An analysis of the role of polar (phospholipid) membranes, polyunsaturated fatty acids and sterols in the thermo adaptation of entomopathogenic nematode (*Steinernema*) species

Erika Virágh[1], Elfridea Fodor[1], David J. Chitwood[3], Tibor Farkas[1] and András Fodor[2]

[1]*Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary;*
[2]*Department of Genetics, Eötvös University, Budapest, Hungary,*
[3]*Nematology Unit, USDA ARS, Beltsville, MD, USA*
fodorandras@yahoo.com

SUMMARY

As in other organisms, the survival of entomopathogenic nematodes depends on the ability of membrane fluidity to adjust appropriately to changes in environmental temperature. Therefore, we performed a series of investigations examining the chemical composition and specific biophysical parameters of the phospholipids from numerous *Steinernema* isolates from different geographical origins, including *Steinernema* species of long dauer phenotype. In some cases, the nematodes were cultured at cold (18 ºC) and warm (25 ºC) temperatures. Interestingly, the phospholipids of steinernematids of the long dauer phenotype contained higher proportions of saturated fatty acids and lower proportions of polyunsaturated acids than did phospholipids from *S. carpocapsae* and *S. feltiae*. The differences were reflected in the saturated/unsaturated acid ratios among the isolates. When four nematode strains of different geographic origins belonging to *S. feltiae* and *S. carpocapsae* were propagated at low and warm temperatures, the warm climate strains increased the proportion of the eicosapentaenoic acid (EPA) more than the boreal ones. This result may indicate that strains inhabiting colder regions do not require as much EPA to maintain structural and functional integrity of their membranes as do strains from warmer areas when grown at lower temperature. In two different measures of membrane fluidity—Fourier transform infrared spectroscopy and fluorescence depolarization—membranes prepared from phospholipids form the boreal nematode *S. feltiae* VIJE (from Norway) were more fluid than those from *S. feltiae* IS6 (from Israel), regardless of the culture temperature of the nematodes. Membrane fluidity in *Steinernema* may be further modified by the observed differences in membrane cholesterol and other sterols.

INTRODUCTION

In entomopathogenic nematodes, similarly to other organisms exposed to changing environmental conditions, the maintenance of the functional integrity and proper physicochemical properties of biological membranes is an essential factor for survival (Fig. 1).

Fig. 1. Ecotype related response to temperature of phospholipids in entomopathogenic nematodes

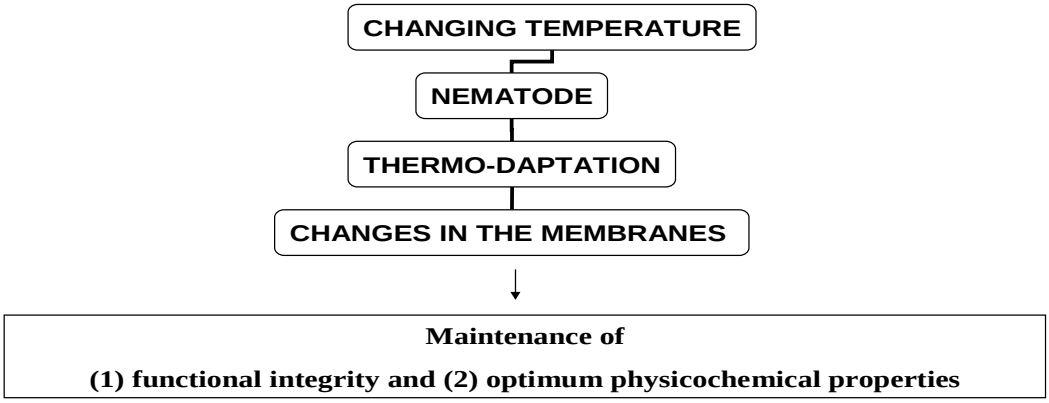

Fig 1 A Diagram indicating the role of cell membranes in maintaining functional integrity and proper physicochemical properties in temperature shift in entomopathogenic nematodes (EPN)

Cell membranes are amongst the cellular structures which are most sensitive to changes in temperature. In poikilotherm organisms, the ability to adjust membrane fluidity to the prevailing temperature determines activity, developmental stage as well as geographical location. Changes in membrane fluidity usually result from changes in the structure of membrane lipids, which form the structural backbone of membranes. The major components of membrane lipids are summarized in Figure 2.

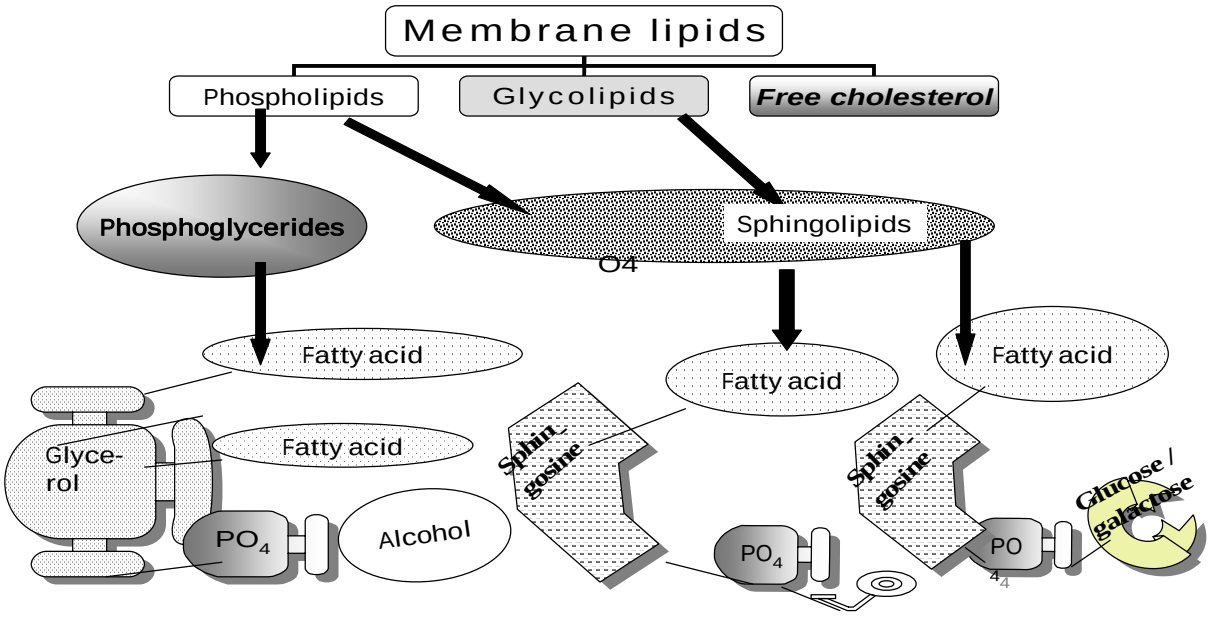

Fig 2 Phospholipids play a key role in thermo adaptation because of the nature of individual phospholipid molecules, wherein glycerol or sphingosine is esterified by two fatty acids and a phosphoric acid. The latter is esterified by an alcohol and together they form the polar head group of the molecule. The apolar side comprises two fatty acids. (There is also another fatty acid attached in an amide linkage to the sphingosine, not shown.)

A detailed schematic structure of a typical phospholipid molecule is presented in Figure. 3.

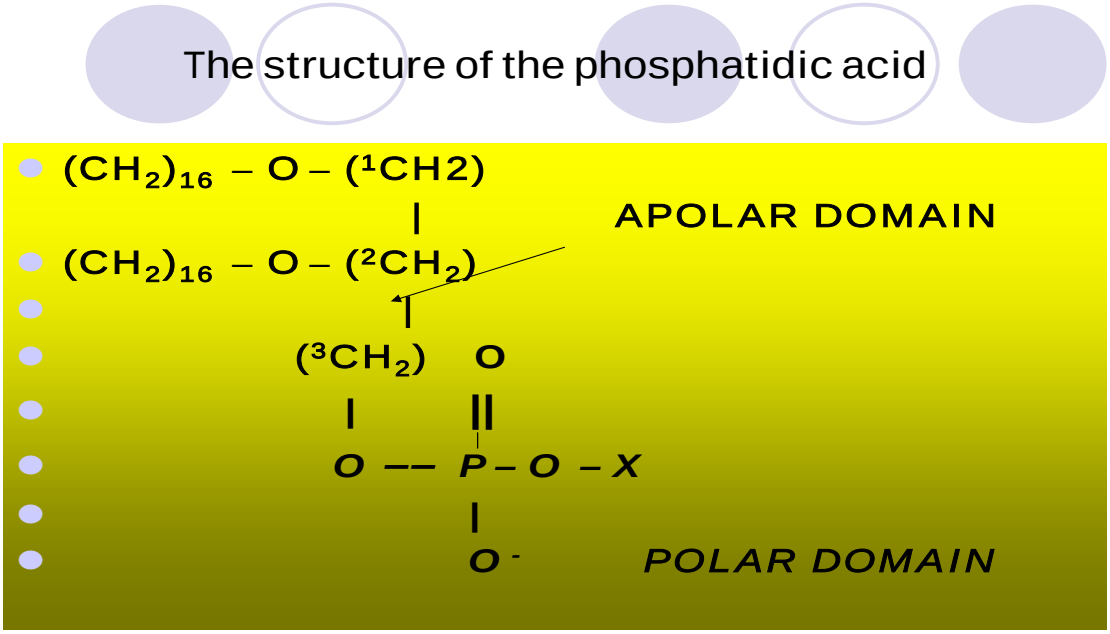

Fig 3 the detailed structure of a typical phosphoglycerid molecule, phosphatidic acid. Like all phospholipids, it contains a polar and an apolar domain. The apolar domain comprises two fatty acids. "X" represents a hydrogen atom, which is replaced by an alcohol in most other phospholipids.

It is important to note that the length and degree of saturation of the fatty acids determines the physical properties of the molecule and in turn the fluidity of the membrane itself. (Fig 4) To create a highly fluid membrane, polyunsaturated fatty acids (PUFA) are necessary.

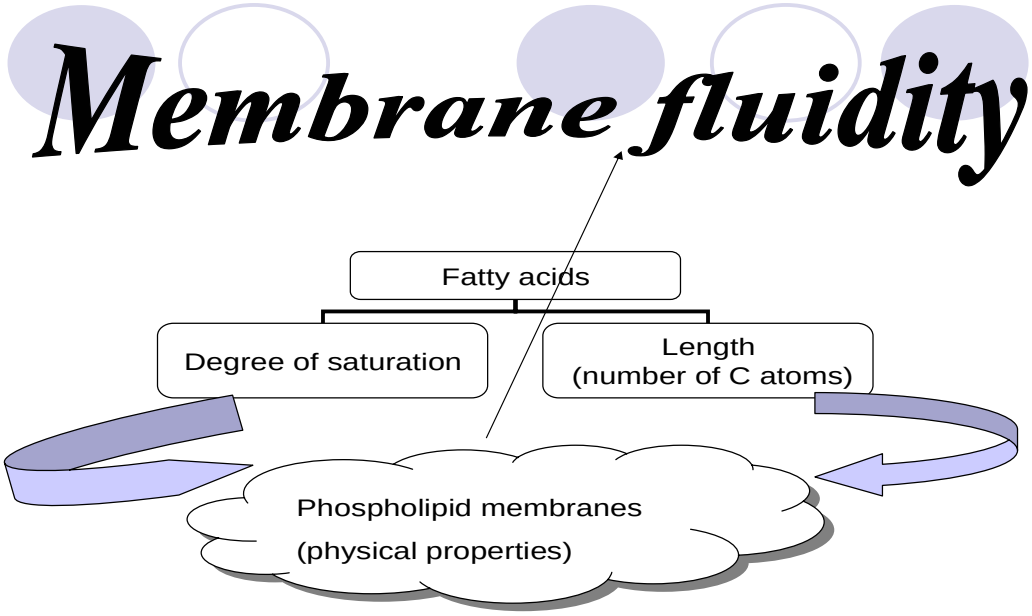

Fig 4 The degree of saturation as well as the number of carbon atoms of the fatty acids determine the physical properties of the phospholipid molecule and ultimately the membrane fluidity.

Several lower organisms have been reported to increase the number of double bonds in order to adapt to decreasing temperature, (Bell et al., 2001; Dey et al., 1993, Farkas et. al, 1994, Lahdes et al., 2000, Bell et al., 2001), including nematode species (*Caenorhabditis elegans, Turbatrix aceti*, and *Steinernema carpocapsae* (Fodor et al., 1994).

In this study, we compared the fatty acid composition and membrane fluidity of different entomopathogenic nematode isolates of the genus *Steinernema* species from different locations of different climate. We consider them as strains evolutionary adapted to different (cold and warm) temperatures. This is not a complete analysis of all available *Steinernema* strains, but the results may answer the questions of (i) whether differences among species are more significant than intraspecific variations and (ii) whether the differences between strains evolutionarily adapted to different environmental temperatures predominate. Other aims were to determine if all species of the *Steinernema* genus are capable of adapting to different ambient temperatures by altering the fatty acid composition of their membranes and, if so, which mechanisms the nematodes switch and adjust to alter their membrane structure in extreme environmental conditions.

MATERIAL AND METHODS

Nematodes: the list of the name, origin and location of the EPN strains we worked on are presented in Table 1.

Table 1 *Steinernema* species /strains adapted to warm and cold climate

Steinernema species	Strain	Geographic location	Provided by
Steinernema glaseri	**NC I**	**North Carolina, USA**	**Michael G. Klein**
	Kmd 15	**Ohio**	**Attila Lucskai**
	NC 513	**North Carolina, USA**	**Attila Lucskai**
	AZ26	**Portugal Azores**	**Nelson Simoes**
Steinernema feltiae	**VIJE**	**Norway**	**?**
	SF22	**Finland**	**Aana Vainio**
	IS6	**Israel**	**Itamar Glazer**
Steinernema carpocapsae	**All**	**Northern USA**	**Ramon Georgis**
	Mexicana	**Mexico**	**Jim Lindegreen**
Steinernema arenarium **(strains of long dauer phenotype)**	**Anomali Biosys**	**Russia via Biosys**	**Ramon Georgis**
	Anomali AZ	**Russia via N. Simoes**	**Nelson Simoes**
	GEL	**Russia**	**Sergei Spiridonov**
	Russian	**Russia via Z. Mracek**	**Zdenek Mracek**
Taxonomically unidentified steinernematids of long dauer phenotype	**"Polish"**	**Poland**	**Marek Tomalak**
	"Italy"	**Italy**	**Oreste**
	"Morocco"	**Morocco**	**Ralf-Udo Ehlers**

The taxonomic position of the *Steinernema* genus was determined by Blaxter et al. (2000). The strains Kmd15, (Triga et al., 2000; Pamjav et al, 2000), AZ26, NC513, NCI belong to the same phylogenetic species (Adams, 1998) *S. glaseri* **(**Steiner, G. 1929). They can be crossed and produce fertile progeny (Arvinbayar Bataar et al., in this issue), but NC513 is probably not the original isolate described by Curran (1989). All strains of *S. feltiae* (Filipjev, 1934) can be crossed freely and progeny are fertile.

Culturing of the nematodes: Nematodes were cultured routinely on caterpillars of the wax moth *Galleria mellonella* and were stored in autoclaved "tap water conserves" at 15 ^{o}C. For experimental purposes, nematodes were cultured in last-instar larvae of *G. mellonella* both at low (18 ^{o}C) and high (25 ^{o}C) temperatures. For biochemical analyses, freshly harvested infective dauer juveniles were centrifuged or vacuum filtered, weighed and then stored at –70 ^{o}C in as small an amount of water as possible.

Analysis of fatty acids: For the phospholipid analysis we extracted the lipids from the samples with chloroform: methanol mix (2:1) (Folch et al.). Phospholipids were separated from the total lipid extract by column chromatography on silicic acid. Chloroform was used to elute neutral lipids and methanol was employed to elute the polar lipids. Polar lipids were trans-esterified in the presence of absolute methanol containing 5% HCl under CO_2 at 80 ^{0}C for 2.5 hours. Methyl esters were separated by gas –liquid chromatography using 30 m x 0.32 x 0.25µm column (NUKOL^{TM} Supelco) and Hewlett Packard instrument. Peaks were identified with the aid of secondary standard (Supelco, Bellefonte PA, Cat. No 4-7885).

Determination of fluidity of phospholipid vesicles: Fourier transformed infrared spectroscopy (Ferrearo and Basile, 1982, Mantsch et al., 1983, Casal and Mantsch, 1984) was used to

determine the conformational state of fatty acyl chains of phospholipid vesicles obtained from nematodes grown at the two temperatures.0.5 mg of total phospholipid hydrated in D20 was used in all measurements. The suspension was placed between standard CaF2 spectroscopic windows (Wilmad Glass Co., Inc., USA) using a Teflon spacer of 25 μm. Spectra were recorded with a Philips PU9800 spectrometer at 2 cm-1 spectral resolution and adding 128 interferograms. Measurements were performed in a heating cycle between 5 and 65 ^{o}C in a 3 degree step. At each temperature a background spectrum was collected and subtracted from the sample spectrum. A linear baseline was subtracted from the spectral region corresponding to the entire range of carbon-hydrogen starching vibration and the spectra were fitted with Lorentzian component bands. The shift in the position of the CH_2 symmetric vibration scan was followed for each sample.

Another (alternative) method utilized to determine membrane fluidity was fluorescence anisotropy. We added 2 μl of 2 mM 1,6-diphenyl-1,3,5-hexatriene (DPH; Molecular Probes) in tetrahydrofurane to phospholipid vesicles (phospholipid:DPH, 1,000:1 vol/vol), which were then dried on the wall of a glass test tube under high vacuum. Multilamellar vesicles were prepared by rehydrating the lipid film by vigorous vortexing in 3 ml of 20 mM Tris·HCl (pH 7.4). The Steady-state anisotropy parameter was measured with a T format fluorescence spectrometer (Quanta Master QM-1, Photon Technology International, Princeton) as $R_{ss} = (R_V/R_H - 1)/(R_V/R_H + 2)$, where R_V and R_H represent the ratios of intensities detected in the two emission channels with the excitation polarizer in vertical and horizontal positions, respectively. Excitation and emission wavelengths were 360 and 430 nm, respectively. Sample temperatures were controlled by a circulating water bath and were measured directly in the cuvettes with a platinum electrode. Measurements were obtained between 5 and 40 °C with a heating rate of 0.4 °C/min.

RESULTS

Figure 5 shows the fatty acids found in all EPN strains so far.

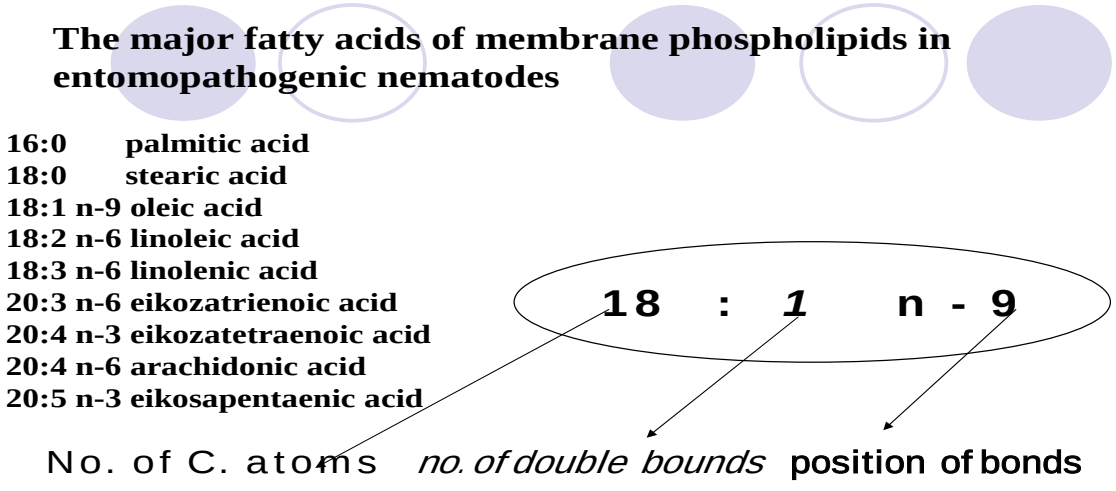

Fig 5 The major fatty acids (names, number of carbon atoms and degree of saturation) found in phospholipid membranes of the EPN strains studied so far.

Figure 6 depicts the relative amounts of fatty acids found in the polar membranes of the EPN strains we studied. The first interesting finding was that the phospholipid membranes of steinernematids of the long dauer phenotype contain significantly higher amounts of saturated (16:0, 18:0) fatty acids than *S. carpocapsae* and *S. feltiae*. In the latter, there is more polyunsaturated fatty acid (20:5 n-3, 18:2 n-6). Table 2 contains unsaturated/saturated fatty acid ratios found for the phospholipid membranes of the different EPN strains.

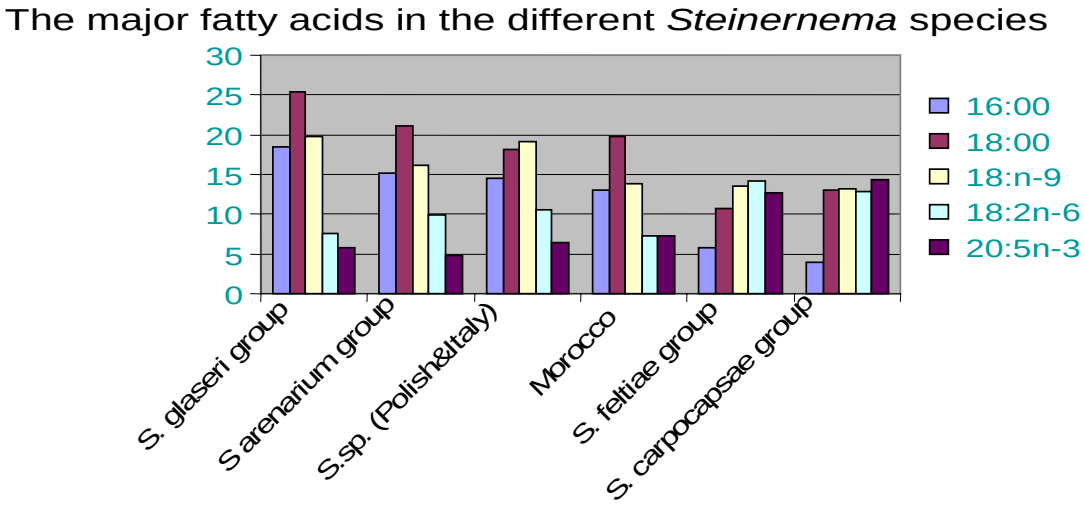

Fig 6. Quantitative data of the amount of different fatty acids in the phospholipide membranes o EPN strains studied.

Table 2 Saturated/unsaturated fatty acid ratio (S/U) in *Steinernema* species at reared at 25 °C

***Steinernema* species**	**strain**	**Saturated (S)**	**Unsaturated (U)**	**S/U**
***Steinernema feltiae* (short dauer phenotype)**	**VIJE**	**22.74**	**62.57**	0.36
	SF22	**29.34**	**63.96**	0.46
	IS6	**28.17**	**60.32**	0.47
***Steinernema carpocapsae* (short dauer phenotype)**	**All**	**29.53**	**61.95**	0.48
	Mexicana	**32.44**	**64.99**	0.50
***Steinernema glaseri* (EPN species of long dauer phenotype)**	**NC I**	**48.83**	**45.22**	**1.07**
	Kmd 15	**60.13**	**35.32**	**1.70**
	NC 513	**40.90**	**53.98**	**0.75**
	AZ26	**41.21**	**41.23**	**0.99**
***Steinernema arenarium* (strains of long dauer phenotype)**	**Anomali Biosys**	**39.61**	**43.72**	**0.90**
	Anomali AZ	**49.17**	**28.13**	**1.74**
	GEL	**39.84**	**33.19**	**1.20**
	Russian	**36.13**	**41.78**	**0.86**
Taxonomically unidentified steinernematids of long dauer phenotype	**“Polish”**	**33.76**	**48.37**	**0.69**
	“Italy”	**46.16**	**38.99**	**1.18**
	“Morocco”	**36.17**	**35.94**	**1.01**

Table 2 The relative amounts of saturated (S) and unsaturated (U) fatty acids as well as their ratios (S/U) for phospholipid membranes from different EPN strains. Although there are many differences between strains, even for those belonging to the same species and origin (e.g., Russian, Gel and the two Anomali strains of *S. arenarium*), the nematode species short of dauer phenotypes (*S. feltiae, S, carpocapsae*) have significantly lower S/U values. The abundance of unsaturated fatty acids in the membranes of the latter suggests a plausible explanation of the fact that each strain of these species is capable of changing the S/U ratio and the fluidity of their membrane in response to ambient temperature (Fodor et al., 1994).

Our results show that in the EPN species *S. carpocapsae* and *S. feltiae*, eicosapentaenoic (EPA) (20:5 n-3), linoleic (18:2 n-6), oleic (18:1n-9) and stearic (18:0) acids are the major phospholipid components. On the other hand, in the EPN species of long dauer phenotype (*S. glaseri, S. arenarium, Steinernema* sp. Morocco, as well as in the Polish and Italian strains) two saturated acids, stearic (18:0) and palmitic (16:0), and the monounsaturated oleic acid (18:1 n-9) were the major phospholipid components. Although each strain of all the studied species synthesizes polyunsaturated fatty acids (PUFA), there are significant differences in the amount of PUFA in each species. Because the saturated/unsaturated ratio affects the degree of fluidity of the membrane, we suppose that *S. feltiae* and *S. carpocapsae* have a genetically determined capability of thermo adaptation through changing the membrane fluidity.

As Table 2 shows, the steinernematids of long dauer phenotype do not incorporate as much PUFA into their membranes as the studied strains of *S. feltiae* or *S. carpocapsae* strains. Amongst the nematode strains of long dauer phenotype, only the *Polish* and *NC513* strains shad a relatively lower but still rather high (respectively 0,69, 0.75) ratio. On the other hand, neither any *S. feltiae* nor any *S. carpocapsae* strain had an S/U ratio above 0.50.

It has been reported earlier that among the membrane components, the polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA), reflects the changes of temperature. Since strains of *S. feltiae* and *S. carpocapsae* accumulated high amount of EPA, we wondered how warm and cold adapted ecotypes of these two species respond to changes in temperature.

There are the saturated (or mono-unsaturated) precursors of the polyunsaturated ones (products) synthesized in the different ecotypes of *S. carpocapsae* and *S. feltiae* species at two temperatures shown in Fig 7.

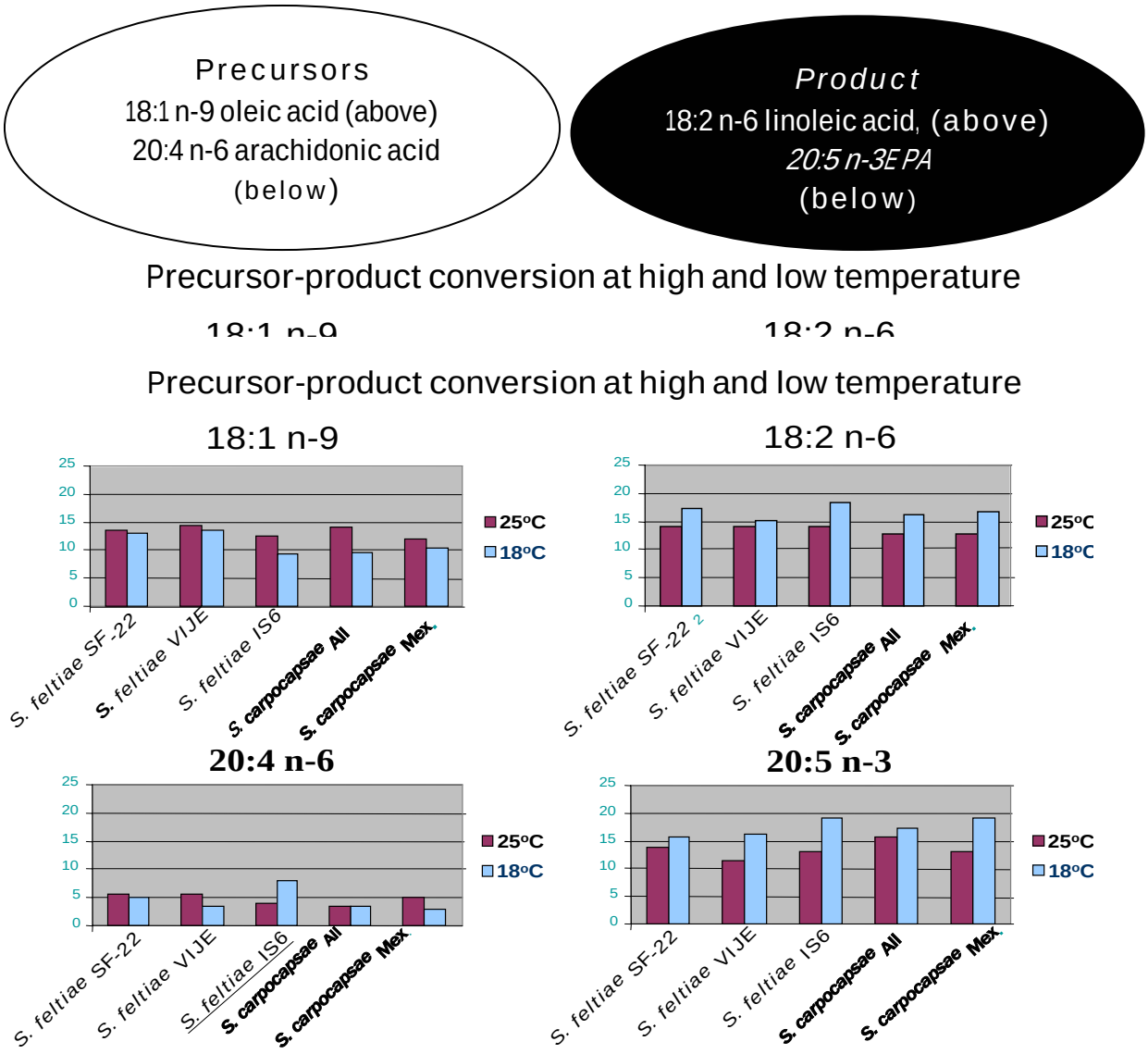

Fig. 7 Relative proportions of precursors (saturated or monounsaturated acids) and product (polyunsaturated acids) in the phospholipid membranes of entomopathogenic nematode strains belonging to *S. feltiae* and *S. carpocapsae* ("non-long" dauer phenotype species) propagated at low (18 °C) and high (25 °C) temperature.

The levels of oleic (18:1 n-9) and arachidonic (20:4 n-6) acid were decreased and the level of linolenic (18:2 n-6) and eicosapentaenoic (20:5 n-3) (EPA) acid were increased with lowering of the growth temperature. The only exception was the warm adapted IS6. In this strain the level of arachidonic (20:4n-6) acid was higher at low temperature. It is interesting that the warm climate strains increased the proportion of the EPA more than the boreal ones. These results may indicate that strains inhabiting colder regions do not require as much EPA to maintain structural and functional integrity of their membranes as do strains from warmer areas when grown at lower temperature.

We also compared the S/U ratio of the different ecotypes grown at 18°C and 25°C. At 25°C, the cold-adapted VIJE strain incorporated a higher proportion of PUFA into its membranes. As expected, S/U was lower in each strain at lower temperature. VIJE, however, was an exception. In this strain the S/U ratio did not reflect the changes of the temperature at all. This finding is surprising, since this would suggest a relatively rigid membrane structure at low temperature in this strain which was expected to be adapted to a cold climate. To clarify this

point, the fluidity of phospholipid vesicles obtained from both the warm adapted IS6 and the cold-adapted VIJE were determined by two different methods (see Materials and Methods).

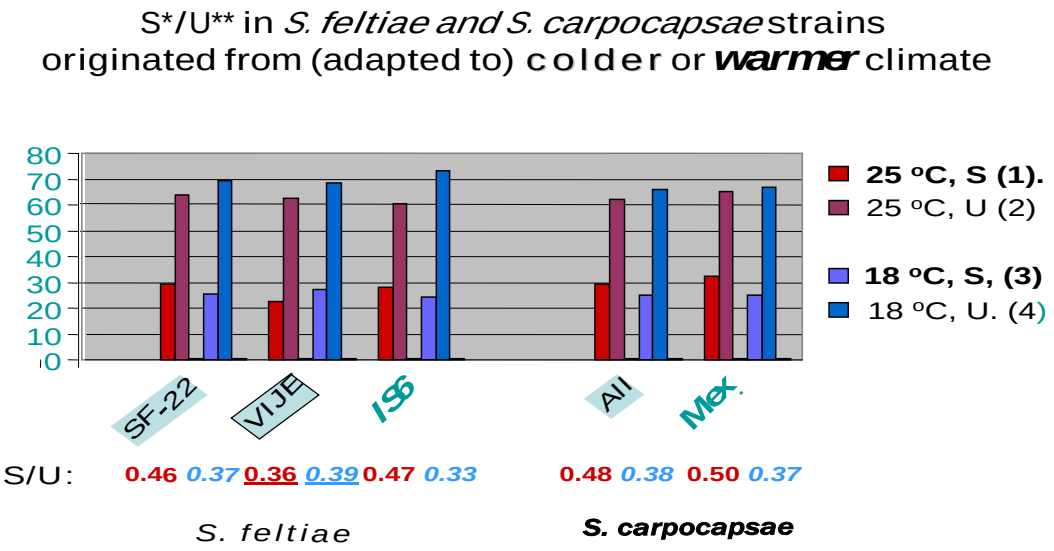

Fig 8 Changes in the relative amounts of saturated (S) and unsaturated (U) fatty acids as well as in the S/U ratio of *S. feltiae* and *S. carpocapsae*. *Steinernema feltiae* strains SF22 (from Finland) and VIJE (from Norway) are supposed to be cold adapted, whereas IS6 (*S. feltiae*) and Mexicana (*S. carpocapsae*) are presumably warm-adapted isolates. The S/U ratio was always 0.5 or lower, and it was lower at the cold (18 °C) temperature than at 25 °C, with the exception of VIJE.

At 25°C, the cold adapted *VIJE* built a higher amount of PUFA into its membranes. As expected, S/U was lower in all but one strain at lower temperature. The cold adapted *VIJE*, however, is an exception. In this strain the S/U ratio did not reflect the changes of the temperature at all. This finding is surprising, because on the basis of this observation a relatively rigid membrane structure could be expected at low temperature. To clarify this point, the fluidity of phospholipid vesicles obtained from both the warm adapted *IS6* and the cold-adapted *VIJE* were determined.

Figure 9 contains two representative spectra comprising the spectral region of carbon-hydrogen stretching vibration arising from the fatty acid chains of lipids of IS6 and VIJE grown at 18 °C. This peak is the most sensitive indicator of the membrane fluidity. The shifts of its position towards higher frequencies in the case of VIJE lead to the conclusion that VIJE has a more fluid membrane structure than IS6.

We recorded the position of this peak in a temperature scan for the lipids of these two strains (Fig 10). This scan revealed that the lipid membranes of VIJE were more fluid than those of IS6 at each temperature tested, regardless of the growing temperature of the nematodes.

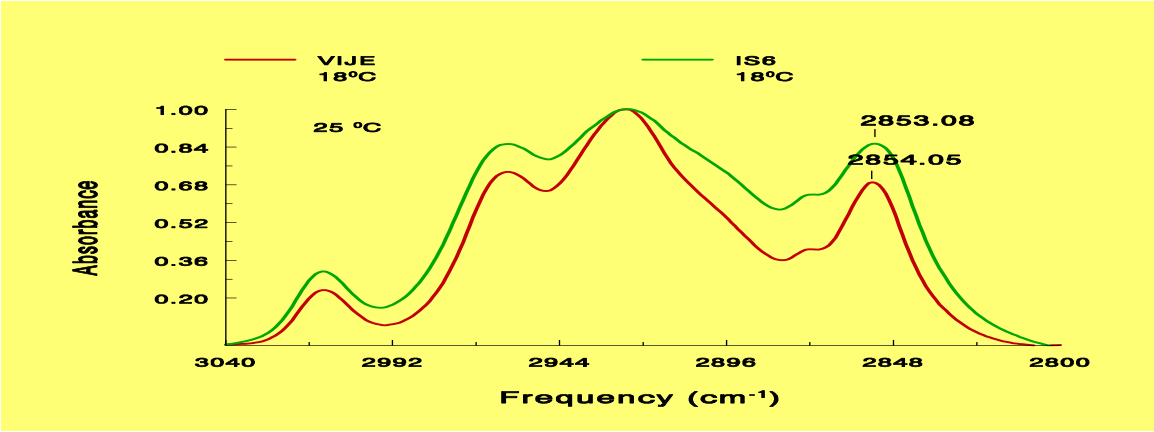

Fig 9 The C-H stretching vibrations of the hydrocarbon chains in the infrared spectra of phospholipids isolated from *S. feltiae Vije* (cold adapted) and IS6 (warm adapted) strains grown at 18 °C. The measurements were carried out at 25 °C. The most right peak of the spectrum comprises the spectral region of the C-H vibration. The difference between the data of IS8 (2853.08) and that of Vije (2854.05) indicate a significant difference concerning membrane fluidity.

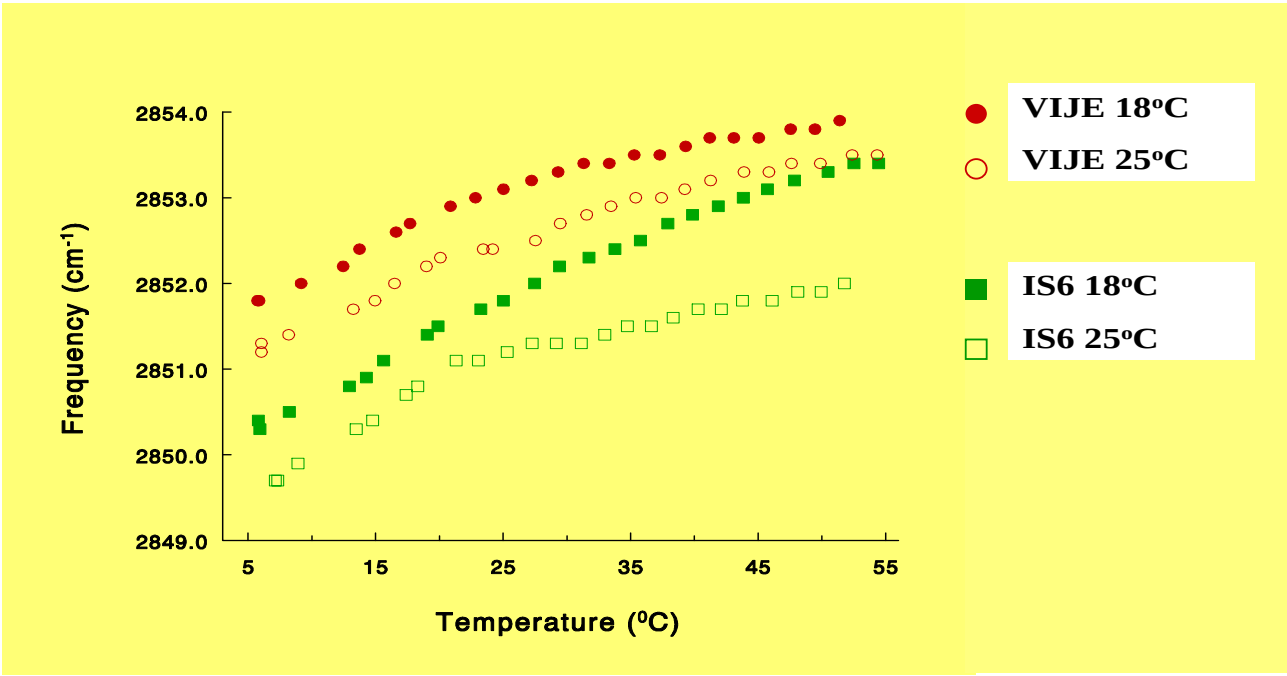

Fig 10 Temperature profiles of the CH_2-symmetric stretching vibration in the isolated lipids of the investigated cold-adapted (VIJE) and warm adapted (IS6) *S. feltiae* nematodes grown at 18 and 25 °C, respectively. The higher the frequency (ordinate) the more fluid the membranes are.

Sterols (Morrison and Ritter, 1986, Ritter, 1988) have a key role in cellular membranes in *S. feltiae* and effect its development. The cholesterol has a key role in altering the membrane fluidity, because of its capability of linking to the phospholipids. Cholesterol makes a fluid membrane structure much more ordered in a mostly PUFA environment. The cholesterol has

a key role in altering the membrane fluidity, because of its capability of linking to the phospholipids. Cholesterol makes a fluid membrane structure much more ordered in a mostly PUFA environment. To clarify the mechanism by which the cold adapted *VIJE* keeps its membrane in a "quasi steady state" at low temperature, we analyzed the neutral lipid fractions of *VIJE* and *IS6*.The results of sterol analysis can be seen on Fig.11.

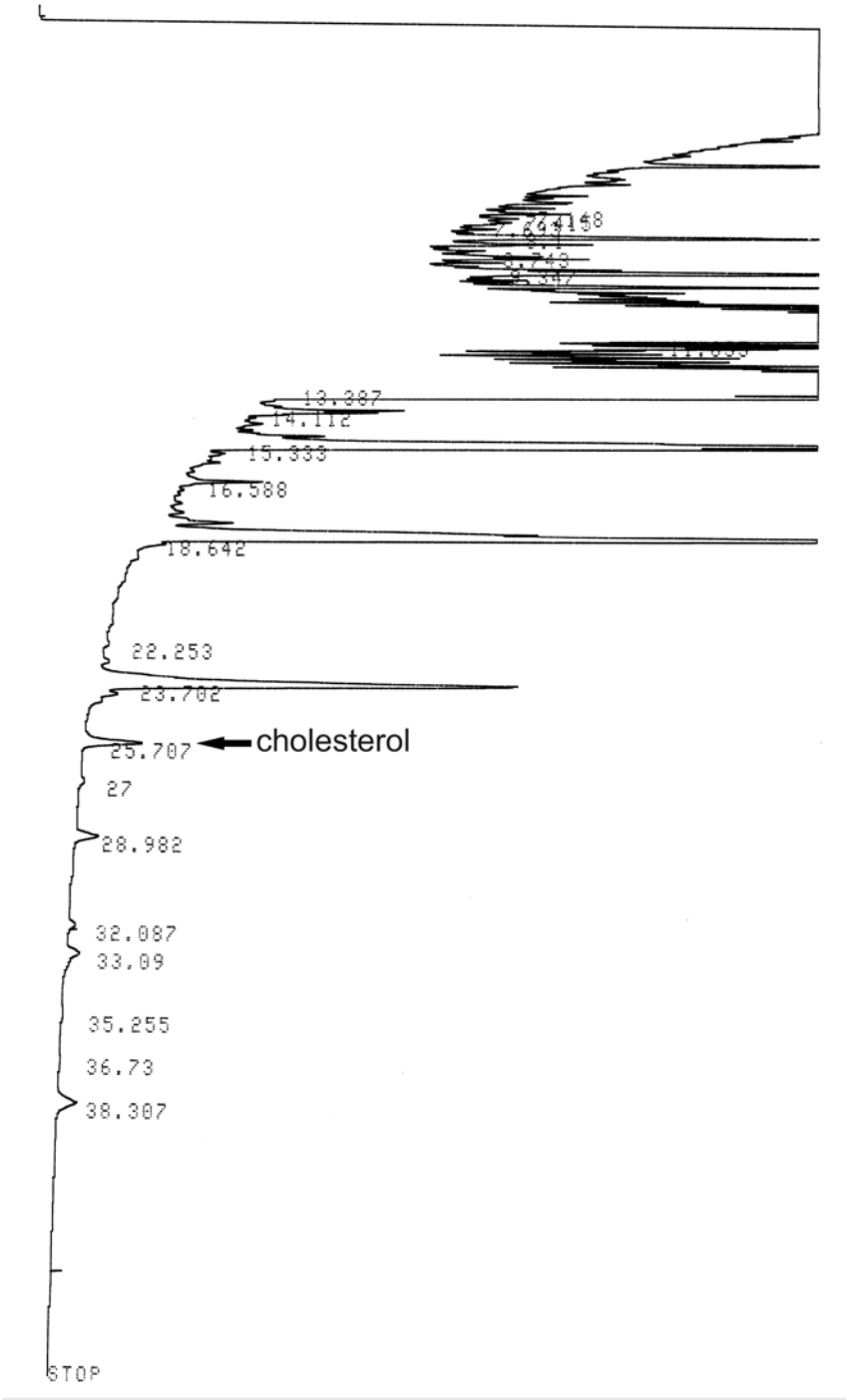

Fig 11 A representative chromatogram of sterol analysis. Cholesterol is in fact emerging at 25.7 minutes. When nematodes are grown at 25 ^{o}C, the sterol fraction contains approximately the same proportion of cholesterol (25% for IS6 and 23.4% for VIJE, respectively). When grown at 18^{o}C, however, sterols of the warm-adapted IS6 are composed of 32% cholesterol, while the cold-adapted VIJE contains hardly any (0.07%).

DISCUSSION

We analyzed the fatty acid compositions of strains of different *Steinernema* species, including warm- and cold-adapted isolates of the same species (*S. carpocapsae, S. feltiae*). As far as we know, no strains of *S. glaseri* occur in cold environments. *Steinernema* sp. Morocco is unambiguously warm adapted, growing well at 28 ^{o}C and 25 ^{o}C but not at lower temperatures. *Steinernema arenarium* (strains Anomali, Gel, Russian) originated from an Eastern European environment (Russia), where the temperature conditions are rather different according to the season and may be rather extreme. We also investigated *S. arenarium*-like strains of long dauer phenotype originating from different climates: Poland, Slovakia, and Italy.

The role of the membrane, and fatty acids and sterol is important in several physiological processes, such as desiccation survival (Patel, Perry & Wright, 1997); assessment (Patel. Stolinski & Wright, 1997); energy reserves (Patel and Wright, 1997a,b; Patel et al., 1998, Wright et al., 1998), osmotic tolerance, permeability (Qui et al., 2000) and anhydrobiotic potential (Grewal, 2000) of infective dauer juveniles as well as growth in liquid culture (Abu Hatab et al., 1998, Abu Hatab end Grewal, 2000l), but we do focus only on the role of the membranes as well as fatty acids and sterols in the thermo-adaptation mechanisms of entomopathogenic nematodes.

We discovered that each *Steinernema* strain examined so far contains polyunsaturated fatty acids. The nematode species of long dauer phenotype synthesize a smaller amount of linoleic (18:2 n-6) acid than *S. feltiae* or *S. carpocapsae*. The amount of EPA found in EPN species of long dauer phenotype is less than half of that found in *S. feltiae* or *S. carpocapsae*. The *S. feltiae* and *S. carpocapsae* strains increase the amount of PUFA at low temperature. At 25 ^{o}C, which is considered to be physiological for all the investigated strains, the S/U ratio was high for the nematode strains of long dauer phenotype, including the warm-adapted NCI, AZ26, Italy, and Morocco strains, the S/U ratio is high. But it is also true for *S. arenarium* and the Polish strain, which cannot be considered as warm adapted but thermo-tolerant. In the *S. feltiae* and *S. carpocapsae* strains, this ratio is lower and appears to be ecotype-independent. However, the level of EPA varied according to the geographical location of origin of the isolates.

It is interesting that *S. feltiae* and *S. carpocapsae* strains from colder areas contain a relatively lower proportion of EPA than those found in warmer climate. However, this relative lack of EPA did not influence the ability to regulate membrane fluidity.

The Norwegian (Vije) strain provide an example of another kind of thermo adaptation and adjusting their membrane fluidity due to temperature. Vije can keep its membrane more fluid, despite its unexpectedly low amount of EPA even at lower temperature, by lowering the amount of cholesterol built in its membrane. Our results suggest that the ability of thermo adaptation effects the geographical distribution of these nematodes. Strains of *S. feltiae* and *S. carpocapsae* are well represented at different climatic areas. Strains of *S. glaseri* and *S. arenarium* appear to be less adaptive based on their fatty acid composition. Further experiments are needed to investigate their behaviour at extreme temperatures.

Perspectives: The temperature optimum as well as the cold, heat and desiccation tolerance of the strains should be precisely described. It should be learnt whether the *Steinernema* species of long dauer phenotypes had a capability of changing their membrane fluidity due to the

ambient temperature, and if they do, which mechanism thy use. We found, that all the *S. feltiae* strains we examined, the "cold adapted" SF22 was almost the most active at high (25 ^{o}C) temperature. The classical and molecular genetic background behind the regulation of membrane fluidity should be elucidated. The cross IS6 and Vije and the Mendelian analysis of the membrane phenotypes of selected and inbred progeny lines is a realistic way to do so. Finally, we want to know how the FA profile change in desiccation tolerant transgenic S. feltiae strains (Vellai and Fodor, in preparation).

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REFERENCES

Abu Hatab M, Gaugler R, and Ehlers R-U. (1998). Influence of culture method on *Steinernema glaseri* lipids. J. Parasitol. 84, 215-221.

Abu Hatab MA, and Gaugler R. (1997). Growth-mediated variations in fatty acids of *Xenorhabdus* sp. J Appl. Microbiol. 82, 351-358.

Adams, B.J. (1998). Species concepts and the evolutionary paradigm in modern nematology. J. Nematology 30, 1-21.

Akhurst, R.J., and G.B. Dunphy. (1993). Tripartite interactions between symbiotically associated entomopathogenic bacteria, nematodes, and their insect hosts. *pp*.1-23. *In*: N. Beckage, S. Thompson, and B. Federici (ed.), *Parasites and pathogens of insects* vol.2, *Academic Press, New York.*

Arvinbayar, Baatar, Szállás, E., Füredi, S., Lengyel, K., Somogyi, E. Saskõi A., and A. Fodor (2003). Cross fertility, gnotobiology and molecular identification of steinernematids of long dauer phenotype: comparative evaluation of the results obtained by the GeneGel Excel kit.

Bell, M. V., Dick, J.R., and Kelly, M.S. (2001). Biosynthesis of eicosapentaenoic acid in the sea urchin *Psammechinus miliaris*. Lipids 36, 79-82.

Blaxter, M., and D. Bird. (1997). Parasitic nematodes, pp. 851-880. *In* C. elegans II. Cold Spring Harbor Laboratory, Cold Spring Harbor.

Casal, H.L., and Mantsch, H.H. (1984). Polymorphic phase behaviour of phospholipid membranes studied by infrared spectroscopy. Biochimica et Biophysica Acta 779, 381-401.

Curran, J. (1989). Chromosome numbers of Steinernema and Heterorhabditis species. Revue de Nematologie 12, 145-148.

Dey, I., Buda, C., Wiik, T., Halver, J.E., and Farkas T. (1993). Molecular and structural composition of phospholipid membranes in livers of marine and freshwater fish in relation to temperature. Proc. Natl. Acad. Sci. U S A. 90, 7498-7502.

Farkas, T., Dey, I., Buda, C., and Halver, J.E (1994) Role of phospholipid molecular species in maintaining lipid membrane structure in response to temperature. Biophys. Chem. 50, 147-155.

Filipjev, I.N. (1934). Miscellanea Nematologica 1. Eine neue Art der Gattung Neoaplectana Steiner nebst Bermerkungen über die systematische Stellung der letzteren, Mag. Parasitol. Instit. Zool. Acad. USSR 4, 229.

Fine, J., and Spretcher, H. (1982). One-dimensional thin-layer chromatography of phospholipids on boric acid-impregnated plates. J. Lipid Res. 23, 660-663.

Fodor, A., Vecseri G., and Farkas, T. (1990). *C. elegans*, as a model for studying entomopathogenic nematodes. *pp*. 285-300. *In*: R. Gaugler and H. Kaya (eds.) *Entomopathogenic Nematodes in Biological Contro*l. CRC Press, Boca Raton.

Fodor, A., Dey, I.., G., Farkas, T., and Chitwood, D. J. (1994). Effects of temperature and dietary lipids on phospholipid fatty acids and membrane fluidity in *Steinernema carpocapsae*. J. Nematol. 26, 278-285.

Folch, J.I., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:496-509.

Gál, T.Z, Solomon, A, Glazer, I., and Koltai, H. (2001). Alterations in the levels of glycogen and glycogen synthase transcripts during desiccation in the insect-killing nematode *Steinernema feltiae IS-6.* J Parasitol. 87, 725-732.

Grewal, P.S., Gaugler R., and Shupe, C. (1996). Rapid Changes in Thermal Sensitivity of Entomopathogenic Nematodes in Response to Selection at Temperature Extremes J Invertebr Pathol. 68, 65-73.

Grewal PS. (2000). Anhydrobiotic potential and long-term storage of entomopathogenic nematodes (Rhabditida: Steinernematidae). Int. J Parasitol. 30, 995-1000.

Lahdes, E., Balogh, G., Fodor, E., and Farkas, T. (2000). Adaptation of composition and biophysical properties of phospholipids to temperature by the Crustacean, *Gammarus* spp. Lipids 35, 1093-1098.

Mantsh, H.H., His, S.C., Butler, K.W., and Cameron, D.G. (1983). Studies on the thermo tropic behaviour of aqueous phosphatidylethanolamines. Biochim. Biophys. Acta 728, 325-330.

Morrison, A.H., and Ritter, K.S. (1986).Effect of host insect sterols on the development and sterol composition of *Steinernema feltiae*. Mol. Biochem. Parasitol. 19, 135-142.

Pamjav, H., D. Triga, Z. Buzás, T. Vellai, A. Lucskai, B. Adams, A.P. Reid, A. Burnell, C. Griffin, I. Glazer, M. Klein, and A. Fodor. (1999) Novel application of PhastSystem PAGE using RFLP-ITS patterns of individuals for molecular identification of EPNs. Electrophoresis 20, 1266-1273.

Patel, M.N, Perry, R.N., and Wright, D.J. (1997). Desiccation survival and water contents of entomopathogenic nematodes, *Steinernema* spp. Rhabditida: Steinernematidae). In. J Parasitol. 27, 61-70.

Patel, M.N., Stolinski, M., and Wright, D.J. (1997). Neutral lipids and the assessment of infectivity in entomopathogenic nematodes: observations on four *Steinernema* species. Parasitology 114, 489-496.

Patel M. N., and Wright D. J. (1997a). Fatty acid composition of neutral lipid energy reserves in infective juveniles of entomopathogenic nematodes. Comp. Biochem. Physiol. B Biochem Mol Biol. 118, 341-348.

Patel, M.N., and Wright, D.J. (1997b). Glycogen: its importance in the infectivity of aged juveniles of Steinernema carpocapsae. Parasitology 114, 591-596.

Patel, M.N., and Wright, D.J. (1997c). Phospholipids and their fatty acids in infective juveniles of entomopathogenic steinernematid nematodes. Com. Biochem. Physiol. B Biochem. Mol. Biol. 118, 649-657.

Patel, M.N., and Wright, D.J. (1998). The ultrastructure of the cuticle and sheath of infective juveniles of entomopathogenic steinernematid nematodes. J. Helminthol. 72, 257-66.

Qiu, L., Lacey, M.J., and Bedding, R.A. (2000). Permeability of the infective juveniles of Steinernema carpocapsae to glycerol during osmotic dehydration and its effect on biochemical adaptation and energy metabolism. Comp Biochem Physiol B Biochem Mol Biol. 125, 411-419.

Ritter, K.S. (1988). Steinernema feltiae (= Neoaplectana carpocapsae): effect of sterols and hypolipidemic agents on development. Exp Parasitol. 67, 257-67.

Steiner, G. 1929. *Neoaplectana glaseri*, n.g., n.sp. (Oxyuridae), a new nemic parasite of the Japanese beetle (Popilla japonica Newm.), J. Wash. Acad. Sci. 19, 436.

Takamura, M., and Kito, M. (1991). A highly sensitive method for quantitative analysis of phospholipid molecular species by high-performance liquid chromatography. J Biochem (Tokyo). 109, 436-439.

Triga, D., H. Pamjav, T. Vellai, A. Fodor, and Z. Buzás (1999). Gel electrophoretic restriction fragment length polymorphism analysis of DNA derived from individual nematodes, using the PhastSystem. Electrophoresis 20, 1274-1279.

Wright, D.J., Grewal, P.S., and Stolinski, M. (1997). Relative importance of neutral lipids and glycogen as energy stores in dauer larvae of two entomopathogenic nematodes, Steinernema carpocapsae and *Steinernema feltiae*. Comp. Biochem Physiol B Biochem. Mol Biol. 118, 269-273.