

An approach towards an improvement of genetic structure of a wisent (*Bison bonasus*) population in the Carpathians

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Abstract: An important aspect in maintaining small, closed populations, is conservation of their genetic variability. The wisent (European bison) belongs to species with extremely narrow gene pool, since its present world population originates from only 12 founders. About 40% of wisent population maintained in captivity, is included into a breeding program depending on an exchange of individuals based upon pedigree analyses. The genetic structure of free ranging populations though, can be assessed only with molecular markers. Therefore the genetic enrichment of free ranging wisent populations is performed through an import of animals, mostly from captivity. An interesting example of such population, where the program for an improvement of genetic pool is carried out, are wisent herds at Bieszczady Mountains (south-east of Polish Carpathians), created in 60s and 80s, with animals originating from Polish breeding centers. The evaluation of the genetic structure of their initial groups revealed the lack or very low participation of several founders of the contemporary species. In order to improve the gene pool, between 2000–2014, over 30 individuals with known pedigree, were brought there from foreign breeding centers. On the basis of pedigree analysis, assessed was a potential change in genetic structure of this population, resulting from those introductions. The true effect though, will depend on the reproductive success achieved by introduced individuals.

Introduction

Each population originating from a small number of founders consists of genetically similar individuals. High level of kinship is in the long term dangerous for the population, because of reduced potential for adaptation to environmental changes. Also, mating between closely related individuals increases the risk of revealing the effects of lethal, recessive alleles. Symptoms of inbreeding depression are more serious and frequent in small populations, being more prone to faster increase of the inbreeding, and with high probability of the loss of some alleles (Mace & Hudson 1986; Mills & Smouse 1994; Frankham 2015).

By the end of 20s of 20th century, only 54 individuals of European bison (29 males and 25 females) survived. Not all of them were able to take part in reproduction, thus the current gene pool of this species includes genes of only 12 individuals (Olech 2009; Olech & Perzanowski 2011). The lowland-Caucasian line (LC), created by crossbreeding of two subspecies – *Bison bonasus bonasus* and *Bison bonasus caucasicus* (only one representative – male No. 100: KAUKASUS) is regarded as an open line, i.e. crossbreeds of both lines (LB – lowland and LC) are regarded as belonging to LC line (Wojciechowska *et al.* 2012). The free living population of LC line in Poland (all those animals dwell in Bieszczady Mtns.) counts currently more than 650 individuals, which constitutes almost one third of all free ranging European bison in the country (Raczyński 2020). This population was created between 1963 and 1980 starting from 33 individuals, and since then, its numbers increased by 20 times. Although it is not completely isolated spatially, the possibility of genetic exchange in a natural way is limited there. There are some contacts confirmed with the adjacent small population in Slovakia, but so far none migrations were observed between Bieszczady and weakly developing population in western Ukraine (Perzanowski 2014; Marszałek & Perzanowski 2018).

Therefore, since the future of this population was considered as uncertain, some attempts towards improvement of its genetic structure through “blood refreshment” were recently undertaken (Olech & Perzanowski 2016).

Genetic variability conservation as an element of population management

One of the essential components of the species’ conservation is the maintenance of its genetic variability. The enrichment of the gene pool of the wisent population in the Carpathians, can be achieved through an increase of the percentage of founders typical for LC line, by supplementing individuals from underrepresented female and male lines, “blood refreshing”, and creation of new subpopulations. In the gene pool of European bison there is exceptionally unequal contribution of particular founders’ genes, with high domination of just two of them (PLANTA and PLEBEJER) and marginal contribution of others (Perzanowski *et al.* 2009; Olech & Perzanowski 2013). Therefore, so important for the restitution program of this species is the genetic enrichment of existing population through an introduction of individuals selected from breeding centers (Olech & Perzanowski 2002). This should mitigate the decrease of genetic diversity through an increase of contribution of rare founders’ genes in the focal population. Proper selection of individuals suitable for such purpose can be based upon data from European Bison Pedigree Book, allowing

to track the genes flow from the founders to contemporary individuals. The effect of exchange of individuals between subpopulations can be verified by the assessment of their polymorphism and heterogeneity level (Olech & Perzanowski 2011; Raczyński 2020; Wojciechowska *et al.* 2017).

The maintenance of genetic diversity is crucial to the achievement of a major goal in the species' conservation – the existence of its viable population. This requires however also an increase of its effective numbers. For the European bison, a minimal, genetically safe population is estimated for about 100 individuals. For the home range of such population, a vast forested area of several tens of square kilometers is required, but presently, such conditions are hardly available in Western and Central Europe. They can be still found in the eastern parts of Europe (e.g. Russia) but so far, only few such sites were actually used for the restitution of European bison there (Klich & Perzanowski 2012; 2013). Habitats of such quality are also available within the Carpathian Range, but due to its structure, and limits connected with carrying capacity of forest stands according to forestry requirements, only few new herds counting several dozen individuals each, could be still established there. So small populations would not be able to maintain their genetic and demographic stability for longer periods of time. This could be however achieved through their management as a metapopulation, where the gene flow would be ensured by periodic, prescribed exchange of individuals between particular subpopulations (Olech & Perzanowski 2011).

Therefore the maintenance of *ex situ* populations is so important for such management strategy. In breeding centers, there is an access to complete genetic profile of all individuals and the reproduction pattern is fully controlled. Hence, in captive conditions it is possible to select animals that are optimal for the enrichment of free living populations in genes of rare founders (Perzanowski & Olech 2003).

The genetic management of Carpathian wisents

Free living populations of European bison in the Carpathians occur in Poland, Ukraine, Slovakia and Romania. All of them consist of individuals belonging to LC line (Olech *et al.* 2009). The reintroduction of wisents to the Carpathians was started in 1963 by releasing first individuals to the eastern part of Polish Bieszczady Mountains (Perzanowski & Marszałek 2012). In 60s and 80s of 20th century, subsequent introductions were performed in the western part of Polish Bieszczady Mountains and in Ukrainian Skolivsky Beskyd. All individuals introduced to Bieszczady came from breeding centers in Poland, while those in Ukraine from former Soviet Union, where European

bison of LC line were almost exclusively derived from male named BORUSSE, great-grandson of KAUKASUS (Olech & Perzanowski 2013).

Starting from 2001, efforts to enrich the gene pool of Carpathian populations have been made in cooperation of Warsaw University of Life Sciences, Polish Academy of Sciences and State Forests. Animals selected for this purpose originated from breeding centers of 12 countries in Western Europe (Fig. 1).

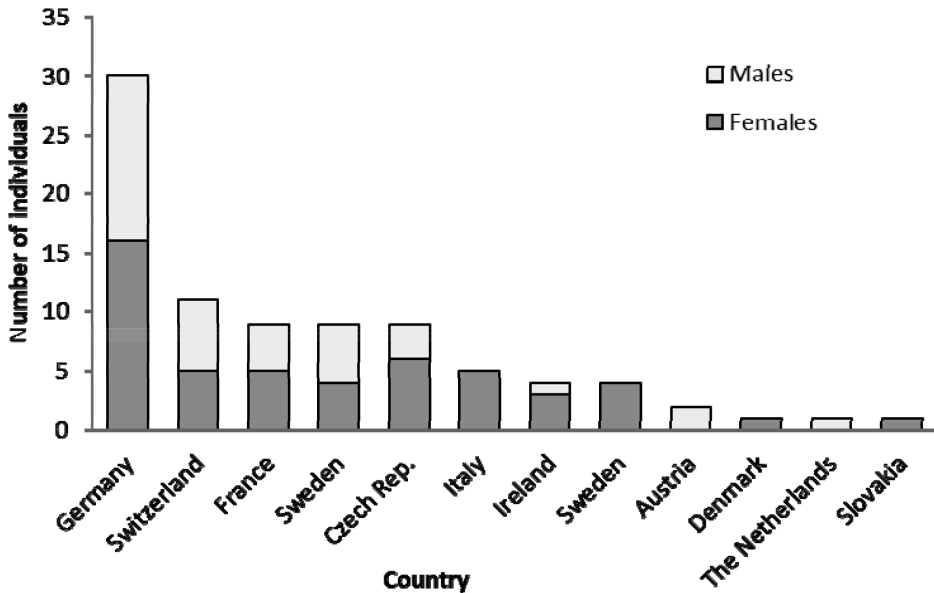


Fig. 1. Countries of origin of European bison transferred to free ranging herds in the Carpathians in years 2001–2014

First transport of 4 individuals was organized by Scandinavian zoos (Olech & Perzanowski 2013). Since then, 85 European bison were transported to various parts of the Carpathians: in Poland, Slovakia, Ukraine and Romania. The Polish population was supplemented with 37 individuals (Fig. 2).

The pedigree and DNA polymorphism (12 microsatellite loci) of each individual were analyzed before the transportation (Olech *et al.* 2009). This information was crucial for the optimal choice of animals bearing genes being rare or lacking in free ranging population. According to pedigree data, the contribution of particular founders differs between populations *in situ* and *ex situ*, and in free living populations some of the founders had very low contribution in their gene pool. For the population in Bieszczady Mountains characteristic was generally low contribution of the typical founders of LC line, lack of genes of the founder no. #46, scarce occurrence of founders no. #35 and #147, but also #95 and #96 in western subpopulation (Fig. 3), thus its actual heterogeneity was much

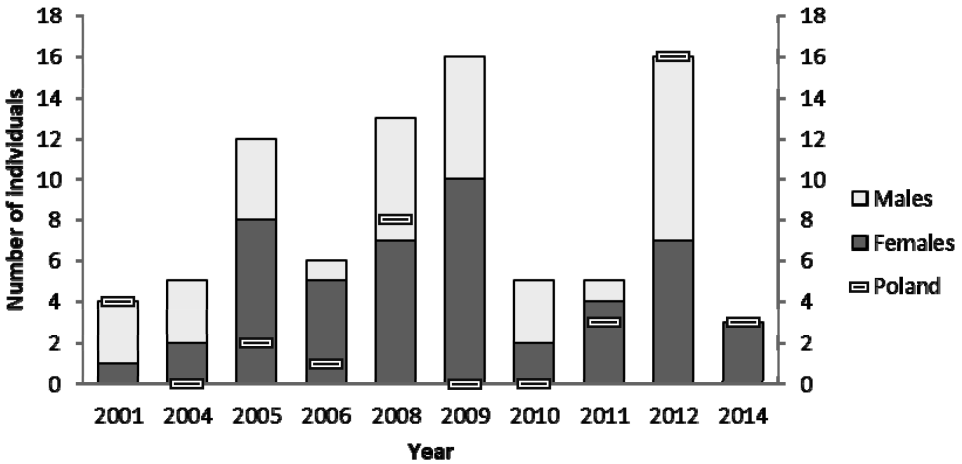


Fig. 2. Numbers of European bison transported to the Carpathians between 2001–2014. Number of individuals imported to the population in Bieszczady Mountains (Poland) is indicated with a black bar

lower than expected (Olech & Perzanowski 2013). In general, the western subpopulation located around Baligród Forest District presents higher domination of lowland founders, especially #45 and #42 (Fig. 3). For this reason, many individuals selected for introduction to Bieszczady were transported from German breeding centers offering animals with quite high contribution of genes of founders #46 and #35. Moreover, there were also valuable males with rare chromosome Y of #15 BEGRUNDER and #100 KAUKASUS (Perzanowski & Olech 2003).

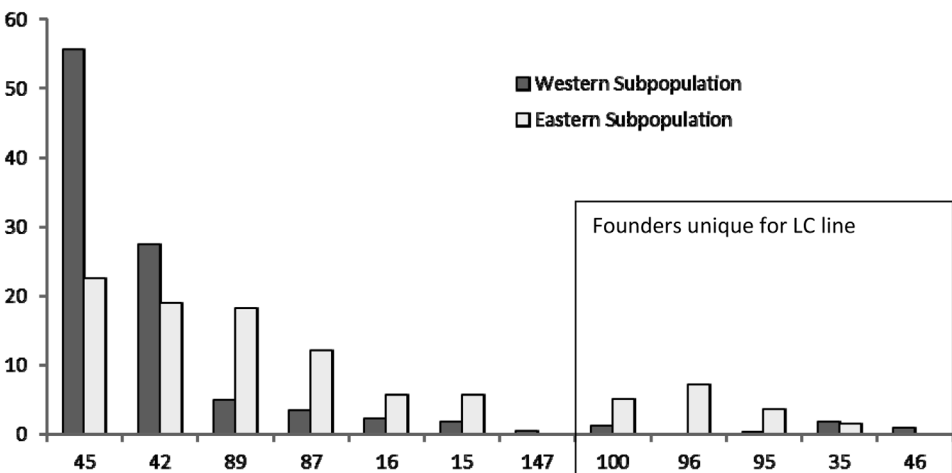


Fig. 3. Mean percentage of founders contribution estimated for a wisent population in Bieszczady Mountains prior to transports of new animals (Olech & Perzanowski 2002)

The supplementation of selected individuals from breeding centers was supposed to enrich the free ranging population in rare genes. Comparing to the existing population, the gene pool of the group selected for reintroduction had much higher contribution of founders typical for LC line: 22.09% comparing to only 10.75% in local population. Also the contribution of founders rare for the whole species was significantly higher in introduced animals: 3.47 vs. 1.1% in the case of founder #35 and 0.55 vs. 0.1% in the case of founder #147. In this group provided were also genes of the founder #46, so far absent in this population. Increased was as well the contribution of the founder KAUKASUS #100 with 7.26% of his genes in this group, comparing to only 3.15% in population existing so far in Bieszczady (Fig 4). In the group of transferred individuals more numerous were females, which gives higher probability for the spread of genes, because almost all adult wisent females participate in reproduction, while males have to fight for an access to females. In general, selected for this program were young individuals; mean age of females and males equaled to 2.5 and 2 years respectively. This is also beneficial due to potentially longer time available for reproduction which should allow for more numerous offspring.

Such genetic selection of animals for introduction was possible because pedigrees of captive individuals are known in a high percentage: 87.8 and 89.2% respectively for females and males. Although in males, the contribution of founders typical for LC line is higher than in females, the difference is not high. The genetic structure of wisent population in Bieszczady Mountains, as a result of introductions in the 60s and 80s of XX century, was incomplete and biased, comparing to the genetic pool of the whole LC line (Olech & Perzanowski 2013). Genetically selected individuals, that were brought there between 2001 and 2014 are supposed to improve this situation, providing they will be able to reproduce. The measure of the success of this program will be changes in the genetic structure of successive generations of this population. Due to the genetic structure of *ex situ* population, zoos and breeding centers of western Europe remain the source for further transports. Only in Germany there are 63 locations where LC line European bison are kept, which gives in total 498 individuals, of which 387 remain in captivity and 111 in semi free breeding centers (Raczyński 2020). Such resources are potentially sufficient for the continuation of genetic improvement of Carpathian wisent populations. Currently the free ranging population in Carpathians is enriched mostly in Romania, where numerous individuals were brought from breeding centers in western Europe. These populations may constitute a good initial genetic basis which, after the final formation of the Carpathian metapopulation, will be beneficial for its development.

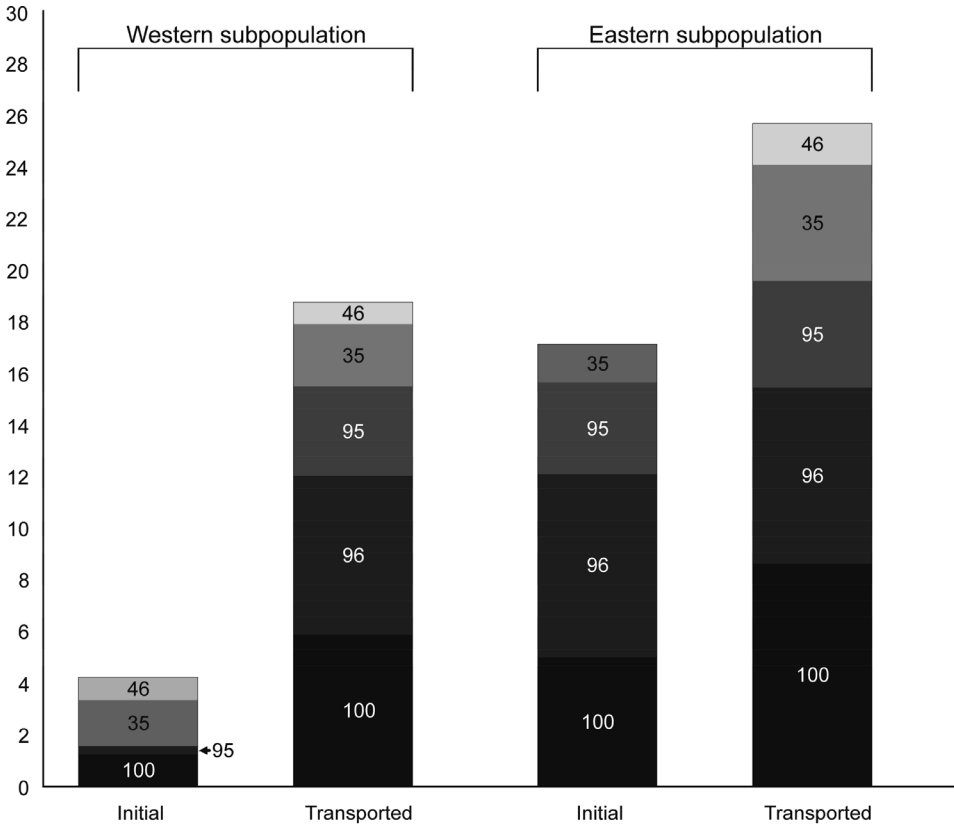


Fig. 4. Mean percentage of typical for LC line founders contribution for the initial group of animals in Bieszczady Mountains (Initial) and transferred to Carpathians (transported) in two subpopulations in Bieszczady Mountains, pedigree number of each founder indicated at bars

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Działania w celu poprawy struktury genetycznej populacji żubra (*Bison bonasus*) w Karpatach

Streszczenie: Ważnym aspektem utrzymania małych, zamkniętych populacji jest ochrona ich zmienności genetycznej. Żubr należy do gatunków z wyjątkowo wąską pulą genetyczną, gdyż jego obecna populacja światowa wywodzi się od zaledwie 12 przodków założycieli (Olech 2009; Olech & Perzanowski 2011). Około 40% populacji żubra utrzymywanej w niewoli jest włączone do programu hodowlanego polegającego na wymianie osobników w oparciu o analizy rodowodu. Natomiast struktura genetyczna populacji wolnościowej może być oceniana jedynie przy użyciu markerów molekularnych. Dlatego też, wzbogacenie genetyczne populacji wolnościowych żubra jest dokonywane poprzez import osobników, głównie pochodzących z niewoli (Perzanowski & Olech 2003, Olech & Perzanowski 2011).

Interesującym przykładem takiej populacji, gdzie prowadzony jest program wzbogacania puli genetycznej są stada żubrów w Bieszczadach (płd. wsch. część polskich Karpat), utworzone w latach 60 tych i 70-tych, z użyciem zwierząt pochodzących z polskich centrów hodowlanych (Perzanowski & Marszałek 2012; Marszałek & Perzanowski 2018). Ocena struktury genetycznej ich grup inicjalnych, wykazała brak lub bardzo niski udział kilku przodków założycieli współczesnych żubrów (Olech *et al.* 2009). W celu poprawy puli genetycznej, pomiędzy 2000 i 2014, ponad 30 osobników o znanym rodowodzie zostało tu przywiezionych z zagranicznych centrów hodowlanych (Ryc. 1). W oparciu o analizy rodowodu oceniono potencjalne zmiany w strukturze genetycznej tej populacji, które mogą zajść w wyniku tych introdukcji (Ryc. 3,4) (Olech & Perzanowski 2013). Prawdziwy ich efekt jednak, będzie zależał od sukcesu reprodukcyjnego osiągniętego przez wsiedlone osobniki.
