms & Heinicke, 1985) and restriction enzyme digests of genomic DNA (Schnick *et al.*, 1990). Unfortunately, only qualitative differences were recorded in these studies. Since potato cyst nematode populations are often neither fixed for their (a)virulence alleles nor for alleles revealed by molecular techniques, qualitative data are insufficient

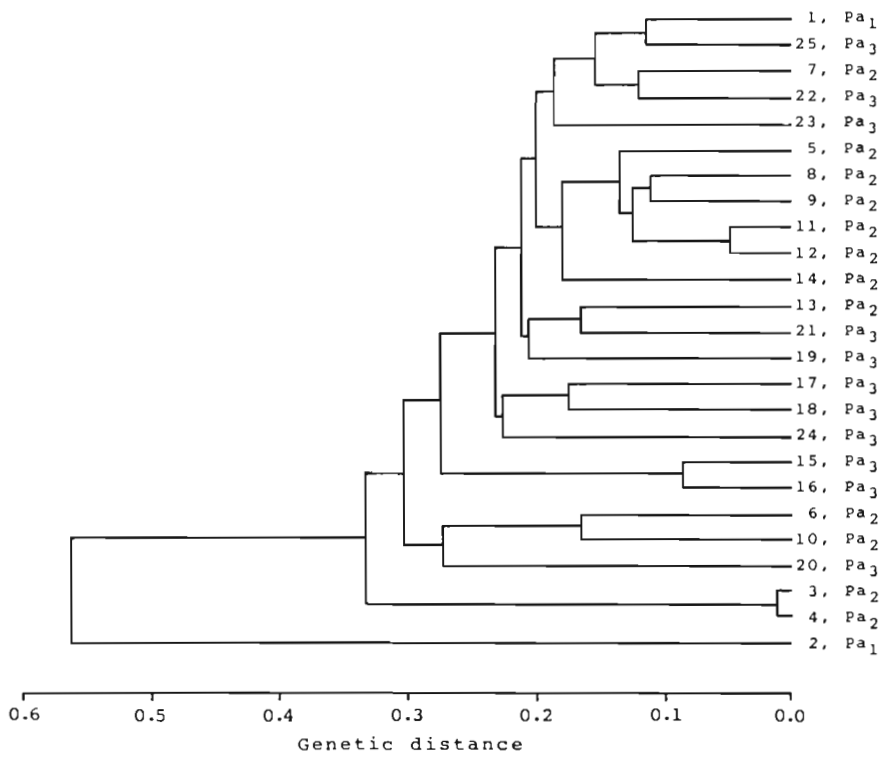

Fig. 3. Similarity dendrogram of 25 *Globodera pallida* populations constructed from the genetic distances at 11 IP-variant loci following the UPGMA method. Populations are numbered as in Table 1.

for a refined assessment of the genetic relationships between pathotypes.

In this report we advanced an efficient method for estimating allele frequencies at polymorphic loci. 2-DGE patterns were made by electrophoresing a homogenate of 100 individuals and the ratio between the protein quantities produced by the alleles at a given locus was used as a measure for the allele frequencies. This approach is reasonably accurate as was demonstrated by electrophoresis of single individuals using a mini 2-DGE system (De Boer *et al.*, 1992). Electrophoresing individuals separately is evidently more accurate, but too laborious for characterizing a large number of populations.

Primary founders, random genetic drift and gene flow

The protein variation between the 25 *G. pallida* populations is in part generated by the various unknown provenances of the primary founders. Evidently, it is not possible to estimate the number of different introductions involved with the 25 *G. pallida* populations, because the share of random genetic drift and gene flow cannot be recovered. Many descendants of the initial populations have probably diverged by random genetic drift due to reductions in population sizes caused by secondary founding events and control measures. In such cases there is also a fair chance that alleles are lost or fixed. Nevertheless, it seems that population sizes in the past were sufficiently large to maintain genetic polymorphisms at several loci. Scrutiny of the 11 IP-variant loci listed in Table 1 shows, that the number of polymorphic loci ranges from two to nine with an average of 5.5 polymorphic loci per population. Of course, polymorphic loci can also be the result of immigration. Inherent to the passive ways of spread of cyst-nematodes, gene flow will depend on factors such as, soil transported by seed tubers, machineries and wind. However, it is evident that the extent of these events is limited, because a uniform distribution of the IP-variants over a large area was not observed. The Dutch populations were collected in a relatively small area and were often discriminated from one another by various qualitative differences (Table 1; Fig. 3).

Pathotypes

Current pathotype classification is incapable of reflecting the genetic diversity among European potato cyst nematode populations. First, the number of differentials used is too limited. For example, the three *G. pallida* pathotypes are discriminated by only two differentials and additional test clones have already differen-

tiated populations classified as the same pathotypes (Fuller & Howard, 1977). Second, populations are designated as virulent or avirulent for a certain differential if the multiplication factor is > 1 or $\leqslant 1$, respectively (Kort *et al.*, 1978). However, in compatible combinations the multiplication factors may range from 1 to 70 (Kort *et al.*, 1978), indicating that the numbers of virulent genotypes may vary considerably among populations classified as identical pathotypes. In addition it is noted that an unambiguous classification is hampered by a variable expression of the nematode and host genotypes.

In view of this classification it is not surprising that populations of the same pathotype are often discriminated at various IP-variant loci and that genetic distances within pathotypes are not necessarily smaller than between pathotypes.

Breeding for resistance

The majority of the IP-variants recorded here, and maybe all, are not encoded by or linked to (a)virulence alleles. Nevertheless, these variants are highly informative for the control of potato cyst nematodes by means of resistance. It is hypothesized, that in the absence of the relevant genes for resistance in Europe, both variation in virulence and proteins are predominantly determined by the same three processes : the genotypes of the primary founders, random genetic drift and gene flow. Because those processes influence the entire gene pool of a population, degrees of similarity revealed by 2-DGE or other molecular techniques will also be reflected at (a)virulence loci. In case populations have large genetic distances at IP-variant loci there is an increased chance that they behave differently towards various genes for resistance, e.g. because they have distinct native ancestors, which have been differentially exposed to genes for resistance. For instance, population no. 4 and no. 5 (Fig. 3), both classified as Pa_2 in the current pathotype scheme, may be discriminated in distinct pathotypes by supplementary genes for resistance. On the other hand populations that are identical or nearly so, e.g. population no. 3 and no. 4 (Fig. 3), will show resemblance at all virulence loci, because they are probably the descendants of the same primary founders and have hardly diverged by gene flow and random genetic drift during the time they have been geographically separated. Despite the arbitrary way populations are classified in the current pathotype scheme, the feasibility of the approach advocated here is supported by the virulence data. All populations linked at a distance less than 0.1 in the similarity dendrogram are classified as identical pathotypes.

2-DGE can be a valuable adjunctive tool in breeding for resistance. The genetic relationships between potato cyst nematode populations in an area can be used to test the effectiveness of any source of resistance. Once an area is investigated by 2-DGE of a substantial number of populations, the affinities can be used as a guidance for a representative survey to estimate the proportion of virulent and avirulent populations. Representatives of each group should be included. How to delineate groups in such a way that they represent useful entities, i.e. a manageable number of groups with an acceptable interpopulation variation in virulence, needs further investigation.

Finally, although we proceed from stochastic processes in explaining the spatial variation in allele frequencies, we do not exclude other mechanisms. However, in case additional genetic mechanisms are operating, e.g. balanced polymorphisms or selection due to environmental conditions, it is very unlikely that such processes are strong enough and act on sufficient loci to impair the value of 2-DGE data for a rational breeding strategy.

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