Trophic niche differentiation, sex ratio and phylogeography of European Collembola

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Masoumeh Chahartaghi-Abnieh (M.Sc.)

aus Tehran, Iran

Referent: Prof. Dr. Stefan Scheu
Koreferent: Dr.-habil. Liliane Ruess

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D17

“You thought Collembola were not popular, didn't you?”

Sminthurus pilleatus: lateral view

Jewellery design by Sophia Chen, 2000

From: Checklist of the Collembola of the World by Bellinger, P.F., Christiansen, K.A. and Janssens, F. 1996-2007.

http://www.collembola.org

Publications resulting from this dissertation

Chapter 2

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Chapter 3

Chahartaghi, M., Scheu, S., Ruess, L., 2006. Sex ratio and mode of reproduction in Collembola of an oak-beech forest. Pedobiologia 50, 331-340.

Chapter 4

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Further Publication

Chapter 5

Chahartaghi, M., Schaefer, I., Scheu, S., 2007. Phylogeography of European Collembola.

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit, abgesehen von den in ihr ausdrücklich genannten Hilfen, selbständig verfasst habe.

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Datum Unterschrift

SUMMARY

Collembola are important soil-dwelling animals reaching high diversity and density. For understanding driving factors for Collembola density and diversity this study investigated (1) trophic niche differentiation of Collembola species using stable isotope analysis, (2) mode of reproduction and sex ratios in the field, (3) colonization of new habitats by parthenogenetic and sexual species and (4) the genetic variation in parthenogenetic and sexual species in Europe.

To evaluate trophic niche differentiation the natural variation in nitrogen isotopes was assessed in 20 Collembola taxa from three deciduous forests stands. The $\delta^{15}N$ gradient spanned over 9 δ units, which implies a wide range in food sources used. Assuming a shift in ^{15}N of about 3 ‰ per trophic level, the results indicate a range of three trophic levels. The $\delta^{15}N$ signature formed a continuum from phycophages/herbivores to primary and secondary decomposers, reflecting a gradual shift from more detrital to more microbial diets. These results suggest that trophic niche differentiation is an important mechanism for the maintenance of the high number of Collembola species in forst ecosystems.

The sex ratios of Collembola species were assessed in a temperate oak-beech forest in two months intervals during one year. A total of 6 species, including the abundant *Mesaphorura machrochaeta, Parisotoma notabilis, Neanura muscorum* and *Isotomiella minor* formed pure female populations suggesting that they reproduced by parthenogenesis. A total of 22 species including the common species *Folsomia quadrioculata, Protaphorura fimata* and *Lepidocyrtus lignorum* formed bisexual populations, suggesting that sexual reproduction predominates in

Collembola of the studied forest. In agreement with earlier studies parthenogenetic species predominated deeper in the soil (euedaphic species), but some parthenogenetic species were hemiedaphic. The sex ratio of bisexual Collembola species in the litter layer generally was more female biased than that in the mineral soil. Presumably, females concentrate at sites with high density of resources whereas males are relatively more abundant at sites more favourable for spermatophore placement.

Sexual and parthenogenetic species may respond differently to environmental changes, e.g. to the availability of resources. We hypothesized that parthenogenetic species are more sensitive to resource depletion than sexual species, and that they will colonize available habitats faster due to their faster mode of reproduction. In contrast to our hypotheses, parthenogenetic and sexual Collembola species were similarly affected by resource depletion. In agreement with our hypothesis, the proportion of parthenogenetic species increased with time when free habitats and plenty of resources were available, indicating that parthenogenetic species are faster colonizers.

Intraspecefic genetic variation was investigated using molecular markers, mtDNA (COI) in two sexual species, *Folsomia quadrioculata* and *Ceratophysella denticulata*, and two parthenogenetic species, *Parisotoma notabilis* and *Isotomiella minor*. The variation of mtDNA (COI) showed Collembola species comprises of ancient lineages which colonized Europe in particular southern and central Europe in the pre-Pleistocene irrespective of the mode of reproduction. In each of the species studied lineages were separated by deep splits, especially in central and southern regions, suggesting that the colonization of Europe by these species

predates the Pleistocene, potentially dating back to the lower Tertiary. Recent colonization by Collembola species of some locations especially in the north including Scandinavia, Marion Island, Ringnes Island and Siberia was inferred. The hypothesis that central European Collembola populations originated from southern refugia after the last glaciation was rejected. The deep splits in each of the four Collembola species studied indicate that Collembola species in general constitute of a number of cryptic species with complex phylogeographic history.

Zusammenfassung

Springschwänze (Collembola) sind wichtige bodenlebende Tiere, die eine hohe Diversität und Dichte erreichen. Um die Faktoren, die für Dichte und Diversität verantwortlich sind zu verstehen, wurden im Rahmen dieser Arbeit folgende Untersuchungen vorgenommen (1) trophische Nischendifferenzierung, (2) Art und Weise der Reproduktion und Geschlechterverhältnisse im Feld, (3) Kolonisierung von neuen Habitaten durch parthenogenetische und sexuelle Arten und (4) die genetische Variation in parthenogenetischen und sexuellen Arten in Europa.

Zur Untersuchung der trophischen Nischendifferenzierung wurde die natürliche Variation von Stickstoffisotopen in 20 Springschwanztaxa von drei Laubwäldern gemessen. Der $\delta^{15}N$ Gradient schloss 9 δ-Einheiten ein, was auf die Nutzung einer großen Auswahl von Nahrungsquellen hindeutet. Unter der Annahme, dass pro trophischer Ebene eine ^{15}N Anreicherung von 3 ‰ stattfindet, zeigen unsere Ergebnisse, dass Springschwänze drei trophische Ebenen einnehmen. Die $\delta^{15}N$ Signatur zeigte ein Kontinuum von Phycophagie/Herbivorie zu primären und sekundären Zersetzern, und spiegelt einen abgestuften Wechsel von einer Ernährungsweise als Zersetzer zu einer mikrobiellen Ernährung wider. Diese Ergebnisse zeigen, dass trophische Nischendifferenzierung ein wichtiger Mechanismus für den Erhalt der hohen Diversität von Springschwanzarten in Waldökosystemen ist.

Die Geschlechterverhältnisse von Springschwanzarten wurden über ein Jahr in einem temperierten Eichen-Buchen-Wald im Intervall von zwei Monaten aufgenommen. Insgesamt sechs Arten, darunter die abundanten Arten *Mesaphorura macrochaeta*, *Parisotoma notabilis*, *Neanura muscorum* und *Isotomiella minor*

bildeten reine Weibchenpopulationen, was auf parthenogenetische Reproduktion hinweist. Insgesamt 22 Arten, darunter die häufigen Arten *Folsomia quadrioculata*, *Protaphorura fimata* und *Lepidocyrtus lignorum* bildeten zweigeschlechtliche Populationen, was auf darauf hinweist, dass sexuelle Reproduktion bei Springschwänzen im untersuchten Wald vorherrscht. In Übereinstimmung mit früheren Untersuchungen dominierten parthenogenetische Arten in tieferen Bodenschichten (euedaphische Arten), aber einige parthenogenetische Arten waren hemiedaphisch. Bei zweigeschlechtlichen Springschwanzarten war das Geschlechterverhältnis in der Streuschicht im Vergleich zu tieferen Bodenschichten allgemein zu mehr Weibchen verschoben. Vermutlich sammeln sich Weibchen an Orten mit hoher Ressourcendichte, während Männchen eher an Orten häufig sind, die für die Ablage von Spermatophoren geeignet sind.

Wir hypothetisierten, dass parthenogenetische Arten sensibler auf die Erschöpfung von Ressourcen reagieren als sexuelle Arten und dass parthenogenetische Arten aufgrund ihrer schnelleren Reproduktion zugängliche Habitate schneller kolonisieren als sexuelle Arten. Im Gegensatz zu unserer Hypothese waren parthenogenetische und sexuelle Springschwanzarten durch Erschöpfung von Ressourcen ähnlich beeinflusst. In Übereinstimmung mit unserer Hypothese stieg der Anteil parthenogenetischer Arten an wenn freie Habitate und viele Ressourcen zur Verfügung standen, was auf das schnellere Kolonisierungspotenzial von parthenogenetischen Arten hinweist.

Intraspezifische genetische Variation wurde mit molekularen Markern, mtDNA (COI), in zwei sexuellen Arten, *Folsomia quadrioculata* und *Ceratophysella denticulata*, und zwei parthenogenetischen Arten, *Parisotoma notabili*s und

Isotomiella minor, untersucht. Die Variation von mtDNA (COI) zeigte, dass Springschwanzarten aus alten Linien bestehen, die insbesondere Süd- und Mitteleuropa vor dem Pleistozän besiedelten; dies erfolgte unabhängig von ihrem Reproduktionstyp. In jeder der untersuchten Arten wurden Linien durch tiefe Splits getrennt, besonders in Individuen aus Zentral- und Südeuropa. Dies deutet darauf hin, dass die Kolonisierung Europas durch diese Arten vor dem Pleistozän geschah, möglicherweise bereits im unteren Tertiär. Regionen im Norden, darunter Skandinavien, Ringness Island und Sibirien, wurden dagegen erst in jüngerer Zeit besiedelt. Die Hypothese, dass die Springschwanzpopulationen Mitteleuropas aus südlichen Refugien stammen, die nach der letzten Eiszeit eingewandert sind, wurde verworfen. Die tiefen Splits in jeder der vier untersuchten Springschwanzarten zeigten, dass diese Arten aus einer Anzahl von kryptischen Arten mit komplexer phylogeographischen Geschichte bestehen.

Contents

Chapter 1

1 General introduction

Soil is a complex environment colonized by an extreme diversity of organisms. Soil organisms collectively referred to as soil biota include soil viruses, bacteria, actinomycetes, fungi, protozoa, nematodes, mites, Collembola and other microarthropods. The surface layers of soil contain the highest numbers and variety of microorganisms, because these layers receive the largest amounts of resources from plants and animals. Soil biota form a belowground system based on the energy and nutrients that they receive from the decomposition of plant and animal tissues. In fact, aboveground systems are responsible for most of the production (carbon input) in an ecosystem, whereas belowground systems are responsible for most of decomposition (carbon loss). After plants have established, decomposition by microorganisms recycles carbon and nutrients in dead plant and animal tissues into forms usable by plants (Wardle et al., 2004). Bacteria and fungi constitute the basal trophic level of most food chains in soil classified as primary decomposers (Bardgett, 2005). They are directly involved in the cycling of elements, such as nitrogen, carbon, sulfur, phosphorus, iron, and micronutrient trace elements. By feeding and comminuting dead plant material soil invertebrates, such as mites and Collembola, in indirect ways affect decomposition

processes and nutrient cycling (Behan and Hill, 1978; Seastedt, 1984; Moore et al., 1988; Maraun et al., 1998). Therefore, soil biota play a key role in the processing of materials that maintain life on the Earth. Despite the vital role of soil biota the structure and functioning of decomposer communities are little understood (Young and Crawford, 2004; Bardgett, 2005). One of the most important soil biota are Collembola which are present in large numbers in virtually any soil. Collembola have been shown to affect decomposition processes and nutrient cycling (Parkinson, 1988; Visser, 1985; Petersen, 2002; Filser et al., 2002).

The present study investigates focal ecological and evolutionary aspects of Collembola. The investigations focus on four topics (1) trophic niche differentiation, (2) sex ratio, (3) recolonization of new habitats, and (4) phylogeography of European Collembola. Collembola reach high diversity (average of about 40-50 species per site; Hopkin, 1977) and high density (up to several million individuals per square meter in forest soils, Petersen and Luxton, 1982) in soil. However, there is no reliable evidence for niche differentiation on the major niche axes, such as food, time and space (Luxton, 1972; Walter and Proctor, 1999). Collembola are regarded general feeders suggesting that trophic niches of Collembola species overlap widely. To test this assumption natural variation in stable isotopes (^{15}N) in Collembola were investigated.

Most of the Collembola species reproduce sexually; however there are several reports for the presence of parthenogenesis. In fact large number of Collembola in terrestrial ecosystems likely reproduces by parthenogenesis (Goto, 1960; Petersen, 1978; Petersen, 2000). This mode of reproduction permits the animals to increase their density quickly in a stable soil environment. Investigation of the sex ratio of Collembola was performed to evaluate how many species in a deciduous forest site

(Kranichstein, Germany) reproduce by parthenogenesis. In systems where food resources deplete over time, parthenogenetic species likely suffer more than sexual species. Generally, sexual species have higher genetic diversity than parthenogenetic species because of recombination. On the other hand, parthenogenetic species might be faster colonizers than sexual species due to the absence of males. I investigated whether parthenogenetic and sexual species suffer differently from the depletion of food resources. Further, the speed of colonization of species with different reproductive strategies into defaunated soil and litter was investigated.

The variability of the mitochondrial DNA (COI) of parthenogenetic and sexual species from different locations of Europe was studied to reconstruct phylogenetic relationships between different populations and to get insight into the postglacial colonization of southern, central and northern Europe by Collembola.

1.1 Collembola

Collembola are among the most widespread and abundant terrestrial microarthropods. They are small (0.12-10 mm), entognathous (mouthparts located within a gnathal pouch), wingless hexapods with antennae always present. Most but not all Collembola may be recognised by a posterior ventral forked abdominal appendage, the furca. They are a very old taxon; indirect fossils date Collembola back to 412 million years suggesting that Collembola were an important component of the earliest terrestrial ecosystems (Hopkin, 1997).

There is a little doubt that Collembola are a monophyletic group. Collembola, Protura and Diplura (Entognatha) together with Archaeognatha and Zygentoma (Thysanura)

constitute the group of Apterygota which is paraphyletic (Hopkin, 1977). Recent molecular analysis indicates that Ellipura (Protura and Collembola) and Entognatha form monophyletic assemblages and also suggests close relationship between Collembola and Diplura (Carapelli et al., 2000). Worldwide about 7,000 species of Collembola are described, and species richness per site ranges from 3 to 60 depending on the ecosystem (Rusek, 1998). They occur everywhere, from seashores to mountain tops, from tropical rainforests to the arctic. Except few reports indicating Collembola being pest on crops (see e.g., Hopkin, 1977), they are considered beneficial. Collembola can be used as bioindicators in polluted areas or as test organisms for new chemicals (Hopkin, 1977). Recent studies showed that Collembola influence root morphology of plants, roots grow longer and thinner and have more root tips (Endlweber and Scheu, 2006). These changes likely are related to the exploitation of nutrient rich patches in soil such as Collembola faecal pellets. By feeding on dead plant material and the resulting comminution of it, Collembola play an important role in decomposition processes, nutrient cycling, in forming soil microstructures and in modifying plant growth, and thus received considerable attention (Parkinson, 1988; Visser, 1985; Petersen, 2002; Filser et al., 2002).

1.2 Diversity and trophic niche differentiation

The large number of microbes and animals that live in soil constitute the soil food web which plays an important role in terrestrial ecosystems (Bardgett, 2005). The high diversity and density of soil animals without apparent competition is one of the great enigmas of soil biology (Anderson 1975a). Species diversity can be measured as local (alpha) and regional (gamma) diversity (MacArthur 1965, Whittaker 1972). Alpha-

diversity is the number of species in a certain habitat whereas gamma-diversity is the total number of species occurring in all habitats within a region. In forest soils, microarthropods, such as Collembola and oribatid mites, have high alpha-diversity. Niche differentiation is one of the most prominent mechanisms responsible for the high diversity of animals. The niche space is defined as an n-dimensional space which comprises dimension such as space, time, temperature and resources (Hutchinson, 1957). Absence of niche differentiation increases the competitions among animals and therefore likely increases extinction of species. The partitioning of resources, time and space is one way to reduce competition and allow the coexistence of large numbers of species in soil (Solem, 1984; Gittenberger, 1991).

Different methods have been established to evaluate trophic niche differentiation in soil animals. Different feeding guilds have been established based on direct observations and morphological characteristics. Due to small size of most soil animals and their cryptic way of life, most studies have been done in the laboratory with the result obtained difficult to prove under field conditions. More recently, new methodologies, such as fatty acid analysis and stable isotopic ratios, have been used to investigate feeding strategies in soil animals. Fatty acid (FA) analysis is used as qualitative markers to trace or confirm predator-pray relationship whereas isotope ratios are used to ascribe animals to trophic guilds (Ruess et. al., 2002, 2004, 2005; Haubert et. al., 2006).

Naturally occurring stable isotopes of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) provide insight into dietary and trophic relationships within food webs (Minagawa and Wada, 1984; Gannes et. al., 1998, Peterson, 1999). In the metabolism of nitrogen, the light isotope is concentrated in nitrogenous excretion products while the heavy isotope

is discriminated against and retained in body tissues (Peterson and Fry, 1987; Peterson, 1999). Therefore, animals higher in food chains are enriched in the heavier isotope compared to animals at lower trophic levels. Measured as per mill deviation from a standard (see Chapter 2.2.3) this enrichment of the heavier nitrogen isotope is in the range of 2-5‰ per trophic level but in most studies an enrichment of 3.4‰ is used to ascribe animals to rophic levels (Minagawa and Wada, 1984; Wada et al., 1991; Eggers and Jones, 2000, Post 2002). This method frequently has been used for aboveground animals. For the first time, Scheu and Falca (2000) and Ponsard and Arditi (2000) used variations in nitrogen stable isotope ratios to differenciate trophic levels in soil animal communities. Fig. 1.1 shows the trophic organization of the soil animal community of a beech forest as indicated by $^{15}N/^{14}N$ ratios in animal tissue. In contrast to other investigations on feeding strategies, such as gut content analyses and food choice experiments, nitrogen stable isotope ratios reflect the long-term trophic relationship of animals (Scheu and Falca, 2000).

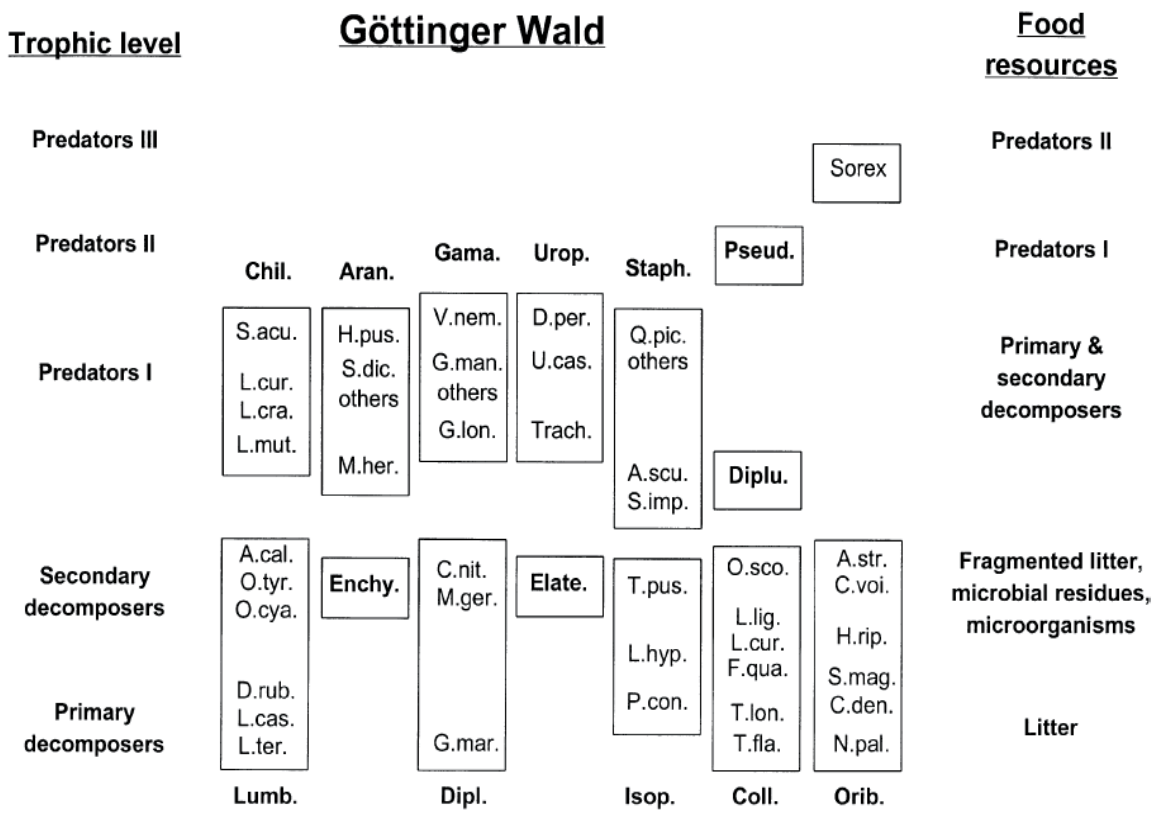


Fig. 1-1: Trophic structure of the decomposer community of a beech wood on limestone (‘Göttinger Wald') as indicated by variations in the natural abundance of ^{15}N in animal tissue (from Scheu and Falca, 2000). Aran: Araneida; Chil: Chilopoda; Coll: Collembola; Dipl: Diplopoda; Diplu: Diplura; Elate: elaterid larvae (Coleoptera); Enchy: Enchytraeidae; Gama: Gamasina; Isop: Isopoda; Lumb: Lumbricidae; Orib: Oribatida; Pseud: Pseudoscorpionida; Staph: Staphylinidae (Coleoptera); Urop: Uropodina; for full species names see Scheu and Falca (2000).

1.3 Sex ratio and population dynamics of parthenogenetic and sexual species

Sexual and parthenonenetic reproduction are two different ways to produce offspring. Parthenogenetic species are generally rare in the animal kingdom; most animals reproduce bisexually (Koivisto and Braig, 2003). A number of theories have been put

forward to explain the prevalence of sexual reproduction (Maynard Smith, 1978; Bell, 1982; Barraclough et al., 2003; Omilian et al., 2006). Advantages of sexual reproduction are assumed to be due to recombination and the resulting genetic diversity of progeny (Bell, 1982; Pound et al., 2002). On the other hand parthenogenesis is advantageous, e.g. by doubling the number of offspring due to not producing males (Maynard Smith, 1978; Bell, 1982; Butlin et al., 1998).

Parthenogenesis is a common mode of the reproduction among soil-living animals including macrofauna such as earthworm and mesofauna such as Collembola and oribatid mites (Goto, 1960; Petersen, 1978; Norton and Palmer 1991; Siepel 1994; Niklasson et al. 2000; Petersen, 2002; Bloszyk et al. 2004). Due to differential speed of reproduction of sexual and parthenogenetic species the mode of reproduction needs to be considered for understanding population dynamics and for disentangling the reasons of high density of mesofauna such as Collembola and oribatid mites in soil.

Collembola are small, many of them are r-selected, produce many eggs suggesting that many species reproduce by parthenogenesis (Goto 1960; Suomalainen et al., 1987). For evaluating this hypothesis the sex ratio of Collembola population in the field needs to be investigated. Furthermore, knowledge of the reproductive mode may allow a better understanding of how Collembola species respond to changes in ecological factors. Ecological factors such as food availability affect the population dynamics of parthenogenetic species and bisexual species in different ways. Parthenogenetic species may suffer more from low availability of food resources due to low genetic diversity as compared to sexual species. Due to recombination and the resulting increase in genetic diversity sexual species are likely to react more flexible to changes in the amount and

quality of resources. On the other hand, due to faster reproduction parthenogenetic species are likely faster colonizers than sexual species.

1.4 Phylogeography of Collembola

Distribution of animal and plant in many parts of the world have remarkably modified after the last glaciation (Stewart and Lister, 2001). In southen but also central Europe refugia played an important role harbouring species during glaciation. Later they played an important role as origin of species during northward expansion. In fact, the present species of north Europe comprise of those that survived in the north and those returning from central and southern refugia. Different methods, such as molecular markers, have been used to investigate the postglacial colonization of plants and animals (Hewitt, 1993; Harrison, 2004; Allegrucci et al., 2005). Molecular phylogeography is a powerful method because it allows formally testing of evolutionary hypotheses on the distribution of species (Byun et al., 1997; Strange and Burr, 1997; Wenink et al., 1996; Zamudio et al., 1997). Most of the studies concentrated on animals in aboveground systems, little attention has been paid to postglacial recolonization of soil animals.

The mitochondrial DNA (COI) as molecular marker has been used in many studies for evaluating phylogenetic relationships. In this study I used COI to investigate genetic variability between countries and to reconstruct the colonization of Europe by Collembola after the last glaciation. The mode of reproduction strongly impacts the colonization of new habitats by animals and plants. Parthenogenetic species usually are faster colonizers (Williams, 1975; Bell, 1982; Scheu and Schulz, 1996; Lindberg and Bengtsson, 2005) while sexual species may be more vigorous in colonizing habitats with fluctuating environmental conditions (Bell, 1982; Pound et al., 2002). I include

two sexual and two parthenogenetic species to investigate whether the mode of reproduction affected post-glacial recolonization patterns and present-day within-species genetic variability.

Chapter 2

2 Feeding guilds in Collembola based on nitrogen stable isotope ratios

Summary

In soil a high number of species exist without extensive niche differentiation, which was assigned as “the enigma of soil animal species diversity”. In particular, the detritivores are regarded as food generalists. We have investigated nitrogen stable isotope ratios ($^{15}N/^{14}N$) of a major decomposer group, the Collembola, to evaluate trophic relationship and determine feeding guilds. Additionally, the $\delta^{15}N$ values of potential food sources such as mosses, lichens and other plant derived material (bark, nuts, and leaves) were analysed. The natural variation in nitrogen isotopes was assessed in 20 Collembola taxa from three deciduous forest stands. The $\delta^{15}N$ signature formed a continuum from phycophages/herbivores to primary and secondary decomposers, reflecting a gradual shift from more detrital to more microbial diets. The $\delta^{15}N$ gradient spanned over 9 δ units, which implies a wide range in food sources used. Assuming a shift in ^{15}N of about 3 ‰ per trophic level, the results indicate a range of three trophic

levels. These variations in $^{15}N/^{14}N$ ratios suggest that trophic niches of Collembola species differ and this likely contributes to Collembola species diversity.

2.1 Introduction

Collembola are among the most abundant soil-dwelling arthropods with densities up to several million individuals per square metre in forest soils (Petersen and Luxton, 1982). Worldwide about 7,000 species are described, and species richness per site ranges from 3 to 60 depending on the ecosystem (Rusek, 1998). Although decomposition is mainly due to microbial activity, the soil fauna is an important driver of these processes by conditioning the litter and stimulating microbial activity. Collembola play an important role in plant litter decomposition and in forming soil microstructure (Visser, 1985; Klironomos and Kendrick, 1995; Rusek, 1998). They affect nutrient cycling through litter comminution, dissemination of microorganisms and grazing (Moore et al., 1987; Addison et al., 2003).

Generally, soil detritivores are regarded as food generalists with a low degree in nutritional specialisation (Scheu and Setälä, 2002). Studies on feeding strategies in Collembola concluded that the majority of euedaphic and hemiedaphic species feed unselectively on a wide variety of food materials (Hopkin, 1997). Depending on the resources available, they ingest bacteria, fungi, algae, plant litter, or other soil animals, such as protozoa, nematodes, rotifers, and enchytraeids (Parkinson, 1988; Rusek, 1998; Scheu 2002). Several studies have demonstrated the importance of fungi in Collembolan nutrition (Visser et al., 1987; Chen et al., 1995; Klironomos and Kendrick, 1995). However, other studies documented preferences for certain types of fungi in

some species of Collembola (Visser and Whittaker, 1977; Hiol et al., 1994; Thimm and Larink, 1995; Sadaka-Laulan et al., 1998). Additionally, Lee and Widden (1996) showed that species such as *Folsomia candida,* which commonly are assumed to consume fungi, preferentially feed on nematodes when offered a choice. Overall, this suggests that trophic relationships are unspecific with a broad overlap in resources and that to ascribe Collembola species to trophic levels or feeding guilds is difficult.

Collembola diets are usually verified by analysis of gut contents or by observations of feeding behaviour in laboratory experiments. Due to this the assigned feeding guilds often reflect more taxonomic rather than functional relationships. Besides, feeding preferences found in the laboratory are difficult to prove under field conditions. Over the last decade nitrogen stable isotope analysis has been used as powerful tool in food web studies (Gannes et al., 1997, 1998; Ponsard and Arditi, 2000). Isotopic ratios of $^{15}N/^{14}N$ were applied to ascribe animals to trophic levels, as the $^{15}N/^{14}N$ ratios of consumers exceed those of their diets. Within food chains this results in a stepwise enrichment of the heavier nitrogen isotope of about 3‰ per trophic level (Minagawa and Wada, 1984; Wada et al., 1991; Eggers and Jones, 2000, Post 2002). Furthermore, in contrast to gut content analyses and food choice experiments, nitrogen stable isotope ratios reflect the long-term trophic relationship of animals (Scheu and Falca, 2000). In sum this technique allows *in situ* investigation of diet history within a broader window of time.

In this study we determined the $^{15}N/^{14}N$ signatures in Collembola collected from three different deciduous forest sites. The aim of the study was to analyse if *(i)* various species in a community differ in their stable isotope ratio indicating separate feeding strategies, *(ii)* different niches with respect to food resource are irrespective of forest

type, and *(iii)* species can be classified in consistent feeding guilds according to stable isotope ratios.

2.2 Materials and methods

2.2.1 Study sites

The Collembola were obtained from litter samples of three forest stands, the Kranichsteiner Wald (K), the Göttinger Wald (G) and the Solling (S). The Kranichsteiner Wald is an oak-beech forest located 8 km northeast of Darmstadt, South Germany, at 150-175 m a.s.l. Parent rock is rothliegendes covered with sand. The soil types are dystric gleysols and orthic luvisols (FAO-UNESCO classification); the humus form is moder. The pH of the soil varies between 3.6 and 4.3. The tree layer is dominated by oak (*Quercus robur*), about 190 years old, with interspersed beech (*Fagus sylvatica*). The understory is dominated by hornbeam (*Carpinus betulus*), about 125 years old. The herb layer is dominated by *Luzula luzuloides*, *Milium effusum*, *Anemone nemorosa*, *Oxalis acetosella*, *Deschampsia cespitosa*, *Stellaria holostea*, *Melampyrum pratense* and *Polytrichum formosum*.

The Göttinger Wald is a 130 years old beech forest (*F. sylvatica*) located on a limestone plateau east of Göttingen (Lower Saxony, Germany) at 420 m a.s.l. The soil is an orthic rendzina type with mull humus. The soil pH ranges from 4.4 to 7.0 with an average of 5.3. Among the beech, maple (*Acer platanoides* and *A. pseudoplatanus*) and ash trees (*Fraxinus excelsior*) are interspersed. The species rich herb layer is dominated by *Allium ursinum*, *Mercurialis perennis* and *Anemone nemorosa*.

The Solling is a mature beech stand about 135 years old, located 50 km northwest of Göttingen on a mountain range at 500 m a.s.l. Parent rock is sandstone covered with

a loess layer of about 1 m. The soil type is a dystric cambisol, the humus form is moder. The soil pH ranges between 3.3 and 4.4. The understory is formed mainly by small patches of *Luzulua luzuloides*.

2.2.2 Sampling

The sampling was carried out in all three forest stands in the L/F layer in October 2003. Collembola were extracted by heat at 45 °C for two days with a Kempson Extractor (Kempson et al., 1963). Animals were collected in water and separated under a dissecting microscope. Collembola were determined to species or higher taxonomic level, and stored in a concentrated NaCl solution until analysis.

For measurement of nitrogen isotope ratios and total N content the specimens were transferred into tin capsules and dried at 60 °C for 48 h. Samples were weighed and stored in a desiccator. Generally three replicates were analysed, whereby each sample consisted of pooled individuals (between 1 to 120 specimens based on body size of the species) to obtain sufficient material for ^{15}N analysis.

Samples of potential food sources for Collembola were taken in the forest stand in Kranichstein. Algae, lichens and different materials originating from trees (bark, branches, rooting wood, nuts, leaves) were collected, dried at 60 °C for 72 h, and ground by a blender. Three replicates each were analysed for nitrogen stable isotopes.

2.2.3 ^{15}N analysis

The $^{15}N/^{14}N$ ratios and total N content of samples were determined by a coupled system of an elemental analyser (NA 1500, Carlo Erba, Milan) and a mass spectrometer (MAT 251, Finnigan). Stable isotope abundance is expressed using the δ notation with

$\delta^{15}N$ (‰) = ($R_{sample} - R_{standard}$) / $R_{standard}$ x 1000. R_{sample} and $R_{standard}$ represent the $^{15}N/^{14}N$ ratios of the sample and standard, respectively. Atmospheric nitrogen served as the primary standard and acetanilide (C_8H_9NO, Merck, Darmstadt) for internal calibration.

2.2.4 Statistical analysis

Data on body weight, N content and $\delta^{15}N$ were subjected to correlation analysis using Pearson´s correlation coefficient. Additionally, $\delta^{15}N$ in Collembolan species was analysed using ANOVA. If significant differences were found, pairs of treatments were compared by Tukey´s HSD test. Statistical analyses were performed using JMP 3.1 for Macintosh (SAS Institute Inc., Cary, USA).

2.3 Results

2.3.1 Nitrogen stable isotopes of potential food sources

Potential plant derived food sources for Collembola were investigated at the Kranichsteiner Wald. Algae and lichens, collected from the bark of trees close to the ground, had the lowest content in $\delta^{15}N$ with –10.4 and –12.8, respectively (Table 2.1). The $\delta^{15}N$ signals in mosses as well as most other plant originating specimens (e.g. bark, nuts) were in the range between –6.2 and –6.7, whereas rotting wood was slightly enriched (-5.8) and entire leaves slightly depleated (-7.3). The ^{15}N signature of the L/F soil horizon from the Solling, the Göttinger Wald and the Kranichsteiner Wald were -3.6 ± 0.1, -3.7 ± 0.3 and -4.2 ± 1.0, respectively (see Schneider et al., 2004).

Table 2-1: ^{15}N (‰ ± s.d.) of potential food sources for Collembola collected at the forest stand in Kranichstein.

	$\delta^{15}N$
Algae	-10.4 ± 0.4
Lichens	-12.8 ± 0.1
Mosses (on tree trunk)	-6.5 ± 0.1
Mosses (on the ground)	-6.2 ± 0.1
Rotting wood	-5.8 ± 0.4
Bark	-6.5 ± 0.1
Woody branches	-6.4 ± 0.3
Acorns	-6.5 ± 0.1
Beechnuts	-6.6 ± 0.1
Entire leaves (without visible damage)	-7.3 ± 0.1
Skeletonized leaves (gnawed, brocken up)	-6.7 ± 0.1

2.3.2 Nitrogen content and $\delta^{15}N$ signature of Collembola

A total of 20 different Collembola taxa were studied in the different forest stands (Table 2.2). The mean biomass of individuals varied greatly from 1.2 to 212 μg ind^{-1}. The smallest species were *Isotoma violacea* and *Sminthurinus aureus* and the biggest was *Tomocerus longicornis*. Most taxa had a body weight between 10 and 25 μg ind^{-1}. The mean total nitrogen content was fairly homogeneous across taxa with 8.8 to 15.3%, whereas the mean $\delta^{15}N$ signatures spanned from –5.6 to 1.2‰. The genus *Neanura* showed the highest nitrogen concentration with 12.8 to 15.3%, and a high $\delta^{15}N$ signature with 0.3 and 1.2‰ for *Neanura muscorum* and *Neanura villosa*, respectively. However, total N and $\delta^{15}N$ in Collembola were not correlated (r = -0.0445, P = 0.747).

Comparably body weight showed no correlation to nitrogen content (r = 0.0254, P = 0.854) and only a weak negative relationship to $\delta^{15}N$ (r = -0.2622, P = 0.053).

Table 2-2: Dry weight per indivdiual (µg ± s.d.), total amount of nitrogen (% ± s.d.) and $\delta^{15}N$ (‰ ± s.d.) in different Collembolan taxa. Means of the three different forest sites studied. n.d. – not determined.

Taxa	Dry weight/ind (mg)	N content (%)	$\delta^{15}N$
Ceratophysella denticulata	10.7 ± 3.3	10.5 ± 1.0	-0.8 ± 0.3
Ceratophysella spp.	10.9 ± 4.0	11.1 ± 0.4	0.3 ± 1.9
Dicyrtoma fusca	11.4 ± 0.9	n.d	-4.8 ± 0.7
Dicyrtomina minuta	7.6 ± 1.2	13.9 ± 0.2	-5.0 ± 1.6
Entomobrya corticalis	15.4 ± 5.1	9.8	-0.4 ± 0.1
Entomobrya muscorum	41.4 ± 24.5	9.7 ± 2.2	-2.5 ± 1.1
Folsomia quadrioculata	6.7 ± 3.3	9.2 ± 2.6	-3.8 ± 1.4
Hypogastrura burkilli	39.0	9.2	-1.6
Isotoma violacea	1.9 ± 0.8	n.d	-2.2 ± 0.6
Lepidocyrtus lignorum	23.1 ± 8.4	10.0 ± 1.0	-1.9 ± 0.5
Lepidocyrtus sp.	19.7 ± 4.9	10.3	-1.7 ± 1.0
Neanura muscorum	26.6 ± 13.5	12.8 ± 1.4	0.3 ± 0.2
Neanura villosa	20.0	15.3	1.2
Fam. Onychiuridae	17.5 ± 4.6	10.5 ± 1.1	-1.1 ± 0.9
Orchesella flavescens	80.7 ± 22.5	11.5 ± 1.0	-3.6 ± 0.6
Parisotoma notabilis	7.5 ± 8.7	8.8	-1.6 ± 0.5
Pseudachorutes sp.	21.3	11.5	0.8
Sminthurinus aureus	1.2 ± 0.5	n.d	-5.6 ± 0.9
Tomocerus longicornis	212.2 ± 103.2	11.6 ± 1.6	-3.3 ± 0.9
Tomocerus flavescens	42.1 ± 7.7	12.4 ± 1.1	-2.4 ± 0.1

Of the 20 Collembola taxa analysed, seven occurred in all three, and four in two of the forests stands investigated, see Fig. 2.1. To allow comparison of ^{15}N values for species from the different forest stands, the ^{15}N values of the animals from the Kranichsteiner and the Göttinger Wald were calibrated to that of the ^{15}N signature of the L/F-layer in the Solling (-3.6). Overall, the $\delta^{15}N$ values formed a continuous gradient from –7.2 to 1.7‰, spanning in total 9 δ units. In the Göttinger Wald, $\delta^{15}N$ ranged

between -3.2‰ in *Orchesella flavescens* and 1.7‰ in *Ceratophysella* sp. In the Solling $\delta^{15}N$ signatures varied between -7.2‰ in *Dicrytomina minuta* and 0.8 in *Pseudachorutes* sp. In the Kranichsteiner Wald the range was between -5.6‰ in *S. aureus* and 1.2‰ in *N. villosa*. The $\delta^{15}N$ values in Collembola communities differed significantly between forest stands with the Kranichsteiner Wald most depleted in the heavier isotope ($F_{2,71}$ = 3.678, P = 0.03).

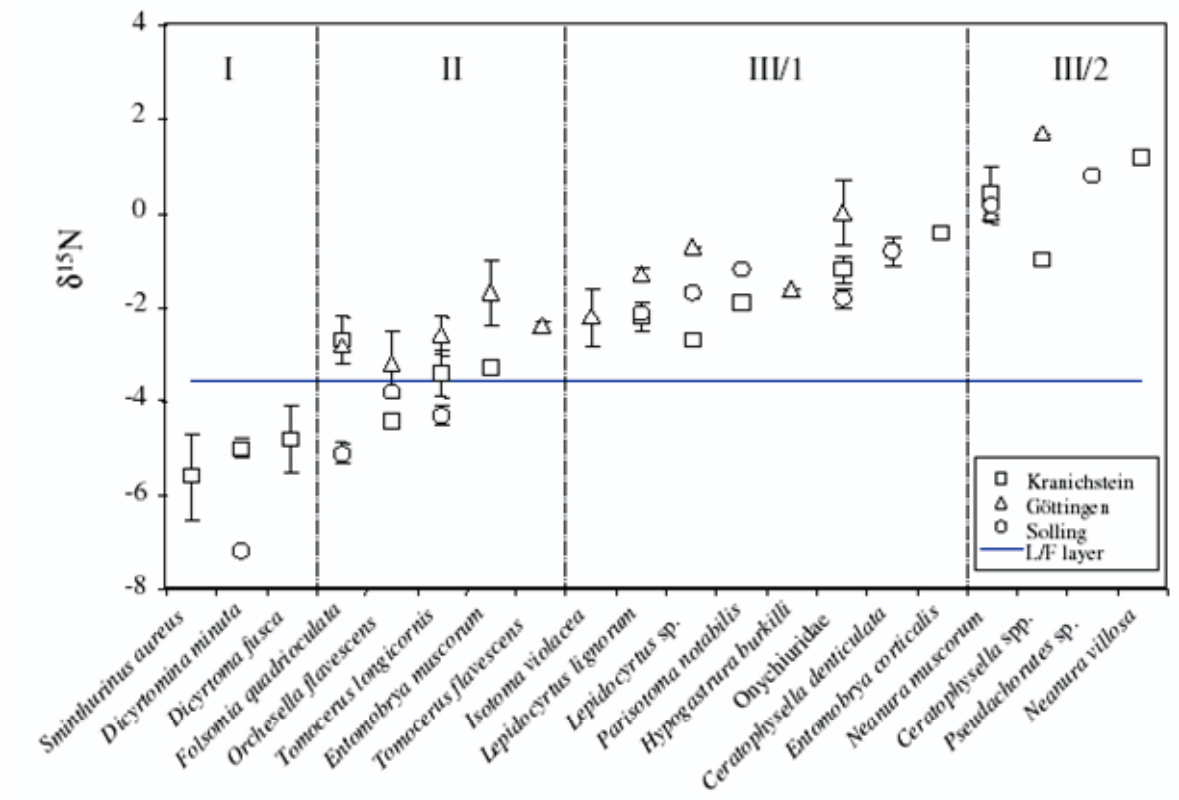


Fig. 2-1: Variation in $\delta^{15}N$ values (± s.d., n= 1-3) of Collembola taxa from three different deciduous forest stands. Data are calibrated to the L/F layer of the Solling. I – phycophages/herbivores, II – primary decomposers, III/1 – secondary decomposers, III/2 – secondary decomposers/predators.

Depleted in $\delta^{15}N$ by -3.7 to -0.7 compared the litter substrate (L/F layer in Solling as baseline) in the different forests sites were the species *S. aureus* (K), *D. minuta* (S, K), *Dicyrtoma fusca* (K), *Folsomia quadrioculata* (S), *O. flavescens* (S, K) and *T.*

longicornis (S). Most Collembola were moderately increased in δ^5N by about 0.3 to 3.2‰ compared to the L/F layer including *Entomobrya muscorum, Tomocerus flavescens, I. violacea, Lepidocyrtus lignorum, Lepidocyrtus* sp.*, Parisotoma notabilis, Hypogastrura burkilli,* Onychiuridae, *Ceratophysella denticulata* and *Entomobrya corticalis.* Strongly enriched in $\delta^{15}N$ by 3.6 to 5.3 compared to the soil were *N. muscorum* (at all three sites), *Ceratophysella* sp., (G) *Pseudachorutes* sp. (S) and *N. villosa* (K).

The range of $\delta^{15}N$ signatures varied within a genus or species among the different forest sites (data calibrated to the L/F layer in Solling as baseline). In *T. longicornis* $\delta^{15}N$ was low in the Solling (-4.3‰), intermediate in the Kranichsteiner Wald (-3.4‰) and highest in the Göttinger Wald (-2.6‰) ($F_{2,5}$ = 9.821, $P < 0.02$). Similarly was the pattern in $\delta^{15}N$ for Onychiuridae ($F_{2,5}$ = 14.077, $P < 0.01$). *F. quadrioculata* had almost the same $\delta^{15}N$ values in Kranichstein and Göttingen, but was strongly depleted in $\delta^{15}N$ in the Solling ($F_{2,3}$ = 114.549, $P < 0.02$). *L. lignorum* had similar $\delta^{15}N$ signatures in Kranichstein and Solling, but was more enriched in the Göttinger Wald ($F_{2,6}$ = 21.173, $P < 0.002$). In contrast, $\delta^{15}N$ signatures in *N. muscorum* were similar in all three forests stands.

2.4 Discussion

2.4.1 Trophic niche differentiation

Belowground systems comprise of a high diversity of decomposer animals, which is difficult to explain via the classical Hutchinsonian niche theory, as there is little evidence for spatial or temporal separation. A general mechanism to avoid niche

overlap is dietary specialisation (Hopkin, 1997), but omnivory is common belowground, particularly among microarthropods (Maraun et al., 2003). Attempts have been made to elucidate the diets of Collembola and to explain the co-existence of diverse communities in soil. Two methods frequently used are food choice experiments and the examination of gut contents (Newell, 1984; Bardgett et al., 1993; Addison et al., 2003; Scheu and Simmerling, 2004). These studies indicate that Collembola are general grazers, and that there is little specialisation in food resources. Our study contradicts this assumption via stable isotope analysis and documents that Collembola species occupy very different trophic niches. The $\delta^{15}N$ signature of the Collembola in the investigated forest stands spanned over 9 ẟ units indicating three different trophic levels. In contrast, Schneider et al. (2004), who examined $^{15}N/^{14}N$ ratios in oribatid mites at the same sites found a gradient over 12 δ units, indicating a more diverse feeding behaviour in this microarthropod group. This is in line with the results of Siepel and de Ruiter-Dijkman (1993) who suggested that Collembola are less diversified in resource utilisation compared to oribatid mites.

The nitrogen stable isotope composition of several Collembola species differed little between forest stands (e.g. in *N. muscorum*, *L. lignorum*) indicating a distinct trophic niche differentiation within taxonomic groups. In contrast, other species differed considerably in the $^{15}N/^{14}N$ ratio between sites (e.g. *F. quadrioculata*, *D. minuta, T. longicornis*) suggesting that they are food generalists and capable to live on different diets depending on the habitat and the available resources. For instance *F. quadrioculata* was most depleted in ^{15}N in the Solling (-5.1‰) in comparison to the Kranichsteiner (-2.7‰) and Göttinger Wald (-2.8‰). This is in line with Scheu and Falca (2000), who found a lower $\delta^{15}N$ signature in this species in the Solling compared

to the Göttinger Wald with a $\delta^{15}N$ of –3.3 and –2.5‰, respectively. Investigations in soil macro-invertebrates (earthworms, enchytraeids, slugs) revealed a comparable continuum in $\delta^{15}N$, but also intrapopulation variation reflecting spatial variation in food sources (Schmidt et al., 2004). Assuming an enrichment of about 3‰ per trophic level (Minagawa and Wada, 1984; Ponsard and Averbuch, 1999) we assigned three feeding guilds in Collembola:

I. Phycophages/herbivores: feeding mainly on lichens, algae and plant tissues
II. Primary decomposers: feeding on litter/detritus with adhering fungi and bacteria
III. Secondary decomposers: predominantly feeding on microorganisms, in particular fungi

2.4.2 Phycophages/herbivores

In the investigated forest stands Collembola of the Symphypleona group (Sminthuridae and Dicyrtomidae) were most depleted in ^{15}N with – 7.2 to –4.8‰, suggesting algae and lichens as major food source ($\delta^{15}N$ with -10.4 and -12.8, respectively). Mosses ($\delta\ ^{15}N$ –6.5 to –6.2) or plant derived tissues ($\delta\ ^{15}N$ –7.3 to –5.8) may also have served as diet. *D. minuta* and *D. fusca* are large-bodied species with chewing mouthparts (Bretfeld, 1999), which can be used to feed on the solid tissue of lichens or algae. Sminthuridae live hemiedaphic or epigeic (Hopkin, 1997) and have frequent access to the proposed food sources. Analysis of the enzymatic capabilities in Sminthuridae by Berg et al. (2004) showed trehalase and cellulase but not chitinase activity. Due to this they classified the genus as opportunistic herbofungivores, which is confirmed by the observed $^{15}N/^{14}N$ ratios in our study.

2.4.3 Primary decomposers

We assign the genera *Entomobrya*, *Folsomia*, *Orchesella* and *Tomocerus* to this group. These Collembola with $\delta^{15}N$ values from -2.4 to -4.4 that are near or similar to the signature of the L/F layer are suggested to function as primary decomposers, feeding on litter material and the adhering fungi and bacteria. Detritus feeders show a lower trophic shift in $\delta^{15}N$ due to the generally high C:N ratio of the resource, that results in lower fractionation rates between 0.5 and 2‰ (McCutchan et al., 2003; Vanderklift and Ponsard, 2003). Comparable low $\delta^{15}N$ values for *T. flavescens* and *T. longicornis* were observed by Scheu and Falca (2000) in the Solling and Göttinger Wald, who similarly assigned these Collembola as primary decomposers. This group comprises particularly large, epigeic species, such as *T. longicornis*, *T. flavescens* and *O. flavescens*, which were found to ingest soil fungi and large amounts of decaying plant material (Poole, 1959). However, Knight and Angel (1967) reported that although fungal spores were the preferred food in the laboratory, they did not form the dominant food compounds in the gut of *T. flavescens* in the field. This could be a consequence of the low availability of the preferred food in the soil (Shaw, 1988), but may also indicate differences in Collembola food choice between laboratory systems and field situations. *F. quadrioculata* was observed to have nearly exclusively humus as gut content (Ponge, 2000). More chitinase than trehalase and cellulase activity suggests this species to feed on fungi, but also to have ability to digest plant materials (Berg et al., 2004). Fungal hyphae can be considerably depleted in $\delta^{15}N$ compared to the soil they inhabit (Wallander et al., 2004). This suggests that regardless of their $\delta^{15}N$ signature close to the L/F layer of the soil, fungal feeding may occur in the primary decomposers, as they graze on the more depleted fungi in the litter.

2.4.4 Secondary decomposers

In our study, secondary decomposers consisted of species with $\delta^{15}N$ values spanning from ca.1.2 to –2.2 ‰. This is the largest group including *E. corticalis*, *C. denticulate,* Onychiuridae, *Hypogastrura burkilli*, *I. violacea, P. notabilis, L. lignorum* and *Lepidocyrtus* sp. Food choice experiments and gut content investigations indicate that these Collembola predominantly feed on fungi (Shaw, 1988; Walsh and Bolger, 1990; Kaneko et al., 1995; Sadaka-Laulan et al., 1998; Chen et al., 1995). Species of Onychiuridae may graze on saprophytic, endomycorrhizal and ectomycorrhizal fungi (Newell, 1984, Walter, 1987; Maraun et al., 2003). Ponge (2000) found fungal material as major gut content in *L. lignorum* collected in the field. For *P. notabilis,* an abundant species in our study, gut content investigations showed fungal hyphae and colloidal material to be frequent (Chen et al., 1996). Also, *E. corticalis* lives in fresh leaf litter and digests saprophytic fungal hyphae and spores (Faber, 1991).

The secondary decomposers include a subgroup of four taxa, *Ceratophysella* spp., *N. villosa*, *Pseudachorutes* sp., and *N. muscorum*, which were distinguished by the others by distinctly higher $\delta^{15}N$ values ranging from 0 to 1.7. Presumably, these Collembola feed on a range of diets including other soil animals (nematodes, rotifers, protozoa), animal body parts and eggs. Recent studies suggest that soil arthropods, which are frequently nitrogen-limited, can enhance their nitrogen intake by broadening their diet to include animal prey (Dennon and Fagan, 2003). Collembola in this subgroup belong to the Hypogastruridae, where several species are reported to be carnivorous and prey on the eggs of other Collembola or ingest tardigrades and rotifers (Hopkin, 1997). However, the $^{15}N/^{14}N$ ratios do not separate them distinctly as

predators, but indicate that they also feed on fungi. For instance *N. muscorum* has piercing and sucking mandibles (Wolter, 1963) and rarely any visible gut content suggesting carnivory (Poole, 1959). However, fungal material and spores have been observed by Singh (1969). Besides, this genus had the highest N content and studies of Ruess et al. (2004) indicate a higher shift in $\delta^{15}N$ in Collembola feeding on high protein diet (i.e. predators), which supports our findings that *Neanura* lives to a substantial extent on animal diet. This is in line with results of Berg et al. (2004), who report the lack of chitinase activity, necessary to digest fungal cell walls, in *N. muscorum* and signify this species as opportunistic herbofungivore or predator.

2.4.5 Variation in trophic shift for $\delta^{15}N$

A general drawback in stable isotope analysis of soil decomposer food webs is that the basis is dead organic matter, which complicates the assignment of an isotopic baseline. Bulk measurements of litter and soil are confounded by differences in digestibility of organic matter. Also Collembola may feed selectively on the microflora therein, such as fungal hyphae that are depleted (Wallander et al., 2004) or fungal rhizomorphs that are enriched (Högberg et al., 1999) compared to the surrounding environment. Another factor is the soil horizon inhabited by Collembola as the $\delta^{15}N$ values for fungal mycelia and tree roots increase with depth (Wallander et al., 2004). Additionally, food preferences may change with depth according to the availability of resources. Ponge (2000) showed a consistent association between the vertical distribution of gut contents and that of food resources in Collembola.

On the other hand, the range in $\delta^{15}N$ signatures observed may partly be caused by differences in isotope fractionation. Recent laboratory experiments showed that

discrimination for ^{15}N in Collembola is affected by food quality (N concentration) and metabolism (starvation, life stage) (Ruess et al., 2004; Scheu and Folger, 2004; Haubert et al., 2005). The C:N ratio of the diet has a major impact on fractionation rates and consumers raised on nutrient poor diets show a lower trophic shift in $\delta^{15}N$ (McCutchan et al., 2003). Organisms with carnivorous, herbivorous and mixed diets displayed similar estimates (2.6 to 3.0‰) while organisms consuming detritus yielded significantly lower estimates in $\delta^{15}N$ (0.5‰) (Vanderklift and Ponsard, 2003). This implies primary consumers in detrital food webs to have lower fractionation rates. As the above mentioned studies investigated only few feeding links the mean fractionation for nitrogen of about 3 δ units across one trophic level may still be a valid approximation when applied on entire food webs with multitrophic pathways and many species (Post, 2002). However, our study indicates that the use of stable isotopes to infer animal diets in belowground systems requires the confirmation by traditional approaches such as food choice experiments or gut content analysis.

2.5 Conclusion

In this study we used the composition in nitrogen stable isotopes to rank Collembola into different feeding guilds, which were phycophages/herbivores, primary and secondary decomposers. Generally, the assigned groups could be confirmed by knowledge from food choice experiments, gut content observations or enzymatic analyses. However, in some species results from stable isotope analyses contradicted data from laboratory experiments. The wide range $^{15}N/^{14}N$ ratios strongly suggest that Collembola species occupy distinctly different trophic niches. Closely related or even the same individual species may have different $\delta^{15}N$ signatures in different habitats,

which indicates that they have the ability to switch their diets if other resources become available. In general, nitrogen stable isotope analysis revealed to be a useful tool to delineate feeding guilds in Collembola.

Chapter 3

3 Sex ratio and mode of reproduction in collembola of an oak-beach forest

Summary

Sex ratios of 31 species of Collembola of a temperate oak-beech forest were investigated in two months intervals during one year. For the estimation of population dynamics, the abundance and dominance of taxa in the litter ($O_{L,F,H}$), the 0-3 cm and 3-6 cm soil layer (A_h) were assessed. A total of six species, including the abundant *Mesaphorura machrochaeta, Parisotoma notabilis, Neanura muscorum* and *Isotomiella minor* formed pure female populations suggesting that they reproduced by parthenogenesis. In three species, *Lepidocyrtus cyaneus, Orchesella flavescens* and *Tomocerus longicornis*, the mode of reproduction remained uncertain. The remaining 22 species including the widespread *Folsomia quadrioculata, Protaphorura fimata* and *Lepidocyrtus lignorum* formed bisexual populations, suggesting that sexual reproduction predominates in Collembola of the studied forest. Sex ratios of sexual species ranged between 33% and 90%. In five species the sex ratio was balanced (ca. 50%), in eight species males made up about one third of the population and in seven species males were rare constituting less than 25% of adults. Females generally

comprised 77% of adult Collembola. Densities were highest in late summer and autumn. Except for *M. machrochaeta*, the density of Collembola deeper in soil (3-6 cm) was low. Consistent with earlier studies parthenogenetic species predominated deeper in the soil (euedaphic species), but some parthenogenetic species were hemiedaphic. The sex ratio of bisexual Collembola species in the litter layer generally was more female biased than that in 0-3 cm of the mineral soil. Presumably, females concentrate at sites with high density of resources whereas males are relatively more abundant at sites more favourable for spermatophore placement. Neither climatic factors (i.e. season) nor population parameters (i.e. density) correlated with the sex ratio of Collembola.

3.1 Introduction

Parthenogenesis, the development of unfertilised eggs into new offspring, is a widespread deviation from the generally dominating sexual reproduction in animals. Parthenogenetic species presumably account for about 1% of the total number of extant species (Koivisto and Braig, 2003). They usually are taxonomically disjunct, i.e. occur scattered throughout the animal kingdom. Ecologically, however, there are marked similarities. Parthenogenetic species predominantly occur at higher latitudes or altitudes, and in transient, disturbed or marginal environments, or at boundaries of ranges, a pattern called geographic parthenogenesis (Kearney, 2003). Parthenogenesis also is more common in xeric compared to mesic habitats and in freshwater rather than in the sea (Glesener and Tilman, 1978; Bell 1982; Lively et al., 1998). Sexual reproduction has been related to environmental uncertainty, being favoured by temporal and spatial habitat fluctuations (Waxman and Peck, 1999). Parthenogenic reproduction

also is more common in small animals, such as rotifers, tradigrades and nematodes, in particular in terrestrial ecosystems (Bell, 1982). Also, in soil many mesofuna species, such as springtails and mites (Petersen, 1978; Siepel, 1994; Norton et al; 1993), and some macrofauna species, such as earthworms and isopods (Jaenike and Selander, 1979; Terhivuo and Suara, 1996), reproduce by parthenogenesis. Sexual animal species may have parthenogenic sister taxa but usually with non-overlapping ranges (Norton and Palmer, 1991). However, sexual and parthenogenetic forms of the same species may also coexist locally (Hopkin, 1997; Niklasson et al., 2000).

Springtails (Collembola) are among the most abundant soil invertebrates, and among arthropods they are one of the earliest colonizers of terrestrial systems. They play an important role in plant litter decomposition, nutrient cycling, in forming soil microstructures and in modifying plant growth, and thus received considerable attention (Parkinson, 1988; Rusek, 1998; Visser, 1985; Gange, 2000; Petersen, 2002; Filser et al., 2002). In contrast, from an evolutionary biology viewpoint Collembola received little attention, e.g. little is known on Collembola sex ratios and parthenogenesis. Information about sex ratios and the lack of males is essential for understanding population dynamics and the response of Collembola to environmental perturbations. In early work parthenogenesis has been neglected (Strebel, 1932, 1938; Falkenhan, 1932; Schaller, 1953; Mayer, 1957). Parthenogenesis in *Orchesella cincta* (Linne) and *O. villosa* (Geoffroy) demonstrated by Lindenmann (1950) was disregarded by assuming that spermatophores were introduced accidentally into culture chambers with food (Schaller 1953; Mayer 1957). Hale (1966) and Sharma and Kevan (1963) suggested parthenogenetic reproduction in two common species, *Tullbergia krausbaueri* (Börner) and *Isotoma notabilis* (Schäffer), and this was confirmed by Petersen (1971, 1978) by

rearing single individuals from isolated eggs for several generations in laboratory cultures.

Based on field studies it is now well established that beside bisexual reproduction parthenogenesis is common in Collembola. A number of species form "all-female populations", i.e. entirely lack males (Petersen 1978, 1980). Other species appear to facultatively reproduce by parthenogenesis. Niklasson et al. (2000) investigated the predominantly parthenogenetic Collembola species *Mesaphorura macrochaeta* at a sandy beach and a copper polluted field in Jutland, Denmark. The sex ratio differed distinctly between both sites. There were also males in the outer exposed zone of the beach. Presumably, apart from geographic parthenogenesis, local environmental factors promote parthenogenetic reproduction in Collembola as in other animal taxa (Christensen, 1983).

The present study investigates sex ratios in Collembola species of a temperate oak-beech forest to evaluate if parthenogenesis in Collembola is as widespread as previously assumed. Multiple sampling allowed evaluating potential changes in sex ratios with season. Also, we proved the hypothesis that most of the epedaphic species of Collembola are bisexual while most parthenogenetic species are euedaphic. The mode of reproduction of Collembola is related to population density to evaluate if the incidence of bisexual and parthenogenetic reproduction varies with population density.

3.2 Materials and methods

3.2.1 Study site

Investigations were performed in an oak-beech forest, the Kranichsteiner Wald, located 8 km northeast of Darmstadt, south Germany, at 150-175 m a.s.l. Parent rock is

Rotliegend covered with sand. The soil types are dystric gleysols and orthic luvisols (FAO-UNESCO classification); the humus form is moder. The pH of the soil varies between 3.6 and 4.3. The tree layer is dominated by oak (*Quercus robur*), about 190 years old, with interspersed beech (*Fagus sylvatica*). The understory is dominated by hornbeam (*Carpinus betulus*), about 125 years old. The herb layer is formed mainly by *Luzula luzuloides*, *Milium effusum*, *Oxalis acetosella*, *Deschampsia cespitosa*, *Stellaria holostea*, and *Polytrichum formosum*.

3.2.2 Collembola

Collembola populations were investigated at two month intervals. Samples were taken in May, July, September and November of 2004, and in January and March of 2005. For sampling animals to be sexed the litter layer ($O_{L,F,H}$) and the upper part of the A_h horizon (0-3 cm) was removed with a shovel. To gain a sufficient number of animals about 2 to 3 kg of litter and soil was collected as bulk sample. Samples were taken from three localities spaced ca. 10 m. Collembola were extracted by heat at 45ºC for two to three days (Kempson et al., 1963). Animals were collected in water, separated under a dissecting microscope, mounted on microscope slides, determined to species and sexed.

For estimating the structure of the Collembola community (density of species in different soil layers) additional samples of the litter layer ($O_{L,F,H}$), the 0-3 and 3-6 cm soil horizon (A_h) were taken. Eight replicate samples were taken with a soil corer (5 cm diameter) spaced from each other by at least 8 m close to where samples for the sex ratio analysis had been taken. Collembola were extracted by heat (Macfadyen, 1961). The samples were exposed to a temperature gradient starting at 20ºC and increasing by 2.5ºC per day for four days and then by 5ºC per day for another four days. Collembola

were collected in glycerol, transferred to ethanol (70%), determined to species and counted.

The sex ratio is expressed as proportion of females of adults and given as percentages. The diversity of species was calculated according to Shannon-Wiener (Pielou, 1971) with $H' = -\sum p_i \ln p_i$, with p_i the ratio between the number of the *i*th species and the total number of Collembola.

3.2.3 Sex determination

Collembola were determined to species using the keys of Gisin (1960), Fjellberg (1980, 1998) and Bretfeld (1999). The general shape of the genital opening as described in Petersen (1978) and Hopkin (1997), and the systematic keys were used for sex determination. The genital opening in females is transversally oriented, whereas males have longitudinal genital apertures. Individual slides of Collembola specimens were prepared in lactic acid, examined and specimens were assigned to female, male or juvenile. For rare species all individuals and for abundant species a representative sample of specimens (up to 100 per sampling date) was sexed. The individuals without an obvious genital opening were considered juveniles. In addition to the genital opening other morphological characters, including appendices anales in females of *Sminthurinus aureus* and *Arrhopalites sericus* and male antennal clasping organs in *Sphaeridia pumilis* were used for sex determination.

The effort necessary for sex identification varies between species (Petersen, 1978). For example, assigning the sex in Collembola with relatively straight body, such as Onychiuridae and Hypogasturidae, which are good to handle on microscopic slides, is rather quick. Isotomidae with *Folsomia* and *Isotoma,* have an obvious genital aperture

and therefore can easily be sexed. On the other hand, sex identification in entomobryid species needs more attentions. In addition to the structure of the genital opening, internal genital structures (visible in sexually active males) were used for sex determination in Entomobryidae and Symphypleona. Placing Collembola in lactic acid followed by heating removes the pigmentation and fat, and inner male genital structures (ductus ejaculatorius) are visible as long tube connected to the genital opening.

3.2.4 Statistical analysis

A likelihood ratio test statistic was computed for evaluating changes in sex ratio (sexual species only) with season, with a Wald test for effects in the model. Linear regressions were carried out to relate changes in sex ratio to density. Statistical analyses were performed with JMP 3.0 for Macintosh (SAS Institute Inc., Cary, USA).

3.3 Results

3.3.1 Sex ratio

In sum, 4541 individuals comprising 31 species were collected during the one year study and sexed, see Table 3.1. Most of the species were present in high numbers, allowing reliable determination of sex ratios. However, in rare species, such as *Lepidocyrtus cyaneus, Tomocerus longicornis* and *Xenylla acauda* the estimated sex ratios need confirmation.

Based on sex ratios, Collembola species were classified into two groups, “without males recorded” and “with males recorded”.

Table 3-1: Ecomophological life form of species, number of specimens inspected, number of adults inspected, percentages of juvenile and sex ratios in Collembola species as percentages of females of an oak-beech forest. Samples were taken in bimonthly intervals from May 2004 to March 2005 in the litter ($O_{L,F,H}$) and 0-3 cm of the mineral soil (A_h). Species without males recorded are ordered alphabetically, species with males recorded are ordered by percentages of females.

Collembola species	Ecomophological life form[$]	Number of specimens inspected	Number of adults	% juvenile	% females
Without males recorded					
Arrhopalites sericus Gisin, 1947	Euedaphic	41	33	20	100
Isotomiella minor (Schäffer, 1896)	Euedaphic	201	128	36	100
Lepidocyrtus cyaneus Tullberg, 1871	Epedaphic	7	6	14	100
Mesaphorura macrochaeta Rusek, 1976	Euedaphic	490	319	35	100
Neanura muscorum (Templeton, 1835)	Hemiedaphic	299	127	58	100
Orchesella flavescens (Bourlet, 1839)	Epedaphic	201	42	79	100
Parisotoma notabilis (Schäffer, 1896)	Hemiedaphic	361	261	28	100
Tomocerus longicornis (Müller, 1776)	Epedaphic	51	5	90	100
Willemia aspinata Stach, 1949	Euedaphic	17	15	12	100
With males recorded					
Sphaeridia pumilis (Krausbauer, 1898)	Hemiedaphic	110	98	11	90
Entomobrya muscorum (Nicolet, 1842)	Epedaphic	246	152	38	86
Sminthurinus aureus (Lubbock, 1862)	Epedaphic or hemiedaphic	98	98	0	83
Tomocerus vulgaris (Tullberg, 1871)	Epedaphic	38	22	42	82
Lepidocyrtus lignorum (Fabricius, 1781)	Epedaphic	365	255	30	79
Orchesella bifasciata Nicolet, 1841	Epedaphic or hemiedaphic	132	105	20	78
Protaphorura cancellata (Gisin, 1956)	Hemiedaphic	19	17	11	76
Friesea mirabilis (Tullberg, 1871)	Hemiedaphic	123	74	40	73
Xenylla humicola (Fabricius, 1780)	Hemiedaphic	21	15	29	73
Protaphorura fimata (Gisin, 1952)	Hemiedaphic	459	390	15	72
Tomocerus minor (Lubbock, 1862)	Epedaphic	20	20	0	70
Pseudachorutes asigillatus Börner, 1901	Hemiedaphic	8	6	25	67
Tomocerus flavescens (Tullberg, 1871)	Epedaphic	4	3	25	67
Folsomia quadrioculata (Tullberg, 1871)	Hemiedaphic	459	348	24	63
Dicyrtomina ornata (Nicolet, 1842)	Epedaphic or hemiedaphic	73	56	23	61
Sminthurinus niger (Lubbock, 1876)	Epedaphic or hemiedaphic	58	24	59	58
Ceratophysella denticulata (Bagnall, 1941)	Hemiedaphic	239	128	46	55
Entomobrya corticalis (Nicolet, 1841)	Epedaphic or hemiedaphic	55	42	24	52
Pseudachorutes dubius Krausbauer, 1898	Hemiedaphic	16	12	25	50
Protaphorura quadriocellata (Gisin, 1947)	Hemiedaphic	295	250	15	49
Odontella lamellifera (Axelson, 1903)	Hemiedaphic	29	22	24	41
Xenylla acauda Gisin, 1947	Hemiedaphic	6	3	50	33

[$] According to Fjellberg, (1998), Gisin, (1960), Bretfeld, (1999), Petersen (1978) and result of this study.

The first group consisted of nine species including the abundant *M. macrochaeta*, *P. notabilis*, *N. muscorum*, and *I. minor* (Table 3.1). The remaining 22 species formed mixed populations. Despite being bisexual, in most of these species the proportion of females considerably exceeded that of males, see Fig. 3.1. However, in *Odontella lamellifera* and *X. acauda* sex ratios were low with only 41 and 33% females, respectively. A balanced number of males and females (49-58%) was found in five species including the abundant *Protaphorura quadriocellata* and *Ceratophysella denticulata*. In nine species, including the abundant *F. quadrioculata* and *P. fimata*, 61-73% of the adults comprised females. In species including the abundant *L. lignorum* and *Entomobrya muscorum*, less than 25% of the individuals were males. *L. lignorum*, *Orchesella bifasciata*, *Sphaeridia pumilis* and *E. muscorum* comprised more than 80% females all over the year. In *E. muscorum* and *F. quadrioculata* there were fewer females in summer whereas in *L. lignorum* there were less females in winter. Overall, females in sexual species comprised more than 65% of adult Collembola and the proportion of females was generally higher in the litter layer compared to 0-3 cm soil depth (Table 3.2). In the other abundant Collembola species, sex ratios varied little or inconsistently with season. Juveniles made up between 19 and 79% of the total number of Collembola. Their proportion was low during winter and spring (40-41%) but high in autumn (61-74%). Generally, as indicated by the likelihood ratio test, the proportion of females did not significantly vary with season, neither in the litter layer ($X^2 = 94.93$, $P > 0.52$) nor in 0-3 cm soil depth ($X^2 = 54.60$, $P > 0.67$). Also, as indicated by linear regression, the proportion of females did not significantly vary with density of Collembola, neither in the litter layer ($r^2 < 0.001$, $P = 0.97$) nor in 0-3 cm soil depth ($r^2 = 0.002$, $P = 0.84$).

Fig. 3-1: Changes in sex ratio of dominant Collembola species during one year; nd=not detected. Samples were taken in bimonthly intervals from May 2004 to March 2005 in litter layers ($O_{L,F,H}$) and 0-3 cm of mineral soil (A_h). Species are ordered according to constancy of occurrence in the sex ratio samples.

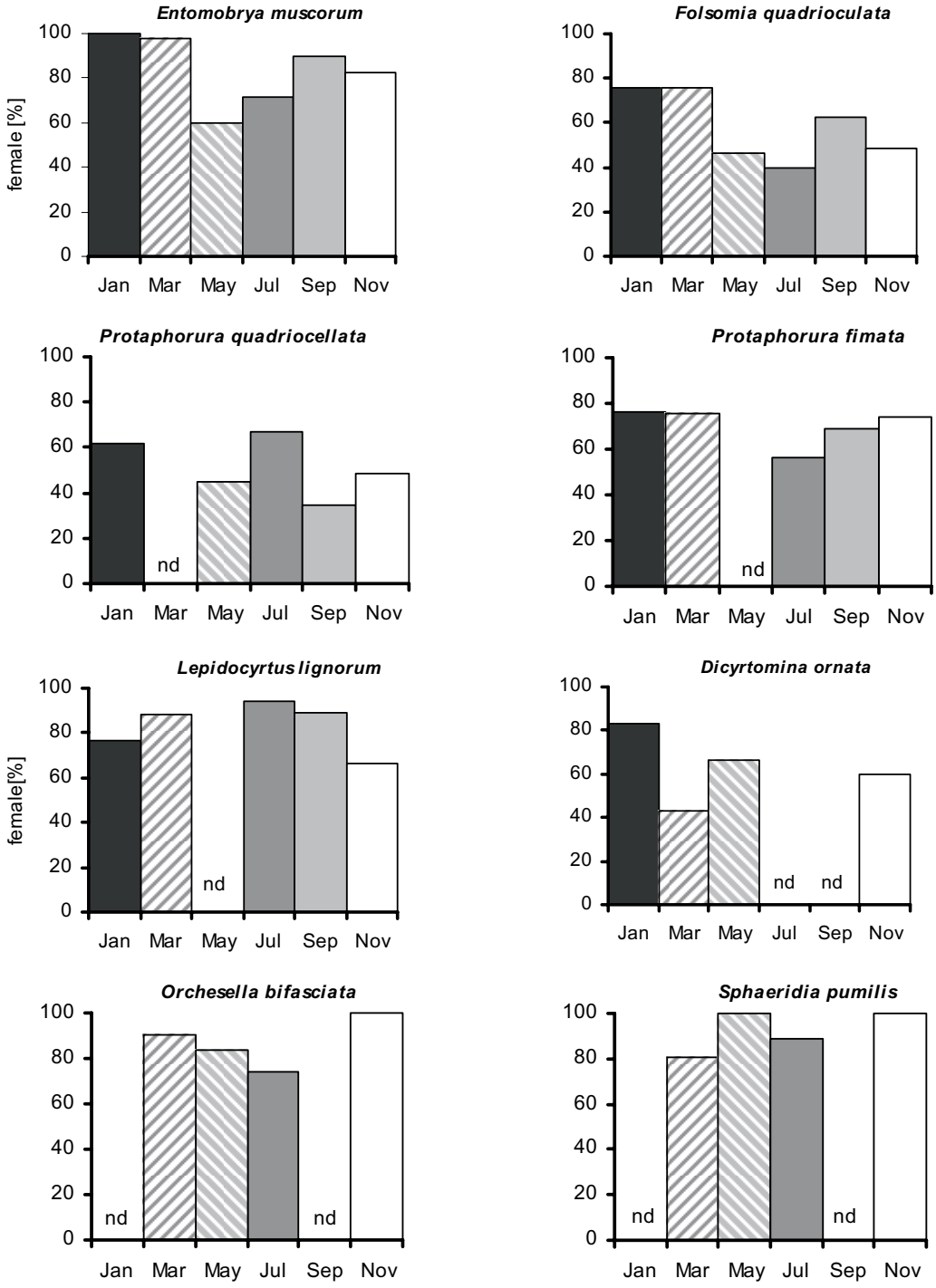


Table 3-2: Seasonal changes in the population structure of Collembola in the studied oak-beech forest (means ± s.d.). Samples were taken in bimonthly intervals from May 2004 until March 2005 in the litter layer ($O_{L,F,H}$), in 0-3 and 3-6 cm soil depth (A_h). nd – not determined. Data on 3-6 cm soil depth not presented.

	January	March	May	July	September	November	Average
Density (Ind m^{-2})							
Litter	6433±4306	10127±5876	7834±6300	14586±8426	15223±9019	24777±11806	13163±6694
0-3 cm	6815±6917	3631±5070	11338±9239	8917±8652	7261±6535	15287±13130	8875±4038
Total	13376±9028	14013±7761	21274±8850	25478±12419	22994±13860	40828±11942	22994±10003
Diversity (H´)							
Litter	0.35 ± 0.16	0.52 ± 0.25	0.47 ± 0.31	0.72 ± 0.28	0.62 ± 0.20	0.87 ± 0.30	0.59 ± 0.19
0-3 cm	0.37 ± 0.33	0.21 ± 0.24	0.50 ± 0.33	0.44 ± 0.36	0.32 ± 0.21	0.51 ± 0.29	0.39 ± 0.12
Total	0.62 ± 0.33	0.67 ± 0.29	0.90 ± 0.20	1.05 ± 0.30	0.84 ± 0.20	1.20 ± 0.16	0.88 ± 0.22
Females (% of adults)							
Litter	80 ± 18	86 ± 11	nd	63 ± 29	73 ± 22	77 ± 10	76 ± 9
0-3 cm	60 ± 40	46 ± 49	nd	50 ± 38	54 ± 50	91 ± 13	60 ± 18
Total	76 ± 9	86 ± 11	nd	62 ± 29	78 ± 21	84 ± 7	77 ± 9
Females (% of adults in sexual species)							
Litter	78 ± 20	83 ± 16	nd	60 ± 27	71 ± 19	71 ± 14	73 ± 9
0-3 cm	56 ± 38	45 ± 49	nd	46 ± 38	4 ± 12	43 ± 47	39 ± 20
Total	57 ± 35	84 ± 11	nd	62 ± 15	66 ± 15	66 ± 31	67 ± 10
Juveniles (% of total population)							
Litter	36 ± 30	43 ± 18	nd	61 ± 21	79 ± 6	69 ± 6	58 ± 18
0-3 cm	39 ± 28	19 ± 25	nd	57 ± 30	76 ± 23	43 ± 21	47 ± 21
Total	40 ± 28	41 ± 16	nd	54 ± 11	74 ± 5	61 ± 6	54 ± 14

3.3.2 Density and diversity

Population density varied from 6,433 to 24,777 ind m^{-2} in the litter layer and from 3,631 to 15,287 ind m^{-2} in 0-3 cm soil depth (Table 3.2). Except for *M. macrochaeta* none of the species frequently occurred in 3-6 cm soil depth. Density of Collembola in total and of most Collembola species was highest in late summer and autumn. Diversity of Collembola in the litter layer considerably exceeded that in 0-3 cm soil. In soil it varied little throughout the year whereas in litter it was at a maximum in late summer and autumn, see Table 3.2.

According to their vertical distribution in soil *F. quadrioculata, L. lignorum,* and *C. denticulata* were classified as epedaphic or hemiedaphic species and *M. macrochaeta* as euedaphic species. The density of *P. notabilis* and *I. minor* was similar in litter and soil. *F. quadrioculata* was the dominant Collembola species with a peak in density in the litter layer in November (Table 3.3). *P. fimata* was also dominant throughout the year except in May and September. Among the deeper soil living Collembola species *M. macrochaeta* was most abundant in May and autumn.

3.4 Discussion

3.4.1 Parthenogenesis

Most Collembola are bisexual (Christiansen, 2003) but several species reproduce by facultative or obligate parthenogenesis (Goto, 1960, Petersen, 1978). Collembola are small, many species are cosmopolitan, and r-selected, suggesting that parthenogenesis might be frequent (Suomalainen et al., 1987).

Table 3-3: Abundance (ind m^{-2} ± s.d.) of dominant Collembola species in the studied oak-beech forest. Samples were taken in bimonthly intervals from May 2004 untill March 2005 in the litter layer ($O_{L,F,H}$), in 0-3 and 3-6 cm soil depth (A_h). Data on 3-6 cm soil depth not presented.

Species	January	March	May	July	September	November	Average
With males recorded							
Folsomia quadrioculata							
Litter	3121±3014	5096±2696	2675±3801	1975±2576	9554±5653	16369±5926	6465±5572
0-3 cm	1975±2381	573±1070	3567±4872	510±721	828±717	2293±3211	1624±1210
Total	5096±4298	5669±3170	6306±4834	2484±2633	10446±5917	18790±5825	8132±5822
Protaphorura fimata							
Litter	2675±1673	2866±2310	318±605	5159±5361	0	1146±1299	2027±1936
0-3 cm	2166±2448	1083±1261	127±236	4713±5244	0	764±903	1476±1767
Total	4841±3045	4076±2710	446±691	10255±9318	0	1975±1795	3599±3784
Lepidocyrtus lignorum							
Litter	64±180	446±574	1592±1137	3439±3464	1783±2179	191±379	1253±1294
0-3 cm	0	64±180	127±236	510±1055	0	64±180	128±193
Total	64±180	573±505	1783±1019	3949±3421	1783±2179	255±384	1401±1454
Friesea mirabilis							
Litter	64±180	64±180	64±180	1083±1621	0	2420±3220	616±976
0-3 cm	1210±1465	127±360	64±180	1465±1896	0	1019±1362	648±656
Total	1338±1610	191±379	127±236	2611±2914	0	3567±3624	1306±1494
Ceratophysella denticulata							
Litter	64±180	127±236	0	191±540	0	446±743	138±168
0-3 cm	127±236	0	0	446±921	0	0	96±179
Total	191±264	127±236	0	637±973	0	446±743	234±257
Without males recorded							
Mesaphorura macrochaeta							
Litter	0	637±1210	1592±1732	318±605	1529±3530	1401±1460	913±684
0-3 cm	1210±1700	510±1248	3631±2473	573±837	5096±5759	10637±9558	3610±3903
Total	1274±1807	1210±2073	6815±5139	2038±2526	6815±8483	12357±8799	5085±4420
Parisotoma notabilis							
Litter	191±379	0	510±903	701±1154	573±635	191±379	361±272
0-3 cm	64±180	0	2484±4007	255±545	191±379	0	499±978
Total	255±472	0	2994±3788	955±1527	828±814	191±379	871±1106
Isotomiella minor							
Litter	0	318±717	701±901	0	828±980	382±527	372±345
0-3 cm	0	64±180	1274±2213	0	955±1503	64±180	393±569
Total	0	382±708	2102±2730	0	1783±1565	446±574	786±921

Petersen (1978) found no males in populations of ten Collembola species which comprised 72% of the total number of Collembola in a Danish beechwood. In the present study, 31 species have been recorded in an oak-beech forest of which in nine no males were recorded. These nine species comprised 30% of the total number of Collembola. However, due to the fact that bisexual populations were also female biased a total of 77% of adult Collembola were female.

Parthenogenetic reproduction was prevalent among some abundant species, including *M. macrochaeta*, *P. notabilis* and *I. minor*. The large number of individuals examined throughout one year suggests that these species indeed exist without any males and this is in line with observations of Petersen (1978).

No males were found for *O. flavescens* at our study site, but its classification as parthenogenetic species needs further investigation; inspecting a nearby population at a forest boundary males were found questioning that the species exclusively reproduces by parthenogenesis. Supporting our quest for further investigation, the report of Lindenmann (1950) on parthenogenetic reproduction in two other *Orchesella* species, *O. cincta* (Linne) and *O. villosa* (Geoffroy), have been disregarded by Schaller, 1953, Mayer, 1957 and Gols et al. 2004.

N. muscorum also generally appears to reproduce by parthenogenesis; however, bisexual populations exist at two localities in the French Pyrénées (Cassagnau, 1972). Several degrees of ploidy (tri-, tetra-, penta-, up to 20-ploid) have been reported in this species and polyploidy is often associated with parthenogenesis or an asexual mode of reproduction (Cassagnau, 1972). The species *Arrhopalites sericus*, *L. cyaneus*, *T. longicornis* and *Willemia aspinata* were examined in low numbers only. Further investigations are needed to confirm the mode of reproduction in these species.

However, *W. aspinata* is known to form all-female populations (Petersen 1978) and for *A. sericus* only females have been reported by Bretfeld (1999) supporting our conclusion that these two species reproduce by parthenogenesis. In contrast, there is no evidence for parthenogenetic reproduction in *L. cyaneus* and *T. longicornis* from other investigations.

According to Petersen (1980) the majority of parthenogenetic Collembola are euedaphic suggesting that parthenogenesis is common among species living deeper in the soil. Presumably, at constant and predictable environmental conditions in deeper soil layers parthenogenesis is beneficial while at more variable conditions in the litter layer sexual reproduction prevails. Most of the parthenogenetic species in the investigated forest were euedaphic. According to Petersen (2002) euedaphic species are small, produce few large eggs and reproduce throughout the year. Most of the parthenogenetic species in our study conform these patterns; however, e.g. *A. sericus* was not present all over the year and other parthenogenetic species were hemiedaphic, including *N. muscorum* and *P. notabilis,* suggesting that parthenogenesis may also be advantageous for species living in the uppermost soil layers.

Generally, the predominance of bisexual Collembola species upper in the soil suggests that at the soil surface the production of genetically diverse offspring is advantageous to cope with fluctuating environmental conditions (Tangled bank hypothesis; Williams, 1975; Bell 1982). However, parthenogenetic reproduction is not necessarily associated with genetic uniformity and this is also true for Collembola; genetic diversity has even been found to be higher in parthenogenetic than bisexual populations of Collembola (Niklasson et al. 2000). Similarly, Simonsen et al. (2004)

found considerable genetic variation among clones of *P. notabilis* along a copper gradient.

Recently, it has been suggested that the infection by *Wolbachia* causes male killing and the formation of all-female populations in Collembola; parthenogenesis therefore may not be related to environmental conditions. Presence of *Wolbachia* was proven for five geographically independent *F. candida* breeding stocks and three parthenogenetic species of Tullbergiinae whereas no *Wolbachia* were found in two sexually reproducing species, *Isotoma viridis* and *P. fimata* (Czarnetzki and Tebbe 2004). Also, Frati et al. (2004) did not find infections by *Wolbachia* in a bisexual population of *F. candida*. Further attempts are needed to assess whether parthenogenesis in Collembola species is induced by *Wolbachia* infections.

3.4.2 Sex ratios in bisexual species

The number of bisexual Collembola species at our study site exceeded that of parthenogenetic species by more than a factor of two. Most of the sexual species were hemi- or epedaphic and present all over the year. Their sex ratio ranged between 33% and 90%. In five species it was close to 50%, in eight species males made up about one third of the population and in seven species males were rare constituting less than 25% of adults. Female biased sex ratios have been reported before for *F. quadrioculata*, *S. aureus* and *L. lignorum*, however, they were less pronounced than in the present study (Petersen, 1978).

The accuracy of sex ratio estimates strongly depends on the number of individuals investigated. In most species analysed in the present study the number of individuals inspected allowed a reliably estimate of sex ratios. Shorter life-span of males than

females may have led to female biased sex ratios as demonstrated for *Smithurides aquaticus* (Falkenhan, 1932). Also, sexual and parthenogenetic reproduction may alternate as known for other invertebrate taxa (Vrijenhoek, 1979, 1984).

In addition to the strong variation in sex ratios between sexual species, a remarkable pattern was that sex ratios changed with soil depth. In the litter layer sex ratios of sexual species were strongly female biased with an annual average of 73% females. In contrast, in 0-3 cm soil depth males predominated with an annual average of 61%. Presumably, females concentrate at sites with high density of resources, i.e. in layers containing freshly fallen or little decomposed organic matter and the associated high density of microorganisms. In contrast, males are relatively more abundant at sites more favourable for spermatophore placement, i.e. deeper soil layers which are constantly moist and therefore prevent spermatophores from desiccation.

Sex and the resulting recombination of parental genomes in offspring may be advantageous at variable environmental conditions (Bell, 1982). High genetic variation may enable sexual species to replace parthenogenetic/asexual sister species. On the other hand, sexual reproduction is a costly mode of reproduction in comparison to parthenogenesis, due to the production of males and genome dilution (Maynard Smith 1978; Williams, 1975). Factors which affect the mode of reproduction and ratios of female and male in Collembola populations are not yet clear. Likely, environmental conditions (e.g. pollution) and climate (e.g. temperature) are important. The same species may exist as bisexual form in disturbed habitats and as parthenogenetic form in constant habitats (Niklasson et al., 2000). Temperature may affect sex ratios, as suggested for *Onychiurus imperfectus*, with the sex ratio being more female biased at

14ºC than at 20ºC (Hopkin, 1997). However, in our investigations of a whole year we found no correlation between season and sex ratios.

Generally, sex ratio in Collembola is genetically determined with some exceptions e.g. in Neanuridae (White, 1973). Female biased sex ratios in the present study possibly resulted from disproportionately high mortality of males. The most important factor appears to be soil depth and the related life history strategy, suggesting that shifts in sex ratio in Collembola might be adaptive. Studies investigating primary sex ratios at different environmental settings are urgently needed.

3.5 Conclusion

Most Collembola species in the studied mature oak-hornbeam forest reproduced sexually but several species reproduced by parthenogenesis. The parthenogenetic species were small and euedaphic, however, some other species in which no males were recorded were hemiedaphic. Bisexual species, such as *F. quadrioculata,* reached high population density in the litter layer, while in deeper soil layers parthenogenetic species, such as *M. macrochaeta,* dominated. In general, the sex ratio of Collembola in the litter layer was more female biased than deeper in the soil. Factors which likely are responsible for the high incidence of all-female populations in Collembola and the dominance of parthenogenetic species in deeper soil layers, such as the accessibility of males (spermatophores) and the necessity of recombination (i.e. the production of genetically diverse offspring) need closer investigation. In the sexual species sex ratio varied considerably. Overall, the number of females considerably exceeded that of males. Unfortunately, knowledge on factors affecting sex ratios in Collembola species is

minute and it remains unknown if Collembola are able to alter the sex ratio of offspring in an adaptive manner.

Chapter 4

4 Parthenogenetic Collembola species suffer to a similar extent from resource depletion than sexual species but are faster colonizers

Summary

Sexual reproduction is the common mode of reproduction in Collembola but several species reproduce by parthenogenesis. Both reproductive modes have advantages and disadvantages which are related to environmental conditions. Under uncertain habitat conditions and when resources are in limited supply sexual species are dominant, whereas parthenogenetic species may prevail in stable habitats. This implies that sexual and parthenogenetic species respond differently to environmental changes, e.g. to the availability of resources. We investigated if sexual and parthenogenetic Collembola species are affected differently by resource depletion and studied their recolonization of defaunated soil and litter. We hypothesized that parthenogenetic species are more sensitive to resource depletion than sexual species, and that they will colonize available habitats faster due to their faster mode of reproduction. In contrast to

our hypotheses, parthenogenetic and sexual Collembola were equally affected by resource depletion. In agreement with our hypothesis, the proportion of parthenogenetic species increased with time when free habitats and plenty of resources were available, indicating that parthenogenetic species are faster colonizers.

4.1 Introduction

Sexual reproduction is the common mode of producing progeny in animals and factors responsible for the maintenance of sexual reproduction have been investigated over decades (Maynard Smith, 1978; Butlin et al., 1998; Barraclough et al., 2003; Omilian et al., 2006). Although sexuality is widespread, several species reproduce by parthenogenesis (the production of progeny without males). Parthenogenesis has several advantages over sexual reproduction and therefore parthenogenetic species should eliminate sexual species at a wide range of environmental conditions (Glesener and Tilman, 1978; Maynard Smith, 1978; Peck et al., 1998; Schön and Martens, 2003). The most obvious difference between the two reproductive modes is the absence of male offspring in parthenogenetic species resulting in higher growth rates of parthenogenetic populations as compared to sexual populations (Maynard Smith, 1978; Bell, 1982; Butlin et al., 1998). While in parthenogenetic species one reproductive female is enough to establish a new population, in sexual species both males and females are necessary. Therefore, parthenogenetic species usually are faster colonizers and the reproductive mode may have a great impact on the colonization ability (Williams, 1975; Bell, 1982; Scheu and Schulz, 1996; Lindberg and Bengtsson, 2005). On the other hand, sex and the resulting recombination of parental genomes in the offspring are advantageous under variable environmental conditions since genetically diverse progeny can exploit a

wider range of resources (Bell, 1982; Pound et al., 2002). Therefore, sexual reproduction has been related to environmental uncertainty while parthenogenetic reproduction may prevail in stable habitats (Glesener and Tilman, 1978; Bell, 1982; Waxman and Peck, 1999; Scheu and Drossel, 2007).

Parthenogenesis is a common reproductive mode in many soil dwelling mesofauna and macrofauna species of Collembola, mites, earthworms and isopods (Petersen, 1978; Jaenike and Selander, 1979; Norton et al, 1993; Siepel, 1994; Terhivuo and Suara, 1996). Collembola are an abundant and important soil dwelling mesofauna group in most terrestrial ecosystems. They comprise about 7000 species and reach densities up to 1,000,000 ind.m^{-2} in forest soil (Peterson and Luxton, 1982; Rusek, 1998). They play an important role in plant litter decomposition, forming soil microstructure, nutrient cycling, and dissemination and grazing on microorganisms (Visser, 1985; Moore et al., 1987; Klironomos and Kendrick, 1995; Rusek, 1998; Addison et al., 2003). Although most Collembola species are sexual (Christiansen, 2003), parthenogenesis is widespread (Goto, 1960; Petersen, 1978; Chahartaghi et al., 2006).

The reproductive mode of animal species is correlated with environmental conditions and ecological factors, such as resource quality and quantity. Changes in these factors cause dynamics in animal communities and these may vary with reproductive modes (Korpelainen 1990). Resource availability often correlates with parthenogenetic reproduction whereas resource shortage is usually associated with sexual reproduction (Redfield, 1999). Forest soils, especially moder systems, are characterised by the permanent availability of rather uniform resources (litter and detritus), i.e. conditions under which parthenogenesis prevails (Bell, 1982; Scheu and Drossel, 2007). Therefore, the percentage of parthenogenetic taxa in e.g. mites, Collembola, enchytraeids and

nematodes in forest soils is high compared with other habitats (Norton and Palmer 1991; Siepel 1994; Niklasson et al. 2000; Bloszyk et al. 2004). If resources become limiting parthenogenetic species may suffer more than sexual species since they are more restricted in adapting to changes in resource supply.

For the recolonization of new habitats sexual species need male and female individuals whereas parthenogenetic species only need a single specimen. Therefore, beside factors such as mobility, fertility, resource availability and habitat characteristics the mode of reproduction affects the colonization of new habitats by animal and plant species with parthenogenetic species usually being the faster colonisers (Williams 1975; Bell 1982; Scheu and Schulz 1996; Lindberg and Bengtsson 2005).

We investigated the effect of resource depletion on the density and community structure of sexual and parthenogenetic Collembola species. Laboratory microcosms were established where food resources (litter material) declined with time. After ten months, the density and community structure of Collembola species were investigated. We hypothesized that due to resource depletion the number of specimens will decline with time and sexual species will become more dominant because higher genetic diversity allows sexual species to exploit limited resources more efficiently. To investigate if parthenogenetic species are faster colonizers than sexual species, we established defaunated microcosms (soil and litter) inoculated with fresh litter and soil material, and traced the recolonization by sexual and parthenogenetic Collembola species. We expected that due to the production of all female progeny parthenogenetic species are faster colonizers than sexual species.

4.2 Materials and Methods

4.2.1 Study site

Soil samples were taken from the Kranichstein forest, located about 8 km northeast of Darmstadt (Germany). The Kranichstein forest is dominated by beech (*Fagus sylvatica*) interspersed with ca. 190 y old oak (*Quercus robur*) and hornbeam (*Carpinus betulus*). The herb layer is dominated by *Luzula luzuloides, Milium effusum, Anemone nemorosa* and *Polytrichum formosum*. Parent rock is Rotliegend covered with sand; the humus form is moder (FAO-UNESCO classification).

4.2.2 Resource depletion experiment

Five soil cores (Ø 21 cm; L, F, H layer and the upper 3 cm of the Ah layer) were taken in the Kranichstein forest and placed in laboratory microcosms. The microcosms were closed with plastic at the bottom and with gauze on top and kept in darkness at 15°C; loss of water was evaluated by weighing and replaced by adding about 100 ml distilled water every week. After 2, 11, 21 and 44 weeks soil cores (Ø 5 cm) were taken from the microcosms (holes were filled with sand), separated into litter and soil (0-3 cm depth) and Collembola were extracted by heat (Macfadyen, 1961; Kempson et al., 1963).

Collembola were counted and determined to species level using the keys of Gisin, (1960), Fjellberg, (1980, 1998) and Bretfeld (1999). In addition, Collembola were sexed inspecting the shape of the genital opening as described in Petersen (1978).

4.2.3 Recolonization experiment

Fifteen soil cores (Ø 21 cm; see above) were taken from the field and placed in laboratory microcosms. Before the start of the experiment, the soil cores were defaunated by drying at 60°C for eight weeks. Then, five of the soil cores were re-inoculated with fresh soil and another five with fresh litter material. The fresh soil and litter was taken from three pooled soil samples (Ø 5 cm) from the study site. Additionally, five control microcosms without inoculation were established (defaunated control). The microcosms were closed with plastic on the bottom and with gauze on top and kept at 15°C in darkness; loss of water was evaluated and replaced as described above. Samples were taken at the same time intervals as in the resource depletion experiment and the same dependent variables were measured. At the end of the experiment the defaunated control was free of microarthropods indicating that drying at 60°C effectively killed microarthropods including eggs.

4.2.4 Statistical analysis

Abundances of Collembola and percentage of parthenogenetic individuals and percentage of parthenogenetic species were analysed by repeated measures analysis of variance (RM-ANOVA) in SAS 9.13 (SAS Institute Inc., Cary, USA) with the fixed factors time and treatment (Scheiner and Gurevitch 2001). Abundances of Collembola were log(x+1) transformed, percentages of parthenogenetic individuals and percentage of parthenogenetic species were arcsin-transformed prior to statistical analysis to increase homogeneity of variances.

4.3 Results

4.3.1 Resource depletion experiment

In sum, 873 individuals were collected and sexed; most individuals were juvenile (69%) and most Collembola colonized the litter layer (75%). Since few individuals were found in the mineral soil layer (about 25% of total), data of both layers were pooled for further analyses. In total, 18 species were present; four species, including *Folsomides angularis, Isotomiella minor, Mesaphorura macrochaeta* and *Parisotoma notabilis,* were parthenogenetic with 100% females (Table 4.1). *Neanura muscorum* was rare (only one juvenile) but has been show to reproduce by parthenogenesis in the Kranichstein forest (Chahartaghi et al., 2006).

Total abundances of adult Collembola decreased significantly with time ($F_{3,12}$=25.11, P<0.0001; Fig. 4.1), mainly from week 2 to 11 ($F_{1,4}$=60, P=0.002) and from week 21 to 44 ($F_{1,4}$=22.34, P=0.009) whereas abundances remained almost constant from week 11 to 21 ($F_{1,4}$=1.38, P=0.30). Total abundances of juvenile Collembola also decreased significantly with time ($F_{3,12}$=7.63, P=0.004) with the dynamics being very similar to adults (Fig. 4.1).

Pa. notabilis and *I. minor* were the most common parthenogenetic species and *Folsomia quadrioculata* was the most common sexual species. The decrease in the number of adult Collembola was mainly caused by *I. minor* and *Fa. quadrioculata* ($F_{3,12}$=7.85, P=0.004 and $F_{3,12}$=24.24, P<0.0001, respectively).

The fraction of both parthenogenetic individuals (46%) and parthenogenetic species (46%) remained constant over time ($F_{3,12}$=0.49, P=0.69 and $F_{3,12}$=0.99, P=0.42, resepectively).

Table 4-1: Number of females, males, juveniles and adults of Collembola and Collembola sex ratio at the end of the resource depletion and recolonization experiments. Species captured in less than three individuals (*Isotoma viridis, Isotoma* sp., *Lepidocyrtus* sp., *Sminthurinus* sp.) are not shown.

	Resource depletion experiment						Recolonization experiment					
Species	Female	Male	Juvenile	adult	Total	sex ratio	Female	Male	Juvenile	adult	Total	sex ratio
Ceratophysella denticulata (Bagnall, 1941)	2	4	28	6	34	33	2	1	9	3	12	67
Dicyrtoma fusca (Lucas, 1812)	0	0	6	0	6	n.d	0	0	4	0	4	n.d
Entomobrya corticalis (Nicolet, 1841)	2	1	0	3	3	67	1	1	2	2	4	50
*Folsomia quadrioculata (*Tullberg, 1871*)*	34	20	235	54	289	63	19	7	74	26	100	73
Folsomides angularis (Axelson, 1905)	3	0	1	3	4	100	341	0	156	341	497	100
*Friesea mirabilis (*Tullberg, 1871*)*	3	1	2	4	6	75	4	4	5	8	13	50
Isotomiella minor (Schäffer, 1896)	61	0	101	61	162	100	35	0	45	35	80	100
Lepidocyrtus cyaneus Tullberg, 1871	9	2	10	11	21	82	17	3	13	20	33	85
*Lepidocyrtus lignorum (*Fabricius, 1781*)*	5	1	25	6	31	83	4	1	17	4	22	80
Mesaphorura macrochaeta Rusek, 1976	39	0	29	39	68	100	117	0	93	117	210	100
Neanura muscorum (Templeton, 1835*)*	0	0	1	0	1	n.d	3	0	2	3	5	100
Odontella lamellifera (Axelson, 1903)	2	1	2	3	5	67	-	-	-	-	-	-
Parisotoma notabilis (Schäffer, 1896)	23	0	107	23	130	100	77	0	269	77	346	100
Protaphorura fimata (Gisin, 1952)	8	1	33	9	42	89	36	15	113	51	164	71
Sphaeridia pumilis (Krausbauer, 1898)	12	2	5	14	19	86	6	2	3	8	11	75
*Sphyrotheca lubbocki (*Tullberg,1872*)*	0	0	3	0	3	n.d	2	1	0	3	3	67
Xenylla humicola (Fabricius, 1780)	-	-	-	-	-	-	0	1	3	1	4	0
Xenylla tullbergi Börner, 1903	17	10	9	27	36	63	-	-	-	-	-	-

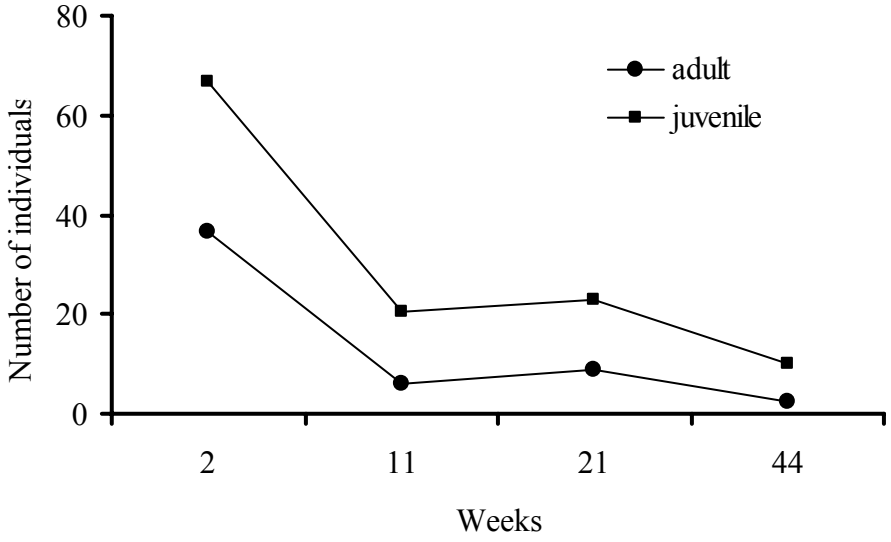


Fig. 4-1: Total abundances of adult and juvenile Collembola 2, 11, 21 and 44 weeks after initiation of the resource depletion experiment (individuals per soil core of 5 cm diameter); for statistical analysis see text.

4.3.2 Recolonization experiment

In the recolonization experiment a total of 1524 Collembola were found and sexed; half of the individuals were juvenile (53%) and most Collembola colonized the litter layer (77%). Since few individuals occurred in the mineral soil layer (about 23% of total), data of both layers were pooled for further analyses. In total, 16 species were present; five species, including *Fs. angularis, I. minor, M. macrochaeta, N. muscorum* and *Pa. notabilis* were parthenogenetic with 100% females (Table 4.1). The density of adult Collembola in the treatment with recolonization from soil increased dramatically at the end of experiment compared to the treatment with recolonization from litter (Fig. 4.2).

In the treatment with recolonization from litter, the number of adult and juvenile Collembola significantly increased with time ($F_{3,12}$= 10.77, P=0.001 and $F_{3,12}$=22.72, P<0.0001, respectively; Fig. 4.2); densities changed little from week 2 to week 11 ($F_{1,4}$=0.13, P=0.73 and $F_{1,4}$=1.83, P=0.24 for adults and juveniles, respectively), increased strongly from week 11 to week 21 ($F_{1,4}$=13.07, P=0.02 and $F_{1,4}$=53.07, P=0.002), but then decreased to week 44 ($F_{1,4}$=5.97, P=0.07 and $F_{1,4}$=39.06, P=0.003). In the treatment with recolonization from soil, the number of adult Collembola increased steadily until week 44, but the number of juveniles decreased after week 21 ($F_{3,12}$= 10.37, P=0.001 and $F_{3,12}$=13.57, P=0.0004 for adults and juveniles respectively; Fig. 4.2).

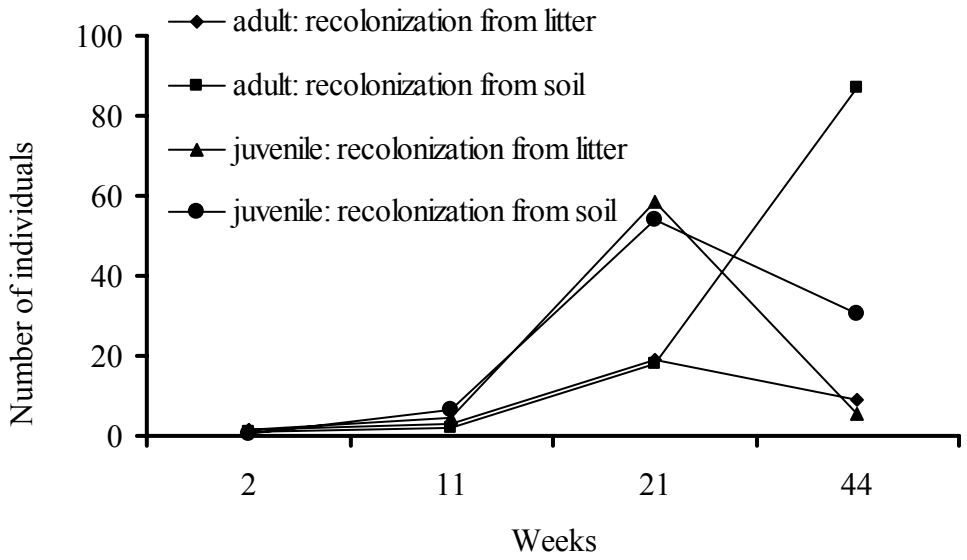


Fig. 4-2: Total abundances of adult and juvenile Collembola in the treatments with recolonization from litter and recolonization from soil (individuals per soil core of 5 cm diameter); for statistical analysis see text.

In the treatment with recolonization from soil, the increase in adult Collembola was mainly due to the parthenogenetic species *Fs. angularis* and *Pa. notabilis* ($F_{3,12}$=3.59, P=0.05 and $F_{3,12}$=20.03, P<0.0001, respectively) but in the treatment with recolonization from litter, the increase was mainly caused by the sexual species *Fa. quadrioculata, Protaphorura fimata* and the parthenogenetic species *Pa. notabilis* ($F_{3,12}$=5.88, P=0.01, $F_{3,12}$=16.58, P=0.0001 and $F_{3,12}$=63.33, P<0.0001, respectively).

The fraction of parthenogenetic individuals increased significantly with time in both treatments ($F_{3,12}$=15.57, P=0.0002 and $F_{3,12}$=8.53, P=0.003 for the treatments with recolonization from litter and soil, respectively) with an average of 27% parthenogenetic individuals in the treatment with recolonization from litter and 22% in the treatment with recolonization from soil (Fig. 4.3). The fraction of parthenogenetic species also increased with time in both treatments ($F_{3,12}$=7.12, P=0.005 and $F_{3,12}$=6.31, P=0.008 for the treatments with recolonization from litter and soil, respectively), with an average of 24% parthenogenetic species in the treatment with recolonization from litter and 38% parthenogenetic species in the treatment with recolonization from soil (Fig. 4.3).

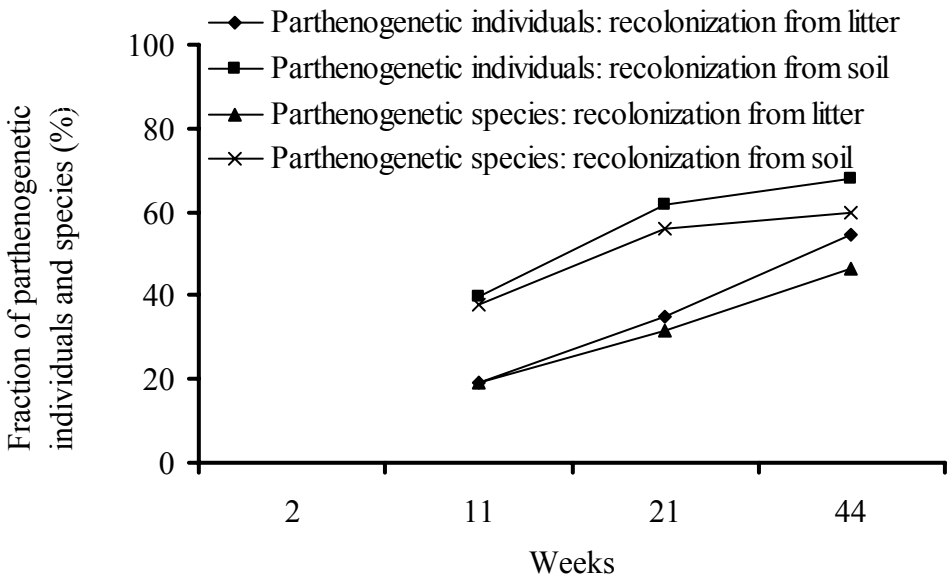


Fig. 4-3: Percentages of parthenogenetic individuals and species of Collembola in the treatments with recolonization from litter and recolonization from soil. Data of week two are not included since no adults of parthenogenetic species were found.

4.4 Discussion

4.4.1 Resource depletion experiment

Collembola are generalistic grazers feeding on a broad spectrum of food materials, including decomposed plant material, fungal spores and hyphae, pollen, algae, diatoms, bacteria, arthropod faeces and exuvia, nematodes, animal body parts (including scales and seta) and amorphous materials (Poole, 1959; Bödvarsson, 1970; Gilmore, 1970; McMillan and Healey, 1971; Sauer and Ponge, 1988). However, stable isotope analyses indicate that Collembola species occupy very different trophic niches suggesting that they exploit different parts of the resource spectrum (Chahartaghi et al., 2005). Conform to our hypothesis the density of Collembola significantly decreased with time indicating that resource availability indeed declined. Initially, the density of Collembola decreased

strongly but later the decrease diminished, indicating that resource depletion slowed down.

In contrast to our hypothesis, the percentage of parthenogenetic individuals and the percentage of parthenogenetic species remained almost constant. The decline in density of Collembola was mainly due to *I. minor* (parthenogenetic species) and *Fa. quadriculata* (sexual species) indicating that both parthenogenetic and sexual species were affected to a similar extent by resource depletion.

The genetical variability of sexually produced offspring is usually higher than that of parthenogenetically produced offspring due to genetic recombination in sexual species. Genetically diverse populations may exploit a wider range of resources and therefore sexual species likely suffer less from resource depletion (Williams 1975; Glesener and Tilman 1978; Scheu and Drossel, 2007). On the other hand, parthenogenetic species are not necessarily genetically uniform; in *M. macrochaeta* the genetic diversity, measured by RAPDs, was higher in parthenogenetic than in sexual populations (Niklasson et al., 2000). In addition, Simonsen et al. (2004) reported genetic differentiation between different colonies of the parthenogenetic species *Pa. notabilis* along a copper pollution gradient. Therefore, since parthenogenetic and sexual Collembola species may have similar genetic diversity, both may have responded similar to resource depletion.

4.4.2 Recolonization experiment

In the recolonization experiment the density of Collembola (adult and juvenile) increased significantly at the beginning of the experiment in both treatments irrespective from which material the recolonization started (litter or soil), indicating that following the defaunation plenty of resources were available. After 21 weeks the density of

Collembola decreased indicating that they are less efficient in exploiting food resources as compared to other mesofauna organisms such as oribatid mites. In contrast to Collembola, the density of oribatid mites continued to increase until the end of the recolonization experiment (Domes et al., 2006).

The increase in density of Collembola in the treatment with recolonization from litter was mainly due to sexual species, such as *Fa. quadrioculata* and *Pr. fimata*, which are common in the litter layer (Chahartaghi et al., 2006). In contrast, the increase in density of adult Collembola in the treatment with recolonization from soil was mainly due to parthenogenetic species, such as *Fs. angularis,* and *Pa. notabilis*, indicating that parthenogenetic species are more abundant in soil as compared to litter (Petersen, 1978). In general, Collembola mainly colonized the litter layer suggesting that resources are more abundant in litter than in soil.

The percentage of parthenogenetic species and the percentage of parthenogenetic individuals increased with time in both treatments suggesting that recolonization of parthenogenetic species was faster than that of sexual species, which agrees with our hypothesis. The results therefore confirm that parthenogenetic species are faster colonizers and become dominant when plenty of resources are available. Therefore, the high density of parthenogenetic Collembola in temperate forests likely is favoured by fast colonization of fresh leaf litter materials by parthenogenetic species (Petersen, 1978; Chahartaghi et al., 2006).

Among the 18 species found in the present study five were parthenogenetic with 100% females. Of these four species including *I. minor*, *M. macrochaeta*, *N. muscorum* and *Pa. notabilis* have been reported to reproduce by parthenogenesis before (Petersen, 1978; Chahartaghi et al., 2006). The fifth species, *Fs. angularis*, has not been reported

to reproduce by parthenogenesis before; according to the large number of individuals examined (341) and the complete absence of males this species likely also reproduces by parthenogenesis.

Remarkably, the sex ratio of sexual Collembola species also was strongly female biased with most species comprising more than 70% females. However, this was similar in the resource depletion experiment and is also similar to the field situation (Chahartaghi et al., 2006) suggesting that the female biased sex ratio in the recolonization experiment was not caused by a disproportionate production of female offspring due to high availability of resources. Rather, it suggests that mortality of males is higher than that of females in many Collembola species.

4.5 Conclusion

Results of this study suggest that parthenogenetic and sexual Collembola species suffer to a similar extent from resource depletion; potentially, parthenogenetic species are genetically diverse allowing them to compete and coexist with sexual species. On the other hand, the results suggest that parthenogenetic and sexual species differ in the speed they recover from disturbances and in recolonization of new habitats (and resources) from litter and soil; parthenogenetic species are faster colonizers than sexual species indicating that parthenogenetic species indeed benefit from the abandonment of producing males, in particular when fresh resources are becoming available for being exploited, e.g. when leaf litter materials enter the soil.

Chapter 5

5 Phylogeography of European Collembola

Summary

The Pleistocene ice ages considerably modified the distribution of plants and animals. The present species of north and central Europe comprise of those that survived in the north and those returning from southern refugia. This study investigated the genetic variation and phylogeography of four Collembola species in order to get insight into the postglacial recolonization of central and northern Europe. Intraspecefic genetic variation was investigated using molecular markers, mtDNA (COI). Two sexual species, *Folsomia quadrioculata* and *Ceratophysella denticulata*, and two parthenogenetic species, *Parisotoma notabilis* and *Isotomiella minor,* were included to analyse if the mode of reproduction affected recolonization patterns. Some populations from Ringnes Island, Marion Island and Siberia were also included. The results indicate recent colonization by Collembola species of some locations especially in the north including Scandinavia, Marion Island, Ringnes Island and Siberia. In contrast, populations of each of the four Collembola species of central and southern Europe were separated by deep splits irrespective of the mode of reproduction suggesting that the colonization of

Europe by these species considerably predates the Pleistocene, potentially dating back to the lower Tertiary (Eocene/Oligocene). This refutes the hypothesis that central European Collembola populations originated from southern refugia after the last glaciation. The deep splits of the two parthenogenetic species studied suggest that similar to other soil invertebrate taxa, such as Oribatida, there also exist "ancient asexual" Collembola species. The deep splits in each of the four Collembola species studied indicate that Collembola species in general constitute of a number of cryptic species with complex phylogeographic history.

5.1 Introduction

The ice ages during the Pleistocene are the most dominant paleoclimatic features. At different stages during the Pleistocene, thick ice sheets (glacial episodes) covered Canada and parts of the United States, northern Europe and Asia (Hewitt, 1999; Cox and Moore, 2005). Warm episodes (interglacials) sandwiched between glacial events with the current interglacial (Holocene) dating back ca. 10,000 years. Global temperature fluctuations during the Pleistocene strongly impacted the distribution of animals and plants (Cox and Moore, 2005). In fact, the expansion of the ice sheets during glaciation eradicated animals and plants from large areas of the northern hemisphere. The situation in Europe was particularly complex due to additional centres of glaciation in the Alps and the Pyrenees (Cox and Moore, 2005).

Population structure is the distribution of genotypes in space and time and is the result of both present processes and past history (Hewitt and Butlin, 1997). Viewed from an evolutionary perspective, present-day animal and plant communities in many parts of the world have a remarkably short history starting with the last glaciation (Stewart and Lister, 2001). Present-day communities in the boreal and temperate zone have been

assembled from both species that survived in the north during the Pleistocene and those returning from southern refugia (Stewart and Lister, 2001). Generally, glacial refugia harbour higher levels of genetic diversity than areas that have been colonized after the retreat of glaciers because colonization involved only some of the individuals in the refugia (Widmer and Lexer, 2001).

In recent years molecular studies have been utilized for the reconstruction of evolutionary relationships and colonization patterns of animal and plant species (Avise, 1998). Molecular phylogeography is a powerful tool for investigating the effect of past events on the today distribution of animals and plants. Most frequently markers of organelle DNA, such as mitochondrial DNA and chloroplast DNA, have been used but also non-coding nuclear DNA (Hewitt, 2004). These studies confirmed southern peninsulas of Europe as major ice age refugia and demonstrated that in most cases genetically distinct taxa derived from them (Hewitt, 1999, 2000, 2001). DNA sequence data indicate that some species have diverged after the last glaciation, while distinct lineages in other species suggest more ancient separation (Hewitt, 1999). Mitochondrial DNA (mtDNA) has a relatively fast rate of nucleotide divergence and therefore is well suited to examine events over the last few million years (Hewitt, 2004) in particular effects of glaciation on present-day distribution of animals (Weisrock and Janzen, 1999; Stevens and Hogg, 2003, 2006). Notably, however, virtually all information on recolonization and phylogeography comes from plant and vertebrate animal species such as oak trees, common beech, black alder, silver fir, hedgehog, brown bear, newts, shrews, water vole and house mice (Hewitt, 1999); little attention has been paid to invertebrate animals in particular those living belowground. Belowground systems harbour old and widespread taxa, such as Collembola and Oribatida, which provide the

opportunity to investigate the role of glacial but also pre-Pleistocene events on present-day colonization patterns.

Collembola are among the most abundant and oldest terrestrial soil-living animals with the first fossils dating back to the Devonian (Greenslade, 1988; Edwards et al., 1995). They live in virtually all terrestrial ecosystems from the seashore to the top of mountains. Collembola species have adapted to harsh environmental conditions, such as low temperatures in the Antarctic and high temperatures in tropical rainforests. The phylogeography of Collembola presumably has undergone dramatic changes during the long evolutionary history, however, this has been hardly studied. Stevens and Hogg (2006) investigated mtDNA variation in one species of Collembola (*Gomphiocephalus hodgsoni*: Hypogasturidae) in the Antarctic and suggested that its present geographic and haplotype distribution results from long-term glacial habitat fragmentation.

The mode of reproduction strongly impacts the colonization of new habitats by animals and plants. Parthenogenetic species are faster colonizers (Williams, 1975; Bell, 1982; Scheu and Schulz, 1996; Lindberg and Bengtsson, 2005) while sexual species presumably are more vigorous in colonizing habitats with fluctuating environmental conditions (Bell, 1982; Pound et al., 2002). Although most Collembola species are sexual (Christiansen, 2003), parthenogenesis is widespread (Goto, 1960; Petersen, 1978; Chahartaghi et al., 2006). Considering the different strengths in colonizing new habitats, parthenogenetic species and sexual species may have differentially colonized deglaciated regions resulting in present-day differences in their genetic structure, e.g. populations of parthenogentic species are likely to be genetically more depauperate than sexual species.

There is no comprehensive study investigating postglacial recolonization of Europe by Collembola. Also, there is no information whether the mode of reproduction affected the postglacial recolonization. Using molecular markers, mtDNA (COI), the present study investigates the genetic variation of Collembola species for understanding phylogeographic relationships and postglacial recolonization of central and northern Europe by four Collembola species. We include two sexual and two parthenogenetic species to investigate whether the mode of reproduction affected recolonization patterns and present-day within-species genetic variability.

5.2 Materials and Methods

5.2.1 Collembola

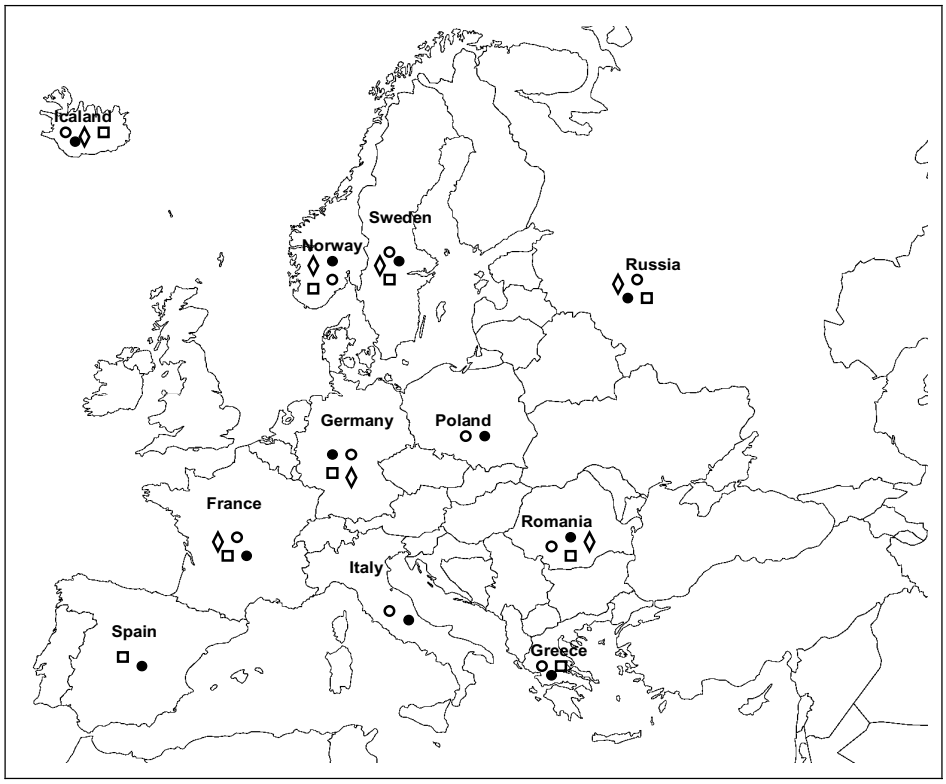
Populations of two sexual species *Folsomia quadrioculata* (Tullberg, 1871) and *Ceratophysella denticulata* (Bagnall, 1941), and two parthenogenetic species *Parisotoma notabilis* (Schäffer, 1896) and *Isotomiella minor* (Schäffer, 1896), were analysed. These species are cosmopolitan and can be identified easily. Most of the specimens originated from North, Central and South Europe and were collected by the authors or provided by collaborators as detailed in Fig. 5.1 and Table 5.1. Further, specimens of *F. quadrioculata* and *P. notabilis* from South and East Russia were included. Additionally, some specimens of *C. denticulata* and *P. notabilis* from Marion Island and some specimens of *F. quadrioculata* from Ringnes Island were included. *F. quadrioculata* in some regions coexist with a very similar species, *F. manolachei*. For investigating their phylogenetic relationship and taxonomic status some individuals of *F. manolachei* from Russia (Moscow) were also included and compared to *F.*

quadrioculata from the same region. *Podura aquatica* was used as an outgroup (GenBank AN: AY665306).

5.2.2 DNA extraction and PCR

Collembola species were preserved in >70 % v/v ethanol until preparation. DNA was extracted by placing single individuals in an Eppendorf tube, frozen in liquid nitrogen and crushed against the tube wall with a plastic rod. Total genomic DNA was obtained from entire Collembola individuals (1-10 individuals from each locality) using the DNeasy Tissue Kit for animal tissue following the manufacturer's protocol (Qiagen, Oberursel, Germany).

A fragment of the mtDNA (COI) of 657 bp was amplified using the forward primer LCO1490 (5`-GGTCAACAAATCATAAAGATATTGG-3´) and the reverse primer HCO2198 (5´-TAAACTTCAGGGTGACCAAAAAATC-3´) (Folmer et al., 1994). Amplification of the COI fragment was performed in 50 µL volumes containing 2 µL of each primer (100 pmol μl^{-1}), 5 µL DNA and 25 µL HotStarTaq Mastermix (2.5 units HotStarTaq® Polymerase, 200 µM of each dNTP and 15 mM $MgCl_2$ buffer solution; Qiagen). Amplification was performed with an initial denaturation step at 95 °C for 15 min, 5 cycles of 1 min at 94 °C, 1 min at 45 °C and 1 min at 72 °C, 35 cycles of 1 min at 94 °C, 1 min at 51 °C, 1 min at 72 °C, and 10 min at 72 °C for final extension (Hogg and Hebert, 2004). PCR products were purified on 2 % agarose gels; reaction products were purified using the Qiaquick PCR Purification Kit (Qiagen) or if it was necessary bands were excised from the gel, recovered and purified using Qiaquick Gel Extraction Kit (Qiagen). The DNA was sequenced in both directions by Macrogen Inc. (Seoul, South Korea) or by Scientific Research and Development GmbH (Oberursel, Germany).



(a: above and b: below)

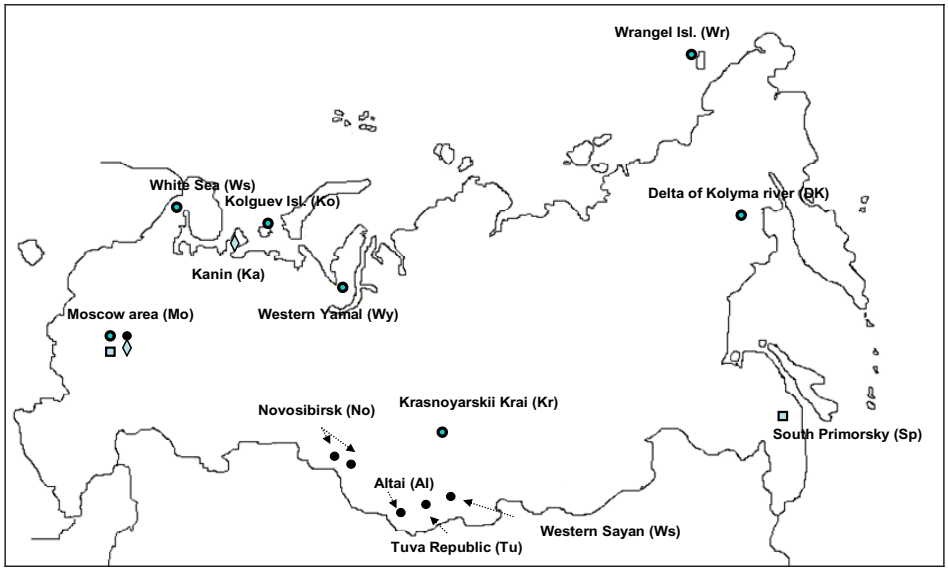


Fig. 1-1. Map of Europe (a) and northern Asia (b) with sampling sites four Collembola species studied; *Folsomia quarioculata* (○), *Ceratophysella denticulata* (□), *Parisotoma notabilis* (●), and *Isotomiella minor* (◊).

5.2.3 Alignment and phylogenetic analysis

All sequences were verified as being derived from Arthropoda using the GenBank BLAST algorithm (Altschul et al., 1997). DNA sequences were aligned with the multiple alignment algorithms in the program Clustal X (Thompson et al., 1994, 1997) using 10 and 0.1 as parameters for gap opening and gap extension penalty, respectively. Models for sequence evolution and corresponding parameters were estimated using likelihood ratio tests with MODELTEST 3.7 (Posada and Crandall, 1998) and corrected and uncorrected maximum likelihood distances were calculated in PAUP (Swofford, 1999). Phylogenetic trees were constructed using neighbourjoining (NJ: from 10,000 bootstrap replicates), maximum parsimony (MP: from 1000 bootstrap replicates) and likelihood based (ML from 100 bootstrap replicates) algorithms in PAUP (Swofford, 1999). χ^2-tests (implemented in PAUP) were used to test the hypothesis of homogeneity of base frequencies among sequences. Additionally, statistical parsimony (Templeton et al., 1992) implemented in the TCS software (Clement et al., 2000) with a 150-step connection limit was used to estimate phylogeographical networks of COI haplotypes for each of the Collembola species. Statistical parsimony analysis for *F. quadrioculata* and *P. notabilis* was done with two connection limits: (1) a 100-step connection limit for the whole data set and (2) a 150-step connection limit for the reduced dataset. The reduced dataset comprised 25 and 26 haplotypes of *F. quadrioculata* and *P. notabilis*, respectively, including 1-3 haplotypes as representatives of each geographical region. Genetic distances were calculated in PAUP. Nucleotide sequences were translated into amino acids in MEGA 3 using the invertebrate mitochondrial genetic code (Clary and Wolstenholme, 1985).

5.3 Results

A 657-bp fragment of the COI gene was sequenced from a total of 63 individuals of *F. quadrioculata*, 48 individuals of *C. denticulata*, 45 individuals of *P. notabilis* and 23 individuals of *I. minor*. The alignment was free of gaps and unambiguous. The selected model for the whole dataset of *F. quadrioculata* and *C. denticulata* was TVM+I+G and for the whole dataset of *P. notabilis* and *I. minor* it was GTR+I+G and HKY+G, respectively (Table 5.1). All sequences were free of stop codons and represented 5, 8, 11 and 4 different functional proteins in *F. quadrioculata, C. denticulata*, *P. notabilis* and *I. minor*, respectively (Table 5.2). The analysed part of the protein comprised 219 amino acids with the number of variable sites differing between species (Table 5.2). Base frequencies were homogeneously distributed among all species (Table 5.2). Nucleotide composition averaged over all species showed an A-T bias (A=25%, T=35%, C=22%, G=18%). The gross topology of phylogenetic trees using corrected distances with neighbour joining, maximum likelihood and maximum parsimony was similar; therefore, only phylogenetic trees based on maximum likelihood are presented. The major clades in phylogenetic trees for each of the Collembola species were also recovered using statistical parsimony as implemented in TCS. Haplotypes of each the four Collembola species from different regions in particular in southern and central Europe were separated by a large numbers of substitutions. Haplotypes from the same region were also separated by a large numbers of substitutions in TCS plots. In contrast, some haplotypes from different regions in particular in northern Europe were separated by a small number of substitutions. Further, haplotypes of species from Marion and Ringnes Islans were separated by a small number of substitutions from haplotypes of the respective species from Europe. Overall, the general patterns of colonization were

similar for each of the Collembola species but the phylogenetic trees and TCS plots also indicated some differences.

5.3.1 Sexual species

Folsomia quadrioculata

In *F. quadrioculata* maximum corrected distances of the 63 analysed specimens were between White Sea and Wrangel Island populations (85%). Maximum likelihood analysis separated geographical distinct clades: an eastern clade including Poland and Siberia, two southern clades including Italy and Greece, a central clade including Germany and France, and a northern clade including Iceland, Moscow and Sweden (Fig. 5.2). In addition to these clades which corresponded to geographic regions others were interspersed. Specimens from Norway formed three lineages, the one close to Greece, and the second in between the Romania and southern clade (Italy) and the third within the northern clade (between Moscow and Iceland). Similarly, specimens from Ringnes Island formed two widely separated lineages, the one closely associated with the eastern clade, the other associated close to the northern clade. Further, specimens from France also constituted of two lineages, the one close to the German clade the other very close to the Poland clade. Remarkably, the specimen from Romania clustered together with a specimen from Russia (White Sea). Clustering of specimens from single countries with different lineages was also reflected by high genetic divergences within countries.

Table 5-1. Country of collection, location, collector, number of analysed specimens and abbreviations of analysed collembola species studied.

Species	Country	Collection locality	Collector	Number	Abbreviation
Folsomia quadrioculata	Iceland	Eyjafjördur, Rímar	A. Fjellberg	4	Fq_Ic_Fj_n
	Norway	Oslo	P. Leinaas	6	Fq_No_Le_n
		Larvik, Holtsetra	A. Fjellberg	2	Fq_No_Fj_n
	Sweden	Lund, Dalby Hage	A. Fjellberg	3	Fq_Sw_Fj_n
	Russia	Delta of Kolyma river	A.Babenko	1	Fq_Ru_Dk_Ba_n
		Wrangel Island, Mammoth river	A. Babenko	2	Fq_Ru_Wr_Ba_n
		Krasnoyarskii Krai	A. Babenko	1	Fq_Ru_Kr_Ba_n
		Kolguev Island	A. Babenko	1	Fq_Ru_Ko_Ba_n
		Western Yamal	A. Babenko	1	Fq_Ru_Wy_Ba_n
		White Sea, near Pon'goma	M. Potapov	1	Fq_Ru_Ws_Pot_n
		Moscow	A. Uvarov	5	Fq_Ru_Mo_Al_n
	France	Paris, Rambouillet forest	L. Deharveng	1	Fq_Fr_De_n
		Brunoy	J.F. Pong	3	Fq_Fr_Po_n
	Germany	Darmstadt, Kranichstein forest	M. Chahartaghi	10	Fq_Ge_Ch_n
	Poland	Krakau	S. Scheu	6	Fq_Po_n
	Italy	Monte Argentario, Grosseto	F. Frati	4	Fq_It_Fr_n
	Romania	Sinaia	M. Falca	1	Fq_Ro_Fa_n
	Greece	North of Thessaloniki	M. Tsiafouli	2	Fq_Gr_Ts_n
	North Pole	Ringnes Island	P. Leinaas	9	Fq_Ri_Le_n
Folsomia manolachei	Russia	Moscow	A. Uvarov	3	Fm_Ru_Mo_Al_n
Ceratophysella denticulata	Iceland	Eyjafjördur, Vaglaskogur	A. Fjellberg	3	Cd_Ic_Fj_n
	Norway	Larvik, Holtsetra	A. Fjellberg	3	Cd_No_Fj_n
	Sweden	Örtofta	A. Fjellberg	8	Cd_Sw_Fj_n
	Russia	East of Russia, South Primor'ye	M. Potapov	2	Cd_Ru_Sp_Pot_n
		Moscow area	M. Potapov	3	Cd_Ru_Mo_Pot_n
	France	Ariege, near Saint-Girons	L. Deharveng	5	Cd_Fr_De_n
	Germany	Darmstadt, Kranichstein forest	M. Chahartaghi	5	Cd_Ge_Ch_n
	Romania	Sinaia	M. Falca	5	Cd_Ro_Fa_n
	Spain	Sierra de Huétor	A. Rodriguez	3	Cd_Sp_Ro_n
	Greece	North of Thessaloniki	M. Tsiafouli	4	Cd_Gr_Ts_n

Table 5-1-continued

Species	Country	Collection locality	Collector	Number	Abbreviation
	North Pole	Marion Island, Sub Antarctic South Africa	P. Leinaas	7	Ca_Ma_Le_n
Parisotoma notabilis	Iceland	Eyjafjördur, Vaglaskogur	A. Fjellberg	3	Pn_Ic_Fj_n
	Norway	Larvik, Holtsetra	A. Fjellberg	3	Pn_No_Fj_n
	Sweden	Lund, Dalby Hage	A. Fjellberg	2	Pn_Sw_Fj_n
	Russia	Altai	S. Stebaeva	2	Pn_Ru_Al_St_n
		Mountains of Southern Siberia	S. Stebaeva	1	Pn_Ru_Ms_St_n
		Western Siberia, Novosibirsk	S. Stebaeva	4	Pn_Ru_No_St_n
		Tuva Republic	S. Stebaeva	1	Pn_Ru_Tu_St_n
		Western Sayan	S. Stebaeva	2	Pn_Ru_Ws_St_n
		Moscow	A. Uvarov	2	Pn_Ru_Mo_Al_n
	France	South-east of Paris	J.F. Pong	4	Pn_Fr_Po_n
	Germany	Darmstadt, Kranichstein forest	M. Chahartaghi	6	Pn_Ge_Ch_n
	Poland	Krakau	S. Scheu	2	Pn_Po_n
	Spain	Sierra de Huétor	A. Rodriguez	3	Pn_Sp_Ro_n
	Italy	Siena	F. Frati	2	Pn_It_Fr_n
		Apenninen	S. Scheu et al.	1	Pn_It_n
	Romania	Sinaia	M. Falca	2	Pn_Ro_Fa_n
	Greece	North of Thessaloniki	M. Tsiafouli	2	Pn_Gr_Ts_n
	North Pole	Marion Island, Sub Antarctic South Africa	P. Leinaas	3	Pn_Ma_Le_n
Isotomiiella minor	Iceland	Öxarfjördur, Kópasker	A. Fjellberg	3	Im_Ic_Fj_n
	Norway	Oslo	P. Leinaas	2	Im_No_Le_n
	Sweden	Halland, Halmstad, Holkåsen	A. Fjellberg	2	Im_Sw_Fj_n
	Russia	Kanin, North European part	M. Potapov	2	Im_Ru_Ka_Pot_n
		Moscow	A. Uvarov	3	Im_Ru_Mo_Al_n
	France	Le Racou, Pyrenees-orientales	L. Deharveng	1	Im_Fr_De_n
		South-east of Paris	J.F. Pong	5	Im_Fr_Po_n
	Germany	Darmstadt, Kranichstein forest	M. Chahartaghi	4	Im_Ge_Ch_n
	Romania	Sinaia	M. Falca	1	Im_Ro_Fa_n

Table 5-2. Sequence variation, distance, base frequency, amino acids and estimated parameters of the four Collembola species studied.

	Folsomia quadrioculata	*Ceratophysella denticulata*	*Parisotoma notabilis*	*Isotomiella minor*
Sequences (n)	63	48	45	23
Variable sites (n)	288	266	289	255
Localities (n)	19	11	18	9
Reproduction mode	Sexual	Sexual	Parthenogenetic	Parthenogenetic
MODELTEST hlrt				
Selected model	TVM+I+G	TVM+I+G	GTR+I+G	HKY+G
Frequence (A)	0.2949	0.2817	0.2721	0.2602
Frequence (C)	0.2064	0.2268	0.2220	0.2302
Frequence (G)	0.1375	0.1543	0.1377	0.1621
Frequence (T)	0.3612	0.3371	0.3682	0.3475
Invariable sites (I)	0.5184	0.5284	0.4942	0
Gamma shape	0.9717	10.343	10.783	0.2985
Distances				
Uncorrected (averaged)	0.1566	0.1402	0.1684	0.1176
Uncorrected (maximum)	0.2481	0.2177	0.2588	0.2554
Corrected (averaged)	0.3660	0.3486	0.4100	0.2507
Corrected (maximum)	0.8531	0.7265	0.9309	0.7145
Base frequencies				
Nucleotide A	0.2627	0.2472	0.2585	0.2521
Nucleotide C	0.1951	0.2255	0.2034	0.2165
Nucleotide G	0.1827	0.1866	0.1831	0.1858
Nucleotide T	0.3595	0.3406	0.3551	0.3456
Ki-Squar	92.96	45.31	54.00	28.85
P	1	1	1	0.99
Amino acids				
Number of amino acids (n)	5	8	11	4
Variable sites (n)	33	16	34	25

The clades separated by phylogenetic methods were also separated by statistical parsimony as implemented in TCS (Fig. 5.3). The COI sequences of the 63 individuals consisted of 44 haplotypes in statistical parsimony (TCS) with a connection limit of 100. Statistical parsimony with a connection limit of 150 of the reduced dataset separated geographical regions by 100-192 substitutions with the highest number of suggested substitutions being more than 300. Within geographical regions, haplotypes were separated by 1-102 substitutions (Fig. 5.3). High genetic divergence between and within countries was also reflected in the TCS plot. Italy, Romania, Greece, Poland, France, and Siberia were separated from others by a large number of substitutions. The separate clades in the phylogenetic tree correspond to species or groups of species separated by large numbers of substitution in the TCS plot. The Siberia clade was separated from the European clade by a large number of substitutions (more than 300; not connected in the TCS plot with connection limit of 150). The two southern clades, Italy and Greece, were separated from each other by 110-115 substitutions. Romania was separated from Italy and Greece by a large number of substitutions (more than 100). The central clade of Germany and France was separated from each other by a maximum of 186 substitutions. Poland was separated from other geographical regions by a large number of substitutions (more than 100). In contrast, lineages of the northern clade, e.g. Iceland and Moscow, were separated by a small number of substitutions (20) while Sweden was separated by 74 substitutions from Iceland and Moscow. Haplotypes from Ringnes Island were widely separated from the northern clade (Iceland, Moscow and Sweden) but were closely related to the Siberia clade (Wrangel Island). Three lineages were separated by phylogenetic methods for Norway and also by a large number of substitutions from each other in the TCS plot.

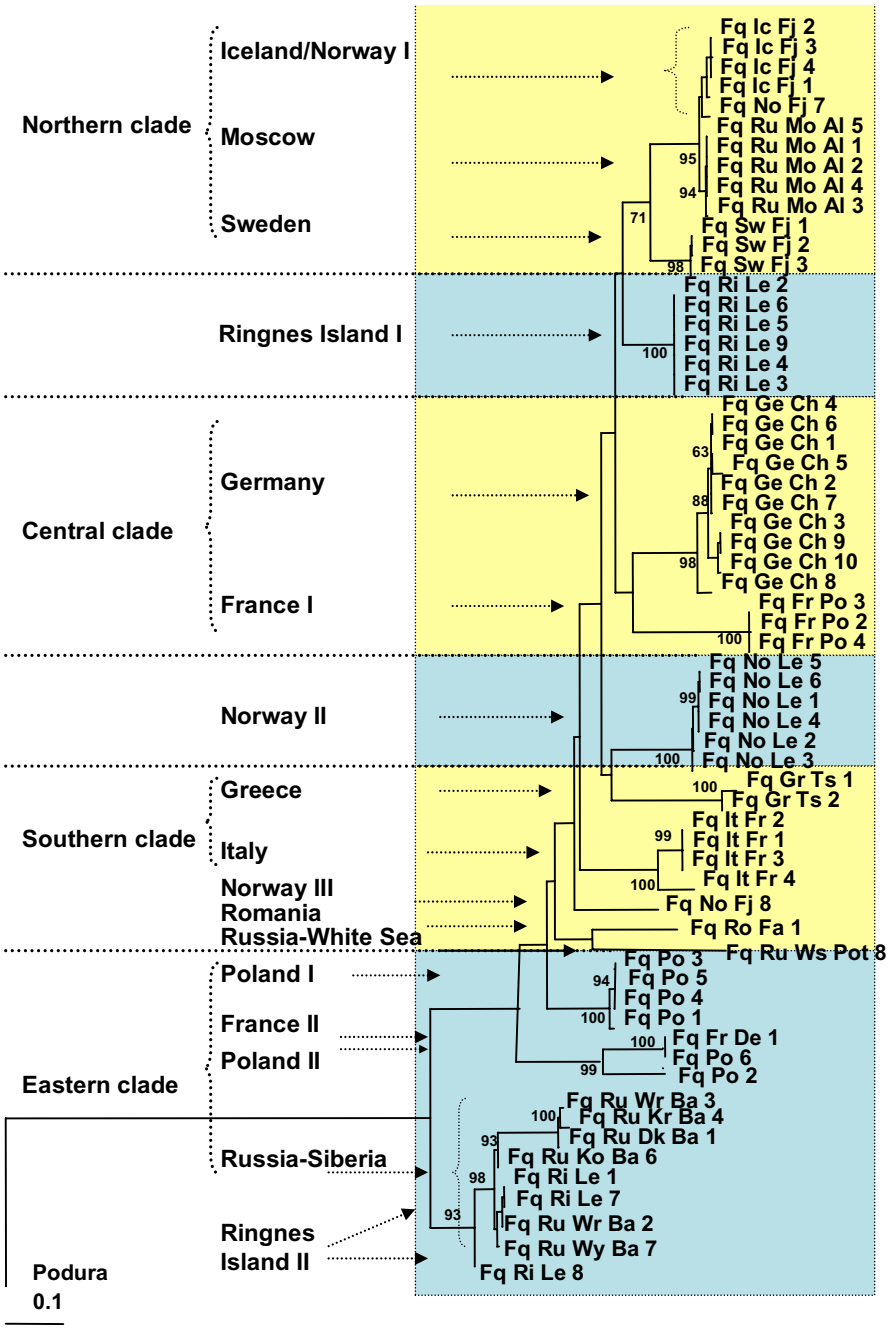


Fig. 5-2. Maximum likelihood tree of 63 specimens of *Folsomia quadrioculata* calculated in PAUP. Numbers at nodes indicate bootstrap values from 100 replicates. For abbreviations, see Table 5-1.

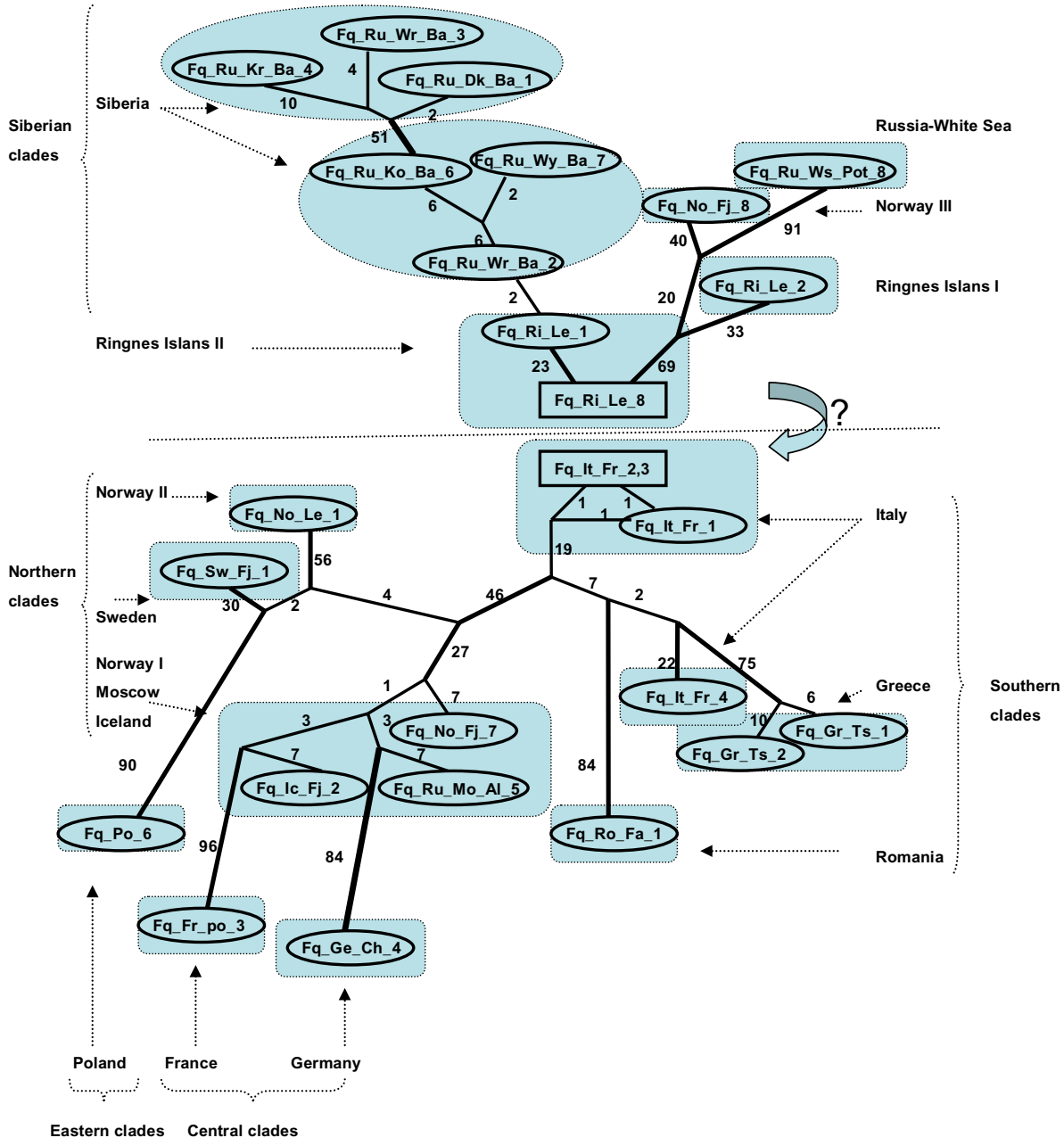
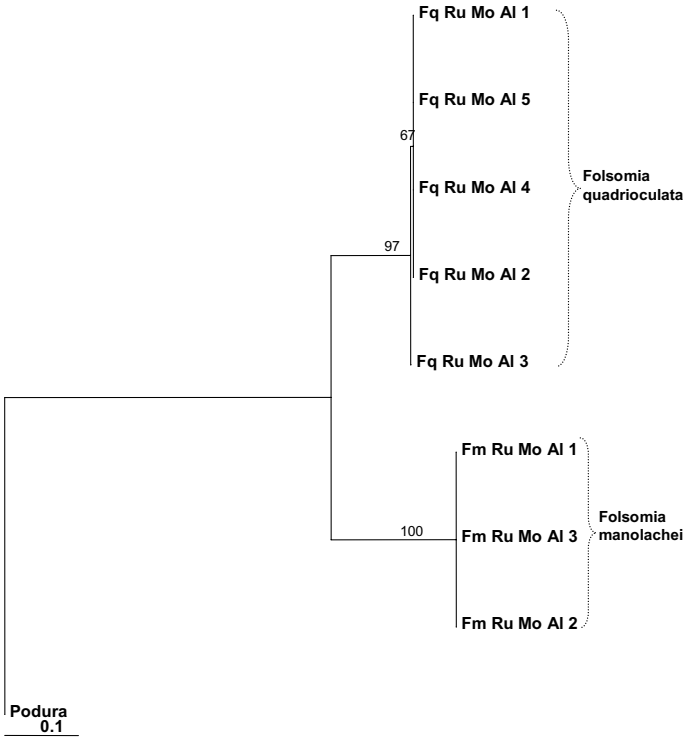
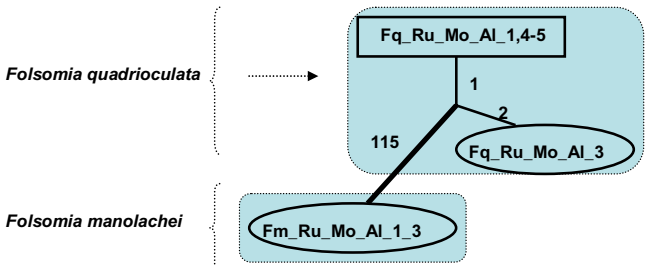


Fig. 5-3. Reconstructed TCS plot of 14 COI haplotypes of *Folsomia quadrioculata* from Europe and 11 COI haplotypes from Russia and Ringnes Island; connection limit set to 150 steps. The length of branches is meaningless, thicker lines indicate number of substitutions ≥ 20.

Folsomia manolachei and *F. quadrioculata* were widely separated as two different lineages in phylogenetic trees (maximum corrected distances 30%) and statistical parsimony (116 substitutions) (Fig. 5.4). However, the number of substitutions separating the two lineages was similar to that between lineages of *F. quadrioculata* between countries (Fig. 5.3).



(a: abow and b:below)



(b)

Fig. 5-4. Maximum likelihood tree of 5 specimens of *Folsomia quadrioculata* and 3 specimens of *Folsomia manolachei* from Russia (Moscow) calculated in PAUP (a) and reconstructed TCS plot of 2 COI haplotypes of *F. quadrioculata* and single COI haplotypes of *F. manolachei* (b); connection limit set to 150 steps. The length of branches is meaningless, thicker lines indicate number of substitutions ≥ 20.

Ceratophysella denticulata

In *C. denticulata* maximum corrected distances of the 48 analysed specimens were between Germany and Romania (73%). Maximum likelihood analysis separated geographically distinct clades: three southern clades including Romania, Greece and Spain, two central clades including Germany and France, a northern clade (northeast clade) including Moscow, Sweden, Norway, and Iceland (Fig. 5.5). In addition to these clades which correspond to geographic regions others were interspersed. The specimens from south Primor'ye (southeast of Russia: Siberia) and Marion Island (south Africa) were associated with the northern clade.

The clades separated by phylogenetic methods were also separated by statistical parsimony (Fig. 5.6). The COI sequences of the 48 specimens consisted of 34 haplotypes. Haplotypes between geographical regions were separated by 19-121 substitutions with the highest number of suggested substitutions being more than 400. Within the geographical regions, haplotypes were separated by 1-52 substitutions (Fig. 5.6). The distinct geographical clades in the phylogenetic tree including three southern clades (Romania, Greece and Spain), two central clades (Germany and Frace) and a northern clade (Moscow, Sweden, Norway and Iceland) were separated by a large number of substitutions in the TCS plot, e.g. Greece and Spain were separated by at least 112 substitutions. Romania constituted an isolated lineage separated by at least 121 substitutions from lineages of other European countries. Also, lineages within single countries such as Greece, Spain, Moscow and Sweden were separated by a large numbers of substitutions. Within the northern clade, Sweden, Iceland, Norway and Moscow were separated by 2-61 substitutions. The haplotypes from Marion Island and

Primor'ye were separated by 1-11 substitutions from lineages of northern European countries.

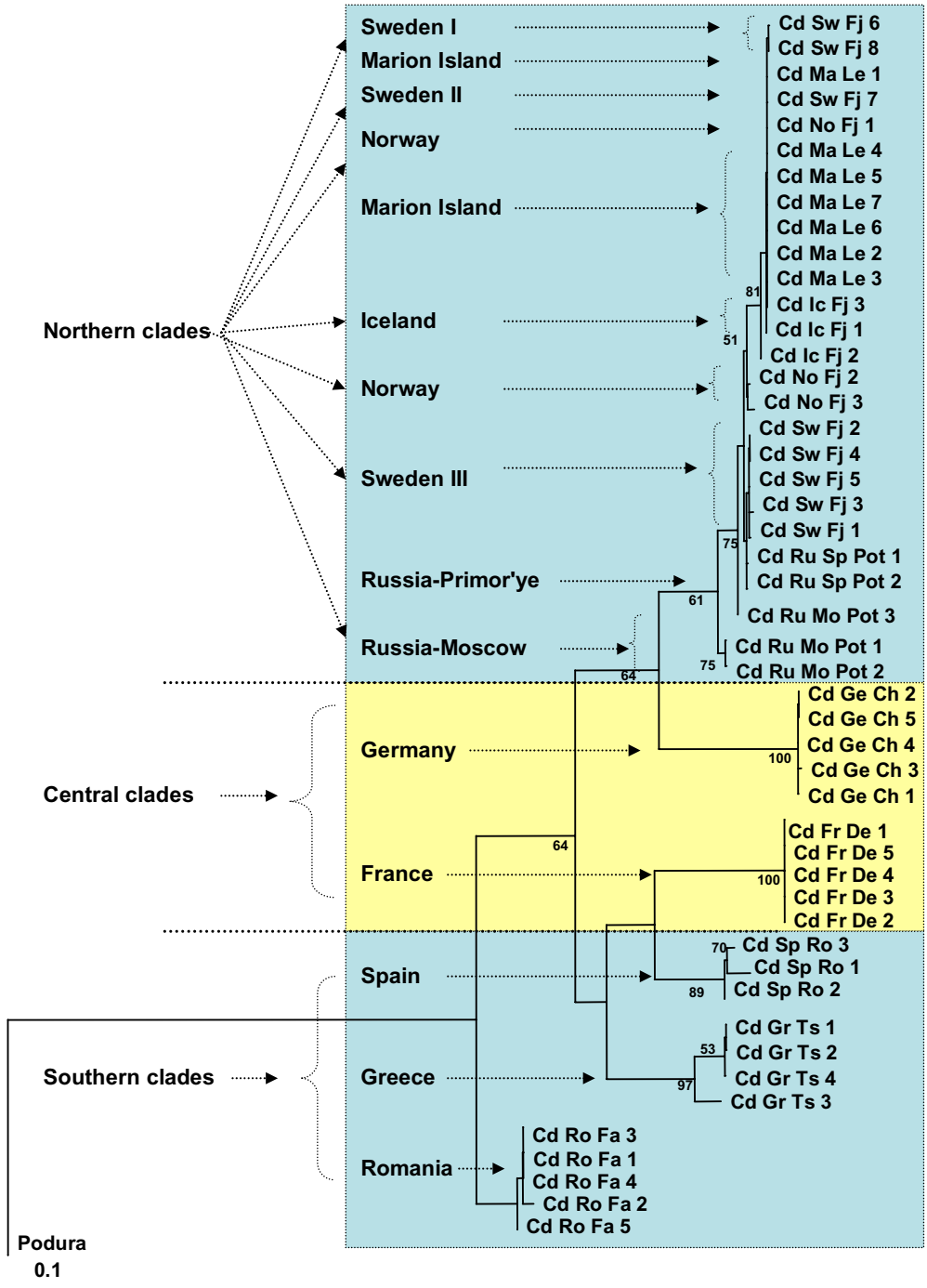


Fig. 5-5. Maximum likelihood tree of 48 specimens of *Ceratophysella denticulata* calculated in PAUP. Numbers at nodes indicate bootstrap values from 100 replicates. For abbreviations, see Table 5-1.

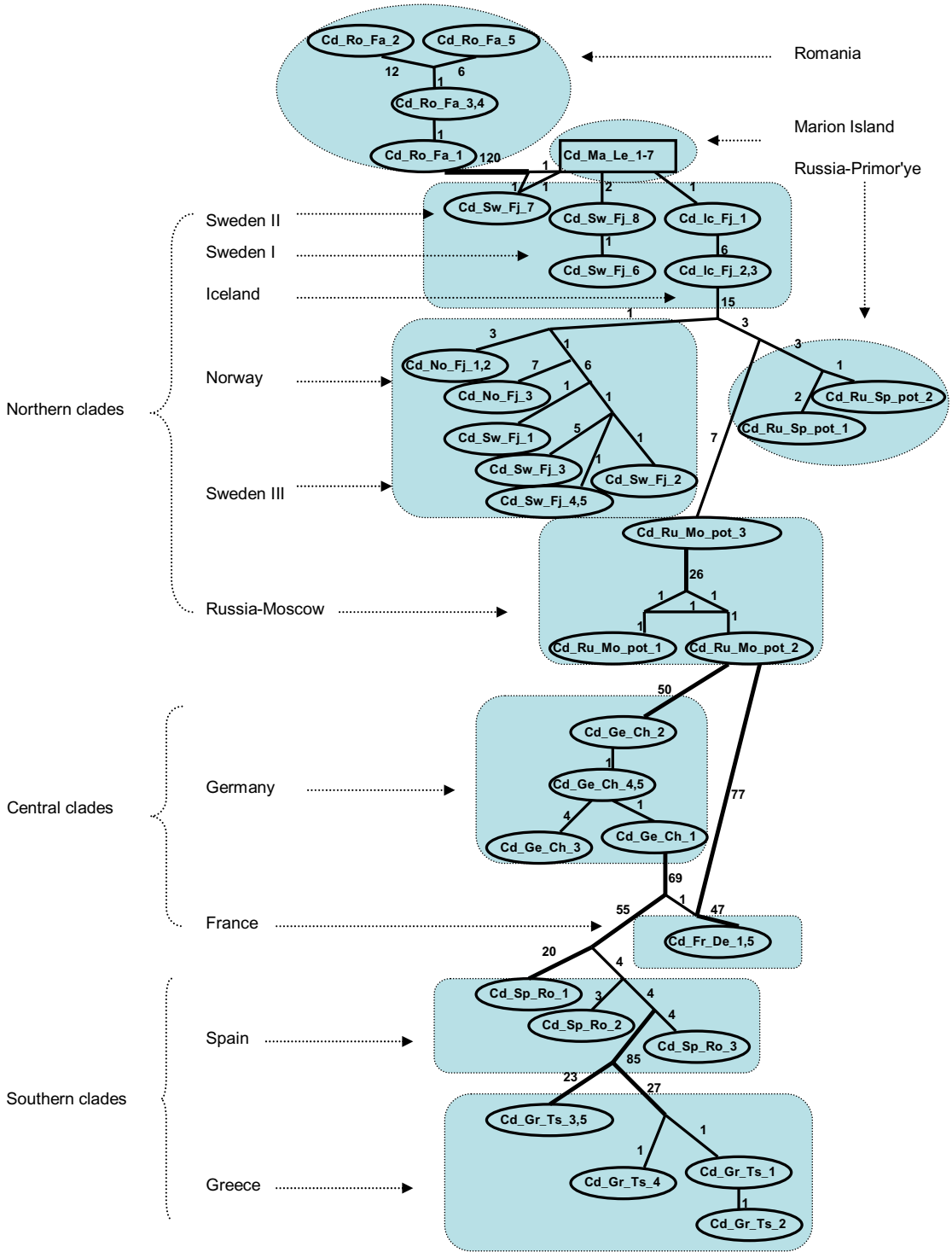


Fig. 5-6. Reconstructed TCS plot of 33 COI haplotypes of *Ceratophysella denticulata*; connection limit set to 150 steps. The length of branches is meaningless, thicker lines indicate number of substitutions ≥ 20.

5.3.2 Parthenogenetic species

Parisotoma notabilis

In *P. notabilis* maximum corrected distance of the 45 analysed specimens was between Moscow and Romania (93%). Maximum likelihood analysis separated geographically distinct clades: a Siberian clade, two southern clades including Italy and Romania, a central clade including France, a central-eastern clade including Germany and Poland, and a northern clade including Norway, Moscow and Iceland (Fig. 5.7). In addition to these clades which correspond to geographic regions, other clades were interspersed. Specimens from Siena and Apennin (Italy) were separated widely, the first clustered close to Greece and Romania, the second close to France. Similarly, the two specimens from Greece each formed separate lineages, one close to Romania, the second close to Spain. The specimens from Spain formed one cluster which was located between the central-eastern clade and the northern clade. Specimens from Marion Island formed one lineage with France.

The clades separated by phylogenetic methods were also separated by statistical parsimony (Fig. 5.8). The COI sequences of the 45 specimens consisted of 32 haplotypes. Statistical parsimony with a connection limit of 150 of the reduced dataset separated geographical regions by more than 100 substitutions with the highest number of suggested substitutions being more than 400. Within geographical regions, haplotypes were separated by 1-49 substitutions (Fig. 5.8). Italy, Romania, Germany, France and Spain were separated by a large number of substitutions from others. Further, haplotypes from Italy were separated by a large number of substitutions from each other. Haplotypes from Greece and Spain formed one linage by phylogenetic methods but were separated by 81 substitutions in the TCS plot. Lineages of the

northern clade of the phylogenetic tree were separated by 5-48 substitutions in the TCS plot. Specimens from Siberia were separated by 1-7 substitutions only. Similarly, lineages from Germany and Poland were separated by a single substitution. Specimens from Marion Island were separated by 2 substitutions from those of France.

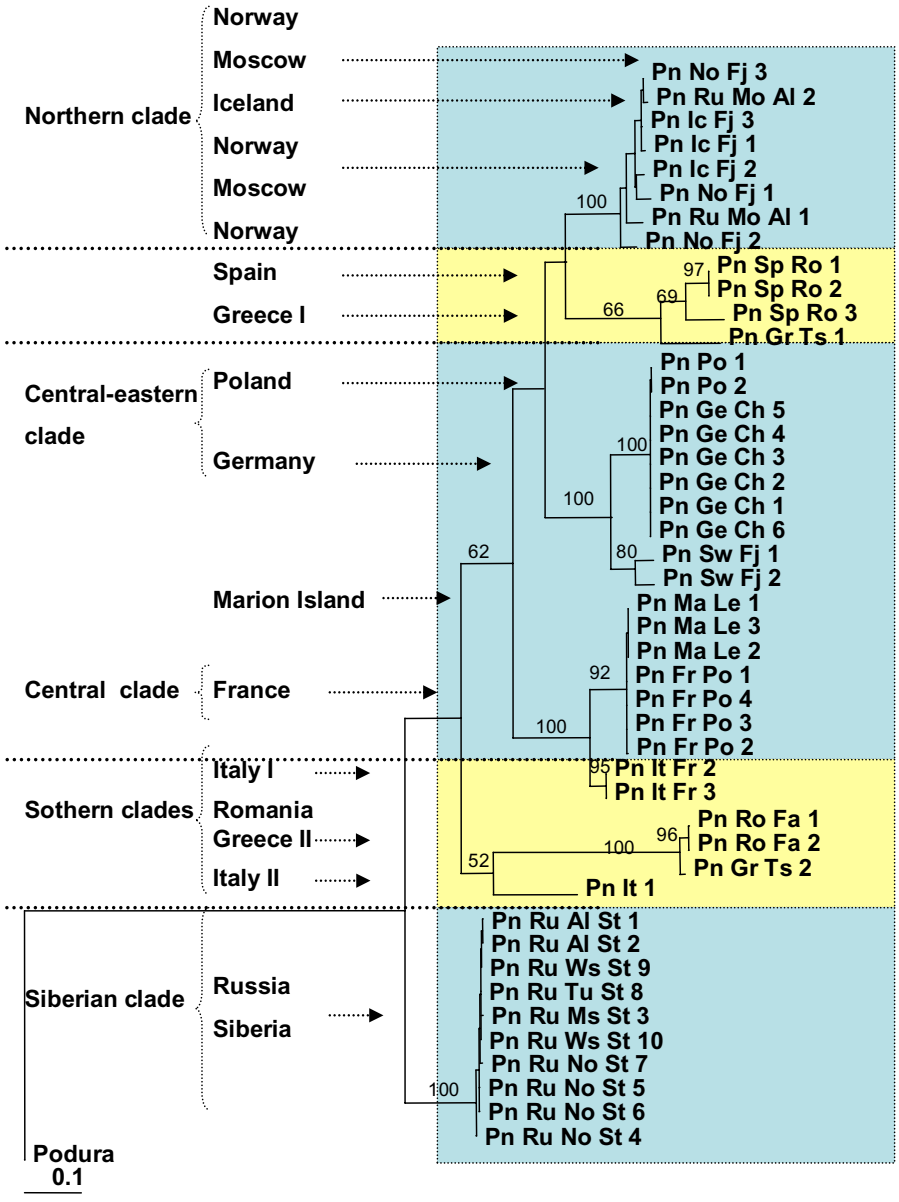


Fig. 5-7. Maximum likelihood tree of 45 specimens of *Parisotoma notabilis* calculated in PAUP. Numbers at nodes indicate bootstrap values from 100 replicates. For abbreviations, see Table 5-1.

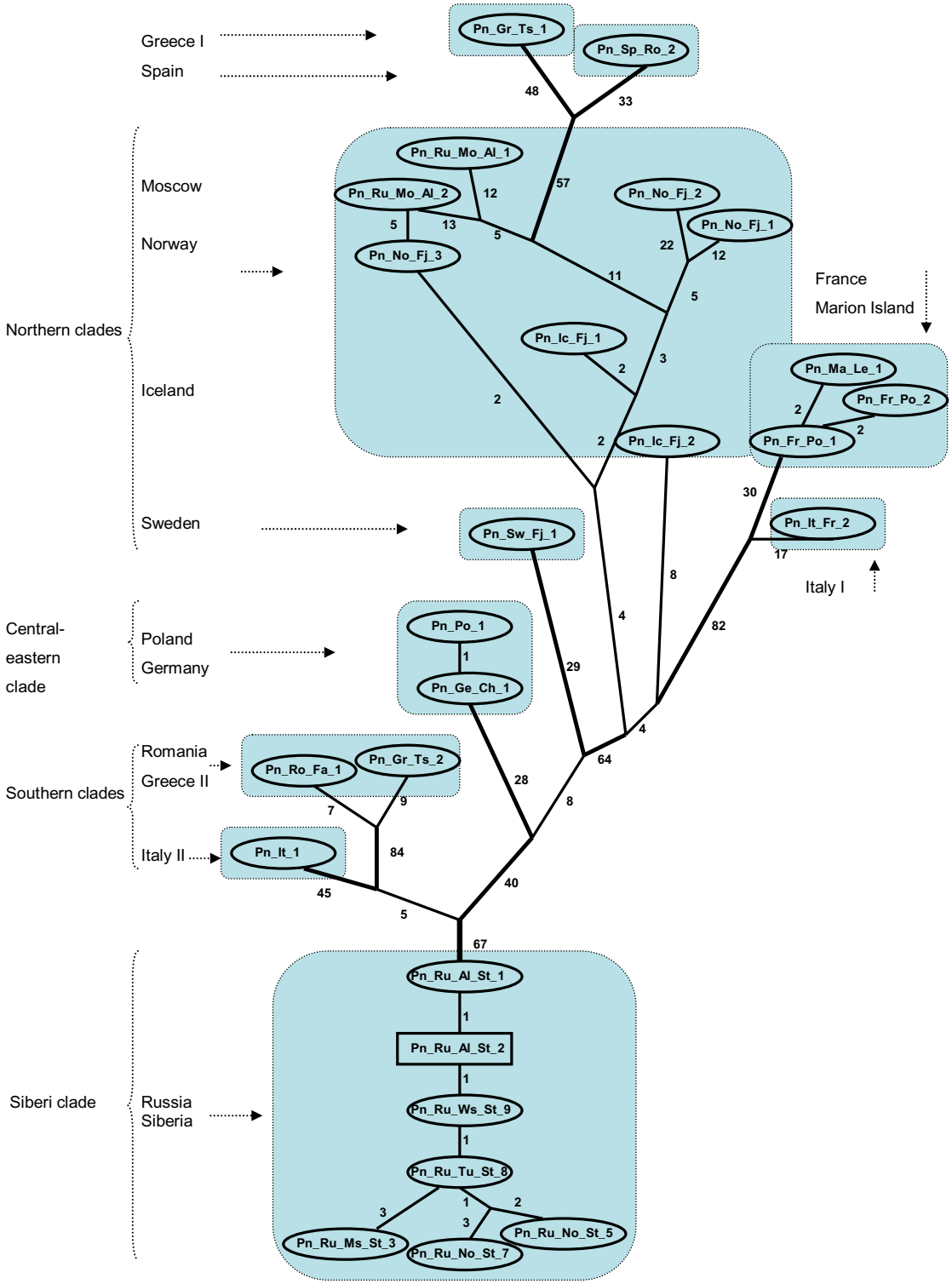


Fig. 5-8. Reconstructed TCS plot of 26 COI haplotypes of *Parisotoma notabilis*; connection limit set to 150 steps. The length of branches is meaningless, thicker lines indicate number of substitutions ≥ 20.

Isotomiella minor

In *I. minor* maximum corrected distance of the 23 analysed specimens were between two populations of Russia, Moscow and Kanin (71%). Maximum likelihood analysis separated four distinct clades: two northern clades including Kanin (north Russia) and Iceland, a central clade including Germany and France, and a north-central clade including Sweden, Moscow, France and Germany (Fig. 5.9). Specimens from Norway formed two lineages, one close to the central clade and the other close to northern-central clade. The specimen from Romania was associated with the central clade.

The clades separated by phylogenetic methods were also separated by statistical parsimony (Fig. 5.10). The COI sequences of the 23 specimens consisted of 14 haplotypes. Haplotypes between the geographical regions were separated by more than 39-144 substitutions with the highest number of suggested substitutions being more than 300. Within geographical regions, haplotypes were separated by 1-51 substitutions (Fig. 5.10). Haplotypes from Norway, Moscow, Kanin and Iceland were separated by a large number of substitutions from others. Haplotypes from Iceland clustered in two lineages, which were separated by a large number of substitutions (157) from each other. In contrast, the haplotypes from Sweden, Germany and France were separated by only 1-2 substitutions.

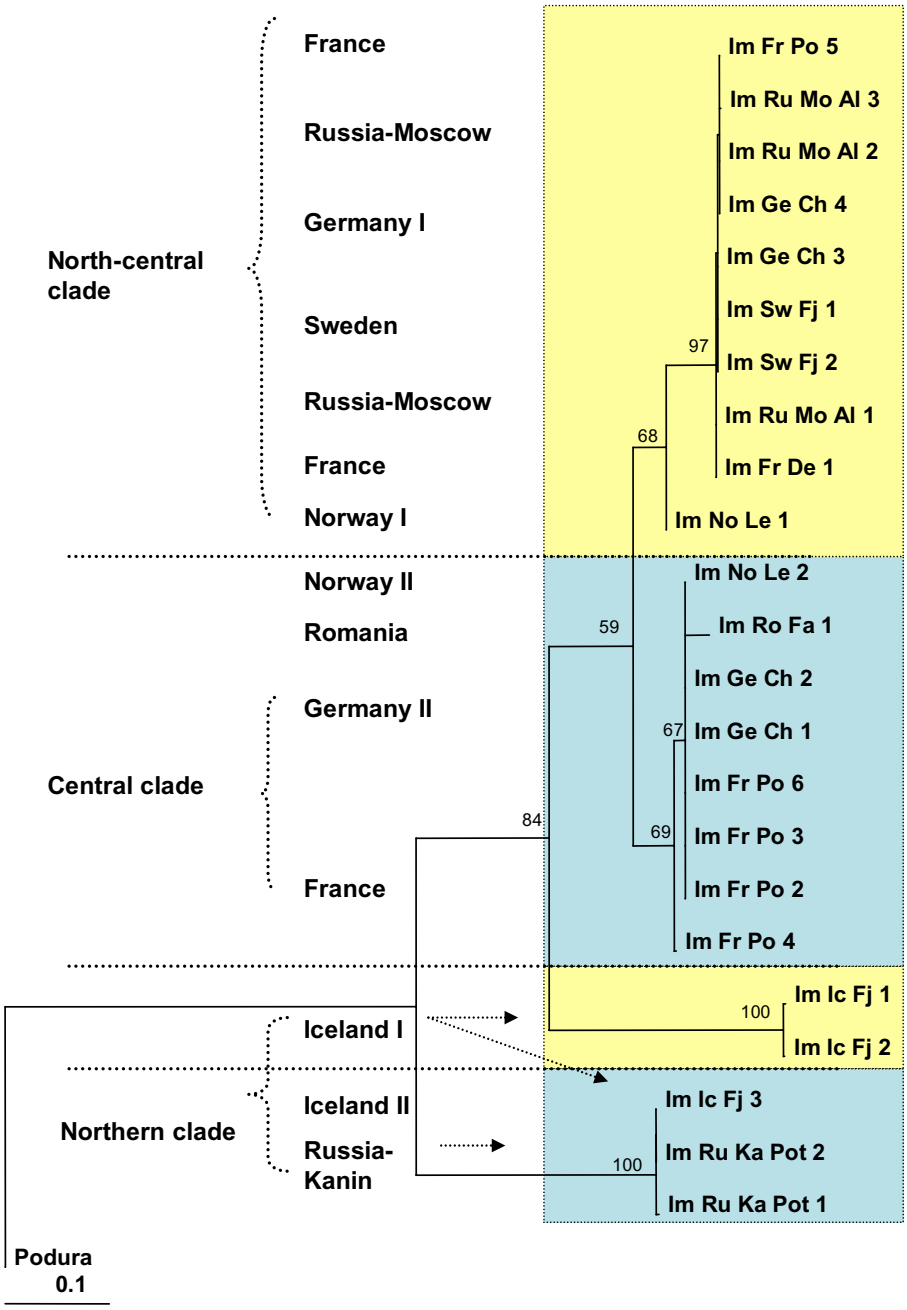


Fig. 5-9. Maximum likelihood tree of 23 specimens of *Isotomiella minor* calculated in PAUP. Numbers at nodes indicate bootstrap values from 100 replicates. For abbreviations, see Table 5-1.

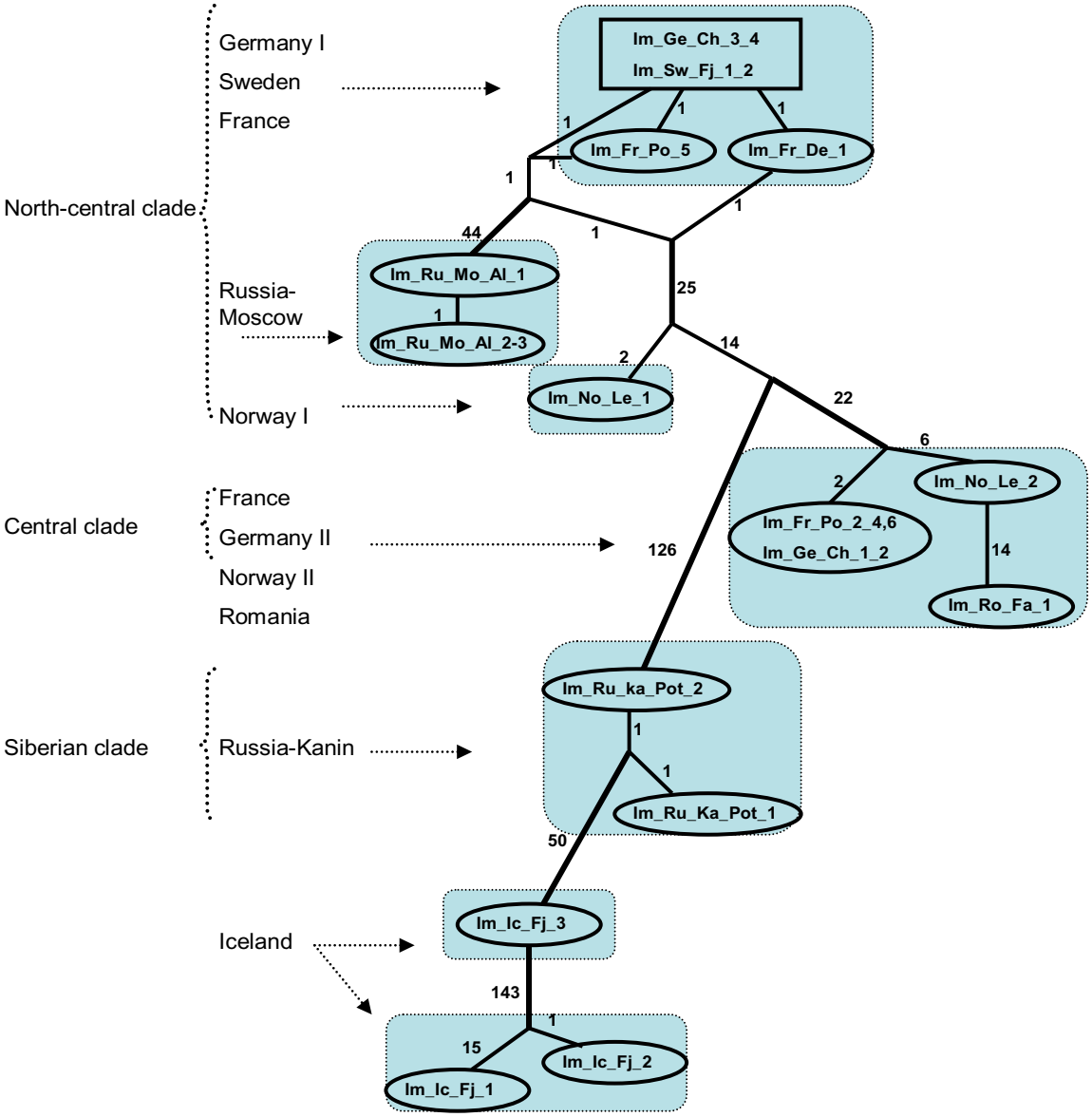


Fig. 5-10. Reconstructed TCS plot of 14 COI haplotypes of *Isotomiella minor*; connection limit set to 150 steps. The length of branches is meaningless, thicker lines indicate number of substitutions ≥ 20.

5.4 Discussion

About 180 million years ago the northern continents separated from Pangea and broke up to form present-day continents (Storey, 1995). During this period continents have undergone fundamental changes in climate. Dramatic climate changes occurred during the last glaciation at the end of the Pleistocene. During the Pleistocene northern regions including Scandinavia and Siberia were glaciated (Hewitt, 1999; Cox and Moore, 2005). Therefore, in particular species of higher latitudes have experienced recent shrinking and expansion of biogeographic ranges (Austerlitz et al., 1997). The present-day communities in the boreal and temperate zone assembled during the post-glacial era from species that survived in the north and those returning from southern refugia (Stewart and Lister, 2001).

In recent years molecular techniques have been used to investigate the postglacial recolonization of glaciated regions by plants and animals. mtDNA is a suitable gene to reconstruct the colonization by extant species of animals after the last glaciation and has been used in a number of phylogeographic studies (Hewitt, 1993; Avise, 1998; Hewitt, 2001; Harrison, 2004; Allegrucci et al., 2005). We used mtDNA (COI) to reconstruct the colonization of Europe by Collembola species. We expected that north and central Europe were colonized from southern refugia as has been shown for other animals (Hewitt, 1999, 2002, 2004; Stewart and Lister, 2001).

The two sexual and two parthenogenetic species used in this study are widespread. *Folsomia quadrioculata* has been recorded from all European and northern Asian countries (Potapow, 2001). *Ceratophysella denticulata* is also a cosmopolitan species and widely distributed in the Palearctic; it has been recorded from most European countries (Gisin, 1960; Fjellberg, 1978, 1998). *Parisotoma notabilis* and *I. minor* are

widespread litter-dwelling species with *P. notabilis* reaching high abundances virtually all over the Holartic including Arctic Islands. *I. minor* also reaches high abundances in the Palearctic, in particular in Central and Northern Europe (Potapow, 2001). No males have been recorded in these two species indicating that they reproduce by parthenogenesis (Petersen, 1978; Chahartaghi et al., 2006).

5.4.1 Divergences between countries

The length of branches in phylogenetic trees and the number of substitutions in TCS plots reflected high levels of divergence between geographical lineages in each of the four species irrespective of the mode of reproduction. Considering 1.5-2.3 % divergence per Myr derived from comparisons between geological and molecular data (Brower, 1994; Gaunt and Miles, 2002; Quek et al., 2004) the maximum corrected distances indicated ancient splits of lineages for each of the four Collembola species studied, i.e. independent of the mode of reproduction. Divergence levels were remarkably high between southern countries of Europe such as Greece, Spain and Romania. Maximum divergence of 85% among *F. quadrioculata* populations suggests that the lineages split at least 40 Myr ago. The large divergence between countries such as Italy, Greece, Germany and France indicate ancient colonization by *F. quadrioculata*. The corrected maximum pairwise distance between *C. denticulata* populations (73%) also corresponds to an ancient split about 35-40 Myr ago. Again, divergences were most pronounced between southern countries such as Greece, Spain and Romania. Maximum genetic divergences between populations of *P. notabilis* (93%) again indicated ancient splits more than 40 Myr ago; splits again were most pronounced between southern countries such as Italy, Romania, Germany, Spain and Greece. Further, corrected maximum

pairwise distances between the lineages of *I. minor* (71%) suggest ancient splits about 35-40 Myr ago. In particular Siberia appears to have been colonized by distinct old lineages, presumably at least twice.

Ancient splits of lineages between species (11-21 Myr) have been reported previously for Antarctic Collembola species (*Cryptopygus* spp.; Isotomidae) by Stevens et al., (2006). Our data suggest that most geographical regions of Europe have been colonized by Collembola tens of millions of years ago considerably predating the Pleistocene. In particular, this applies to southern but also to central Europe. Each of the four Collembola species likely colonized southern Europe almost 40 million years ago back in the Tertiary. This refutes the hypothesis that Collembola colonized central Europe from southern refugia after the last glacition; rather, Collembola appear to have survived in these regions.

The different lineages within each of the four Collembola species can be viewed as cryptic species. Morphologically cryptic species are known in many Collembola species (Carapelli et al., 1995; Stevens and Hogg, 2003). In fact, the different lineages of the sexual species may represent genetically separated biological species as appears to be the case in *F. quadrioculata* and *F. manolachei*. These two species are morphologically very similar and have long been confused; both the phylogenetic tree and TCS plot suggest that they indeed are separated species which presumably were separated for tens of millions of years.

5.4.2 Divergences within countries

Our data also showed large divergences within populations of single countries in each of the four Collembola species suggesting that these populations constitute of lineages

of different biogeographic origin. In part they consisted of separate (endemic) lineages and lineages closely associated to those of other biogeographic regions, suggesting that they colonized these localities recently, presumably by long-distance dispersion. In *F. quadrioculata* high divergences within various countries, including Poland, Norway, Germany, Russia and Italy, suggest colonization by populations originating from very different regions. Hogg and Hebert (2004) also reported high divergence levels within populations of the same country for *F. quadrioculata* (13%) when studying COI as barcoding gene for molecular identification of Collembola species. High genetic divergences within populations of the other Collembola species from single countries suggest independent colonization of countries by Collembola species, i.e. Russia, Sweden, Romania, Spain and Greece by *C. denticulata*, Italy, Norway and Russia by different lineages of *P. notabilis*, and Iceland, Norway and France by different lineages of *I. minor*. Potentially, the different lineages of sexual Collembola species in these countries interbreed thereby forming hybrid zones; however, some may also be genetically separated, i.e. consisting of cryptic species.

5.4.3 Recent colonizations

During glaciation northern Europe and Siberia were covered by thick ice sheets and were colonized by animals and plants after glacial retreat. Indeed, phylogeographical analysis and statistical parsimony reflected this recent colonization for several countries/regions including Iceland, Norway, Sweden and regions from Siberia. Therefore, Collembola species surviving glaciation in central and southern Europe and Asia indeed likely colonized deglaciated regions of northern countries/regions after the last glaciation. Recent colonization of northern Europe was most obvious by

phylogenetic trees and a small number of substitutions in the TCS plot in *C. denticulata* (sexual species) and *P. notabilis* (parthenogenetic species). In contrast, phylogenetic trees indicated close relationship of populations of *F. quadrioculata* from Iceland, Norway, and Sweden whereas statistical parsimony indicated that these lineages in fact are very different, i.e. colonized these countries independently. The length of braches of phylogenetic trees and the small number of substitutions between locations of lineages of *F. quadrioculata* and *P. notabilis* also suggest recent colonization of Siberia by these species. However, genetic divergences among the Siberian clades of *F. quadrioculata* exceeded those within the Siberian clades of *P. notabilis* suggesting that colonization by *P. notabilis* was more uniform. Phylogenetic trees and statistical parsimony also indicate that northern and central European countries, including Sweden, Germany and France, have been colonized by *I. minor* more recently.

5.4.4 Colonization of islands

In contrast to the ancient colonization of southern and central Europe, phylogenetic trees and TCS plots suggested recent colonization of Islands including Marion, Ringnes, Wrangel and Kolguev Islands. Recent colonization (< 2 Myr) by Collembola species (*Cryptopygus* spp. Isotomidae) between some subantartic islands, such as Marion Island, has been reported previously by Stevens et al. (2006). They suggested that the colonization of these Islands occured during Pleistocene glacial cycles. Marion Island is young (~ 300 Kyr) (Stevens et al., 2006) and the low divergence levels between lineages of this Island and Europe indicate recent colonization originating from Europe. According to TCS plots Marion Island was colonized by *C. denticulata* originating from northern European countries, potentially from Iceland or Sweden, and by *P. notabilis*

potentially originating from France. Further, TCS plots suggest that colonization of Ringnes, Wrangel and Kolguev Island by *F. quadrioculata* occurred recently. Presumably, Collembola species have been transported to Islands by wind, water, migrating birds and humans. In fact, dispersion of Collembola by human activity from one side of the world to the other has been documented for a number of Collembola species (Christiansen and Bellinger, 1988; Hopkin, 1997).

5.4.5 Colonization by sexual vs. parthenogenetic species

Parthenogenetic species are faster colonizers than sexual species (Williams, 1975; Bell, 1982; Scheu and Schulz, 1996; Pound et al., 2002Lindberg and Bengtsson, 2005). Therefore, the pattern of colonization of deglaciated regions by sexual and parthenogenetic species likely differed after the last glaciation. In contrast to this assumption, the general pattern of colonization of northern Europe and Siberia by Collembola species was similar irrespective of the mode of reproduction. Phylogenetic trees and statistical parsimony indicated high genetic divergences between and within regions, and recent colonization of northern Europe, Siberia and Islands for both sexual and parthenogenetic species.

Additionally, large divergences of lineages of both parthenogenetic species studied indicate that they colonized Europe while being parthenogenetic. This contrasts the assumption that parthenogenetic lineages are short-lived evolutionary dead ends (Muller, 1964; Maynard Smith, 1978; Kondrashov, 1988, 1993; West et al., 1999). The existence of “ancient asexual” scandals such as bdelloid rotifers (Welch and Meselson, 2000), darwinulid ostracods (Martens et al., 2003), and certain lineages of oribatid mites (Norton and Palmer, 1991; Maraun et al., 2003, 2004; Schaefer et al., 2006; Heethoff et

al., 2006) challenge this dogma. The deep splits of the two parthenogenetic species studied suggest that similar to other soil invertebrate taxa, there likely exist “ancient asexual” Collembola species.

5.5 Conclusion

Results of this study indicate that each of the four Collembola species consists of ancient lineages which colonized southern but also central Europe in the pre-Pleistocene, presumably even the lower Tertiary (Eocene/Oligocene). The deep splits within populations of single countries in each of the four Collembola species suggest that Collembola species in general constitute of a number of cryptic species with complex phylogeographic history. The results suggest that many species survived the harsh conditions in central European countries during the last glaciation refuting the hypothesis that central European populations originated from southern refugia. Further, the data reflected post-glacial recolonization by Collembola of northern Europe (Scandinavia and northwest Russia) and Siberia. Also, colonization of islands including Marion Island, Ringnes Island, Wrangel and Kolguev Island by Collembola species occurred more recently. Deep splits of lineages of the two parthenogenetic species studied suggest that similar to other soil invertebrate taxa, such as Oribatida, there are also exist “ancient asexual” Collembola species.

Chapter 6

6 General discussion

Collembola are one of the most diverse and abundant group of soil microarthropods; until today about 7000 species have been described, however, according to the most recent estimate of the ratio of described and undescribed species of all organisms on the earth by Heywood (1995), there might be more than 50,000 species (Hopkin, 1977). Collembola presumably reach maximum diversity in tropical rainforests (Hopkin, 1977). In contrast, maximum density of more than one million individuals per square metre is reached in forest soils of the temperate and boreal zone (Petersen and Luxton, 1982). This great diversity and density of Collembola make them an important component of the decomposer system. The high diversity of soil animals such as Collembola in the rather homogeneous environment of the soil is one of the great riddles in soil biology (Schaefer, 1991; Giller, 1996). Until now, many attempts have been made to understand this riddle. One explanation for the high diversity of Collembola is adaptive radiation. Adaptive radiation is defined as the outcome of divergent natural selection due to environmental factors, resources and resource

competition (Schluter, 2000). This ecological specialization results in species occupying different niches (Gittenberger, 1991), and therefore coexisting (sympatric diversity; Solem, 1984). Specialization in food resources can be one explanation for the high diversity of Collembola species in one habitat. Therefore, in the present study the natural variation in nitrogen isotopes was assessed in Collembola taxa to investigate trophic niche differentiation.

The density of Collembola especially in tropical rainforests may also be related to the mode of reproduction. The stable habitat of the soil provides an ideal environment for the prevalence of parthenogenesis (Bell, 1982). Most Collembola species are sexual (Christiansen, 2003), but parthenogenesis also is widespread (Goto, 1960; Petersen, 1978; Chahartaghi, 2006). Knowledge on mode of reproduction and sex ratios is necessary to understand population dynamics and the colonization of new habitats by different species. In parthenogenetic species one female is enough to establish a population while in sexual species presence of both sexes (female and male) is necessary (Williams, 1975; Bell, 1982; Scheu and Schulz, 1996; Lindberg and Bengtsson, 2005). Theoretically at similar envioromental conditions the density of parthenogenetic species increases at least twice as fast as that of sexual species. On the other hand at harsh envioromental conditions parthenogenetic species may suffer more than sexual species due to the absence of recombination.

Population structure is the result of both present processes and historical events. Collembola as an old component of soil systems all over the world represent ideal models to investigate present processes as well as historical events. The most recent dramatic historical event was the glaciation at the end of the Pleistocene. At different stages during the Pleistocene, thick ice sheets covered the earth that expanded from

north to south (Hewitt, 1999; Cox and Moore, 2005). With the expansion of the ice sheets the global distribution of animals and plants was considerably disturbed. Animal and plant communities of the boreal and temperate zone assembled after glacial retreat thereby combining both species that survived the cold environment in the north and those returning from southern refugia (Stewart and Lister, 2001). Molecular markers allow to uncover these processes and therefore to understand their respective contributions to the present-day community composition (Hewitt, 1999). Recently, DNA sequence information has been used to analyse the genetic variation of species across Europe and to investigate the colonization after the last glaciation (Hewitt, 1999; Stewart and Lister, 2001). Molecular data confirmed that southern regions of Europe functioned as major ice age refugia, and demonstrated that genetically distinct taxa emerged from them (Hewitt, 1999). The genetic variation of Collembola species was investigated using molecular markers, mtDNA (COI), to understand phylogenetic relationships of different populations in Europe, and also in order to get insight into the postglacial recolonization of central and northern Europe.

6.1 Trophic niche differentiation in Collembola

Overlapping of food resources results in competition between animal species; separated food resources therefore contribute to the coexistence of species, i.e. to local species diversity. Trophic niche differentiation therefore may allow the coexistence of Collembola species in a homogeneous habitat, such as the soil. Different attempts have been made to elucidate the diets of Collembola and to explain the co-existence of the high number of species in soil. Collembola feed on various food materials and therefore have been assumed to be generalistic feeders (Newell, 1984; Bardgett et al., 1993;

Addison et al., 2003; Scheu and Simmerling, 2004). New methodologies, such as stable isotope analysis, provide unique opportunities to analyse food selectivity and trophic niche differentiation in Collembola. Scheu and Falca (2000) and Ponsard and Arditi (2000) were the first to analyse soil animal communities using nitrogen stable isotope ratios ($^{15}N/^{14}N$).

My investigations are the first detailed analyses of trophic niche differentiation of Collembola of a large number species (20 species/taxa) using $^{15}N/^{14}N$ ratio (Chapter 2). The $\delta^{15}N$ signature formed a gradient spanning over 9 delta units suggesting that different species occupy very different trophic niches. According to the $\delta^{15}N$ signature three feeding guilds have been assigned, i.e. phycophages/herbivores, primary and secondary decomposers. The assigned groups were confirmed by results from food choice experiments, gut content observations and fatty acid and enzymatic analyses (Scheu, 2002; Maraun et al., 2003; Berg et. al., 2004; Ruess et. al., 2005a; Haubert et. al., 2006). In contrast to the assumption that Collembola are generalistic feeders the results of this study suggest that Collembola species feed on very different food materials. Therefore, trophic niche differentiation likely contributes to the high diversity of Collembola species in terrestrial systems. On the other hand, closely related or even the same species may have different $\delta^{15}N$ signatures in different habitats, which indicates that they have the ability to switch diets if other resources become available. Overall, the investigation of trophic niche differentiation of Collembola species supports the hypothesis that Collembola underwent an adaptive radiation. However, the co-evolutionary relationship between Collembola and food resources presumably was weaker than that between e.g. plants and herbivores.

6.2 Impact of reproductive mode on recolonization and response of Collembola to resource depletion

Parthenogenesis is the production of new progenies from unfertilised eggs. Parthenogenetic species occur scattered throughout the animal kingdom and only about 1% of the total numbers of extant species reproduce by parthenogenesis (Koivisto and Braig, 2003). Parthenogenetic reproduction has several advantages over sexual reproduction that theoretically should result in the elimination of sexuality in a wide range of habitats (Maynard Smith 1978). The most obvious difference between sexual and parthenogenetic reproduction is that parthenogenetic organisms do not produce male offspring (Neiman, 2004). This results in a doubling of population growth rates compared to sexual taxa (Williams, 1975; Maynard Smith, 1978; Bell, 1982; Butlin et al. 1998). On the other hand, non-recombining parthenogenetic species should accumulate mutations and therefore the doomed to extinction in the long-term (Muller, 1964; Kondrashov, 1988; Paland and Lynch 2006). Despite the two-fold reproductive advantage of asexual over sexual reproduction, the majority of eukaryotic species are sexual (Neiman, 2004). Why sex is so widespread is still unknown and remains one of the most prominent unanswered questions in evolutionary biology. The reproductive mode of most animal species is correlated with ecological factors. Theory predicts sexuality to be superior to parthenogenesis in unstable habitats since higher genetic diversity allows a faster reaction to changing environmental conditions (Williams, 1975; Hamilton, 1980). Consequently, parthenogenetic taxa should dominate in stable habitats because there is no need to adapt to changing environmental conditions. Forests appear to be rather stable habitats. In fact, the percentage of parthenogenetic taxa in mites,

Collembola, enchytraeids and nematodes in forest soils is high compared with other habitats (Norton and Palmer, 1991; Siepel, 1994; Niklasson et al. 2000; Bloszyk et al., 2004).

Collembola are one of the most important micro-arthropods which have high density in soil especially in forests. This high density partly might be due to mode of reproduction. Based on field studies it is now well established that beside bisexual reproduction parthenogenesis is common in Collembola (Goto, 1960; Petersen, 1978). There are two ways to confirm if certain species reproduce by parthenogenesis; first, by showing that field populations of certain species exist only as females. Second, by maintaining female only populations of a species in the laboratory for several generations (Hopkin, 1977).

This study investigated sex ratios in Collembola species to evaluate if parthenogenesis in Collembola is as widespread as previously assumed (Chapter 3). Most Collembola species in the studied forest reproduced sexually but several species reproduced by parthenogenesis. The parthenogenetic species were small and euedaphic, however, some species in which no males were recorded were hemiedaphic. Generally, the results showed that the community sex ratio was strongly female biased. Several factors can be responsible for the high incidence of all-female populations in Collembola and the dominance of parthenogenetic species in deeper soil layers, such as the accessibility of male spermatophores.

Two further studies were conducted to investigate if the mode of reproduction corresponds to the availability of food resources. We expected that due to resource depletion the number of specimens will decline and bisexual species will be favoured

over parthenogenetic species, due to greater genetic diversity. Further, we expected that parthenogenetic species will colonize empty habitats faster than bisexual species. The

results suggest that parthenogenetic and bisexual Collembola suffered to a similar extent from resource depletion; potentially, parthenogenetic species are genetically diverse allowing them to compete and coexist with bisexual species. On the other hand, parthenogenetic and bisexual species differed in the speed they recovered from disturbances and in recolonization from litter and soil; parthenogenetic species are faster colonizers than bisexual species indicating that parthenogenetic species indeed benefit from the abandonment of producing males, in particular when fresh resources are becoming available for being exploited.

6.2.1 Phylogeography of European Collembola

Distribution of animals and plants species in the world has undergone dramatic changes during their evolutionary history. The most recent change occurred at the end of Pleitocene during glaciation. Some species survied the harsh conditions during glaciation while others migrated to southern refugia. In fact, the present communities of animals and plant in the northern hemisphere assembled during the post-glacial era by species that survived in the north and those returning from southern refugia (Stewart and Lister, 2001). Collembola were strongly affected from these hash climate changes during glaciation.

Based on previous studies on the post-glacial recolonization of animal and plant species (Hewitt, 1999, 2000, 2001), we assumed that present-day distribution of Collembola species in the central Europe originated from species of southern Europe.

Parthenogenetic species are faster colonizer than sexual species, therefore, we also assumed sexual and partheonegetic species have to have different pattern of post-glacial recolonization.

The genetic variation of Collembola species were investigated using a 657-bp fragment of the COI gene for understanding phylogeographic relationships and postglacial recolonization of central and northern Europe by Collembola species. Two sexual species, *Folsomia quadrioculata* and *Ceratophysella denticulata*, and two parthenogenetic species, *Parisotoma notabilis* and *Isotomiella minor,* from different regions of Europe were included to analyse if the mode of reproduction affected recolonization patterns. Some populations from Islands including Ringnes Island and Marion Island and from Siberia were also included.

My investigations are the first comprehensive analyses of phylogenetic relationship of Collembola from different countries (Chapter 5). The results indicated recent colonization dating back to the end of Pleistocene by Collembola species in some regions of northern Europe including Norway, Iceland and Sweden. The regions of Siberia also were colonized by Collembola species more recently. Further, our data showed recent colonization in Islands including Marion Island and Ringnes Island. In contrast, populations of each of the four Collembola species of central and southern Europe were separated by deep splits irrespective of the mode of reproduction. Deep splits between different populations of Collembola species suggest that the colonization of Europe by these species considerably predates the Pleistocene, potentially dating back to the lower Tertiary. This refutes the hypothesis that central European Collembola populations originated from southern refugia after the last glaciation. The deep splits of the two parthenogenetic species studied suggest that similar to other soil invertebrate

taxa, such as Oribatida, there also exist “ancient asexual” Collembola species. The deep splits in each of the four Collembola species studied indicate that Collembola species in general constitute of a number of cryptic species with complex phylogeographic history.

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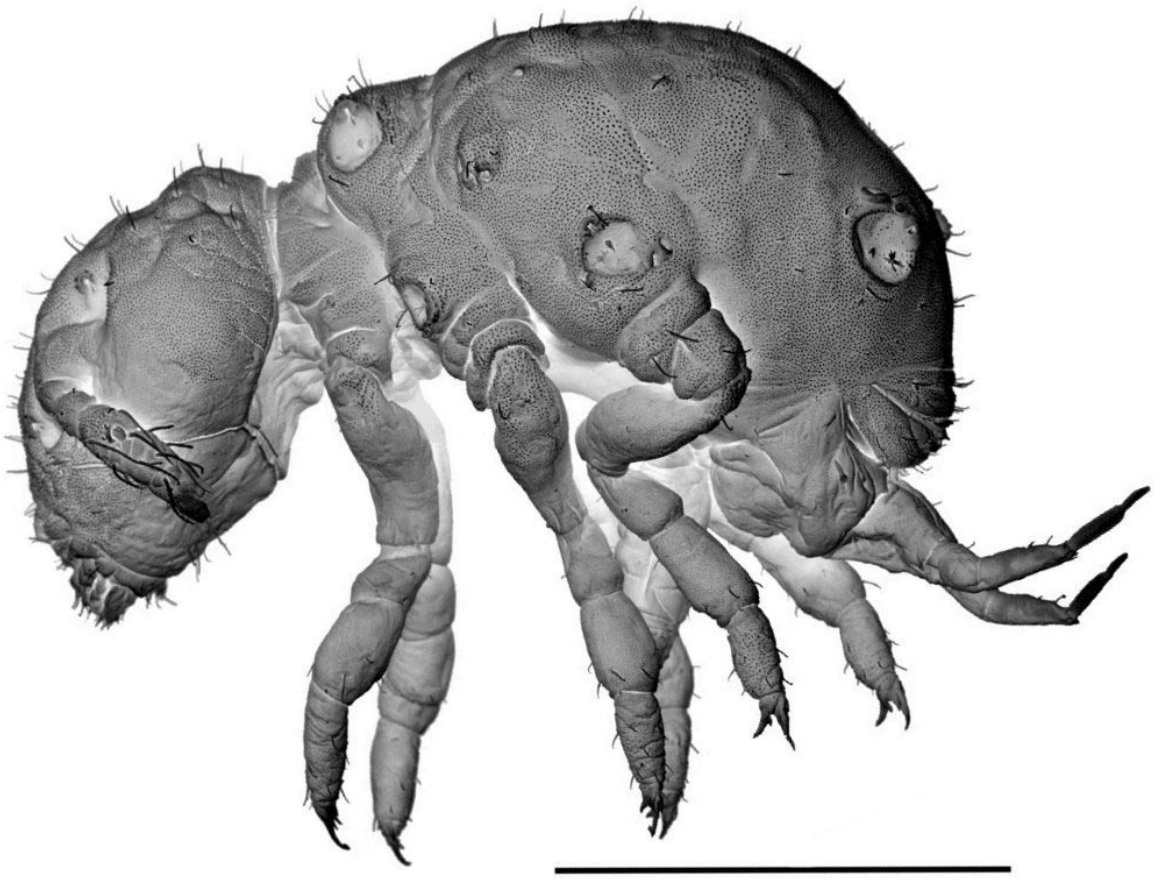

Note: The scale bar=0.1 mm

Lateral habitus of *Megalothorax* sp. (Neelidae)
Scaning electron microscope picture by D.E. Walter, 1999
From: Checklist of the Collembola of the World by Bellinger, P.F., Christiansen, K.A. and Janssens, F. 1996-2007. http://www.collembola.org

Curriculum Vitae

1-Personal Details:

Masoumeh Chahartaghi

Date and Place of Birth: Dec 10, 1976, Tehran.

Martial Status: Married, No child.

2- Address:

Present address (Germany): Schöneweibergasse 6, 64347- Griesheim, Germany,

Tel: +49 (0)61555-848698

Former address (Iran): Pirbodaghi Alley. No.21, Sianaki Ave, Doolatabad, Tehran, Iran.

Tel: +98-021-33762670

chahar@bio.tu-darmstadt.de , chahartaghi@yahoo.com

3-Educational Background:

M.Sc: University of Tehran, Tehran-Iran. (G.P.A = 18.12 out of 20.00)

B.Sc: University of Bahonar, Kerman-Iran. (G.P.A = 17.34 out of 20.00)

Publications:

Journal Papers:

Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005. Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biology and Biochemistry 37, 1718-1725.

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My Research Interests:

1. Molecular systematic of insects
2. Biogeography of insects
3. Soil animal ecology
4. Systematic of Scoleidae (hymenoptera)