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Effects of temperature and life stage on the fatty acid composition of Collembola

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ABSTRACT

Fatty acid (FA) analysis is used as a promising tool to investigate trophic interactions in soil food webs. The FA profile of neutral lipids in consumers is affected by the diet, and the occurrence and amount of certain FAs can reflect feeding strategies. We investigated the lipid composition of the Collembola *Folsomia candida*, *Heteromurus nitidus* and *Protaphorura fimata* with the fungus *Chaetomium globosum* as food source. The impact of environmental temperature and life stage was assessed, with special respect to linoleic acid (18:2ω6,9) as a marker FA for fungal feeding. In all Collembola species the ratio of C16/C18 in neutral lipid fatty acids (NLFAs) increased with decreasing temperature. In the NLFAs of *F. candida* and *H. nitidus* the Unsaturation Index and the amount of 18:2ω6,9 decreased with temperature, whereas in *P. fimata* effects were the opposite. The composition of phospholipid fatty acids (PLFAs) differed between species, but was little affected by temperature. The degree of unsaturation in NLFAs increased with the age of Collembola, mainly due to higher amounts of 18:2ω6,9 and a lower proportion of 18:1ω9. The biomarker linoleic acid represented over 20% of FAs in all fungal feeding Collembola. Despite considerable influence of temperature and life stage on its proportion, the amount was always higher than in individuals reared on other diets. This suggests that linoleic acid can serve as marker for fungal feeding independent of such physiological variations in Collembola.

1. Introduction

Cellular fatty acid composition has been frequently used to investigate food webs in aquatic systems [6,23,24]. Only recently this approach was applied in soil food webs (e.g. [5,3,29,30]). Several studies investigated the feeding strategies of Collembola, a widespread and abundant group of soil animals, which play an important role in decomposition processes [38]. Laboratory experiments showed that specific FAs are typically found in the neutral lipid fatty acids (NLFAs) of consumers, when reared on a certain diet [13,30]. For bacterial feeding, specific marker FAs indicate consumption of either Gram-positive (i14:0, i15:0, a15:0, i17:0) or Gram-negative (cy17:0) bacteria [13]. The FA 20:1ω9 was only present in Collembola feeding on nematodes, whereas on fungal diet they contained a higher proportion of 18:2ω6,9 and on leaf diet a higher proportion of 18:1ω9 [31].

However, not only diet can influence the FA composition of an animal [8,16]. The relative abundance of FAs in hexapods is also determined by the specific type of biosynthetic pathway of the given species [10,12], life stage [25,33], and environmental conditions [18]. Especially thermal adaptation is a known

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factor in alterations of fatty acid composition [14], but most studies deal with influence on phospholipid fatty acids (PLFAs), the main components of cell membranes, whereas changes in NLFAs are not well documented.

The changes during cold acclimation to maintain membrane viscosity and subsequently membrane transport and cell functionality are termed homeoviscous adaptation [14]. Two major metabolic compensation mechanisms exist. First, short-chain PLFAs result in higher membrane fluidity than long-chain PLFAs, leading to temperature induced changes in the ratio of C16 to C18. Second, unsaturated PLFAs result in more fluidity than saturated PLFAs, and the Unsaturation Index (UI) therefore increases with lower temperature [15].

In contrast, NLFAs have an important role as energy reserves and are closely related to nutritional requirements and metabolism [34]. The composition of NLFAs in the fat body results from different processes including the storage of dietary lipids, de novo synthesis, degradation and subsequent release for mobilisation to sites where they are metabolised [2]. Due to their use to investigate trophic relationships [13,30,31], it is important to assess if effects known to alter PLFA composition may also influence the NLFA pattern.

Collembola are known to feed on a large variety of food sources [32], but fungi are generally regarded as the most important diet [4,22]. The biomarker FA linoleic acid (18:2ω6,9) is found in high amounts in Collembola feeding on fungi, but is also found in Collembola feeding on other diets, although in lower amounts [3,13,31]. It is therefore considered as a relative biomarker for fungivory in Collembola. Physiological changes in Collembola metabolism may affect the abundance of linoleic acid, as well as related precursors and products. Linoleic acid is the precursor for eicosanoids, which play an important role in insect physiology. They influence reproduction, mediate cellular immune response and are involved in temperature regulation [35,37]. In addition, the amount of linoleic acid may be altered to maintain membrane fluidity at different environmental temperatures. We have analysed the influence of temperature and life stage on the lipid pattern in three Collembola species (*Protaphorura fimata*, *Heteromurus nitidus* and *Folsomia candida*) reared on fungal diet focusing on linoleic acid, and its reliability as trophic biomarker.

2. Materials and methods

2.1. *Fungi*

The soil decomposer fungus *Chaetomium globosum* Kunze was cultivated at 10 °C on Potato Dextrose Agar (PDA, Merck, Darmstadt). As food source for Collembola round pieces of fungal mats (diameter 10 mm) were cut out of the agar cultures under sterile conditions and offered to animals. For analysis of fungal FA composition *C. globosum* was grown on PDA covered by a membrane filter (Millipore, 0.8 μm). Cultures were kept at 5, 10 or 15 °C for 3 days before harvest to simulate the incubation conditions for fungi in the feeding experiment. To gain fungal biomass membrane filters were stripped of the agar with the adhering fungal mats. Hyphae were scrapped from the filter with a sterile scalpel and frozen at −20 °C until analysis.

2.2. *Collembola*

The Collembola species *Protaphorura fimata* (Gisin, 1952), *Heteromurus nitidus* (Templeton, 1835) and *Folsomia candida* (Willem, 1902) were taken from laboratory cultures fed with bakers yeast. Specimens were kept in plastic vessels (diameter 7 cm, height 4.5 cm) with a layer of plaster mixed with activated charcoal (2:1) at the bottom. Each vessel contained ten individuals. This low density was chosen to avoid cannibalism due to crowding. Vessels were kept in darkness and under moist conditions with distilled water (pH 7). Eggs, pellets and exuvia were removed once a week. To investigate the effect of temperature, Collembola were incubated at 5, 10 and 15 °C. Animals were fed with fungal diet ad libitum, thereby the fungal food source was renewed three times a week. After 6 weeks Collembola were sampled destructively and frozen at −20 °C until analysis. Three replicates (with 30 individuals each) per temperature were performed.

To study the influence of different life stages, eggs of *P. fimata* were kept in plastic vessels until hatching. Collembola hatched within one week were joined into the same life stage group. Eggs and newly hatched Collembola were removed once a week to insure cohorts of comparable age. Specimens were fed with *C. globosum* for 4, 8 and 12 weeks after hatching. Three replicates (with 30 individuals each) per developmental stage were performed and harvested destructively. Collembola were frozen at −20 °C until analysis.

2.3. *Analysis of fatty acid patterns*

Whole cellular lipids of fungi were extracted, whereas lipids of Collembola were divided into NLFA and PLFA fractions. Collembolan lipids were extracted by shaking in 5 ml single phase extraction solvent (chloroform/methanol/0.05 M phosphate buffer (pH 7.4) 1:2:0.8) overnight. The solvent was then transferred to new tubes and samples were re-extracted by shaking for 2–3 h with additional 2.5 ml. Extraction solvents of both steps were combined, 0.8 ml distilled water and 0.8 ml $CHCl_3$ were added and samples centrifuged at 1500 rpm for 5 min. Samples were allowed to stand and separate. Then the top two phases were removed and the chloroform fraction of each sample was transferred to a silica acid column (0.5 g silicic acid, mesh size 100–200 μm). Lipids were eluted with 5 ml chloroform (neutral lipids), 8 ml acetone (glycolipids) and 5 ml methanol (phospholipids). Neutral lipids and phospholipids were analysed further. The chloroform and methanol fraction was reduced by evaporation (50 °C, vacuum 200 hPa) in a Labconco RapidVap (Labconco Corp., Kansas City).

Chloroform and methanol fractions of the Collembola samples and total fungal biomass were saponified and methylated following the procedures given for the Sherlock Microbial Identification System (MIDI Inc., Newark, DE). Saponification of lipids was conducted in a sodium hydroxide/methanol solution (45 g sodium hydroxide, 150 ml methanol, 150 ml distilled water) at 100 °C for 30 min, followed by acid methanolysis in HCl/methanol (325 ml 6.0 N hydrochloric