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ORIGINAL PAPER

Dispersal and clonal diversity of North-European parthenogenetic earthworms

Juhani Terhivuo · Anssi Saura

Abstract At least 15 earthworm species are known to reproduce parthenogenetically. Most of these retain meiosis but the chromosome set is duplicated before meiosis; alternatively there is mitosis instead of meiosis. In both cases the offspring are genetic copies of the parent worm. Parthenogens are always polyploid. Parthenogenesis is associated with a dispersal advantage: a single propagule suffices to establish a new population. We have studied the clone pool structure and dispersal of ecologically dissimilar polyploid parthenogenetic lumbricids in northern Europe using enzyme electrophoresis. The anthropochorous *Octolasion cyaneum* has a very low number of clones in populations that are located far away from each other. The opposite is the eurytopic *Dendrobaena octaedra* that has a wide array of clones in each population. The ripicolous *Eiseniella tetraedra* disperses with flowing water and possibly also through zoochory. On subarctic North-European mountains its clone pool decreases with increasing elevation. At the top there are a few but persistent clones. Small brooks carry propagules downstream, so that at the mouths of brooks clone pools are more diverse than higher up; again larger rivers carry clones downstream. Clone dispersal is relatively free in a freely flowing river, while dams stop propagules in harnessed rivers. The mouths of rivers have high *E. tetraedra* clone diversity. Clones disperse from these clone centers to islands formed through land uplift along the northern Baltic Sea. The annual turnover of clones is high on these islands. A survey of epigeic and endogeic parthenogens on the Åland islands which serve as stepping stones between Estonia, Finland and Sweden shows an invasion route of clones across the Baltic Sea. Anthropochory (*Aporrectodea rosea* and *Octolasion cyaneum*) and hydrochory (*E. tetraedra* and *Dendrobaena octaedra*) seem to play important roles in the clone pool formation on the Åland islands. Quite recently an exotic parthenogen *Dichogaster bolaui* has found a curious habitat in human settlements viz., the sewer pipe system. Many clonal earthworms show significant morphological and morphometric diversity in and between sample localities but we have failed to associate this variation with the clonal variability. It seems that local factors modify the morphometrics and morphology ultimately determined by the genotype of parthenogenetic earthworms.

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Introduction

Partenogenesis in earthworms

Parthenogenesis entails a two-fold advantage over sexual reproduction (Williams 1975; Maynard Smith 1978). In sexually reproducing (amphigonic) organisms it normally takes two individuals to produce one offspring per unit time, while each parthenogenetic individual is a reproducing female. Accordingly, two parthenogens produce two offspring at the same time as a male and a female produce just one. Earthworms are largely hermaphroditic, and the advantage of dispensing with sex is not as clear as in otherwise amphigonic animals, which do away with the male sex altogether. In some earthworm species with biparental reproduction there is an exchange of sperm between the partners whereas in some species certain individuals function mostly as males and others again as females (Meyer and Bouwman 1997). Parthenogens can allocate resources efficiently to egg production. Some of them retain, however, unnecessary or potentially costly attributes of sex such as copulation, male genitalia and production of spermatophores, even though there seems to be no sperm production and the male pores may not always open (e.g. Casellato 1987; Terhivuo 1988a). These earthworms share many of these evidently unnecessary features with asexual plants such as dandelions that produce flowers, nectar and empty pollen. Parthenogenetic reproduction in plants goes with the collective name of apomixis. We may here refer to the monograph by Asker and Jerling (1992) that covers the cytological and evolutionary attributes of plant apomixis.

Most parthenogenetic lumbricids retain meiosis. The chromosome number is doubled before meiosis at the last oogonial divisions. This premeiotic doubling is accomplished through a mitosis, in which all resulting chromosomes become included in a single nuclear membrane at telophase. The next step is regular meiosis. In the first meiotic division the chromosomes pair and form bivalents, the number of which is equal to the chromosome number in the somatic cells. The chromosome number is restored and all offspring are genetically copies of their mother (Suomalainen et al. 1987). *Dendrobaena octaedra* produces eggs through mitosis instead of meiosis, i.e. through cloning in the proper sense of the word (Omodeo 1955; Casellato and Rodighiero 1972). Amphigonic *D. octaedra* populations have never been encountered. Most populations are hexaploid (6×) but there are also populations that have high uneven chromosome numbers (Casellato 1987; Suomalainen et al. 1987).

Chromosomal sex determination is the major obstacle for polyploidy in animals. Polyploid animals reproduce in general parthenogenetically (Suomalainen et al. 1987). Hermaphroditic earthworms lack sex chromosomes, and biparentally reproducing earthworms may be either diploid or polyploid while all parthenogenetic earthworms appear to be polyploid or aneuploid (Muldal 1952; Omodeo 1951a–e, 1952, 1953a,b, 1955; Casellato and Rodighiero 1972; Vedovini 1973; Casellato 1987; Terhivuo et al. 1987, 1994; Viktorov 1997. Odd degrees of ploidy (e.g. 3×, 5× etc.) are not compatible with sexual reproduction but do not constitute an obstacle for clonality. Accordingly, clonal earthworms can have either an odd or even degree of ploidy, while sexually reproducing polyploids always have an even multiple of the haploid set.

Parthenogenesis is a condition derived from sexual reproduction. In general sexual forms inhabit the central area of distribution of a species and the parthenogenetic derivatives are found in marginal or disturbed habitats around the sexuals in a pattern called geographic parthenogenesis (Vandel 1928). The asexual polyploids form, likewise, concentric rings around the central diploid populations, so that the lowest degrees are found close to the diploid sexuals and the highest ones farthest away from them, a pattern called geographic polyploidy by Stenberg et al. (2003). Amphigonic polyploid earthworms seem to display a somewhat similar pattern (Omodeo 1951b). Perel and Graphodatsky (1982) have found degrees of polyploidy all the way up to dodecaploidy (12×) in *Eisenia nordenskioldi*. Viktorov (1997) has shown that different polyploid forms have discrete geographic distributions and ecological attributes. Deeply burrowing parthenogenetic *E. nordenskioldi* polyploids inhabit the southern margins and amphigonic epigeic polyploids the northern margins of the total area of distribution across Siberia (also see Tiunov et al. this issue).

Clone diversity, dispersal and occupation of habitats

Dispersal modes and pathways between earthworm populations are not easy to trace, particularly if no obvious human agency is involved. Enzyme electrophoresis is an efficient tool in surveying genetic structure, clonal adaptation and dispersal affinities between populations of polyploid and parthenogenetic earthworms. A clone is identified through an overall enzyme phenotype coded by several independent loci. Clonal offspring are copies of their parent worm. Mutations and eventual effects of transposition will, however, accumulate in the clones. Sexual reproduction in animals is usually tied to diploidy, meiosis, fertilization and Mendelian inheritance. These processes produce new gene combinations in each generation. Some combinations may confer to their bearers a higher fitness, i.e. a potential to produce more surviving offspring than either parent. On the other hand, genetic recombination disrupts adapted gene complexes. Local adaptation can be maintained through inbreeding, low mobility and other attributes of population structure. Alternatively, an animal can dispense with sex altogether and propagate a well-adapted genotype through cloning.

We surveyed parthenogenetic polyploid earthworm species in northern Europe. We have tried to work out how ecologically dissimilar parthenogenetic earthworm species disperse in relation to their genetic variability (i.e. clones) and what kind of clone pool variability they show in northern Europe where they live at the northern limits of their range. The latter area was completely covered with ice during the last glaciation called Weichsel. Earthworms must have colonized it in the course of the past 10,000 years or so.

Terhivuo and Valovirta (1978) and Terhivuo (1988b) have shown that the habitat spectra of earthworm species diminish from the south to the north, i.e. towards the margins of their range in Finland. This also holds true to the parthenogenetic endogeic *Aporrectodea rosea* and the epigeic *D. octaedra* and *Octolasion tyrtaeum* as well as the rather stenotopic epigeic *Eiseniella tetraedra* that we have studied in the Nordic and Baltic countries and in Russia.

In our studies, we have also focused on working out postglacial dispersal routes by means of clone pool affinities between parthenogens along the shores of the northern Baltic Sea and on the Åland Islands lying between Finland and Sweden and Estonia. Their clone pool structure shows that there are dispersal ties between continental populations of the Baltic basin and that the Åland Islands form a stepping stone for the dispersal of many clones across the Baltic sea. We have also posed the questions of how do the clones disperse from high subarctic mountains down to the Baltic sea and how do they disperse to islands that emerge from the northern Baltic Sea at a rate of about 1 cm/year. In this study, *E. tetraedra* has been our target worm.

In general, we have identified variable numbers of clones for the different species by using starch gel enzyme electrophoresis. The technique is simple and repeatable and is to be preferred when one is dealing with polyploids that are difficult to study in other routine molecular methods (see e.g. Saura et al. 1979; Parker and Selander 1980; Stille et al. 1980). Functional alleles at each genetic locus produce a distinct enzyme phenotype and the overall phenotype over all loci is used to identify a clone. Clone pool diversities were estimated using the distribution-free rarefaction method, which avoids sample size differences among populations.

Morphological diversity

We have also studied morphological variability and made morphometric measurements on adults of parthenogenetic lumbricids. By doing this we have aimed at relating observed variability to clone diversity in North Europe. Our surveys often revealed significant differences in somatic and reproductive characters between local populations but with few exceptions we have not recorded any regular pattern of geographical variability (Terhivuo 1988a; Terhivuo and Saura 1993a, b, 1996, 1997). This emphasizes the key role of local environmental factors on the genotype of the worm that ultimately determine the structural characteristics of the species.

Results

About 20 species of earthworms have been recorded in Norway, Sweden, Estonia, Finland and European parts of Russia (Julin 1949; Stöp-Bowitz 1969; Perel 1979; Terhivuo 1988b; Timm 1999). At least nine of these are known to reproduce by facultative or obligate parthenogenesis (Casellato 1987).

We have surveyed a variety of North-European parthenogenetic earthworms that are dissimilar in many ways (Table 1). In Finland *Octolasion cyaneum* is recorded only in southern parts of the country but in North Sweden it is found as far north as the city of Umeå. *Aporrectodea rosea* and *O. tyrtaeum* extend their ranges to central Finland. *D. octaedra* and *E. tetraedra* occur all over the country (Terhivuo 1988b). *Dichogaster bolaui* is an exotic invader found only indoors in Finland (Terhivuo 1991). Some species such as *O. cyaneum*, *A. rosea* and North American populations of *O. tyrtaeum*, appear to be primarily dispersed by human activity but vary in clone diversity in a given locality. On the other hand, northern European *O. tyrtaeum*, *D. octaedra* and *E. tetraedra* have less dependence on anthropochorous dispersal. These species also vary in clone diversity and patterns of clone distribution according to their primary means of dispersal (Table 1). Therefore, we will consider them below one by one.

Octolasion cyaneum and anthropochory

Terhivuo and Saura (1993a, 1996, 2003) studied clonal variation in this endogeic and highly (9× and 10×) polyploid (Muldal 1952; Vedovini 1973) worm with obligate parthenogenesis. The material is geographically extensive. We have worms from the northernmost population ever found (Umeå, Sweden at 63°55′), through central Sweden and southern Finland, England, northern Germany through Switzerland and we have worms from a population in Canberra, Australia.

The number of clones is in general low (mostly one or two), even though some samples are large (ranging from 20 to 42 worms). The sample of

Table 1 Ecological and other characters in peregrine parthenogenetic earthworm species considered in this study

Species	Ecological plasticity			Dispersal and distribution	Clone pool diversity
	1	2	3	4	5
O. cyaneum[a]	end	steno.	anth	Eur, NAm, Me, SAm, Austr,	1/2/1
A. rosea[b]	epi	±eury.	anth	WPa, Ne, SAm, SAfr, Austr,	7.2/7.3/7.0
O. tyrtaeum[a]	end.	±eury.	spont	Eur, NAm, SAm, MiE, NAfr, Austr	3.9/4.2
D. octaedra[a]	epi	eury.	spont	Pa, Me, Ind, Co	7.6/8.3/6.7
E. tetraedra[a]	epi	steno.	spont	WPa, NNe, SAm, SAfr, Aus, Me, Ind	6.4/8.0/5.3
D. bolaui[a]	epi	indoors	anth	Afr, Ind, Austr, Eur	–

[a] Only parthenogenetic strains are known

[b] Besides parthenogenetic strains amphigonic populations are reported from central and southern Europe (Casellato 1987)

Symbols: (1) Vertical distribution in soil; end = endogeic, epi = epigeic. (2) Habitat spectrum; steno = stenotopic, eury = eurytopic, indoors (in N Europe). (3) Main mode of dispersal; anth = anthropochorous, spont = spontaneous (methods not related to human agency). (4) World distribution (introductions included) (Stöp-Bowitz 1969; Sims and Gerard 1985); Eur = Europe, NAm = North America, Me = Mexico, SAm = South America, SAfr = South Africa, NAfr = North Africa, Austr = Australasia (India, Pakistan, Australia, oceanic islands, etc), WPa = Western Palearctic, Ne = Nearctic, SAf = South Africa, Pa = whole Palearctic, Ind = India, Co = Columbia, NNe = Northern Nearctic, Aus = Australia. (5) Clone pool diversity i.e. expected number of clones in a sample of 10 individuals correspondingly from Finland/Sweden/Estonia (Terhivuo and Saura 1996, 1997) nine worms from Switzerland had five clones and the one of 24 worms from Australia had 4 clones. The extent of variation among populations was low so that the northern samples mostly had just one and the same clone. There is no overall pattern that would support a concept of a specific multipurpose genotype. Morphometric variation showed local heterogeneity rather than regional trends; this variation was not associated with the clone(s) at each locality.

Amphigonic forms of *O. cyaneum* are not known but the overall pattern of variation agrees to some extent with the one observed in other clonal organisms, e.g. flightless weevils (Saura et al. 1976; Stenberg et al. 2003). There is high diversity in the area of origin i.e., the Alps and low diversity in the area colonized since the Ice Age. *Octolasion cyaneum* is a commensal of horticulture and it is evidently transported with garden plants, tools or soil. This gives rise to founder effects with the result of low clone diversity. Many of the sites, where we found *O. cyaneum*, were old, some even with continuous cultivation since Roman or Middle ages. Random factors related to the activities of man are the likely explanation for the rather high number of clones in the city of Canberra in Australia, which these worms have reached several times through the agency of man through time.

Today *O. cyaneum* is widening its range in Finland. This takes place mostly through human agency. For instance, at the Lammi Biological Station located about 100 km to the north of the city of Helsinki, these worms increase rapidly in numbers and spread to nearby soils where they were absent some tens of years ago (Karppinen and Nurminen 1964; Terhivuo 1988a). They also spread within the Helsinki and Umeå city areas by means of soil transport (Terhivuo and Saura unpublished).

A. rosea and agriculture

We have sampled populations from the northern margin of distribution of the endogeic *A. rosea* (Terhivuo and Saura 1993b, 1996, 1997). The material comes from Estonia, central Sweden, the Åland islands between Sweden and Finland and southern Finland. *A. rosea* is tied to agriculture but it may be found also in biotopes other than fields and meadows. This indicates that it is less intimately connected with human culture than *O. cyaneum* and can evidently disperse to nearby soils irrespective of human transport. Clone pool diversity was high. A sample of 50 worms could be expected to include about 20 clones in Sweden and Finland, while the Åland populations had about half of that diversity. Åland had few clones that were not found elsewhere. Using the Ochiai and Renkonen number similarity measures we found that the Åland populations shared clones more with Sweden than the Finnish mainland (Fig. 1). This agrees with the cultural and commercial ties that Åland has had with the Swedish mainland. Clone diversity is highest in areas where agriculture is old. We found some evidence for morphological differentiation tied to the genetic constitution of populations. Whether or not that was associated with the level of polyploidy in a given locality is not known (Terhivuo and Saura 1993b).

Octolasion cyaneum and *A. rosea* are often found in the same habitat but the latter has a far wider niche in northern Europe. It almost always has a high clone diversity, which indicates effective clone flow and powers of dispersal. The ties between populations as well as with history of agriculture show that human activity is the main agent of dispersal. The northern limit of distribution is quite sharp in Finland (Terhivuo 1988b). It is difficult to envision any historical or other pattern in agricultural practice that would give rise to such a border. The likely explanation is that the clones share a more or less identical reaction norm that does not allow colonization beyond a certain ecological threshold. One may hypothesize the possibility of multiple evolution of clones in *A. rosea*, combined with clone flow from different sites that would build up a diversity of clones in old agricultural and horticultural sites.

We may mention here that we have found copulating *A. rosea* individuals. We have considered this as a behavioural remnant of an earlier mode of amphigonic reproduction (Terhivuo and Saura 1993b). Evidently pointless sexual reproduction is a common feature in many parthenogenetic animals, such as lizards etc.

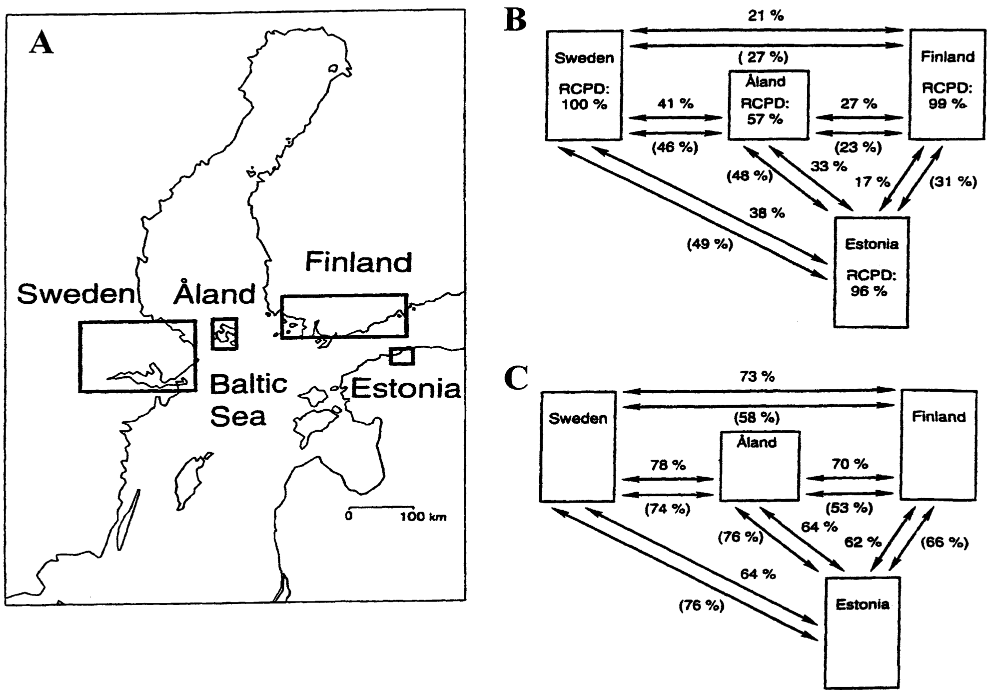

Fig. 1 Study area (**A**), proportions of clones (**B**) and enzyme variants (**C**) shared by the clone pools of the parthenogenetic earthworm *Aporrectodea rosea* on the Åland Islands and the mainlands of Finland, Sweden and Estonia as indicated by the Ochiai and the Renkonen Number (in parentheses) similarity measures (based on Terhivuo and Saura 1997). RCPD = relative clone pool diversity by the rarefaction values for a sample size of 10 worms (the highest regional value denotes 100% and the other values are calculated in relation to it)

O. tyrtaeum, an epigeic of waterlogged and moist soils

These worms seem to be triploid and parthenogenetic (Casellato 1987). In northwestern Europe they inhabit waterlogged soils such as shore meadows and banks of rivers and lakes. *O. tyrtaeum* seems to be capable of dispersing without human agency, i.e. by hydrochory. The populations may reach high densities at shores and shore alder thickets from which they may have dispersed into less moist habitats. Quite recently we have found that some upland *O. tyrtaeum* populations in Finland comprise specimens smaller in size and with more brightly yellow coloured rear end of the body than at wetland populations.

Terhivuo and Saura (1993a, 1997) studied *O. tyrtaeum* clone diversity in an extensive material from Sweden, Finland, Estonia and the Åland islands in between them. Clone diversity was rather low, from about 5–6 clones in a sample of 50 individuals. The amount of clones shared among the three regions was the same. Some clones shared among the regions were remarkably abundant on Åland. There was no clear-cut morphological differentiation among either regions or clones.

The North American continent appears to be a natural laboratory to study the invasion and dispersal of parthenogenetic earthworms. Jaenike and Selander (1979, 1985) and Jaenike et al. (1982) have studied the clonal diversity in *O. tyrtaeum*. While these worms are rather stenotopic inhabitants of wetlands and rivers in northern parts of the Old World, they have successfully invaded all kinds of habitats, including ploughed fields in the eastern United States. In fact, they are the most common earthworms in many localities there. Nevertheless, the clonal diversity is lower than in our material that comes from the northern margin of the species in its European home. Two genotypes, A and B, predominate in an extensive American material that has a total of eight recognized genotypes. Both A and B have a broad niche and their distributions overlap across a range of habitat and soil types. Jaenike and Selander (1985) argue that fluctuating population sizes and stochastic factors explain the coexistence of these two abundant types. Consequently, they have a very broad niche. The low number of clones across the eastern USA seems to indicate the effect of one or more founder events. It also shows that there has evidently been little evolution either through mutation or recombination through some unknown sexual process.

D. octaedra, an eurytopic epigeic with wide clone pools

This hardy worm occupies the widest scope of habitats among north-European lumbricids (Terhivuo and Valovirta 1978). It is by no means dependent on anthropogenic soils but does not avoid them. In Fennoscandia it can be found in the north as far as there is dry land on the coast of Arctic Norway. We have explored its clone composition and morphometrics in a set of papers (Terhivuo et al. 1987; Terhivuo and Saura 1990, 1996, 1997). The worms have been collected from an extensive area extending from Estonia and central Sweden in the south to northernmost Norway.

Clone diversity was always high, from 22 clones up to 26 clones in a sample of 50 worms. It may be pointed out that the sample from Estonia had a slightly lower diversity. In general the number of clones is high and relatively few clones were shared among localities. In Finland, the species seems to be hexaploid (Hongell and Terhivuo 1989). About every third worm sampled had a new overall genotype. All populations sampled had a high diversity, including geologically young islands far from the mainland (Terhivuo and Saura 1990 and unpublished). This shows that *D. octaedra* disperses with mechanisms that are highly efficient and does not rely on the active movements of the worm. The absence of male pores in many specimens and the derived mode of parthenogenesis do not support biparental reproduction as an explanation for high clone diversity.

The *D. octaedra* clone pool on the Åland islands was more similar to Finland than to Sweden. Åland is tied to Finland through thousands of stepping-stone islands, while the sea separating it from Sweden (which is geographically closer) is open and relatively deep. As stated above, cultural contacts to Sweden have been more active than to Finland, a circumstance seen in the clone pool affinities of culture commensals but not with *D. octaedra*.

A transect from northern Norway through southern Finland and another one through the Åland islands in the west to eastern Finland did not show any clear-cut affinities between the *D. octaedra* clone pools. In general, the clone pool in Finland comprised less than ten abundant clones that were recorded along most transects and of a swarm of less abundant clones with restricted distributions. Clone pool diversity increased northwards in open habitats but decreased in forests. The conclusion is that the populations of these worms are always made up of a wide variety of clones. Whether any of them differ in niche parameters remains to be seen. The clone pools along the transects show apparent clinal patterns that may indicate adaptation. The clones in the north deviated more in their enzyme compilations from a norm clone than the ones in the south. There was no evidence for clinal variation in the east-west transect (Fig. 2).

D. octaedra shows extensive morphological variability in North America where the species has come from Europe through human activity Gates (1974). In northern Europe it also has a wide variability in somatic as well as in reproductive characters (Terhivuo 1988a). Though morphological studies on parthenogenetic earthworms often reveal local differences between populations these differences are seldom reported to show any clinal pattern.

D. octaedra adults in southern and central Finland have the same mean number of segments as those in northern Finland and Norway. They show, however, a clinal pattern of increasing body size in different types of biotopes towards the north. Adult *D. octaedra* may lack or possess rudimentary male pore terminalia. The ones that have the pores, have them sometimes (in Sweden and Estonia) on segment XIV but this was never

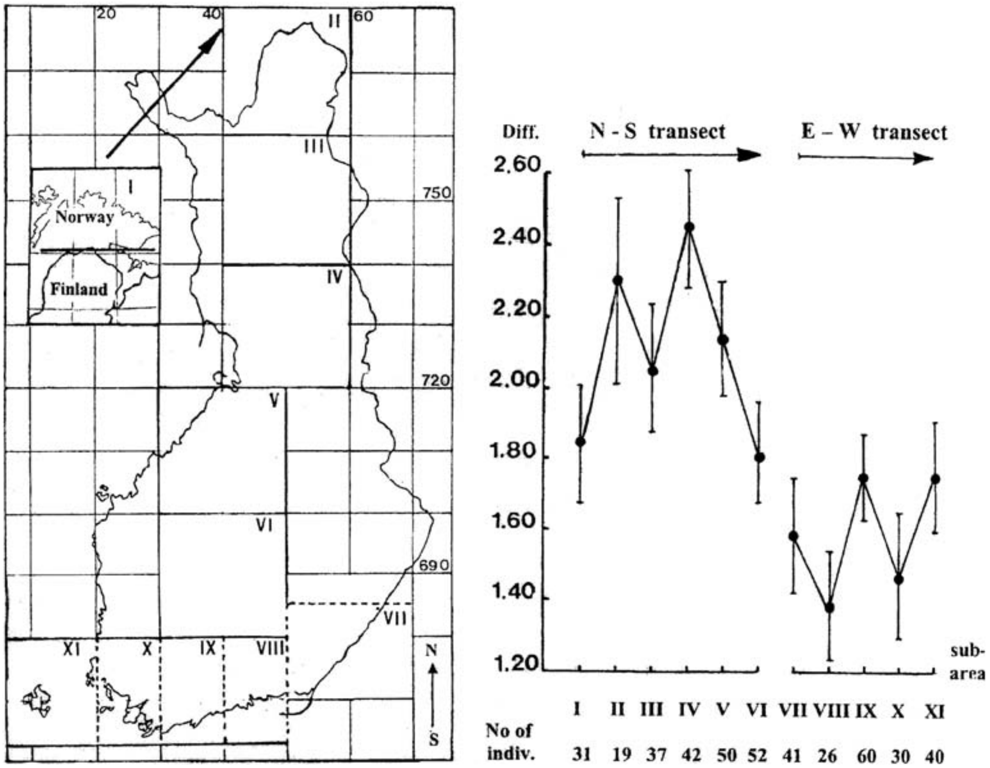

Fig. 2 (**A**) Study area with the north-south transect (subareas I–IV) and the east-west transect (subareas VII–XI). (**B**) Mean enzyme variant differences (mean - SE) among *Dendrobaena octaedra* specimens as measured by the numbers of the enzyme variants deviating from those in the norm clone. Individuals of the norm clone are considered equal to 0. See also the text (based on Terhivuo and Saura 1990)

observed in Finland or Åland. The scope of variation on Åland resembles more that in Finland than in Sweden.

E. tetraedra, a stenotopic epigeic dispersing by hydrochory and zoochory

E. tetraedra is a common inhabitant of shores all the way to Arctic Norway. In the mountains of northern Scandinavia it reaches the middle alpine zone, about 1,000 m above sea level. We may add that glaciers form there at an altitude of 1,300 m. These small worms are short-lived, with a life span of about one and a half years. They are tetraploid (Casellato 1987; Terhivuo et al. 1994).

We (Terhivuo et al. 1994) first compared clone pools in southern Finland with ones in northern Norway. Populations had rather low diversity in general, so that in a sample of 100 worms one can expect to find about 12 clones. The corresponding figure for *D. octaedra* is about 28 clones (Terhivuo and Saura 1990). In the Baltic area, clone diversity is highest on the Swedish mainland, so that a sample of 50 *E. tetraedra* is expected to include about 24 clones, on the Åland islands about 16, and on the Finnish mainland about 12.5 clones. There was considerable variation (presence versus absence and location) in the non-functional male organs among and within populations. Such traits need not be subject to selection (Suomalainen et al. 1987). In the survey by Terhivuo et al. (1994) it turned out that as in *D. octaedra* there are only a few abundant clones and a swarm of less common ones. Moreover, the two most frequent clones A and B were recorded both in the north and in the south. Using these as norm clones, comparisons showed that the clones in North Norway deviated on average less from clone A than from clone B. The opposite was true for clones in South Finland. This may indicate differential adaptation of *E. tetraedra* clone pools in South Finland and North Norway.

A comparison of clone pool affinities between Estonia, the Åland islands and Sweden and Finland, respectively, showed that similarity was highest between Åland and Sweden, followed by Sweden and Finland, whereas Åland and Finland and Estonia had lower similarities to the above (Terhivuo and Saura 1996, 1997). The results agree roughly with linear distance and suggested that *E. tetraedra* disperses with water.

Northern Scandinavia is subject to the phenomenon of land uplift, a consequence of a rebound of the crust of the earth after having been pressed down by the weight of ice in the not too distant past. In the environs of the town of Umeå in northern Sweden the rate of uplift is about 9 mm a year. This means that new islands rise from the sea at a constant rate, while older islands merge to form larger islands and these join gradually with the mainland. We followed the clone constitution of islands at the Skeppsvik archipelago (Terhivuo and Saura 1999). The river Sävarån feeds clones to the area in the northern part of the Baltic Sea where salinity is low (about 0.3%) and *E. tetraedra* lives all along the shores of the mainland. We first estimated clone diversity along the Sävarån close to the river mouth and then inspected clone diversity on islands of different ages with differing distances from the river mouth. We also followed the turnover of clones within this area for 3 years. A total of 94 *E. tetraedra* clones were found among 449 worms collected during this period. Eighty-one percent of the clones found on the islands was found also along the river but these clones made up a minority (35%) in the river sample. Clone diversities on new islands were low (8–13 clones in a sample of 25 worms) in comparison with the riverside samples (16–21 clones in a sample of 25 worms) and the turnover rate between years was high (about 80%) on the islands. The newest and also the most isolated islands did not have any worms. The structure of the clone pool on the islands differs clearly from that of the mainland and it is subject to a rapid turnover. However, the river feeds new clones to the sea all the time and these fugitive clones colonize the islands. New islands are occasionally submerged through floods and the worms are subject to fish predation. The final stage is a permanent and quite diverse clone assemblage that characterizes the shores of the Baltic Sea.

Northern Sweden has a set of parallel rivers that run from the Scandes mountains to the Baltic sea. Two rivers next to each other constitute an ecological theater, where the process of colonization and adaptation can be observed. We have studied the Ume and Vindel rivers and compared them with the much smaller Sävar river. The Vindel river is one of the few remaining free-flowing large rivers in Europe, while the Ume is harnessed with 20 major dams. The two rivers join before they meet the sea (Terhivuo et al. 2002). The two river mouths (the Ume–Vindel and the Sävar) had highest clone diversities in terms of Swedish clone diversity, i. e. about 18 clones in a sample of 20 worms (Fig. 3). The result seems to be unexpected, as the Sävar is a much smaller river. It is, however, in line with the results of river ecology stating that small rivers behave like large ones. The river mouths did not appreciably have clones in common. This means that they do not represent samples of a large coastal clone pool. In fact, the river mouths experience a rapid turnover of clones between years (Terhivuo et al. 2002).

Populations along the free-flowing Vindel river had higher diversities than corresponding sites along the Ume river. The Vindel river contributed about three times more clones to the common river mouth than the harnessed Ume river. In addition, populations along the Vindel had more clones in common than populations along the Ume river. A stepwise pattern of shared clones is seen along the Vindel. The locality highest upstream shares clones with the next one downstream, which again shares clones with the next one, etc. The localities along the harnessed Ume river do not share any clones with each other. In other words, worms disperse down the Vindel but the dams stop the dispersal along the Ume river. The morphometric characters of *E. tetraedra* adults do not show any association with an area or river. For example mountain brooks could have large worms as well as small ones. There was, however, a between-year difference in samples taken in two consecutive years at the Ume–Vindel mouth. In conclusion, the

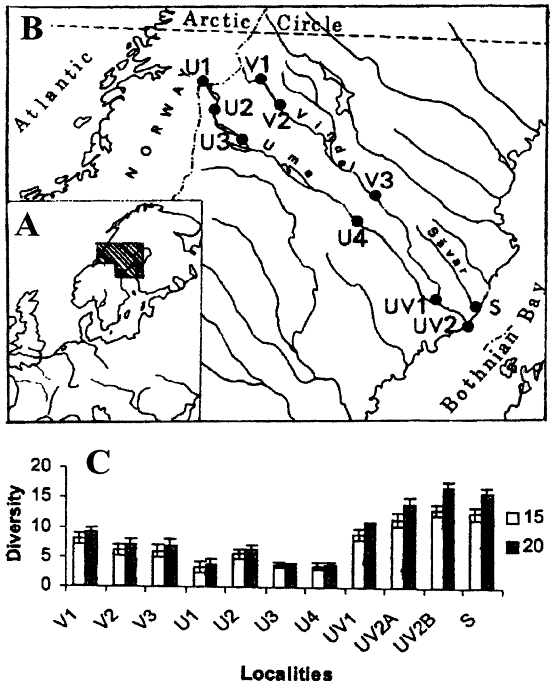

Fig. 3 Study area (**A**) and sample localities (**B**) along the free running Vindel river (V1–V3), the harnessed Ume river (U1–U4), the part of the two rivers joined (UV1–UV2A), and the Sävar river (S). *Eiseniella tetraedra* clone pool diversities (**C**) are indicated according to the rarefaction method. The expected numbers of clones are calculated for sample sizes of 15 and 20 individuals. The bars indicate ±1SD (based on Terhivuo et al. 2002)

results show that worm cocoons float down a free river. Small colored wood cubes do the same; they are also stopped in water reservoirs of a harnessed river (Terhivuo et al. 2002) (Fig. 3).

We have continued the studies of *E. tetraedra* clones along small mountain brooks in the watershed area of the Ume river (Terhivuo and Saura unpublished) on the Scandes. We sampled several brooks as far as up the subarctic mountains where *E. tetraedra* was found and took samples with about even intervals down. Many brooks did not have any worms. On arctic mountains of North Sweden clone pool diversity decreases with increasing elevation. It seems that small brooks carry propagules from wide areas of mountain slopes especially in periods of heavy rainfall and melting snow. Small brooks join and make wider brooks prior to running to large rivers or lakes. At the mouth of the brooks *E. tetraedra* clone pools are considerably more diverse than they are at the top of arctic mountains. Herds of reindeer wander along the mountain slopes, cross brooks, and probably disperse cocoons. Propagules can be spread in pieces of soil attached to the feet of mammals and maybe birds also contribute to their dispersal. Sport fishermen can also spread earthworms along the rivers in Northern Scandinavia but the mountain brooks where we have studied *E. tetraedra* are so small that they are devoid of fish. Our material of *E. tetraedra* clones from northern parts of Russian taiga shows that there are riverside localities with very low clonal variability thus resembling the clone pools at higher elevations of subarctic mountains (Terhivuo and Saura unpublished).

In summary, our surveys point out that *E. tetraedra* is an inhabitant of river and shore soils extending its range up to the Ice Coast in northern Europe. It disperses with flowing water and possibly also through zoochory. On subarctic North-European mountains its clone pools decrease with increasing elevation so that at the top there are a few but persistent clones. Small brooks carry propagules downstream, and at the mouths of brooks clone pools are more diverse than higher up; again larger rivers carry clones downstream. Clone dispersal is relatively free in a freely flowing river, while dams stop propagules in harnessed rivers. The mouths of rivers have high *E. tetraedra* clone diversity. Clones disperse from these clone centers to islands formed through land uplift along the northern Baltic Sea. The annual turnover of clones is high on these islands that occasionally can be submerged. The Åland Islands serve as a stepping stone for the invasion of clones between Estonia, Finland and Sweden across the Baltic Sea.

D. bolaui, a curious parthenogenetic inhabitant of human settlements

D. bolaui (Acanthodrilidae) originates either from India and the nearby areas (Sims and Gerard 1985) or more probably from Africa (Lee 1985; Jamieson et al. 2002). Terhivuo (1991) reported that it has established itself in the sewer systems in Finland. Later similar records have been made in several European cities. It may be considered a minor nuisance as small pink worms appear in swimming pools and toilet bowls. Lee

(1985) proposed *D. bolaui* to be a parthenogen and the preliminary analysis by Terhivuo and Saura (unpublished) showed that in Finland there may be just a single clone of the species.

In Finland the *D. bolaui* individuals recorded in flowerpots of decorative plants imply the arrival of the species with imported plant material. Probably it first arrived in greenhouses and from there was transported with flowerpots to separate localities in Finland. Total lack of *D. bolaui* records prior to 1988 and numerous ones thereafter indicates that it is a rather late arrival among the Finnish earthworm fauna. Its presence in urban sewer pipe systems seems to due to the habit of showering decorative plants in toilets so that individuals or cocoons can easily be carried with water to the sewer pipe system where the species seems to thrive because many of the worms found were adults with well-demarcated secondary reproductive organs (Terhivuo 1991).

Discussion

Dispersal methods

Earthworm movements in soil or on the soil surface are called spontaneous or active dispersal. It is, however, an inefficient method since it does not disperse the worm more than 10–15 m/year (Ghilarov and Perel 1984; Hoogerkamp et al. 1983). If this were the only means of dispersal for lumbricids, northern Scandinavia and Finland should still be devoid of Lumbricidae; since the retreat of the ice sheet after the culmination of the Weichsel glaciation some 15,000 years ago, lumbricids should have spread spontaneously no more than some 200 km northwards from the southernmost edge of the ice sheet.

Stephenson (1930) stressed the importance of anthropochory in earthworm dispersal. Human introductions, either intentional or unconscious, play a key role in earthworm invasions as is well demonstrated by the presence of numerous European Lumbricidae in North America, Asia, New Zealand, Australia, Central and South America and Africa (see e.g. Gates 1982; Lee 1985; Sims and Gerard 1985; also see Baker et al., Frelich et al., and Hendrix et al., this issue).

According to (Schwert and Dance 1979) hydrochory makes an efficient dispersal mechanism especially for earthworms not associated with anthropomorphic soils. Our surveys on *E. tetraedra*, *D. octaedra* and *O. tyrtaeum* also highlight the importance of hydrochory. Moreover, zoochory may be involved as well but we lack actual observations of it.

There are few reports on the role of anemochory in earthworm dispersal in general. However, Stöp-Bowitz (1969) reports an occasion when heavy storm had lifted dead leaves with earthworms and carried them on the ice of a Norwegian lake. Such occasions are hard to detect in other seasons of a year.

The evolution and adaptation of clones

A clone is a reproductively isolated unit. Formation of a new clone through mutation and lack of genetic recombination is a fast process in comparison to the formation of a new species characterized by isolation and cessation of gene flow between populations. Assuming a constant mutation rate *per* locus *per* generation, a hexaploid should have three times as many mutations *per* generation as a diploid with the same haploid number. Thus polyploid clones accumulate mutations and become highly heterozygous for alleles in any locus. Aneuploidy will also change expression of alleles if the chromosome with the allele is lost in a clone. In general, polyploidy complicates the picture since each chromosome in a polyploid may have differentiated widely from its multiple homologs through mutations and transposition. The result is a swarm of new clones that can be derived from one or few frequent and widely distributed parent clones. We do not know, however, whether the clones are monophyletic or how old they are. An old clone or a widely distributed common clone may be monophyletic or through mutations it may result in a huge assemblage of minor derivatives (Terhivuo and Saura 1990; Loxdale and Lushai 2003). In fact, Lushai et al. (2003) have stressed that molecular data from prokaryotes and eukaryotes support the argument that clones possess a highly dynamic and adaptive genome. It should be pointed out that it is likely that more and more clones will be found along with a growing number of polymorphic enzyme systems used to identify clones (Terhivuo and Saura 1990). The detailed phylogeny of clones would be very interesting when done in concert with the geographical data, rivers system data, etc, presented in this paper. Rapidly evolving methods of DNA sequencing could be used together with our enzyme data.

In Central and southern Europe amphigonic strains of *A. rosea* have been recorded but the other lumbricid species studied by us are considered to reproduce by obligate parthenogenesis (Casellato 1987).

Given that a study is done in a sufficiently fine scale, one may expect to find evidence for or against a concept that clones would be ecologically equivalent. Christensen (1980) and Christensen et al. (1978) studied the distribution and long-term changes in parthenogenetic and polyploid populations of the enchytraeid *Lumbricillus lineatus* along short ecological transects on a shore. Seasonal and long-term changes were observed in the genotype distributions. Several genotypes coexisted in a single population. The triploids inhabited the areas highest up on the shore, pentaploids intermediate localities and tetraploids dominated at the lowest sites. Different genotypes divided the transect among themselves and the distributions were similar at two transects. This means that genotypes actively select and use different parts of their niche in a heterogenous environment. Christensen et al. (2002) have shown that the clone pool of the enchytraeid *Buccholzia appendiculata* is stable and can persist for a long time.

In any case, clonal organisms are reproductively isolated from each other and many authors (e.g. Viktorov 1997) point out that clones or degrees of ploidy correspond to good taxonomic entities. In fact, botanists treat clones as good species. Clones should then be expected to share the same niche and be subject to competitive exclusion. Studies on clonal animals have seldom shown such an effect (Suomalainen et al. 1987); clones and degrees of ploidy seem to coexist freely, as shown by the results described above and e.g. by Stenberg et al. (2003).

We have seen that morphological variation exists independently of genetic variation in clonal earthworms. This is an argument against competitive exclusion on the basis of genotype, given that phenotypes are poorly correlated with genotype. The effect of this should be the maintenance of clonal diversity because selection is expected to be somewhat random with genotype in a situation like this. All evidence that we have implies that competitive exclusion does not operate among clonal worms and certainly more work is needed to understand the factors that determine their population structure and geographical range.

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