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GENETIC VARIATION AND PATHOTYPE RESPONSE IN POTATO CYST-NEMATODES FROM CYPRUS

by

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Summary. Thirty-six samples of potato cyst-nematodes (PCN) from Cyprus (Xylophagou and Troodos regions) were examined for pathotype composition and genetic variation using differential potato clones and isozyme analysis. Isoelectric focusing (IEF) analysis revealed *Globodera rostochiensis* to be predominant in the samples, with 32 out of 36 populations consisting of this species. Of the remaining four samples, three were *G. rostochiensis/G. pallida* mixtures and one was pure *G. pallida*. These analyses were confirmed in the pathotyping tests and subsequent electrophoretic analysis of cysts produced in these tests.

The distribution pattern of pathotypes of the potato cyst nematodes (PCN) apparent today is the result of genetic changes which occur with each new infestation and the mixing of different infestations. As a result of an ongoing study of PCN gene pools, and the genetic changes which have occurred during the spread of nematode populations, a number of discreet geographical areas are being studied. One such area in the Mediterranean is Cyprus, about 9251 km^2 in size. Panayi (1974) first reported PCN in the Xylophagou area of Cyprus. It was originally thought that only *G. rostochiensis* was present, having been introduced into the country during the last two or three decades in seed potatoes from Northern Europe. Later, Philis (1981) reported the presence of *G. rostochiensis* and *G. pallida* through morphometric studies. This paper reports the results of a study into the pathotype response and genetic variation in Cyprus potato cyst-nematodes.

Materials and methods

Thirty-six populations of PCN were collected from potato fields in the Xylophagou and Troodos regions of Cyprus. Upon arrival in N. Ireland, populations were increased on cv. Desiree (*Solanum tuberosum* L.) until sufficient material

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was available for further analysis. Because of the very low viability of some populations this process took up to four years in certain cases. Populations were stored at 4 °C prior to pathotype and virulence testing.

Isoelectric focusing and silver staining were used to determine the species present in each sample (Fleming and Dolan, 1986). Cysts were also examined morphologically by using cyst characteristics (Stone, 1975). The pathotype responses of the populations were tested following the scheme of Kort *et al.* (1977) although where cyst numbers were limiting P55/7 was excluded. In addition to the Cyprus populations, one standard population each of Pa2/Pa3 and Ro1 from N. Ireland (Trudgill, 1985) were also tested for comparative purposes.

Genetic variability among 17 of the populations was examined by polyacrylamide gel isoelectric focusing and specific enzyme staining. Gels (125x245 mm and 0.5 mm thick) were cast on PAG gel bond sheets (FMC BioProduct) using a glass gel casting kit (Pharmacia). Gel solutions were composed of 12 ml distilled water, 3.5 ml acrylamide solution (29.1% w/v), 3.5 ml bisacrylamide solution (0.9% w/v) and 0.8 ml pharmalytes pH range 3-10 (Pharmacia). This solution was deaerated for 2 min. in a Buchner flask and 0.5 ml ammonium persulphate (1% w/v) and 20 µl 'temed' were added to the solution prior to filling the cassette.

Sample application was as described by Fleming and Marks (1983) and gels were run at 8 °C for 90 min. at 2000 V, 150 mA and 15 W. Prior to staining, the pH gradient across each gel was measured using a surface pH electrode. This permitted the isoelectric point (pl) of each enzyme band to be determined after staining (Righetti, 1983). In order to optimise the staining reactions, pH gradients were removed prior to addition of the stain. This was achieved by soaking the gel in the appropriate staining buffer for 10 min.

Gels were stained for 15 enzymes: aldolase (ALD), cholinesterase (CHO), enolase (ENO), esterase (EST), fumarate hydratase (FUM), hexokinase (HEX), isocitrate dehydrogenase (IDH), malic enzyme (ME), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), glucose-6-phosphate dehydrogenase (G-6-PDH), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), phosphomannose isomerase (PMI) and peptidase (PEP).

Enzymes were localized using the staining mixtures and buffers described by Allendorf *et al.* (1977) except for cholinesterase and enolase where the procedures of Harris and Hopkinson (1976) were followed. After incubation and development of the enzyme bands, gels were fixed and stored in a mixture of acetic acid, ethanol and water (20:3:1 v/v). Enzyme patterns were photographed and the pl of each band estimated from the previously determined pH gradient. Variation in enzyme banding was used to assess genetic identity among the populations (Nei, 1971; Hubby and Throckmorton, 1965). Genetic groupings were then determined using UPGMA cluster analysis (Sneath and Sokal, 1973).

Results and discussion

Results of the electrophoretic species identification and pathotyping tests for the Cyprus populations are shown in Table I. Initial IEF analysis revealed that *G. rostochiensis* (Woll.) was present in 32 out of 36 populations. Of the remaining four samples, three were *G. rostochiensis/G. pallida* (Stone) mixtures and one was a pure *G. pallida* population. These analyses were confirmed in the pathotyping tests and in subsequent electrophoretic analysis of cysts produced in those tests.

The pure *G. pallida* population 43 gave a Pa3 reaction. However, determining the pathotype of the *G. pallida* populations when mixed with *G. rostochiensis* was difficult due to the low proportion of the former species in the samples. The high Pf/Pi on potato (*Solanum tuberosum* L.) line 62.33.3 of population 40 indi-

TABLE I - *Multiplication rates (Pf/Pi) for Cyprus potato cyst nematodes populations tested against differential potato clones.*

Population	Species[1]	Desiree	M. Piper (Rol, 4)	65346/19 (Rol, 4)	62.33.3 (Rol-4 Pal, 2)	60.21.19 (Rol, 2)	581642/4 (Rol, 3)	P55/7 (Pal)	Pathotype
		Potato genotype and PCN resistance (in brackets)							
1	Ro/Pa	39.4	1.2	.0	2.2	2.8	.4		Rol/Pa?
2	Ro	38.6	.0	.0	.4	.6	.1		Rol
3	Ro	16.6	.2	.0	.1	1.0	1.2	3.8	Rol
4	Ro	25.8	.0	.0	.0	1.0	.0		Rol
5	Ro	26.3	.3	.0	1.2	1.1	.2	7.6	Rol
6	Ro	27.9	.0	.0	1.6	2.5	.0		Rol
7	Ro	16.8	.2	.0	.8	1.1	.2		Rol
10	Ro	26.9	.1	.0	.7	.7	.1	10.1	Rol
11	Ro	45.7	.0	.0	1.3	2.0	.0	7.2	Rol
12	Ro	25.1	.0	.1	.0	1.3	.2	13.1	Rol
13	Ro	29.7	.0	.0	.1	1.1	.4	8.1	Rol
14	Ro/Pa	13.9	1.3	.0	.4	1.0	.2		Rol/Pa?
15	Ro	18.6	.3	.0	1.2	1.3	.1		Rol
17	Ro	41.6	.1	.0	5.0	1.6	.3		Rol/5?
18	Ro	18.7	.1	.0	.1	1.1	.1		Rol
19	Ro	36.7	1.2	.0	2.1	3.1	.1		Rol/3?
25	Ro	18.2	.0	.0	.2	.1	.3		Rol
27	Ro	26.1	.0	.0	.2	1.5	.2		Rol
29	Ro	17.3	.8	.0	2.1	2.6	.3		Rol/3?
30	Ro	17.6	.0	.0	1.1	1.5	.2	11.9	Rol
31	Ro	12.7	11.1	.0	.3	2.6	.1	2.7	Rol/3
32	Ro	16.1	.0	.0	.1	.0	.1	3.5	Rol
33	Ro	15.1	.1	.0	.1	2.5	.3		Rol
39	Ro	12.9	.7	.0	1.5	1.5	.2		Rol/Ro?
40	Ro/Pa	60.1	45.2	2.1	29.6	38.0	24.3	6.4	Rol/Pa2-3
42	Ro	35.1	.7	.0	1.0	2.6	.1		Rol/Ro?
43	Pa	24.8	25.7	2.1	6.0	18.1	17.9	15.1	Pa2-3
45	Ro	45.1	.1	.0	.1	1.5	.1		Rol
46	Ro	46.3	.7	.0	2.9	1.0	.1	7.8	Rol/Ro?
47	Ro	50.2	.0	.0	.5	.9	.1	31.7	Rol
48	Ro	21.4	.1	.0	1.0	3.0	.1		Rol
52	Ro	28.5	.2	.2	.1	.5	.2		Rol
55	Ro	31.6	.0	.0	1.0	2.1	.1		Rol
58	Ro	12.6	.1	.0	5.6	.8	.0	6.2	Rol
60	Ro	17.6	.1	.0	.5	.5	.1	4.8	Rol
61	Ro	21.2	.0	.0	.5	2.9	.1	12.7	Rol
Pa3NI	Pa	32.6	35.1	35.1	12.1	29.6	21.9	6.8	Pa2-3
Ro1NI	Ro	41.6	.1	.1	.6	.4	.1	8.1	Rol

[1] Species determined from IEF analysis of inocula and confirmed on new cysts.

cated that, at least in this case, the *G. pallida* was likely to be Pa3. Within the *G. rostochiensis* samples, pathotype Ro1 was the most common reaction, but some gene H1 resistance-breaking populations were evident. In a number of cases, and due to a mixture with Ro1, it was not possible to determine the additional pathotype present. Nevertheless, populations 19, 29 and 31 appeared to consist of mixtures of Ro1 and Ro3. This assessment was made on the basis of their higher than expected reproduction on potato Maris Piper and *S. kurtzianum* bybrid 60.21.19, although accurate identification in the case of pathotype mixtures is not always certain. The relatively high multiplication of population 17 on potato 62.33.3 suggested that this population was a mixture of Ro1 and Ro5, although this interpretation must be tentative, taking into account the low Pf/Pi on Maris Piper.

Sufficient material was available from Cypriot populations to permit an isozyme analysis of 17 of the populations. Only three enzyme systems exhibited variations among these populations (Table II). The resulting mean genetic identity values showed that low levels of genetic heterogeneity were present among the majority of Cypriot populations (Table III). In the dendrogram in Fig. 1 two main population groups were apparent. The largest, containing 14 populations, exhibited only minor differences among the populations (1>0.99). A second smaller group, consisting of three populations, clustered away from this group at an identity level of 0.96.

A comparison of seven Cypriot populations, with a selection of N. Ireland *G. rostochiensis* Ro1 populations, revealed that N. Ireland populations showed a greater affinity with the major genetic grouping in Cyprus (Fig. 2). The mean genetic identities for this comparison are presented in Table IV.

Genetic diversity among *G. rostochiensis* in Cyprus is of a low level, the obvious conclusion from this being that the original PCN introduction into Cyprus was of a very limited gene pool, either consisting of a small number of

TABLE II - *Isozyme banding patterns from 17 Cyprus* Globodera rostochiensis *populations of three polymorphic enzyme systems (CHO-Cholinesterase, PEP=Peptidase, EST=Esterase)*

Populations													
(1) (8) (15)	C6 C41 C9	(2) (9) (16)	C29 C19 C42	(3) (10) (17)	C2 C39 C12	(4) (11)	C14 C13	(5) (12)	C38 C27	(6) (13)	C62 C61	(7) (14)	C55 C48

Enzyme	Band	pI	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
CHO	A	3.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	H	9.25														–			
	I	9.75																–	
	J	9.85											–	–	–		–	–	
PEP	A	9.99	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	B	6.4														–			
EST	A	4.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	B	4.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	C	4.95	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	D	5.45	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	E	6.65	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	F	8.65									–	–							
	G	9.2														–			

TABLE III - *Matrix of mean genetic identity values for 17 populations of* G. rostochiensis *from Cyprus*

1. C6, 2. C29, 3. C2, 4. C14, 5. C38, 6. C62, 7. C55, 8. C41, 9. C19, 10. C39, 11. C13, 12. C27 13. C61, 14. C48, 15. C9, 16. C42, 17. C12.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1000	1000	1000	1000	1000	1000	1000	992	992	988	988	988	988	964	963	959
2		1000	1000	1000	1000	1000	1000	992	992	988	988	988	988	964	963	959
3			1000	1000	1000	1000	1000	992	992	988	988	988	988	964	963	959
4				1000	1000	1000	1000	992	992	988	988	988	988	964	963	959
5					1000	1000	1000	992	992	988	988	988	988	964	963	959
6						1000	1000	992	992	988	988	988	988	964	863	959
7							1000	992	992	988	988	988	988	954	963	959
8								992	992	988	988	988	988	964	963	959
9									1000	995	995	995	995	963	963	961
10										995	995	995	995	963	963	961
11											1000	1000	987	965	964	959
12												1000	987	965	964	959
13													987	965	964	959
14														965	964	959
15															962	986
16																992

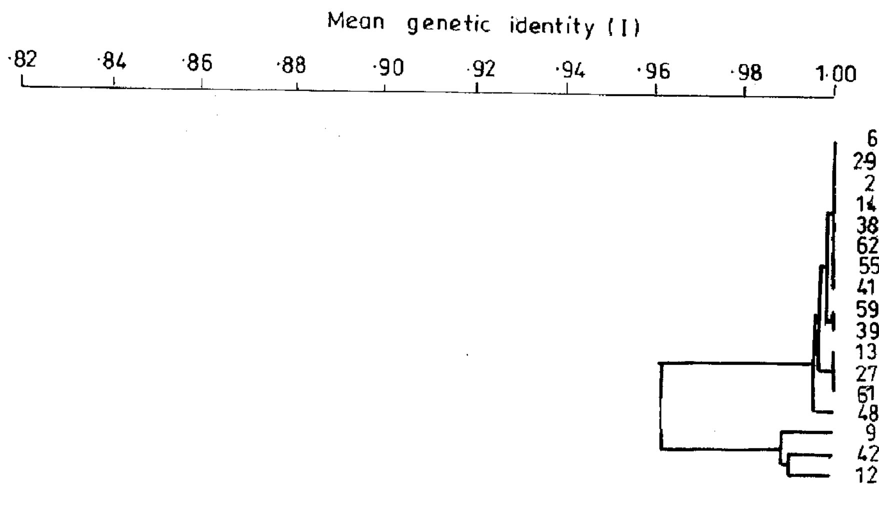

Fig. 1 - Genetic relationships among 17 *Globodera rostochiensis* populations from Cyprus based on Nei's (1972) mean genetic identity across 15 enzyme systems.

cysts or a more extensive introduction of a very homozygous gene pool. As Ro1 was the major pathotype in the samples this might be expected. Bakker (1987) has demonstrated the similarity of Ro1 populations from various parts of Northern Europe and the distinctiveness of the pathotype from Ro2, Ro3, Ro4 and Ro5 from Germany and The Netherlands. The low level of non-Ro1 individuals in Cypriot populations made it impossible to assess the genetic diver-

TABLE IV - *Matrix of mean genetic identiy values for 12 populations of* G. rostochiensis *from Cyprus and N. Ireland*

1. C2, 2. C48, 3. C13, 4. C6, 5. C9, 6. C12, 7. C42, 8. 606605 9. 622496, 10. A2, 11. 606604, 12. 606606.

	2	3	4	5	6	7	8	9	10	11	12
1	1000	1000	999	965	964	959	994	995	995	995	995
2		1000	999	965	964	959	994	995	995	995	995
3			999	965	964	959	994	995	995	994	994
4				964	963	959	996	993	963	965	963
5					992	986	960	964	963	965	964
6						992	964	963	963	965	964
7							960	964	963	994	964
8								965	995	995	995
9									998	998	1000
10										997	998
11											999

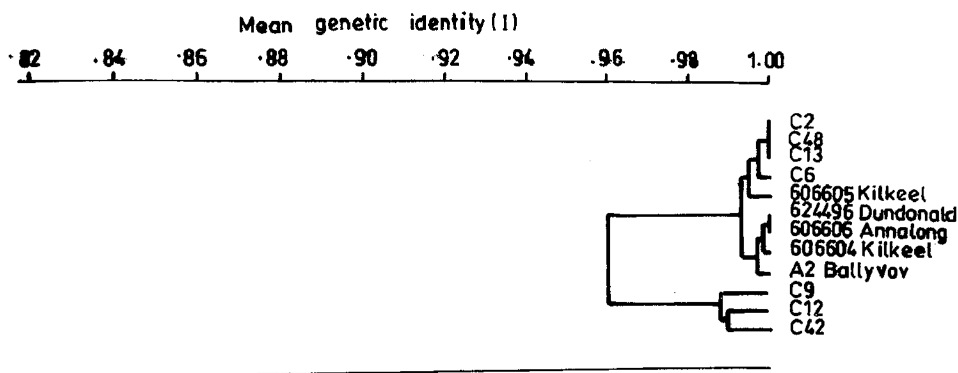

Fig. 2 - Genetic relationships among 12 *G. rostochiensis* populations from Cyprus and N. Ireland based on Nei's (1972) mean genetic identity across 15 enzyme systems.

sity of these pathotypes but material is being bulked up to facilitate this study in the future.

Fig. 2 highlights the probable influence of initial founding events on the genetic composition of subsequent populations. The genetic structure of the founding population is the major influence on the genetic structure of any secondary infestations. Thus, some degree of geographical heterogeneity among populations would be expected. The comparison between N. Ireland and Cypriot *G. rostochiensis* showed such a pattern, with four out of five N. Ireland populations clustering together and separate from the Cyprus groups. Detailed studies of the Cyprus potato cyst nematodes response on potato 62.33.3 as well as other interactions have already been initiated at this laboratory in order to determine the relative importance of genetic and environmental factors in influencing the reactions.

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