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RESEARCH ARTICLE

The genetic characterization of an isolated remnant population of an endangered rodent (*Cricetus cricetus* L.) using comparative data: implications for conservation

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Abstract Estimates of genetic diversity and phylogenetic affiliation represent an important resource for biodiversity assessment and a valuable guide to conservation and management. We have found a new population (Jawor—JW) of the common hamster *Cricetus cricetus* in western Poland that is remote from the nearest populations by 235–300 km. With the objective of genetically characterizing of this population, we compared it with other populations from Poland and Germany by taking into account sequences of four mitochondrial DNA genes and variation at 10 microsatellite loci. The JW population exhibited low levels of genetic diversity and allelic and haplotype richness, which likely reflects its extreme isolation. This factor, coupled with inbreeding and genetic drift, are major threats to JW. A neighbor-joining tree based on mtDNA haplotypes shows that JW clusters among samples representing the *Central* subgroup that is known from central Germany but that has not yet been identified in Poland. Findings presented here improve our understanding of the spread and diversification of the common hamster. We offer the following hypotheses to explain the observed pattern of mtDNA haplotype distribution: JW could be a byproduct of postglacial migrations or back-migrations from eastern refugia to the western part of Europe, or/and be a result of population and habitat fragmentation. We recommend translocation of individuals as an effective management strategy, both at the level of Central phylogeographic group and at the species level, to overcome the negative consequences of inbreeding and geographical isolation of the JW population.

Keywords *Cricetus cricetus* · Central Europe · Approximate Bayesian computation · Translocation · *Central* sublineage

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Introduction

In the escalating extinction crisis, both the range and the local abundance of many mammalian species have sharply decreased (Janzen 2001; Pekin and Pijanowski 2012). The common hamster *Cricetus cricetus* (L. 1758) is not an example of a "charismatic vertebrate". Thus, it is not surprising that there has been almost no attention paid to its preservation until the early 1990s (Weinhold 2008). In the past several decades, this small rodent, which inhabits agrarian ecosystems and steppe habitats, has undergone a major decline in numbers to near-extinction in western

and some central parts of its European range (e.g., Nechay 2000; Neumann et al. 2004, 2005; Ziomek and Banaszek 2007; Banaszek et al. 2011; Schröder et al. 2014; La Haye et al. 2014; Reiners et al. 2014; Surov et al. 2016). Interestingly, the common hamster has shown some potential for recovery following intensive ex situ and in situ conservation actions undertaken recently (e.g., Weinhold 2004; La Haye et al. 2010; Villerney et al. 2013). These management actions involve techniques such as: protective legislation, habitat restoration, fencing, establishing of captive-breeding programs for supplementation of wild populations, translocation, and monitoring (e.g., Kayser and Stubbe 2002; Jordan 2002; Weinhold 2004, 2008; Geske 2008; Kupfernagel 2008; La Haye et al. 2010; Villerney et al. 2013; O`Brien 2015). For example, on the local scale, attempts have been made to restock a population *via* translocation, i.e. the release of animals taken from a source population to enhance viability of a recipient population, known as augmentation, with the goal of genetic rescue or genetic restoration (see also Weeks et al. 2011). However, the genetic properties of donor and recipient populations, as well as the genetic consequences of such translocations, have not been extensively studied.

Genetic research on the common hamster has long been of interest (e.g. Neumann et al. 2004, 2005) and has generated a compelling picture of its evolutionary relationships and possible migration flows. Current genetic structure of *C. cricetus* is proposed to be an effect of several phenomena, such as partial extinctions, (re-)immigration, bottleneck events, in situ survival in refugia, genetic drift, and different migration routes. The negative consequences of small population size, inbreeding, lack of gene flow, and founder effects are more pronounced in populations from western Europe compared with populations from central and eastern regions (Neumann et al. 2005).

The currently observed range of *C. cricetus* has resulted mainly from a postglacial westward recolonization of Europe from different eastern refugia (Neumann et al. 2005). The history of its settlement in central Europe is however complicated due to repeated range expansions during the Quaternary climate oscillations. The trace fossils indicate that the common hamster was present in Poland from the Eemian interglacial (Nadachowski 1989). The westward migration from the European steppe zone was probably *via* two routes: a northern route across the European plains, and a southern route to the Carpathian Basin (Neumann et al. 2005). At the beginning of the Würm glacial stage, when the presence of steppe areas increased, the surge of westward expansion began to western Europe. When the glaciers expanded, a severe range retreat occurred, but the common hamster survived in the Carpathian Basin (Jánossy 1986; Neumann et al. 2005). At the end of the Würm glaciation, and in an interglacial that began 10,000–12,000 years ago, the range expanded again to encompass central and western Europe. At that time Poland became inhabited by new haplotypes derived from eastern refugia as well as from the Carpathian Basin (Banaszek et al. 2010; Korbut and Banaszek 2016).

Cricetus cricetus comprises several mitochondrial haplogroups representing western, eastern, and southern phylogeographic lineages (Neumann et al. 2005; Banaszek et al. 2012). Representatives of the *North* lineage in western Europe are organized into two clades: populations from western Europe (*West*) and populations from the central regions of Germany (including Saxony-Anhalt and Thuringia) (*Central*), harboring multiple mtDNA haplotypes (Neumann et al. 2004, 2005; Schröder et al. 2014). However, many aspects of the phylogenetic relationships are still unresolved, concerning for example the branching pattern in the eastern part of the species range. One group, denoted as *E*, spans several lineages with a western distribution border in central Europe; a *Pannonia* group is restricted to the Carpathian Basin and the southern part of Poland (Banaszek et al. 2010).

In Poland, individuals representing two lineages have been described to date: *P3*, which clusters inside the *Pannonia* group, and *E1*, one of a number of *E* lineages, whose western border lies in eastern Poland. This latter lineage is also present in the neighboring Ukraine from where it probably originates (Neumann et al. 2005; Banaszek et al. 2010). The *P3* populations inhabited Polish areas from source populations in Moravia and Slovakia using the natural route through the Moravian Gate valley between the Sudetes and the western Carpathian Mountains in the Czech Republic (Ziomek and Banaszek 2007; Banaszek et al. 2009, 2010, 2011). In Poland, individuals representing these two lineages are found in close proximity, but the central part of Małopolska Upland creates an effective topographic and ecological barrier between them (Banaszek et al. 2012). In the majority of cases, Polish populations are geographically isolated, but some of them may have had contact with populations from western Ukraine (Korbut et al. 2013). Polish populations have diversity indices intermediate between genetically strong *Pannonian* and *Central* populations and genetically depauperate *Western* populations (Banaszek et al. 2011).

We focused on one particular Polish population located in the western part of the country (Jawor population, hereafter JW), representing the only Polish population remaining west of the river Oder. Geographically situated among different phylogroups (*Central, Pannonia*, and *E1*), this population may harbor unique haplotypes and may constitute a group of immigrants distinct from those already identified in Poland. Moreover, compared with populations occurring in core areas of distribution, this significantly isolated population offers

an ideal model for studying levels of inbreeding and the potential loss of genetic diversity. Genetic properties of this remnant population need to be considered when making conservation decisions.

The aims of this study were (1) to define the mtDNA phylogeographic group to which JW belongs and to characterize evolutionary relationships among the populations examined representing the *Pannonia, West, Central* and *E1* phylogroups, (2) to provide input regarding genetic parameters estimated from microsatellite data for analyzed populations, including the level of genetic diversity, structure, and effective population size, (3) using evidence from these data, to provide information useful for prioritizing the allocation of limited conservation resources in order to preserve populations of the common hamster. This information might be valuable in (1) determining a potential donor population for a translocation program, (2) future evaluation of translocation.

Materials and methods

Material and sampling

Here, we present a re-analysis of a previously published SSR data set comprising 219 individuals from Poland and Germany (Banaszek and Ziomek 2011; Banaszek et al. 2012; Reiners et al. 2011a, 2014) and 63 newly scored individuals from two Polish populations (Silesian Region) using the information provided in the ten SSR marker genotypes, Table 1, Table S1.

We sequenced four coding and non-coding mtDNA regions (in total 226 sequences) in 61 accessions, from 1 to 22 samples from each sampled field population (10) and from four samples denoted as MAM1-4, obtained from pelts of specimens collected in 2012–2015 and deposited in 2015 by collectors K. & U. Mammen in the Berlin Museum, Germany. As only a few individuals were available for each MAM sample, no SSR analysis was performed. However, the SSR analysis was completed on individuals

Table 1 Characteristics of a population sample set of the common hamster *Cricetus cricetus* used in this study

Country	Field ID	STRUCT-URE cluster	Locality	code	n*	Lat./ Long.	mtDNA lineage
Poland	1	1	Jaworzno	JRZ	45/6	N: 50.17°–50.19° E: 19.30°–19.38°	*Pannonia*
	2	2	Jawor	JW	18/22	N:51.05°–51.27° E: 16.14°–17.16°	*Central*
	3	3	Opatów	OP	38/5	N: 50.80° E: 21.42°	*E1*
	6	6	Radymno	RAD	10/5	N: 49.95° E: 22.82°	*Pannonia*
	7	6	Szczyglice	SZ	10/5	N: 50.67° E: 21.32°	*E1*
Germany	4	4	Borsum	BOR	20/2	N: 51.10°–52.21° E: 10.01°–10.04°	*Central*
	5	5	Quedlinburg	QL	24/1	N: 51.78°– E: 11.22°	*Central*
	8	7	Dexheim	KIL	19/2	N: 49.78°–49.85° E: 8.23°–8.32°	*West*
	9	8	Flörsheim	FL	27/1	N: 50.02°–50.03° E: 8.41°–8.44°	*West*
	10	8	MKK	MKK	71/2	N: 50.16°–50.22° E: 8.78°–8.92°	*West*
Germany	11	–	Klitschmar	MAM 1	0/4	N: 51.46°–50.22° E: 12.23°–12.27°	*Central*
	12	–	Kroppenstedt	MAM 2	0/3	N: 51.78°–52.14° E: 11.31°–11.44°	*Central*
	13	–	Nordhausen	MAM 3	0/2	N: 51.47°–51. 50° E: 10.78°–11.29°	*Central*
	14	–	Querfurt	MAM 4	0/1	N: 51.38° E: 11.60°	*Central*

*The first number in the column refers to the number of individuals analyzed using the simple sequence repeat analysis, and the second number (after slash) refers to the number of individuals analyzed by sequencing mitochondrial DNA (for details, see text and Table S1)

from the closest locality in Quedlinburg (Germany), ca. 12 km E from MAM2, Table 1, Table S1.

From field populations of *C. cricetus* hair samples were collected from individuals using noninvasive hamster-specific hair traps in 2015 (Reiners et al. 2011a, Fig. 1; Table 1). DNA was extracted from at least ten hairs per individual using a standard Chelex 100 (Bio-Rad) protocol (Walsh et al. 1991) modified by Reiners et al. (2011b). Samples are preserved at the Department of Genetics, Adam Mickiewicz University, in Poznań, Poland.

Mitochondrial sequencing

We used four partial mitochondrial genes [control region (ctr), 16 S rRNA (16 S), cytochrome *b* (Cyt b), and a subunit of cytochrome *c* oxidase (*COI*)] to identify the lineages to which the analyzed individuals belong. We identified all newly scored individuals using the ctr control region. It was not possible to obtain a full data set from all accessions across all four loci. We performed DNA fragment assembly and a quality assessment with the help of SeqTrace (Stucky 2012). The final corrections were done by hand. To concatenate gene datasets, we employed Sequence Matrix v. 1.8 (Vaidya et al. 2011). For the final alignment, we trimmed sequences to the greatest length of 2072 base pairs (bp) and then used the data set to construct neighbor-joining (NJ) and maximum likelihood (ML) trees (Saitou and Nei 1987) with the help of MEGA v. 6.0. (Tamura et al. 2013).

We used the most suitable distance measure, the T92 + 0.05G distance model (Tamura 1992), for a neighbor-joining tree construction based on mtDNA sequences, and HKG + G for maximum likelihood tree construction. We chose the model using MEGA v 6.0 (Tamura et al. 2013), treating *COI* (1–642bp) and Cyt *b* (643–1380) genes as two coding regions, and 16 S (1381–1910bp) and ctr (1911–2072bp) as coding rRNAs and a non-coding DNA region, respectively; we used a gaps/missing data approach "all sites," and the number of discrete gamma categories equal four.

A bootstrap analysis, based on 1000 replicates, compared similarities and differences between trees using TOPD/FMTS (Puigbò et al. 2007). We performed a translation into amino acid sequences using MEGA v. 6.0 to check for stop codons and non-functional coding sequences and to identify amino acid substitutions in protein-coding regions.

We conducted a statistical parsimony analysis with TCS v. 1.21 (Clement et al. 2000) to generate a haplotype network between mtDNA sequences. The connection limit was set to 95% as proposed by Hart and Sunday (2007). We performed a visualization of the network using tcsBU (Santos et al. 2015).

The mtDNA sequences have been deposited into the GenBank database (http://www.ncbi.nlm.nih.gov/GenBank), Table 2 using the submission tool BankIt (http://www.ncbi.nlm.nih.gov/BankIt/.

Population genetics analysis based on microsatellite markers

To characterize analyzed populations genetically, we employed a data set of 10 microsatellite markers (Ccrμ 10, 11, 12, 15, 17, 19, 20, IPK 03, 05, 06) (Neumann

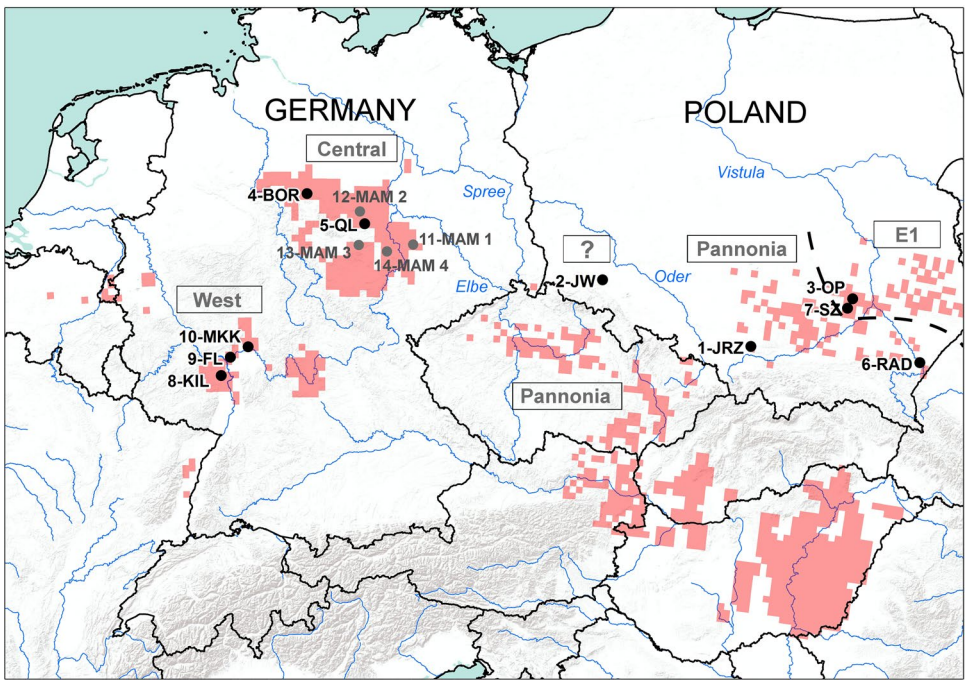

Fig. 1 Location of sample sites throughout Poland and Germany. Samples analyzed using microsatellite markers and mtDNA are black. Populations represented by 1–4 individuals used only in mtDNA phylogenetic analysis are grey. A Polish unique JW population was indicated by a question mark. Distribution of the common hamster after Reiners et al. (2014) modified based on information stored in The Institute of Nature Conservation PAS, http://www.iop.krakow.pl/ssaki/. Table 1 provides numbers referring to sampling sites

Table 2 Genbank accession numbers for four Sanger sequenced mitochondrial genes (control region (ctr), 16 S rRNA (16 S), cytochrome *b* (Cyt *b*), and a subunit of cytochrome *c* oxidase (*COI*))

Field population mtDNA	Structure cluster SSR	Cyt *b*	*COI*	16 S	ctr
1	1	KX827011-15	KX827059-64	KX827118-23	KX827176-81
2	2	KX827016-37	KX827065-85	KX827124-45	KX827182-203
3	3	KX827038-41	KX827086-9	KX827146-50	KX827204-8
6	6	–	KX827093-7	KX827154-8	KX827212-16
7	6	KX827045-9	KX827098-102	KX827159-60	KX827217-21
4	4	KX827042-3	KX827090-1	KX827151-2	KX827209-10
5	5	KX827044	KX827092	KX827153	KX827211
8	7	KX827050-1	KX827103-4	KX827161-2	KX827222-3
9	8	KX827052	KX827105	KX827163	KX827224
10	8	KX827053-4	KX827106-7	KX827164-5	KX827225-6
11	–	KX827055	KX827108-9, KX827114, KX827117	KX827166-7, KX827172, KX827175	KX827227-8, KX827233, KX827236
12	–	KX827056	KX827112-3, KX827116	KX827170-1, KX827174	KX827231-2, KX827235
13	–	KX827057	KX827110-1	KX827168-9	KX827229-30
14	–	KX827058	KX827115	KX827173	KX827234

and Jansman 2004; Jakob and Mammen 2006). Protocols S1 presents all PCR reaction condition guidelines with primer sequences for mtDNA PCR amplification and details concerning the SSR and mtDNA analyses.

Using PowerMaker v. 3.25 (Liu and Muse 2005), we calculated average values of summary statistics for loci within each sampled population; we then used the same program to test the Hardy–Weinberg equilibrium for each locus. We employed ADZE (Allelic Diversity Analyzer) to calculate allelic richness within the sampled populations corrected for sample size (Szpiech et al. 2008). We tested a genotypic linkage disequilibrium with the Fisher`s method using a Markov chain (dememorization 10,000, batches 100, iterations per batch 5000) with the help of Genepop v. 4.2 (Raymond and Rousset 1995) and estimated the null allele frequency with the help of ML-NullFreq (Kalinowski and Taper 2006). With the help of FreeNa (http://www.montpellier.intra.fr/CBGP/software/FreeNa), we used the so-called ENA method (Chapuis and Estoup 2007) to perform F_{ST}-refined estimation by excluding null alleles.

To identify population structure and assign individuals to populations of their origin, we employed STRUCTURE v. 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). To imagine relationships between sampled populations, we undertook several distance-based approaches with the help of TREEFIT (Kalinowski 2009); details of this analysis are presented in Supplementary materials S1. We compared observed genetic distances between populations with the fitted genetic distances within the NJ tree and UPGMA using TREEFIT (Kalinowski 2009). We quantified the proportions of variation in the matrix of these distances explained by the NJ or UPGMA trees as R^2, displaying the resulting NJ tree in TreeView v. 1.6.6.

We assessed the effect of drift within each sampled population by employing pairwise F_{ST} for each STRUCTURE cluster against all the other following standard AMOVA as in Weir and Cockerham (1984) with the help of GenPop v. 4.2 (Rousset 2008), presenting average estimates. Because gene flow mitigates the negative effect of drift in small populations by restoring genetic variation and preventing inbreeding, we assessed recent migrations among the distinguished STRUCTURE groups using a Bayesian Markov chain Monte Carlo approach as implemented in BAYESASS v. 3.0 (Wilson and Rannala 2003). We visually assessed MCMC mixing and convergence using TRACER v. 1.6. (Rambaut et al. 2007). The isolation by distance pattern (IBD) was evaluated by assessing the correlation matrix between genetic distance and geographic distance using a Mantel`s test, as implemented in GenAlEx v. 6.5 (Peakall and Smouse 2006, 2012).

Approximate bayesian computation (ABC) analysis

We inferred effective population sizes of the STRUCTURE clusters based on an SSR data set using ABC as implemented in DIYABC v. 1.0.4.37 (Cornuet et al. 2008, 2010), available from http://www1.montpellier.inra.fr/CBGP/diyabc/. Beaumont et al. (2002) provide a full description of the ABC method. Figure 2 provides a graphic representation of competing scenarios. Models` formulation were based on our preliminary analysis (not shown). We confronted a broad spectrum of ABC explanatory models which differ in a level of complexity and a pattern of

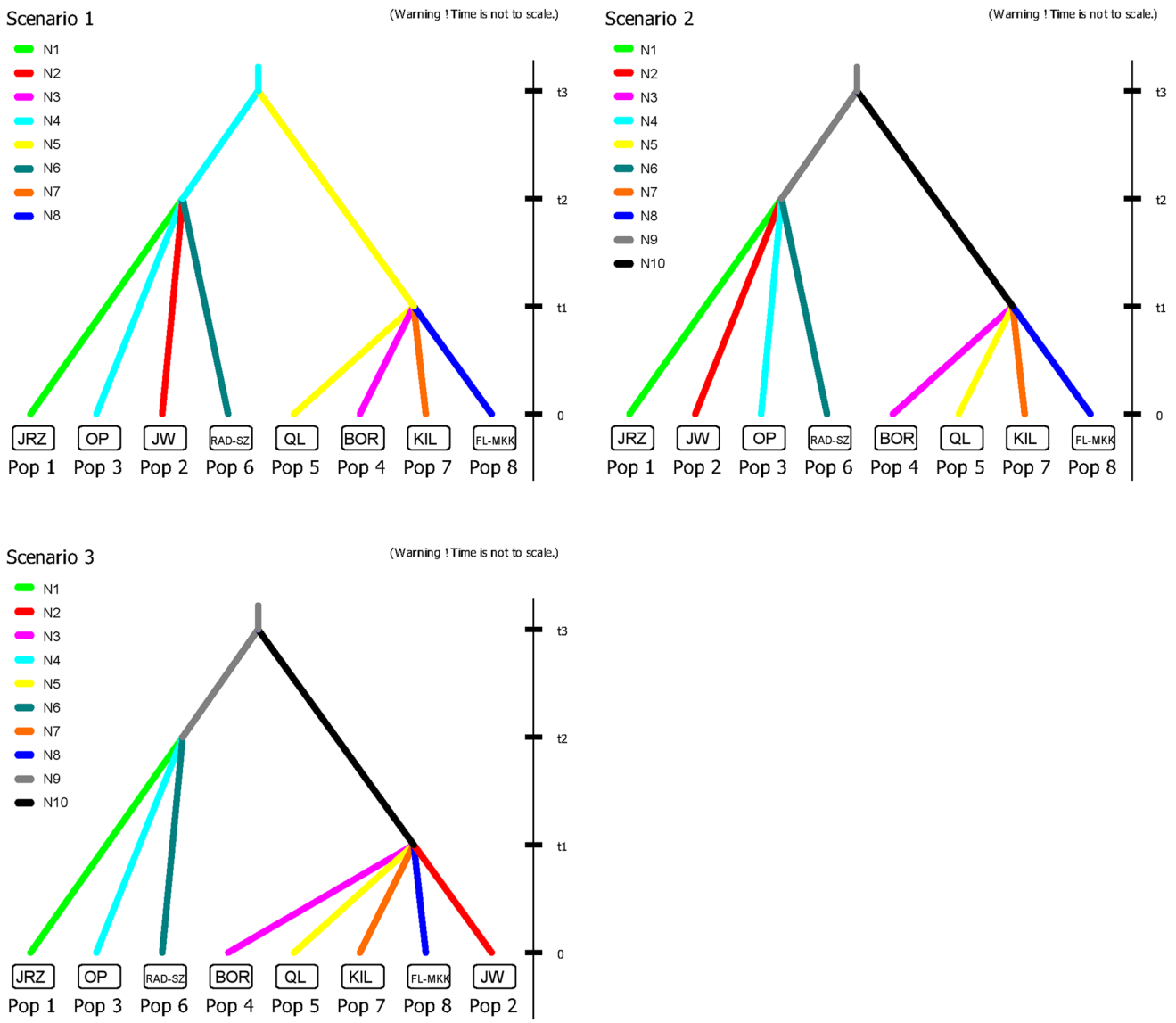

Fig. 2 Graphic representation of competing scenarios (sc.) modelled in DIYABC for *Cricetus cricetus*, focusing on the origin of Polish JW population and splits (sc. 1) Population split model assuming the origin of Polish and German populations (1–3, 6 and 4–5, 7–8, respectively) from currently sampled populations (3 and 5); population 2 (JW) is situated among Polish populations; (sc. 2) model assuming the origin of currently sampled populations from two unsampled ancestral populations (*N9* and *N10*); JW population is situated among Polish populations; (sc. 3) is modified sc. 2, simulating the origin of JW among German populations. The populations can have different effective population sizes (N_{1-10}); the split events occurred at t_1–t_3 generations ago, t_o-time of sampling. Time is not at scale. STRUCTURE populations, see Table 1. Each scenario is assumed to be equally probable, meaning that these models equally likely explain the data. (Color figure online)

diversification. Based on the posterior probability distribution and a credibility interval for the parameter of interest, we chose three models (presented), that best fit to the data. Table S2 presents prior distribution of the simulated parameters (demographic, historical, and mutational) used in the ABC analysis. We tested different prior interval specifications for the mean microsatellite mutation rate across loci because these priors likely strongly influenced estimates of effective population size (see Lye et al. 2011). Supplementary materials S1 describe the remaining ABC analysis details.

To compare the results of the ABC calculations concerning effective population size with another method we used a linkage disequilibrium method, which is powerful for small populations (Waples and Do 2010). An algorithm was implemented in LDNe (Waples and Do 2008). The *Ne* was calculated using the jackknife method for determination of 95% confidence intervals of *Ne*.

Results

Mitochondrial sequences were unambiguously aligned to 2076 base pairs (bp) comprising 530 bp (16 S), 645 bp (*COI*), 739 bp (Cyt *b*), and 162 bp (ctr). The 18 haplogroups comprised unique concatenated sequences in the entire data set of 226 sequences, under the 95% "parsimony" criterion, of which 63 positions were variable and 32 were parsimony informative. The mean base frequencies were as follows: A = 28.5%, C = 23.5%, G = 20.9%, T = 27.1%. Table 3 presents basic mtDNA statistics.

The phylogenetic analyses using NJ and ML approaches (the latter available upon request) produced congruent topologies regarding three well-supported groups: (1) those containing almost all samples from Germany and JW haplotype; (2) samples from Poland belonging to *E1*, and (3) samples from Poland belonging to *Pannonia* (Fig. 3a, Table S1). In the first group, it is interesting to discover that the JW population shares mitochondrial similarity with populations MAM1, 3 from central Germany, some 300 km away. The sample belonging to the *E1* lineage appeared to be more closely related to the *Central* samples than the *Pannonian* samples. The data also lend support (bs

Table 3 Basic statistics of mtDNA data set used in this study

mtDNA	n	Length [bp]	π	[SE]	C	V	Pi	S
16 S	18	530	0.0073	0.0026	517	13	8	5
COI	18	642	0.0118	0.0039	621	21	13	8
ctr	18	162	0.0238	0.0212	156	6	6	0
Cyt *b*	11	738	0.0083	0.0026	719	19	6	13
total	18*	2072	0.0102	0.0021	2013	59	33	26

n number of different sequences, *number of haplotypes based on concatenated sequences, π *[SE]* nucleotide diversity [standard error], *C* conserved sites, *V* variable sites, *Pi* parsimony-informative sites, *S* singleton sites

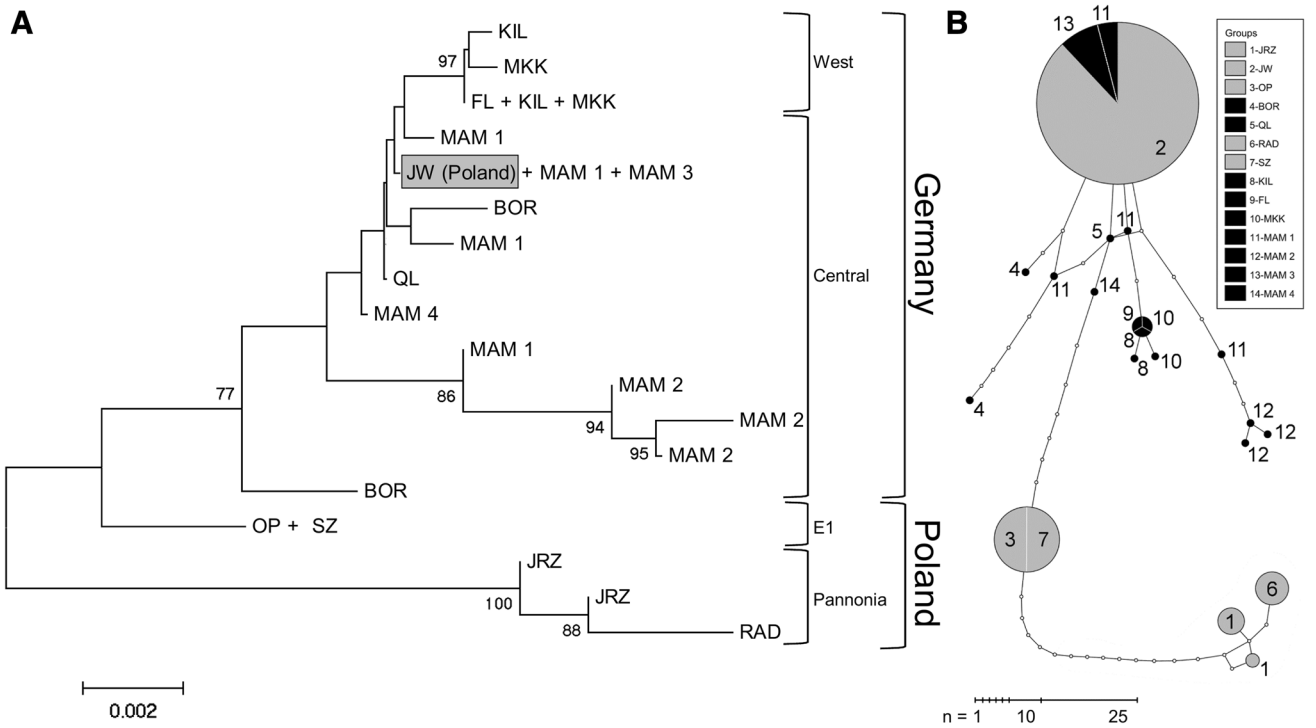

Fig. 3 **a** Neighbor-joining tree of based on mtDNA haplogroups of the common hamster; tree is based on the Tamura 3-parameter distance measure and bootstrap method with 1000 bootstrap replicates with gap/missing data treatment—pairwise deletion. For more details, see Fig. 1 and Table 1, S1. Abbreviations *E1* eastern phylogeographic group; **b** Haplotype network of concatenated sequences for the mitochondrial genes ctr, 16 S, Cyt *b*, and *COI*. *Circle sizes* correlate with haplotype frequencies, note that the frequencies are related to the number of individuals sequenced. *Empty circles* refer to missing intermediates. Poland: *grey*; German: black

96%) to grouping of western populations (*West*) within the first group. The mean distance between analyzed 18 haplogroups is 1.05% (range from 0.0005 to 0.03), as shown in Table S3. Nevertheless, ML and NJ trees also include polytomies, which render the trees unsuitable for statistically evaluating their similarities and differences. The dataset (~2000 bp) is inapplicable for inferring accurate phylogenetic relationships for samples from central Germany and JW, although within the *Pannonia* and *E1* (Fig. 3a) a deep node can be resolved using only a single region (16 S rRNA). In summary, the JW sample clusters unsupported among the samples from central Germany representing the *Central* phylogeographic subgroup. There is a strict correspondence between the results of the NJ tree and parsimony network (Fig. 3b). The network connects JW with closely related, geographically cohesive haplotypes from central Germany (MAM1, 3) representing the *Central* phylogeographic group.

A number of mtDNA haplotypes were unique to sampling locations, thus geographic clustering is observed. The most frequent haplotype 6 (41%) was noted in three sampling locations JW, MAM 1 and MAM 3, and required six unsampled haplotypes to connect to its closest Polish sampled haplotype (see also Table S1). The mtDNA haplotypes found in JW were previously published for samples originated in Germany, e.g., 16S: GenBank accession number: AJ633741.1 (Neumann et al. 2005), Cyt *b*: AJ633762.1 (Neumann et al. 2005), ctr: e.g., AJ550197.1 (Neumann et al. 2005) or KC953782 (Schröder et al. 2014), and *COI*: e.g. KC953805.1 (Schröder et al. 2014).

The alignment of coding sequences appears to be in frame, i.e. it does not contain premature and terminal stop codons or non-functional coding sequences. The lengths of amino acid sequences of Cyt *b* and *COI* genes are 245 and 214, respectively. The amino acid translations produced five unique protein sequences for Cyt *b* and four for *COI*, similar to those previously described in the GenBank database (similarity ranged from 99 to 100%). Individuals from JW and two other German populations show one unique amino-acid change (Cyt *b*: leucine instead of methionine or valine), as shown in Table S4.

Populations—genetic differentiation

The analysis of the population genetic structure based on SSR markers showed eight STRUCTURE clusters, one of which is JW. We found that the delta K value was highest at K = 8 and we found consistent results between runs at K = 8 (Fig. 4). The clusters identified in the run with the highest estimated probability reflect the samples' close geographic proximity. The STRUCTURE results suggest also the presence of a small number of individuals with admixed ancestry. Based on Q-matrix of the 283 individuals studied, 92.58% had a coefficient of membership equal to or higher than 0.80 to their identified cluster. The presence of these individuals is attributable to shared ancestral polymorphism, gene flow, admixture events in the past, or unsampled populations.

To infer relationships between field populations (10), we also measured and graphically displayed pairwise genetic distances using a clustering algorithm on NJ. The highest average value of R2 (R2 = 0.978) was for the NJ tree based on Theta (θ) (see Table S5, Fig. 5). The NJ tree derived from corrected genotype data for null alleles shares the same tree topology (not shown). The NJ tree showed two distinct groups (Fig. 5) clearly associated with their geographical distribution and partially reflecting the samples' phylogeographic affinities. The first group comprises samples originating from Poland and representing *Pannonia, E1* and the JW population, whereas the second group contains samples from Germany that are considered the *West* and *Central* subgroups. Incongruences are visible between relationship hypotheses regarding the origin of Polish populations based on two types of markers (mtDNA and SSR).

Populations—genetic diversity

We calculated genetic summary statistics for eight STRUCTURE clusters (Table 4). The clusters appeared highly correlated with the sampling locations. Mean *He* values over all loci varied between 0.346 (JW) and 0.743 (QL) with an average of 0.587. Observed heterozygosity (*Ho*) was lower than *He* in all clusters, as it was extremely low in JW. All loci were in H–W disequilibrium in the JW and JRZ clusters: nine loci in FL and MKK; six in KL; four in BOR, OP, and in a cluster composed of RAD and SZ, and two in QL. Nine pairs of loci across all clusters (20%) showed highly significant linkage disequilibrium and thus signs of hidden genetic structure. We found a consistent pattern in the occurrence of null alleles across loci for three STRUCTURE clusters (1) JRZ, (2) FL and MKK, and (3) RAD and SZ, indicating a Wahlund effect. Table S6 presents values of F_{ST} estimations for each pair of populations with and without the ENA correction for null alleles. Regardless of the correction, F_{ST} values indicate isolation between populations. The parameter F_{ST} standardized by the maximum value, which has been shown to provide more accurate measures when polymorphism is high, indicates a strong population structure and near-fixation of different alleles within populations (F'_{ST} = 0.842).

All analyzed STRUCTURE clusters showed moderate allelic richness values (mean A_r = 4.01), but the value was the lowest for JW. The number of private alleles in JW was higher than that in the clusters representing the *West* subgroup, but lower than those in the other populations, Table 4.

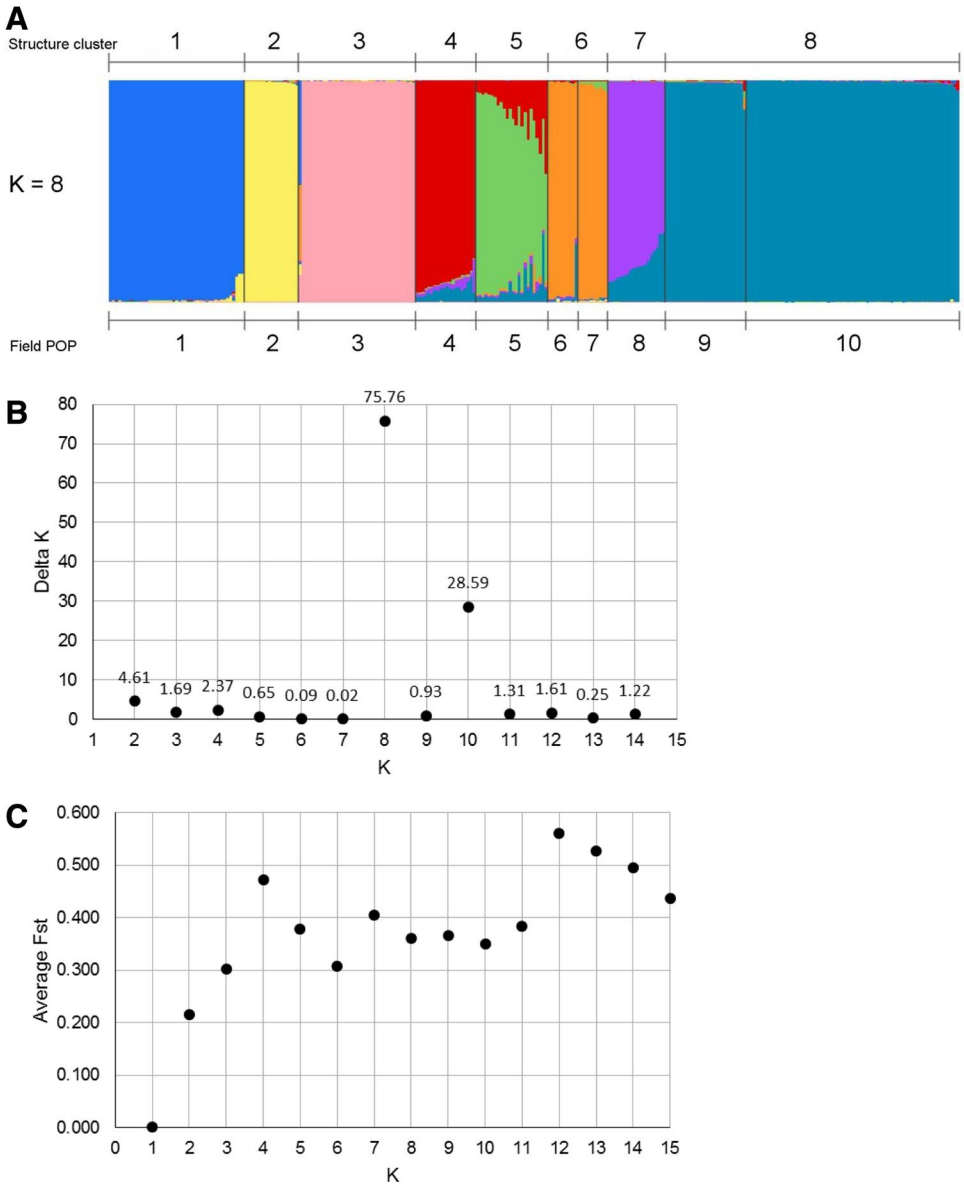

Fig. 4 **a** Bayesian clustering analysis, inferred from STRUCTURE and STRUCTURE HARVESTER, of the common hamster (*C. cricetus)* data (283 individuals/10 SSR loci) collected from Poland and Germany (see Fig. 1). Different colors indicate the assignment probability to different demes (K = 8); each individual is represented by vertical bars shaded in proportion to its ancestry; **b** Evanno`s Delta K values of the K values tested; **c** average F_{ST} values of the K values tested. (Color figure online)

Migrations and drift

All treatments performed based on the SSR data set indicate substantial differences among analyzed STRUCTURE clusters and a very low level of gene flow. The pairwise F_{ST} values between a given cluster and the others varied between 0.091 and 0.296, but the highest values were observed for samples obtained from three Polish clusters (JW, JRZ and OP), indicating barriers to gene flow and the most pronounced effect of drift.

The pattern of migration rates within a few generations indirectly estimated by BAYESASS showed that the proportion of individuals originating from within each identified STRUCTURE cluster (non-migrants) varied from 89.97 to 97.82%, with the highest and lowest values found in the German populations. The general picture that emerges is that the analyzed clusters are strongly isolated (Table S7). Significant isolation by distance – based on the Mantel′s test – was found (r = 0.608, $P < 0.010$ from 10,000 randomizations).

Inferring effective population sizes with DIY ABC

In the first step, ABC based on the SSR data set yielded the strongest support, using both the direct and logistic approach, for scenario 2. The use of larger datasets 3×10^6 did not improve the best scenario's probability over the use of 3×10^5 datasets. In scenario 2 the genetic STRUCTURE clusters, correlated to some extent with geographical location and characterized by different effective population sizes, were founded independently from two undetected and thus unsampled ancestral populations.

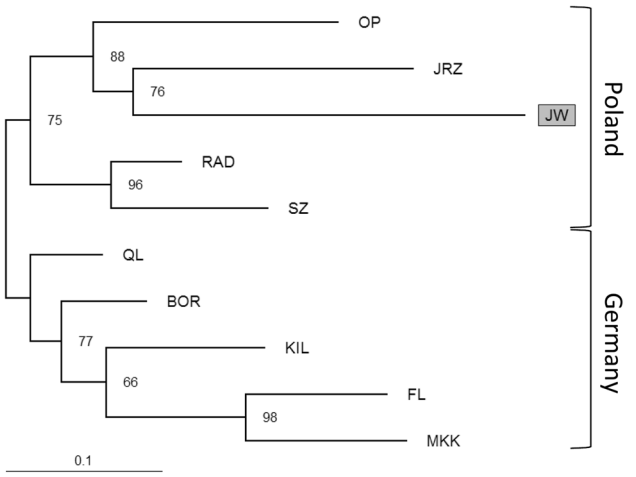

Fig. 5 Neighbor-joining tree based on the Theta distance (θ) distance matrix of microsatellite genotypes and bootstrap method with 10,000 bootstrap replicates. For detailed information related to populations, see Fig. 1 and Table 1, S1

Relative posterior probabilities of investigated scenarios do not show strong conflictual differences between the logistic and direct approach. Nevertheless, when we used the logistic approach, which is more discriminant than the direct approach, the best-supported model appeared to be scenario 2 (set 1 priors), but when we applied the direct approach, the same scenario was preferred, but with the set 4 of prior values. Scenario 2's posterior probabilities ranged from 0.81 (95% CI: 0.78–0.83) (set 3 priors) to 0.90 (0.88–0.915) (set 1 priors) using the logistic approach and from 0.44 (0.001–0.87) (set 3 of priors) to 0.50 (0.06–0.94) (set 4 of priors) using the direct approach (Table S8). The remaining scenarios, which assumed different origins of the JW population and different ancestral lineages, were poorly supported statistically regardless of the priors used.

Given that the results clearly favored scenario 2 (Table S8–9), we inferred the posterior distributions of parameters for this model only. The goodness-of-fit test of the scenario 2 parameter posterior combinations found five statistical differences between observed and simulated summary statistics (SuSt), (set 1 priors). Discrepancies in four of 116 of SuSt were found using the second set of prior values, whereas seven differences were found with the third set and only two using the fourth set of priors, Table S10. Table 5 presents results of the posterior distributions of parameters.

According to the methods of Cornuet et al. (2010), we estimated confidence in the model choice as Type I and II error rates from the 500 pseudo-observed data sets (PODs), (Table S9). Type I error was determined by calculating the number of cases in which the selected scenario did not have the highest posterior probability, while 500 PODs were accomplished under the best-supported scenario. Type II error rates were estimated as the proportion of cases in which the best scenario had the highest probability, while the 500 PODs were modeled with another scenario. Using the direct estimates and logistic approach methods, Type I error amounts to 11.6–18.3%, whereas Type II errors ranged from 0.00 to 12.9%.

Regardless of priors used, the mean effective population size of JW was the smallest among analyzed populations, estimated as 539–864 individuals (see Table 5). We obtained the largest values of *Ne* (~6000–7000 individuals) for the populations from central Germany (QL, BOR) and the STRUCTURE cluster, containing Polish samples belonging to two different mtDNA lineages [RAD and SZ], (see Figs. 3a, 5). Between ABC parameter estimates of *Ne*

Table 4 Summary statistics for the STRUCTURE clusters (1–8) of the common hamster *C. cricetus* from Poland and Germany based on 10 microsatellite markers

STRUCTURE cluster (n)	MAF mean	Mean # of genotypes	$P_{A\ (SE)}$	*Ar* (SE)	Mean Gene Div	Mean *Ho*	Mean PIC	*F*	F_{ST}
1. JRZ (45)	0.578	7.2	0.92 (0.163)	3.44 (0.217)	0.564	0.235	0.517	0.5926 (0.0338)	0.2368
2. JW (18)	0.750	3.5	0.245 (0.082)	2.30 (0.254)	0.346	0.128	0.3175	0.7699 (0.0434)	0.2962
3. OP (38)	0.458	11.2	1.79 (0.286)	4.08 (0.293)	0.660	0.6395	0.616	0.0607 (0.0291)	0.2301
4. BOR (20)	0.420	11.3	0.45 (0.180)	4.89 (0.366)	0.710	0.615	0.682	0.1641 (0.0453)	0.0988
5. QL (24)	0.375	12.4	0.66 (0.127)	5.52 (0.353)	0.743	0.692	0.711	0.1113 (0.0378)	0.0901
6. RAD/SZ (20)	0.410	10.3	0.71 (0.184)	5.20 (0.439)	0.708	0.630	0.672	0.1852 (0.0421)	0.1344
7. KIL (19)	0.5395	6.1	0.07 (0.051)	3.43 (0.322)	0.571	0.453	0.522	0.3569 (0.0590)	0.1306
8. MKK/FL (98)	0.582	10.5	0.07 (0.025)	3.21 (0.262)	0.534	0.355	0.491	0.3006(0.0265)	0.2035

Abbreviations: average values: *MAF* Major Allele Frequency, *PIC* Polymorphism information content (POWERMAKER), *PA* mean number of private alleles per STRUCTURE population, *Ar* allelic richness, *P*A and A*r* were calculated using a rarefaction approach (subsample of size g=20), (ADZE); *F* inbreeding coefficients (BAYESASS), *FST i* population vs the other populations

Table 5 Results of ABC analysis for estimating demographic and mutational parameters of *Cricetus cricetus* populations for scenario with the strongest support employing four sets of prior values; for detailed specification of priors, see Table S2

Set 1									
Parameter	Mean	Median	Mode	q025	q050	q250	q750	q950	q975
N1	2070	2110	2180	1690	1760	2000	2180	2240	2260
N2	782	810	824	401	452	659	921	1030	1060
N3	2600	2490	2220	1550	1650	2060	3080	3870	4100
N4	5760	5630	5370	5290	5310	5440	5970	6600	6770
N5	5930	6070	6280	4510	4980	5820	6220	6320	6340
N6	6140	6080	5930	4940	5020	5600	6660	7430	7560
N7	1530	1490	1380	928	1010	1250	1770	2210	2300
N8	1900	1810	1610	1520	1540	1650	2020	2600	2790
N9	16,500	15,200	12,800	12,900	12,900	13,600	18,300	24,700	26,000
N10	3830	3620	2620	312	498	1970	5620	7730	8030
Âµmic_1	3.02×10^{-4}	3.06×10^{-4}	3.39×10^{-4}	1.99×10^{-4}	2.12×10^{-4}	2.65×10^{-4}	3.42×10^{-4}	3.76×10^{-4}	3.81×10^{-4}
pmic_1	0.193	0.19	0.183	0.114	0.119	0.159	0.226	0.273	0.281
Set 2									
Parameter	Mean	Median	Mode	q025	q050	q250	q750	q950	q975
N1	1540	1360	1020	662	737	1040	1820	2870	3390
N2	539	514	500	242	279	408	638	895	981
N3	3140	3050	2900	1590	1770	2470	3720	4740	5100
N4	4500	4470	4910	2020	2340	3530	5430	6690	7010
N5	5470	5510	5600	3690	3990	4880	6110	6860	7050
N6	5950	6280	7390	2490	3120	5000	7110	7770	7880
N7	1900	1820	1560	1000	1090	1470	2240	2980	3210
N8	2400	2220	2120	1000	1160	1760	2810	4210	4960
N9	13,900	13,000	7330	3420	4070	7880	19,400	26,600	28,000
N10	2250	1680	1110	1030	1050	1260	2610	5780	7220
Âµmic_1	3.03×10^{-4}	3.31×10^{-4}	3.31×10^{-4}	1.93×10^{-4}	2.04×10^{-4}	2.64×10^{-4}	3.46×10^{-4}	3.78×10^{-4}	3.82×10^{-4}
pmic_1	0.205	0.205	0.174	0.118	0.129	0.17	0.24	0.28	0.287
Set 3									
Parameter	Mean	Median	Mode	q025	q050	q250	q750	q950	q975
N1	2510	2340	2160	1030	1180	1790	2990	4260	5390
N2	704	669	589	307	348	524	864	1150	1270
N3	3380	3310	3460	1750	1930	2720	4000	5090	5360
N4	6930	7030	7070	5380	5600	6590	7400	7730	7790
N5	6300	6370	6630	4580	4990	5900	6770	7310	7460
N6	6730	7010	7630	4010	4710	6160	7540	7870	7920
N7	2160	2080	2030	1120	1250	1690	2520	3360	3730
N8	2120	1890	1420	663	804	1370	2530	4250	5370
N9	23,800	25,200	27,900	10,600	12,800	21,000	27,800	29,500	29,700
N10	4160	3750	2920	1200	1320	2290	5700	8460	9060
Âµmic_1	2.06×10^{-4}	1.89×10^{-4}	1.58×10^{-4}	1.16×10^{-4}	1.23×10^{-4}	1.57×10^{-4}	2.36×10^{-4}	3.36×10^{-4}	3.84×10^{-4}
pmic_1	0.188	0.182	0.16	0.115	0.122	0.153	0.22	0.268	0.283
Set 4									
Parameter	Mean	Median	Mode	q025	q050	q250	q750	q950	q975
N1	2170	1960	1710	848	976	1510	2560	4090	5040
N2	864	820	643	382	440	641	1040	1440	1580
N3	3890	3820	3710	2180	2390	3190	4520	5600	5970

Table 5 (continued)

Set 4									
Parameter	Mean	Median	Mode	q025	q050	q250	q750	q950	q975
N4	7020	7120	7280	5640	5930	6690	7450	7780	7840
N5	6280	6330	6300	4790	5050	5840	6780	7310	7460
N6	7270	7430	7960	5650	6020	6960	7750	7960	7980
N7	2350	2250	2030	1190	1320	1820	2760	3650	4080
N8	3220	3040	2650	1400	1600	2390	3820	5510	6400
N9	23,400	24,600	27,900	10,600	12,900	20,500	27,500	29,500	29,700
N10	3550	2930	1220	1090	1180	1860	4730	8110	8930
Âµmic_1	2.29×10^{-4}	2.13×10^{-4}	1.81×10^{-4}	1.24×10^{-4}	1.35×10^{-4}	1.74×10^{-4}	2.66×10^{-4}	3.74×10^{-4}	4.25×10^{-4}
pmic_1	0.23	0.235	0.256	0.131	0.145	0.198	0.267	0.295	0.3

and sample size $p < -0.26$, we found no correlations. Using the LD method, we got undefined *Ne* estimates for the JW population (negative values); for the remaining STRUCTURE clusters, *Ne* values were one to three-fold lower than those obtained by the ABC calculations (not shown).

Accuracy and precision varied between parameters. The mean relative bias of the ABC analysis was low (Table 6). The effective population size for unsampled ancestral population *N10*, from which German populations diverged, appeared minimally biased when we employed the second set of priors (MRB = 1.605).

Discussion

From where did the Jawor population come?

A phylogenetic analysis of central European populations of *Cricetus cricetus* has been performed using data from approximately 2000 nucleotide-long sequences of the mtDNA four regions to define a (sub)group to which JW belongs. To our knowledge, this is the first report showing that isolates from Poland (JW) cluster together with samples from Germany. There are no recent reports of the presence of the common hamster in the western part of Poland, suggesting that JW is probably the last one and that contact with populations belonging to the *Central* lineage have been completely disrupted. The JW population was found 300 km east of the previously detected range edge of the *Central* subgroup in Europe.

The shared mtDNA haplotypes occur in JW as well as in samples belonging to the *Central* phylogeographical group. This might reflect historical events, i.e. repeated postglacial immigration of *C. cricetus* to the western parts of Europe from eastern refugia, thus supporting the hypothesis of Neumann et al. (2005). This hypothesis is indirectly supported by several other lines of evidence. Firstly, the reconstructed NJ tree suggests that mt haplotypes representing

Table 6 Mean relative bias of the ABC analysis for estimated demographic and mutational parameters of *Cricetus cricetus* based on the SSR data set (see text and Table 1 for details)

Parameter	Priors Set 1			Priors Set 2			Priors Set 3			Priors Set 4		
	Means	Medians	Modes	Means	Medians	Modes	Means	Medians	Modes	Means	Medians	Modes
N1	−0.097	−0.082	−0.0508	0.247	0.1837	0.1062	0.040	−0.012	−0.0781	0.064	−0.0018	−0.0813
N2	−0.067	−0.066	−0.0653	0.099	0.0435	−0.0506	−0.133	−0.1851	−0.2747	−0.100	−0.1485	−0.2418
N3	0.069	0.051	0.017	0.119	0.1028	0.0712	0.021	0.0067	−0.0189	−0.010	−0.0266	−0.0585
N4	0.029	0.0293	0.0296	0.063	0.0768	0.1012	−0.118	−0.1045	−0.0777	−0.105	−0.0935	−0.0639
N5	−0.090	−0.0823	−0.0662	−0.047	−0.0469	−0.0484	−0.208	−0.2059	−0.2035	−0.190	−0.1898	−0.192
N6	0.047	0.0582	0.0836	0.023	0.0487	0.0978	−0.06	−0.0341	0.0252	−0.041	−0.0186	0.056
N7	0.004	−0.0222	−0.0725	0.097	0.0552	−0.0074	0.017	−0.0212	−0.081	−0.028	−0.0738	−0.1488
N8	0.162	0.1394	0.0887	0.321	0.2674	0.1867	0.160	0.1035	0.0265	0.173	0.1234	0.0535
N9	0.225	0.2117	0.1846	0.573	0.5671	0.546	−0.228	−0.2239	−0.2117	−0.141	−0.1288	−0.0864
N10	0.687	0.568	0.2758	1.605	1.4439	0.9849	0.158	0.0507	−0.1948	0.462	0.2868	−0.1707
Âµmic_1	−0.028	−0.0233	0.0048	−0.037	−0.0404	−0.029	0.291	0.207	0.0656	0.323	0.2446	0.0973
pmic_1	0.038	0.0248	−0.0008	0.034	0.0206	−0.012	0.100	0.0856	0.0517	−0.074	−0.0755	−0.0642

E1 are more closely related to the *Central* samples than to the *Pannonian* samples. Secondly, there are literary records describing the presence of hamsters on both sides of the Germany-Poland border in the nineteenth and twentieth centuries (Surdacki 1971; Weinhold 2008). Thirdly, there are also reports of hamsters crossing rivers and of the same hamster population inhabiting both banks of a given river (Banaszek et al. 2009; La Haye et al. 2012). This suggests that migration is possible even in a landscape bisected by a major European river like the Elbe or the Spree. Furthermore, the possibility of a contraflow from the central part of Germany to the western part of Poland cannot be excluded. There are reports of simultaneous surges of hamster numbers in central Germany until 1984, which may have led to increased migration (Nechay 2008). Moreover, such increases in numbers may have impelled hamsters to migrate in an east-southerly direction as has been observed in the northern part of Saxony by Zimmermann (1923), after Stubbe, Stubbe (1998). In summary, JW could constitute the last link in a chain of populations, reflecting the migration track that connected large distribution centers in both countries, but direction of migration remains unknown and requires further investigations.

An alternative explanation is that the observed haplotype distribution in JW and in the Central lineage may have arisen from habitat and population fragmentation. One combined ctr-*COI*-16S-Cyt *b* haplotype was found to be characteristic of both JW and populations located in the central part of Germany. A combined ctr-*COI* haplotype is considered the ancestral and most common haplotype of those identified in western Germany (Schröder et al. 2014 see also; Neumann et al. 2005). This finding might indicate that JW and at least some populations from those two parts of the species range descended from the same, geographically-widespread maternal progenitor (see Avise 2000). At present, a previously large, continuous area inhabited by the common hamster is shrinking in size, being divided into scattered and significantly isolated areas (Fig. 1, see e.g., Werth 1936; Weidling and Stubbe 1998; Nechay 2000; Neumann et al. 2004, 2005; Ziomek and Banaszek 2007; Tkadlec et al. 2012; Reiners et al. 2014; Korbut and Banaszek 2016). In tracking both the range expansion and its contraction, JW can be viewed as a genetic footprint of range dynamics.

Incongruences between molecular markers are commonly identified (Toews and Brelsword, 2012), as demonstrated in some *C. cricetus* populations (Neumann et al. (2004, 2005) and in this study. On the one hand, nuclear SSR markers showed extensive sharing of genotypes between JRZ (*Pannonian*) and JW, on the other hand, JW clusters among mitochondrial *Central* lineages. This disparity may be due to (1) differences in characteristics of molecular markers (effective population size and mutation rate), (Moore 1995; Charlesworth 1998; Hedrick 1999), or (2) long-term female philopatry and male-biased dispersal (Kayser and Stubbe 2003; Banaszek and Ziomek 2012; Banaszek et al. 2012). The extensive sharing of SSR genotypes between distant localities is expected when male-biased gene flow is common. Employing nuclear data, a significant isolation by distance based on the Mantel`s test was found ($P < 0.01$), which argues against male mediated gene flow, but is in accordance with the habitat and population fragmentation. Nevertheless, this pattern might be also produced in the situation when migration is constrained due to distance (Van Strien et al. 2015). Thus, analyzed SSR markers, showing clear geographic structuring, may or may not reflect the true demographic history of JW. More intensive fine-scale sampling and genotyping more loci could reveal a better resolution of the Central populations and facilitate a clearer approach to the problem of disparity in molecular markers.

Genetic characteristics of the Jawor population in comparison to neighboring populations from Poland and Germany

In the analyzed populations` set the genetic diversity value for JW was the lowest, reflecting a high degree of isolation and the lowest population size. The JW population contains only one mtDNA haplotype (6, Table S1), indicating that the population is under the strong influence of genetic drift. Through genetic drift, some alleles can disappear from a population (Wright 1931), which also may explain the low mean number of private alleles in JW. On the other hand, the almost total lack of private alleles in some German populations indicates that the positive gene flow among these populations has been disrupted relatively recently. Heterozygote deficiency was detected in all analyzed populations. The stronger deficit is in small and isolated populations rather than in larger populations occurring in close proximity to each other. This indicates non-random mating (e.g. in JW, $F = 0.769$, see e.g. Wright 1921). Breeding of related individuals is a consequence of restricted gene flow due to habitat fragmentation, relatively small population size, and biological constraints (e.g. Melosik et al. 2016). The common hamster does not disperse far, and its travel distance depends on sex, age, season, the location of food sources, and the incidence of reproduction outbreaks (Berdyugin and Bolshakov 1998; Nechay 2000; Kupfernagel 2008). These factors may contribute to segregation of populations into isolated reproductive units (Wahlund effect), which subsequently may lead to departures from HW proportions. Such signs of hidden genetic structure were also found in this study, but the STRUCTURE approach appeared to be insufficient to detect it, presumably due

to an overwhelming effect of geographic partitioning of genetic variation (see also Evanno et al. 2005).

The results of the study are interesting from a conservation standpoint, as we provided the first single-sample effective population size estimates using a model-choice approach (ABC). Banaszek and Ziomek (2012) and Rainers et al. 2014 provided *Ne* estimates for the common hamster using temporal sampling. In this study, the *Ne* estimates ranged from a few 1000 individuals to a few 100 having the smallest value in JW (539–864 individuals). However, our estimations of *Ne* are at least one order of magnitude larger than those based on a single-sample linkage disequilibrium method (NeLD), (the latter not shown). The last method appeared to be sensitive, for example to persistent population fragmentation (England et al. 2010) or declines in population size (Antao et al. 2011). The model used by this method (the infinite island model under drift and migration equilibrium), requires a constant population size and migration rate over a long period of time (Wang 2005). This is probably violated in our sample due to cyclical fluctuations of population size in the common hamster and changes in the migration rates. Additional studies are required to gain better understanding of the *Ne* estimators using this method. Discordant results of *Ne* estimates depending upon the method used were also found by Leroy et al. (2013). Regardless of the method employed, several other largely unknown processes influence effective size, for example, fluctuations in size, overlapping generations, life-history traits (e.g., repeated breeders), non-random sampling (kin sampling) (Waples and Yokota 2007; Serbezov et al. 2012; Holleley et al. 2014), or sampling method used (Marucco et al. 2011). In conclusion, due to the significant variability in results depending on the method used in *Ne* estimations, the precise evaluation of extinction risk of JW is presently difficult (see also Franklin 1980; Soulé 1980; Gilpin and Soulé 1986; Lande 1995). It is clear, nonetheless, that JW has the smallest effective size and is subject to the heaviest inbreeding stress among the analyzed populations.

Implications for conservation

To restore genetic variability of JW and to mitigate the negative effect of inbreeding, translocations of individuals is being proposed at the level of the *Central* phylogeographic group as well as at the species level. Any plans of translocation should not include transfer of individuals representing different evolutionarily significant units (ESU) to avoid diminishing differently adapted variants and to prevent potentially detrimental consequences of outbreeding depression (e.g. La Haye et al. 2012, see also; Banaszek et al. 2009–2010, 2012; Neumann 2013; Schröder et al. 2014). Taking into account this position, translocation should be between JW and the *Central* population(s). All analyses, based on neutral genetic markers, show considerable amounts of genetic differentiation between analyzed populations, indicating uniqueness of these populations. This uniqueness most likely stems from genetic drift whose largest impact manifests in the most fragmented and isolated population. Therefore, focusing only on a few local populations as potential translocation units might further increase this fragmentation and might be ineffective in maintaining or increasing their adaptive potential in a time of progressing global warming and human pressure (Moritz 1994; Weeks et al. 2016).

Although increasing neutral genetic diversity does not guarantee that the adaptive potential of species will be protected or increased, according to Weeks et al. (2016), translocation experiments should be considered at the species level, rather than at a population, subspecies, or the ESUs levels (but see La Haye et al. 2012). If so, translocation experiments between JW and *Pannonian* populations should be at least preliminary assessed in captivity given that: (1) genetic similarities between the currently geographically separated populations exist, both in mitochondrial and nuclear markers, e.g. between the *Pannonia* and *West* lineages (Smulders et al. 2003) or between the *Pannonia* and *Central* lineages (Neumann et al. 2004, 2005, and results of this study); (2) reports exist showing that the contemporary hamsters have reduced fitness relative to those alive prior to the population crisis (e.g. Pucek 1981; Monecke et al. 2013), thus the beneficial effect of proposed outcrossing, such as heterosis, may be observed, and (3) biological importance of postulated ESUs is not always clearly defined and understood, and subspecies designation is not broadly accepted (Neumann et al. 2004; Banaszek et al. 2009–2010; Schröder et al. 2014). In conclusion, if the decline in genetic diversity is strictly due to neutral processes in JW, introducing a new genetic variant would be beneficial. However, the direction of translocation remains an open question until potential risks of extinction of analyzed populations and environmental sustainability are evaluated and measured (Daimler plans to build a Mercedes engine plant in Jawor). A successful outcome of this intervention would be long-term survival of the population. This would, however, depend on how quickly after translocation the effective population size reaches a minimum threshold of 1000 individuals, the number considered sufficient for maintaining its adaptive potential and evaluability (Weeks et al. 2011). Therefore, pre- and post-translocation monitoring of genetic diversity in the donors and recipient populations should be performed.

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