Contents lists available at ScienceDirect

Mammalian Biology

Original investigation

Genetic diversity and extinction risk in a small, declining Polish common hamster (*Cricetus cricetus*) population

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ABSTRACT

Losses of common hamster populations (*Cricetus cricetus*) have become a major conservation issue in Poland and Europe. The species' range has declined by approximately 75% since 1971 in Poland. We aimed to retrace a recent history and to characterize the genetic status of a small, declining Polish population to develop the best management strategy for this isolated population. By combining field observations with genetic information from 13 microsatellite markers genotyped in 45 individuals and a population viability analysis, we computed the projected time to extinction using models with varying migration rates, first-year mortality (FYM), and supplementation.

Environmental changes in recent years have negatively affected the size of the population (burrow density dropped from 5.5 burrows per hectare to 0.6). The population is spatially structured in demes, with each deme having a small effective population size. The population exhibits an overall deficiency of heterozygosity. Restricted gene flow (m ranging from 0.01 to 0.05) induces inbreeding and spatial clustering of closely related individuals.

This study encompasses the effect of deme supplementation as the best strategy to increase the population's median time to extinction (MTE), gene diversity, and the number of alleles. Varying FYM does not greatly affect these parameters when demes are not supplemented, but it becomes more important when supplementation is occurring. A 5% increase in migration rates does not influence the parameters. Based on the deterministic scenarios, a positive growth rate is observed when FYM is reduced. This study contributes to ongoing efforts to ensure the persistence of the endangered *C. cricetus*.

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Introduction

The common hamster *Cricetus cricetus* (Linnaeus, 1758) was previously widespread throughout the Eurasian steppes and agricultural areas, occasionally becoming a very abundant component of these ecosystems (Nechay, 2000). Today, this small rodent is regionally rare and threatened (e.g., Hegyeli et al., 2015), and in Poland, it is protected under the Nature Conservation Act (April 16, 2004). The possible reasons for its decline may include habitat loss and fragmentation, land development, deaths caused by human activities (e.g., Nechay et al., 1977; Nechay, 2000; Banaszek et al., 2010), and climate change (Neumann et al., 2004, 2005). Also, population fluctuations observed under natural conditions could have been compounded by destruction caused by human threats.

Western European populations of the common hamster have experienced population reductions that are much more serious than those of the Central European and western and central Asiatic regions; however, even in these latter regions, some population shrinkage is noted (Neumann et al., 2004, 2005; Reiners et al., 2014; Schröder et al., 2014; Hegyeli et al., 2015). In Poland the common hamster belongs to two phylogeographic groups: Pannonia and E1 (Banaszek et al., 2011), surviving as small and isolated populations only in the southeastern parts of the country (Ziomek and Banaszek, 2007). Some populations representing the Pannonia phylogroup, occurring also in Hungary and Czech Republic, are genetically comparable to the genetically depauperate populations from the western part of the European range (Banaszek et al., 2010; see also Neumann et al., 2005; Banaszek et al., 2009).

Theory predicts that small, isolated, and fragmented populations experience inbreeding depression (Hedrick, 1994) and high genetic load (Charlesworth et al., 1993), lose the ability to adapt to changing environments (Franklin and Frankham, 1998), and are highly vulnerable to extinction. Such negative trends are reflected

in the time elapsing before extinction occurs, which becomes exponentially smaller; this is a process referred to as an "extinction vortex" (Gilpin and Soulé, 1986; Lacy and Pollak, 2015). These predictions have been verified empirically in a large number of studies (e.g., Crnokrak and Roff, 1999). Loss of allelic diversity has also been documented among common hamster populations in the western part of the European range (Smulders et al., 2003; La Haye et al., 2012).

To increase the common hamster population, various conservation actions have been postulated or undertaken, including captive breeding and reintroduction, establishing corridors between fragmented populations, and optimizing living conditions to decrease mortality (Weinhold, 2004; La Haye et al., 2012; Villerney et al., 2013). Applying population genetics and population viability analysis (PVA; Brook et al., 2000; Reed et al., 2002) can help to assess the extinction risk of these populations and improve understanding of how human population management influences their time to extinction. This approach allows then to develop more effective strategies for recovery of endangered populations.

This study summarizes genetic data, field records, and species-specific information to predict the fate of a population of *C. cricetus* representing the Pannonia lineage from Upper Silesia (Poland). The population was chosen because it constitutes an important entity of the total set of populations of *C. cricetus* representing this lineage in Poland. It is located at an edge of the southwestern limit of the lineage distribution range, and the potential loss of this population would result in a significant gap in the range boundaries. This population is isolated from the other populations in the Province of Upper Silesia and in Poland. It is located in an area that was traditionally cultivated until the 1980s, but now this population is threatened by changes in agricultural practices, the abandonment of cultivation, and the impact of urban pressure. This area had an abundance of suitable habitats for the common hamster because of the presence of a mosaic of arable fields, pastures, and meadows, which were cultivated under a mixed farming system and crop rotation. This means that the fields were crossed by a network of hamster-friendly balks and that not all crops were harvested at the same time; thus, the fields provided shelter for the animals. However, over the past several years the number of small, cultivated fields has decreased, with those fields being replaced by large blocks of arable fields, and the proportion of abandoned wastelands has continually increased. Moreover, for the studied population, genetic and field observations exist based on materials collected in 2007 (Banaszek and Ziomek, 2012; see also Banaszek et al., 2010). This gives us a better view about the current state of the population and future trends. There are also management options available to rescue this population. The population is now being studied under a program devoted to the protection and restoration of the common hamster. Among other purposes, this unique national-scale program is aimed at establishing a conservation area to maintain the common hamster population in Jaworzno.

The purposes of this study are (1) to provide current information about genetic conditions of the population and (2) to assess the extinction risk of the population by employing a Vortex scenario analysis to identify the most effective conservation actions. Specifically, we aim to answer the following questions: How long is this small population likely to last without human intervention and under alternative management activities, including enhancement of its first-year survival by such strategies as captive breeding programs or facilitating movement of individuals (e.g., via establishing corridors) and population supplementation? What is the most important variable that influences the median time to extinction (MTE) of the population under study?

A two-step analysis was employed to realize the objectives, beginning with recognizing the genetic structure and contemporary gene flow patterns among demes within the analyzed population based on microsatellite markers. In the second step, the extinction risk of that population was calculated by employing PVA modeling based on the results of genetic analyses and literature searches as well as on site-specific observations.

Material and methods

Site description and monitoring of burrow density

The Jaworzno (JRZ) population of *C. cricetus* is located in the Upper Silesia, a region in the southern part of Poland (Fig. 1). The hamsters' burrows were found in districts around Jaworzno (i.e., Wilkoszyn, Cezarówka, Byczyna, Jeziorki, and Balin).

Material and sampling

A part of the area inhabited by the JRZ population (i.e., an area of 10 ha) was monitored for burrows two times per year (with some exceptions) from 2007 to 2015 (see Fig. 1, Fig. S1). Details related to the plot description and the burrow-monitoring procedure are presented in Supplementary information S1. In terms of burrow counts and density, the quality of soil structure, a form of cultivation, and shrinkage of the common hamster ecological niche, this 10-ha area is representative of the whole area of the JRZ population (in total ca. 377 ha); field sampling for genetic research studies has been performed for the whole area.

The hairs (including roots) of 45 individual hamsters were collected for genetic analyses using noninvasive hamster-specific hair traps (Reiners et al., 2011a,b) from August 8–17, 2015. These traps were placed in burrow entrances during late afternoon and surveyed for hairs the next morning.

DNA preparation and PCR amplification

Total DNA was isolated from the hair roots using a modified Chelex 100 protocol, following Walsh et al. (1991) and Reiners et al. (2011a). The microsatellite primers used for polymerase chain reaction (PCR) amplification were those developed for *C. cricetus* by Neumann and Jansman (2004) and Jakob and Mammen (2006) and with the same modifications of Reiners et al. (2011a). The following loci were studied: Ccrμ 6, 10–12, 15, 17, 19, and 20, and IPK 03, 05, 06, 09, and 12. DNA sequences were amplified to obtain a 162 bp segment of the control region (*ctr*) for species identification. PCRs were performed using a primer pair combination published by Neumann et al. (2005). PCR reaction condition guidelines are presented in Protocols S1.

Microsatellite diversity, Hardy–Weinberg, and linkage equilibrium

The Micro-Checker v. 2.2.3 (Van Oosterhout et al., 2006) program was used to test for the presence of null alleles, stuttering during PCR amplification, and large allele dropout. The frequency of null alleles was also estimated with the help of the program ML-NullFreq (Kalinowski and Taper, 2006; Wagner et al., 2006).

The program ADZE (Allelic Diversity Analyzer) calculated allelic richness and private allelic richness within identified STRUCTURE clusters (hereafter demes) that had been corrected for sample size (Szpiech et al., 2008). The program employs a rarefaction procedure to adjust differences in sample size. Summary statistics for each locus were calculated in the program PowerMaker v. 3.25 (Liu and Muse, 2005). Deviations from the Hardy–Weinberg equilibrium were tested using three different tests: χ^2 goodness-of-fit test, likelihood-ratio test (LRT), and exact test, as implemented in the same program.