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Feeding on Different Host Plants Alters the Natural Abundances of $\delta^{13}C$ and $\delta^{15}N$ in Longidoridae (Nemata)[1]

Roy Neilson[2,3] and Derek J. F. Brown[2]

Abstract: Natural abundances of the stable isotope pairs $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) have been used previously to study food sources and trophic relationships in soil invertebrates. In this study, $\delta^{13}C$ and $\delta^{15}N$ were measured in five species of Longidoridae to investigate the effect of transferring nematodes from one plant host to another. *Longidorus elongatus, Paralongidorus maximus, Xiphinema diversicaudatum, X. index,* and *X. vuittenezi* were cultured initially on *Lolium perenne, Petunia hybrida, Rubus ideaus, Ficus carica,* and *Rubus ideaus,* respectively, and subsequently transferred to 4-week-old *P. hybrida* seedlings. After feeding on *P. hybrida* for 28 days, whole body $\delta^{13}C$ and $\delta^{15}N$ values of the three *Xiphinema* species were depleted ($P = 0.001$) and enriched ($P = 0.001$), respectively, compared to nematode populations that had fed solely on the original plant hosts. Similar changes in *L. elongatus* and *P. maximus* whole body $\delta^{13}C$ and $\delta^{15}N$ were not detected. Changes in whole body $\delta^{13}C$ are considered to be indicative of the new plant host (*P. hybrida*), whereas differences in whole body $\delta^{15}N$ are probably related to the different feeding strategies used by the longidorid nematodes in this study.

Key words: $\delta^{13}C$, $\delta^{15}N$, carbon isotope, feeding behavior, Longidoridae, *Longidorus,* metabolism, natural abundance, nematode, nitrogen isotope, *Paralongidorus,* stable isotopes, *Xiphinema.*

Measurement of stable isotope natural abundances can provide information regarding energy sources and flows through ecosystems (Hamilton et al., 1992; McCarthy et al., 1997; Spies et al., 1989; Wada et al., 1987), trophic relationships (Boutton et al., 1983; Lepage et al., 1993; Rau et al., 1991; Tayasu et al., 1997), predator-prey interactions (Fry et al., 1978), and effects of land management on soil invertebrates (Neilson et al., 1998a).

Ratios of the natural abundance levels of stable isotope pairs such as $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in a sample are measured by mass spectrometry and compared with that of a standard. This is expressed as the parts per thousand (‰) difference from the standard using the delta notation, e.g. $\delta^{13}C$ and $\delta^{15}N$.

Natural abundances of $\delta^{13}C$ in animal tissues reflect the $\delta^{13}C$ of the diet (DeNiro and Epstein, 1978; Wada et al., 1993), although small ^{13}C-enrichments of ≤ 1‰ between body and dietary $\delta^{13}C$ may occur at the lowest trophic levels of a food web. However, $\delta^{15}N$ in animal tissue is typically 0‰ to 6‰ more ^{15}N-enriched than the nitrogen in the diet, but large whole-body ^{15}N-enrichments can also occur due to starvation (Minagawa and Wada, 1984; Scrimgeour et al., 1995; Wada et al., 1993). A stepwise increase of whole animal $\delta^{15}N$ relative to diet occurs at each trophic level; thus $\delta^{15}N$ is useful for ranking animals into their relative trophic levels. In contrast, $\delta^{13}C$ is more useful than $\delta^{15}N$ for directly tracing energy sources. Additionally, previous mammalian and avian studies have used changes in $\delta^{15}N$ and $\delta^{13}C$ to measure turnover rate of tissues (Hilderbrand et al., 1996; Hobson and Clark, 1992a, 1992b; Tieszen et al., 1983).

Few nematological studies have utilized stable isotope analyses. However, Couch (1989) and Riera et al. (1996) used stable isotopes to identify dietary sources of marine nematodes (at the taxon level), whereas Boag et al. (1998) showed trophic-level relationships between species of parasitic intestinal nematodes and their wild rabbit host. Neilson et al. (1998b) demonstrated, using plant $\delta^{15}N$ values, that feeding by *Xiphinema diversicaudatum* (Micoletzky) Thorne and *Longidorus elongatus* (de Man) Micoletzky induced a physiological response in the plant host. Furthermore, a multiple pathogen

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[2] Nematology Unit, Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland, UK.

[3] Unit for Stable Isotope Studies in Biology, Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland, UK.

E-mail: Roy.Neilson@scri.sari.ac.uk

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load, i.e. nematodes feeding on roots accompanied by virus infection, induced a greater physiological response in the host plant.

Plant responses to parasitism and interactions between hosts and endoparasitic nematodes have been investigated previously (Gravato Nobre and Evans, 1998; Hussey and Williamson, 1998; Sijmons, 1993). However, effects on the physiology of nematodes feeding successively on different hosts have been poorly studied. Our main objective, using nematode $\delta^{15}N$ and $\delta^{13}C$, was to determine whether a physiological or metabolic reaction could be detected in species of ectoparasitic Longidoridae when transferred from one plant host species to another. A second objective was to determine the feasibility of using stable isotope analyses in nematological studies at species level.

Materials and Methods

Nematode feeding and sample preparation

Experiment one: Individual 4-week-old seedlings of *Petunia hybrida* cv. Blue Picotee growing in sterile compost were individually transplanted into a series of 25-cm^3 plastic polypots containing air-dried silica river sand (Brown, 1997). Groups of ca. 50 adult *L. elongatus* from a local field (sown with *Lolium perenne*) population were hand-picked and added to the individual plastic pots. Aliquots of similar numbers of adult *X. diversicaudatum,* maintained on *Rubus ideaus* L. cv. Glen Moy (raspberry), and *X. index* Thorne and Allen, maintained on *Ficus carica,* in a glasshouse, were added to similar plastic pots, giving five replicates per nematode species. The small number of nematodes added per replicate ensured that sufficient root tips were available for feeding. Additional aliquots of nematodes and samples of root tissues were also sampled and processed, as described elsewhere, to provide baseline values for comparison at the end of the experiment and dietary information, respectively. Pots containing seedlings were placed in a glasshouse and carefully watered to prevent waterlogging or drought. After 28 days, nematodes were extracted (Brown and Boag, 1988) and immediately transferred to pre-weighed 6 × 5 mm, smooth-walled tin capsules (Elemental Microanalysis, Okehampton, UK) to which 0.5 cm^3 of distilled water had been added. Capsules were placed in a freeze-dryer for ca. 18 hours, and thereafter removed and re-weighed to determine the nematode dry weight. Root tips of the *Petunia* seedlings were checked for the presence of galls, an indicator of nematode feeding activity.

To achieve a valid $\delta^{15}N$ analysis, the N content of the sample should be comparable with that of the reference standard. Preliminary studies (R. Neilson and D. J. F. Brown, unpublished data) determined that the minimum nematode dry weight required to achieve this was 0.4 mg/sample. If the number of nematodes recovered from each seedling after 28 days was less than this weight, samples were combined.

Experiment two: A second experiment using the same techniques as described for experiment one was done to provide evidence of the reproducibility of data obtained from experiment one. However, in this second experiment, 10 replicate *P. hybrida* seedlings for each of the following nematode species were used: *L. elongatus, X. diversicaudatum,* and *X. index* from the same populations as in experiment one; *Paralongidorus maximus* (Butschli) from glasshouse-grown mature *Petunia hybrida* cv. Blue Picotee; and *X. vuittenezi* Luc, Lima, Weischer and Flegg from glasshouse-grown *Rubus ideaus* cv. Glen Moy. As in experiment one, whenever the weight of nematodes extracted from a seedling was less than 0.4 mg, samples were combined.

Root analyses

Whole root samples were taken from host plants, as noted above, at time of nematode extraction immediately prior to the commencement of both experiments. Similarly, whole-root samples of 4-week-old *P. hybrida* seedlings were also taken, prior to the commencement of both experiments, to determine the differences between the putative nematode food sources. Roots were thoroughly washed to remove any residual growing medium, oven-dried at 60 °C for 24

hours, then ground to a fine powder with a mortar and pestle. Samples of 1 mg were used for $\delta^{13}C$ and $\delta^{15}N$ analyses (Handley et al., 1993).

Isotope analyses

Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ in a continuous-flow isotope ratio mass spectrometer (Europa Scientific, Crewe, UK) (Handley et al., 1993). The δ values are expressed as the parts per thousand (‰) difference from the standard using the 'delta' notation: $\delta = ((R_{sample} - R_{standard})/R_{standard}) \times 1000$ (‰), where R_{sample} is the ratio of heavy to light isotopes in the sample and $R_{standard}$ is that of the reference standard. By definition, the δ value of a standard is 0‰. If the sample's δ value is >0, it is enriched in the heavier isotope compared with the standard; if it is <0, the sample is relatively depleted in the heavier isotope. Values of $\delta^{13}C$ and $\delta^{15}N$ are expressed relative to the International Atomic Energy Agency (Vienna), International Standards, V-PDB, and atmospheric nitrogen. Sample precision (SD) from both experiments were 0.2‰ and 0.1‰ for nematode and plant root $\delta^{15}N$ and $\delta^{13}C$, respectively.

Statistical analysis

A one-way analysis of variance (ANOVA) using Minitab (Minitab, State College, PA) was done to compare nematode δ values after they had fed on a new host (*P. hybrida* seedlings) with values obtained from nematodes taken directly from the original hosts, which acted as the experimental control.

Results

Isotopic data for *L. elongatus, X. diversicaudatum,* and *X. index* from experiments one and two were not significantly different and therefore were combined for each species.

Root $\delta^{13}C$ and $\delta^{15}N$ values: Root $\delta^{15}N$ values of *P. hybrida* seedlings used as the second host for all nematode populations were 6.7‰ to 7.7‰ more ^{15}N-enriched than the roots of the various hosts on which nematodes were cultured previously, including *P. hybrida* from the *P. maximus* culture (Table 1). Only small differences (≤1‰) in root $\delta^{15}N$ were recorded among original hosts (Table 1), but $\delta^{13}C$ differences were greater. For example, roots of *R. ideaus* cv. Glen Moy from the *X. vuittenezi* culture were 2.9‰ more ^{13}C-enriched than roots of *P. hybrida* from the *P. maximus* culture (Table 1). Root $\delta^{13}C$ of the *P. hybrida* seedlings used as the second host for all nematode populations was 0.1‰ to 4.3‰ ^{13}C-depleted compared with roots from the original host plants (Table 1).

Nematode $\delta^{13}C$: After feeding on *P. hybrida* seedlings for 28 days, whole-body $\delta^{13}C$ values of all nematode species, except *L. elongatus,* were depleted compared with nematodes from the original hosts (Table 1). The $\delta^{13}C$ value for *P. maximus* was depleted by 0.5‰ ($P = 0.05$), whereas *X. diversicaudatum, X. index,* and *X. vuittenezi* were depleted by 2.3, 1.1, and 3.2‰, respectively ($P = 0.001$) (Table 1).

Nematode $\delta^{15}N$: Feeding on *P. hybrida* seedlings for 28 days did not significantly alter the whole-body $\delta^{15}N$ values of *L. elongatus* and *P. maximus* compared with nematodes taken directly from the original hosts (Table 1). However, after feeding on *P. hybrida* seedlings, all three *Xiphinema* species were significantly ($P = 0.001$) ^{15}N-enriched compared with nematodes from the original hosts (Table 1), with *X. vuittenezi* having the greatest (2.3‰) and *X. diversicaudatum* the least (1.2‰) ^{15}N-enrichment, respectively.

Discussion

Stable isotope analyses have been rarely used in nematological studies (Boag et al., 1998; Couch, 1989; Neilson et al., 1998b; Riera et al., 1996). Although DeNiro and Epstein (1978, 1981) published $\delta^{13}C$ and $\delta^{15}N$ values for *Caenorhabditis* fed on artificial diets, to our knowledge, this is the first report of whole-body $\delta^{15}N$ and $\delta^{13}C$ values for plant-parasitic nematodes. Nematode whole-body δ values presented here are within the range previously reported for other soil-dwelling or C3-plant-feeding organisms (Fry et al., 1978; Martin et al., 1992a,b; Neilson et al., 1998a; Schmidt et al., 1997).

TABLE 1. Stable isotope ratios for whole-body preparations of longidorid nematode populations and for roots of the species of host plant on which each had been maintained (first host), and for the same nematode populations and roots of *Petunia hybrida* seedlings (second host) 28 days after transferring nematodes to *P. hybrida* seedlings in a glasshouse.

Isotope data	*Longidorus elongatus* on *Lolium perenne*	*Paralongidorus maximus* on *Petunia hybrida* 'Blue Picotee'	*Xiphinema diversicaudatum* on *Rubus ideaus* 'Glen Moy'	*X. index* on *Ficus carica*	*X. vuittenezi* on *R. ideaus* 'Glen Moy'
Nitrogen: $\delta^{15}N$ (*n*, SE)					
Roots					
From 1st host species	0.7 (10, 0.18)	1.7 (10, 0.08)	0.8 (10, 0.12)	1.1 (10, 0.08)	1.5 (10, 0.10)
From 2nd host (*P. hybrida*)	8.4 (10, 0.61)	8.4 (10, 0.61)	8.4 (10, 0.61)	8.4 (10, 0.61)	8.4 (10, 0.61)
Nematodes					
From 1st host species	2.7 (10, 0.21)	−2.9 (10, 0.19)	3.8 (10, 0.14)	2.2 (10, 0.22)	0.1 (10, 0.15)
From 2nd host (*P. hybrida*)	2.3 (6, 0.28)	−2.4 (9, 0.25)	5.0 (14, 0.16)	4.0 (14, 0.16)	2.4 (3, 0.21)
Change	−0.4 NS	+0.5 NS	+1.2***	+1.8***	+2.3***
Carbon: $\delta^{13}C$ (*n*, SE)					
Roots					
From 1st host species	−29.6 (10, 0.05)	−28.3 (10, 0.04)	−27.3 (10, 0.07)	−25.5 (10, 0.06)	−25.4 (10, 0.04)
From 2nd host (*P. hybrida*)	−29.7 (10, 0.10)	−29.7 (10, 0.10)	−29.7 (10, 0.10)	−29.7 (10, 0.10)	−29.7 (10, 0.10)
Nematodes					
From 1st host species	−28.0 (10, 0.19)	−27.0 (10, 0.05)	−24.1 (10, 0.29)	−23.5 (10, 0.06)	−24.9 (10, 0.12)
From 2nd host (*P. hybrida*)	−28.0 (6, 0.12)	−27.5 (9, 0.22)	−26.4 (14, 0.10)	−24.6 (14, 0.38)	−28.1 (3, 0.25)
Change	0.0 NS	−0.5*	−2.3***	−1.1***	−3.2***

*,*** indicate significant differences for nematode isotope values between first and second host at $P = 0.05$ and $P = 0.001$, respectively.

Animal tissue $\delta^{13}C$ reflects the dietary source $\delta^{13}C$ (DeNiro and Epstein, 1978; Wada et al., 1993). Thus, in general, if an animal feeds on a new enriched or depleted ^{13}C dietary source, with time the $\delta^{13}C$ of the animal will move toward the source $\delta^{13}C$ value. Root $\delta^{13}C$ of the *P. hybrida* seedlings used as the second host for all nematode populations was 0.1‰ to 4.3‰ ^{13}C-depleted relative to the original hosts. It is therefore probable that the measured ^{13}C-depletion of the nematode species after 28 days was indicative of the new dietary source (*P. hybrida* seedlings). In support of this argument, only the $\delta^{13}C$ value for *L. elongatus* was unaltered after 28 days, as the roots of the original hosts (*L. perenne*) had a similar $\delta^{13}C$ value to that of the second host (*P. hybrida* seedlings). Similarly, the greatest change in nematode $\delta^{13}C$ was for *X. vuittenezi,* which had the largest difference in root $\delta^{13}C$ between the first and second host plants.

Root $\delta^{15}N$ of the *P. hybrida* seedlings used as the second host for all nematodes was ca. 7‰ ^{15}N-enriched relative to roots from all original hosts. Therefore, unlike nematode $\delta^{13}C$, differential changes in nematode $\delta^{15}N$ after 28 days feeding on *P. hybrida* seedlings cannot be attributed directly to this second $\delta^{15}N$ source.

After feeding on a new host for 28 days, there were no significant differences in whole-body $\delta^{15}N$ of *L. elongatus* or *P. maximus* compared with whole-body $\delta^{15}N$ values from nematodes feeding on their original hosts. In contrast, all *Xiphinema* species were ^{15}N-enriched by 1.2‰ to 2.3‰ compared to nematodes from their original hosts. There are several possible explanations for the changes in $\delta^{15}N$ among the different nematode species. First, if all the nematode species in this study ingest similar nutrients, each species may assimilate and metabolize them in different ways, producing the observed differences in $\delta^{15}N$ values. Second, the apparent species-specific change in $\delta^{15}N$ when these nematodes feed on *P. hybrida* may reflect an alteration in their reproductive capacity. Food quality directly affects the reproductive capacity of longidorid nematodes (Brown and Coiro, 1983, 1985; Coiro and Brown, 1984; Griffiths and Trudgill, 1983). *Petunia hybrida* is regarded as a ''good'' host for both *L. elongatus* and *P. maximus* but is less suitable for culturing the three *Xiphinema* species, compared with *Rubus ideaus* and *Ficus carica.* Starvation is known to cause an increase in invertebrate $\delta^{15}N$ (Scrimgeour et al., 1995). Although *P. hybrida* is considered a poor host for *Xiphinema,* we observed that all nematodes that had fed on *P. hybrida* for 28 days had relatively full intestinal tracts, indicative of recent feeding. Additionally, there was no significant difference in the number of root galls produced as a result of feeding on *P. hybrida* by the different nematode species. Therefore, we concluded that starvation was not a factor in this study.

Third, and most likley, is that changes in whole-body $\delta^{15}N$ are a consequence of different feeding strategies used by the nematodes, which may affect the availability of certain compounds or bio-molecules. With the exception of *Xiphinema americanum*-group species, feeding by longidorid nematodes results in terminal root-tip galling. However, the mechanisms by which different longidorid species derive nutrients from their plant hosts differ markedly (Bleve-Zacheo et al., 1979; Taylor and Brown, 1997). *Longidorus* spp. simply remove cytoplasmic contents of modified cells (Griffiths and Robertson, 1984; Robertson et al., 1984). In contrast, *Xiphinema* spp. usually induce coenocyte formation and remove products from the end of metabolic pathways that are continually replenished by the host plant, which is considered indicative of a balanced host-parasite relationship (Zacheo and Bleve-Zacheo, 1995). In general, feeding by *Paralongidorus* spp. is more similar to that of *Longidorus* than *Xiphinema* (Zacheo and Bleve-Zacheo, 1995). Therefore, due to these different feeding strategies, the compounds and bio-molecules available to *Longidorus* and *Paralongidorus* species are likely to be different from those available to *Xiphinema,* resulting in the different nematode $\delta^{15}N$, even when they feed on the same host plant. This explanation parallels data from other invertebrate stud-

ies. For example, within insect-induced galls, cells of so-called nutritive tissue, adjacent to the insect pathogen, have markedly different levels of amino acids and sugars than surrounding plant tissue and can also be insect species-specific (Birch et al., 1992; Hartley, 1998). For this final explanation to be unequivocally tested, a true factorial experiment would be required, e.g. using *Xiphinema* spp. from *P. hybrida* as a first host. However, this is not possible as *P. hybrida* is a good host for only one of the three *Xiphinema* species in this study (Brown and Coiro, 1983).

Our study has shown that the stable isotopes, $\delta^{13}C$ and $\delta^{15}N$, can be used to analyze the feeding behavior and metabolism of plant-parasitic nematodes at species level. Stable isotope analyses may also have applications in the study of host preferences, nutrient assimilation, protein turnover (Gannes et al., 1998), and energy flows within soil ecosystems as has previously been investigated for marine systems (Fry and Sherr, 1984; Hamilton et al., 1992). Preliminary laboratory studies have shown also that stable isotopes can be used to determine the role(s) of nematodes in micro-food webs. For example, predatory *Anatonchus tridentatus* (de Man) de Coninck were ca. 5‰ ^{15}N-enriched compared with their prey, *L. elongatus* (R. Neilson, unpublished data), which is within the typical range found in trophic studies (Scrimgeour et al., 1995). Furthermore, as with insects (Ostrom et al., 1997), it should be possible to quantify the energy transfer between nematode and host to provide novel data on host-parasite interactions (Neilson et al., 1998b). These studies will be further enhanced by measuring δ values of nematode-respired CO_2, excretory products, and specific metabolites extracted from the host during feeding.

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