The effect of Pleistocene glacial morphogenesis on the genetic structure of the humid- and cold-tolerant root vole *Microtus oeconomus* (Rodentia, Cricetidae) in Poland, central Europe

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Abstract

During the Pleistocene in the northern part of Europe and Asia, the presence of ice sheets not only limited the range of species but also influenced landscape and thus the contemporary habitat system that determines the pattern of biodiversity. The aim of the research was to find out whether and how a lowland landscape, which formed as a result of subsequent Pleistocene glaciations (five) that in Eurasia covered various and generally successively smaller areas, affected the genetic differentiation of a species. The research was carried out in eastern Poland on the root vole *Microtus oeconomus* (Arvicolinae, Rodentia), a model boreal and hygrophilous species. Samples were collected from 549 vole individuals at 33 locations. Based on the analysis of 12 microsatellite loci and the 908 bp of cytochrome b sequences (mitochondrial DNA), the genetic structure of *M. oeconomus* in the landscape zones of the Polish Lowlands was determined. The results show that the latitudinal variability of the relief in eastern Poland (resulting from different ranges of Pleistocene ice sheets) and the related specific configuration of hydrogenic habitats are reflected in the genetic differentiation of the root vole. Therefore, it may be concluded that the history of landscape development affects the genetic structure of hydrophilic species.

Keywords: Pleistocene glaciations; Lowland; Morphogenesis; Habitat connectivity; Hygrophilous species; Genetic differentiation; Phylogeography

INTRODUCTION

A significant role in the distribution of species is attributed to historical paleoclimate changes and the associated periodic existence of appropriate living conditions, tectonic movements, and processes of terrain evolution (e.g., Hrbek et al., 2004; Lister and Stuart, 2008; Markova and Kolfschoten, 2008; Fløjgaard et al., 2009; Conroy et al., 2016; Gottscho, 2016; Baca et al., 2017; Stojak et al., 2019). Geologic processes may affect ecological (climatic, soil, and habitat) conditions of different areas by shaping their contemporary topography and lithology. This favors the formation or disappearance of habitats, preferring (or not) the occurrence of species with specific requirements. Such interconnections have been analyzed in recent years, and molecular analyses of genetic variability in populations and the genetic diversity of species have become tools for assessing landscape differentiation. Owing to such analyses, it is possible to identify even indistinct, "hidden" diversity within a population of the same species (Hrbek et al., 2004).

Today, such relationships have been documented for numerous plant and animal species in areas of strong tectonic and erosional activity, including North America (along the San Andreas Fault), Central America, Tenerife, Turkey, and New Zealand (Hrbek et al., 2002; Daza et al., 2010; Gutiérrez-García and Vázquez-Domínguez, 2013; Waters et al., 2015; Gottscho, 2016; Puppo et al., 2016). Molecular methods indicate that highly diverse relief forms—ranging from river valleys to mountain chains, which developed because of tectonic movements or erosional processes, such as river captures or karst processes—are barriers for the migration of individuals of species living in those areas (Waters et al., 2001; Hrbek et al., 2004, 2014; Burridge et al., 2008; Craw et al., 2008; White et al., 2009). However, less work has focused on the relationship of genetic diversity and genetic differentiation of populations

with geomorphological processes and changes occurring in the Pleistocene in repeatedly glaciated lowland areas.

The greatest role in shaping the distribution of contemporary species and their genetic diversity in northern and central Europe and Asia in the Pleistocene is generally attributed to the last glaciation (e.g., Jaarola et al., 1999; Brunhoff et al., 2003, 2006; Kotlík et al., 2006; Wójcik et al., 2010; Haring et al., 2011; Herman et al., 2014; García-Vázquez et al., 2017). Postglacial colonization by species and their genetic variability are considered to have been primarily controlled by climatic processes that took place in the Pleistocene and Holocene. These studies focused on several related species of voles, including the root vole (*Microtus oeconomus*) (Brunhoff et al., 2003, 2006), the bank vole (*Myodes glareolus*) (e.g., Wójcik et al., 2010), the common vole (*Microtus arvalis*) (Ratkiewicz and Borkowska, 2006; Stojak et al., 2015), and the field vole *(Microtus agrestis)* (e.g., Jaarola and Searle, 2002). In this context, however, the impact of previous glacial episodes on the development of various habitat types should also be considered.

In the middle and late Quaternary, successive glaciations in the Northern Hemisphere covered various and generally successively smaller areas of Eurasia (Różycki, 1972; Baltrūnas et al., 2007; Astakhov, 2013). The resulting landscape shows zonation, well marked in Germany, Poland, Belarus, Lithuania, Latvia, Estonia, and part of Russia (Lindner, 1987; Marks, 2011; Astakhov, 2013).

In central and eastern Europe, a good example of postglacial landscape diversity is eastern Poland (Fig. 1), where different types of postglacial landscape are present over a relatively short distance of several hundred kilometers with a latitudinal arrangement in accordance with the ranges of successive Scandinavian ice sheets (Różycki, 1972; Marks et al., 2016) (Fig. 1). Thus, the landscape maturity level, understood as transformation and even obliteration of glacial landscape elements, increases from north to south. This maturity was also influenced by the advance of denudation processes.

Considering the glaciation history and landscape diversity, related to the modern distribution of many plant and animal species, it can be assumed that in Europe and Asia: (1) glaciation history that varies across latitudes contributes to (a) different numbers and characters (e.g., different degrees of connectivity) of favorable habitats and (b) different colonization times of the postglacial landscape by species because of the presence of small local refugia in the ice-marginal zone; (2) different numbers of favorable habitats and different degrees of their communication/isolation results in different courses of in situ evolution, which should be reflected in the genetic structure; and (3) different connectivity of favorable habitats, their variability in attractiveness, and different availabilities contribute to different directions and intensities of contemporary species migration.

In postglacial areas, moisture-loving species are good for testing the impact of geologic history on their genetic differentiation because of specific habitat requirements. Postglacial humid (aqueous and boggy) environments undergo relatively fast evolution in a temperate climate, which in turn forces quick reaction of the inhabiting species to the changing environment.

The aim of this research was to determine whether and how the landscape shaped during subsequent Pleistocene glaciations affects the diversity and persistence of populations, expressed by the genetic differentiation of species. We focused our research on the root vole (tundra vole) *Microtus oeconomus* (Arvicolinae, Rodentia). We know that (1) this model species shows clear preferences for inhabiting wetlands (e.g., Tast, 1966; Gliwicz and Jancewicz, 2004), (2) the southern limit of its contemporary natural range runs across southern Poland (Sałata-Piłacińska, 1990; van Appeldorn, 1999), and (3) the history of postglacial colonization of the species in this area is complex (Jancewicz et al., 2015).

GEOMORPHOLOGICAL CHARACTERISTICS OF THE STUDY AREA

The geologic history of eastern Poland has resulted in differences in the features and character of the land relief. From north to south, the following features clearly change: lake density, acreage and percentage of wetlands, the character of rivers, land surface gradients, and relief variations. Postglacial landscape zones showing different geomorphological and landscape features can be distinguished, consistent with the limits of the individual glaciations: Vistulian zone (V-Z), Wartanian zone (Wa-Z), Odranian zone (O-Z), and Sanian 1 and Sanian 2 zones (herein referred to as Sanian zone S-Z) (Marks, 2005) (Fig. 1, Table 1).

Vistulian/Weichselian zone (V-Z)

This is a young glacial area of the East Baltic Lake District (Kondracki, 2011). The "fresh landscape" (Marks, 2005) of this area is characterized by highly varied terrain relief, complicated geologic structure, and the presence of numerous lakes and boglands (Table 1). The surface relief of this area was ultimately formed during the Vistulian glaciation (115–11.7 ka BP; Marine Oxygen Isotope Stage [MIS] 2–5d) (e.g., Słowański, 1981; Szumański and Laskowski, 1993; Lisicki and Rychel, 2006; Marks et al., 2016). Young glacial relief landforms, such as strings of frontal moraine hills and consecutive glaciofluvial accumulation plains (e.g., Ber, 2006), document the consecutive deglaciation stages in the individual phases of this glaciation.

A characteristic feature of this zone is the occurrence of extensive and numerous small melt-out depressions. Their surface is occupied by lakes and peat plains that developed from lakes being overgrown. In this zone, there are also elongated subglacial tunnel valleys or meltwater valleys, narrow and deeply incised in the upland areas. Nowadays, there are lakes or wetlands in these tunnel valleys (Szumański and Laskowski, 1993; Lisicki and Rychel, 2006). They are bounded by steep slopes attaining a height of 20–70 m.
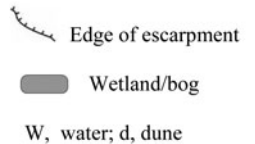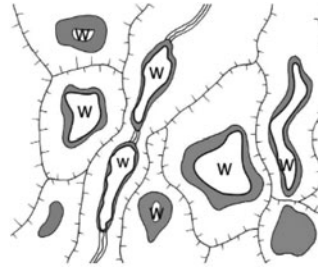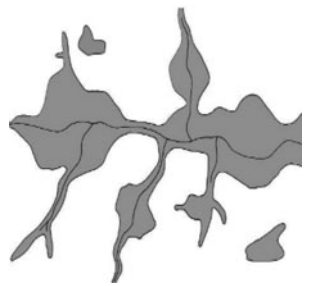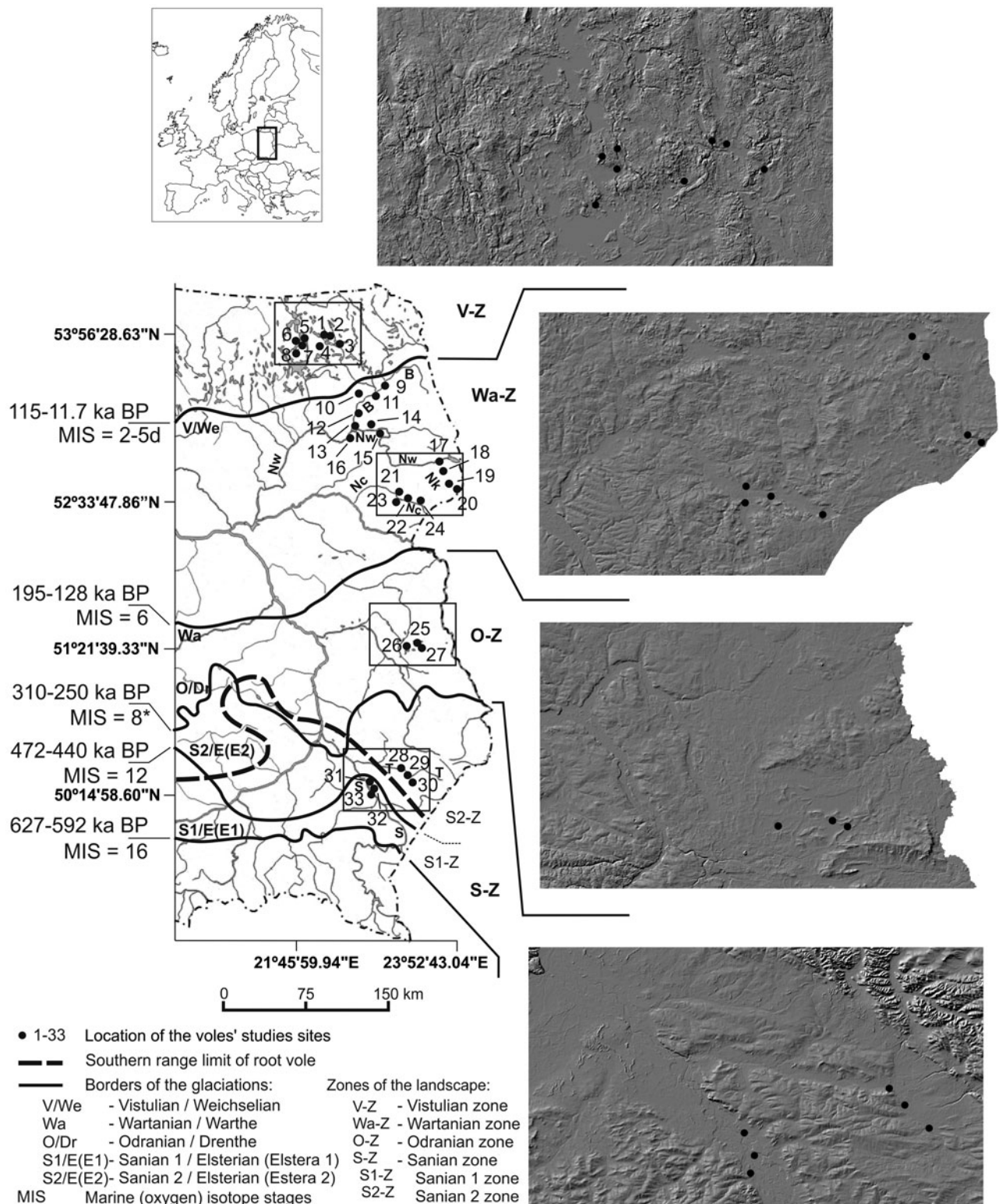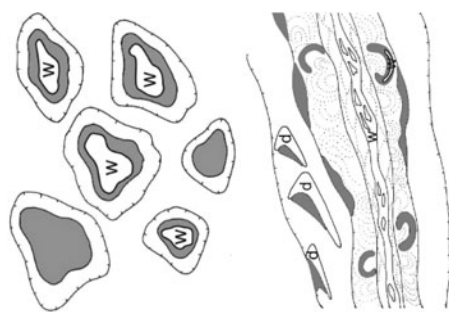
Figure 1. Location of the research sites in eastern Poland on the background of Pleistocene glaciations limits and the characteristics of surface relief shown on a digital elevation model. Limits of the main Pleistocene glaciations after Marks (2005, 2011). Southern range limit of the root vole in Poland after Sałata-Piłacińska (1990). B, Biebrza River; Nw, Narew River; Nk, Narewka River; Nc, Nurzec River; T, Tanew River; S, San River. *MIS 6 according to Marks et al. (2016)

Such landforms often exist as isolated elements of the landscape. The relief diversity of the East Baltic Lake District, which results from glacial morphogenesis, and the poor degree of denudation processes are the reasons for the presence of many habitats preferred by the root vole, which are potential sites of occurrence of this rodent species. These

Table 1. Geomorphological characteristics of the landscape zones analyzed. Data sources: [a]Mojski (2005), [b]Harasimiuk et al. (2002), [c]based on various authors, after Lindner (1987) and Mojski (2005), [d]Dembek et al. (2000), [e]Choiński (2007), [f]Majdanowski (1954). Drawn by T. Falkowski.

Zones of the landscape	Main landscape elements[a, b]	Main morphogenetic factors[a] Age of landscape zone[c]	Average land surface gradients (°)[b, d]	Total area of wetlands (km²)[d] % Wetlands in the macroregion area[d]	Average area of deposit bogs (ha)[d] % Peat bogs in the macroregion area[d]	% Share of wetlands in the area of Poland[d] % Nonpeat wetlands in the macroregion area[d]	Lake density (%)[e, f] Density of small lakes (N/100 km²)[e]	Schematic layout of habitats
Vistulian zone V-Z	Hills of terminal/frontal moraines and dead-ice moraines, hilly and undulated morainic uplands, kame, eskers, extensive melt-out depressions, numerous small isolated melt-out depressions without drainage, elongated subglacial tunnel valleys or meltwater valleys (contemporarily forming lakes or wetlands), glaciofluvial accumulation plains, lakes	Deglaciation of the Vistulian glaciation ice sheet 11.7 ka	11–12 (locally max. 20)	12,991 11.40	16.65 5.88	4.1 5.15	3.05 (Masurian Lake District) 79.6	
Wartanian zone Wa-Z	Extensive moraine uplands with gentle slopes, melt-out depression forming the currently vast boggy bottoms of river valleys and their side valleys (polygenic river valleys), boggy small melt-out depressions within uplands, extensive levels of glaciofluvial accumulation plains, ablation covers, strongly denuded moraine hills, dunes, kames, eskers, outwash plains	Deglaciation of the Wartanian glaciation ice sheet, denudation 128 ka	3.5	18,762 17.94	57.78 3.80	6.0 14.19	<0.1 5.5	

Schematic layout of habitats legend: Edge of escarpment; Wetland/bog; W, water; d, dune.

Zone	Landscape / landforms	Deglaciation / processes								
Odranian zone O-Z	Extensive flat moraine uplands with small morainic hills, outwash and lacustrine plains, peat plains and bogs, depressions of glacial and thermokarst origin occupied by lakes, denuded plains, dunes, eskers, kames	Deglaciation of the Odranian glaciation ice sheet, denudation 250 ka	2	2723 41.5	n. d. 11.29	0.9 30.22	3 n. d.
Sanian zone S-Z (Sanian 1 zone S1-Z & Sanian 2 zone S2-Z)	Undulated denuded morainic plains, denuded plateaus built of Miocene sediments covered by glaciofluvial plains or ground moraines, mature river valleys with river terraces and sandy floodplains, loess covers, dunes	Deglaciation of the Sanian 1,2 glaciation ice sheets, periglacial processes, denudation S1: 592 ka S2: 440 ka	8	8890 10.66	47.47 1.01	2.8 9.64	<0.1

are mainly lakeshores (still not overgrown by vegetation) or partially boggy and peaty melt-out depressions of various sizes. Many of these depressions represent isolated landscape patches. This area is subject to rapid evolution: lakes are being overgrown and turned into peat bogs. A measure of these changes is the decrease in lake density from 1.1% in 1954 to 0.9% in 1991 (Dembek et al., 2000; Choiński, 2007).

Wartanian/Warthe zone (Wa-Z)

This area developed mainly during the Wartanian glaciation and then was partly denuded during the Eemian interglacial and the Vistulian glaciation. It comprises the Podlasie-Belarus Uplands and the eastern part of the Middle Polish Lowland (Kondracki, 2011). In some publications, the Warta glaciation is termed the Odra glaciation stade (Marks et al., 2016). Despite the differences in the stratigraphic interpretation, this area is distinguished by a different landscape relief than other zones discussed here.

The area is characterized by a higher level of landscape maturity in relation to the northern zone: relief variations are smaller, and the geomorphological boundaries are gentler and less easily visible (Table 1, Wa-Z). Denudation processes are more advanced here than in the Vistulian zone. For this reason, the level of communication between humid landscape elements is higher. This area has developed natural habitats preferred by the vole. These are mainly peaty melt-out depressions, which were originally lakes, then included in the runoff system of surface waters at later stages of landscape development, and used by rivers as polygenetic sections of their valleys (Falkowska, 2001; Falkowski, 2003). For this reason, the valleys of the Biebrza (B), Narew (Nw), Narewka (Nk), and Nurzec (Nc) Rivers form a network of connected wetlands (Fig. 1, Table 1, Wa-Z). Melt-out landforms that are not included in the surface runoff system are now isolated boggy and peaty depressions. A feature that distinguishes this part of the area of Poland analyzed from the V-Z zone is the lack of lakes (Choiński, 2007) (Table 1).

In the area adjacent to melt-out depressions and river valleys of the glaciogenically modified glacial upland, there are also small depressions after minor dead-ice blocks. These are often filled with deluvial material deposited here mainly in periglacial conditions. Currently, peat deposition takes place within them. Such landforms are usually the routes of surface water runoff to rivers that appear periodically during precipitation events (Falkowska, 2009).

The relief of the area is enriched in landforms whose development was related to the mode of deglaciation. These are kames and eskers occurring within the melt-out areas and uplands, kame terraces on the upland slopes, extensive levels of glaciofluvial plains, and ablation covers, as well as strongly denuded moraine hills and dunes formed in periglacial conditions (Bałuk, 1991; Musiał, 1992; Falkowska, 2009; Marks et al., 2019).

Odranian/Drenthe zone (O-Z)

The relief of the Polish part of Polesie has been shaped mainly by glacial processes (deposition and glaciotectonics) during the Odranian glaciation (Fig. 1, O-Z) (Lindner and Marks, 1994; Marks, 2005) and subsequent periglacial processes, as well as denudation processes associated with warming periods of various stratigraphic ranks. For this reason, Polesie shows a higher level of landscape maturity in relation to both the northern zone and the boggy river valleys in the central zone (Fig. 1, Table 1) of the study area. The land surface relief is characterized by low diversity. Generally, this is a flat low-land area with relief variations reaching only a few meters and average gradients smaller than 1° (occasionally up to 3°). Larger hills are formed by outcrops of both older basement deposits and glacial forms: kames, eskers, moraine uplands, and single moraine hills. The most characteristic feature of this area is the presence of vast peat plains and bogs (as in Wa-Z) and numerous lakes (unlike in Wa-Z). The formation of these landforms was associated with glacial and thermo-karst processes (Wojtanowicz, 1994) that took place in conditions of the shallow occurrence of Cretaceous poorly permeable marls and limestones under the glacial deposits. The watershed zones between these depressions form strings of eskers and kames. Large areas in this zone are occupied by flat, sandy, glacial (outwash) plains (Harasimiuk et al., 2002).

South Polish glaciations zone, Sanian1/Elsterian (Elstera1), Sanian2/Elsterian (Elstera2) zones (S1-Z, S2-Z; herein referred to as Sanian zone S-Z)

The South Polish glaciation zone of the study area is located within the Sandomierz Valley (Kondracki, 2011) and is characterized by the highest level of landscape maturity (Fig. 1, Table 1, S-Z). The landscape shows signs of denudation here. The major features of the relief have developed because of the activity of the Sanian 1 and Sanian 2 ice sheets (Kwapisz, 1998; Wieczorek, 2006). The surface relief was also shaped by processes occurring in the periglacial zone during the later glaciations and by river erosion and accumulation processes that operated from the Mazovian interglacial to the present day (Starkel, 1988; Gębica, 2004). During this long period, denudation of the upland areas was accompanied by the filling of melt-out depressions and shaping of valleys. In many cases, signs of valley polygenesis, easily visible in the northern and central parts of the entire study area, have been completely obliterated in the terrain morphology here. Deposition of the largest loess succession and calm fluvial sedimentation, which led to the deposition of sandy alluvial sediments of considerable thickness, occurred in this area during the Vistulian glaciation (Kwapisz, 1998). Hydrogenic habitats, suitable for the root vole (Tast, 1966), are associated within the S-Z zone mainly with river valleys (these occur predominantly at oxbow lakes or in near-slope boggy areas) (Falkowska and Falkowski, 2015; Falkowska et al., 2016) and with deflation zones on Pleistocene terrace surfaces overlain by eolian sands. Their formation might have been related

to changes in climate humidity and a regional groundwater-level rise in the Holocene (e.g., in the Tanew River valley [T], Fig. 1) (Kwapisz, 1998). The moraine upland that occurs in the southern zone is characterized by a poorly diverse relief and the presence of various Pleistocene glaciofluvial land-forms and dunes. Its surface zone is composed of glacial deposits and loess (Kwapisz, 1998). There are a few boggy areas (Table 1, S-Z).

MATERIALS AND METHODS

Animal trapping

In the period of 2008–2010, intense trapping of root voles was carried out at 33 sites in eastern Poland in four zones located within the limits of the individual Pleistocene glaciations (Fig. 1). Environmental conditions favorable for the occurrence of this rodent species were selected as research sites, including *Carex* wetlands, local boggy areas, lake-shores, and meadows in river valleys. The number of selected sampling sites in each zone was adequate to the proportion of habitats appropriate for the vole. The geographic distance between the trapping sites was 3.5–405 km.

Animal trapping was conducted from June to September or October—that is, in the growing season and breeding season, when the density of herbivorous rodents (including root voles) is the highest (Gliwicz and Jancewicz, 2004, 2016). To catch animals, wooden single-capture live traps for rodents ($8 \times 9.5 \times 16.5$ cm) with bait and food (oat grains and pieces of juicy cucumber) were used. The traps were spaced every 10 m in lines (Jancewicz and Gliwicz, 2017) at the most likely sites of vole occurrence. They were checked once a day. At a given site (20 traps), 7–23 individuals of *M. oeconomus* were captured. From each newly captured *M. oeconomus* individual, tissues were collected for examination (from the ear, as recommended by the ethics committee), and the animal was released at the point of capture. Recaptured individuals and those of other species were released at the trapping site immediately. The tissue samples were preserved in 70% alcohol and kept at –20°C until the analysis started. A total of 549 samples were collected from various *M. oeconomus* individuals from 33 sites.

All procedures of capture of root voles and sampling tissues were in accordance with Polish law and were accepted by the Third Local Ethical Commission in Warsaw (permission no. 45/2007). Trapping site locations were approved by the administrators of the area. All animals were released at the point of capture.

Laboratory analysis and analysis of genetic data

DNA was extracted from tissue samples of 549 individuals of *M. oeconomus* with the Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland). For all individuals, 12 microsatellite loci were established according to the method described by Czajkowska et al. (2010). FSTAT (Goudet, 1995) was used to estimate the number of alleles per locus and allelic

size range. Observed (Ho) heterozygosity was calculated in ARLEQUIN 3.11 (Excoffier et al., 2005). Departures from Hardy-Weinberg equilibrium (HWE) were calculated using Markov chain Monte Carlo with 1 million steps and 100,000 dememorization steps (ARLEQUIN 3.11).

STRUCTURE 2.3.4 was used to infer population structure and assign individuals to subpopulations (clusters) (Pritchard et al., 2000; Falush et al., 2003). The number of genetic groups (K) was tested from 1 to 10. The results were summarized using the STRUCTURE HARVESTER v. 06.94 (Earl and Vonholdt, 2012). The same program was used to calculate the posterior probability of the data [LnP(D)] and delta K (ΔK) (Evanno et al., 2005).

Mitochondrial DNA cytochrome b (mtDNA cyt b), based on the 908 bp fragment of the gene, was established for 427 individuals using the method described by Jancewicz et al. (2015). The relationships between the mtDNA haplotypes (GenBank accession number KP684101-KP684121; Jancewicz et al., 2015) were illustrated by the minimum spanning network using ARLEQUIN 3.11.

ARLEQUIN 3.11 was also used to estimate the number of haplotypes (N_h), haplotype diversity (h), and nucleotide diversity (π). Genetic differentiation between populations was assessed as pairwise F_{st} for both markers, and statistically tested using this program (1000 permutations).

In GENALEX v. 6.0 (Peakall and Smouse, 2006), principal component analysis (PCA) was performed for microsatellite DNA based on F_{st} and mtDNA F_{st} data.

All genetic parameters were averaged for four the geomorphological zones analyzed. Genetic parameters/genetic data/genetic variability of *M. oeconomus*—the number of alleles per locus (N_a) and allelic size range, expected heterozygosity (H_e), number of haplotypes (N_h), haplotype diversity (h) and nucleotide diversity (π), genetic differentiation (F_{st}), and minimum spanning network of cytochrome b gene sequences—from eastern Poland were analyzed taking into account the zonal variability of the landscape, especially the zonal variability of the distribution and character of wetland habitats.

Statistical relations between geographic coordinates of the population and both the haplotype diversity (h) and nucleotide diversity (π) in these populations were determined by means of a regression curve in STATISTICA 10 (StatSoft Inc., 2012).

RESULTS

Genetic variability within geomorphological zones

Microsatellite DNA

Average allelic richness for different landscape zones and average allelic size range and average heterogeneity were determined. The lowest values for all parameters calculated were found in the Sanian zone (S-Z), and the highest values in the Wartanian zone (Wa-Z) (Table 2). The average values of the parameters calculated for a single population in the Wartanian zone (Wa-Z) and the Vistulian zone (V-Z) were always significantly higher, and the average values for the Sanian zone (S-Z) were always significantly lower than the average for the whole study area. In the case of the Odranian zone (O-Z), only the average allelic size range was higher than the average value for the whole study area. The remaining parameters did not exceed the average values for the whole study area. All populations were in HWE.

According to Wright's scale (Hartl and Clark, 1997), the average pairwise F_{st} between the populations in the landscape zones for nuclear DNA was low in the Vistulian zone (V-Z) and moderate in the remaining landscape zones. The average F_{st} values increase across zones from north to south (Table 2).

Because of reaching a constant value by LnP(D) at K = 5, STRUCTURE analysis was carried out for divisions up to K = 5 (Fig. 2). ΔK showed the highest support for K = 3. For this value of K, STRUCTURE identified 3 subpopulations: (1) Vistulian zone, (2) Wartanian and Odranian zone, and (3) Sanian zone. The Odranian zone was distinctly separated only for K = 5. Division at K = 2 was also very clear and indicated clusters: the Sanian zone and the rest of the populations analyzed. In mtDNA, these two clusters correspond to the central European phylogroup (CE) and South Polish (CE-PL S) group described by Jancewicz et al. (2015) (Table 3). For K from 2 to 4, the Odranian zone is only slightly marked, and that shows that it may be a mixing zone for voles from the Sanian and Wartanian zones.

mtDNA cytochrome b

The haplotype diversity h and nucleotide diversity π were highly variable within the zones. The highest values of both parameters were recorded in the Wartanian zone (Wa-Z, Table 3, Fig. 3). In the Sanian zone (S-Z), determination of F_{st} was impossible because of the fixation of one haplotype (for more information, see Jancewicz et al., 2015). We found correlations (P < 0.0001) between the latitude and the values of both parameters (h and π) here. A correlation between these values and the longitude, and an increase along the west-to-east axis (P < 0.001), were found only for the nucleotide diversity (Fig. 3, right side).

The average pairwise F_{st} between the populations within the landscape zones was high in the Wartanian zone (Wa-Z) and very high in the Vistulian zone (V-Z) and the Odranian zone (O-Z) (Table 3). The average F_{st} was greater than the average value for the whole study area only in the Odranian zone.

The glacial landscape zones differed in the number of recorded haplotypes: from 1 in the Sanian zone (S-Z) to 17 in the Wartanian zone (Wa-Z) (with an average of 1 to 4.2 for each locality, and an overall average of 2.9, as reduced to a single locality) (Table 3). In the Wartanian zone (Wa-Z) and Odranian zone (O-Z), both haplotypes were recorded, which belong to the central European clade CE (Table 3, Fig. 4). The haplotypes of these groups show different distribution patterns. In the most southerly Sanian zone (S-Z), there is only the dominant haplotype from the South Polish group CE-PL S. Haplotypes of this group are absent

Table 2. Genetic variability parameters for the root vole (*Microtus oeconomus*) within the geomorphological zones analyzed, genotyped at 12 microsatellite loci (division into zones and places of research on the Fig. 1). Because the populations were in Hardy-Weinberg equilibrium (HWE), only H_e values have been given. F_{st}, genetic differentiation value for zones; H_e, expected heterozygosity; N_a, number of different alleles per locus; N_{ind}, number of individuals analyzed. F_{st} according to the Wright scale: 0.000–0.05, little differentiation; 0.05–0.15, moderate differentiation; 0.15–0.25, great differentiation; >0.25, very great differentiation (Hartl and Clark, 1997).

Zones of the landscape (locations in Fig. 1)	N_{ind}	N_a	Allelic size range	H_e	F_{st}
Vistulian zone	163	8.021	23.312	0.811	0.049
V-Z (1–8)		(5–14)	(10–51)		(0.021–0.075)
Wartanian zone	268	8.599	24.703	0.838	0.053
Wa-Z (9–24)		(5–16)	(13–59)		(0.005–0.099)
Odranian zone	39	6.361	23.889	0.767	0.093
O-Z (25–27)		(2–11)	(2–51)		(0.050–0.117)
Sanian zone	79	4.819	14.889	0.687	0.105
S-Z (28–33)		(2–8)	(2–36)		(0.026–0.163)
All study areas	549	7.568	22.508	0.798	0.099
		(2–16)	(2–51)		(0.005–0.163)

from the peripheral, most northerly Vistulian zone (V-Z). In the remaining landscape zones—the Wartanian zone (Wa-Z) and the Odranian zone (O-Z)—haplotypes from both phylogenetic groups are recorded (Table 3). Out of 21 haplotypes described (for details, see Jancewicz et al., 2015), only 5 occur in at least two distinguished landscape zones: 4 haplotypes were found in two zones, and only 1 haplotype in three zones (Fig. 4). The distribution of the remaining 16 haplotypes, commonly of low and very low frequency, is limited to a single landscape zone. Widely distributed haplotypes were usually found in landscape zones neighboring each other. Only haplotype PL-9 (Fig. 4), dominant in the South Polish group CE-PL S, had a different distribution pattern: its presence was recorded in the Wartanian zone (Wa-Z) and the Sanian zone (S-Z), but it was absent from the intervening Odranian zone (O-Z).

Genetic differentiation between the geomorphological zones

The average pairwise F_{st} between the zones was very high (Wright scale) in all mtDNA pairwise comparisons (Table 4, above the diagonal). The highest values were between the Sanian zone (S-Z) and the remaining zones. For microsatellite DNA, the pairwise F_{st} was low between the neighboring Vistulian zone (V-Z) and the Wartanian zone (Wa-Z), and large between the Sanian zone (S-Z) and the Wartanian zone (Wa-Z) and between the Sanian zone (S-Z) and the Odranian zone (O-Z). In the remaining three comparisons, the pairwise F_{st} was moderate (Table 4, below the diagonal).

PCA performed on the F_{st} values reflects the subdivision into the four postglacial landscape zones but with overlap between some populations, particularly those from the Wartanian and Odranian zones (Fig. 5). This subdivision is more clearly pronounced for the nuclear marker (Fig. 5, upper image), although one population from the Odranian zone (O-Z) shows similarity to the population from the Wartanian zone (Wa-Z). The PCA based on F_{st} values for mtDNA

is slightly different: the zones are clearly distinct, but the populations of the Odranian zone are contained within the Wartanian zone. In addition, two populations from the Vistulian zone were grouped with populations from the neighboring Wartanian zone, and one population from the Wartanian zone was grouped within the Sanian zone (Fig. 5, lower graph).

DISCUSSION

The linking of geologic processes with the occurrence of species, unequivocally postulated relatively recently by Conroy et al. (2016) and Gottscho (2016), seems to be necessary to explain genetic variability in populations of contemporary animal species. The results presented in our work suggest that—apart from tectonic and erosional processes—Pleistocene glacial processes can also affect the genetic differentiation of a population. Most phylogeographic studies make general reference to the effects of glaciations and do not consider geomorphology in any detail (e.g., Brunhoff et al., 2003; Haring et al., 2011; Hulejová Sládkovičová et al., 2018). The results of our research indicate a relationship between the genetic structure of *M. oeconomus* and the diversity of landscape relief, geologic structures, and geologic history of the glacial area. Genetic differences between populations inhabiting different glacial zones are visible despite the fact that the voles studied are included by mtDNA into a single phylogenetic group—the central European phylogroup CE (Brunhoff et al., 2003; Jancewicz et al., 2015), in which the voles from the southern zone differentiate as a separate branch, CE-PL S, that survived the last glaciation in the local northern latitude refugium situated in Poland (Jancewicz et al., 2015). The separate origin of voles from the southern part of Poland living close to the range of occurrence of the species is also supported by results of the microsatellite analysis that include determination of basic genetic variability parameters: number of alleles per locus, allelic size range, expected heterozygosity (Table 2), PCA grouping analysis
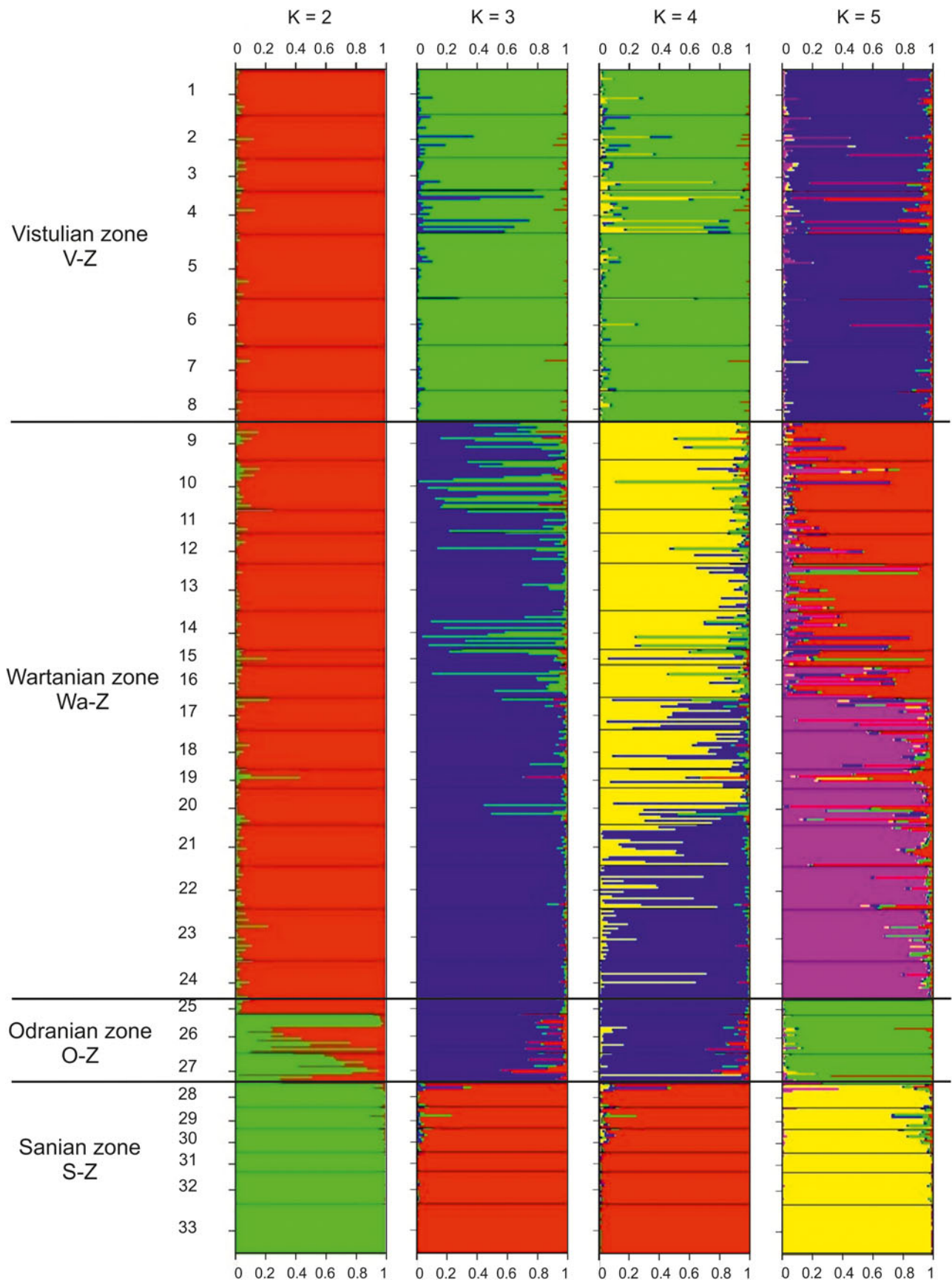
Figure 2. (color online) Results of STRUCTURE analysis of root vole data based on 12 microsatellite loci assuming K = 2 to 5 genetic clusters. Vertical lines separate voles from different geomorphological zones.

Table 3. Genetic variability parameters of mitochondrial DNA cytochrome b of the root vole (*Microtus oeconomus*) within the geomorphological zones analyzed (see Fig. 1). CE, central European haplotype group; CE-PL S, South Polish group–distinct haplotype group within the CE phylogroup (see Fig. 4); F_{st}, genetic differentiation value for zones; h, haplotype diversity; N_h, number of haplotypes within the zones; n_h, number of haplotypes at a single study site; N_{ind}, number of individuals analyzed; π, nucleotide diversity. Scale for F_{st} as in Table 2.

| Zones of the landscape	N_{ind}	N_h All	N_h Without CE-PL S group	N_h Only CE-PL S group	n_h	h	π	F_{st}
Vistulian zone V-Z	109	6	6	0	2.2 (1–4)	0.376 (0–0.636)	0.118 (0–0.310)	0.350 (−0.014–0.793)
Wartanian zone Wa-Z	211	17	13	4	4.2 (1–7)	0.647 (0–0.872)	0.389 (0–0.620)	0.238 (−0.001–0.904)
Odranian zone O-Z	33	3	1	2	2.0 (1–3)	0.221 (0–0.530)	0.189 (0–0.420)	0.529 (0.125–0.904)
Sanian zone S-Z	74	1	0	1	1.0 (1)	NA	NA	NA
All study areas	427	21	17	4	2.9 (1–7)	0.425 (0–0.872)	0.234 (0–0.620)	0.456 (−0.001–1.000)]

(Fig. 5, upper figure), and clustering for two clusters (for K = 2) in STRUCTURE (Fig. 2).

There are many studies showing the influence of contemporary environmental and landscape fragmentation and the impact of various types (geographic, topographic, geologic, environmental, and climatic) of barriers on the genetic structure of plant and animal populations (e.g., Gerlach and Musolf, 2000; Ratkiewicz and Borkowska, 2006; Banaszek et al., 2011; Huck et al., 2012; Smissen et al., 2013; Fietz et al., 2014; Czarnomska et al., 2018). However, there have
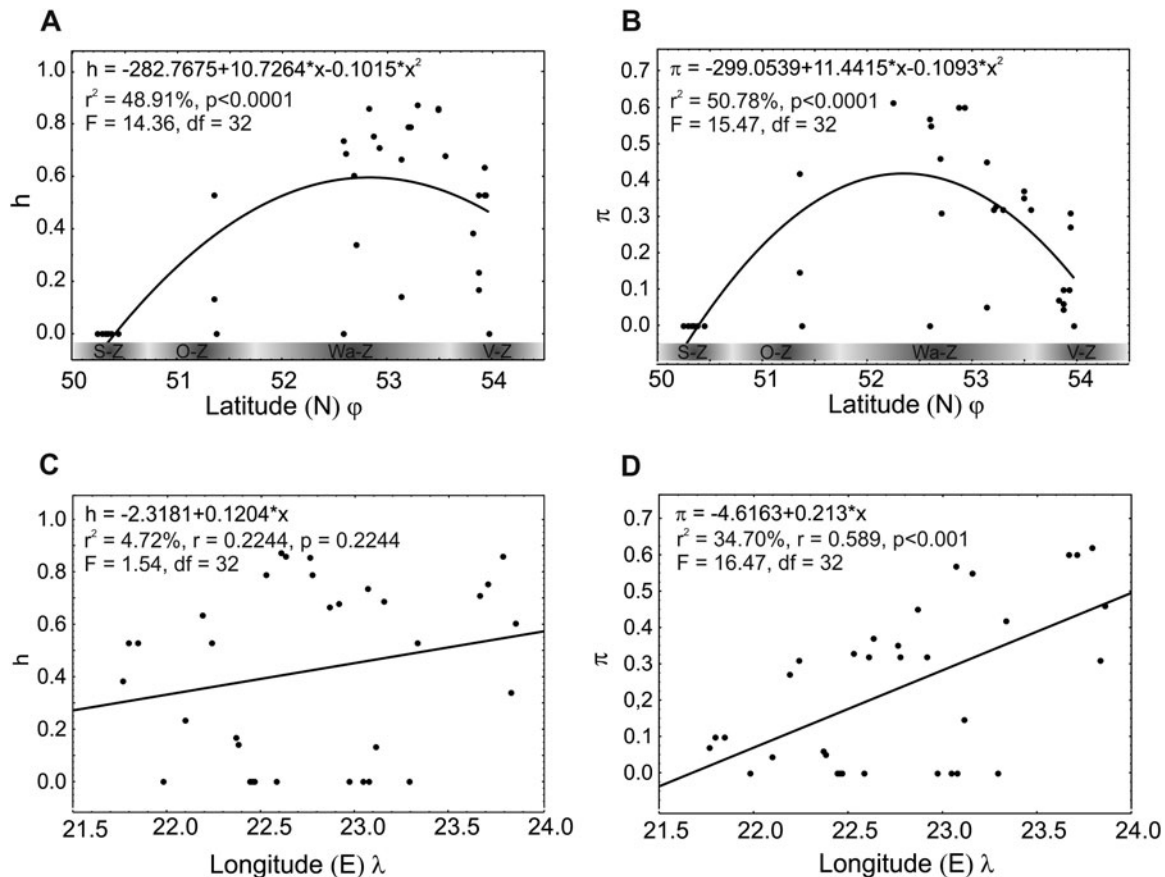

Figure 3. The relationship between haplotype diversity (h; A, C) and nucleotide diversity (π; B, D) for mitochondrial DNA of the root vole (*Microtus oeconomus*) and the geographic location of 33 research sites (λ, latitude [A, B]; φ, longitude [C, D]). O-Z, Odranian zone; S-Z, Sanian zone; V-Z, Vistulian zone; Wa-Z, Wartanian zone.
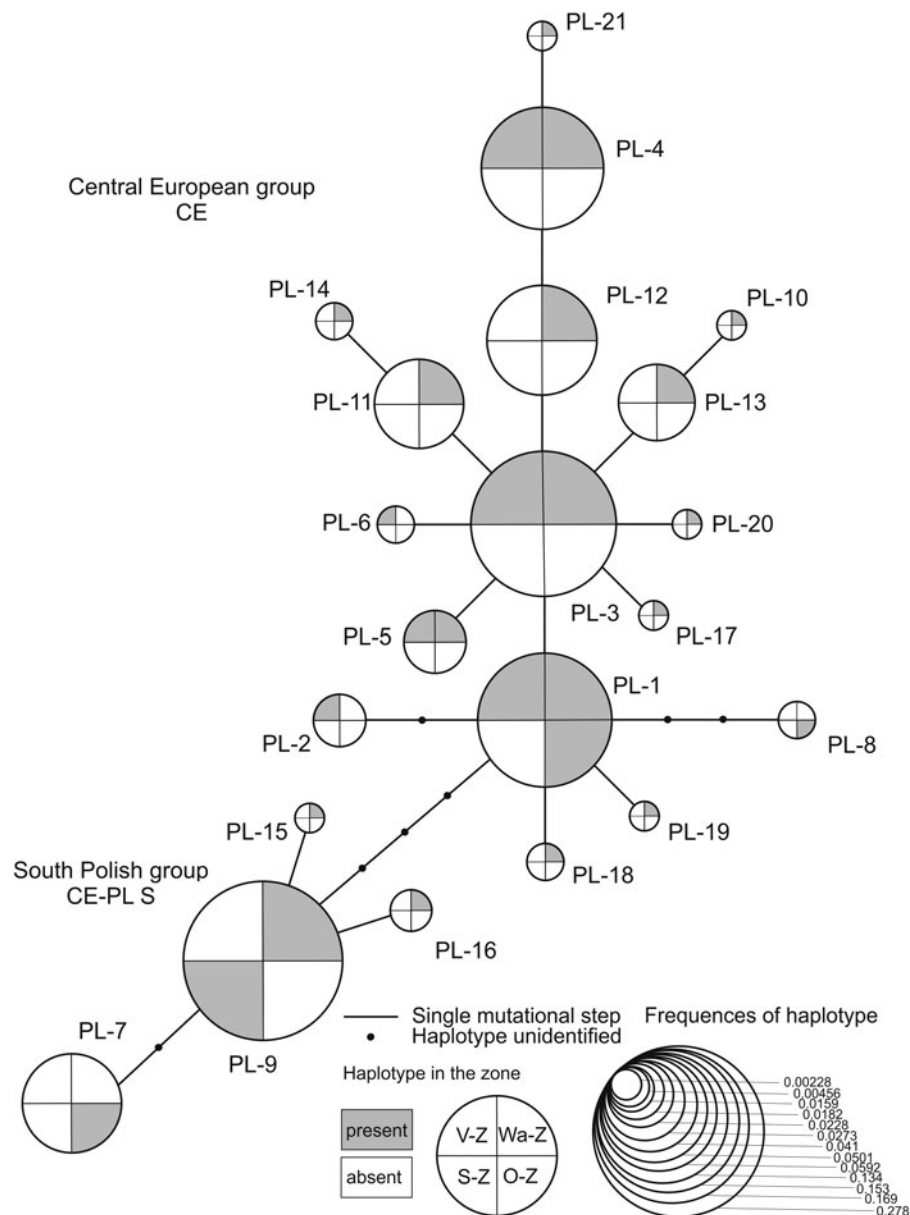
Figure 4. Minimum spanning network of 21 cytochrome b gene sequences of the root vole (*Microtus oeconomus*) in eastern Poland. Phylogenetic groups (central European haplotype group [CE] and South Polish group–distinct haplotype group within the CE phylogroup [CE-PL S]) after Jancewicz et al. (2015). The size of the circles corresponds with the frequency of haplotypes in the entire sample of 427 individuals analyzed. The presence of haplotypes in the geomorphological zones analyzed (Fig. 1) is marked. O-Z, Odranian zone; S-Z, Sanian zone; V-Z, Vistulian zone; Wa-Z, Wartanian zone.).

Table 4. Pairwise genetic differentiation F_{st} (average ± standard deviation) for voles computed between geomorphological zones: below the diagonal, for microsatellite markers; above the diagonal, for mitochondrial DNA (mtDNA). F_{st} according to the Wright's scale as in Table 2.

	Vistulian zone V-Z	Wartanian zone Wa-Z	Odranian zone O-Z	Sanian zone S-Z	
V-Z	–	0.456 ± 0.195	0.647 ± 0.196	0.806 ± 0.109	
Wa-Z	0.077 ± 0.017	–	0.493 ± 0.197	0.507 ± 0.228	mtDNA
O-Z	0.113 ± 0.026	0.096 ± 0.029	–	0.885 ± 0.118	
S-Z	0.166 ± 0.036	0.152 ± 0.038	0.162 ± 0.061	–	
	Microsatellite DNA				
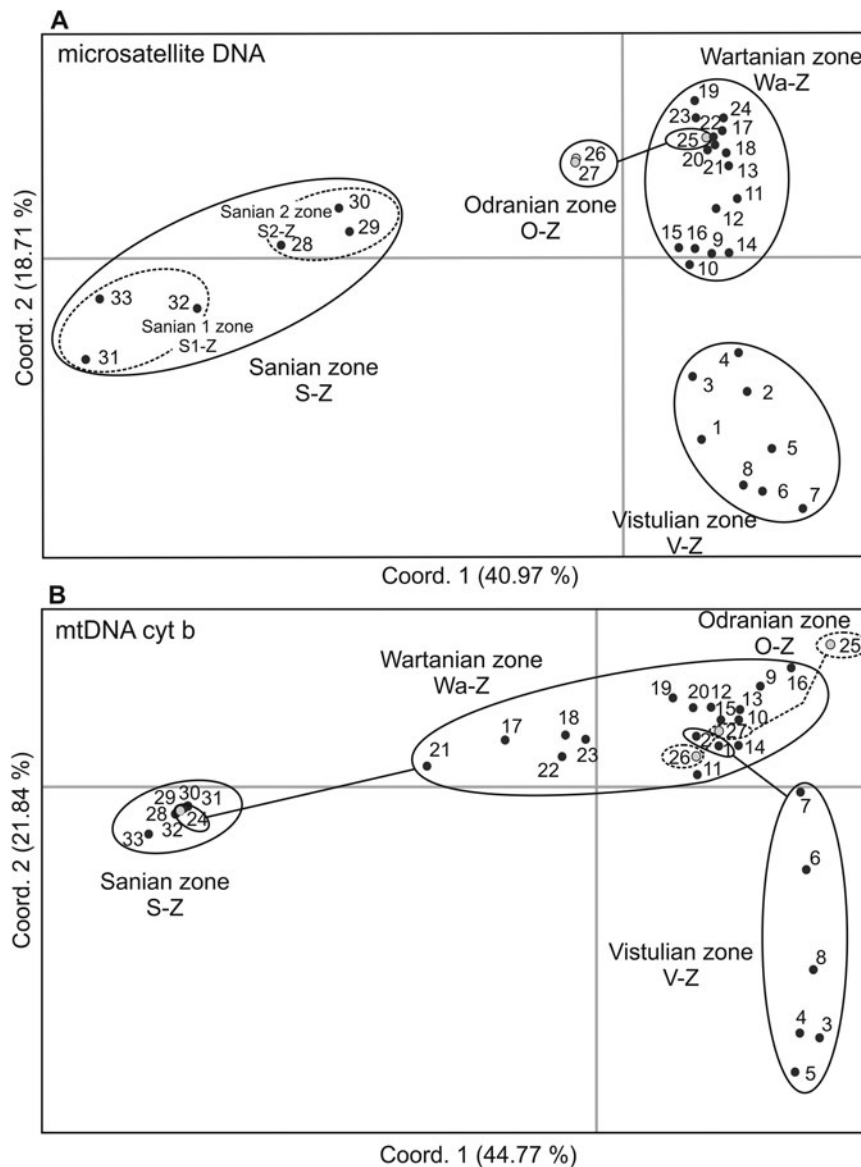
Figure 5. Principal component analysis of the root vole (*Microtus oeconomus*) representing the geomorphological zones analyzed for F_{st} for microsatellite DNA (A) and mitochondrial DNA cytochrome b (mtDNA cyt b; B).

been no studies so far that would focus on explaining the role of postglacial landscape differentiation on a larger temporal and spatial scale. The research presented here discusses this problem based on a very large sample (549 individuals) and a large amount of data collected from a morphologically diverse area, shaped over several Pleistocene glaciations and intervening warming periods.

The study area has been shaped by geologic processes occurring during these glaciations and by periglacial and interglacial processes (Lindner and Marks, 1994, 1999; Marks et al., 2019). The clear subdivision of the area of eastern Poland into four zones, consistent with the extents of consecutive glaciations (Fig. 1, Table 1), allowed testing the genetic-landscape relations in differently developed environments. The impact of modern climate, which is very important in biogeographic research, was less significant here,

because the study area lies within one climate zone—the temperate climate zone. The diversity of climatic conditions is not high enough to affect the contemporary distribution of the boreal root vole. In terms of climatic conditions, an exception is the Polesie region (within the Odranian zone), in which a slight influence of continental climate is observed (Kaszewski, 2002).

The PCA (Fig. 5) enabled grouping the populations based on F_{st} between the populations and divided them into groups largely according to the division of the landscape into zones. This division is very clearly visible in analyses of microsatellite DNA (Fig. 5, upper graph), which is a marker inherited from both parents and reflects the spatial distribution of the voles. However, this distribution is not so clearly visible in mtDNA, which is inherited only in the maternal line (Fig. 4, lower graph). In PCA grouping of mtDNA, the lack

of a clear separation of populations of the Odranian zone from the Wartanian zone may result from the fact that the landscapes of these zones could have been shaped in a similar period and under similar conditions. The stratigraphic position of these zones is not unambiguous because for some authors (Marks et al., 2016) the Wartanian glaciation is considered to be the stade of the Odranian glaciation (MIS 6). This pattern and the way of mtDNA grouping in PCA can be partly explained by the haplotype network and by the presence or absence of these haplotypes in populations within the zones analyzed (Fig. 4). Despite such a result of haplogrouping of mtDNA in PCA, the pairwise comparison of the zones shows very large differences in F_{st} (Table 4), which suggests limited flow of haplotypes between the zones. This is also visible in the haplotypes network (Fig. 4), in which the occurrence of haplotypes in different landscape zones is additionally marked.

STRUCTURE (Bayesian clustering) shows very similar results. In this analysis, the highest fit (probability) was found for division of the populations into three clusters in accordance with the Vistulian, Wartanian and Odranian, and Sanian zones (Fig. 2). However, populations from the Odranian zone form a separate cluster for K = 5 (Fig. 2). This could be caused by the presence of a mixed zone there. In addition, the distinctness of the Odranian zone is visible in parameters from Table 2. The number of alleles per locus and allelic size range for voles from this zone reveal a very wide range of values (minimum to maximum): from low values characteristic for the Sanian zone to very high ones that were found in the Vistulian zone.

As we expected, the vole finds its best conditions for living in the area of vast boggy valleys of the Biebrza, Narew, Narewka, and Nurzec Rivers (Fig. 1) in the Wartanian zone. High genetic diversity and usually low F_{st} values between populations in this area indicate free mating of large numbers of individuals and free flow of genes within a zone. The Wartanian zone landscape (Wa-Z, Table 1) began to be shaped earlier than the Vistulian zone landscape (V-Z, Table 1); it developed during the Odranian and Wartanian glaciations and then was modified by denudation processes in the Eemian interglacial and the Vistula glaciation. These processes resulted in the "levelling" of the terrain relief. The lakes that remained in melt-out depressions were later partly inundated with sediments from interglacial rivers and then overgrown, forming extensive peat bogs. Most of these depressions have later become river valleys (Falkowski, 1975; Falkowska, 2001, 2009). On the other hand, the hills were largely denuded, which has limited the number of natural barriers for the migration of individuals of this small rodent. The landscape of this zone favors the presence of moisture-loving species, a free spread of individuals, stability of spatial relations between them, and free mixing of genes. In the case of the root vole, this is indicated mainly by the highest number of alleles per locus, the range of allelic size range, and high heterozygosity (Table 2).

The nature and distribution of habitats (including extensive boggy river valleys) in the landscape of this zone translate into the continuity of habitats optimal for voles and thus promote the free spread of individuals, stability of spatial relations between them, and free mixing of genes between populations. The lack of permanent isolation in the landscape is the result of its maturity level. This type of environment favors free migration of the root vole, although this species has very low dispersal capability (Steen, 1994). Relations between haplotypes, visible in the haplotype network, and the distribution of haplotypes closely related to each other within the zones (Fig. 4) reflect the sedentariness of females, characteristic of this species (e.g., Gliwicz and Jancewicz, 2004; Pilot el al., 2010). In stable and heterogeneous environments, which are suitable for this rodent, daughters settle near mothers and other closely related females, which causes an increase in the number of haplotypes (because of fixed mutations and a relatively large number of individuals) in a small space. This is more possible in the extensive favorable environments of the Wartanian zone (Wa-Z), resulting in an increase in the gene diversity. This population parameter, seemingly insignificant to landscape researchers, is important for understanding both the mechanism of functioning of the population and the effects of environmental changes for the population in the past and in the future.

Because of this, genetic diversity in stable and rich environments of the Wartanian zone (Wa-Z), expressed in the diversity of haplotypes, nucleotides, and alleles, is high (Tables 2 and 3). It is higher than in the other zones where other types of landscape predominate, and the environments have different features. It can be assumed that this diversity will continue to be high, even if there were periodic decreases in the population size, or rapid reductions, in the past (Pilot et al., 2010), because only rich environments guarantee the appropriate level of genetic differentiation. Similarly high allelic diversity was observed in other single populations of root vole within the Wartanian zone—in the Narewka valley (Pilot et al., 2010; Hulejová Sládkovičová et al., 2018) and the Biebrza valley (Czajkowska et al., 2010).

Because of a different geologic history of the Odranian zone and the impermeable basement (Cretaceous marls) to the glacial deposits, bogs and lakes occupy a large part of the area (O-Z, Table 1). High lake density makes this area similar to the northern Vistulian zone (V-Z), but because of the extensive areas of bogs and much lower relief gradients than in the north, this area is similar to the Wartanian zone (Wa-Z) in terms of landscape features. The Odranian zone (O-Z) was shaped in a specific way (thermokarst process) during the Odranian glaciation, on the basement composed of marls (Harasimiuk et al., 2002). This difference can also be seen in the mtDNA genetic structure of the local root vole population. No PL-9 haplotype from the separate South Polish branch of the central European clade, CE-PL S, was found here (Jancewicz et al., 2015), which is dominant in eastern Poland (Fig. 4) and fixed in the immediately neighboring Sanian zone (S-Z). However, the presence of two other haplotypes, which have not been found in the other zones, was recorded only in the O-Z: haplotype PL-7, directly originating from PL-9, and PL-8, a rare (present in this area only)

haplotype from the central European group (Fig. 4). Perhaps the PL-9 haplotype, although most numerous among the 21 haplotypes found in this area, has not been revealed during this study, yet still exists here. A similar situation was previously observed in the root vole population studied for many years in eastern Poland (Dąbrowski et al., 2013). In spite of some geologic differences of the Polesie region, spatial analysis of molecular variance (SAMOVA) of mtDNA includes the root vole population from this area to the common group of populations from the Vistulian, Wartanian, and Odranian zones (Jancewicz et al., 2015). To some extent, this is also suggested by the mtDNA PCA analysis (Fig. 5, lower graph). However, based on analyses of microsatellite DNA transmitted to progeny by mothers and fathers, we can distinguish the vole populations from the Odranian zone as a separate population group (Fig. 5, upper graph). This population was also distinguished in STRUCTURE analysis for K = 5 (Fig. 2).

In the most northerly and southerly zones (i.e., in the Vistulian zone [V-Z] and the Sanian zone [S-Z]), moist habitats are scattered and the fragmentation is more pronounced. However, the lack of habitat continuity differs in character between both these zones. In the oldest landscape of the southern zone, the habitat discontinuity is ordered and linear (S-Z, Fig. 1, Table 1). Moist habitats are concentrated along river valleys: in oxbow lakes and near-scarp boggy areas, and in deflation depressions on upper river terraces overlain by eolian sands. In the northerly located Vistulian zone, the terrain relief gives an image of random and chaotic fragmentation, which results from the young age of the landscape and the short period of its evolution. Here, in topographic lows, moist places are more frequent, but their mutual arrangement seems to be relatively disordered (V-Z, Fig. 1, Table 1). However, a system of connections exists between the moist terrain depressions that can be convenient migration routes despite considerable relief variations and gradients. The evolution of this young glacial area, already observed nowadays, consists of denudation of uplands, shallowing of lakes and their transformation into bogs (a change of lake density by 0.2% over 37 yr; Dembek et al., 2000; Choiński, 2007), and their incorporation into the surface outflow system. This will increase both the area occupied by hydrogenic habitats and the communication between them. It can be expected that these phenomena will increase the genetic diversity of the root vole, which has already been observed to some extent (six haplotypes and heterozygosity of 0.8021; Tables 2 and 3). If this is the direction of its evolution, it is possible that, in the future, the system of habitats in the Vistulian zone will be similar to the system observed currently in the Wartanian zone.

The present-day differences in the terrain relief and the system of habitats in the Vistulian zone (V-Z) and the Sanian zone (S-Z) give both peripheral zones different characters in terms of root vole requirements. The southern zone of the landscape studied (S-Z) is special also because it is currently inhabited by voles that probably originate from a small northern glacial refugium located in the territory of Poland (Jancewicz et al., 2015). This different genesis, previously described/postulated on the basis of analysis of mtDNA (inherited only in the maternal line), is supported by the results of the analysis of the other genetic marker provided herein—the nuclear (microsatellite) DNA. The allelic size range, which is clearly lower than in other zones (Table 2), indicates the different origin of voles currently inhabiting the zone located close to the limit of its present-day occurrence (Sałata-Piłacińska, 1990; Linzey et al., 2016; see also Fig. 1). In addition, this thesis is supported by the high pairwise F_{st} between the S-Z and O-Z and between the S-Z and Wa-Z zones for microsatellite DNA (Table 4), clearly higher than between the S-Z and V-Z.

The voles studied from the south of Poland (Fig. 1, localities 28–33) do not show mtDNA genetic variability (Table 3). All individuals have descended from a common (pra)female. The loss of genetic variability by the fixation of one haplotype indicates a strong reduction in abundance in the past, which is undoubtedly because of the limited availability of suitable habitats and the lack of, or difficult, communication between them in the Pleistocene. This may be because of the origin of the local refugium (Jancewicz et al., 2015). Paleozoological research provided evidence for the presence of rodents in southern Poland during the Vistulian. On the Kraków-Częstochowa Upland, in the Holy Cross Mountains and in the Carpathians, fossil remains of root voles, common voles, field voles, bank voles, and other mammals were found (e.g., Madeyska, 1981; Nadachowski, 1989; Nadachowski et al., 1993; Sommer and Nadachowski, 2006; Żarski et al., 2017). In small and isolated populations, rapid decreases in abundance are dangerous, because these populations quickly lose their genetic variability because of the fixation of alleles (Leijs et al., 1999; van de Zande, 2000; Báldi et al., 2001). Heterozygosity in the Sanian zone (S-Z) is relatively low (0.687, Table 2) and is comparable to that found in the endangered *M. oeconomus* populations in isolated and fragmented environments of the Netherlands and Hungary (van de Zande et al., 2000; Hulejová Sládkovičová et al., 2018). The contemporary Sanian zone environment, although severe in terms of the existence of moisture-loving species, provides the possibility of migration and exchange of genes between the vole populations. This is reflected in the genetic differentiation of nuclear DNA inherited from both parents.

In the Sanian zone (S-Z), hygrophilous species have a limited number of places to settle. This "dilution" of the distribution, as we move from the center of the species range to its borders, is a universal phenomenon related to the decreasing number of habitable environments (Brown, 1984). From the point of view of *M. oeconomus*, the mature river valleys in a highly denuded landscape do not provide enough places and space to live, and the limited number of potential places to colonize increases the isolation between populations. It also impedes the migration of individuals, thus restricting the free mixing of genes. This phenomenon overlaps with the universal model of distribution and functioning of species at the range margins (Hampe and Petit, 2005), because the

range limit of *M. oeconomus* runs across this zone. It seems, therefore, that the lack of mtDNA variability in the Sanian zone (Table 3) and relatively low heterozygosity (Table 2) are a result of several factors—the lack of appropriate habitats and a strong reduction in abundance in the past, which may also result from the lack of habitats in a highly mature and transformed landscape. The result is probably the current course of the range limit of this boreal species. Paleozoological data show a completely different (wider) range of the root vole in Europe (Madeyska, 1981; Nadachowski, 1989; Kowalski, 2001; Falkowska, E., Jancewicz, E., unpublished manuscript), which was undoubtedly related to the distribution of favorable habitats.

CONCLUSIONS

The landscape of eastern Poland, shaped during consecutive Pleistocene glaciations, forms a specific system of hydrogenic habitats in a zonal pattern. This geographic patterning is reflected in the genetic structure of the moisture-loving root vole.

The genetic structure of the root vole shows that the best living conditions for the root vole persist in the extensive habitats of the Wartanian zone with a high degree of connectivity—in boggy river valley systems. Such conditions promote free migration, random mixing of genes, and increase of genetic diversity. The lowest genetic diversity indicates that the most severe conditions for the vole occur in the Sanian zone, which offers a completely different pattern of habitats preferred by the vole. Its original landscape developed during the oldest glaciations. Changes in both the nature and connectivity of environments, resulting from the geologic evolution of postglacial areas, may be the beginning of a change in species ranges. This is supported by the results of molecular research.

The relationships found as a result of the studies may become to some extent an indicator useful in explaining the geologic history of the postglacial region. This issue requires further analysis concerning other species with different habitat preferences. Thus, it is appropriate to combine geologic knowledge with biological data in order to understand fully the biogeographic history and contemporary distribution of a species in postglacial areas.

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