A strategy in searching for stress tolerance-correlated characteristics in nematodes while accounting for phylogenetic interdependence

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Summary – Biological indicators are highly relevant for assessing the condition of a soil as they are integrative; they reflect the overall impact of physical, chemical and biological changes. Indigenous soil organisms are preferable to other test organisms because the diversity and condition of indigenous soil organisms reflect both acute and chronic effects of soil disturbances. Nematodes are ubiquitous, speciose, easily extractable and present in extremely high numbers. Given the ever increasing amount of sequence data, DNA barcode-based community analysis will soon be possible and a next step would be to define objective criteria for the ecological grouping of soil nematodes. Here, we present a framework to ascertain which traits are correlated with a tolerance to stress. For this, a field study on the effects of pH and copper on nematode communities was re-analysed. Changes in abundances of individual genera were correlated with a number of potentially stress tolerance-related characteristics. The generalised least squares (GLS) method was used to account for the phylogenetic dependence of the data. Only the relationship between the ability to enter a survival stage and tolerance to copper at pH 6.1 was found to be significant, but the quantity of missing data probably had a negative impact on the analyses. This study did, however, clearly demonstrate the importance of accounting for the effects of phylogenetic dependence in the data. When the phylogeny was taken into account, we observed an average change in P value of 0.196 (and in some cases as much as 0.6) for the correlations of possible stress-related characteristics and Cu or pH tolerance. This research constitutes a proof-of-principle for a transparent method to relate stress tolerance to (ecological) characteristics. The usefulness of this powerful method should become even clearer when substantially higher numbers of individuals are analysed (as facilitated by using DNA barcodes) and when missing data are filled in.

Keywords – comparative method, interdependence, maturity index, nematodes, phylogenetic stress tolerance.

Because of their abundance and their (trophic) diversity, nematodes occupy important positions in the soil foodweb (De Ruiter et al., 1998). They also display a great variability in sensitivity to environmental stress (Bongers, 1990). As such, the composition of nematode communities is considered to be an informative indicator for soil health. However, little is known about biological characteristics that underlie the very wide variation in stress (in)tolerance. Here, we propose a strategy to investigate what traits are correlated with stress tolerance in nematodes.

The monitoring of nematode communities as indicators for soil health conditions is widely applied. However, sample size and taxonomic resolution are currently dictated by practical limitations (and are respectively smaller and lower than desired). The morphology of nematodes is
relatively conserved and, therefore, community analysis is time-consuming and requires ample expert knowledge. Recent advances in the use of molecular characteristics for the analysis of nematode communities may remove (in part) these practical obstacles (Holterman et al., 2008).

Several indices can be used to describe community diversity, such as the Shannon-Wiener index (Shannon, 1948), Simpson’s diversity index (Simpson, 1949) or the species richness index ($S$; the number of species present in an ecosystem). An important characteristic of the Maturity Index (MI) as proposed by Bongers (1990) is the inclusion of ecological characteristics, and, over the last decade, this MI has been widely used for nematode community analyses. The Maturity Index assigns each nematode family a so-called cp-value. This ‘coloniser-persister’ scale ranges from 1 to 5 and corresponds roughly to r-K strategies, with values of 1 and 2 being assigned to the most tolerant r-strategists and values of 4 and 5 to the most sensitive K-strategists. Families assigned to cp-class 1 are enrichment opportunists and, while they can outlast periods of adverse conditions, they are mainly found in food-rich conditions. For this reason they are often excluded from the MI (Bongers, 1999). The MI was demonstrated to be an effective means for assessing and quantifying the impact of soil pollution on nematode communities (Bongers & Ferris, 1999). However, families were assigned to cp-classes mainly on the basis of expert knowledge and some general observations (Bongers, 1999), and there are few clear criteria for each cp-class. In addition, it is known that genera in a single family do not always react similarly to stress (Ettema & Bongers, 1993) and, for some nematode families, a refinement of the MI down to genus, or even species, level would be appropriate. Identification of traits correlated with specific forms of stress tolerance would be helpful for the refinement of the MI or related indices.

When looking for correlations between traits using data from different species, it is important to realise that one of the basic assumptions of the comparative method, i.e., the data are independent, is often violated (Felsenstein, 1985). If a strong phylogenetic correlation exists in the data, it is important to take this into account. Felsenstein designed a method, named phylogenetically independent contrasts, which takes these confounding effects into account (Felsenstein, 1985). However, this method may overestimate the effects of phylogeny (Harvey & Rambaut, 2000). Another (intermediate) approach was proposed by Pagel (1999): the generalised least squares (GLS) method.

To study traits correlated with stress tolerance in nematodes we focused on the effects of copper and pH on nematode communities. The traits involved in this study are body size, reproductive potential, (a)sexual reproduction, feeding type, the ability to form survival stages and cuticle permeability. Body size is an important life history trait which correlates with many processes (Peters, 1983). Reproduction can be expected to play a role in stress tolerance since a high reproduction rate could partially compensate for increased mortality and enhance population recovery from the stress. It is noted that stress tolerant nematodes are often small, have short generation times and produce large numbers of offspring (Bongers, 1999). Unfortunately, generation times and number of offspring are not determinable for most nematodes, since most nematodes cannot be cultured or are barely culturable. Therefore, we looked to the gonad size relative to body size as an alternative measure for fecundity. To the best of our knowledge this has never been done for nematodes, but it has been done in various other animals, such as tardigrades (Guidetti et al., 2007), flat worms (Schärer et al., 2005) and tree frogs (Rodrigues et al., 2005, 2007). Asexual reproduction is often considered a trait for opportunists but this does not necessarily seem to be the case for nematodes (Bongers, 1999). Feeding type could also be related to stress tolerance. In the MI, most bacterial feeders belong to low cp-classes while members of cp-classes 4 and 5 are often predators or omnivores (Bongers, 1999). Entering a survival stage can help to outlast long periods of stress and, finally, changing the cuticle permeability could be an expedient to prevent pollutants from entering the body.

Until now, nematode families have been assigned to cp-classes for the Maturity Index on the basis of expert knowledge. Identifying traits which are correlated with a tolerance to stress is a first step towards defining clear objective criteria for the assigning of nematode taxa to cp-classes. Furthermore, having clear criteria will allow for the refinement of the MI from a family to a genus level index. In this study we set out a framework to identify traits important to stress tolerance. We will demonstrate the importance of taking the effects of phylogeny on the data into account. Finally, the effectiveness of our framework to identify relevant traits will be discussed.