The Genetic Structure Features of the Amphimictic Earthworm *Aporrectodea caliginosa* (Oligochaeta, Lumbricidae) Populations within Ukraine

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Abstract—Genetic differentiation of the amphimictic earthworm *A. caliginosa* populations was investigated in the territory of Ukraine by analyzing the variability of the polyallelic locus *Es*-4. It was established that the settlements of this species are characterized as $F_{st} = 0.13$, which means genetic heterogeneity above the average level. The values of this index depend on the size of the population groups. Moreover, these changes can be represented as a leap from insignificant interdeme differences to statistically significant ones, obtained in the analysis of geographically remote populations, with the stabilization of the F_{st} index values on the macroscale. This situation is reasonable for the model of a genetically homogeneous settlement that arose once over a large area, the secondary differentiation of which was caused by internal migrations and the founder effect, which is in agreement with the regularities observed for populations of this species in North America. The comparison of the spatial differentiation of *A. caliginosa* populations with the genetically, ecologically, and arealogically close parthenogenetic *A. trapezoides* species within Ukraine showed that the apomictic species has a different type of geographical differentiation of populations. It is characterized by a vicarious structure of settlements in which one clonal form replaces another and the genotypes of clones of distant populations differ the most. This means that there are no migrations between remote settlements of earthworms within Ukraine, and the reasons for the alternative nature of the genetic subdivision of amphimictic and apomictic species settlements are associated with the mechanisms of forming genetic diversity. In amphimictic species populations, the maximum genotypic diversity is achieved through recombinations and is realized at the individual level within populations, while it is due to mutations in clonal species and appears as intergroup variability.

INTRODUCTION

Earthworms of the family Lumbricidae are popular objects of evolutionary and population genetic research. The reasons are their mass and ecological significance, clonal reproduction of numerous parthenogenetic species, and invasive processes on the scale of the globe, which became one of the factors of ecosystem changes (Hendrix et al., 2008). Traditional interest of geneticists is caused by clonal populations of parthenogenetic allopolyploid species (Janike and Selander, 1979; Tehivua and Saura, 2008; Mezhzherin et al., 2017; Shekhovtsov et al., 2020), while the peculiarities of the genetic structure of settlements of diploid amphimictic species remain poorly studied. In fact, there is only one publication that describes the genetic structure of the settlements of one of the most abundant earthworm species in the temperate zone of North America, *Apoirectodea tuberculata* (Stille et al.,

1980). Analysis of the genetic structure of this species by allozyme analysis revealed a fairly significant level of differentiation of its settlements. The main contribution to spatial heterogeneity belongs to populations separated by several tens of kilometers. The genetic similarity of neighboring deme-structured populations is expected, but the revealed low contribution to the spatial differentiation of geographically remote populations was quite unexpected and explained by the authors by migration processes.

Phylogenetic analysis showed (Perez-Losada et al., 2009, 2012) that *A. tuberculata* is a conspecific with amphimictic cosmopolitan species *A. caliginosa*, which is one of the most widespread members of the family Lumbricidae within Ukraine. This fact allows us to conduct a comparative study of the genetic structure of settlements of the species in the vast range that covers the extratropical regions of the Old and New
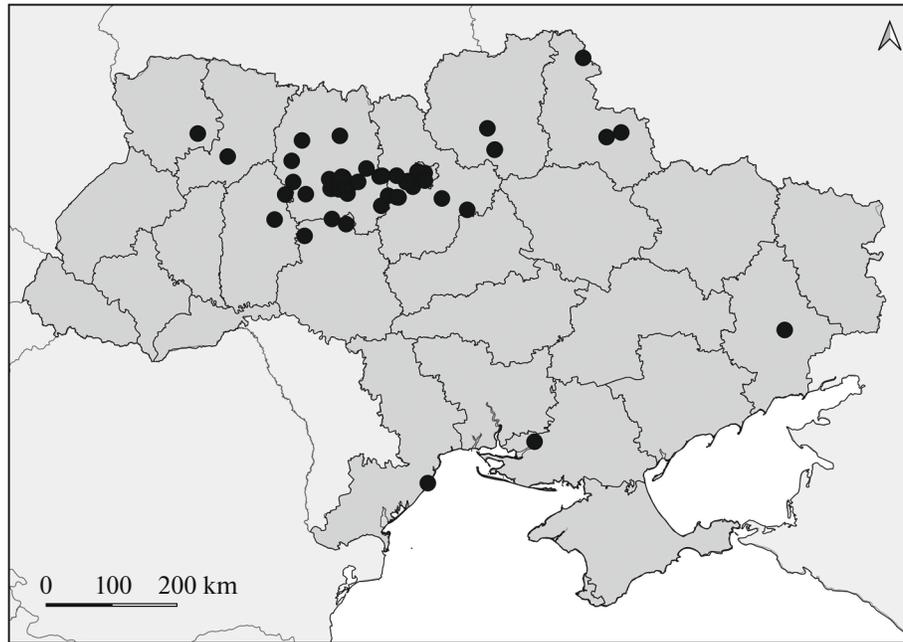
Fig. 1. Places where earthworm *A. caliginosa* samples were collected.

World. In the case of reproduction of the result obtained in the North American part of the range, the features of spatial differentiation of *A. caliginosa populations* can be extrapolated to other amphimictic species of earthworms, which is quite adequate since it is one of the most common and most widespread species in the Holarctic.

The possibility of direct comparison of the spatial structure of *A. caliginosa* with the peculiarities of geographical differentiation of ecologically and genetically close to it *A. trapezoides* provides particular intrigue to this study. This is a parthenogenetic allotriploid species, one of the parent species of which is *A. caliginosa* (Mezhzherin et al., 2017), inhabiting, together with the latter, in open landscapes throughout Ukraine. The genetic structure of its settlements has been studied in detail before (Mezhzherin et al., 2008). As a result, it was found that *A. trapezoides* populations are characterized by polyclonality and a high degree of spatial genetic differentiation, which consists in the vicarious structure of settlements in which one clonal form replaces another. Comparative analysis of the spatial genetic structure of these two ecologically and arealogically similar species is valuable not only as an independent study but it also allows us to reveal the features of microevolution of amphimictic and apomictic species as well as solve the problem of evolutionary predominance of amphimixis.

MATERIALS AND METHODS

The actual basis of this study was 1083 specimens of *A. caliginosa*, comprising 61 samples collected from all

regions of Ukraine (Fig. 1). The largest number of samples was taken from the Continental Biogeographical Zone, which covers the Forest and Forest-Steppe geographical zones of Ukraine, where this species is much more numerous than in the Steppe Biogeographical Zone, where it is sporadic.

The species affiliation was determined by a multilocus analysis of four enzyme systems: aspartate amino transferase, malate dehydrogenase, nonspecific esterases and superoxide dismutase. Only the *Es-4* locus, represented by five alleles, three of which occur with a high frequency, was highly polymorphic, which allows it to be fully used to analyze the spatial structure of *A. caliginosa* populations.

Gel electrophoresis followed by the staining of gels according to standard methods was performed in 7.5% polyacrylamide gel and a Tris-EDTA-borate buffer system (Peacock et al., 1965).

Two standard indices of *F*-statistics (Wright, 1965; Nei, 1977) applied to an estimation of genetic structure of populations were used as parameters of genetic structure of populations.

The genotype fixation index (F_I) was calculated according to the formula $F_I = 1 - Het\,(\text{obs})/Het\,(\text{exp})$, where *Het* (obs) is the actual heterozygosity and *Het* (exp) is the expected heterozygosity. The value of the index varied in the range from −1 to 1. Negative values indicated an excess of heterozygotes and the complete absence of homozygotes in extreme cases. Positive values indicated a deficiency of heterozygotes, and the extreme situation $F_I = 1$ indicated their absence in the population. The reasons for the deviations from zero,

Table 1. Statistical indicators of variability of alleles of the *Es*-4 locus in populations of the earthworm *A. caliginosa* in Ukraine

Allele	N	M	σ	Min-max	F_{st}
Es-4a	61	0.36	0.17	0−0.78	0.13*
Es-4b	61	0.31	0.17	0.01−0.86	0.13*
Es-4c	61	0.34	0.17	0.08−0.77	0.13*

N—number of subpopulations; M—average population frequency of the allele; χ^2—its variance within the study area; Min−max—minimum and maximum frequencies. * The population structure has possible values of the heterogeneity index F_{st} at the level of $p < 0.001$.

which corresponds to the equilibrium state of the population, are related to the limitation of amphimixis. An excess of heterozygotes is a rare situation, usually caused by cloning, while deficiency is a more common phenomenon due to restrictions on the crossing of certain genotypes. This can be due to both self-fertilization and the Wahlund effect: an excess of homozygotes caused by mixing of populations with different gene frequencies. The degree of heterogeneity of regional population groups can be assessed by the degree of manifestation of the Wahlund effect. The probability of disequilibrium was checked by comparing the actual and expected distributions of genotypes by criterion χ^2.

Standardized genetic variance or F_{st}-index is used to assess the differentiation of population groups. It is the ratio of the two variances: empirical variance, which is obtained based on variance calculations at the level of subpopulations composing the total population, and theoretical variance calculated based on averages for the total population. The formula used for calculations is:

$$F_{st} = \sigma p^2 / (P - p_i)^2,$$

where P is the average frequency of the allele, and p_i is the frequency of the allele in the *i*-th sample. The value of σp^2 is calculated according to the following formula:

$$\sigma p^2 = \Sigma (P - p_i)^2 / k,$$

where k is the number of samples. The null hypothesis, according to which $F_{st} = 0$, is refuted by the criterion χ^2, calculated using the formula $\chi^2 = 2NF_{st}$, where N is the total number of specimens, and the number of degrees of freedom is $k - 1$. The value of the index varies in the range from 0 to 1. In the first case, it is the complete genetic identity of the population, while that in the second is the maximum differentiation associated with the fixation of alternative alleles in different populations.

RESULTS

Statistical analysis of the variability of the three main alleles of the *Es*-4 locus in the generalized *A. caliginosa* sample from the territory of Ukraine showed equal values of mean frequency, close limits of variation for subpopulations, and the same variance and degree of differentiation of populations of this species throughout Ukraine, estimated according to F_{st} index (Table 1), which had high possible values in all three cases. This indicates the existing spatial and genetic heterogeneity of settlements of this species within Ukraine.

At the same time, it was not possible within the territory of Ukraine to identify trends in the geographical variability of gene frequencies. In particular, there were no reliable values of correlation coefficients between allele frequencies in samples with longitude or latitude. This indicates that there is no clinal variability and there are no gene flows within the study area and the genetic heterogeneity of the settlement of this species within Ukraine is mosaic.

The check of the correspondence of empirical and theoretical distributions of genotypes of the *Es*-4 locus indicates their equilibrium in most of the studied populations. The only exception was a sample from the Kyiv oblast with a deficit of heterozygotes. However, the analysis of the distribution of the index F_I in 61 studied samples revealed the shift towards positive values (Fig. 2), which was confirmed by statistical calculations ($F_I = 0.08 \pm 0.027$, $t = 2.74$, $p < 0.01$). This indicates a certain tendency to heterozygote deficiency in populations, which is most likely caused by a certain level of self-fertilization inherent in most hermaphroditic animals (Ramm et al., 2015), including annelides (Diaz Cosin et al., 2011).

Analysis of the values of F_{st} index showed that it varied from 0.03 to 0.15 (Table 2) in the groups of samples of different degrees of spatial separation and the possible values start with $F_{st} = 0.08$. At a distance separating the subpopulations of one group, up to 30 km, the values of this index are in the range from 0.03 to 0.13 with an average $F_{st} = 0.07$ (Table 3). Genetic heterogeneity of population groups with subpopulations separated by a distance of 30−200 km was significantly higher and average F_{st} values were 0.10. The highest value did not exceed the maximum value of the previous level. At a distance between the samples of 201−400 km, the values of the F_{st} index ranged from 0.13 to 0.15, while the index stabilized at the level of 0.13−0.14 in the range of 401−800 km. This means that there is a tendency to increased heterogeneity of populations as
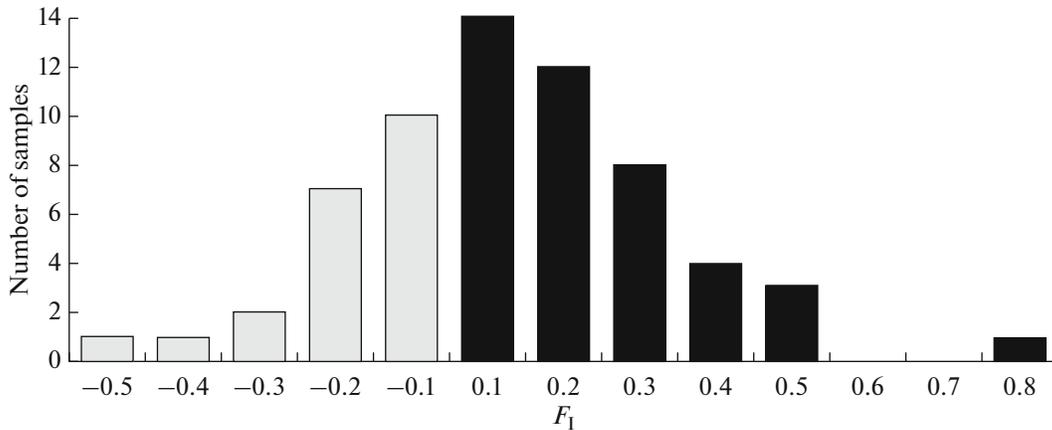
Fig. 2. Distribution of the fixation index (F_I) in *A. caliginosa* subpopulations within Ukraine.

the area occupied by the group of populations increases, which was confirmed by the correlation analysis ($r = 0.51$; $p < 0.05$). However, this process lasts only to a certain extent. The tendency to increase in the genetic heterogeneity of a group of populations with the increase in the area they occupy does not have a clear linear character. The largest changes in the F_{st} index occur during the transition from the smallest to medium-sized groups, whose subpopulations are separated by a distance of 30 to 200 km. In the most massive groups, covering several regions and even the entire territory of Ukraine (range of 200–800 km), the F_{st} index stabilized at 0.13. F_I index changes in a similar way, however with less expressive values (Table 2).

Estimation of genetic heterogeneity of *A. caliginosa* populations obtained in Ukraine corresponds to the importance of genetic differentiation of settlements of this species of earthworms in North America (Stille et al., 1980). Thus, the average index of genetic heterogeneity of settlements, calculated for four alleles of the homologous locus of nonspecific esterases, in American populations provides the same values as in Ukraine $F_{st} = 0.13$. For two other *Got-1* and *Got-2* loci, polymorphic in American populations, the index values were slightly lower than 0.09 and 0.1, respectively. The slight decrease in the index calculated for the last two loci was due to a significantly lower level of their heterozygosity compared to the *Es*-4 locus (*Es*-5 according to the nomenclature of American researchers).

DISCUSSION

Summarizing the results, it can be concluded that the settlements of *A. caliginosa*, one of the most widespread amphimictic species of earthworms, within Ukraine correspond to a mosaic model of genetic differentiation since the nature of differences in its populations cannot be explained by clinal variability or gene flows. Peculiarities of structuring settlements of this species should be considered: (1) the leap in the values

of genetic heterogeneity from minimal to high values in the transition from deme settlements in geographical populations is expected to some extent and (2) unexpected stabilization of the heterogeneity index in large population groups covering large areas. This type of spatial differentiation of a population group is adequate to the model of a single primary genetically homogeneous and large settlement area, the differentiation of which has a secondary nature and is determined only by migration processes and founder effects within it (Altukhov, 2003).

The results of the study of *A. caliginosa* populations within Ukraine generally correspond to the nature and degree of genetic differentiation of this species in North America (Stille et al., 1980). Changes in the level of genetic differentiation as the size of settlements increased in both cases were nonlinear and can be described as a leap from insignificant interdeme differences to statistically significant ones between geographical populations with macroscale stabilization. The indicators of genetic heterogeneity of American and European settlements of this species, which were evaluated by F_{st}, were also very close. In both cases, the assessment of genetic heterogeneity, carried out at homologous loci, was at the level of $F_{st} = 0.13$. This level of differentiation should be considered above average. It significantly exceeds the indicators of genetic differentiation of geographical populations of insects (Eanes et al., 1978), freshwater fish of neighboring river systems (Avise and Felley, 1979), demes of house mice (Selander, 1970), where the F_{st} index varied between 0.01–0.05. The obtained level of differentiation of earthworm populations corresponds to the distribution of territorial groups of sedentary organisms. For example, in the settlement of the land snail *Helix aspersa* from a metropolis, this index was 0.11, while it reached 0.16 in a group of populations from several large cities (Selander Kaufman, 1975). The F_{st} index of earthworms within Ukraine was not much lower than the value of 0.15, which was obtained for

Table 2. Indices (F_I, F_{st}), reflecting the spatial heterogeneity of groups of *A. caliginosa* subpopulations of different geographical scale

Group of populations	D	N	n	F_I	F_{st}
Village of Kozhukhivka (Vasylkiv district)	1	3	66	0.11	0.04
Zhytomyr	10	6	154	0.05	0.03
Kyiv	15	3	72	0.23	0.10
Zhytomyrskyi district (southern part)	15	5	99	0.08	0.07
Zhytomyrskyi district and the city of Zhytomyr	30	18	353	0.09	0.08
Nizhynskyi district	30	3	58	0.05	0.04
Fastivskyi district	10	3	48	0.36	0.13
Makarivskyi district	40	4	53	0.19	0.08
Vinnytsia oblast	70	3	31	0.48	0.13
Sumy oblast	120	3	36	0.04	0.05
Kyiv oblast	140	17	295	0.22	0.14
Zhytomyr oblast	150	29	592	0.11	0.13
Sumy and Chernihiv oblast	200	6	94	0.06	0.04
Volyn + Podillya	275	7	88	0.33	0.15
Zhytomyr and Kyiv oblast	281	45	872	0.15	0.13
Volyn, Podillya and Zhytomyr oblast	295	37	680	0.14	0.13
Continental biogeographical zone of the Right Bank	400	52	960	0.17	0.14
Continental biogeographical zone as a whole	600	58	1054	0.16	0.13
Ukraine as a whole	900	61	1083	0.20	0.13

D—maximum distance between subpopulations; *N*—number of samples; *n*—number of specimens. Possible values are marked in bold.

the entire human population (Cavalli-Sforza and Bodmer, 1971).

The relationship between the indicators and the nature of the genetic differentiation of *A. caliginosa* populations in Europe and North America can obviously be considered as a certain standard for other wide-range amphimictic species of earthworms in the Holarctic.

The comparative analysis of the genetic differentiation of settlements of amphimictic *A. caliginosa* and closely related allotriploid parthenogenetic species *A. trapezoides* within Ukraine is of great interest; the geographical structure of the latter settlements was studied in detail earlier (Mezhzherin et al., 2008). The compared species are cosmopolitan and genetically, areologically, and ecologically similar. These are typical for earthworms in the territory of Ukraine, whose populations are confined to moderately humid landscapes, where they form common settlements. The genetic structure of *A. trapezoides* populations is radically different from *A. caliginosa*, not only by clonal organization but also by the nature of spatial-genetic differentiation. Apomictic species *A. trapezoides* is characterized by a vicarious structure of settlements. If deme-structured populations of this species do not differ from each other in the composition of clonal biotypes, in a reasonably distant population, there is most often a change in the dominant biotype, while

there is a complete replacement of the composition of biotypes in populations separated by considerable geographical distance. Thus, the spatial genetic structure of *A. trapezoides*, in contrast to *A. caliginosa*, clearly corresponds to the concept: the greater the distance that separates populations, the higher the level of their genetic differentiation.

The stable vicarious structure of settlements, when one clone of *A. trapezoides* in geographical space replaces another, proves the absence of migration flows in settlements of this species within Ukraine. This provision can be applied to *A. caliginosa*, which

Table 3. Average values of the F_{st} heterogeneity index of earthworm *A. caliginosa* populations in population groups of different sizes

D, km	N	M	Min−max
1−30	7	0.07	0.03−0.13
31−100	2	0.11	0.08−0.13
101−200	4	0.09	0.04−0.14
201−400	3	0.14	0.13−0.15
401−800	3	0.13	0.103−0.14

D—maximum distance between subpopulations within the spatial group; *N*—number of samples; *M*—average value of F_{st} index; Min−max—limit of variation of the index.

has the same type of range and practically the same landscape-biotope confinement with *A. trapezoides*. This means that the formation of features of the genetic structure of amphimictic and apomictic species of earthworms is not associated with migratory processes but occurs due to various reproductive and evolutionary strategies. In the amphimictic species, genetic diversity is manifested at the level of specimens and is concentrated within populations, while it has the character of group variability in the apomictic species.

Differences in the organization of genetic diversity of populations of amphimictic and apomictic species allow discussing the reasons for the dominance of amphimixis in highly organized animals. On the one hand, amphimixis is a distinctive feature of evolutionarily advanced groups of animals, which, from the standpoint of a selective evolutionary paradigm, should mean greater adaptive efficiency of this method of reproduction. On the other hand, the loss of half of the reproductive potential of the population at the expense of males makes the cost of bisexual amphimixis very high (Maynard Smith, 1978). In most cases, this discrepancy is resolved by explaining the need for amphimictic reproduction by the benefits of recombination variability (Maynard Smith, 1978; Steams, 1987; Barton and Charlesworth, 1998; Gandon and Otto, 2007). It is believed that successful genotypic combinations resulting from recombination allow organisms to provide rapid and effective responses to adverse factors, including, in particular, evolutionary changes in pathogenic unicellular organisms, the rate of evolution of which is higher than the rate of evolution of multicellular hosts.

In such interpretations based on the opposition of amphimixis and apomixis, however, there is a weakness. It is believed that the genetic diversity of organisms with clonal reproduction, is very limited because apomixis produces genetically identical offspring and, accordingly, homogeneous settlements. However, this is not so. Studies of the genetic structure of clonal species indicate their enormous genotypic diversity. As a result, in apomictic species, including earthworms, polyclonality is the norm (Mezhzherin et al., 2017). Moreover, the number of clones can be so significant that such cases are interpreted as evolutionary hypervariability (Cywinska and Hebert 2002), which can sometimes occur within a single population and always occurs throughout the range.

In the context of this study, this means that the species *A. caliginosa* with amphimictic reproduction experiences the benefits of recombination and individual genetic diversity mainly within populations, with the relative homogeneity of populations over a large area. The genetic diversity of the parthenogenetic species *A. trapezoides* has a group character and is achieved in the form of polyclonal settlements along the range. The implementation of one or another strategy makes earthworms with different types of reproduction equally adapted. After all, both *A. caliginosa* and *A. trapezoides* achieve biological progress: they have huge cosmopolitan ranges and high population density. This allows us to conclude that meiotic recombination, as such, is not a key cause of the evolutionary predominance of amphimixis in the most evolutionarily advanced systematic groups of animals.

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REFERENCES

Altukhov, Yu.P., *Genetic Processes in Populations*, Moscow: Academperiodic, 2003.

Avise, J.C. and Felley, I., Population structure of freshwater fishes I. Genetic variation of bluegill (*Lepomis macrochirus*) population in man-made reservoirs, *Evolution*, 1979, vol. 33, no. 1, pp. 15–26.
https://doi.org/10.1111/j.1558-5646.1979.tb04658.x

Barton, N.H. and Charlesworth, B., Why sex and recombination, *Science*, 1998, vol. 281, pp. 1986–1990.
https://doi.org/10.1126/ science.281.5385.1986

Cavali-Sforza, L.L. and Bodmer, W.F., *The Genetics of Human Populations*, San Francisco: Freeman, 1971.

Ciwinska, A. and Hebert, P.D.W., Origins of clonal diversity in the hypervariable asexual ostaracod *Cyprodopsis vidua, J. Evol. Biol.*, 2002, vol. 15, no. 1, pp. 134–145.
https://doi.org/10.1046/j.1420-9101.2002.00362.x

Diaz Cosin, D.J., Novo, M., and Femandy, R., Reproduction of earthworms: sexual selection and parthenogenesis, in *Biology of Earthworms*, Caraca, A., Ed., Heidelberg: Springer, 2011, vol 24, pp. 69–86.
https://doi.org/10.1007/978-3-642-14636-7_5

Eanes, W.F. and Koehn, R.K., An analysis of genetic structure in the monarch bitterfly, *Danaus plexippus* L., *Evolution*, 1978, vol. 32, no. 4, pp. 784–797.
https://doi.org/10.1111/j.1558-5646.1978.tb04633.x

Gandon, S. and Otto, S.P., The evolution of sex and recombination in response to abiotic or coevolutionary fluctuations in epistasis, *Genetics*, 2007, vol. 175, no. 4, pp. 1835–1853.
https://doi.org/10.1534/genetics.106.066399

Hendrix, P.F., Mac Callaham, A., Drake, J.M., Huang, Ch.Y., James, S.W., Snyde, B.A., and Zhang, W., Pandora's Box contained bait: the global problem of introduced earthworms, *Ann. Rev. Ecol., Evol., Syst.*, 2008, vol. 39, pp. 593–613.
https://doi.org/10.1146/annurev.ecolsys.39.110707.173426

Jaenike, J. and Selander, R.K., Evolution and ecology of parthenogenesis in earthworms, *Am. Zool.*, 1979, vol. 19, no. 3, pp. 729–737.
https://doi.org/10.1093/icb/19.3.729

Maynard Smith, J., *The Evolution of Sex*, Cambridge: Cambridge Univ. Press, 1978.

Mezhzherin, S.V., Vlasenko, R.P., and Garbar, A.V., Features of genetic structure of the earthworm *Aporrectodea* (superspecies) *caliginosa* (Oligochaeta, Lumbricidae) complex in Ukraine, *Cytol. Genet.*, 2008, vol. 42, no. 4, pp. 256—262.

Mezhzherin, S.V., Harbar, A.V., Vlasenko, R.P., Onishchuk, I.P., Kotsyuba, I.Yu., and Zhalay, E.I., *Evolutionary Paradox of Parthenogenetic Earthworms*, Kiev: Naukova Dumka, 2018.

Nei, M., F-statistics and the analysis of gene diversity in subdivided populations, *Ann. Hum. Genet.*, 1977, vol. 41, pp. 225—233.
https://doi.org/10.1111/j.1469-1809.1977.tb01918.x

Peacock, F.C., Bunting, S.L., and Queen, K.G., Serum protein electrophoresis in acrylamyde gel patterns from normal human subjects, *Science*, 1965, vol. 147, pp. 1451—1455.

Perez-Losada, M., Ricoy, M., Marshall, J.C., and Dominguez, J., Phylogenetic assessment of the earthworm *Aporrectodea caliginosa* species complex (Oligochaeta: Lumbricidae) based on mitochondrial and nuclear DNA sequences, *Mol. Phylogenet. Evol.*, 2009, vol. 52, no. 2, pp. 293—302.
https://doi.org/10.1016/j.ympev.2009.04.003

Perez-Losada, M., Bloch, R., Breinholt, J.W., Pfenninger, M., and Domнnguez, J., Taxonomic assessment of Lumbricidae (Oligochaeta) earthworm genera using DNA barcodes, *Eur. J. Soil Biol.*, 2012, vol. 48, pp. 41—47.
https://doi.org/10.1016/j.ejsobi.2011.10.003

Ramm, S.A., Schlatter, A., Poirier, M., and Schärer, L., Hypodermic self-insemination as reproductive assurance strategy, *Proc., Biol. Sci.*, 2015, vol. 282, no. 1811, p. 20150660.
https://doi.org/10.1098/rspb.2015.0660

Selander, R.K. and Kaufman, D.W., Genetic structure of populations of the brown snail (*Helix aspersa*), *Evolution*, 1975, vol. 29, no. 3, pp. 385—401.
https://doi.org/10.1111/j.1558-5646.1975.tb00829.x

Shekhovtsov, S.V., Derzhinsky, Ye.A., Golovanova, E.V., and Peltek, S.E., Phylogeography and genetic lineages of *Aporrectodea rosea* (Lumbricidae, Annelida), *Eur. J. Soil Biol.*, 2020, vol. 99, p. 103191.
https://doi.org/10.1016/j.ejsobi.2020.103191

Stearns, S.C., Why sex evolved and the differences it makes, in *The Evolution of Sex and its Consequences*, Basel: Birkhouser, 1987, pp. 15—31.
https://doi.org/10.1007/978-3-0348-6273-8

Stille, B., Ochman, H., and Selander, R.K., Genetic structure of populations of the earthworm *Aporrectodea tuberculata*, Oikos, 1980, vol. 34, no. 2, pp. 195—201.
doi 10.2307/3544182

Terhivuo, J. and Saura, A., Dispersal and clonal diversity of North-European parthenogenetic earthworms, *Biol. Invasions*, 2006, vol. 8, no. 6, pp. 1205—1218.
https://doi.org/10.1007/978-1-4020-5429-7_2

Wright, S., The interpretation of population structure by F-statistics with special regards to system of mating, *Evolution*, 1965, vol. 9, no. 3, pp. 395—420.
https://doi.org/10.2307/2406450

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