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Bacterial traits and quality contribute to the diet choice and survival of bacterial-feeding nematodes

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ABSTRACT

The dietary choices of bacterial-feeding nematodes could control the structure and ecological functions of soil bacterial communities. However, the physiological basis for the selection of particular bacterial species as food, and the consequences of these dietary choices for the survival of bacterial-feeding nematodes, is poorly understood. The objectives of this study were (1) to determine how nematode feeding preference was related to bacterial traits (cell size, gram stain and growth rate) and quality (water content, carbohydrate content, protein content and metabolite concentration), and (2) to evaluate how dietary choices affected the reproduction and lifespan of two soil-dwelling bacterial-feeding nematodes of the species *Mesorhabditis* and *Acrobeloides*. Their food sources included one model bacterium, *Escherichia coli* OP50, and four soil-dwelling bacterial species: *Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Variovorax paradoxus* and *Pseudomonas fluorescens*. Both nematode species exhibited a similar hierarchy of diet choice, with *P. fluorescens* and *E. coli* OP50 being the most preferred food, whereas *B. megaterium* was the least preferred bacteria. Nematode feeding preference was strongly related to the water content, growth rate and metabolite concentration of bacterial cells, which explained 63–75% of the variation in the feeding preference index (PI, which indicates the number of nematodes attracted to specific bacteria), and the rest of the variation was attributed to bacterial cell size, gram stain, carbohydrate content and protein content. We propose two physiological mechanisms to explain dietary choices of bacterial-feeding nematodes: 1) chemical attraction to higher carbon dioxide levels around rapidly-growing bacteria or repulsion to volatile organic molecules released from bacterial cells, and 2) selective ingestion of bacterial cells with preferred characteristics (e.g., high water content in cells). Nematodes feeding on preferred bacteria always had higher reproduction, but dietary choices were not a good predictor of their lifespan. For example, *Acrobeloides* feeding on their preferred food *P. fluorescens* had the largest brood size but a moderate survival time. However, when *Acrobeloides* consumed their least-preferred food *B. megaterium*, they produced the smallest brood size and had the shortest survival time. This may be due to the fact that dietary resources are allocated first towards reproduction, and second to prolong the lifespan of bacterial-feeding nematodes. Our findings suggest that dietary choices are important for the survival of bacterial-feeding nematodes, and their ability to find and selectively ingest preferred bacterial species may have implications for soil bacterial community structure and ecological functions.

1. Introduction

Bacterial-feeding nematodes, one of the primary grazers of soil bacteria, are often considered as a single trophic group in model soil food webs. However, nematodes evolved like other terrestrial animals to exhibit dietary choice, and they show preference for

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certain soil bacterial species (Salinas et al., 2007; Shtonda and Avery, 2006; Venette and Ferris, 1998). Nematode food-seeking behavior may be related to their morphological features and nutritional requirements. For example, the buccal morphology of bacterial-feeding nematodes are always funnel-shaped (e.g., family Cephalobidae) or rod-shaped (e.g., family Rhabditidae) (Ferris et al., 1996), and the buccal shape affects their ability to capture and ingest bacterial cells of various sizes and motility (Bongers and Ferris, 1999).

Bacterial cell size is another factor contributing to the feeding preference of bacterial-feeding nematodes. Small-sized bacteria are always preferred because they are easy to swallow and nutrient-rich, allowing more efficient ingestion and nutrient acquisition, whereas large-sized bacteria are considered to be a lower quality food (Avery and Shtonda, 2003; Avery and You, 2012; Shtonda and Avery, 2006) and harder to pass through the narrow buccal cavity (Salinas et al., 2007). Bacteria with high respiration and growth rates are attractive to *C. elegans*, presumably because the CO_2 concentration is higher in the vicinity of their cells (Yu et al., 2015). Nematodes can also distinguish pathogenic bacteria from healthy bacteria by experience and learn to avoid feeding on the pathogenic species (Beale et al., 2006; Zhang et al., 2005). Bacterial-feeding nematodes show a preference for gram-negative bacteria rather than gram-positive bacteria, which is attributed to the fact that gram-negative bacteria have a thinner cell wall and thus may be easier to digest (Salinas et al., 2007; Xiao et al., 2010). Soil protists are also reported to prefer gram-negative bacteria over gram-positive bacteria (Rønn et al., 2002).

The selective feeding behavior of bacterial-feeding nematodes may be motivated by other characteristics of bacterial cells, which we refer to as 'food quality'. Food quality could be determined by the water content, nutritious compounds (e.g., protein and carbohydrate) and metabolites released from bacterial cells as attractant or repellant compounds. Water is needed for digestion and metabolic processes, and bacterial-feeding nematodes may selectively consume bacterial cells with higher water content. While mammals discriminate between protein-free diets and a diet containing low to moderate level of protein (Fromentin et al., 2012), and soil invertebrates like earthworms showed a strong preference for litter with high soluble carbohydrate content (Curry and Schmidt, 2007), it is not known if bacterial-feeding nematodes have similar dietary cues. Soil-dwelling *Pseudomonas* produce metabolites such as small alcohols, ketones, diacetyl, and esters, which could act as natural chemoattractants or repellents for *C. elegans* (Bargmann et al., 1993). However, there is currently a lack of knowledge about how food quality is related to bacterial traits (e.g., cell size, gram stain and growth rates) and to what extent these food quality attributes influence the diet choice of bacterial-feeding nematodes.

Feeding preference has implications for the survival of bacterial-feeding nematodes. Food is a physiological need for basal metabolism throughout the lifespan of the organism and for secondary production, also referred to as reproduction, leading to trade-offs between reproduction and survival when there are food shortages (Partridge et al., 2005). The bacteria-feeder *C. elegans* produced more eggs after consuming its preferred food, but reduced reproduction to ensure survival when the food supply was limited (Mukhopadhyay and Tissenbaum, 2007). Yu et al. (2015) reported that *C. elegans* produced more offspring and had a shorter lifespan when supplied with preferred food resources (*P. fluorescens* and *E.coli* OP50), but the brood size decreased and the lifespan increased when *C. elegans* was given less-preferred *B. megaterium.* In contrast, Coolon et al. (2009) found that *C. elegans* preferred *Pseudomonas* sp. over *B. megaterium* and it had a shorter lifespan when feeding on *B. megaterium*. While dietary choices affect nematode life traits, it is unclear from the literature how bacterial-feeding nematodes make trade-offs between reproduction and lifespan when feeding on preferred and less-preferred bacterial species. It is difficult to generalize results from *C. elegans* to all soil-dwelling bacterial-feeding nematodes because *C. elegans* is a colonizer of microbe-rich habitats, in particular decaying plant matter (Félix and Braendle, 2010). Thus, we chose to study the dietary choices of two widely distributed soil-dwelling bacterial-feeding nematodes, *Mesorhabditis* sp. and *Acrobeloides* sp., rather than *C. elegans*.

The objective of this study was to determine i) the relative contribution of food quality, based on bacterial traits (i.e., cell size, gram stain and growth rate) and chemical composition (i.e., water content, carbohydrate content, protein content and metabolite concentration) to the dietary preference of bacterial-feeding nematodes; and ii) if food selection will cause bacterial-feeding nematodes to make a tradeoff between reproduction and longevity. We hypothesized that nematodes feeding on preferred bacteria will produce more offspring but shorten their lifespan, whereas those consuming the less-preferred bacteria will have fewer offspring but a longer lifespan.

2. Materials and methods

2.1. Preparation of bacterial-feeding nematodes

Two bacterial-feeding nematodes *Mesorhabditis* sp. and *Acrobeloides* sp. were obtained from a sandy loam alluvial soil collected from Banqiao Town, Nanjing City, Jiangsu Province, China. Nematodes were extracted from soil using a modified Baermann method (Liu et al., 2008) and observed under the microscope to select the bacterial-feeding nematodes based on morphological features. Selected bacterial-feeding nematodes were rinsed with sterile water and dark-cultivated on freshly prepared nematode growth medium (NGM) inoculated with *E. coli* OP50 in a 20 °C incubator (see supplementary information for details). Selected organisms were transferred sequentially to new culture plates with NGM until a single bacterial-feeding nematode species was present in the culture plate. This procedure isolated two dominant nematode species, whose morphological characteristics head, tail, genitalia and excretory pore were visualized with a scanning electron microscope (Fig. S1 and Fig. S2). Finally, the dominant nematode species were sent to BGI Company (Shenzhen, China) for 18S rRNA sequencing and analysis of molecular data with BLAST in the NCBI database followed by phylogenetic analysis with the Neighbor-Joining method in MEGA software (Fig. S3). The morphological and molecular analyses confirmed that the bacterial-feeding nematodes were the species *Mesorhabditis* and *Acrobeloides*.

2.2. Preparation of bacterial species

Five bacterial species were selected for this study, including *Bacillus amyloliquefaciens* (*B.a*), *Bacillus megaterium* (*B.m*), *Variovorax paradoxus* (*V.p*), *Pseudomonas fluorescens* (*P.f*) and *Escherichia coli* OP50 (*E.c*). In a pre-feeding trial, these bacteria were confirmed to support the growth of bacterial-feeding *Mesorhabditis* and *Acrobeloides*. As the standard food for *C. elegans* in laboratory studies, the *E.coli* OP50 was obtained from CGC (Caenorhabditis Genetics Center, USA), whereas the other four bacteria were collected from the same field as bacterial-feeding nematodes. The bacteria were obtained by first plating a serial dilution (to 10^{-6}) of soil suspension onto LB medium dishes (10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 10 g L^{-1} NaCl, and 17 g L^{-1} agar, pH 7.0). Next, bacteria were purified by transferring a single colony onto LB media in a fresh dish, growing cells at 20 °C, and repeating the procedure (transfer of a single colony, growing cells at 20 °C) for 7–8