THE GENUS *NACOBBUS* IN ARGENTINA. 4. PRELIMINARY COMPARISON OF POPULATIONS OF *N. ABERRANS* (THORNE, 1935) THORNE & ALLEN, 1944 BY MEANS OF ISOENZYME PHENOTYPES

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RESUMEN

Doucet M. E. y C. N. Gardenal. 1992. El género *Nacobbus* en Argentina. 4. Comparación preliminar entre poblaciones de *N. aberrans* (Thorne 1935) Thorne & Allen, 1944 por medio de fenotipos de isozimas. Nematrópica 22:243–246.

Siete poblaciones de *Nacobbus aberrans* provenientes de diferentes regiones y hospederos de Argentina fueron comparadas en base a los fenotipos electroforéticos de seis enzimas extraídas de hembras endoparásitas del nematodo. Se detectó variabilidad intra e interpoblacional. Se confirma la existencia de diferencias entre poblaciones Argentinas de *N. aberrans*, previamente observada al evaluar caracteres morfométricos y de comportamiento de este nematodo.

Palabras clave: electroforesis, *Nacobbus aberrans*, taxonomía.

Nacobbus aberrans (Thorne, 1935) Thorne & Allen, 1944 is a plant parasitic nematode widely distributed in Argentina, inhabiting very different agroecological environments (7). It parasites a variety of plant species and is considered a serious pest in several vegetable and field crops (2,7). Recently, significant morphometric variation among populations from various geographical areas has been observed (8). There also is evidence that populations of *N. aberrans* can differ pathologically and reproductively on the same horticultural or field crop (3,4). For this reason, it will be valuable to widen the spectrum of criteria used to detect genetic differences among populations. A preliminary study based on enzyme phenotypes showed that populations from two distant regions of Argentina had different esterase phenotypes (6). In order to increase our knowledge of genetic variation in *N. aberrans*, we have analyzed electrophoretic patterns in additional populations from different geographical areas of Argentina, parasiting a variety of hosts (Table 1).

Plant roots infected with *N. aberrans* were collected from six localities in Argentina. Living endoparasitic adult females were dissected from root galls under a stereoscopic microscope. Samples of 20 females were collected in microcentrifugue tubes, suspended in 10 µm of Ringer's solution and frozen at –20 C until electrophoretic analysis was performed. Depending on the number of individuals available, three, four, or five pools of 20 females from each population were processed separately for each buffer system. Samples were thawed and ground at 4 C in the same microcentrifugue tube with an adapted glass rod. The resulting suspensions were adsorbed onto Whatman No. 3 filter paper (2 × 6 mm) and inserted in starch blocks (1 cm

Table 1. General information on distribution of Argentine populations of *Nacobbus aberrans* characterized by electrophoresis.

Population code	Province	Locality	Geographical coordinates	Altitude (m)	Host
A	Buenos Aires	La Plata	34 55 S; 57 57 W	19	*Lycopersicon esculentum*
B	Catamarca	Las Mesadas	27 26 S; 62 02 W	1 400	*Solanum tuberosum*
C	Catamarca	Las Mesadas	27 26 S; 62 02 W	1 400	*Amaranthus versicolor*
D	Córdoba	Río Cuarto	33 08 S; 64 21 W	436	*Chenopodium album*
E	Santa Fé	Rosario	32 57 S; 60 40 W	25	*Lycopersicon esculentum*
F	Tucumán	Lules	26 56 S; 65 21 W	300	*Lycopersicon esculentum*
G	Tucumán	Tafí del Valle	26 52 S; 65 41 W	2 000	*Amaranthus versicolor*

thick, 12 cm wide). Horizontal electrophoresis was carried out at 4 C (1,10). Samples from all origins and hosts were not available simultaneously, so two of the most abundant populations (*C* and *D*) were used as reference standards in each gel to determine relative electrophoretic mobility. A total of eight enzymes were analyzed. Two electrophoretic buffer systems were employed: 1) Tris-boric-EDTA, 6 mA for 15 hr, pH 8.6 (11) for the separation of catalases (CAT), malic enzyme (ME), hexoquinase (HK), esterases (ES), aspartate aminotransferase (AAT), glucose-6-phosphate dehydrogenase (G-6-PDH), and isocitrate dehydrogenase (ICD); and 2) discontinuous Tris-citric, 15 mA for 5 hr, pH 6.7 for gels and pH 6.3 for electrode cells (15), for the separation of malate dehydrogenase (MDH) and isocitrate dehydrogenase (ICD). Standard staining procedures were used to visualize isozymes after electrophoresis (14). Paired comparisons of shared electromorphs were performed by the simple matching coefficient (5) which is derived from a matrix of digital data where 1 = presence of a single band (or intensely stained zone) of constant mobility and 0 = absence of the band. Interpopulation relationships were determined by cluster analysis based on similarity values (16).

None of the samples revealed activity for G-6-PDH and diffuse unreproducible bands were obtained for ICD; these enzymes were not considered further.

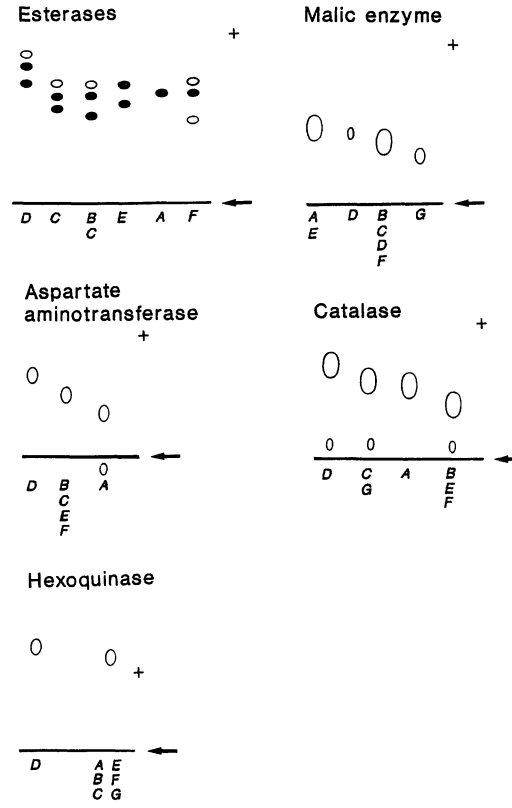

Fig. 1. Schematic representation of isoenzyme electrophoretic patterns of *Nacobbus aberrans* from Argentina. Capital letters denote populations given in Table 1.

Isozymes of CAT, ME, HK, ES, AAT, and MDH, however, provided highly reproducible zymograms for all populations except *G* in which only the first three enzymes were studied. Malate dehydrogenase had two well-defined bands of identical cathodic mobility in all the samples (Fig. 1). For ME, two different electromorphs were detected among samples of population *D*. The same was observed for esterases in population *C*. Twenty-four characters (bands of different electrophoretic mobility) were computed for populations *A* to *F*. In population *G*, a total of 10 characters were considered. The similarity dendrogram based on the single linkage clustering method (16) discriminated population *D* from the rest (Fig. 2). Populations *A* and *B* had the highest similarity values.

Since analyses were carried out on extracts from composites of 20 females it was not possible to evaluate the level of intrapopulation variability. Nevertheless, results suggested polymorphism within a population for esterases and malic enzyme, which had distinct patterns for different pools of the same population. Use of microtechniques with sufficient sensitivity to study one individual (9) would permit genetic analysis of different electromorphs and quantitation of genetic variability within populations based on allele frequencies. Among populations, enzyme polimorphism was indicated for six enzymes, probably representing a greater number of genetic loci.

This is the first comparison of *N. aberrans* populations by a biochemical criterion. Biochemical criteria have shown considerable usefulness in the taxonomy of other nematode species (12,13). Electrophoretic patterns of *N. aberrans* populations from identical hosts (for example, those associated with *Lycopersicon esculentum* and *Amaranthus versicolor*) did not show the highest degree of similarity. The same phenomenon has been observed when comparing populations of *N. aberrans* based on morphometric characters (8). Thus, the morphometric and enzymatic variability that has been observed among *N. aberrans* populations is partly unrelated to the hosts from which they were collected. There was some similarity among populations from the same geographic region, such as those from Las Mesadas and Lules, even though they were obtained in areas with different climatic and soil conditions. Therefore, the existence of differences among Argentine populations of *N. aberrans* is supported by these data, and it is possible that some populations represent distinct races whose characteristics are not yet well defined.

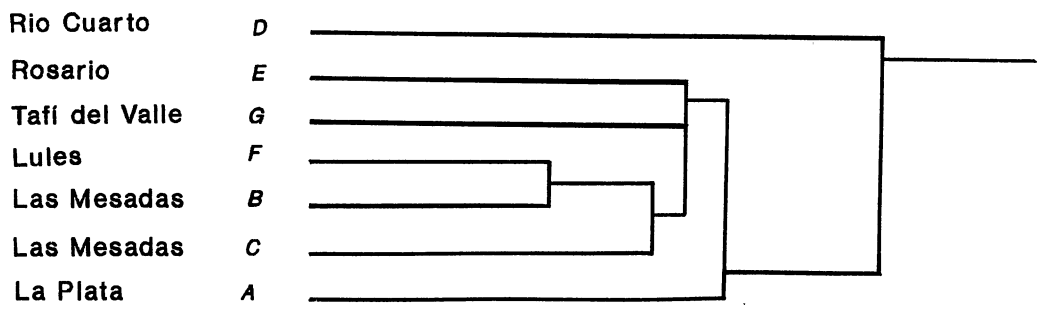

Fig. 2. Dendrogram showing relationships among *Nacobbus aberrans* populations from Argentina based on electrophoretic characters. Capital letters denote populations given in Table 1.

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