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Genic and morphological variation of the parthenogenetic earthworm *Aporrectodea rosea* in southern Finland (Oligochaeta, Lumbricidae)

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The parthenogenetic earthworm *Aporrectodea rosea* (Savigny) showed a wide electromorph ('clonal') heterogeneity in southern Finland with 46 'clones' in a sample of 155 individuals. Individual clones were not randomly distributed along an east-north-west sample transect. Clonal heterogeneity was highest in Inkoo in the West, where agriculture and horticulture have been practised longer and more intensively than in the other sample localities. Morphological characters related to soma and secondary reproductive organs varied according to locality, region, and clone. With the exception of the protuberance of male pore terminalia, no clear-cut clinal trends were observed in the patterns of morphological variability. However, application of a detrended correlation analysis, which groups the clones according to their enzyme variant constitution, indicated a separate cluster of clones, all of which were sampled in Inkoo. The worms representing these clustered clones were significantly smaller, with a lower number of segments than the worms of the other clones sampled in Inkoo and in the other localities. This implies that electrophoretic differences can be correlated with differences in morphological characters in parthenogenetic *A. rosea*.

1. Introduction

Dendrobaena octaedra, *Eiseniella tetraedra*, *Octolasion tyrtaeum* and *O. cyaneum* are obligatorily parthenogenetic lumbricids that show very dissimilar patterns of genotypic variation in southern Finland. In the eurytopic *D. octaedra*, about every third individual represented a clone new to the sample. In the stenotopic *E. tetraedra*, clone diversity was lower, viz. one out of seven individuals, and in *O. tyrtaeum* it was one out of ten individuals. *O. cyaneum*, an anthropochorously distributed lumbricid and a recent invader to the fauna of Finland, had only two clones (Terhivuo & Saura 1990, Terhivuo & Saura 1993, Terhivuo et al. 1993).

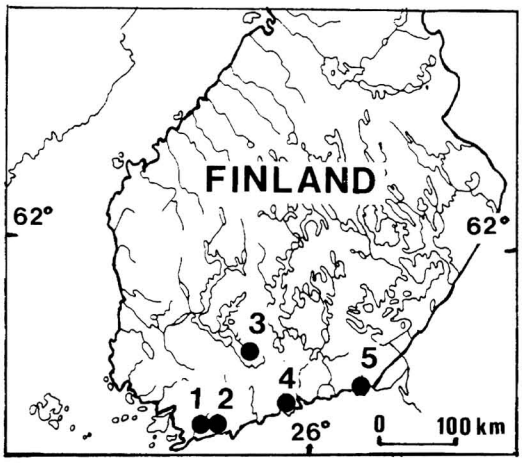

Fig. 1. Regions and sample localities (1–5). West: 1 = Tenhola, 2 = Inkoo. North: 3 = Tuulos. East: 4 = Porvoo, 5 = Virolahti.

A comparison of morphological characters related to soma and secondary reproductive organs indicated dissimilar patterns of variation and evolutionary trends among the four lumbricids in Finland and northern Norway. For instance, about 40% of *D. octaedra* adults lacked male pore terminalia, and there was an increase in body size as their habitat shifted northwards (Terhivuo 1988a). In *E. tetraedra* no clinal pattern in body size was observed, and in *O. cyaneum* and *O. tyrtaeum* characters related to secondary reproductive organs varied very little (Terhivuo & Saura 1993).

Aporrectodea rosea (Savigny), an obligatorily parthenogenetic lumbricid, lives in southern Finland at the northern margin of its European range. Its dispersal seems to be facilitated by agricultural and horticultural practices, but its presence in soils with lesser human impact implies that it can also disperse spontaneously. In central and northern parts of Finland *A. rosea* seems to be unable to establish itself (Terhivuo 1988b).

In southern Finland *A. rosea* is often abundant in fertile clayey soils where *E. tetraedra* and *O. tyrtaeum* are seldom found. It is very rare in acid podsolic soils where *D. octaedra* often occurs in great numbers. Unlike the epigeic *D. octaedra* and *E. tetraedra*, *A. rosea* occupies subsoil layers where it is less exposed to forces that facilitate dispersal of topsoil and litter-dwelling lumbricids (Terhivuo 1989).

This article examines electrophoretic and morphological variations in *A. rosea* from southern Finland and attemps to correlate them with each other. Moreover, if we suppose that the *A. rosea* population in southern Finland originates from one or a few anthropochorously distributed individuals, the clone pool of *A. rosea* can be expected to resemble that of *O. cyaneum*. If the clone pool were diverse, it might be surmised that the clones had established themselves through repeated introduction by man. Postglacial dispersal events unrelated to human agency may also have occurred. In the latter case local and regional differences can be expected between the populations.

2. Material and methods

2.1. Sample localities and regions

We collected *A. rosea* individuals by digging them in the following localities and habitats during the fall of 1991 (numbered as in Fig. 1);

1) Tenhola, Skarpkulla, October 3, 1991. A clayey soil at the edge of a cultivated field near a deciduous wood. The field layer is covered with dense vegetation of grasses and herbs.
2) Inkoo, Fagervik, October 3, 1991. An old field lying fallow with a dense field layer vegetation of grasses and herbs on a clayey soil. The field belongs to the manor of Fagervik, known for agricultural and horticultural pioneering that includes the introduction of foreign plants since the 1600's. The localities in Tenhola and Inkoo represent western *A. rosea* populations in southern Finland. In regional comparisons localities 1 and 2 represent a unit called the West.
3) Tuulos, Oksjärvi, September 22, 1991. The upper edge of a shoreside *Alnus* thicket by Lake Oksjärvi. There were extensive growths of *Rubus idaeus*, *Filipendula ulmaria*, *Urtica dioeca* and Poaceae spp. A dense layer of mosses covered the surface of the soil. There was mull and some litter in the topsoil, but the subsoil was composed of clay. In regional

comparisons the population in Tuulos is called the North.

4) Porvoo, Tarkkinen, October 10, 1991. An old abandoned field with a dense growth of grasses and herbs. The clayey soil contained patches of mull due to agricultural activity some years ago.
5) Virolahti, Ravijoki, October 17, 1991. A fertile clay soil with a layer of mull at the top near the bank of a ditch at the edge of a cultivated field. The site was densely covered by grasses and herbs.

Porvoo and Virolahti are called the East in regional comparisons.

2.2. Laboratory procedures

In the laboratory the worms were quickly washed and dried on paper towels for about one minute prior to weighing the adult worms. A set of some anterior-most segments was cut off from each worm and stored at –70°C prior to using it for electrophoresis. The remaining parts of the worms were preserved in 70% ethanol for morphological examination. Most of the worms subjected to electrophoresis were adults. An additional sample of juveniles was taken from each locality for morphological examination.

2.2.1. Electrophoresis and definition of clones

The starch gel electrophoresis and enzyme assay methods are in common use. Phosphoglugose isomerase (Pgi), esterase (Est), phosphoglucomutase (Pgm) and leucine aminopeptidase (Lap) proved to be polymorphic. In addition, some 15 other systems were tried: these were disregarded as monomorphic or poorly reproducible. The four polymorphic enzyme systems were used to establish the enzyme phenotype for each individual worm; every variant of an enzyme was provided with a serial number, and the combinations of variants of the four enzymes denoted an individual phenotype which we call a clone for short. We are aware that a clone so defined is an assemblage that might be subdivided further by adopting additional polymorphic enzyme systems. Enzyme variant compilations and numbers of individuals for the most common clones are given in Table 1. The clones were assigned symbols in alphabetical order according to frequency, with A representing the most frequent clone.

2.2.2. Morphological characters

We examined morphological variation in characters related to soma and to secondary reproductive organs. The former group of characters involved fresh body weight, the posterior length of the body (clitellum + postclitellar part of the body) and the number of segments. The latter group of characters comprised the location of clitellum and tubercula pubertatis and the presence/absence and protuberance of male pore terminalia. Protuberance of male pore terminalia was visually estimated according to the following scale: 1 = inconspicuous and poorly demarcated, 2 = rather protuberant and demarcated and 3 = very conspicuous and well demarcated.

There was some intra-segment variation in the first and the last segment of the clitellum, but we did not take that into account and, on average, the clitellum was wider dorsally than laterally. We always recorded the maximum length of the clitellum.

2.3. Statistics

Clone pool diversity was studied by means of the rarefaction method (Simberloff 1978, 1979) and similarities between the clone assemblages were calculated according to the indices of Ochiai (1957) and Renkonen (see e.g. Wallwork 1970). Rarefaction is a distribution-free method which calculates the expected number of clones for a random sample of n individuals derived from a larger sample with more than n individuals. Standard deviation for the mean number of expected clones was obtained by repeating random sampling of n individuals with replacement.

The index of Ochiai is based on the number of clones, but it does not take into account the number of individuals that make up the clones. In Renkonen's formula the lowest relative proportions of the clones in common are summed up in pairwise comparisons made both between lo-

calities and between regions. For additional information on the methods see for example Terhivuo & Saura (1990, 1993).

In order to group the clones according to their enzyme variant composition, a detrended correlation analysis (DCA) was applied to the clones. Morphological characters of the worms were inspected according to the groups of clones produced by this method.

Statistical significances are indicated as follows: ns, not significant; o, $0.10 > P > 0.05$; * $0.05 > P > 0.01$, ** $0.01 > P > 0.001$ and *** $P < 0.001$.

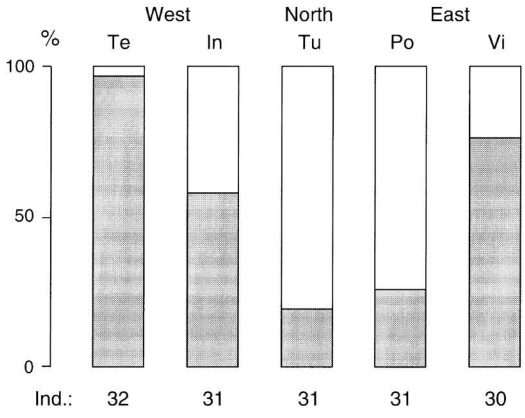

Fig. 2. Proportions of *A. rosea* individuals belonging to clones found in only one sample locality (hatched parts of columns) and those of worms recorded in at least two localities. Localities: Te = Tenhola, In = Inkoo, Tu = Tuulos, Po = Porvoo and Vi = Virolahti.

3. Results

3.1. Clones

3.1.1. Distribution of clones

We recorded 46 clones in the total of 155 *A. rosea* individuals from five localities in southern Finland. This indicates that approximately every third worm represented a clone new to the sample. Clones with five or more individuals were considered to be common clones. They made up 24% of all the clones and 62% of the individuals (Table 1). The worms representing clones found in one sample locality only were not equally distributed between localities ($\chi^2 = 55.2^{***}$). As Fig. 2 shows, these individuals made up a higher proportion in the West and in the East than in the North.

In order to work out whether our total sample catch could be derived by chance from a population where free recombination of alleles takes place, we made the following calculations: based on the information on the numbers of variants in each enzyme system and on the total number of individuals, one can expect to have $3 \times 4 \times 4 \times 6 = 288$ clones in a hypothetical population of $155 \times 155 \times 155 \times 155 = 577\,200\,625$ worms. Based on the frequencies of worms with an individual enzyme variant in the total sample catch of 155 worms, we first counted the expected number of worms in each clone within the hypothetical population. For instance, clone A would be represented by $79 \times 48 \times 91 \times 105 = 36\,232\,560$ worms in it (in the original data set there were 79 individuals with Pgi variant-1, 48 with Est variant-5, 91 with Pgm variant-10 and 105 with Lap variant-14). The sum total of all the correspondingly calculated

Table 1. Enzyme variant combinations (1–16) and numbers of individuals for *A. rosea* clones (A–W) represented by at least two individuals.

Enzyme system	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
Pgi	1	1	1	1	1	2	3	2	1	4	1	1	2	2	2	2	3	3	4	1	3	3	3
Est	5	6	7	8	9	5	5	6	8	7	6	5	6	7	7	5	6	8	5	8	5	7	8
Pgm	10	11	10	11	10	10	10	10	12	10	10	11	11	10	11	11	13	10	10	12	11	10	10
Lap	14	14	14	15	14	14	14	14	15	14	14	15	15	14	14	15	16	16	14	16	15	14	14
Indiv.	15	12	10	10	9	9	7	7	6	6	5	4	4	4	4	3	3	3	3	2	2	2	2
%	10	8	6	6	6	6	5	5	4	4	3	3	3	3	3	2	2	2	2	1	1	1	1

numbers of individuals of the clones recorded in our original sample is 304 006 275 worms. These worms correspond to 52.7% of all the worms in the hypothetical population. One can also expect to have 52.7% of all the clones in this large a sample of worms (= 52.7/100 × 288 = 151.8 clones). Because there were only 46 clones in the original sample set we concluded that it could not represent a random sample from any population with a free recombination of alleles.

According to Table 2 no clone was found in all localities and different clones predominated in different localities and regions. A perusal of Table 2 also reveals that at least some of the common clones are not randomly distributed between the localities. For instance, all 12 individuals of clone B came from Virolahti and all 10 individuals of clone D from Tenhola. Assuming a random distribution of all the clones between the localities, the probability of a sample of 30 individuals where all the individuals of clone B are present by chance is (30/155) × (29/154) × (28/153) × ... (19/144), i.e. $P < 0.001$. Correspondingly, the probability for individuals of clone D in a sample of 32 worms is (32/155) × (31/154) ... (23/146); $P < 0.001$.

3.1.2. Clone pools

The rarefaction method indicated considerable differences in the diversity of local and regional clone assemblages (Table 3). In Inkoo the clone pool was greater than in the other localities, making the clone pool of the West as a whole more diverse than that of the North or the East.

Clone pool similarities implied that western clone assemblages resembled northern more than eastern clone pools (Table 4). The clone assemblages of the East shared more clones with the North than with the West.

An application of the DCA analysis to cluster the clones according to their allozyme composition revealed a group of clones with considerable

Table 2. Proportions (%) of *A. rosea* clones in different sample localities and regions. For localities see Fig. 1.

Region & locality	Clone A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	Unique clones	Indiv. *n*	Clones *n*
West	6	–	3	16	–	3	3	2	10	–	3	6	6	–	–	5	5	5	–	3	3	–	1	19	63	28
Tenhola	–	–	–	31	–	–	–	3	19	–	–	13	13	–	–	9	–	–	–	–	6	–	–	6	32	9
Inkoo	13	–	7	–	–	7	7	–	–	–	7	–	–	–	–	–	10	10	–	7	–	–	3	32	31	19
North	29	–	–	–	7	23	3	3	–	–	7	–	–	13	–	–	–	–	3	–	–	3	3	7	31	12
East	3	20	13	–	12	–	7	8	–	10	2	–	–	–	7	–	–	–	3	–	–	2	–	15	61	20
Porvoo	7	–	20	–	13	–	13	16	–	19	3	–	–	–	–	–	–	–	3	–	–	–	–	7	31	10
Virolahti	–	40	7	–	10	–	–	–	–	–	–	–	–	–	13	–	–	–	3	–	–	3	–	23	30	13

Table 3. Clone pool diversity (mean ± *SD*) according to expected number of *A. rosea* clones in different sample localities and regions, indicated by rarefaction method.

Region & locality	Sample size 25 ind.	50 ind.
West	16.3 ± 1.7	24.8 ± 1.4
Tenhola	8.3 ± 0.7	–
Inkoo	16.7 ± 1.0	–
North	10.6 ± 0.9	–
East	12.5 ± 1.6	18.0 ± 1.2
Porvoo	9.2 ± 0.8	–
Virolahti	11.5 ± 0.9	–

Table 4. Similarities between clone pools according to the indice of Ochiai (right upper portion of matrix) and the index of Renkonen Numbers (left lower portion of matrix).

Region & locality	West	Tenh.	Inkoo	North	East	Porvoo	Virol.
West	–	–	–	0.29	0.22	0.14	0.05
Tenhola	–	–	0.00	0.10	0.00	0.11	0.00
Inkoo	–	0.00	–	0.39	0.21	0.30	0.06
North	0.23	0.03	0.36	–	0.42	0.55	0.25
East	0.13	0.03	0.25	0.23	–	–	–
Porvoo	0.18	0.03	0.23	0.26	–	–	0.26
Virolahti	0.03	0.00	0.07	0.13	–	0.20	–

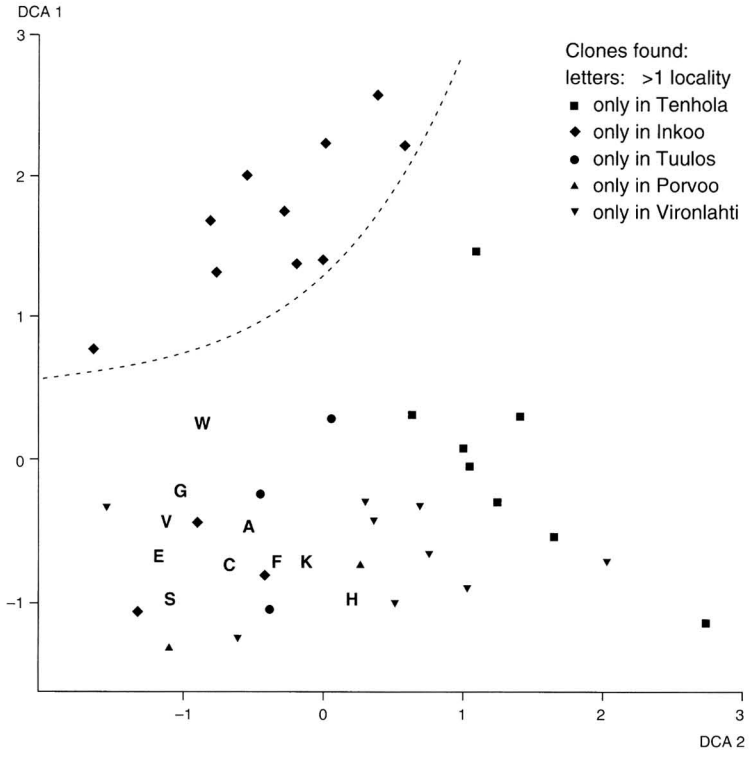

Fig. 3. DCA ordination of clones according to their enzyme variant combinations. Eigenvalue for axis 1 = 0.533 and for axis 2 = 0.476. Solid signs indicate clones found in only one locality and letters those found in at least two localities (for letters see Table 1).

genotype similarities (Fig. 3). It turned out that all the clones in this cluster originated from Inkoo and were not found in any other locality. In addition to these clones the sample from Inkoo also comprised worms representing other clones, but they showed greater affinities with the clones of the other sample sites.

3.2. Variation in soma and in secondary reproductive organs

In the pooled *A. rosea* material the somatic characters in adults (n = 132) correlated significantly with each other (Spearman rank correlation, fresh weight/posterior length: 0.595^{***}, fresh weight/number of segments: 0.276^{**} and posterior length/number of segments: 0.222^{**}). In the juveniles (n = 69), however, the correlation between segment number and total body length was not significant (r_S = 0.122).

Table 5 indicates that local *A. rosea* populations differed significantly from each other in body size, as shown by the fresh body weight, posterior length and total number of segments. An application of ANOVA to the corresponding rank values derived from the Kruskal-Wallis test similarly indicated significant differences between the populations. "A posteriori" comparison of the means by the Bonferroni test (with a rejection level of 0.05) showed that the population of Inkoo deviated most from the other populations. In Inkoo the adult worms were on average smaller and had a lower number of segments than adults from other localities. It is also noteworthy that the juveniles sampled in Inkoo had a significantly lower number of segments than did those from other populations.

Table 5 also indicates that in some populations the number of segments in juveniles was significantly lower than in adults from the same site, whereas in other populations the difference was not significant. A Kruskal-Wallis test applied to localities indicated that depending on the population the juvenile growth varies from population to population.

Table 6 shows the variability observed in secondary reproductive organs. The location of the clitellum varied more than did that of the tubercula pubertatis. No clear-cut local or regional trends were, however, evident. On the other hand, male pore terminalia were on average more prominent in adults of the West than in those of the East or the North.

3.2.3. Morphological variation related to clonal variation

We attempted to correlate clonal and morphological variability (Table 7). Individual clones differed in fresh weight and in total number of segments but not in posterior body length. Unfortunately, the study material was too scanty to

Table 5. Fresh weight (g), posterior length (mm, clitellum + posterior part of body) and total number of segments, in *A. rosea* from South Finland. Segment number in juveniles refers to individuals measuring 13–31 mm. Mean ± *SD*, median. KW = Kruskal-Wallis test, MW = Mann-Whitney test for adult / juv. segments.

Region & locality	Adults fresh weight		posterior length		segments		*n*	Juveniles segments		*n*	MW
West	0.28 ± 0.08	0.29	27.2 ± 4.2	28.0	123.9 ± 5.7	125.0	55	119.3 ± 10.0	118.0	29	*
Tenhola	0.33 ± 0.06	0.32	29.3 ± 3.0	29.0	128.8 ± 2.7	128.0	30	126.1 ± 5.4	127.5	14	ns
Inkoo	0.22 ± 0.06	0.23	24.8 ± 4.1	24.0	119.0 ± 4.4	119.0	25	113.1 ± 9.2	116.0	15	**
North	0.26 ± 0.04	0.25	28.7 ± 2.0	28.0	127.0 ± 3.6	127.0	27	123.0 ± 9.6	126.0	14	ns
East	0.27 ± 0.07	0.25	30.4 ± 3.4	30.0	122.8 ± 7.0	125.0	50	119.8 ± 8.2	121.0	26	*
Porvoo	0.25 ± 0.05	0.24	30.3 ± 3.5	29.5	124.8 ± 5.5	126.0	30	120.0 ± 7.6	122.5	14	*
Virolahti	0.30 ± 0.09	0.27	30.6 ± 3.3	30.0	119.8 ± 8.1	122.5	20	119.6 ± 9.2	121.0	12	ns
KW localities	***		***		***			**			
regions	ns		**		**			ns			

Table 6. Variation (%) in secondary reproductive traits (location of clitellum and tubercula pubertatis and protuberance of male pore terminalia) of *A. rosea* adults from Finnish localities and regions, and from USA and Mexico (Gates 1974).

	Tenhola	Inkoo	West	North	Porvoo	Virolahti	East	Finland	USA&Mexico
Clitellum:									
XXIII–XXXII	–	–	–	–	–	–	–	–	1
XXIII–XXXIII	–	–	–	–	–	–	–	–	1
XXIV–XXXII	–	–	–	–	–	–	–	–	34
XXV–XXX	–	–	–	–	–	–	–	–	<1
XXV–XXXI	–	–	–	–	–	–	–	–	2
XXV–XXXII	17	88	50	80	63	33	51	57	47
XXV–XXXIII	53	12	34	17	17	38	25	27	4
XXVI–XXXII	23	–	12	1	20	24	22	14	11
XXVI–XXXIII	7	–	4	–	–	5	2	2	1
Tubercula pubertatis:									
XXVIII–XXXI	–	–	–	3	–	–	–	<1	–
XXIX–XXX	–	19	9	–	–	–	–	4	–
XXIX–XXXI	100	81	91	97	100	95	98	95	100
XXIX–XXXII	–	–	–	–	–	5	2	<1	–
Male pore terminalia:									
mean±SD	2.0±0.0	2.6±0.5	2.1±0.7	1.2±0.4	1.6±0.5	1.9±0.3	1.7±0.5	1.8±0.6	?
n	30	26	56	30	30	21	51	137	252

test the variation in somatic characters of the worms belonging to the same clone but living in different localities.

A comparison of somatic characters in the worms of the clones ordinated by DCA analysis (Fig. 3) showed that the individuals in the cluster of clones in Inkoo (Inkoo 1, Table 7) were significantly smaller in size and had a significantly lower number of segments than did the worms of the other Inkoo clones (Inkoo 2, Table 7) or the clones in other localities (Others, Table 7). Application of the Bonferoni test (rejection level = 0.05) after application of ANOVA showed that in none of these traits did the means differ significantly between Inkoo 2 and Others; however, they both differed from those in Inkoo 1 (Table 7).

The variation in secondary reproductive organs (Table 6) could not be correlated with electrophoretic variability.

4. Discussion

4.1. Polyploidy and parthenogenesis in *A. rosea*

A. rosea is a polyploid lumbricid with a wide variation of karyotypes. Its basic chromosome number is n = 18. There are amphigonic and parthenogenetic strains in central and southern Europe. The amphigonic strains have 2n, 4n and 10n, whereas parthenogenetic forms can have 3n, 4n+x, 5n, 6n, 8n and 10n+x chromosomes (cf. Casellato 1987).

In the absence of bisexual reference material we could not establish the number of alleles at any locus; a crude estimate shows, however, some 3 to 4 alleles at Pgi and Est. The number of individuals representing the common clones showed that *A. rosea* is parthenogenetic (Tables 1 and 2). In an amphimictic species each worm is a unique individual.

4.2. Morphological variation

In *A. rosea* adults somatic traits correlated with each other, whereas in *D. octaedra* for example, variation in the number of segments was independent of variation in body size (Terhivuo 1988a). Although statistically significant local and regional differences existed in characters related to soma and to secondary reproductive organs in *A. rosea*, the patterns of variation showed no clinal trends. The only exception was the more prominent protuberance of male pore terminalia in the North than elsewhere (Table 6).

Phillipson & Bolton (1977) found "pygmy" *A. rosea* adults together with larger adults in the same sample site. In Inkoo we also secured some

Table 7. Fresh weight (g), posterior body length (mm) and total number of segments in *A. rosea* adults representing most common clones (A–K) and clone groups Inkoo 1, Inkoo 2 and Others (see Section 3.2.3.). Mean ± *SD*, median, coefficient of variation.

Clone & group	Fresh weight			Posterior length			Segments			*n*
A	0.26 ± 0.04	0.25	14	28.6 ± 2.0	29.0	7	125.3 ± 4.9	127.0	4	14
B	0.30 ± 0.09	0.27	29	30.4 ± 4.9	29.0	16	113.0 ± 10.7	117.0	9	5
C	0.27 ± 0.08	0.23	30	32.0 ± 4.5	32.0	14	125.4 ± 1.5	126.0	1	7
D	0.34 ± 0.06	0.36	17	30.2 ± 2.9	30.0	10	128.2 ± 2.3	128.0	2	9
E	0.25 ± 0.05	0.24	18	30.3 ± 2.3	30.0	8	126.9 ± 3.6	126.0	3	9
F	0.27 ± 0.04	0.27	16	28.5 ± 1.9	28.5	8	128.0 ± 3.2	128.0	3	8
G	0.24 ± 0.03	0.24	10	29.0 ± 2.3	28.0	8	122.7 ± 5.2	126.0	4	7
H	0.25 ± 0.04	0.25	14	29.0 ± 2.9	28.0	10	124.3 ± 8.9	127.0	7	7
I	0.37 ± 0.08	0.34	21	29.4 ± 3.0	28.0	10	128.2 ± 0.8	128.0	1	5
J	0.25 ± 0.04	0.24	17	30.7 ± 4.1	29.5	13	125.7 ± 2.9	125.0	2	6
K	0.31 ± 0.03	0.30	10	31.6 ± 2.3	32.0	7	124.6 ± 4.6	125.0	4	5
Kruskal-Wallis:	***			ns			**			
Inkoo 1	0.16 ± 0.02	0.16	15	21.2 ± 1.5	21.0	7	115.6 ± 2.1	116.0	2	12
Inkoo 2	0.27 ± 0.03	0.28	10	28.1 ± 2.5	29.0	9	122.2 ± 3.5	121.0	3	13
Others	0.28 ± 0.07	0.27	24	29.7 ± 3.0	29.5	10	125.3 ± 5.8	127.0	5	107
Kruskal-Wallis:	***			***			***			

small adults in addition to larger ones. Environmental differences can hardly account for this. One possible factor may be a different degree of polyploidy. Phillipson & Bolton (1977) found that the growth rate of the "pygmy" *A. rosea* individuals did not differ from that of other juveniles until the onset of maturity, when their growth ceased. In Inkoo, both adults and juveniles had lower mean and median numbers of segments than did those in other populations (Table 5). A slight bimodality in segment numbers of the adults was also observed in Inkoo: 111–113 segm. / 1 adult, 114–116 segm./ 8 adults, 117–119 segm./ 5 adults, 120–122 segm./ 7 adults, 123–125 segm./ 2 adults and >125 segm. / 2 adults. In juveniles, however, the material was too scanty to allow for any definite conclusions in this respect: < 110 segm./ 2 juv., 111–113 segm./ 0 juv., 114–116 segm./ 7 juv., 117–119 segm./ 5 juv. and 120–122 segm./ 1 juv.

In order to determine whether the differences observed between the localities in Table 5 were solely due to the presence of the Inkoo population in the analysis, we applied the Kruskal-Wallis test once more, but only to the worms from populations other than Inkoo. There were, however, significant differenecs between the populations in both body weight and the number of segments. The posterior body length was, however, similar in all populations. We concluded that all the morphological differences in Table 5 were not due solely to the worms from Inkoo.

Gates (1974) reported 126.7±9.5 (mean ± *SD*) (median 129.0, $n = 120$) segments for *A. rosea* in USA and Mexico, where the species is allochtonous. In the pooled Finnish material, *A. rosea* had 124.1±6.1 (mean ± *SD*) (median = 126.0, $n = 132$) segments, which is significantly different from the material inspected by Gates (1974) (Kruskal-Wallis: $P < 0.001$).

Gates (1974) recorded a wide variability in secondary reproductive traits, notably in the location of the clitellum, in 252 individuals of *A. rosea* from North America and Mexico (Table 6). Intrasegmental variability was not included in this this tabulation. The location of tubercula pubertatis showed some variation in Finland (Table 6), but Gates (1974) gives only segments XXIX–XXXI for them.

In parthenogenetic earthworms male reproductive organs often show signs of reduction (Stephenson 1930). In Finland about 40% of *D. octaedra* adults lacked male pores (Terhivuo 1988a). In *E. tetraedra*, male pores were sometimes located in segments other than XV (Terhivuo et al. 1993). In *O. cyaneum* and *O. tyrtaeum* they were well-demarcated and always present on segment XV (Terhivuo & Saura 1993). In Finland, *A. rosea* adults always had male pores, but their protuberance was less distinct in northern and eastern than in western populations. In Inkoo the estimated mean protuberance index was higher than in other populations, indicating that the small-sized adults found there displayed no reduction.

As noted above, *A. rosea* lacked spermatophores. On the other hand, we have once recorded copulating *A. rosea* in southern Finland. This may not be significant, however, as vestiges of mating behavior are often found in parthenogenetic animals (Suomalainen et al. 1987).

In conclusion, *A. rosea* shows less wide variability in secondary reproductive organs than do the more widely distributed parthenogenetic *D. octaedra* and *E. tetraedra* in southern Finland.

We can also conclude that in *A. rosea* variability in somatic traits correlates with electrophoretic variability.

4.3. Clone pools and dispersal of clones

In southern Finland *A. rosea* shows extensive clonal variability, which seems to decrease towards the north (Fig. 2, Table 3). A random sample of 50 *A. rosea* individuals is expected to contain 18.0–24.8 clones. This is far more than for instance in the parthenogenetic *O. tyrtaeum* with its 11.1–12.6 clones (Terhivuo & Saura 1993) or in *E. tetraedra* with its 7.2 clones (Terhivuo et al. 1993) for the same area. On the other hand, in eurytopic and spontaneously dispersing *Dendrobaena octaedra* in that area a random sample of 40 individuals may be expected to contain 25.0–28.9 clones (Terhivuo & Saura 1990). In all the above investigations we have studied some 15–20 enzyme systems, and of these Est, G-3-pdh, Idh, Lap, Mdh, Pgm and Pgi proved to be polymorphic in at least one. The comparisons shown above are based on the numbers of clones defined by the variability observed in these seven enzyme systems.

In Finland, *A. rosea* occupies fertile soils which are often under cultivation. Its wide clonal heterogeneity may be attributed to efficient dispersal resulting from agricultural and horticultural activities. In any case, *A. rosea* in Finland is unlikely to have originated from a small founder population. The present survey rather suggests that the rate of immigration has been considerably higher in the past than in *O. cyaneum* for example. The latter species has rather recently arrived in southern Finland and was found to have only two clones (Terhivuo & Saura 1993).

Table 2 reveals that clone A was most frequent in the North, where most of the clones seem to originate either from the East or the West. Perhaps clone A does better in the North than in the other two regions where it was less frequent and was recorded only in the localities nearest to Tuulos in the North.

Table 2 and Fig. 2 also show that local and regional clone pools did not comprise random arrays of clones. The high proportion of clones found in only one sample site both in eastern and western parts of the transect may result from individuals of more widely distributed clones at the margin of their wider geographical distribution; perhaps some of the clones represent strains derived through mutations from the more common clones in the area. Their low proportion in the North suggests that this discrepancy is due to differences in dispersal history rather than in unequal rate of mutation.

A. rosea is well adapted to agriculture. It is, in fact, one of the most frequent earthworms of cultivated fields in North America (Gates 1974), and in Finland it is also found in cultivated fields. An endogeic species such as *A. rosea* has a better ability to escape possible detrimental effects of agriculture than do epigeic species. On the other hand, epigeics may be less exposed to forces that promote the dispersal of topsoil species.

In Inkoo the *A. rosea* clone pool was considerably wider than in the other localities. Though all sample localities had been exposed to some agriculture for several years in the past at least, it is possible that the old field sampled in Inkoo has received many new *A. rosea* clones from the nearby manor to which for centuries individuals or cocoons may have been imported along with foreign plants.

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