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Inbreeding in captive bred Przewalski horses from local populations

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A b s t r a c t . The objectives of the study were to estimate the inbreeding level in the population of captive bred Przewalski horses and its changes over time in the biggest conservation centers. The data of 2935 horses extracted from the Studbooks were considered. The average inbreeding coefficient was equal to 9.4%. In general, the inbreeding level decreased over the analyzed period. Average percentage of pedigree information was 40.2%.

Key words: endangered species, geographical distribution**,** inbred, zoo

Introduction

The most important strategy of genetic resource conservation is protection of the natural environment. However, in critical situations it might be necessary to restore population in captive breeding. One of the species extinct in the natural environment and later successfully reintroduced is Przewalski horse (*Equus przewalskii* Poliakov, 1881). The species, which is the last surviving ancestor of the domestic horse, was discovered by Mikołaj P r z e w a l s k i in 1878 in the grasslands and semi-deserts of Mongolia. First attempts at captive breeding were carried out in 1898 in Ascania Nova (Russia) and soon after the first individuals were sold to European and American zoological gardens (B o u m a n 1986). The first animals imported to Europe were thereafter distributed to several zoological gardens. Serious problems with reproduction appeared which is now explained by high dismemberment of the population and high increase in the inbreeding level (B o u m a n & B o u m a n 1988, 1994). The situation changed between 1935–1938 when the first interchanges of individuals were transacted between the zoos in Prague and Munich. A similar situation appeared after the Second World War. The animals that survived were distributed over several breeding institutions. From 31 individuals in 1945 the population size had only been increased to 40 individuals in 1945 whereas after the interchanges between 1955–1965 it was increased to 134 individuals. Subsequently the interchanges were discontinued which was reflected in reduced increase of population size. Since 1970 when interchanges of breeding material were restored constant increase in population size and stabilized inbreeding level have been recorded (B o u m a n & B o u m a n 1988). The current population originates from 13 founders. With a small number of founders it is impossible to avoid mating of relatives. It leads to increase a homozygosity in given population. Therefore such population often affected by inbreeding depression. A number of reports on inbreeding effects has been performed for livestock (P a r l a n d et al. 2007, S ø r e n s e n et al. 2005) and wild animal populations (M a r s h a l l & S p l a n t o n 2000). Early methods for estimation of inbreeding for large populations involved sapling animals from the population, looking for common ancestors. At present, rapid computer algorithm are available that allow estimation of inbreeding coefficients via full relationship

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matrix (Q u a s s 1976). Over last year, a new advances in molecular genetics created a new possibilities for monitoring of inbreeding in animal populations (K i m et al. 2007, D a e t w y l e r et al. 2007).

In undomesticated populations inbreeding decreases their adaptability (M a r s h a l l & S p a l t o n 2000) and in some cases even leads to extinction of populations (S a c c h e r i et al. 1998, K a i s e r 1998, W e s t e m e i e r et al. 1998). Therefore the breeding programs for endangered populations should be permanently verified according to their inbreeding level and conservation of genetic variance. Genetic variance demonstrates the degree to which the genetic pool of the population and its genetic potential of adaptability can be preserved.

The objectives of the study were to estimate the inbreeding level in the population of captive bred Przewalski horses and its changes over time in the biggest conservation centers.

Material and Methods

The data were extracted from the Studbooks which include the pedigrees of all known captive bred Przewalski horses. The data of 1263 stallions 1386 mares and 33 horses of unknown sex born between 1901 and 2004 were included. Within country analysis was restricted to countries from which more than 100 horses were registered (Table 1). Inbreeding coefficients were extracted from the additive relationship matrix (Q u a a s 1976). Thus an inbreeding coefficient of i-th individual (F_i) is expressed as: $F_i = \frac{1}{2}a_{s_id_i}$,where: $a_{s_id_i}$ is additive relationship coefficient between parents of i-th individual. It should be stressed that all known ancestors were included. Seventeen percent of horses had incomplete pedigree information (442 animals both parents unknown, 35-unknown sire, 32-unknown dam). Percentage of pedigree information was calculated by Cassell's method (C a s s e l l et al. 2003). The effective number of founders (f_e) was estimated as $f_e = \frac{1}{\Sigma p_i}$, where p_i is probability that two genes randomly drawn from the reference population (animals born in 2004) originate from the same founder. The PEDIG software (B o i c h a r d 2002) was used for the calculations.

Results

D i s t r i b u t i o n o f P r z e w a l s k i h o r s e p o p u l a t i o n s

The size of captive bred population is relatively small with 524 individuals surviving in July 2004 (with sex ratio of 1.74 females : males). The population was dispersed over 86 breeding units in 27 countries on all continents with biggest national subpopulations in Germany, USA, Great Britain, Australia, Czech Republic, Ukraine and Poland. Until 1960 the reproduction of the species was poor with less than 20 foals born per year all over the world. The postnatal mortality was also on a high level (similar date of birth and death in the data base). After 1960 the number of births was increasing achieving 145 foals in 1992, however since then negative trend in population reproduction has been observed (Fig. 1).

I n b r e e d i n g l e v e l

Average inbreeding rate in the studied population of Przewalski horses was equal to 0.094. The percentage of pedigree information was 40.2 for whole population with 30% of population

Table 1. Distribution of registered births of Przewalski horses.

Australia	Austria	Belgium	Canada	Croatia
79	82	55	45	2
Cuba	Czech Rep.	Denmark	Estonia	Finland
3	215	10	19	10
France	Germany	Great Britain	Japan	Kazakhstan
57	570	512	5	7
Lithuania	Mongolia	Netherlands	Poland	Russia
7	13	91	106	84
South Africa	Slovakia	Spain	Sweden	Switzerland
10	13	3	10	39
Taiwan	U.S.A.	Ukraine	Vietnam	Other
31	415	139	4	16

exceeding 70 percent of pedigree completeness. Missing ancestors might have significantly decreased estimated inbreeding level.

An increase in population size over time was observed (Fig. 1) except for the last two decades which could result from an incomplete updating of the database. As the beginning of captive breeding 1901 was assumed as the year of studbook foundation. Until 1940 the inbreeding level was increasing up to the level of 0.37 due to small population size with no possibility to avoid mating of relatives. During the times of Second World War small number of births was registered, registered breeding was restored after 1950. When the population size was stabilised, after a rapid increase in the 1960s (over 20% for all individuals and 33% for inbred individuals) a decreasing trend in the inbreeding level can be noticed (Fig. 1). In most of the periods inbred individuals outnumbered the non-inbred ones. Since the 1980s this proportion has been improving. The reintroduction programs began in 1990 but they do not seem to affect the inbreeding level in a captive population. The horses were mainly

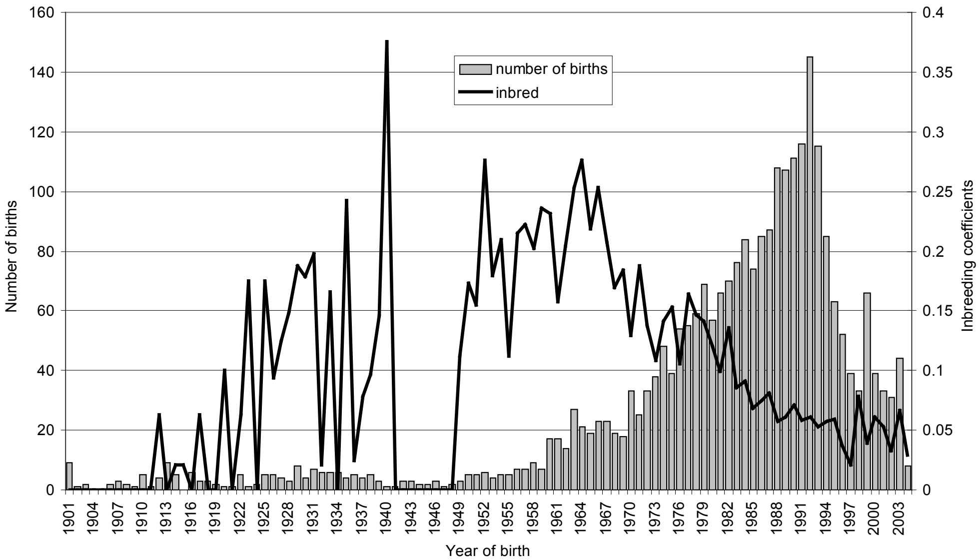

Fig. 1. Inbreeding level and number of births in captive bred Przewalski horse population.

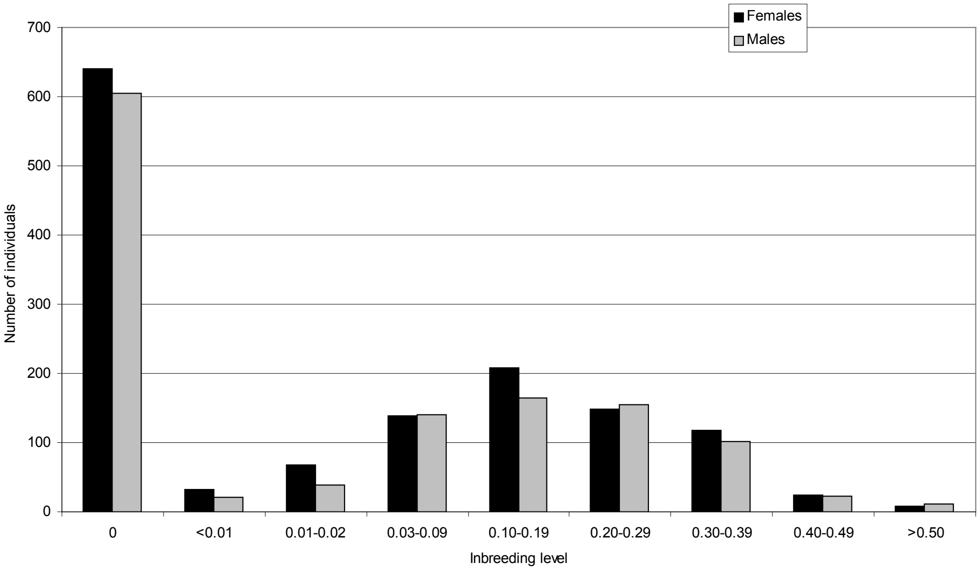

Fig. 2. Inbreeding distribution for male and female Przewalski horses.

mated within country or even zoo subpopulations. Similar level of inbreeding was found for male and female horses (Fig. 2).

The inbreeding level estimated for inbred animals was 19%. Inbred animals had on average 73% of pedigree information. Average inbreeding for the overall population is lower because individuals without full pedigree information (assumed inbreeding coefficient equal to 0) were also included. For horses born in 2004 effective numbers of founders were equal to 7.8 and 17.6 for males and females, respectively.

Inbreeding level in the biggest subpopulations

On Fig. 3 numbers of animals and their inbreeding level was given for the biggest conservation centers.

Czech Republic
Zoological garden in Prague plays important role in conservation of Przewalski horses with 40 individuals surviving in 2004. Additionally 3 horses were registered from Brno. First foal was born in 1933. In the next 20 years inbreeding level stayed on the level of zero however only 5 births were registered. In 1950 3 foals were born with the inbreeding level of 0.24. The increasing tendency was continued until 1966 when the maximum value of 0.39 was reached. Since than inbreeding level has been decreasing with fluctuations in 1981 and 1982.

Germany
The situation in German zoos was similar although this country has the biggest registered population in the world. 21 breeding units contributed to the history of breed preservation 13 of which had horses in 2004. Until 1949 the inbreeding level was rarely increased with the highest level (25%) in 1930–1931. Most significant changes appeared after 1949 with

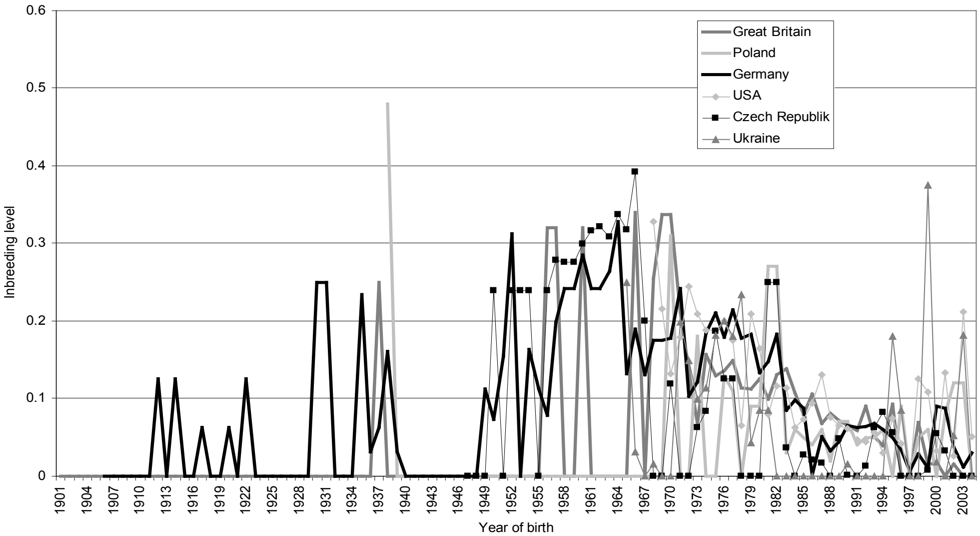

Fig. 3. Average inbreeding coefficients in the biggest conservation centres of Przewalski horse over time.

increasing homozygosity until 1964 (33%). Since then the inbreeding level was decreasing with some fluctuations to achieve 3% in 2004.

Great Britain (GB)

12 breeding institutions in England, 1 in Scotland and 2 in Wales had the second largest national population of Przewalski horses. 78 animals were kept in 2004. In England the studbook was already set in 1901. The highest inbreeding level was recorded in the beginning of 1930s then it was reduced to 0 on which level was kept until 1966. In 1966 inbreeding rapidly increased to 34% but then was again reduced to 0 in 2003.

Poland

In December 1937 two mares and two stallions were imported from Ukraine to Warsaw zoological garden which can be treated as the beginning of Przewalski horse breeding in Poland. The individuals were inbred and related therefore first foal born in 1938 in Poland was highly inbred (48%). Next transfers were performed in 1965 and 1968 when 3 horses were imported from Prague. Next two stallions were born in 1969 with null inbred, however horses born in following year had inbreeding level of 31%. Next increases of homozygosity appeared in 1973–18%, 1976–13%, 1977–11%. The inbreeding was kept low with two fluctuations 1981–82 (27%) and 2002–03 (12%). The foal registered in 2004 was not inbred. Until August 2004 106 Przewalski horses were born in Poland 55 of which were females. 34 non-inbred stallions and 33 non-inbred mares were born. In 2004 Polish population consisted of 13 individuals.

Ukraine

Four zoological gardens in Ukraine (Askania, Kharkov, Kyev, Nikolaev) participated in the breeding program. 26 horses in 2004 were dispersed over these four units. Registered breeding was started in 1905. The inbreeding level began to increase in 1920 (0.19) up to 0.25 in 1935, although also non-inbred individuals were born. Second period of increased inbreeding appeared between 1963–1981 and the third in 1995. The maximal values (0.37) were obtained for animals born in 1939, 1940 and 1999. The average inbreeding level in 2003 was equal to 18%.

United States of America (USA)
Przewalski horses were bred in 22 cities in the USA, in 9 of which more than 10 foals were born. Population of 86 horses in 2004 was dispersed over 12 units. The inbreeding level has never bees as high as in the Ukraine, except from two case it was less then 0.3, mostly below 0.25. The highest level was observed in 1968- 0.33 and 1915–0,31. Generally until 1958 inbreeding was kept on the zero level. Then the homozygosity kept increasing until 1968 when wit was successively reduced to star increasing again in 1995 reaching 0.21 in 2003.

General Discussion and Implications

Mating of relatives leads to an increase of homozygosity, which may result in phenotypic expression of recessive lethal and semi lethal genes. The biological potential of more heterozygous animals can be higher than that of inbred ones (Keller 1994, Launey & Hedgecock 2001). Inbreeding can be advantageous for well-adapted populations in stabilized environment in which well-developed traits become fixed in the homozygous state. Generally, natural selection favours heterozygous individuals, which was confirmed by a negative relationship between inbreeding and longevity in many species (Mousseau & Roff 1987, Shikano et al. 2000, Reed & Bryant 2000). From the perspective of conservation of genetic resources, inbreeding is a negative phenomenon since it increases the mortality of the youngest, immature individuals, favours adaptation to captive environment with degeneration of other traits essential in the wild. It corresponds with report on Przewalski horse population by Ryder (1993) Negative inbreeding effects were extensively studied in livestock (White 1972, Smith et al. 1998, Rodriganez et al. 1998, Thompson et al. 2000). Similar tendencies of decrease in fitness traits (higher mortality and decreased number of progeny) have also been observed in undomesticated species (Laikre 1999, Bales 2001, Rossiter et al. 2001, Hansson 2001). These results were also confirmed in laboratory experiments where mice with inbreeding coefficient equal to 25% showed reproductive performance decreased by 44% (Meagher et al. 2000). Therefore in breeding programs for endangered species several steps are undertaken to avoid the mating of relatives (Chesser et al. 1980, Foose 1980, Lacy 1994, De Boer 1995, Nomura 1998). Unfortunately, this problem appears in most of endangered populations due to a small number of founders and small population size (Spevak 1993, Kalinowski et al. 2000). On the other hand some populations are known not to be affected by high inbreeding. For example, the whole population of the Syrian hamster (*Mesocricetus auratus)* originates from a single dam (Alder 1948) and the animals do not have reproduction problems or other negative consequences of inbreeding. To sum up, inbreeding may be beneficial in stabilized environments, although it might become dangerous if the conditions change. Such a situation may arise in the case of reintroduction when captive bred animals may appear unable to adapt and reproduce in the natural environment.

Average inbreeding level of inbred animals in the analyzed data corresponds to the results of Bouman & Bouman (1988) and Kolter & Zimmermann (1988) but it is lower for all individuals because animals with unknown ancestors were also included. Similar tendencies were also observed in other captive bred populations (Sternicki et al. 2003). The availability of pedigree information was comparable to population of Grade Holsteins reported by C a s s e l l et al. (2003) and was increasing until 1980s. Average inbreeding level in the population under study has been decreasing over the analysed period, which may result from correct implementation of the breeding programs and inclusion of horses from the wild.

A c k n o w l e d g e m e n t s

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