

Genetic rescue and the increase of litter size in the recovery breeding program of the common hamster (*Cricetus cricetus*) in the Netherlands. Relatedness, inbreeding and heritability of litter size in a breeding program of an endangered rodent

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Reduced genetic variation is a severe threat for long-term persistence of endangered animals. Immigration or translocation of new individuals may result in genetic rescue and increase the population viability of the endangered population or species. Unfortunately, studying genetic rescue in wild populations is very difficult, but breeding programs of endangered species can contribute to our knowledge of the diverse effects of genetic rescue.

A recovery breeding program of common hamsters in the Netherlands enabled the study of genetic rescue in an endangered rodent as a few wild hamsters from two nearby and also highly threatened populations were added to the breeding stock.

Litter size increased over the years, but no relation between inbreeding levels and litter size was found. Average litter size benefited from the genetic variation introduced by a hamster from Germany, but hamsters from Belgium had no effect on litter size. Rather than alleviating inbreeding depression the genetic rescue effect observed in this population seems to originate from the introduction of beneficial alleles by the German male. Breeding programs using several populations may increase the success of reintroductions and long-term persistence of these populations.

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Highly threatened species often have a small population size and reduced genetic variation, which can be a severe threat for long-term persistence of populations. Immigration or translocation of new individuals from other populations or from breeding programs may result in a recovery of individual fitness in the hybrid offspring and an increase of the population fitness (WESTEMEIER et al. 1998; MADSEN et al. 1999; HEDRICK 2005; PIMM et al. 2006). This hybrid offspring may also have a positive effect on the success of reintroductions or augmentations (SCHWARTZ and MILLS 2005; FREDRICKSON et al. 2007). However, the outcome of such genetic rescue events is uncertain as genetic rescue can lead to outbreeding depression (EDMANDS and TIMMERMAN 2003) and depends on the genetic quality or genetic constitution of immigrants and the response of recipient populations on these immigrants (RICHARDS 2000). More natural and laboratory experiments are needed to understand the theory

and mechanisms behind genetic rescue (TALLMON et al. 2004). Studying genetic rescue in wild populations is, unfortunately, very difficult, but breeding programs of endangered species can contribute to our knowledge of the diverse effects of genetic rescue. The Dutch recovery breeding program of the common hamster (*Cricetus cricetus canescens*) enabled the evaluation of genetic rescue effects in a natural rodent population in captivity and the exploration of these effects.

The common hamster, a medium sized rodent, has suffered a more than 99% decline in the most western part of its range (Fig. 1) and is nowadays on the brink of extinction in Belgium, the Netherlands and the adjacent German federal state of North Rhine-Westphalia (further referred to as the BNN-region). Only five hamster populations survived in the BNN-region (Fig. 1) and all populations show a severe loss of genetic variation at the molecular level (NEUMANN et al. 2004; LA HAYE et al. 2012).

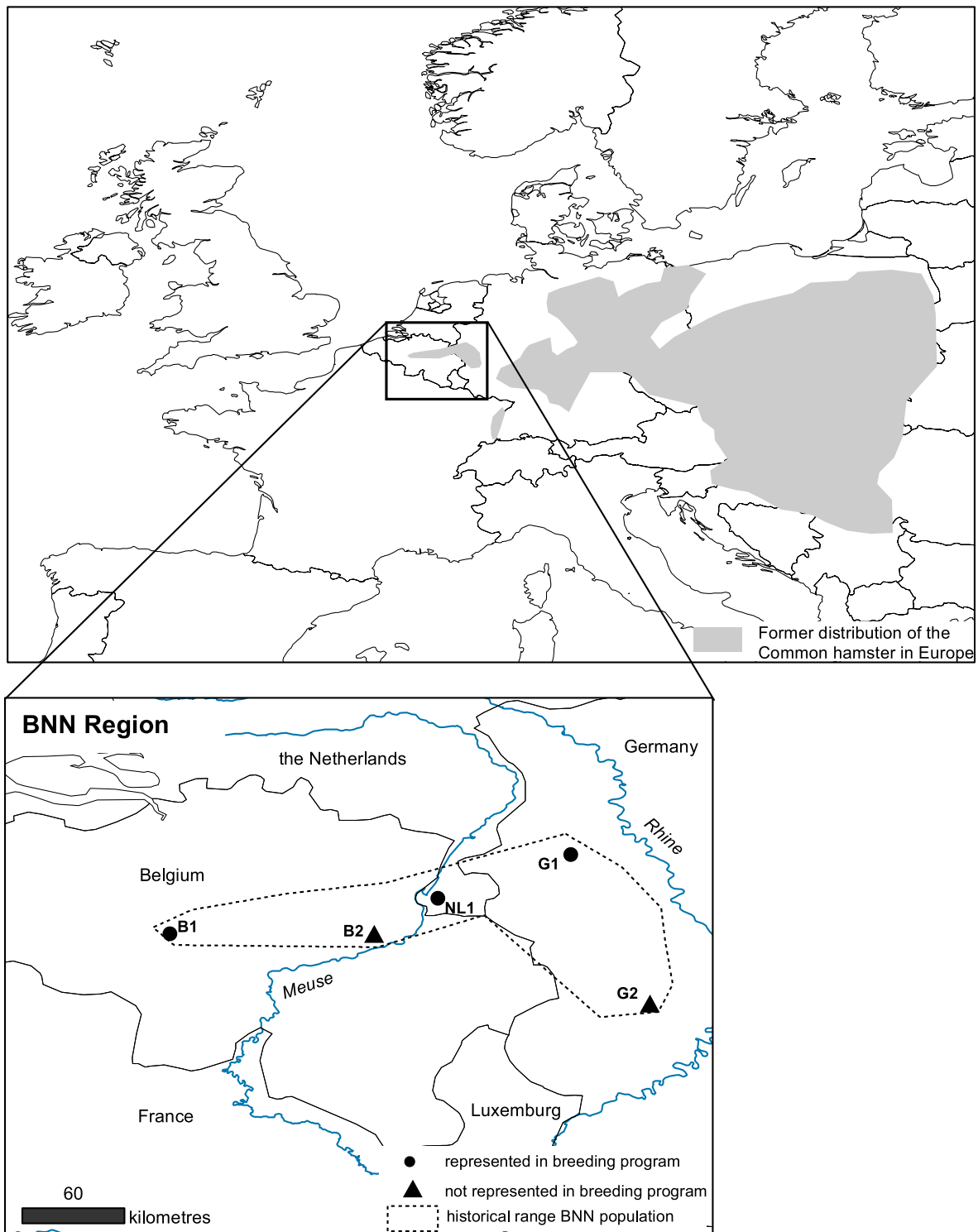


Fig. 1. Former geographical distribution of the common hamster (*Cricetus cricetus*) in Europe (grey area) according to MITCHELL-JONES et al. (1999) and a detailed map of the historical distribution of the common hamster in Belgium, the Netherlands and adjacent Germany (referred to as the BBN region). The historic distribution area in the BNN region is situated within the dotted-line. Currently only five relict populations remain, of which B1, NL1 and G1 are represented in the Dutch breeding program, while B2 and G2 are not represented.

Reduced genetic variation is generally associated with lower population fitness (KELLER and WALLER 2002) and may limit the success of conservation projects (ROBERT 2009). To overcome possible negative consequences of the loss in genetic variation in running conservation projects of the species in the BNN-region, it was advised to increase the genetic variation in all five relict hamster populations by exchanging individuals between the BNN-populations or to use individuals from the Dutch breeding program to augment wild populations (NEUMANN and JANSMAN 2003; LA HAYE 2008).

The Dutch breeding program was founded with 10 individuals from the last wild population in the Netherlands and a few years later three hamsters from two other highly threatened and inbred BNN populations were added to the breeding stock and founded two extra breeding lines. These new individuals can be seen as rare immigrants in the Dutch population and their consecutive successful breeding as a genetic rescue event.

The aim of this paper is to explore the possible genetic rescue effect in the Dutch breeding program of the Common hamster and the potential effects on conservation projects. We used observed litter size in the breeding program as a substitute for population fitness, because this trait is easy to measure in captivity. Litter size is also an important demographic parameter impacting the persistence of populations of short-living rodents such as common hamsters (KUITERS et al. 2007; ULBRICH and KAYSER 2004). We hypothesize a larger litter size in genetically more diverse breeding lines compared to the original genetic depleted NL-breeding line. This increase is caused by a decrease in inbreeding, and less inbred individuals are thus expected to have larger litters. Therefore, observed litter size was analyzed for

1. differences between the Dutch breeding line and hybrid breeding lines,
2. the heritability of litter size to see whether an increase of fitness as a result of selection can be retained over long timescales because it is heritable,
3. effects of the inbreeding level on litter size.

Our analysis of the Dutch recovery breeding program of the hamster can help in understanding the effects of genetic rescue in the field of conservation biology and specifically in conservation of endangered rodents.

MATERIAL AND METHODS

Species description

The common or European hamster (*Cricetus cricetus canescens*) is a small medium sized rodent, which inhabits cultivated fields and farmland on loss and loamy soils across Europe (MITCHELL-JONES et al. 1999),

although the species can also be found in urban areas in some countries (FRANCESCHINI-ZINK and MILLESI 2008). Hamsters can produce two, sometimes three litters in the wild during one reproduction season, which last from May till August (FRANCESCHINI-ZINK and MILLESI 2008; HUFNAGL et al. 2011). Males in the wild rarely survive longer than one year, while females have a better survival and can survive up to two years in the wild (KUITERS et al. 2007). In the Dutch breeding program, hamsters were normally allowed to produce one litter per year, with sometimes a second litter in the following year. In the wild it is possible that young of the first litter already reproduce in the same season (FRANCESCHINI-ZINK and MILLESI 2008; LOSÍK et al. 2007), but in captivity all individuals reproduced only after their first hibernation. In captivity some hamsters reached an age of three years, but breeding with these individuals appeared not to be possible anymore.

Breeding program

The Dutch breeding program was founded in 1999 with five males and five females from the last Dutch population (population NL1, Fig. 1) and with these individuals the Dutch lineage (NL-line) was constructed. In 2003 and 2004 three hamsters were successfully added to the Dutch breeding program, two males from the Belgium population of Bertem-Leefdaal (population B1, Fig. 1) and a male from the German population of Neuss (population G1, Fig. 1). The complete pedigree of all individuals, except wild trapped founders, and litters used in this study was known. The Studbook also include a few individuals with an unknown pedigree, although born in captivity from captive-bred parents, but these individuals were excluded from this analysis and further breeding within the breeding program.

From 2003 onwards three different breeding lines were maintained (Table 1), with:

1. hamsters from the last wild Dutch population with no mixing from other populations: the NL-line,
2. off-spring from matings between the two males from the Belgian population B1 (Bertem-Leefdaal) with Dutch females (n = 9): the B/NL-line,
3. off-spring from matings between the male from the German population G1 with Dutch females (n = 10): the G/NL-line.

In consecutive years breeding was restricted to, genetically hybrid, individuals from the same lineage, although in later years individuals from the NL-line (genetically 100% Dutch) were mated with genetically hybrid individuals from the B/NL- and/or G/NL-line (an overview is presented in Table 2). In 2007 a complete hybrid lineage was constructed, the B/G/NL-line, with individuals from

Table 1. The number of litters (N_{litters}) and the number of surviving juveniles (N_{ind} , until time of separation) per year and per breeding-line. *no. of hamsters born in captivity.

Year	NL/ NL-line		B/ NL-line		G/ NL-line		B/G/ NL-line		Total	
	N_{litters}	N_{ind}	N_{litters}	N_{ind}	N_{litters}	N_{ind}	N_{litters}	N_{ind}	N_{lit}	N_{ind}
2000	7	34							7	34
2001	19	99							19	99
2002	23	115							23	115
2003	17	82							17	82
2004	9	52	5	24	6	33			20	109
2005	8	41	13	70	13	90			34	201
2006	8	54	10	60	7	50			25	164
2007	5	27	3	11			14	76	22	114
2008	2	11					4	20	6	31
Total	98	515	31	165	26	173	18	96	173	949*

all established lineages. The breeding program was managed under a scenario of minimizing kinship within different breeding lines. The number of litters born within the breeding program each year was restricted, normally between 10 and 25 litters were bred during one breeding season (Table 1).

Breeding from 1999 to 2008 took place at three breeding locations in the Netherlands. However, no more than two breeding locations were used in one year. From 1999 to 2008 breeding took place at the Rotterdam Zoo, from 1999 to 2004 also at a private breeding station in Beek-Ubbergen at 'Stichting Das&Boom'. The hamsters kept at the breeding station in Beek-Ubbergen was taken over by the Gaiapark Zoo in Kerkrade in 2005 and breeding was continued in the following years. All breeding locations had more or less the same breeding task each year, producing an equal number of litters per breeding-line. Before each breeding season hamsters were translocated between breeding station if necessary for breeding goals. Although breeding was highly standardized, there were some differences in husbandry between the breeding locations. In Rotterdam Zoo the cages with hamsters were housed in different compartments of one building, while in Beek-Ubbergen and Kerkrade all

cages were housed in one big compartment respectively hangar. The natural daylight rhythm was visible for all hamsters and temperature was not regulated and followed the seasonal variation, although temperatures below freezing were avoided as much as possible by heating the air inside the breeding stations.

Breeding of hamsters was in accordance with the Animal Welfare Law and Flora- and Fauna Act of the Netherlands.

Data collection

The analysis of the data is based on the registration in the Hamster Studbook managed by the Rotterdam Zoo, ver. April 2009. This version of the studbook gives a complete pedigree of all hamsters within the breeding program and includes information on 969 hamsters of which 20 were trapped in the wild and 949 were bred in captivity. Only 13 out of the initial 20 founders trapped in the wild reproduced successfully, 10 individuals from the Dutch population (NL1), two individuals from the Belgian population (B1) and one male from the German population (G1).

Litter size is measured as the number of juveniles alive at an age of six weeks. At six weeks after birth litters are separated and each individual is equipped with a transponder. Before an age of six weeks the exact number of juveniles is unknown, because breeding females are left alone as much as possible to prevent infant-mortality.

All the founders in the breeding program came from three geographic isolated and very small populations (LA HAYE et al. 2012), being relicts from the former non-fragmented hamster population in the BNN-region (Fig. 1). Indicating that relatedness of founders trapped in different populations was probably low, while relatedness of founders trapped in the same population was high. Inbreeding within each of the populations was probably high because of the strong reduction in genetic variation compared with their historic ancestral population (NEUMANN et al. 2004; LA HAYE et al. 2012) and the strong reduction in population numbers. This assumption is based on genetic results as published by LA HAYE et al.

Table 2. Mean litter size (\pm SD) of crossings between unrelated founders ($W \times W$ or $W \times F$) and the mean litter size in consecutive generations (F_1 - F_6). W = founders, F = captive bred offspring.

Breeding-line	Crossings (Sire \times Dame)	Sample size	Mean litter size (\pm SD)
NL-line	Crossings of NL Founders \times NL founders or NL offspring and NL offspring \times NL offspring	98	5.3 \pm 2.0
B-line	Crossings of B founders \times NL offspring or B/NL offspring	9	4.4 \pm 1.9
	Crossings of B/NL offspring \times B/NL offspring or NL offspring	22	5.7 \pm 2.2
G-line	Crossings of G founder \times NL-offspring	10	5.9 \pm 1.6
	Crossings of G/NL offspring \times G/NL offspring or NL offspring	16	7.1 \pm 1.8
B/G/NL-line	Crossings of B/NL offspring \times G/NL offspring and B/G/NL offspring \times B/G/NL offspring	18	5.3 \pm 1.4

(2012). Genotypes of all founders in the Dutch breeding program were included in the same study to measure the loss of genetic diversity in relict populations of hamsters compared to the un-fragmented historic population of the BNN-region. Genetic diversity, number of alleles, differed strongly between populations, with almost no genetic diversity left in the Belgian and German population. An initial high inbreeding coefficient within each of the founders was achieved by creating genetic groups by adding common ancestor pairs for each of the populations (QUAAS 1988): in the pedigree all founders got virtual ancestral parents and grandparents (the same parents and grandparents for individuals from the same population). Furthermore, one of the founding females (W11) in the Dutch breeding line was trapped with six of her young and the father of this offspring was presumed to be a single untrapped individual. This approach resulted in a basic inbreeding coefficient of 0.25 for all founders. See Fig. 2 for an overview of the pedigree. It was not possible to calculate real inbreeding coefficients, because of the low genetic diversity in all individuals.

Analysis

Effects on litter size were analyzed simultaneously for breeding location, breeding year, parity of mother, age of father, inbreeding of the mother, inbreeding of the offspring and genetic origin of the breeding-line (NL, B/NL, G/NL and B/G/NL) using a mixed model with a restricted error maximum likelihood (REML) analysis. The general form of the animal model is $y = X\beta + Zu + e$ with y being the vector of observations of trait y , X and Z the incidence matrices relating respectively the vector

with fixed effects (β) and the vector of additive breeding values (u) to y , and e the residual value. The random effects u and e are assumed to be normally distributed with mean 0 and variance σ^2_A and σ^2_E , respectively the additive genetic variance component and the residual variance component. The heritability h^2 is defined as σ^2_A/σ^2_p , with σ^2_p the total phenotypic variance being equal to $\sigma^2_A + \sigma^2_E$ (FALCONER and MACKAY 1996). In this specific case the model was

$$L = \mu + \text{Fixed} + \text{reg}(F) + a + e$$

where L = litter size of the mother; μ = mean litter size; fixed = fixed effects: parity of mother, age of father, location of breeding or year of birth; $\text{reg}(F)$ = regression on inbreeding coefficient, either of the mother, the father or the offspring; a = random additive genetic effect (i.e. breeding value of mother), with relationship between breeding values of the mothers determined by the numerator relationship matrix calculated from the pedigree; e = random residual effect.

With a and e leading to the estimation of the additive and residual variance components. To test whether the additive variance component and the heritability were significantly different from 0 the model was run twice: once with the additive effect included, and once without. Twice the difference between the maximum likelihood of the two models is χ^2 -distributed with 1 degree of freedom.

Year of birth, and inbreeding of mother, father or offspring were confounded. Inbreeding increased over the years, and consequently in a model with both year of birth and inbreeding of mother (or father or offspring) only one of them will have a significant effect. Therefore, initially each of these effects was analyzed separately, and then compared to a model with both year and the inbreeding included. The other fixed effects were always included. Their significance was similar in all models and will be reported only for the model with inbreeding of the mother.

Analyzing age of mother was not possible, because breeding was restricted to one-year old females in most years. Zoo breeders reported difficult breeding results in two-year old females in the first years of the breeding program: much more mating attempts were needed for a successful litter. From points of efficiency it was decided to exclude two-year old females from breeding as much as possible.

RESULTS

Effect of new males

Mating in the first few years was between individuals from the NL-line only, since individuals from other populations were not available. The mean litter size in this breeding-line was 5.3 ± 2.0 (SD) and ranged from 1 to 10.

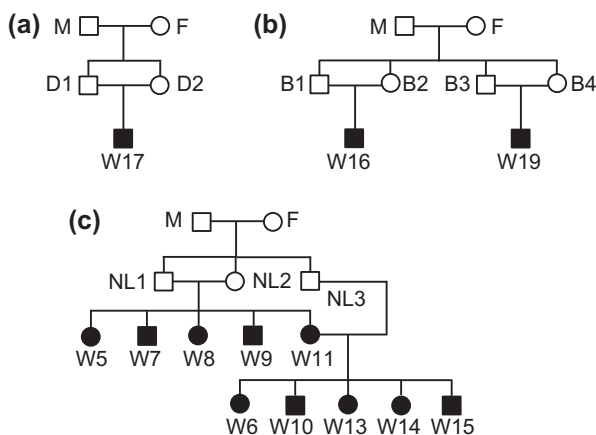


Fig. 2. Virtual pedigree of wild trapped founders (W-individuals) within the Dutch breeding program which successfully reproduced. All founders are indicated with a black symbol and have an initial inbreeding coefficient of 0.25. Females are represented by circles, males by squares.

Litter sizes in the first generation (F_1) of the B/NL-line, where wild Belgian males were crossed with captive-bred Dutch females, was not significantly lower in comparison with the litter sizes of the NL-line (Table 2). The litter sizes of crossings between the F_1 -individuals in the B/NL-line increased to a level that was slightly above that of the NL-line, B/NL-line 5.7 ± 2.2 (SD) and NL-line 5.3 ± 2.0 (SD), and mean litter size remained at a slightly higher level in the B/NL-line in consecutive generations although not significantly different from that of the NL-line.

The F_1 litter size of the G/NL-line was above that of the NL-line (Table 2), but not significantly different: the mean of the G/NL-line was 5.9 ± 1.6 (SD) versus 5.3 ± 2.0 (SD) for the NL-line. The F_2 and consecutive generations of the G/NL-line had a mean litter size of 7.1 ± 1.8 (SD), which is the highest mean litter size in all crossings and breeding lines and significantly higher than in the NL-line ($p = 0.001$).

Genetic analyses

No significant effects were found for age of the father ($p = 0.38$), breeding location ($p = 0.32$), and breeding year ($p = 0.14$), when analyzed in the mixed model (REML). Parity of the mother had a significant effect on litter size ($p = 0.037$) with first parity mothers having larger litters. When analyzed separately regression on either inbreeding level of the mother, father or the offspring had no significant effect (slope of mother -0.44 , $p = 0.28$; father -1.7 , $p = 0.34$ and offspring -0.04 , $p = 0.33$, respectively), nor had year of birth ($p = 0.13$). The additive effect was just significant ($p = 0.0499$), with an estimated heritability (h^2) of 0.275 (estimated phenotypic variance 3.191 and the additive variance component 0.877) for the model with only a regression on inbreeding level of the mother. In a model with both year and inbreeding of the mother effects of year and inbreeding remained insignificant. The h^2 in such a model reduced to 0.121, possibly because year and breeding value were also confounded. Litter sizes tended to be higher in later years as did breeding values for litter size. When the model was restricted to animals of the NL-line only, the h^2 was 0.228. The effect of inbreeding of the mother within the NL-line was positive (i.e. more inbred individuals had larger litter sizes) although not significant. When both regression on the inbreeding level of the mother and year of birth were removed from the model the h^2 increased to 0.368.

DISCUSSION

In our study we analyze litter size in different breeding lines of the Dutch Hamster breeding program in the period

1999 till 2008. This breeding program was founded in 1999 with 10 individuals from the last natural population in the Netherlands. The Dutch breeding population was highly inbred of its own (NEUMANN et al. 2004; LA HAYE et al. 2012), which raised concerns on the genetic viability of the breeding stock and the feasibility of using the offspring for reintroductions in the future. However, it is difficult to measure genetic viability and generally its effect can only be demonstrated by crossings with individuals from other populations (HEDRICK and KALINOWSKI 2000). The arrival of unrelated hamsters from two other populations (populations B1 and G1, Fig. 1) into the breeding program made it possible to make crossings between individuals from different populations and to evaluate the effect of increased genetic variation on litter size.

These new males came from two relict populations, which were in the past part of a large hamster meta-population in the BNN region. This meta-population started to decline and disintegrate some 30 to 40 years ago. It is not clear when gene-flow stopped completely between populations, but an estimated isolation of at least 25 years between Dutch (NL1) and German (G1) populations seems reasonable. The Belgian population (B1) has been isolated even longer, because the river Meuse and the Albert-canal (constructed in the midst of the 20th century) form an almost absolute barrier for natural dispersal of hamsters between Belgium and the Netherlands. These periods are too short to result in clear effects of outbreeding depression once gene-flow is restored (FRANKHAM et al. 2011), but inbreeding depression at the population level was expected based on the time of isolation, small population sizes and low genetic variation in all populations (LA HAYE et al. 2012).

Effect of non-genetic factors on litter size

In our study we focus primarily on the effects of increased genetic variation on litter size as a result of immigration of new genetic variation in an inbred population of an endangered species. However, from other breeding programs it is known that husbandry, diet or other factors may have an effect on breeding results as well (KALINOWSKI et al. 2000; CASIMIR et al. 2007). We therefore tested several non-genetic factors like age of the father, breeding location and breeding year for possible effects on litter size, but no clear effect of non-genetic factors on litter size was found, with exception of parity of the mother. First parity mothers had larger litters, which is in line with a study from Vienna where first litters were bigger than second litters, probably as a result of energetic constraints (HUFNAGL et al. 2011).

It cannot be excluded that there was an effect of age, especially in females in their second reproductive season, on successfully producing a litter (not litter size).

Zoo-keepers reported that it took more time to successfully mate two-year old females, but unsuccessful mating attempts with no offspring were not officially noted and breeding attempts with two-year old females were, based on the advices from the Zoo-keepers, avoided as much as possible after the first years of the breeding program.

Litter sizes tended to be bigger in later years which corresponds with the introduction of new males. We therefore conclude that the differences in litter size in the breeding program between the different breeding lines are the result of a genetic effect.

Effect of new males on litter size

The Dutch population (the NL-line) which founded the breeding program was inbred (LA HAYE et al. 2012), but the first few years breeding went well and no signs of inbreeding depression, for example high mortality of juveniles with external abnormalities, were detected. Although litter size of the NL-line was with a mean of 5.3 ± 2.0 (SD, $n = 98$) somewhat smaller than reported in other studies of the same species. NECHAY (2000) and KAYSER (2002) reported a mean litter size of seven in large wild populations in Hungary respectively Germany. Breeding results of Common hamsters held in laboratory colonies were in the same range as wild populations (MONECKE and WOLLNIK 2008). The litter size in the NL-line was therefore smaller than could be expected. The absence of other signs of inbreeding depression can be the result of an already highly inbred population in which any further inbreeding will have no or only minor effects, but it may also be the result of the more benign environment of captivity.

Hence, hamster populations in the Netherlands and adjacent regions showed such a strong decline in the second half of the nineties, that genetic drift was the main process shaping the current genetic diversity in the surviving populations of the BBN region. Recent population size estimates varied from 5 to 20 individuals in populations in the BNN region, with the not represented population of Zülpich (Fig. 1, population G2) as a relative positive exception with an estimate population size of 150–200 individuals (LA HAYE et al. 2012). Genetic drift may or may not have led to the fixation of (mildly) deleterious alleles reducing litter size in local populations. It is therefore highly unpredictable which alleles with an effect on litter size are preserved in the current highly inbred populations and if genetic load is low or high in these relict populations.

The introduction of the German male in the breeding stock led to an increase in litter size, although the difference with the NL-line was not significant. Litter size increased further in F_1 -crossings of the G/NL-line (Table 2) and this increase was significant ($p = 0.001$)

compared to the NL-line. The contribution of the Belgian males, however, resulted at first in a reduced litter size (Table 2), but litter size was not significantly different compared with the NL-line. In the F_1 -crossings of the B/NL-line litter size increased to values comparable with initial values of the NL-line.

These results can be explained by the presence of alleles with a positive effect on litter size in the German male. Litter size depends on several alleles (e.g. compare ROCHA et al. 2004) and the genetic contribution of the German male probably consisted of beneficial alleles in hybrid G/NL off-spring, while no beneficial alleles were contributed by the Belgian males. Selection in subsequent G/NL generations increased the frequency of beneficial alleles resulting in a further increase of litter size. This would also explain why more inbred animals in later years of the breeding program had increased litter size, contrary to the expectation that inbred animals perform less well. The ultimate test should have been to make crossings between Belgian (population B1) and German individuals (population G1) and to measure litter size in these crossings. Unfortunately it was not possible to make such crossings because only males were available and it was not possible to obtain more individuals from these highly endangered wild populations.

A further change in litter size in consecutive generations within each breeding line was not found, however, breeding lineages were managed under a scenario of minimizing kinship instead of specific crossings between the same generations. Probably this breeding scheme of minimizing kinship within a breeding line has led to stabilization of litter size, because involved alleles with an effect on litter size were randomized (FALCONER and MACKAY 1996).

Effect of heterozygosity, inbreeding coefficients and selection

Introduction of new males from Belgium and Germany had a direct effect on genetic diversity, inbreeding coefficients and heterozygosity in captive-bred offspring. Heterozygosity in captive-bred off-spring increased, but this increase was almost comparable in both mixed breeding-lines and therefore not used for further analysis. The new males were monomorphic and although alleles differed slightly compared to the Dutch population, only one allele (out of nine microsatellites) of the German male was new for the breeding stock (LA HAYE et al. 2012). Despite the little difference in allelic diversity and heterozygosity in mixed breeding lines, a difference in litter size was found between breeding lines. This difference indicates that our observation is not a typical example of heterosis, as mixing of different inbred-populations (B1 and NL1 respectively G1 and NL1) did not show the same effect on litter size.

The introduction of unrelated males decreased inbreeding coefficients in hybrid off-spring substantially. However, litter size was not significantly affected by the inbreeding coefficient of the mother ($F = -0.882$, SE 1.539). The negative slope is an indication that the inbreeding coefficient may have a small negative effect on litter size, but even in the NL-line where inbreeding coefficients were as high as 0.4–0.5 in 2008, there was no significant effect on litter size. When the effect of inbreeding was analyzed simultaneously with the additive effects, inbreeding even had a positive effect on litter size.

The absence of an effect can be caused by, unintended, selection in the breeding program. The number of successful litters per breeding-line were sometimes so small that the next generation came from individuals from three or four different litters. Such a small number of litters may facilitate unintended selection for captivity which can have a profound effect on litter size in further generations. Those individuals with large litter sizes will contribute disproportionately to next generations even under our breeding scheme of maximizing genetic variation in each breeding line by minimizing mean kinship of the population. Minimizing kinship should have prevented a strong adaptation to the captive environment (HEDRICK and KALINOWSKI 2000; FRANKHAM 2008), although some selection must have been unavoidable. This points to a potential extra benefit of captive breeding programs, the possibility to breed against deleterious alleles preventing recovery of endangered populations.

Another explanation for the absence of an effect of inbreeding coefficients on litter size should have been the option that deleterious alleles were already purged in the wild hamster populations. However, this possibility can be ruled out as shown by the increase in litter size in the G/NL-breeding-line as a result of the introduction of beneficial alleles by the German male and the absence of such an effect, despite the introduction of more genetic variation per se, in the B/NL-breeding-line.

Heritability of litter size

The new males had a substantial effect on litter size even in later generations, which indicate that litter size has a heritable component. The heritability for litter size is 28% for the complete breeding stock. Such a heritability of litter size is above the range as found in laboratory mice, where a heritability of 12 to 20% is reported (PERIPATO et al. 2004; FALCONER 1965). The heritability of litter size is somewhat lower in the NL-line with 23%, but without the inbreeding coefficients in the model, the heritability increased to 37% for the NL-line. Our results show that litter size has a component of heritability, which makes it possible that litter size in the long-term is restored to levels as reported in wild hamster populations (NECHAY 2000; KAYSER 2002).

Management dilemmas

Our analysis indicated that only the German male had a positive effect on litter size, while the Belgian males had no effect. This result clearly shows that increasing genetic variation alone will not automatically result in genetic rescue. An effect of increased genetic variation depends on the genetic quality or genetic constitution of immigrants and the response of recipient populations on these immigrants (RICHARDS 2000), which makes it very difficult to predict the outcome of genetic rescue. The Dutch breeding program illustrates once more the complexity and uncertainties involved in genetic rescue (HEDRICK and FREDRICKSON 2010; BIJLSMA et al. 2010; TALLMON et al. 2004).

The most effective way of increasing litter size in the hamster breeding program should have been to focus on breeding with the G/NL-line and exclude the Belgian males from breeding. However, excluding the Belgian males, would have resulted in a much smaller increase of the total genetic variation as well. Alternative possibilities to increase the genetic variation in the breeding program are limited, because the number of potential donor populations is only four in the BNN-region (Fig. 1, NEUMANN et al. 2004; LA HAYE et al. 2012) and all BNN populations are highly endangered, which makes it very difficult to obtain individuals for the breeding program in the first place. As in many other highly endangered species it is impossible to first test the effect of individuals on reproduction or other important life-history traits. And although our results suggests that some deleterious alleles with an effect on litter size were introduced by the Belgian males, it is possible that the Belgian males also introduced important life-history traits with an effect on the population persistence or the success of reintroductions which we did not measure (LEBERG and FIRMIN 2008; ROBERT 2009). Selection of specific individuals with the largest litter size was therefore no option (EDMANDS 2007). We focused in the breeding program on restoring and preserving as much genetic variation as possible and we hope that deleterious alleles will be purged after reintroduction in the wild (HEDRICK 2005). Moreover, it is important to maintain genetic variation in litter size as litter size in the wild will have an optimum depending on the environment. Too large litter sizes cannot be maintained in environments where and in years when food is scarce (HUFNAGL et al. 2011).

Conclusion

Our study showed that the highly endangered Dutch hamster population suffered from inbreeding depression which negatively affected litter size, but this was only clarified after crossings with individuals from other populations as proposed by HEDRICK and KALINOWSKI (2000).

The introduction of new individuals from two other threatened populations, which can be seen as a genetic rescue event, resulted in an increase of the genetic variation in the breeding program and a decrease of inbreeding coefficients in the offspring. However, only the genetic contribution of the single individual of the German population increased litter size, while the two males of the Belgian population had no effect on litter size. This result can be explained by the presence of beneficial alleles in the German population and this result illustrates the complexity and uncertainties involved in genetic rescue (HEDRICK and FREDRICKSON 2010; BIJLSMA et al. 2010; TALLMON et al. 2004). Restoring gene flow between threatened populations will not always lead to an increase of population fitness, but in the case of the common hamster litter size is only one of the traits influencing population persistence in the wild and hopefully other beneficial traits have been introduced by the Belgian males. In general, it is important to keep in mind that more genetic variation in species that are subject of conservation, augmentation or reintroduction projects means that selection can act on as much genetic variation as possible, which hopefully will increase the success and the long-term persistence of the species or populations of concern.

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