Säugetierkundliche Informationen, Jena 8 (2011) 107 - 116 30.01.2011

Genetic diversity in reintroduced and restocked populations of the Common hamster (*Cricetus cricetus*)

Genetische Diversität in wiedereingebürgerten und aufgestockten Populationen des Feldhamsters (*Cricetus cricetus*)

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Zusammenfassung: Der Feldhamster (*Cricetus cricetus*) hat in Europa, besonders im westlichen Teil Europas, den Niederlanden, Belgien, im Westen von Deutschland und Nordosten von Frankreich, drastisch abgenommen. Diese Abnahme hat zum strikten Schutz der Art in Europa geführt. Zur Zeit werden Maßnahmen durchgeführt, um die restlichen Populationen zu schützen. In den Niederlanden ist die Feldhamsterpopulation in Zahl und Verbreitung / Fläche / Areal stark zurückgegangen, so ist die Zahl der Populationen 1999 auf 14 Individuen gefallen. Dies führte zum Fangen der letzten Individuen.

Ein Zuchtprogramm mit diesen letzten niederländischen, mit belgischen und deutschen Feldhamstern wurde begonnen. Seit 2002 sind Populationen mit den Nachkommen dieser Individuen etabliert worden. Dies resultierte in drei unterschiedlichen genetischen Zuchtlinien: niederländische, niederländisch-belgische und niederländisch-deutsche Linie. Diese genetischen Linien wurden in verschiedenen Regionen ausgesetzt. Diese Studie analysiert fünf niederländische Hamsterpopulationen von Amby, Heer, Puth, Sibbe und Sttard und zwei belgische Populationen von Berthem und Widooie. Vier Populationen sind mit in Gefangenschaft gezüchteten Hamstern mit neuen Allelen aufgestockt worden. Diese Studie analysierte drei Fragen die genetische Diversität der wiedereingebürgerten und der aufgestockten Populationen von Feldhamstern: 1) Ist die genetische Diversität der wiedereingebürgerten Populationen vom Gründer-Effekt beeinflusst? 2) Hat sich die genetische Zusammensetzung nach einigen Jahren verändert? 3) Hat die Aufstockung zu einer Erhöhung der genetischen Diversität geführt?

Die genetische Diversität von wiedereingebürgerten Feldhamstern ist nicht durch Gründer-Effekte beeinflusst. Die genetische Variation der wiedereingebürgerten Population (t=0) ist mit der genetischen Variation der ersten Meßperiode (t=1) verglichen worden und genetisch hat sich nicht viel verändert. Keine neuen Allele wurden entdeckt und keine bedeutenden Änderungen in der Heterozygotie wurden festgestellt. Nur ein kleiner Anstieg in Allel Richness (Rs) [Anzahl der Allele] in der Amby und Sibbe Region wurden beobachtet.

Die genetische Struktur in der Puth Region hat sich in vier Jahren nicht verändert. In Sittard ist die Situation anders. Die Population beginnt sich in Bezug auf seinen zunehmenden Fst Wert von der Ursprungspopulation zu unterscheiden. Auch die Heterogozytie ist geringfügig in fünf Jahren gestiegen. Die Zunahme der genetischen Diversität mag durch die Immigration von Individuen einer benachbarten Population bedingt sein.

Die Einführung von Hamstern mit neuen Allelen (Aufstockung) führte zu einem Anstieg der genetischen Diversität in den Regionen von Heer und Widooie. Aufstockung führte zu einer Zunahme der Anzahl von Allelen, Allel richness und Heterozygosität. Weitere Forschungen sind nötig, um den Effekt dieser Zunahme in genetischer Diversität auf das Populationswachstum zu untersuchen. Der Schutz des Feldhamsters ist nach wie vor das wichtigste Ziel, aber Forschung trägt zum Wissen für besseren Schutz der Art bei.

Schlagworte: Feldhamster, *Cricetus cricetus*, genetische Diversität

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Abstract: The Common hamster (*Cricetus cricetus*) has declined dramatically throughout Europe, especially in the most western part of Europe (the Netherlands, Belgium, West-Germany and northeast of France). The decline has led to strict protection of the species within Europe. At this time conservation projects are in progress to protect the remaining populations. In the Netherlands and Belgium the hamster population has drastically declined in number and range. In the Netherlands, for example, the hamster population declined to only 14 individuals in 1999. This has led to rapping of the last remaining individuals.

A breeding program with the last remaining individuals and Belgian and German hamsters has been established. Since 2002 hamster populations are created with the offspring of these individuals. This resulted in three genetically different breeding lines: Dutch; Dutch-Belgian and Dutch-German. These breeding lines have been released in different areas. This study analyses five Dutch hamster populations (Amby, Heer, Puth, Sibbe and Sittard) and two Belgian populations (Berthem and Widooie). Four populations have been restocked with captive-bred hamsters with "new" alleles. This study analysed three questions regarding the genetic diversity in reintroduced and restocked populations of the Common hamster: 1) Is the genetic diversity of the reintroduced populations affected as a result of a founder-effect ? 2) Is the genetic composition changed after some years ? 3) Did the restocking result in an increase in genetic diversity ?

The genetic diversity of reintroduced hamster populations is not affected as the result of any founder effects. The genetic variation of the reintroduced population (t = 0) is compared with the genetic variation of the first measurement period (t = 1). Genetically, not much has changed. No new alleles were detected, and no considerable change in heterozygosity has been found. Just a small increase in Allelic Richness (Rs) in the Amby and Sibbe area has been found.

The genetic composition in the Puth area hasn't changed in four years. In Sittard the situation is different. The population starts to differ from this source, regarding its increasing Fst value. Also the heterozygosity slightly increases during five years. The increase in genetic diversity might be due to immigration of individuals from a neighbouring population.

Introduction of individuals with new alleles (restocking) resulted in an increase of genetic diversity in the Heer, Berthem and Widooie areas. Restocking led to an increase in the number of alleles, Allelic Richness and heterozygosity. Further research is needed to examine the effect of this increase in genetic diversity and the population growth. Conservation of the Common hamster still remains the most important goal, but research contributes to knowledge for better protection of the species.

Key words: Common hamster, *Cricetus cricetus*, genetic diversity, reintroduction, restocking

Introduction

Reintroductions are attempts to restore a species within its historical range. It is a common approach for preserving intraspecific biodiversity in fragmented landscapes. However, in general reintroduced populations are smaller and more isolated than native populations. Reintroductions may result in an increase of genetic erosion initially caused by population fragmentation by reducing the effective population size of both the source and reintroduced populations. Several studies have shown significant reductions in the genetic variability of reintroduced populations relative to their source (FITZSIMMONS et al. 1997; MOCK et al. 2004). Reduced genetic variation may decrease evolutionary potential, reduce ability to fight off disease, and increase other harmful effects of inbreeding. Mixing source populations is a method used in reintroduction programs which can increase genetic variability (HUFF *et al.* 2010). In addition, it can avoid the negative effects of inbreeding. It's thought that genetic mixing increases the degree of adaptive potential for the novel ecological situations that often occur at reintroduction sites. On the other hand, two potential disadvantages are associated with mixed sources within reintroduced populations. First, genetic mixing between genetically different individuals may result in a decline in fitness of the offspring, referred to as outbreeding depression. Second, mixing source populations may disrupt the genetic individuality of populations (MORITZ 1999). From a restoration standpoint, preserving genetic individuality is important to protect the ecological and genetic processes in neighboring communities and in remnant conspecific populations that may be influenced by gene flow from the reintroduced population. Preserving the intraspecific variation present across a species' range is widely accepted as a critical conservation priority, and the loss of genetically distinct populations is considered by some to be as significant as the loss of entire species (EHRLICH 1988; FOSTER *et al.* 2003).

The Common hamster (*Cricetus cricetus)* has declined dramatically throughout Europe (NECHAY 2000), especially in the most western part of Europe (the Netherlands, Belgium, west-Germany and northeast of France). The decline has led to strict protection of the species

within Europe. At this time conservation projects are in progress to protect the remaining populations (KUITERS *et al.* 2010, LA HAYE *et al.* 2010).The hamster population in the Netherlands and Belgium has drastically declined in number and range. In the Netherlands, for example, the hamster population declined to only 14 individuals in 1999. This has led to trapping of the last remaining individuals and the start of a breeding program (DE VRIES 2003). LA HAYE (submitted) has concluded that, historically, the Dutch, Belgian and west-German individuals belonged to one population. The populations have genetically drifted away from each other. In this study we focus on the genetics of the hamster populations in the Netherlands and Belgium. To restore the historical genetic composition, some individuals from Belgium and one male from German were included in the Dutch breeding program. This resulted in three genetically different breeding lines: Dutch; Dutch-Belgian and Dutch-German. Since the start of reintroduction in 2003, five successful populations have been established. This study genetically examined these Dutch reintroduced populations of the Common hamster.

The research area was expanded with two Belgian wild populations (Figure 1). These Belgian populations were restocked with captive-bred hamsters with "new" alleles (Dutch-German) in 2008/2009. Also three Dutch populations have been restocked (Table 1). All these interventions have been taken to boost the declining populations (LA HAYE *et al.* submitted). But did the restocking succeed? This study analyzed three questions regarding the genetic diversity in reintroduced and restocked populations of the Common hamster: 1) Is the genetic diversity of the reintroduced populations affected as a result of a founder-effect? 2) Is the genetic composition changed after some years? 3) Did the restocking result in an increase in genetic diversity?

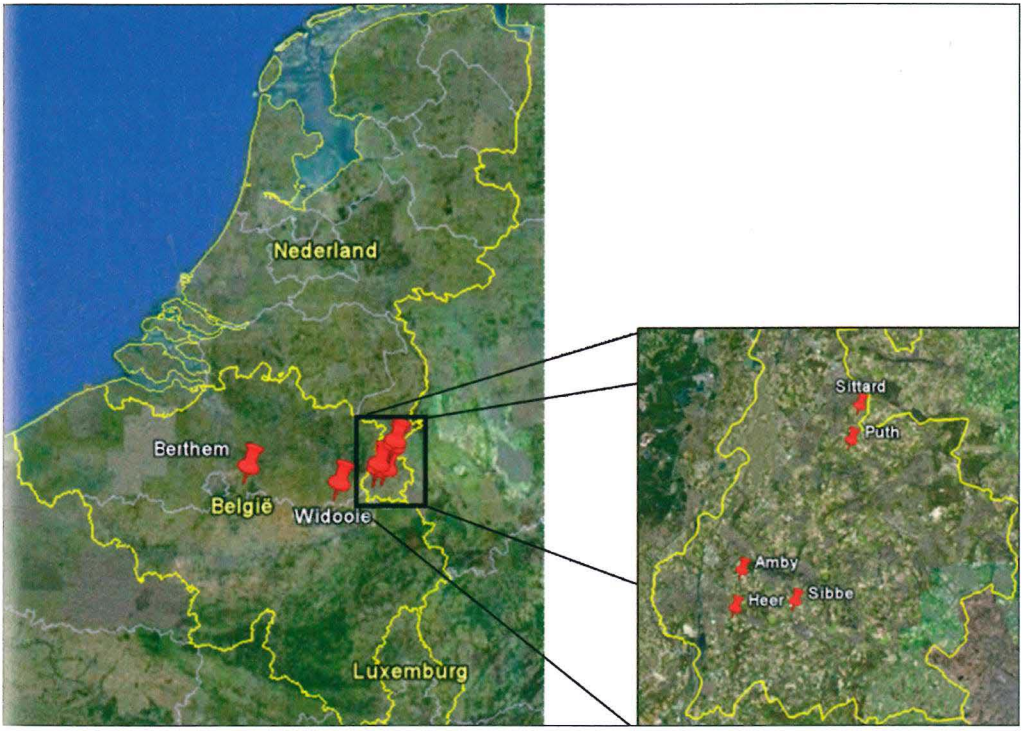

Fig. 1 The geographical distribution of the seven examined hamster populations. The map is created using Google Earth.

Tab. 1 All current populations of Common Hamsters in the Netherlands and Belgium. The Dutch populations are reintroduced populations (*). The start and origin populations are given. For all restocked populations the allelic pattern group (NL, D and B). NL=Netherlands; D=Germany; B=Belgium.

Area	Start	Origin	Restocking	Expected
Sibbe*	2002	NL	NL (,09)	NL
Amby*	2003	NL	NL-B (,06,'08,'09)	NL-B
Heer*	2004	NL	NL-D (,08)	NL-D
Sittard*	2005	NL-D	none	NL-D
Puth*	2006	NL-D	none	NL-D
Berthem (B)	-	B	NL-D (,08,'09)	NL-D-B
Widooie (B)	-	B	NL-D (,08,'09)	NL-D-B

(*) Reintroduced populations

Methods

Species

The Common hamster (*Cricetus cricetus*) is the largest species of the family Cricetidae. It originates from the lowlands of central and Eastern Europe and Siberia, living on steppes, agricultural land and river banks. Its living area extends until the Yenessey River and Altai Mountains, and the Chinese province of Sinkiang (ZHANG 1997). In the past, the species has thrived thanks to the expansion of agriculture, and in Eastern Europe it was (and sometimes still is) considered a pest. The last decades, however, changes in agricultural practices and disappearance of habitats have dramatically reduced the populations of the common Hamster in Western Europe, and currently the Hamster is threatened with extinction in The Netherlands, Germany, Belgium and France.

Study area

The study area is located in the Southern part of the Dutch province Limburg (n=5) and in Belgium (n=2) (figure 1).The study area is characterized by its loess sediments. These were deposited during the last glacial period. Loess tends to develop into highly rich soils. Under appropriate climatic conditions it is some of the most agriculturally productive soil in the world. Therefore farmland dominated this region for centuries. The Common hamster only occurs within these loess-containing areas.

The Dutch populations were founded with individuals from the breeding program. Amby, Heer and Sibbe were founded with individuals from the Dutch breeding line. Puth and Sittard were founded with individuals from the Dutch Dutch-German breeding line (table 1).

Sampling and genotyping

Samples were taken in the period 1997-2010. The study is based on 644 samples: the Netherlands (n=604), Belgium (n=32) and Germany (n=8). These samples contained hair, flesh and bone tissue. The samples were collected from: individuals trapped and released in the field (hair); dead individuals (flesh, bone); individuals living in the field and sampled by hair-trap (hair). DNA isolation was realized at Alterra, Wageningen. For the DNA isolation the DNeasy tissue kit from Qiagen was used. The PCR was performed using 8 different microsatellites.

For PCR amplification 1µl of 1:10 and 1:100 dilutions of DNA was mixed with 24 µl of PCR master mix (5,88 µl dH_20; 1 µl 10x PCR buffer; 0,3 µl $MgCl_2$ 50 mM; 0,16 µl BSA 20 mg/µl; 0,2

µl DNTP and 0,06 µl Taq polymerase 5 U/µl) and 0,4 µl of each primer was added. PCR reactions were performed in a Biometra Tgradient thermocycler (Westburg, Leusden, NL) and started by an initial denaturation at 94 °C for 2 min followed by a 30-cycle amplification (94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min) and a final extension for 20 min at 70 °C. PCR products were examined using a LI-COR 4300 DNA analyzer (LI-COR biosciences).

Genetic analysis

To characterise within-population diversity, we determined the fraction of polymorphic loci, the average number of alleles, and the observed and expected heterozygosity (i.e. genetic diversity), using the program GenAlEx (PEAKALL & SMOUSE 2006). Fst values were calculated with Fstat (JEROME GOUDET).

Tab. 2 Genetic diversity characteristics of the hamster (Cricetus cricetus) populations based on 8 microsatellite loci. Indicated are the sampling period, number of samples, the total number of alleles present in a population, the mean number of alleles per locus (Na), Allelic Richness (Rs), the mean observed heterozygosity (H0), the mean expected heterozygosity (He) and the deviation from Hardy-Weinberg equilibrium.

Population	Period	# samples	Total number of alleles	Na	Allelic Richness (Rs)	Observed Heterozygosity (Ho)	Expected Heterozygosity (He)	Deviation from H-W
Amby (t = 0)	2003	77	12	1.5	1.5	0,195	0,187	-0,04
Amby (t = 1)	2006/2007	17	12	1.5	1.4	0,100	0,127	0,21
Amby (t = 2)	2009/2010	6	13*	1.6	1.6	0,225	0,216	-0,04
Heer (t = 0)	2004	48	12	1.5	1.4	0,179	0,177	-0,01
Heer (t = 1)	2006/2007	20	12	1.5	1.4	0,138	0,165	0,16
Heer (t = 2)	2009/2010	9	14**	1.8	1.7	0,370	0,287	-0,29
Puth (t = 0)	2006	74	15	1.9	1.8	0,429	0,322	-0,33
Puth (t = 1)	2007/2008	35	15	1.9	1.8	0,269	0,329	0,18
Puth (t = 2)	2009/2010	15	15	1.9	1.7	0,285	0,298	0,04
Sibbe (t = 0)	2002	86	12	1.5	1.5	0,199	0,186	-0,07
Sibbe (t = 1)	2005/2006	21	12	1.5	1.4	0,175	0,183	0,04
Sibbe (t = 2)	2009/2010	8	12	1.5	1.5	0,227	0,219	-0,04
Sittard (t = 0)	2005	85	15	1.9	1.7	0,290	0,260	-0,11
Sittard (t = 1)	2007/2008	35	15	1.9	1.8	0,332	0,323	-0,03
Sittard (t = 2)	2009/2010	43	15	1.9	1.9	0,387	0,381	-0,02
Berthem (t = 0)	2001	10	9	1.1	1.1	0,013	0,012	-0,09
Berthem (t = 2)	2009/2010	6	16	2.0	2.0	0,296	0,349	0,15
Widooie (t = 0)	2001	6	8	1.0	1.1	0,000	0,000	0,00
Widooie (t = 2)	2009/2010	10	15	1.9	1.8	0,222	0,308	0,28
NL - Original	1999-2001	25	24	2.7	2.0	0,214	0,258	0,17
D - Original	2003	8	10	1.1	1.1	0,048	0,051	0,06

/** = In the year 2009/2010 two new alleles were detected and one allele was lost.

Tab. 3 Overview of the Fst values calculated between all populations for all measurement periods. The lower part of the table (below yellow) shows the Fst values. The upper part of the table (above yellow)

	Amby (t = 0)	Amby (t = 1)	Amby (t = 2)	Heer (t = 0)	Heer (t = 1)	Heer (t = 2)	Puth (t = 0)	Puth (t = 1)	Puth (t = 2)
Amby (t = 0)		0,003	0,011	0,398	0,049	0,003	0,001	0,001	0,001
Amby (t = 1)	**0,051**		0,238	0,006	0,002	0,007	0,001	0,001	0,001
Amby (t = 2)	**0,086**	0,018		0,004	0,001	0,037	0,010	0,001	0,003
Heer (t = 0)	0,000	0,063	0,105		0,118	0,004	0,001	0,001	0,001
Heer (t = 1)	0,022	0,143	0,172	**0,014**		0,016	0,001	0,001	0,001
Heer (t = 2)	0,101	0,113	0,083	**0,101**	0,094		0,372	0,011	0,139
Puth (t = 0)	0,143	0,134	0,125	0,136	0,142	0,000		0,001	0,108
Puth (t = 1)	0,250	0,226	0,178	0,241	0,245	0,065	**0,040**		0,200
Puth (t = 2)	0,235	0,216	0,144	0,230	0,239	0,024	**0,018**	0,011	
Sibbe (t = 0)	0,000	0,045	0,080	0,000	0,022	0,100	0,145	0,252	0,235
Sibbe (t = 1)	0,021	0,111	0,131	0,023	0,000	0,073	0,136	0,244	0,232
Sibbe (t = 2)	0,000	0,125	0,090	0,006	0,000	0,069	0,128	0,204	0,195
Sittard (t = 0)	0,021	0,047	0,059	0,020	0,044	0,005	0,049	0,131	0,103
Sittard (t = 1)	0,207	0,176	0,142	0,201	0,217	0,017	0,002	0,030	0,004
Sittard (t = 2)	0,228	0,220	0,167	0,220	0,204	0,044	0,038	0,057	0,040
Belgium (t = 0)	0,717	0,861	0,834	0,751	0,816	0,785	0,582	0,590	0,662
Berthem (t = 2)	0,347	0,421	0,210	0,356	0,333	0,213	0,231	0,209	0,180
Widooie (t = 2)	0,413	0,438	0,230	0,417	0,411	0,284	0,278	0,244	0,228

Results

Genetic diversity

Genetic diversity analysis between start population (t=0) and the first measurement (t=1) resulted in Fst values varying from 0.014 till 0.084 (table 3). Heer revealed the least difference in genetic diversity, but significant (Fst=0.014; P=0.003). Puth had the highest Fst value (0.086; P=0.001) also significant. No loss of alleles occurred between start and first measurement. But looking at the allelic richness, Sibbe showed a slight decline. The analysis of the heterozygosity between the start population and first measurements showed that the expected heterozygosity (He) slightly decreased in the Heer area. Puth and Sibbe were stable, only Sittard demonstrated an increase in heterozygosity (He) of 0.063.

Genetic composition in time (without interventions)

Looking at the genetic composition in time, all populations with interventions were excluded. This resulted in two remaining populations: Puth and Sittard. Comparing the Fst values of the three different measurements of each population results in an indication of the change in genetic composition in time. Genetic composition in Puth didn't change. The Fst values of the different measurements were low (Fst $\leq$ 0.040) (table 3). Within the measurements in Sittard the Fst values showed a different pattern. The Fst values comparing the start population with "t=1" and "t=2" are respectively 0.086 and 0.121. It seems that the Sittard population increases its

shows the matching statistic significance value (P) calculated with GenAlex using 999 permutations.

Sibbe (t = 0)	Sibbe (t = 1)	Sibbe (t = 2)	Sittard (t = 0)	Sittard (t = 1)	Sittard (t = 2)	Belgium (t = 0)	Berthem (t = 2)	Widooie (t = 2)	
0,397	0,041	0,390	0,001	0,001	0,001	0,001	0,001	0,001	**Amby (t = 0)**
0,005	0,001	0,005	0,004	0,001	0,001	0,001	0,001	0,001	**Amby (t = 1)**
0,014	0,010	0,038	0,025	0,001	0,001	0,001	0,007	0,003	**Amby (t = 2)**
0,383	0,048	0,313	0,005	0,001	0,001	0,001	0,001	0,001	**Heer (t = 0)**
0,038	0,383	0,389	0,003	0,001	0,001	0,001	0,001	0,001	**Heer (t = 1)**
0,001	0,016	0,045	0,304	0,168	0,030	0,001	0,002	0,001	**Heer (t = 2)**
0,001	0,001	0,002	0,001	0,303	0,002	0,001	0,002	0,001	**Puth (t = 0)**
0,001	0,001	0,001	0,001	0,011	0,001	0,001	0,001	0,001	**Puth (t = 1)**
0,001	0,001	0,001	0,001	0,340	0,009	0,001	0,001	0,001	**Puth (t = 2)**
	0,039	0,403	0,001	0,001	0,001	0,001	0,001	0,001	**Sibbe (t = 0)**
0,021		0,415	0,005	0,001	0,001	0,001	0,001	0,001	**Sibbe (t = 1)**
0,000	0,000		0,094	0,001	0,001	0,001	0,003	0,001	**Sibbe (t = 2)**
0,021	0,040	0,026		0,001	0,001	0,001	0,001	0,001	**Sittard (t = 0)**
0,209	0,206	0,186	**0,086**		0,004	0,001	0,001	0,001	**Sittard (t = 1)**
0,232	0,196	0,155	**0,121**	0,030		0,001	0,002	0,001	**Sittard (t = 2)**
0,714	0,794	0,834	0,630	0,619	0,517		0,001	0,001	**Belgium (t = 0)**
0,350	0,321	0,199	0,257	0,238	0,131	**0,511**		0,3980	**Berthem (t = 2)**
0,414	0,405	0,312	0,318	0,273	0,198	**0,364**	0,000		**Widooie (t = 2)**

difference with the start population in time. The number of alleles (15) and allelic richness (1.9) remained stable. Heterozygosity analysis revealed and increase in observed heterozygosity (Ho) and expected heterozygosity (He) in Sibbe and Sittard (figure 2).

Heterozygosity analysis in Puth resulted in a stable expected heterozygosity but an extreme high observed heterozygosity in the starting year (0.429). This is a result of the reintroduced individuals: at least 68% of the reintroduced animals were first generation heterozygotes (Dutch female x German male).

Restocking

Three Dutch reintroduced hamster populations (Amby, Heer and Sibbe) were restocked using individuals from different breeding lines (table 1). Amby en Heer were restocked with individuals carrying additional alleles: Dutch-Belgian (Amby) and Dutch-German (Heer). Sibbe was restocked with animals from the same breeding line (Dutch). Restocking in Sibbe resulted in an increase in allelic richness (Rs) and heterozygosity (He) (table 2). The number of alleles didn't change, which was according our expectation: no new alleles were added. In the other two Dutch populations restocking led to an increase in the number of alleles (Amby+1; Heer +2). Also an increase in allelic richness was reported. The expected heterozygosity almost doubled in Amby and Heer. Fst analysis of the populations before restocking (t=1) and after restocking (t=2) resulted in values of 0,018 (Amby) and 0,094 (Heer).

The wild Belgian populations were restocked with captive bred hamsters and with wild hamsters with a Dutch-German profile. Before restocking the Belgium populations were almost com-

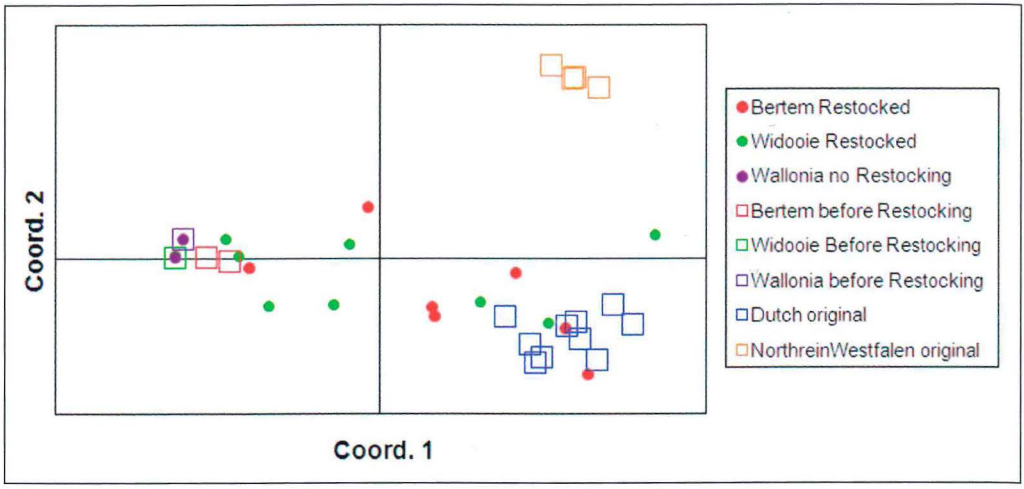

Fig. 2 Principal Coordinate Analysis (PCA) on the genetic distance matrix of the complete Belgian data-set, including the reference populations Nordrhein-Westfalen (Germany) and the Netherlands. Shown are scores of the Belgian populations (Widooie and Berthem) before and after restocking with Dutch-German breeding animals.

pletely homozygote. Restocking resulted in a large increase in the number of alleles (Berthem +7; Widooie +7) (table 2). The allelic richness doubled and the heterozygosity (He) revealed values of 0.349 (Berthem) and 0,308 (Widooie). Fst analysis between the populations before (t=1) and after restocking (t=2) resulted in values of 0,551 (Berthem) and 0,364 (Widooie).

Visualization of the results of restocking is demonstrated by a principle coordinate analysis (PCA). Restocking in Berthem and Widooie resulted in mixing of the wild and restocked population. This is visualized by genetic profiles (dots) centered in between the source populations of both the wild and restocked populations (figure 2). Similar graphs have been made for the Dutch restocked populations. Heer showed the same pattern; mixed populations. Amby didn't show mixed populations.

Discussion

Genetic diversity of reintroduced hamster populations is not affected as a result of a founder-effect. Analysis revealed no considerable differences in Fst values or heterozygosity between the start populations and the next generation. In time, genetic composition in populations without intervention mainly showed an increase in heterozygosity. Restocking with additional alleles increased the genetic diversity in the populations of the Common Hamster. A significant increase in number of alleles and heterozygosity underlines this result.

Genetic diversity

It is important to assess founder effects of reintroduction practices on the genetic variation, because evaluation can help minimize negative effect of founding events and inbreeding in reintroduced populations (LATCH & RHODES 2005, HUFF *et al.* 2010). The genetic variation of our populations is estimated with an Fst analysis and comparison. WRIGHT (1978) suggested that an Fst range of 0-0.05 indicates little differentiation, 0.05-0.15 moderate, and 0.15-0.25 large differentiation and above 0.25 indicates very large differentiation. Comparing start population (t=0) with the first measurement t=1) revealed Fst values varying from 0.014 till 0.086, with an average of 0.042 (table 3). This means, according to WRIGHT (1978), that there is little

differentiation between the start population and the first measurement. Also no loss of alleles was found among the different populations (table 2). But the allelic richness declined in the population of Sibbe. Although this result indicates that there is hardly any difference with the founder population. With the results of this study no bottle-neck could be detected. It might be possible that within the West-European Common Hamster population too little genetic variation is left to detect any bottle-neck effects.

Reduction in heterozygosity was detected in the Heer area, along with a lack of heterzygotes (positive deviation from H-W). Same patterns were found in the Amby and Puth areas (table 2). This reduction of heterozygosity may be caused by the Wahlund effect. The Wahlund effect refers to reduction of heterozygosity in a population caused by subpopulation structure (WAHLUND 1928). If subpopulations have different allele frequencies then the overall heterozygosity is reduced, even if the subpopulations themselves are in a Hardy-Weinberg equilibrium. The underlying causes of this population subdivision could be geographic barriers to gene flow followed by genetic drift in the subpopulations.

Genetic composition in time (without interventions)

The genetic composition of Puth didn't change much in four years. The Fst values indicated little differentiation between the three measurements (table 3). Looking at the heterozygosity (He) development in time, stabilization is reported (table 2). In Sittard the situation is different. The population starts to differ from its source, regarding its increasing Fst value (table 3). Also the heterozygosity slightly increases during five years (table 2), which underlines this possible genetic drift away from their source population. From our data this phenomenon can't be explained. The increase in genetic diversity might be due to immigration of individuals from a neighboring population, in this case would that be the population of Puth.

Restocking

The Belgian populations, Berthem and Widooie, were restocked in 2007 and 2008. Captive-bred hamsters with Dutch alleles and trapped individuals from the Puth/Sittard area were released. The restocking was successful, because allelic diversity showed an increase of almost 100%, and heterozygosity levels rose to similar levels as the Dutch wild populations. Interbreeding was detected between the restocked individuals and the wild population (table 2), but the populations is not completely mixed yet. There are still some individuals left with only Belgian or Dutch genetic characteristics.

Amby was restocked three times (table 1) with individuals from the

Dutch-Belgian breeding line. The first restocking was probably not successful, because no new alleles were detected and there was no sign of increase in heterozygosity (table 2). On the contrary, the other two restockings might be more successful. In the measurements of 2009/2010 a slight increase in allelic richness and heterozygosity has been discovered. Also two new alleles were reported in 2010. Nevertheless, because of these results, the restocking might not be considered successful. One allele could not be detected anymore and the number of individuals sampled in 2009/2010 in Amby is very low (n=6). The group presented might not represent the whole wild population.

However, the Heer population revealed more promising results. Heer was restocked with individuals from the Dutch-German breeding line.

After restocking the analysis showed a large increase in both the allelic richness and heterozygosity (table 2). Principal Coordinate Analysis pointed out that the released individuals interbred with the wild population. This result can be considering pretty outstanding. Heer was restocked with only seven individuals, and four of these individuals were predated within one month.

So what determines if a restocking will be successful? The number of individuals involved in restocking can be excluded. The restocking in Heer was successful with only 7 individuals, while the restocking in Amby in 2006 failed with more than 20 individuals. On the other hand, the population size just before restocking might be more important. Restocking in a small popu-

lation triggers larger effects on the genetic diversity of the population than restocking in a large population. Another possibility might be the origin of the restocked animals. Restocking in the successful areas (Heer, Berthem and Widooie) was carried out with a combination of wild and captive bred hamsters. While restocked animals in Amby all came from the breeding program.

Conclusion

Altogether this study shows the importance of genetic monitoring of reintroduced species to evaluate the success of reintroduction. In case of the Common Hamster, genetic diversity of reintroduced populations in the Netherlands is not affected as a result of any founder effects. No bottle-necks could be detected. Indication of possible genetic drift was found in the Puth area, but can't be explain from the results. But did the restocking succeed? Restocking in the Netherlands and Belgium resulted in an increase in heterozygosity and mixing of wild and restocked individuals. Therefore the restocking is considered successful. Further research is needed to examine the effect of this increase in genetic diversity on the population growth. Conservation of the Common Hamster still remains the most important goal, but research contributes to knowledge for better protection of the species.

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