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ORIGINAL PAPER

What Can an Invasive Species Tell Us about Evolution? A Study of Dental Variation in Disjunctive Populations of *Microtus rossiaemeridionalis* (Arvicolinae, Rodentia)

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Abstract The sibling vole, *Microtus rossiaemeridionalis*, is a North Eurasian rodent that undergoes range expansion via casual introductions by humans. The documented cases of human-mediated spread of *M. rossiaemeridionalis* provide an opportunity to explore phenotypic consequences associated with the invasion events. We present an analysis of dental variability in two recently discovered invasive populations of *M. rossiaemeridionalis* in northern Asia (Surgut and Khabarovsk) and summarize the data on conspecifics within and outside the core range in order to uncover common and specific patterns of dental variation in the disjunctive invasive populations, and to consider the potential evolutionary significance of the phenotypic effects from neontological and paleontological standpoints. The analysis of morphotype dental patterns and inspection for rare traits suggest that the existence of invasive populations under conditions of isolation leads to a release of hidden phenotypic variation, which could be inferred from sharp increases in frequencies of reserve morphotypes and/or rare dental traits, and also from the presence of abnormalities. An atavistic anomaly of the third upper molars revealed in one individual in a fragmented habitat in Surgut recapitulates some features of the prismatic arrangement and occlusal pattern of extinct *Mimomys*-like arvicolines. All variants of released phenotypic variation in the invasive populations of *M. rossiaemeridionalis* under conditions of isolation could be interpreted as de-specialization of dentition. Such de-specialization appears to be maladaptive for a herbivore, though it might favor a transition to a more generalized diet and enhance the success during the transport, colonization, and establishment stages of the invasion.

Keywords Arvicolinae · Dental variation · Phenotype · Biological invasions · Evolution of specialization

Introduction

Evolutionary origins and potential of invasive populations are open research problems at the intersection of evolutionary biology, genetics, ecology, and conservation (Lee and Gelembiuk 2008; Pascal et al. 2010; Handley et al. 2011; Bock et al. 2015). Being considered as 'natural experiments' that can improve our understanding of contemporary evolution (Colautti and Lau 2015), biological invasions provide evidence for rapid evolutionary change during relatively short periods of time (a review in Whitney and Gabler 2008), although evidence for evolutionary stasis is rather scarce (Miehls et al. 2015). The analysis of the reviews on the evolutionary significance of phenotypic change in invasive populations shows that there are few, if any, cases when evolutionary change in the invasive populations is compared to the data on direct or collateral ancestral forms known for the species under study. Comparison of an extent of phenotypic variation in an invasive living species to amounts of evolutionary change in a phyletic lineage or clade, to which an invader belongs, is only possible when the fossil

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record is complete enough and when the same traits are included in assessment of evolutionary change. Molar teeth of mammals that evolved to trophic specialization represent an adequate object for such comparisons, especially when evolutionary trends towards achieving a specialist's type of dentition are well established for a lineage or a clade to which an invader belongs. Relating the changes revealed in invasive populations of modern species to the stages of evolution in a respective clade towards the present-day pattern of specialization may provide novel insights into understanding of the evolutionary potential of biological invasions from both neontological and paleontological standpoints.

A promising model for comparing the extent of phenotypic change during the ongoing invasion to the extent of evolutionary change inferred from the fossil record of the inclusive clade is the sibling vole, an arvicoline rodent that undergoes range expansion via casual introductions by humans.

The sibling vole, *Microtus rossiaemeridionalis* Ognev, 1924 (= *M. mystacinus* De Filippi, 1865, *M. levis* Miller, 1908, *M. epiroticus* Ondrias, 1966, or *M. subarvalis* Meyer et al., 1969) is a temperate North Eurasian rodent, and the continuous part of the species' distribution area lies approximately between 60 and 39°N and 30–60°E. The populations outside this area are usually considered as casually introduced by the Trans-Siberian Railway or by supply ships through discharges of solid ballast (Bolshakov and Shubnikova 1988; Sokolov and Bashenina 1994).

To date, not less than ten persisting populations are found outside the continuous range of *M. rossiaemeridionalis*. Phenotype consequences of the invasion events have been studied in two outlier populations based on dental characters - in the Russian Far East (Tiunov et al. 2013) and on the High Arctic archipelago of Svalbard (Markova et al. 2016). Both outlier populations show significant differences from the conspecifics in the core range, raising a question on evolutionary significance of the phenotypic change in the invasive populations of *M. rossiaemeridionalis*.

Microtus rossiaemeridionalis belongs to the subfamily Arvicolinae, the fossil record of which is studied enough to establish evolutionary stages crucial for the formation of the genus-specific (and sometimes species-specific) dental morphology. Evolutionary history of the species is related to the clade *Allophaiomys*-*Microtus*, which is rather well studied due to biochronological implication in both European (Rekovets 1994; Maul and Markova 2007) and Asian parts of Eurasia (Smirnov et al. 1986; Krukover 1992; Borodin and Ivakina (Pogodina) 2000). Direct ancestors of *Allophaiomys* are not found, but it is generally accepted that they have a common ancestor with the *Mimomys*-*Arvicola* lineage, which had diverged from the Pliocene *Mimomys* stock (Fejfar et al. 2011).

Among Arvicolinae, the *Allophaiomys*-*Microtus* clade is recognized as one of the most specialized to herbivory. Molar teeth in *Microtus* sensu lato are adapted to the mechanical demands of high-fiber diets, which are reflected in structural adaptations common either for all members of subfamily Arvicolinae: 1) alternating prismatic columnar cusps on high-crowned molars, or for members of the *Allophaiomys*-*Microtus* clade, 2) permanent growth of molars; or for members of the genus: 3) topological pattern of prism arrangement, 4) strict regularity of leading and trailing enamel ridges; or for the subgenus *Microtus* sensu stricto: 5) a particular range of molar complexity reflected in the number of additional prisms in anterior parts of the first lower molar and posterior parts of the upper molars.

According to Agadjanian (1996), herbivory is a highly specialized type of phytophagy, which demands highly specialized chewing teeth, thus closing the ways back to a low-fiber diet. We assume here that the five groups of structural adaptations mentioned above may be used to compare the populations of *M. rossiaemeridionalis* by assessing the expression of traits reflecting dental specialization towards herbivory.

Here, we provide an analysis of dental variability in the two recently discovered invasive populations in Surgut (Markova et al. 2014) and Khabarovsk (Lapin 2013) and in previously studied conspecific populations within and outside the core range of the species in order to: 1) uncover common and specific patterns of dental variation in disjunctive populations, and 2) to answer the question on potential evolutionary significance of the dental phenotypic changes revealed in the outlier invasive populations of *M. rossiaemeridionalis* from neontological and paleontological standpoints. To answer the latter question, we employ the concepts of evolutionary ecology (Shvarts 1977) and use the macroevolutionary background inferred from the fossil record of the direct and collateral ancestors of the sibling vole within the Arvicolinae. The current understanding of arvicoline tooth development (Jernvall et al. 2000; Renvoise and Montuire 2015) and concepts of evo-devo (Salazar-Ciudad 2007) are also incorporated into the analysis of dental variation.

Materials and Methods

Specimens and Samples

We examine for the first time the collections of skulls from two recently discovered invasive populations in Surgut and Khabarovsk and re-examine previously studied collections from 19 localities within or outside the species' core range (Markova et al. 2010, 2016; Kartavtseva et al. 2012; Tiunov et al. 2013) using the same methodology.

A total of 53 animals were collected in Surgut City (61°15′ N, 73°26′ E) during April and June–September in 2009–2013 and 2015–2016. The city is located in the taiga zone of western Siberia, on the right bank of the Ob' River, in its middle reach. No findings of the sibling vole are known in the natural landscapes surrounding the city (Starikov and Morozkina 2012). The collection is primarily formed as a result of a small mammal census and is represented by the samples of small size or single individuals from all over the city area (Fig. 1: 1). All animals were captured in urban biotopes including city parks, cemeteries, garden-plots, urban forests, and patches of ruderal vegetation in underexploited areas.

A total of 16 individuals were captured in Khabarovsk City (48°29′ N, 135°04′ E) during March–April and July 2010–2012. The city is situated in the Russian Far East on the high right bank of the Amur River. Since the first record of *M. rossiaemeridionalis* in Khabarovsk dated back to 2010 (Lapin 2013), the species is encountered regularly but at low frequency, being confined to the urban areas and adjacent fallow lands on the right bank of the Amur River. The morphological dataset comes from three plots including commercial and building areas, and a river port (Fig. 1: 3).

Re-examined collections from invasive populations include three datasets. Irkutsk Region (Fig. 1: 2) is represented by the collection obtained near the settlement of Novonukutskoe in 1984 and housed in Zoological Institute RAS, Saint-Petersburg, Russia. The dataset was first analyzed by Markova and co-authors (Markova et al. 2010: sample 65; no information on the trapping plots). A seaport Sovetskaya Gavan' in the Russian Far East is represented by animals captured in September 2008–2009 and in March–November 2010–2011 on eight plots (Fig. 1: 4). The collection was first studied by Tiunov et al. (2013). The Archipelago of Svalbard is characterized by the dataset collected in 1997–2005 near the

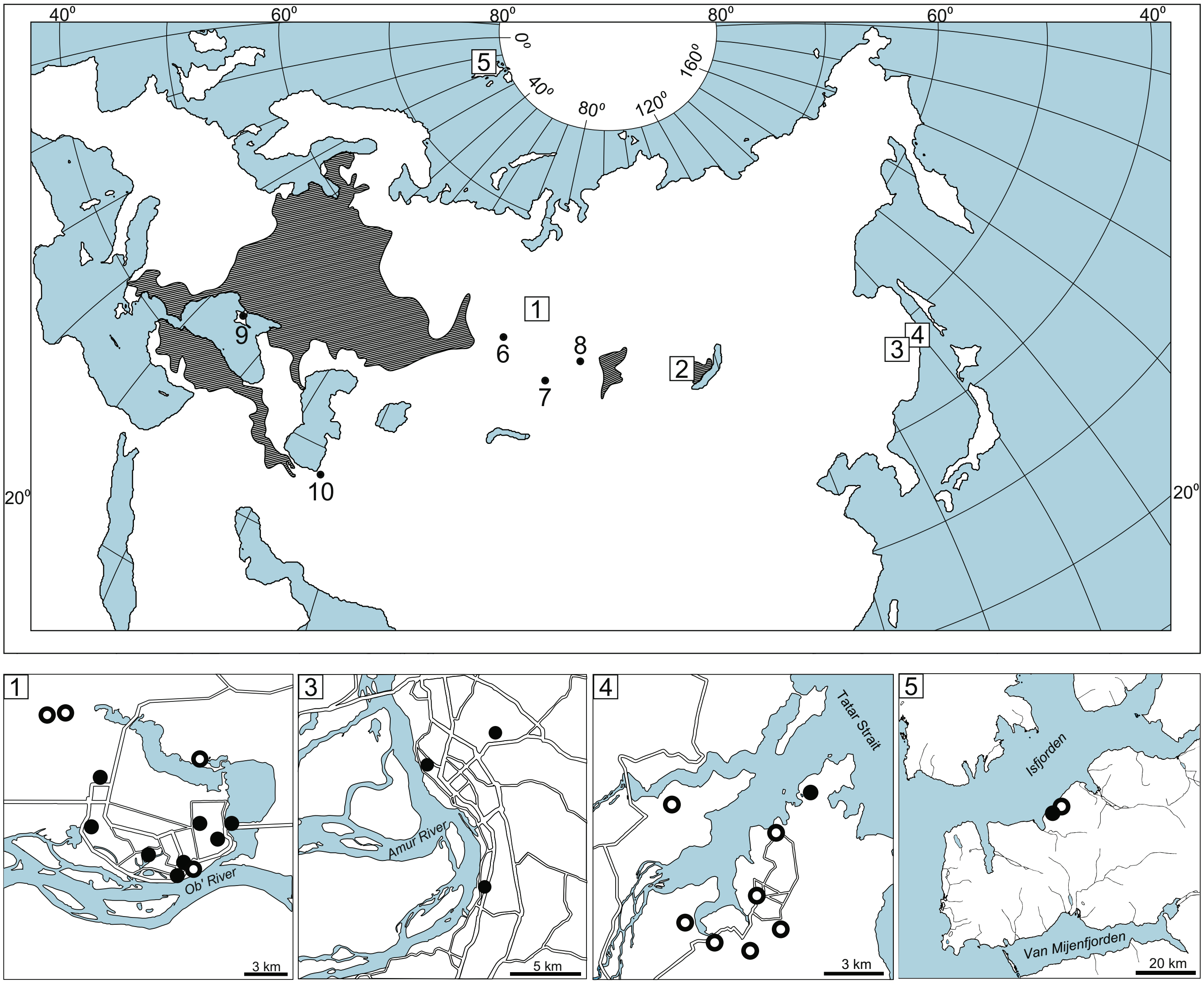

Fig. 1 Geographic location of the disjunctive populations of *M. rossiaemeridionalis* relative to the species' core range (above) and local distribution of sampling plots (below). *Gray* area indicates the core range following Shenbrot and Krasnov (2005); 1–5 – morphologically studied populations listed in Table 1 (1 – Surgut, 2 – Irkutsk Region, 3 – Khabarovsk; 4 – Sovetskaya Gavan'; 5 – Svalbard); 6–10 – other findings outside the core range (Tovpinets 1996; Gashev 1996; Dupal 2010; Ilyinskikh et al. 2010; Mahmoudi et al. 2014; Ghorbani et al. 2015). *Circles* indicate sampling plots and testing subsets 1 (*black*) and 2 (*white*)

abandoned town of Grumant (Fig. 1: 5) and housed in the Agder Natural History Museum, Norway. The collection was first studied by Markova et al. (2016).

The comparative dataset from the continuous part of the species' range includes 16 samples described in Markova et al. (2010). To achieve methodological uniformity and to meet the requirements of statistical testing, five samples were randomly chosen among the representative samples in the comparative dataset ($N \geq 30$), and then reexamined using the approaches presented here (sample numbers: 56, 57, 59–61).

The species distribution area is considered according to Sokolov and Bashenina (1994) and Shenbrot and Krasnov (2005). The continuous range outlined in Shenbrot and Krasnov (2005) is considered to be the species' core range. We also summarize some new findings (Fig. 1), although a detailed description of the species' distribution falls outside the scope of the study. The history of the core range of *M. rossiaemeridionalis* remains elusive. As the native geographical range of the species is not known, it is not clear how much the human-mediated spread contributes to the formation of the present-day continuous range of the species.

Taxonomic Identification

All individuals captured in Surgut in 2013–2016 were identified to species using PCR method applied to ethanol-preserved muscle samples. The dataset collected before 2013 included 18 skull-and-skin specimens, of which nine (at least one for each sampling plot) were identified to species based on dry skin fragments. Several individuals captured in 2013 were also subjected to karyological analysis (Markova et al. 2014).

All individuals from Khabarovsk were identified using PCR method applied to either ethanol-preserved muscle samples or dried tissue fragments collected during preparation of skulls. Complete cytochrome b gene was sequenced for three individuals from Khabarovsk.

Taxonomic identification was conducted using the DNA analysis facilities at the Institute of Plant & Animal Ecology UrB RAS. For PCR identification we extracted total DNA from the samples of cardiac or skeletal muscles fixed in 95% ethanol or from dry tissues using a salt-extraction method (Aljanabi and Martinez 1997). Precipitation of DNA extracted from freshly preserved muscle samples was done using ethanol. DNA extracted from dried specimens was precipitated on silica particles following the protocol developed by Rohland and Hofreiter (2007). Two pairs of primers recommended for identification of sibling species (Nekrutenko et al. 1999) were used for PCR:

Mar14F 5′_CCCCTAAAGATTGTTACAGAAACCATC_3′
Mar14R 5′_AAGCCCAACTTGTAACCAGATAAAGCAC_3′
Mro16F 5′_GCTCGCTGGTAGAGACAGTCACC_3′
Mro16R 5′_GAAGGCAGATTGACCAAGATTTCC_3′

For cytochrome b gene sequencing, a fragment of mtDNA was amplified by PCR with universal primers located in the flanking tRNAs - L7 and H6 (Tougard et al. 2008). PCR products were visualized on 1% agarose gel and purified on silica (Sigma-Aldrich). Sequencing was performed using primers L7 and H6 and the Big Dye Terminator Cycle Sequencing Kit V. 3.1 (Applied Biosystems, USA) following the manufacturer's protocol on the ABI Prism 3130 sequencer (Applied Biosystems). All obtained sequences were compared to the GeneBank sequences that allowed us to identify individuals as *M. rossiaemeridionalis*.

Methods of taxonomic identification applied to the datasets from Svalbard, Sovetskaya Gavan', Irkutsk Region, and to the comparative populations from the core range are described in previous publications (Markova et al. 2010, 2016; Kartavtseva et al. 2012).

Dental Terminology and Abbreviations

Dental terminology for this study is adopted from van der Meulen (1973): T1, T2…Tn – occlusal triangles with their respective numbers, AC – anterior cap of m1, PL – posterior lobe. M1-M3 – upper molars, m1-m3 – lower molars. To designate morphotypes, we use traditional names (e.g., simplex) or abbreviate them after a symbol of a respective molar (e.g., M3_s for simplex*,* M3_t for typica*,* etc.).

Dental Variation

Occlusal patterns of molars are analyzed using a morphotype based approach. Among a variety of morphological methods, this approach is chosen because i) it enables inter-study comparability of results when the morphotypes are defined in the same way, ii) because of the existing evidences for heritability of particular dental morphotypes in *Microtus*, and iii) because of the possibility to define morphotypes based on the characters or character groups, which experience directional change over time, thus reflecting the major evolutionary trends towards progressive specialization.

In this study, we divide dental morphotypes previously described for *M. rossiaemeridionalis* into groups according to their relationship to one of the five groups of structural adaptations to herbivory mentioned in the introduction. We also consider compatibility of the criteria used to establish morphotype groups with the current understanding of tooth development (Jernvall et al. 2000; Renvoise and Montuire 2015).

Morphotype Group 'Complexity'

For inter-study comparability, we use the traditional designation of morphotypes for M3: simplex, typica, duplicata, variabilis (Rörig and Börner 1905), and for m1: *I-IV* (Markova et al. 2016). Those occlusal morphotypes are based on the number of re-entrant and salient angles (and respective dental prisms), and thus reflect the complexity of a tooth crown.

From an evo-devo point of view, complexity should be interpreted as a continuous variable underpinned by an iterative developmental mechanism (e.g., Jernvall et al. 2000). It is shown that main cusps on m1 of *M. rossiaemeridionalis* appear around embryonic day 18 (Jernvall et al. 2000). At the same time, breeding experiments have revealed Mendelian inheritance of the morphotypes distinguished by the number of additional elements and incomplete dominance of more complex morphotypes (e.g., Bolshakov et al. 1980; Stohl 1984). To account for both genetic and developmental mechanisms, we consider complexity as a continuous variable, which is measured at an interval scale by using a morphotype ranking approach (Markova 2014). The ranking approach makes it possible to measure dental complexity at the individual level and allows us to consider tooth complexity as a quantitative variable.

Complexity ranks are evaluated for each molar in the upper and lower tooth rows, except for M1, which exhibits no variation in complexity. Correspondence of traditional morphotypes and complexity ranks is shown on Fig. 2.

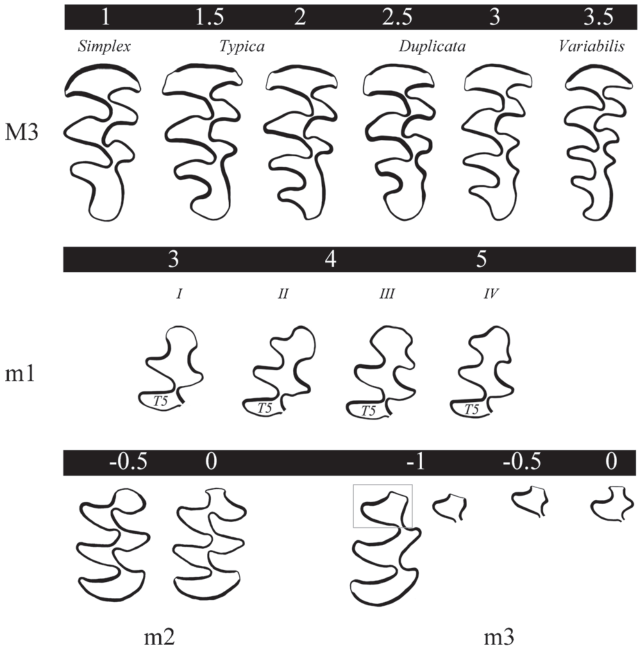

Fig. 2 Morphotype group reflecting crown complexity: morphotypes of M3, m1, m2, m3 arranged according to ranks (on *black bars*) and traditional morphotype designations (below the *bars*)

Morphotype Group 'Regularity of Leading and Trailing Enamel Ridges'

This group includes morphotypes that which differ in the extent of separation between crown prisms of the same molar by re-entrant angles. When the two prisms are completely separated, the opposite enamel ridges of lingual and buccal re-entrant angles nearly adjoin one another, thus enabling the leading and trailing edges of the enamel to occlude in diagonal position. Such diagonal position increases the efficiency of high-fiber food processing (Koenigswald 1980).

We estimate regularity of leading and trailing enamel ridges on m1 and m2. The distance between the opposite re-entrant angles is compared with the maximal width of the anterior (leading) enamel ridge in order to obtain a relative measure of confluence between the adjoining prisms (Fig. 3).

In terms of functional specialization to herbivory, the morphs with strictly regular arrangement of enamel ridges are interpreted as functionally advantageous, and those with a decrease in the regularity of enamel ridges are interpreted as less effective for fibrous food processing during propalinal chewing movements typical for *Microtus* (e.g., Koenigswald 1980).

Developmental background of the characters related to the regularity of enamel ridges is yet to be uncovered. Strictly alternative arrangement of crown prisms is always retained on the morphotypes of this group, so it is not possible to relate

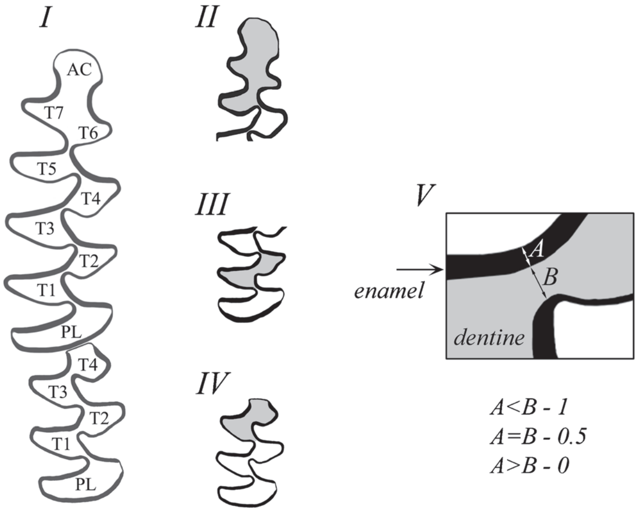

Fig. 3 Morphotype group reflecting regularity of enamel ridges, as assessed by the presence of broadly confluent dentine fields on the occlusal surfaces of lower molars. I – Typical occlusal outlines of m1 and m2 showing no deviations from the usual pattern of complete separation between dentine prisms T1-T6; II – anterior part of m1 with confluent T5-T6 (m1_Oec morphotype); III – posterior part of m1 with confluent T1-T2 (m1_t1t2 morphotype); IV – m1 with confluent T3-T4 (m2_t3t4 morphotype); V - the degrees of confluence between dentine prisms ranging from complete confluence (1) to incomplete (0.5) and complete separation (0). Dentine fields of the confluent prisms are shown with *gray*. AC – anterior lobe, PL – posterior lobe, T1 – T7 – occlusal terminology adopted from van der Meulen 1973

the regularity of enamel ridges with the topological rearrangements of cusps in an antero-posterior direction (Jernvall et al. 2000). Most probably, this character group is related to the formation of the longitudinal crest that is retained in voles from their cricetid ancestors and connects the anterior and posterior cusps. The longitudinal crest appears on about embryonic day 15 and is distinguishable on the unworn occlusal surfaces of juvenile animals.

Rare Morphs and Abnormalities

The group includes dental morphs showing no association with progressive complexity and regularity of enamel ridges, and defined based on rare qualitative traits with no signs of continuous variation. The samples were checked for presence or absence of rare morphs and dental abnormalities, which could be found in common voles sensu lato with extremely low frequencies (Jánossy and Schmidt 1960, 1975; Markova et al. 2010).

Analysis

Statistical analysis was performed using STATISTICA 8 package (Statsoft 2007).

Kolmogorov-Smirnov test (KS-test) was used to determine if the frequency distributions of particular morphotypes differ significantly. To interpret the differences between distributions, we used the classification proposed for studying temporal and spatial variation in arvicolines (Bolshakov et al. 1980). Under this classification, all morphotypes recorded in a sample should be arranged according to their frequency as follows: 36% and more — over-dominant morphotypes, 12–35.9% — dominant and sub-dominant morphotypes, and less than 12% — subordinate (reserve) morphotypes. Over-dominant, dominant, and sub-dominant morphotypes constitute a group of basic morphotypes. Originally, this classification was applied to fossil samples in order to establish evolutionary significant morphological shifts, and it is also applicable when considering hidden and released phenotypic variation in extant populations of arvicolines (Bolshakov et al. 1980).

Spearman's correlation coefficient (R_S) was used to assess relationships between variables. Analysis of variance (ANOVA) was used to test for significant differences between mean values of complexity ranks. We applied a mixed model nested ANOVA to the datasets from two parts of the range (disjunctive vs. continuous) using five populations for each part of the range as subgroups in the hierarchic design. For three disjunctive populations, we also tested the differences among and within populations using testing sets 1 and 2 as subgroups. Non-parametric Kruskal-Wallis test (KW-test) was used for comparing average degrees of regularity of enamel rigdes (morphotype group 'regularity') because the departures from the typical regular pattern were rare, and frequency distributions were extremely skewed to the right.

Sources of Variation

Taking into account the absence of sexual dimorphism in dental characters shown for *M. rossiaemeridionalis* (Markova et al. 2010), we do not consider sex as a source of variation.

All individuals included in the analysis exhibited completely formed occlusal surfaces with no juvenile enamel folding. To test for the presence of age differences at the post-juvenile stages, we determine relative age of the animals with complete skulls based on percentage of skull maturation from 10% to 100% following the approach developed by Larina and Lapshov (1974). Then we divide the samples from five disjunctive populations into age classes 1 and 2 according to the percentages of skull maturation. The class 1 included animals with 30–60% and the class 2 comprised those with 70–100% mature skulls; the animals with immature skulls (10–20%) were not present in the analyzed dataset. Statistical tests revealed no significant differences between age classes in both complexity ranks of m1, m2, m3, M3 (ANOVA, $F = 0.02–3.39$, $p = 0.06–0.902$) and regularity of enamel ridges on m1 and m2 (KW $H = 0.43–1.98$, $p = 0.160–0.511$). Based on that, we do not consider age as a significant source of variation in the studied dataset.

Among-population variability and differences between the disjunctive and continuous portions of the range were assessed for five disjunctive populations (Fig. 1) and five populations within the continuous range (comparative samples 56–57, 59–60 from Markova et al. 2010).

To account for intra-population variability related to the repeated sampling, we split the data from invasive populations 1, 4, 5 into testing sets. The testing sets for population 1 represent the sampling years 2009–2012 (Surgut 1, $N = 36$) and 2013–2016 (Surgut 2, $N = 69$), for population 4 – the sampling years 2008–2009 (Sovetskaya Gavan' 1, $N = 14$) and 2010–2011 (Sovetskaya Gavan' 2, $N = 37$), and for population 5 (Svalbard) the dataset is split according to the sampling year (2001, $N = 42$ and 2003, $N = 164$), where N is the number of molars of the same category. The samples from populations 2 and 3 are not split because of their smaller size.

Systematics and Phylogenetic Background

In this study we use the taxonomy of Abramson and Lissovsky (2012) for extant species, and Fejfar et al. (2011) for their ancestral forms within Arvicolinae.

The sibling vole belongs to the group of closely related species, which is referred to as *Microtus 'arvalis'* species group or as a subgenus *Microtus* sensu stricto within the genus *Microtus*, which in turn is a member of a generic group *Microtus* sensu lato within the tribe Arvicolini (Abramson and Lissovsky 2012). All members of the generic group *Microtus* sensu lato are considered here as a clade emerged from *Allophaiomys* (*Allophaiomys-Microtus* clade), and subgenus *Microtus* sensu stricto is referred to as one of the lineal descendants of *Allophaiomys* (*Allophaiomys*-*Microtus* lineage). The presumed ancestral form common for the members of the 'arvalis' group is *M. arvalinus* Hinton, 1923 (= *M. nivaloides* Major, 1902).

To illustrate ancestral states of dental complexity patterns, we use comparative data on *Allophaiomys* cf. *A. deucalion* Kretzoi, 1969 (Tesakov 2004). The comparative fossil sample includes 61 m1 and 50 M3 from two localities in the south of Eastern Europe dated back to late Pliocene – early Pleistocene (Tesakov 2004). This comparative dataset is chosen because the description allows us to recalculate dental complexity ranks.

To compare the regularity of enamel ridges between recent voles and their ancestors, we use drawings of 120 m1s of *M. arvalinus* from the Tiraspol faunistic complex of the south of Eastern Europe (Rekovets 1994). Because the drawings might differ from the original specimens, we use this dataset for illustrative purposes only, without statistical testing.

Assessing Specialization to Herbivory

Within the framework of this study, we define specialization as a process towards achieving a specialist's type of dentition, which could be traced by the increasing number and/or expression of traits reflecting structural adaptation of cheek teeth to the mechanical demands of a diet. Five structural adaptations of molar teeth to herbivory inherent to *Microtus* sensu stricto are listed in the Introduction. In contrast to specialization, which is directed towards the progressive adjustment of teeth to a particular diet, de-specialization has no intrinsic directionality. We define de-specialization as a process related to a weakened expression of structural adaptations developed in the course of evolutionary specialization.

Specialization as a process is usually discussed at the level of major taxonomic groups (e.g., Agadjanian 1996). Here, we suggest that the concept of specialization might be applied to the study of intraspecific variation in extant species. Knowing that any species diverged within a highly specialized clade retain a particular range of variability in the characters reflecting structural adaptation of teeth, we make an a priori assumption that the shifts in the species-specific range of dental phenotypic variability might be interpreted as either co-directional or counter-directional with the evolutionary trends known for the inclusive clade.

The relevant mandatory conditions for testing this assumption are as follows: i) the fossil record must be complete enough to establish the evolutionary trends; ii) the same methodological approach must be used to compare living and extinct members of the inclusive clade; and iii) the datasets must be representative enough to characterize the species range of variability.

Knowing that *M. rossiaemeridionalis* might exhibit a widening of the range of dental variability in modern isolated populations (Tiunov et al. 2013; Markova et al. 2016) we analyze the data from five disjunctive populations at the background of comparative datasets from the continuous range and from the fossil record in order to determine if the departures from the species-specific range of variability are co-directional or counter-directional with the evolutionary trends towards achieving a specialist's type of dentition.

The dataset generated during the current study is available from the corresponding author on reasonable request.

Results

Frequencies of the morphotype dental patterns reflecting complexity, rare morphs, and dental abnormalities in *M. rossiaemeridionalis* from five invasive populations compared with the conspecifics from the continuous range are shown in Table 1. Frequencies of the morphotypes reflecting regularity of enamel ridges are shown in Appendix 1.

Morphotypes Reflecting Tooth Complexity

We estimated correlation between complexity ranks of different molars in a pooled dataset from two range parts, and in continuous and disjunctive parts separately. Stable correlation patterns are revealed for the estimates of complexity. Complexity ranks of different molars exhibit strong positive correlation (e.g., in the pooled dataset from the core and disjunctive populations: $R_S = 0.17–0.44$, $p < 0.05$), thus suggesting that there should be a common mechanism that underpins complexity of all elements of a tooth row. Even in the disjunctive population 4, where the lower m1s are predominantly complex and upper M3s are predominantly simple (Fig. 7), there is still significant positive correlation between complexity ranks of these molars ($R_S = 0.31$, $p < 0.05$).

Mixed-model nested ANOVA results suggest that differences among populations inferred from complexity ranks of all molars are greater than would be expected by chance (Table 2). The amount of variation between disjunctive and continuous parts of the range is not greater than to be expected on the basis of the observed variation among populations (Table 2).

The differences among populations are more pronounced in the disjunctive range part than in the core. The mean estimates of complexity for m1 and M3 displayed on Fig. 4 clearly show that the most geographically distant populations 4 and 5 are different from one another and from the rest of the dataset. When excluding these two outlier populations, the differences among the disjunctive populations are no longer statistically significant, as assessed on complexity ranks of all molars.

Morphotypes Reflecting Regularity of Enamel Ridges

Analysis of the morphotypes reflecting regularity of enamel ridges indicates that the variation among disjunctive populations is higher than expected by chance (morphotypes

Table 1 Relative frequencies of morphotypes reflecting crown complexity, rare morphs and abnormalities in five disjunctive populations of *M. rossiaemeridionalis* compared with the core range conspecifics. The values falling outside the range of variability within the core range are shown in bold italics. See Fig. 1 for population numbers

Dental morphotype	Populations outside the core range					Core range[a]
	1	2	3	4	5[a]	
Morphotype dental patterns reflecting crown complexity (according to complexity ranks)						
m1_I (3)	0.86	0.85	0.82	***0.24***	0.90	0.39–0.92
m1_II (4)	0.02	0.07	0.12	0.06	0.04	0–0.33
m1_III (4)	0.10	0.07	0.06	***0.36***	0.02	0–0.33
m1_IV (5)	0.02	0	0	***0.34***	0.03	0–0.27
m2_rank-0.5	0.01	0.12	0	0.04	**0.46**	0–0.12
m3_rank-1	0.02	0	0	0.02	**0.50**	0–0.27
m3_rank-0.5	**0.91**	**0.80**	**0.45**	**0.34**	**0.39**	0.46–0.61
m3_rank0	0.07	0.20	**0.55**	**0.64**	0.11	0.18–0.54
M2_rank0.5	+	+	+	+	−	−/+
M3_s (1)	0.015	0.08	0	***0.42***	0.00	0–0.33
M3_t (1.5/2)	0.13/0.26	0.13/0.29	0.31/0.28	0.30/0.17	0.38/0.53	0.14–0.90
M3_d (2.5/3)	0.24/0.34	0.08/0.38	0.41/0	0/0.11	0.02/0.08	0.10–0.65
M3_v (3.5)	0.015	0.04	0	0	0	0–0.43
Rare dental morphs						
m1_g	–	–	0.03	–	–	Single id
m1_h	–	–	–	–	0.004	Single id
Developmental abnormalities						
DPS † of molars	***One id***	–	–	–	–	–
Functional abnormalities						
Overgrown incisors	–	–	–	–	***Single id***	–
N, teeth of the same category (m1)	106	30	33	50	248	580

[a] Compiled from Markova et al. 2010, 2016

†Disordered prismatic structure of molars

m1_Oec, m1_t2t3, m2_t3t4: KW H = 26.0–76.9, $p < 0.01$), except for m1_t1t2 (KW H = 8.2, $p = 0.08$). Morphotypes with irregularities of enamel ridges are significantly more frequent in the disjunctive popualtions than in the core (Fig. 5). Differences are the most obvious when comparing datasets from the continuous range with the island population 5, which exhibits the highest frequency of enamel irregularities on m1 and m2, with the exception of m1_t2t3 morphotype. At the same time, a relatively high proportion of m1_t2t3 morphotype is found in population 4. When excluding the outlying populations 4 and 5, the differences among the disjunctive populations 1–3 are no longer significant, except for m2_t3t4 morphotype (KW H (2, $N = 155$) =16.8, $p < 0.001$)

We estimate correlations between the morphotypes with irregularities of enamel ridges in a pooled dataset from two range parts, and in continuous and disjunctive parts separately. Correlation patterns of the morphotypes reflecting regularity of enamel ridges vary between range parts. In the core, where the morphotypes with irregularities of enamel ridges are rather rare (Table 1), they share a positive correlation ($R_S = 0.16$–0.35, $p < 0.05$). In the disjunctive range, where the frequencies of some morphotypes increase at the level of particular populations, there is no clear pattern of correlation and the R_S coefficients vary from positive (e.g., m1 complexity rank vs. m1_t2t3: $R_S = 0.35$, $p < 0.05$) to negative (m1 complexity rank vs. m1_Oec and m1_t3t4: R_S from -0.24 to -0.11, $p < 0.05$). Such unstable correlation patterns revealed between the range parts suggest that random sorting might contribute to the variation among disjunctive populations.

Collectively, the results of correlation analysis suggest that m1_Oec, m1_t1t2 and m2_t3t4 might follow the common pattern of variation in both parts of the range being more readily present on less complex molars, and m1_t2t3 tends to occur at random.

Rare Morphs and Abnormalities

Of 16 rare variations of m1 known for the '*arvalis*' group, only two morphs revealed in the invasive populations (Table 1), and no new rare morphs are found.

Dental abnormalities have not been detected in the populations within the core range. At the same time, in two of five disjunctive populations of sibling vole, we

Table 2 Mixed-model nested ANOVA results comparing I) dental complexity between disjunctive vs. continuous parts of the sibling vole range (Range part) and among five populations within each part of the range (Population, nested); II) among disjunctive populations 1, 4, 5 (Population) and between two temporal subsamples from each population (Testing set, nested)

Character	Factor	Effect	SS	DF	MS	Den. Syn.		F	p
						DF	MS		
I									
Rank m1	Range part	Fixed	0.06	1	0.06	8.15	5.20	0.01	0.918
	Population, nested	Random	55.74	8	6.97	680	0.19	36.78	0.000
	Error		128.79	680	0.19				
Rank m2	Range part	Fixed	0.20	1	0.20	8.19	0.57	0.35	0.573
	Population, nested	Random	6.05	8	0.76	645	0.03	28.13	0.000
	Error		17.34	645	0.03				
Rank m3	Range part	Fixed	0.01	1	0.01	8.09	3.66	0.00	0.963
	Population, nested	Random	39.46	8	4.93	641	0.08	65.27	0.000
	Error		48.44	641	0.08				
Rank M3	Range part	Fixed	0.79	1	0.79	8.26	5.15	0.15	0.705
	Population, nested	Random	55.45	8	6.93	678	0.31	22.66	0.000
	Error		207.39	678	0.31				
II									
Rank m1	Population	Fixed	0.96	2	20.31	3.26	0.31	66.60	0.002
	Testing set, nested	Random	40.63	3	0.32	336	0.15	2.20	0.088
	Error		48.94	336	0.15				
Rank m2	Population	Fixed	0.17	2	2.39	3.38	0.05	43.84	0.004
	Testing set, nested	Random	4.79	3	0.06	333	0.04	1.56	0.200
	Error		12.05	333	0.04				
Rank m3	Population	Fixed	0.88	2	10.26	3.13	0.27	37.52	0.006
	Testing set, nested	Random	20.52	3	0.29	333	0.07	4.43	0.005
	Error		22.03	333	0.07				
Rank M3	Population	Fixed	5.21	2	16.28	3.08	1.62	10.03	0.045
	Testing set, nested	Random	32.56	3	1.74	334	0.26	6.67	0.000
	Error		87.05	334	0.26				

SS sum of squares, *MS* mean square, *DF* degrees of freedom, *Den. Syn.*synthesized denominator for testing the significance of random effects, *F* Fisher's F statistic, *p* probability values

found single individuals with substantial deviations from normal phenotypic patterns.

Few individuals with functional abnormality caused by overgrown incisors are found in population 5 (Table 1). In one individual, the overgrown incisors caused substantial malocclusion. Absolute number of animals with overgrown incisors is not possible to calculate because of intravital breakage.

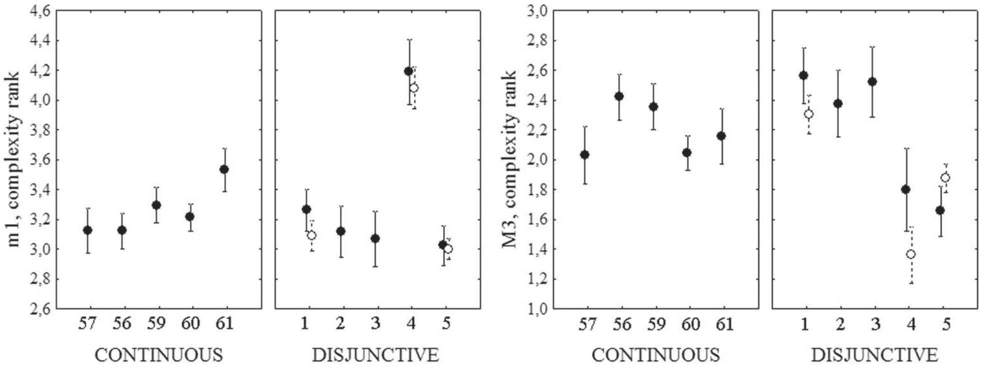

Fig. 4 Variation in dental complexity ranks between continuous and disjunctive portions of the present-day range of *M. rossiaemeridionalis*, among five populations within each portion of the range, and between testing subsets within population, wherever applicable. Means and 0.95 confidence intervals are shown. *Open circles* indicate testing subset 2. See Fig. 1 and Materials and methods section for population numbers

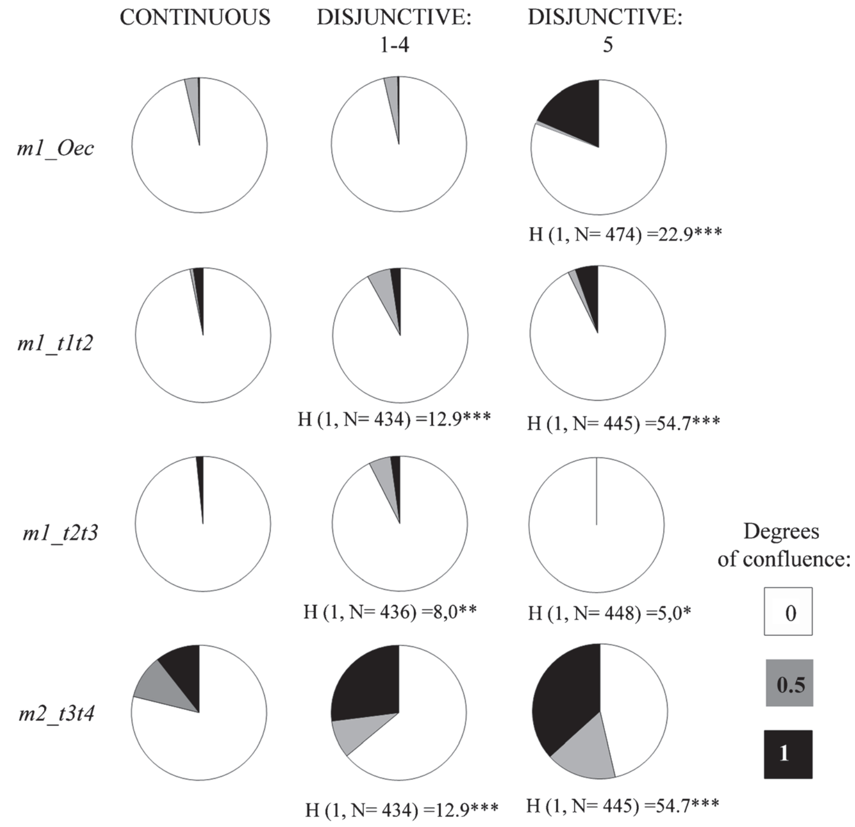

Fig. 5 Relative frequency of the morphotype group 'regularity' in in continuous and disjunctive portions of the present-day range of *M. rossiaemeridionalis*. Disjunctive population 5 is shown separately because of the transition of a reserve morphotype m1_ Oec to a category of basic morphotypes. The results of Kruskal-Wallis H tests are shown under the pie charts when the differences between the core and invasive testing sets are significant at the level of 0.05 (*), 0.01 (**) or 0.001 (***). A degree of confluence for a particular morphotype indicates the extent to which the regularity of enamel ridges is biased from strictly regular arrangement (0) to partial (0.5) or complete confluence (1) between one pair of dentine fields. See Fig. 1 for population numbers and Fig. 3 for morphotype descriptions

One individual (id 38,969) found in Surgut exhibits anomalous M3s. It is an adult male with 100% mature skull captured in 2010 on the Ob' River bank in a small plot of birch and aspen forest on the area subjected to city renovation. Single individuals of *M. rossiaemeridionalis* with no dental abnormalities were captured at the same plot in 2011 suggesting that the species was present at low frequency. After complete removal of the habitat in 2011, the species was encountered on the adjacent plots in 2013 and 2016. The individuals captured after 2010 exhibited no abnormalities.

Upper third teeth of id 38,969 exhibit substantial difference from normal occlusal pattern ever seen in the species (Fig. 6). M3s are prismatic, unrooted, with alternating T2 and T3; however, the number of prisms is reduced compared with normal M3s of *M. rossiaemeridionalis*: both right and left molars of id 38969 are shortened at the expense of the posterior parts, and prismatic arrangement is disordered. In the left maxilla, there is an additional isolated element completely separated from M3 looking like a reduced tooth or a separately developed prism, which is lower than the occlusal surface.

Both left and right M1, M2, m2, m3 of id 38969 show no abnormalities or specific features. Left m1 exhibits broad confluence between T1 and T2, but is absolutely normal in appearance. Right m1 shows severe intravital damage of the anteroconid complex, so that the occlusal pattern is undistinguishable. The alveolar view of this molar shows normal pattern and reveals the m1_Oec morphotype.

Common and Specific Patterns of Dental Variation in Disjunctive Populations

Nested ANOVA results (Table 2) clearly suggest that there are no directional shifts in the disjunctive part of the species range towards more complex or more simple molars. KW- tests reveal significant increases in the frequency of morphotypes with irregularities of enamel ridges in the disjunctive range compared to the core (Fig. 5). However, each population follows its own pattern of variability showing increases in frequencies of different morphotypes (Table 1, Appendix 1). Collectively, these results suggest that disjunctive populations exhibit phenotypic specificity, which is the most pronounced in the most geographically distant populations 4 and 5.

Phenotypic differences among the disjunctive populations assessed on all molars are higher than expected on the basis of variation between testing sets obtained in different years. If we consider variation among testing sets as a measure of within-population genetic drift, we can infer that, despite strong correlation among complexity estimates of different molars, contribution of random variation is higher on posterior teeth in both upper and lower tooth rows than on m1 and m2 (Table 2). As assessed on m1 and m2, there are no statistically significant differences among testing sets in populations 1, 4, 5; however, those testing sets differ significantly when assessed on m3 and M3.

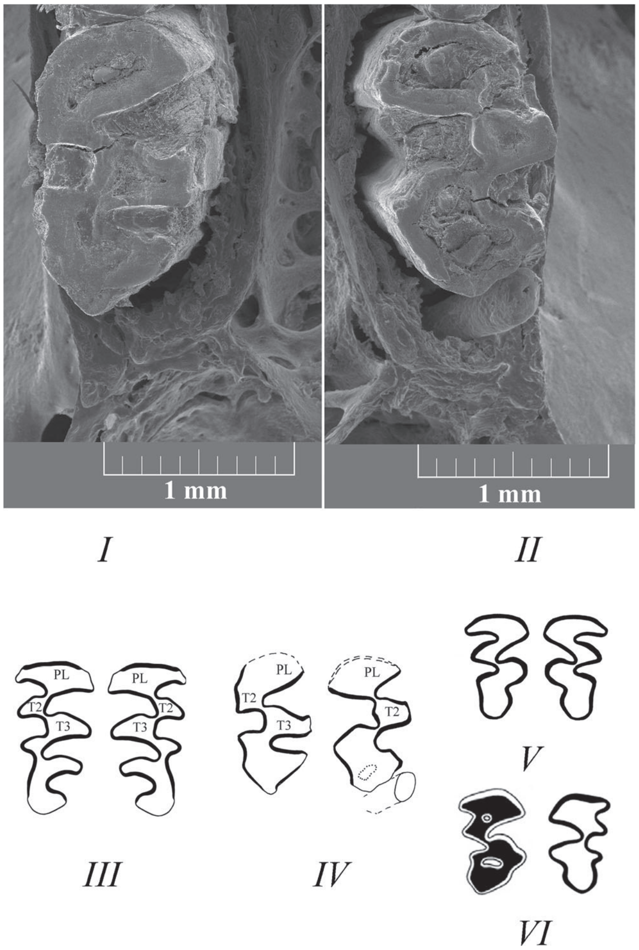

Fig. 6 Atavistic anomaly of the upper third molars detected in one individual in Surgut (id 38969), and its comparison with normal M3 occlusal patterns of *M. rossiaemeridionalis* and some extinct arvicolines. *I-II* - Scanning electron microscope photos of right (*I*) and left (*II*) molars; IV - schematic drawings of occlusal surfaces of the same molars compared to normal right and left M3s of *M. rossiaemeridionalis* (*III*), and of the Pliocene arvicoline species (V – *Mimomys tornensis* showing eosimplex M3 morphotype, which was hypothesized to be ancestral for different lineages of *Microtus* sensu lato (von Rabeder 1986); *VI*, left – *Mimomys vandermeuleni* (Fejfar et al. 2011); *VI*, right - *Pliomys destinatus* (Tesakov 2005) showing no complete separation of T2 from PL

Assessing Specialization to Herbivory

Phenotypic peculiarities found in disjunctive extant populations of *M. rossiaemeridionalis* correspond to three of five groups of structural adaptations to herbivory known for *Microtus* sensu stricto, including topological pattern of prism arrangement, regularity of leading and trailing enamel ridges, and molar complexity.

We suggest that evolutionary trends towards increasing complexity and regularity of enamel ridges known for the members of the *Allophaiomys-Microtus* clade may be used as a background for comparing recent disjunctive populations to the core range conspecifics, and to their ancestral forms (Fig. 7). The departures from typical patterns of variability known in the core could be interpreted as co-directional or counter-directional with the progressive evolutionary trends towards increasing specialization to herbivory as assessed by two groups of structural adaptations (complexity and regularity).

The most obvious phenotypic departure towards the ancestral state is the anomalous M3 found in population 1. The ranks of complexity estimated for these molars (Fig. 7: I) fall into the range of variability of *Allophaiomys* cf. *A. deucalion* (right molar, rank 0) or even outside this range (left molar, rank −1), thus resembling the more simple *Mimomys* morphotype eosimplex (Fig. 6: V). Along with a decrease in complexity, the anomalous individual from population 1 exhibits disordered arrangement of prisms posteriorly and anteriorly of T2-T3, and shows irregularities of enamel ridges, thus representing a departure from the typical character states included in three different groups of structural adaptations, numbered from 3 to 5 in the Introduction.

To check the releases of phenotypic variation revealed in recent disjunctive populations for directionality, we compare frequency distributions in recent samples with the distributions recalculated for the earliest ancestral forms known for *Microtus* sensu lato (Fig. 7: I) and presumed for the ‘*arvalis*’ species group (Fig. 7: II).

No unidirectional shifts towards increasing complexity of both m1 and M3 are revealed in the disjunctive populations of the sibling vole. In population 4, the distribution of ranked morphotypes tends to flatten out as the frequencies of reserve morphotypes increase, regardless of their complexity (Fig. 7: I). The decrease in frequency of typical (basic) morphotypes has led in this population to an unusual combination of predominant morphotypes of upper and lower teeth, which could be interpreted as plesiomorphic (M3 morphotype simplex, rank 1) and derived (m1 morphotypes falling to rank 4).

The increase in frequency of m1_Oec morphotype with enamel irregularities appears to be counter-directional with the tendency to lowering this morphotypes down in the course of evolution from early Tiraspolian to late Tiraspolian *M. arvalinus*, and also from *M. arvalinus* to modern sibling vole (Fig. 7: II).

Thus, in the invasive populations of sibling vole existing outside the continuous range, we find no co-directional phenotypic shifts with the evolutionary trends towards more complex molars and more strict arrangement of enamel ridges. All the departures from the typical range of phenotypic variability revealed in the disjunctive invasive populations are counter-directional with those evolutionary trends, or non-directional.

From a functional point of view, the releases of hidden phenotypic variation revealed in the disjunctive invasive populations lead to accumulation of individuals with less pronounced structural adaptations to herbivory.

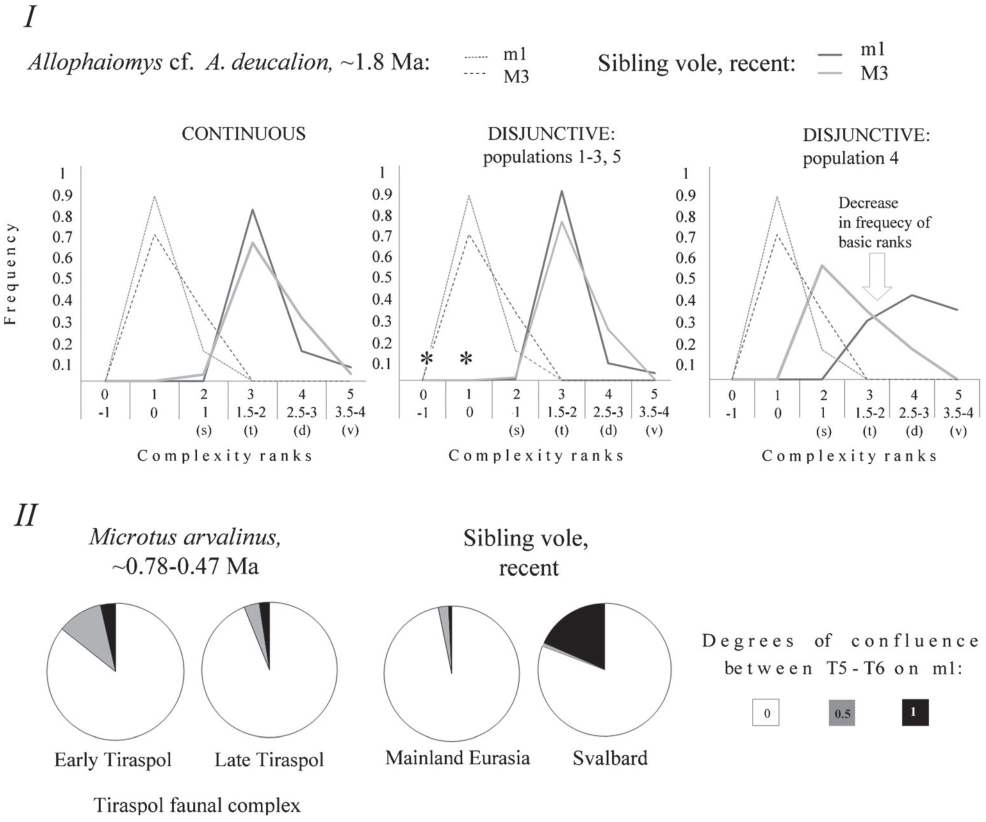

Fig. 7 Comparison of morphotype frequencies in recent sibling vole with its earliest known ancestors to have directly comparable occlusal patterns of m1 and M3. I - Frequency distributions of the morphotype group 'complexity' in continuous and disjunctive portions of the present-day geographic range of *M. rossiaemeridionalis* (this study) compared to the ancestral form *Allophaiomys* cf. *A. deucalion* (recalculated from Tesakov 2004). The first row of x-axis labels indicates complexity ranks for m1 (0–5), and the labels of the second and third rows indicate complexity ranks from -1 to 4 for M3, with abbreviations of traditional M3 morphotypes for recent *Microtus ʻarvalisʼ* species group. *Asterisks* indicate complexity ranks of the anomalous M3s of the individual 38969 from population 1 (left molar - rank −1, right molar - rank 0). *Arrow* points at the reduced frequency of the basic ranks of m1 (rank 3) and M3 (ranks 1.5–2) on the flattened distribution of complexity ranks in population 4. See Fig. 1 for population numbers. II – Morphotype group ʻregularityʼ: frequencies of m1_Oec morphotype with half-confluent (0.5) and fully confluent T5 and T6 (1) compared with m1 showing completely separated T5 and T6 (0) in recent sibling vole (this study) and in *M. arvalinus*, a presumable common ancestor of *Microtus ʻarvalisʼ* species group (recalculated from Rekovets 1994)

Discussion

A common feature of the studied invasive populations of *M. rossiaemeridionalis* is that all dental morphotypes and rare morphs observed in the disjunctive populations are also known in the species' continuous range. At the same time, the drastic increase in frequency of particular reserve morphotypes and rare morphs and/or the occurrence of atavistic dental anomalies are revealed in three of five disjunctive populations. Given a substantial genetic component of dental variation (Zimmermann 1958; Stohl 1984; Rakitin et al. 2009; Anthonappa et al. 2013; Markova et al. 2013), the increased frequency of rare traits and occurrence of atavistic anomalies can be collectively interpreted as a release of cryptic phenotypic variation, which is normally hidden or found with low frequency within the species' core range. It is generally accepted that cryptic variation is invisible under normal conditions but fuel for evolution when circumstances change (Paaby and Rockman 2014).

The releases of hidden phenotypic variation in the disjunctive populations of *M. rossiaemeridionalis* appear to be a result of a founder effect reinforced by conditions of isolation. Genetic drift might either restrict the range of variability in dental complexity (population 5) or flatten the frequency distribution of molar complexity ranks by reducing the over-dominance of basic morphotypes at the expense of reserve ones (population 4).

Comparing the cases of released phenotypic variation with respect to the characters reflecting dental specialization to herbivory summarized from a number of paleontological studies (Guthrie 1971; Koenigswald 1980; Chaline et al. 1999; Borodin 2009; Fejfar et al. 2011), we can conclude that all of those cases in the disjunctive populations of sibling vole are counter-directional with the evolutionary trends or non-directional and might be considered as de-specialization of molar teeth. The tendency towards decreasing expression of the phenotypic traits reflecting adaptations to herbivory is not newly evolved in the invasive populations but pre-existed in the populations within the core range.

From a neontological perspective on evolution (e.g., Shvarts 1977), the differences between the disjunctive populations of sibling vole and the conspecifics within the core range could not yet be regarded evolutionarily significant, although the outlier populations 4 and 5 (Svalbard and Sovetskaya Gavan') have formally passed into the stage preceding a microevolutionary event. The outlier populations of *M. rossiaemeridionalis* accumulate dental phenotypic patterns that do not coincide with the evolutionary trends towards progressive specialization to herbivory inferred from the fossil record of the *Allophaiomys-Microtus* lineage. It means that de-specialization is not yet a trend but a state that could lead the populations to potentially different results. Will the populations retain the de-specialized pattern of dentition or will those signs of de-specialization disappear as maladaptive? We suppose that the answer depends on both extrinsic (environment and the necessity to shift the diet toward less abrasive food) and intrinsic factors (an ability of the species to overcome phylogenetic inertia). Examination of other invasive populations of *M. rossiaemeridionalis* may help asking those questions at the level of particular species.

The number of case studies revealing rapid morphological changes in micromammals invading distant areas and islands has increased during the last years (Renaud et al. 2013; Cucchi et al. 2014; Gargan et al. 2016). An example of taxonomically close species *M. arvalis* colonizing the Orkney Islands suggested that invasion events dated back to about 5000 years ago are characterized by initial rapid evolution followed by stasis (Cucchi et al. 2014). Methodology of geometric morphometrics used in that study is quite informative as an exploratory approach but it reveals no variation in complexity and has only a minor power to infer enamel irregularities. In contrast to geometric morphometrics, the morphotype-based approach allowed us to reveal complex m1 morphotypes III and IV even in a small sample from the Orkney Mainland (Markova et al. 2010). The specimens tend to exhibit quite a regular pattern of enamel ridges with a tendency to increase the degree of separation between the crown prisms at the expense of the anterior cap, thus suggesting that some populations on the Orkney Islands might reveal dental variation patterns that coincide with the evolutionary trends towards a progressive specialization. Principal component analysis on shape variables (Cucchi et al. 2014) does not contradict this hypothesis, although exact conclusions require direct assessment of the characters reflecting complexity and regularity of enamel ridges, and inclusion of other molars to the analysis.

When dealing with extant populations, incorporation of both upper and lower molars in the analysis of dental variation appears to be crucial because different parts of the tooth row show unequal probability of revealing genetic drift. Based on the analysis of complexity ranks, we found that m3 and M3 are more readily subject to random variation than m1 and m2. This result confirms previous reports of developmental modularity of the arvicoline dentition and once again emphasizes the quasi-independence of third molars in an arvicoline tooth row (e.g., Laffont et al. 2009).

A case of anomalous tooth development is revealed in the sibling vole in a fragmented habitat in Surgut City. An adult male exhibits atavistically simple M3s on both sides and a supernumerary tooth-like prismatic structure in the distal part of the upper left tooth row. Supernumerary teeth and tooth-like structures that have either erupted or remain unerupted in addition to the regular number of teeth are described in many taxa of mammal (Miles and Grigson 1990) and appear to have a strong genetic component in their aetiology (Anthonappa et al. 2013). In *Microtus*, supernumerary teeth and their vestiges might be found in the area of the diastema, as shown for *M. agrestis* (Keränen et al. 1999; Witter et al. 2005) and *M. longicaudatus* (Harris and Fleharty 1962). The only evidence of a supernumerary M4 is documented for *M. agrestis* (Winge 1881; Charles 2015). According to its description, the additional tooth was represented by a single prism completely separated from M3 (Winge 1881). In contrast to the vestigial teeth in the mesial part of the diastema (Keränen et al. 1999; Witter et al. 2005), the developmental basis of supernumerary M4s in *Microtus* has never been studied. Based on comparison with normal M3s in *Microtus* and in other avicolines, living and extinct, we suggest that the cylindrical tooth-like element is rather a structure originated from the abnormally developed M3 than a true supernumerary tooth. It appears on the background of the atavistic-looking M3s, which recapitulate some features of the prismatic arrangement and occlusal pattern of M3 known in extinct arvicolines. This anomaly results in a loss of fitness for the animal because of malocclusion and appears to represent a case of reducing complexity typical for mutant mammals (Harjunmaa et al. 2012). Disordered arrangement of prisms, simplified pattern of occlusal surface, and presence of an additional tooth-like element next to the left M3 collectively suggest that the posterior part of M3 is developmentally "handled" as a separate unit, or a developmental module, and the anterior and posterior parts of M3 are capable of changing independently.

Mechanisms underpinning the release of hidden phenotypic variability in the disjunctive populations of sibling vole deserve particular attention. The roles of genetic and developmental factors as well as functional significance are yet to be partitioned. Adaptive plasticity might also contribute to the accumulation of de-specialized phenotypes in the disjunctive populations. Recent studies indicate that environmentally driven plasticity plays a role in shaping mandibular morphology in rodents, in particular, in mice feeding on different types of food (e.g., Renaud et al. 2013; Anderson et al. 2014). Considering plastic and genetic components of dental complexity and regularity of enamel ridges, we can find indirect evidence for a genetic background of these characters in our previous studies. Highly significant differences in complexity of m1 were found among genotypes and among maternal lines in laboratory-bred *Lasiopodomys* (formerly, *Microtus*) *gregalis* kept and nourished under uniform conditions (Markova et al. 2013). The appearance of irregularity of enamel ridges (morphotype m1_Oec) was documented for a laboratory colony of *M. arvalis obscurus* (Rakitin et al. 2009). Being extremely rare in natural populations, m1_Oec increased its frequency in laboratory-bred voles kept under the same conditions. Of 1700 offsprings obtained from four males and ten females, about 9% possessed this morphotype. All individuals with this pattern of m1 originate from one female caught pregnant in the wild, suggesting that genetic factor plays a role in the expression of the m1_Oec pattern.

Increased frequency of rare phenotypic traits and abnormalities is rather common effect in populations of mammals and other vertebrates surviving bottlenecks and living under condition of isolation (e.g., Kovalenko 2003; Korablev 2016). The novelty of our result is that we show for the first time that all the varieties of phenotype changes in disjunctive invasive populations of a species, which has diverged within a highly specialized clade, might be described in terms of de-specialization.

The features of de-specialized dentition are described for the first time in recent Arvicolinae. However, the divergent patterns of trophic specialization among the descendants of the early Pliocene voles of a pro-*Mimomys* organization level have been recently discussed in connection with strong molecular evidence for the monophyletic origin of Arvicolini, mole voles Ellobiini, and steppe lemmings Lagurini (Abramson et al. 2009). This monophyletic group is widely radiative and includes groups with contrasting patterns of trophic specialization from true herbivores (*Microtus* sensu lato, Lagurini) to rhizophagous subterranean forms (Ellobiini). Such divergent patterns of specializations are strictly directional, which distinguishes them from non-directional patterns of de-specialization. However, the ancestral forms for the tribes included in this monophyletic group might have passed through stages of de-specialization, e.g., when the number of de-specialized phenotypes increased under conditions of small population size and/or isolation. Repeated appearance of true herbivores (Lagurini, *Allophaiomys-Microtus* sensu stricto, etc.) and lineages tending to specialize in rhizophagy - obligate (Ellobiini*)* or facultative (*Mimomys-Arvicola, Allophaiomys-Terricola*) - might be interpreted as an indirect evidence for possible accumulation of de-specialized phenotypes in ancestral stocks of some lineages before splitting.

From a paleontological perspective, the cases of de-specialization are of particular interest because they raise a challenge to reconsider once again a classical rule of progressive specialization, which is sometimes referred to as ‘Deperet’s rule of progressive specialization’ (Levit et al. 2006, but see Bokma et al. 2016 for a slightly different definition of the rule). De-specialization was previously described for domesticated animals and humans and was related to neotenic evolution (e.g., Coppinger and Smith 1983). The cases of de-specialization found in our study might not be interpreted as neotenic because they do not recapitulate the juvenile pattern of arvicoline dentition. However, they recapitulate the phylogenetic stages of the respective lineage, which in turn could be related to the stages of tooth morphogenesis (e.g., Renvoise and Montuire 2015). In our opinion, the possibility of a species to accumulate de-specialized phenotypes under condition of isolation illustrated in the example of sibling vole or in the example of domestication does not contradict the rule of progressive specialization but it accentuates that members of highly specialized clades of mammal might retain a capability to overcome the phylogenetic constraints, which orient them toward progressive specialization.

Another intriguing perspective is to test the hypotheses concerning the influence of fallback foods on tooth morphology, and to determine how much the possibility to process alternative foods can contribute to the survival of the species under conditions of food scarcity. It has been recently shown that efficiency of fallback food processing was quite important during the evolution of primates and might also be considered in the context of hominine evolution (Constantino et al. 2009). We can hypothesize that the increased frequency of de-specialized phenotypes revealed in *M. rossiaemeridionalis* appears to be maladaptive for a herbivore, though it might favor a transition to a more generalized diet and enhance the success during the transport, colonization, and establishment stages of the invasion process.

Our results show that the ongoing invasion of *M. rossiaemeridionalis* to distant regions in northern Asia and High Arctic provides unique evidence for the species capability to develop de-specialized phenotypes under conditions of small population size and/or isolation. Further studies are necessary to find out if this is a species-specific pattern or a more general phenomenon occurring in small isolated populations of specialized animals surviving the bottlenecks.

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