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Variable expression of isozymes in entomopathogenic nematodes following laboratory recycling

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Summary – Cellulose acetate electrophoresis was used to determine the degree to which isozyme banding patterns differed among four strains of steinernematid nematodes that had been recycled or stored at 5, 10, 15, 20 and 25 °C: *Steinernema feltiae* Umeå strain, *S. carpocapsae* All strain, *S. riobravis* TX strain and *S. feltiae* NF strain. In all four strains, isozyme banding patterns of malate dehydrogenase (MDH), mannose-6-phosphate isomerase (MPI) and phosphoglucomutase (PGM) were affected by recycling or storage temperatures. The NF and Umeå strains of *S. feltiae* synthesized additional isozymes of MPI and MDH or PGM at cold temperatures, while *S. carpocapsae* All strain synthesized three isozymes of MDH at warm temperatures and an additional isozyme of PGM at cold temperatures. The implications of these findings are discussed in relation to temperature adaptation mechanisms involving synthesis of isozymes and the use of isozyme determinations in steinernematid taxonomy. © Elsevier - ORSTOM

Résumé – ***Expression variable des isoenzymes chez les nématodes entomopathogènes en fonction de leur élevage au laboratoire*** – L'électrophorèse sur acétate de cellulose a été utilisée pour déterminer les différences de profils de bandes d'isoenzymes entre quatre souches de nématodes Steinernematides conservées ou élevées à 5, 10, 15, 20 et 25 °C: *Steinernema feltiae* souche Umeå, *S. carpocapsae* souche All, *S. riobravis* souche TX et *S. feltiae* souche NF. Chez les quatre souches, les profils des bandes d'isoenzymes de malate déshydrogénase (MDH), de mannose-6-phosphate isomérase (MPI) et de phosphoglucomutase (PGM) sont affectés par l'élevage et la température de conservation. Les souches NF et Umeå de *S. feltiae* ont synthétisé des isoenzymes supplémentaires de MDI, de MDH et de PGM aux basses températures, tandis que la souche All de *S. carpocapsae* a synthétisé trois isoenzymes de MDH aux températures élevées et un isoenzyme supplémentaire de PGM aux températures basses. Les conséquences de ces résultats sont discutées en relation avec les mécanismes d'adaptation à la température impliquant la synthèse d'isoenzymes et l'utilisation de ces isoenzymes dans la taxinomie des Steinernematides. © Elsevier - ORSTOM

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Entomopathogenic nematodes (fam. Steinernematidae and Heterorhabditidae) are a globally distributed category of nematodes (Hominick *et al.*, 1996) that is being commercially mass produced for use against soil dwelling or otherwise cryptic insect pests (Ehlers, 1996). However, the degree to which repetitive recycling, necessitated by commercialization, may modify the character of strains selected for pest management has not been fully evaluated. The reproduction, development, dispersal and infectivity of entomopathogenic nematodes are known to be affected by environmental temperature (Griffin, 1993; Grewal *et al.*, 1994; Mason & Hominick, 1995; Steiner, 1996). Thus, it seems reasonable to conclude that the temperature of the mass reproduction system could affect the biological and physiological characteristics of these nematodes in a fashion that could influence field efficacy.

We have recently shown that the temperatures at which four strains of steinernematids were passaged through *Galleria mellonella* larvae affected thermal tolerance (Jagdale & Gordon, 1998) and infectivity (Jagdale & Gordon, 1997*b*) adaptively. Nematodes recycled at a cold temperature displayed a better infectivity and capacity for freezing tolerance than those that had been recycled at warmer temperatures. The specific activities of key metabolic enzymes were elevated in cold adapted nematodes (Jagdale & Gordon, 1997*a*), suggesting that compensatory changes in physiology occurred. Teleosts (Hochachka & Somero, 1984; Somero, 1995), echinoderms (Marcus, 1977) and insect parasitoids (Smith & Hubbes, 1986) synthesize isozymes that are functionally suited to specific temperature regimes. However, the degree to which this temperature adaptation strategy is used by nematodes is unknown.

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In a recent study, we suggested that the isozyme distribution patterns of metabolic enzymes may be used as a taxonomic tool for steinernematid nematodes. Cellulose acetate electrophoresis of eight such enzymes in five nematode strains revealed that the isozyme complements were distinctive for each strain (Jagdale *et al.*, 1996). Given the possibility that modification of isozyme synthesis may be temperature-induced, it is important to determine the degree to which isozyme patterns are affected by recycling temperatures. Such information is needed to fully assess the taxonomic merit of isozyme determinations and potentially, to provide insight into mechanisms of thermal adaptation to the recycling regimes.

The purpose of the present investigation was to determine whether there was a change in the isozyme patterns of three key metabolic enzymes extracted from three species (four strains) of *Steinernema*, which had been recycled or stored in the laboratory for prolonged periods of time at various temperatures.

Materials and methods

Sources of nematodes

Steinernema carpocapsae All strain was provided by Plant Products Ltd., Brampton, Ontario, Canada; *S. riobravis* TX strain by Dr H. E. Cabanillas, USDA, ARS, Crop Insects Research Unit, Weslaco, TX., U.S.A. *Steinernema feltiae* Umeå strain was provided by Dr R. West, Canadian Forest Service, St. John's, NF, Canada from a stock colony that had been initially obtained from Biologic Biocontrol Products, Willow Hill, PA., USA. *Steinernema feltiae* NF strain is a new strain (Jagdale *et al.*, 1996) that we isolated in Summer 1994 from soil on a farm site close to St. John's, NF, using *Galleria* bait traps (Woodring & Kaya, 1988).

Recycling/storage temperature regimes

All nematode strains were recycled at different temperature regimes for 2 years (May 1994-May 1996) by propagation through *Galleria mellonella* larvae (Woodring & Kaya, 1988): NF and Umeå strains of *S. feltiae* at 10, 15, 20 and 25 °C; All strain of *S. carpocapsae* at 15, 20 and 25 °C and TX strain of *S. riobravis* at 20 and 25 °C. The numbers of generations perpetuated at the various temperatures were estimated to be 15-18 (10 °C), 21-25 (15 °C), 30-35 (20 °C) and 48-60 (25 °C). In situations where recycling was not possible, the effects of lower temperatures on isozyme profiles were ascertained by storing infective juveniles washed in distilled water (Gordon *et al.*, 1996) in tissue culture bottles (600 ml) for four months (February-May, 1996): *S. feltiae* at 5 °C, *S. carpocapsae* at 5 and 10 °C, *S. riobravis* at 5, 10 and 15 °C. Each tissue culture bottle contained 200-250 ml of nematode suspension (depth *c.* 2 cm): 4000 infective juveniles/ml. Once per week, the distilled water in the bottles was replaced. In each case, the infective juveniles were initially obtained from cultures that had been recycled at the lowest possible temperatures: *S. feltiae* 10 °C, *S. carpocapsae* 15 °C, *S. riobravis* 20 °C.

Cellulose acetate electrophoresis

The procedures for enzyme extraction, cellulose acetate electrophoresis and enzyme staining were as previously described (Jagdale *et al.*, 1996). The enzymes were extracted from newly harvested infective juveniles of each of the four strains that had been recycled or stored at various temperature regimes. Electrophoresis was conducted on fresh infective juveniles, *i.e.*, they had not been stored or frozen. Samples of each strain (40 mg wet weight) were transferred into separate polypropylene microcentrifuge tubes (1.5 ml), then macerated in the tubes with a motor-driven pellet pestle(R) (Baxter Diagnostic Corporation, Canlab Division, Mississauga, ON, Canada). Three separate homogenates were prepared for each strain. After maceration, 50 ml of distilled water containing bromophenol blue tracking dye (pH 6.0) was added to each tube, the homogenates centrifuged at 3200 *g* for 2 min, then held on ice. Aliquots (10 ml) of the supernatants were transferred from the tubes to separate wells in the sample holder and kept on ice until the loading of the samples was performed on the cellulose acetate plates (Helena Laboratories, Beaumont, TX, USA).

Aliquots (0.6 ml) of the supernatants were transferred from the sample holder to Titan III Zip Zone Cellulose Acetate Plates using a Super Z Applicator (Helena Laboratories). The protein content of newly hatched infective juveniles had already been determined (Jagdale & Gordon, 1997*c*). Based on these values, we estimate that the amounts of the protein loaded onto the plates were: *S. riobravis* TX strain (8 mg), *S. feltiae* Umeå strain (10 mg), *S. carpocapsae* All strain (14 mg) and *S. feltiae* NF strain (30 mg). The amounts of protein loaded onto the plates was constant for each strain, regardless of recycling or storage temperatures. The plates had been pre-soaked (20 min) in Tris-Glycine buffer (3.0 g Tris, 14.4 g glycine, 1 L distilled water, pH 8.5) prior to spotting. Electrophoresis was carried out (2 mA/ plate; 20-30 min) in Tris-Glycine buffer in a horizontal electrophoresis chamber at room temperature (20-25 °C).

Specific stains were used to visualize the following enzymes: glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), malate dehydrogenase (EC 1.1.1.37), mannose-6-phosphate isomerase (EC 5.3.1.8) and phosphoglucomutase (EC 2.7.5.1) (Hebert & Beaton, 1993).

Three replicate plates were prepared for each nematode strain that had been recycled or stored at a specified temperature. Each replicate represented a separate infective juvenile homogenate. The relative electrophoretic mobility (μ) for each isozyme was measured to compare the migration rates (Lehninger, 1979) and isozyme bands among temperature regimes were considered the same if their μ values were within 10 % of one another. This margin of error was selected because the highest and lowest μ values among three replicates of the same isozyme were always found to be within 10 % of one another (Jagdale *et al.*, 1996).

Results

The recycling temperature affected the distribution of isozymes in all enzymes except glycerol-3-phosphate dehydrogenase. In all nematode strains, isozyme banding patterns were consistent for all three replicates of each enzyme at each specified temperature.

GLYCEROL-3-PHOSPHATE DEHYDROGENASE (G3PDH)

Isozymes of this enzyme were not synthesized in response to different recycling temperatures. Each strain that had been recycled or stored at 5, 10, 15, 20 and 25 °C possessed a single band of G3PDH activity that migrated toward the cathode (Fig. 1 A-D; Table 1).

MANNOSE-6-PHOSPHATE ISOMERASE (MPI)

S. carpocapsae All strain and *S. riobravis* TX strain possessed only two cathodal isozyme bands which were common to all temperature regimes (Table 2). In the NF and Umeå strains of *S. feltiae*, the isozyme profile of this enzyme differed among nematodes that had been recycled at the various temperatures. The NF strain of *S. feltiae* possessed six cathodal bands of MPI activity at 5 °C, four bands at 20 °C and only three bands at 10, 15, 20, and 25 °C. These three isozymes were common to all temperature regimes. Thus, three additional isozymes were synthesized at 5 °C, one of which was also synthesized at 20 °C (Fig. 1 E; Table 2). The Umeå strain of *S. feltiae* possessed three common isozyme bands at 5, 10, 15 and 20 °C. At 25 °C, only two of these isozymes were synthesized (Fig. 1 F).

PHOSPHOGLUCOMUTASE (PGM)

S. feltiae Umeå strain and *S. riobravis* TX strain possessed two cathodal bands regardless of the temperature regime at which they were recycled (Table 3). However, in the All strain of *S. carpocapsae* and NF strain of *S. feltiae*, the isozymes of this enzyme differed among nematodes that had been recycled at the various temperatures. *S. carpocapsae* All strain, recycled at 20 and 25 °C, possessed three isozymes. At lower temperatures (5, 10, 15 °C), an additional isozyme (the same one at all three temperatures) was synthesized (Fig. 2 A). *S. feltiae* NF strain possessed four isozymes of PGM common to all recycling and storage temperatures. However, one additional isozyme was synthesized by nematodes recycled at 10 °C or stored at 5 °C (Fig. 2 B). The additional isozyme synthesized at 5 °C had a different electrophoretic mobility from the one synthesized at 10 °C (Table 3).

MALATE DEHYDROGENASE (MDH)

In all four strains, the isozyme distribution patterns of MDH differed among nematodes that had been recycled at the various temperatures. *S. carpocapsae* All strain, recycled at 25 °C, possessed four isozymes, whereas only one such isozyme was produced at the other temperatures (Fig. 2 C; Table 4). The NF strain of *S. feltiae* possessed two (5; 25 °C), three (10; 15 °C), or four (20 °C) cathodal bands of MDH activity, according to storage or recycling temperature; one of these isozymes was common to all temperature regimes (Fig. 2 D; Table 4). The Umeå strain of *S. feltiae* possessed two common isozymes when recycled at 5, 10 and 15 °C, whereas only one isozyme was present when recycled at higher temperature regimes (20, 25 °C) (Fig. 2 E). *S. riobravis,* TX strain recycled at 25 °C had one isozyme, two bands of MDH activity at 5 and 20 °C and three isozymes at 10 and 15 °C (Fig. 2 F; Table 4).

Discussion

The steinernematid strains responded differentially to recycling temperature with respect to synthesis of isozymes. Isozyme patterns of MDH, MPI and PGM changed according to recycling temperatures in four (All, NF, Umeå and TX), two (NF and Umeå) and two (All and NF) strains of steinernematid nematodes, respectively. *Steinernema feltiae* NF strain displayed changes in isozyme synthesis at the specified recycling temperatures for three enzymes (MDH, MPI, PGM), *S. feltiae* Umeå strain for two enzymes (MDH, MPI), *S. carpocapsae* All strain for two enzymes (MDH, PGM) and *S. riobravis* TX strain for one enzyme (MDH). None of the strains showed modifications in the synthesis of isozymes of the enzyme G3PDH resulting from recycling at the various temperatures.

It is tempting to conclude that the changes in isozyme distribution patterns resulting from recycling at different temperatures are adaptive in nature. The production of isozymes, with kinetic properties suited to the ambient temperature, is regarded as an important component of the seasonal temperature adaptation mechanism in several other groups of poi-

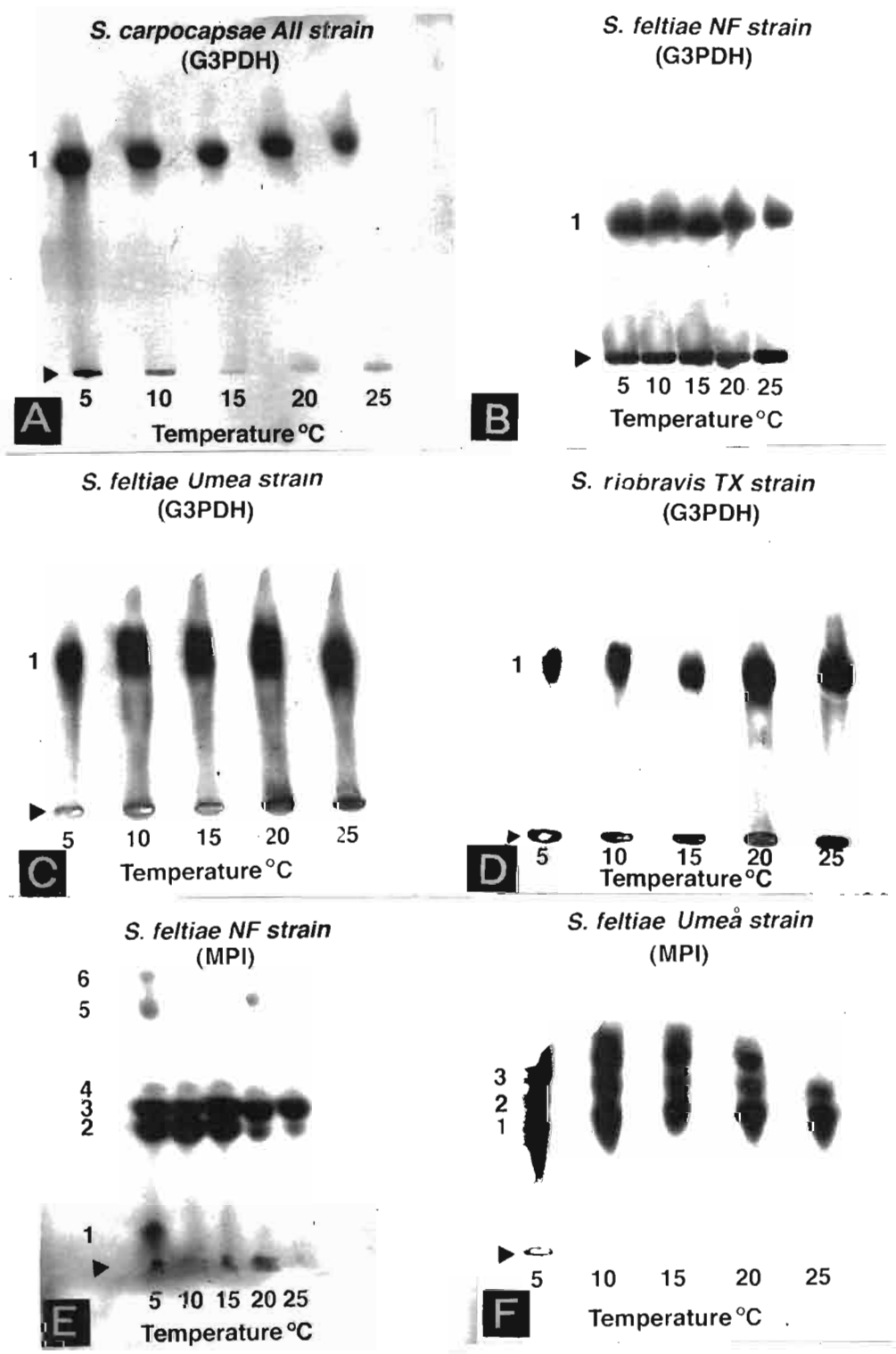

Fig. 1. *Electrophorograms of two enzymes in three species (four isolates) of* Steinernema *recycled/stored at five different temperature regimes. A-D: G3PDH = Glycerol-3-phosphate dehydrogenase; E-F: MPI = Mannose-6-phosphate isomerase. Bands were numbered in increasing numerical order relative to the distance that they migrated cathodally from the origin. Arrow heads indicate points of sample application. In situations (1A, 1E, 1F) where uneven staining intensity resulted at the origin among treatments, this was caused by unequal removal of sample after electrophoresis, during the washing procedure and prior to fixing.*

Table 1. *Mean electrophoretic mobility ($cm^2/s/v$) of isozymes of glycerol-3-phosphate dehydrogenase in four strains of steinernematid nematodes recycled and stored at five temperature regimes.*

No of bands*	Temperature regime (°C)				
	5	10	15	20	25
S. carpocapsae All strain					
1	2.39×10^{-4}	2.26×10^{-4}	2.48×10^{-4}	2.46×10^{-4}	2.46×10^{-4}
S. feltiae NF strain					
1	1.93×10^{-4}	1.91×10^{-4}	1.89×10^{-4}	1.96×10^{-4}	1.93×10^{-4}
S. feltiae Umeå strain					
1	1.98×10^{-4}	2.15×10^{-4}	2.16×10^{-4}	2.15×10^{-4}	2.15×10^{-4}
S. riobravis TX strain					
1	2.35×10^{-4}	2.35×10^{-4}	2.38×10^{-4}	2.38×10^{-4}	2.38×10^{-4}

* For each enzyme of each strain, bands were numbered in increasing numerical order relative to the distance that they migrated cathodally from the origin; they do not necessarily correspond numerically among the strains. Values are the means of three replicate plates, each replicate representing a separate homogenate of infective juveniles.

Table 2. *Mean electrophoretic mobility ($cm^2/s/v$) of isozymes of mannose-6-phosphate isomerase in four strains of steinernematid nematodes recycled and stored at five temperature regimes.*

No of bands*	Temperature regime (°C)				
	5	10	15	20	25
S. carpocapsae All strain					
1	1.54×10^{-4}	1.62×10^{-4}	1.62×10^{-4}	1.65×10^{-4}	1.65×10^{-4}
2	1.83×10^{-4}	1.98×10^{-4}	2.05×10^{-4}	2.05×10^{-4}	2.05×10^{-4}
S. feltiae NF strain					
1	4.59×10^{-5}	-	-	-	-
2	1.64×10^{-4}	1.64×10^{-4}	1.64×10^{-4}	1.64×10^{-4}	1.65×10^{-4}
3	1.90×10^{-4}	1.90×10^{-4}	1.92×10^{-4}	1.88×10^{-4}	1.88×10^{-4}
4	2.06×10^{-4}	2.06×10^{-4}	2.06×10^{-4}	2.04×10^{-4}	2.07×10^{-4}
5	2.84×10^{-4}	-	-	3.04×10^{-4}	-
6	3.51×10^{-4}	-	-	-	-
S. feltiae Umeå strain					
1	1.22×10^{-4}	1.22×10^{-4}	1.24×10^{-4}	1.26×10^{-4}	1.28×10^{-4}
2	1.51×10^{-4}	1.43×10^{-4}	1.50×10^{-4}	1.58×10^{-4}	1.61×10^{-4}
3	1.79×10^{-4}	1.83×10^{-4}	1.87×10^{-4}	1.87×10^{-4}	-
S. riobravis TX strain					
1	1.85×10^{-4}	2.04×10^{-4}	2.00×10^{-4}	1.90×10^{-4}	1.93×10^{-4}
2	2.14×10^{-4}	2.28×10^{-4}	2.24×10^{-4}	2.18×10^{-4}	2.20×10^{-4}

* For each enzyme of each strain, bands were numbered in increasing numerical order relative to the distance that they migrated cathodally from the origin; they do not necessarily correspond numerically among the strains. Dashes indicate absence of bands. Values are the means of three replicate plates, each replicate representing a separate homogenate of infective juveniles.

kilotherms (Marcus, 1977; Hochachka, & Somero, 1984; Smith & Hubbes, 1986; Somero, 1995). In such studies, animals were maintained for relatively short periods of time (usually several weeks) at various temperature regimes and isozyme determinations conducted on the same organisms as were initially set

Table 3. *Mean electrophoretic mobility ($cm^2/s/v$) of isozymes of phosphoglucomutase in four strains of steinernematid nematodes recycled and stored at five temperature regimes.*

No of bands*	Temperature regime (°C)				
	5	10	15	20	25
S. carpocapsae All strain					
1	8.61×10^{-5}	8.81×10^{-5}	9.01×10^{-5}	8.81×10^{-5}	8.81×10^{-5}
2	1.09×10^{-4}	1.15×10^{-4}	1.13×10^{-4}	-	-
3	2.93×10^{-4}	2.93×10^{-4}	2.87×10^{-4}	2.93×10^{-4}	2.95×10^{-4}
4	3.11×10^{-4}	3.11×10^{-4}	3.09×10^{-4}	3.15×10^{-4}	3.13×10^{-4}
S. feltiae NF strain					
1	2.43×10^{-5}	2.43×10^{-5}	2.43×10^{-5}	2.43×10^{-5}	2.43×10^{-5}
2	4.40×10^{-5}	4.46×10^{-5}	4.67×10^{-5}	4.67×10^{-5}	4.67×10^{-5}
3	6.37×10^{-5}	6.37×10^{-5}	6.23×10^{-5}	6.23×10^{-5}	6.23×10^{-5}
4	-	2.06×10^{-4}	-	-	-
5	2.39×10^{-4}	2.31×10^{-4}	2.31×10^{-4}	2.31×10^{-4}	2.31×10^{-4}
6	3.03×10^{-4}	-	-	-	-
S. feltiae Umeå strain					
1	2.60×10^{-4}	2.60×10^{-4}	2.60×10^{-4}	2.60×10^{-4}	2.60×10^{-4}
2	2.73×10^{-4}	2.73×10^{-4}	2.73×10^{-4}	2.73×10^{-4}	2.73×10^{-4}
S. riobravis TX strain					
1	1.08×10^{-4}	1.06×10^{-4}	1.04×10^{-4}	1.10×10^{-4}	1.08×10^{-4}
2	2.22×10^{-4}	2.30×10^{-4}	2.28×10^{-4}	2.28×10^{-4}	2.34×10^{-4}

* For each enzyme of each strain, bands were numbered in increasing numerical order relative to the distance that they migrated cathodally from the origin; they do not necessarily correspond numerically among the strains. Dashes indicate absence of bands. Values are the means of three replicate plates, each replicate representing a separate homogenate of infective juveniles.

up. Accordingly, changes in isozyme profile were attributed to environmentally - induced thermal acclimation. However, in the present study, the nematodes were continuously recycled over a prolonged time frame of 2 years.

Several trends in the data are suggestive of genetically - based artificial selection or purely environmentally - induced changes in isozyme distribution patterns adaptive to the various temperature regimes. For example, it is possible that *S. carpocapsae* All strain synthesized three additional isozymes of MDH at 25 °C as an adaptation to warm temperature and one additional isozyme of PGM at temperatures ≤ 15 °C as an adaptation to colder temperatures. *S. feltiae* NF strain may have synthesized three additional isozymes of MPI at 5 °C and one additional isozyme of PGM at 10 and 5 °C (not the same isozyme at these two temperatures) as adaptations to cold temperature. *S. feltiae* Umeå strain could have synthesized one additional isozyme of MPI at temperatures ≤ 20 °C and one additional isozyme of MDH at temperatures ≤ 15 °C to adapt to colder temperatures.

However, interpretation of other temperature related data is less clear. *S. riobravis* TX strain yielded zymograms for MDH that were identical only at 10 and 15 °C; at temperatures above and below this range, there was a decrease in the number of isozymes. Also with respect to MDH, *S. feltiae* NF strain possessed an isozyme distribution pattern that displayed no observable trend, except that it was the same only for nematodes recycled at 10 and 15 °C. Moreover, comparisons of isozyme distribution patterns of MPI and PGM in nematodes that had been recycled at 25 °C with those of an earlier study, conducted 12 months previously, revealed that changes in isozyme profile had occurred as a consequence of the 24-30 nematode cycles that the nematodes had undergone during the time interval separating the two studies. Only two bands were previously displayed for MPI in *S. feltiae* NF strain (Jagdale *et al.*, 1996), equivalent to bands 3 and 4, compared with three bands in the present study. Similarly, in the previous study, only two bands were apparent for PGM in the NF strain of *S. feltiae* (Jagdale *et al.*, 1996), compared with four bands in the present

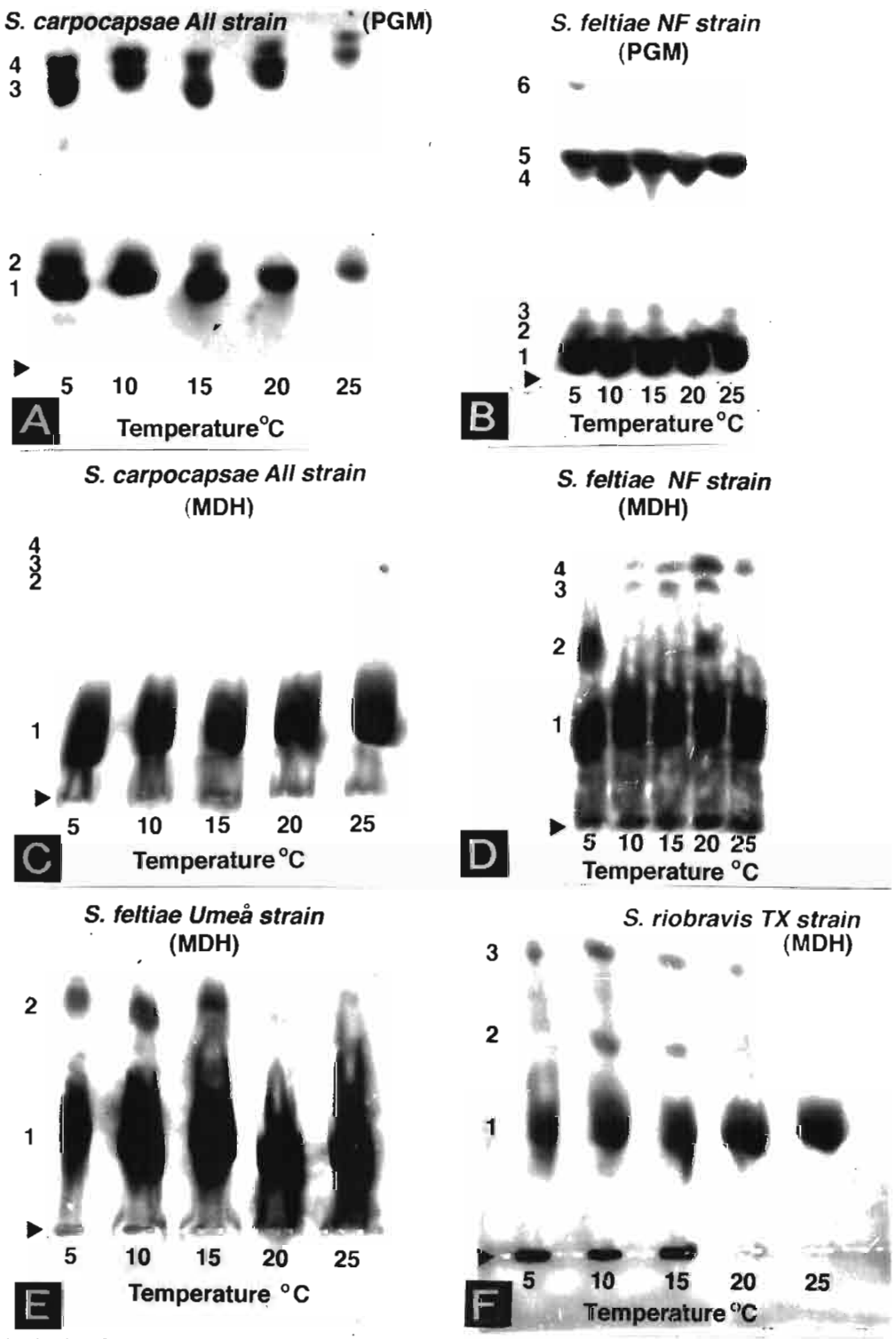

Fig. 2. *Electrophorograms of two enzymes in three species (four isolates) of* Steinernema *recycled/stored at five different temperature regimes. A-B: PGM = Phosphoglucomutase; C-F: MDH = Malate dehydrogenase. Bands were numbered in increasing numerical order relative to the distance that they migrated cathodally from the origin. Arrow heads indicate points of sample application. In situations (2C, 2F) where an uneven staining intensity resulted at the origin among treatments, this was caused by unequal removal of sample after electrophoresis, during the washing procedure and prior to fixing.*

Table 4. *Mean electrophoretic mobility ($cm^2/s/v$) of isozymes of malate dehydrogenase in four strains of steinernematid nematodes recycled and stored at five temperature regimes.*

No of bands*	Temperature regime (°C)				
	5	10	15	20	25
S. carpocapsae All strain					
1	7.45×10^{-5}	7.45×10^{-5}	7.45×10^{-5}	7.45×10^{-5}	7.45×10^{-5}
2	-	-	-	-	2.67×10^{-4}
3	-	-	-	-	2.82×10^{-4}
4	-	-	-	-	3.04×10^{-4}
S. feltiae NF strain					
1	1.22×10^{-4}	1.30×10^{-4}	1.30×10^{-4}	1.32×10^{-4}	1.32×10^{-4}
2	1.67×10^{-4}	-	-	1.67×10^{-4}	-
3	-	2.30×10^{-4}	2.32×10^{-4}	2.41×10^{-4}	-
4	-	2.76×10^{-4}	2.76×10^{-4}	2.79×10^{-4}	2.79×10^{-4}
S. feltiae Umeå strain					
1	1.56×10^{-4}	1.56×10^{-4}	1.58×10^{-4}	1.58×10^{-4}	1.58×10^{-4}
2	3.21×10^{-4}	2.92×10^{-4}	2.92×10^{-4}	-	-
S. riobravis TX strain					
1	1.33×10^{-4}	1.39×10^{-4}	1.33×10^{-4}	1.29×10^{-4}	1.37×10^{-4}
2	-	2.33×10^{-4}	2.33×10^{-4}	-	-
3	2.67×10^{-4}	2.71×10^{-4}	2.58×10^{-4}	2.44×10^{-4}	-

* For each enzyme of each isolate, bands were numbered in increasing numerical order relative to the distance that they migrated cathodally from the origin; they do not necessarily correspond numerically among the strains. Dashes indicate absence of bands. Values are the means of three replicate plates, each replicate representing a separate homogenate of infective juveniles.

study. Four bands were previously displayed for PGM in the Umeå strain of *S. feltiae*, whereas only two bands were shown in this study. Such temporal instability of isozyme banding patterns at a fixed temperature indicates that it is not possible to interpret with certainty the differences in isozymes at the various recycling temperatures as being of a thermally adaptive nature. It is possible that inadvertent genetic selection, resulting in changes unrelated to thermal adaptation, may have occurred within the isolated nematode colonies and at least some of the changes in isozyme banding patterns exhibited at the various recycling temperatures may have such a basis. Further studies are needed to clarify the role of the recycling temperature as the primary stimulus for inducing changes in isozyme complement and the significance of the changes with respect to thermal adaption.

In contrast to the changes resulting from recycling, the synthesis of new isozymes of MPI and PGM by *S. feltiae* NF strain as well as loss of MDH isozyme synthetic capacity by *S. riobravis* at 5 °C cannot be ascribed to genetic changes, because the nematodes were stored but not recycled at this temperature. Whether such changes are due to thermal acclimation or are a physiological consequence of ageing of the infective juveniles is not known with certainty. However, we have established that the infective juveniles display physiological acclimation to cold temperature storage by increasing the degree of saturation of their fatty acids (Jagdale & Gordon, 1997*c*), so it seems likely that the observed changes in isozyme complement constitute part of the overall compensatory mechanism.

The fact that isozyme banding patterns of three of the four enzymes were shown to be affected by recycling temperatures in all four nematode strains and that, independent of temperature, two of the enzymes displayed altered isozyme profiles over time in two of the strains, suggests that the use of cellulose acetate electrophoresis in steinernematid taxonomy should be cautiously undertaken. To minimize the effects of laboratory or commercial recycling, isozyme profiles should be obtained and evaluated for nematodes that are in a condition comparable to those that have been newly isolated from the soil, a requirement that underscores the need to compile an inventory of cryopreserved entomopathogenic nematodes (Akhurst, 1995). The role of isozyme banding patterns in taxonomic assignments may best be considered as confirmatory, rather than diagnostic.

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