Supplementation of genetic structure through introductions of selected animals *ex situ* into free ranging wisent population of Bieszczady Mountains

Wanda Olech¹, Kajetan Perzanowski²

¹ Department of Genetics and Animal Breeding, Faculty of Animal Sciences, Warsaw University of Life Sciences – SGGW, Poland

² Museum and Institute of Zoology PAS, Carpathian Wildlife Research Station, Ustrzyki Dolne, Poland

Abstract: Summarised were data on 37 European bison imported to Poland between 2001 and 2014, regarding their pedigree, age, sex, place of birth, and fate after their release at Bieszczady Mountains. Analysed were effects of earlier reintroductions upon the genetic structure of this population and suggested were possible consequences of an import of genetically selected animals for an increase of genetic variability and better representation of underrepresented founder of the species in its structure.

Key words: European bison, reintroduction, genetic structure, the Carpathians

Introduction

Plans for reintroductions of wisents *Bison bonasus* to the wild initiated in 1952, did not consider genetic constraints, connected with extremely low heterogeneity of the species. Therefore, a group of animals selected for the establishment of new herds, very often had a genetic structure quite different from the profile of the whole population (Olech *et al.* 2008; Nowak *et al.* 2009; Olech and Perzanowski 2013; Krasińska *et al.* 2014; Tokarska *et al.* 2015). Additionally, it is quite impossible to predict the reproductive success of animals introduced from *ex situ* conditions to the wild, so the effects of supplementation of so called "fresh blood" are usually difficult to assess (Soorae 2013).

Pedigree analyses performed for wisent population of Bieszczady Mountains, originating from reintroductions performed between 1963–1980, show quite distinct differences in genetic structure between two major herds of the region, as well as between those parameters for the world population of the species (Olech and Perzanowski 2002; Perzanowski and Olech 2004).

te
E.
q
re
Б
õ
S
n,
Ś
q
E
60
-É
22
÷Ð
Ę
of
5
5
Ň
٥ <u>،</u>
þ
ť.
Ĵ_
ť
H.
9
J
ar
é
\mathbf{r}
p
9
60
S
g.
Ы
Ξ
ō
۳.
Ľ,
13
N
"B
Ň
22
ŝ
ie.
В
0
Ţ
g
te
)r
ă
ľ
Ξ.
ŝ
Jt
eı
Ē.
M
ú
0
g
at
q
e
re L
50
H:
ĕ
2
1
e
9
a
L

Pedigree number	Name	Sex	Breeding center	Year of birth	Number and the year of transport	Fate
9235	Bjiva	Н	Ebeltoft	1999	Nr 1. 2001	Released, shot by a poacher while being pregnant in 2003
9204	Bosse	Μ	Boras	1999		Released
9438	Stockholm Daveron	W	Stockholm	2000		Released, shot because of broken leg in 2011
9448	Bolo	Μ	Boras	2000		Released, died after the fall to ravine in 2004
10018	Presto	Μ	Praha	2003	Nr 2. 2005	Released
10331	Pralinka	F	Praha	2004		Released
10516	Pruda	F	Praha	2005	Nr 3.2006	Released
10127	En 23	Μ	Eriksberg	2004	Nr 4. 2008	Released
10541	En 28	Μ	Eriksberg	2005		Released
10231	FT-LOES	F	Carrigtwohill	2004	Nr 5.2008	Released
10232	FT-BO	Μ	Carrigtwohill	2004		Eliminated in June 2013 because of blindness
10522	FT-LYNDA	F	Carrigtwohill	2005		Released
10763	FT-EVELIJN	F	Carrigtwohill	2006		Released
10662	VALBALLA	F	Vahrendorf	2006	Nr 6.2008	Released
10663	VAMPIR	Μ	Vahrendorf	2006		Released
11525	Prga Ii	F	Praha	2009	Nr 7.2011	Killed because of TB in 2014
11526	PRYCNA	Μ	Praha	2009		Killed because of TB in 2014
11527	PRINCEZNA II	н	Praha	2009		Killed because of TB in 2014

Fate	Released	Released	Released	Died on 24.02013	Released	In breeding enclosure at Muczne	In breeding enclosure at Muczne	Released	In breeding enclosure at Muczne	Released	In breeding enclosure at Muczne	Released	In breeding enclosure at Muczne	In breeding enclosure at Muczne	In breeding enclosure at Muczne	Released	Released	Died during acclimatisation	Released
Number and the year of transport	Nr 8.2012					Nr 9. 2012			Nr 10.2012			Nr 11.2012			Nr 12.2012		Nr 13.2014		
Year of birth	2009	2010	2010	2011	2011	2009	2010	2010	2009	2010	2009	2011	2011	2011	2009	2010	2010	2010	2010
Breeding center	Springe	Hardehausen I	Kiel	Chemnitz	Chemnitz	Thoiry	Thoiry	Thoiry	Bern	Bern	Goldau	Bielefeld	Hanau	Hanau	Karlsruhe	Karlsruhe	Eriksberg	Eriksberg	Eriksberg
Sex	W	W	W	Ч	W	ч	F	W	F	W	F	W	F	F	Μ	W	F	F	ц
Name	Spyridon	Eggeherold	Kımı	Orikana	Origusu	Сн	Сн	Сн	Uroa	Urkan	Tjuli	JONAS	Fabula	FARFALLE	XARIUS	XADJO	ENTRE	Energi	Endora
Pedigree number	11502	11667	11698	12018	12019	11461	11803	11804	11580	11793	11588	12090	12113	12114	11449	11735	11655	11656	11657

_

Therefore, there was necessary to supplement already existing wisent population of Bieszczady (340 in 2016), with individuals bearing genes of underrepresented founders of the species. Under the framework of the program for the restitution of the wisent to the Carpathians, 37 animals selected from various breeding centres of Europe were imported during last 14 years to Bieszczady, and most of them were released there to the wild. This paper summarises those efforts and provides full description of organized transport of selected animals.

Material and methods

We analysed data concerning wisents of known pedigree, imported in 13 transports to Poland between 2001 and 2014, to be released to the wild at Bieszczady Mountains – a range in south-eastern part of the country (about 2000 km²). Wisents (18 males and 19 females) were provided by various European breeding centres from Czech Republic, Denmark, France, Germany, Ireland, Sweden, and Switzerland. Animals were initially placed in acclimatisation enclosures (forestries: Wola Michowa, Stuposiany, and at Bukowiec – Bieszczadzki National Park). Registered were pedigree data (number and name in European Bison Pedigree Book) of those animals, as well as place and year of their birth, the year of transport, and all known circumstances of their further fate (Tabl. 1).

Results

During fourteen years performed were in total 13 transports of wisents, involving 1–5 animals. In several cases that was possible thanks to donations by zoological gardens: Ebeltoft (Denmark), Stockholm and Boras (Sweden), Prague (Czech Republic), Carrigtwohill (Ireland); and transport on their expense. Remaining wisents were brought to Bieszczady thanks to financing provided by the European Bison Friends Society, Warsaw University of Life Sciences or the Foundation Rewilding Europe, and supported by the Regional Directorate of State Forests at Krosno. Quite various was the fate of imported animals. First fifteen wisents (transports 1-6) were release at acclimatisation enclosure of Komańcza Forest District at Wola Michowa, next transport was to the enclosure of Bieszczadzki National Park at Bukowiec, and since the transport No 8, ready was the acclimatisation enclosure at Muczne, within the home range of the eastern part of Bieszczady population. This enclosure consists of two parts - one for acclimatisation and the second for breeding. In the latter, remained six females and one male, forming a breeding group. In this enclosure, nine calves were born, out of which one male already has been released to the wild. In total 26 wisents imported from abroad were released, so far eight cases of death were registered, i.e. almost $\frac{1}{3}$ of total number. Most of them were released within the home range of the western part of Bieszczady population, but several individuals supplemented the eastern herd.

Out of 37 imported animals, one cow died before the release, 3 individuals were eliminated because of tuberculosis, and seven animals remained in breeding enclosure at Muczne. So far, released to the wild were 26 animals (10 cows and 16 bulls). Their age distribution is as follows: three females about 1 year old, three 2 years old, one 3 years old, and three four years old, i.e. all in the early reproductive age (average age below 3 years). Among bulls there were four yearlings, eight 2 years old, two 3 years old and two 4 years old. All released bulls were young (average age below 3 years) to allow for their easier acclimatisation in the wild herd (low level of competition with old bulls).

Discussion

In case of introductions to the wild, it is quite difficult to predict the breeding success of particular individuals. In wisent populations, fairly high are chances of cows (e.g. the case of #9235 BIJVA, that became pregnant already in the first season after the release). For bulls that were bred in captivity, it is rather difficult to compete with males from wild population, and reach a dominant position in a group. Therefore the release of very young bulls gives a chance to be included in mixed groups and later for their participation in breeding only after several years. However, sometimes for individuals of exceptionally good condition such achievement is possible, like in the case of male #9438 STOCKHOLM DAVERON, that was finally eliminated in 2011 because of a broken leg (Januszczak, pers. comm.)

Reintroductions of wisents to Bieszczady Mountains, performed between 1963 and 1980 have lead to establishment of two separate herds, with considerably different genetic structure, especially regarding the representation of genes belonging to founders of Lowland-Caucasian line (Olech and Perzanowski 2002; Nowak *et al.* 2009). Genetic structure of both herds was also significantly different from the structure of LC line being maintained in captivity, i.e. being the source of animals for reintroduction. Obviously, randomly selected animals that provided the base for newly established population, were not genetically representative for the whole contemporarily existing species (Olech and Perzanowski 2002, Perzanowski and Olech 2014) (Fig. 1).

Wisents were imported to Bieszczady from several countries and many different breeding centres, but a majority of them was selected, to be possibly the most representative for the genetic structure of wisent population in captivity (Fig. 1). Among captive animals, the proportion of founders characteristic only for Lowland – Caucasian line is much higher than in herds of Bieszczady. A detailed analysis of an importance of particular individuals is now prepared for publication. However, thanks an import of captive individuals it is possible to increase considerably the participation of three founders i. e. #100 KAUKASUS, #35 PLEWNA and #46 PLACIDA (Fig 1). Particularly valuable are male lines of the founder KAUKASUS. In breeding



Figure 1. A comparison of founders' contribution in two wisent herds of Bieszczady Mountains (western and eastern) with respective parameters of the captive part of Low-land-Caucasian line of the species (according to Olech and Perzanowski 2002)

centers, there are now about 20 bulls with a copy of Y-chromosome of this founder. The analysis of pedigree of males imported to Bieszczady revealed, that 3 out of 18 transferred bulls had the chromosome of this rare founder.

However, the evaluation of genetic structure of a population based upon pedigree analysis, not necessarily reflects correctly the actual state of its genetic structure. The decisive factor, that is not taken in such case into account, is usually not well recognised social structure. Therefore in fact, we do not know which animals actually took part in the reproduction, how often, and with what success. Hence, pedigree analysis provides us only with an estimate of potential genetic structure. Nevertheless, if the pedigree analysis shows, that e.g. contribution of some ancestors into contemporary genetic pool is very low, it would be unreasonable to expect a significant increase of their representation in monitored population. Thus, a true picture of actual genetic structure can be obtained only on the basis of chromosomal and DNA analysis.

Conclusions

Wisent population of Bieszczady Mountains, the largest mountain free ranging population of this species in the world, has considerably biased genetic structure, resulting from the lack of genetic analysis of animals reintroduced there in the second half of XXth century. Precisely, it was an effect of narrow gene pool available for reintroductions in Poland and former Soviet Union, due to the limited contacts and possibilities for animals' exchange with countries of western Europe. Until now it is difficult to assess true effects of prescribed supplementation of this population with genetically selected animals from various European breeding centres that was done in last 14 years. Undoubtedly, a number of imported animals was bearing rare genes of underrepresented founders of the species. An answer to the question whether the enrichment of genetic pool through introductions was successful, would be possible only after performing DNA analyses on a representative sample of wild population.

References

- Krasińska M., Krasiński Z., Olech W., Perzanowski K. 2014. European bison. [In:] Ecology, evolution and behaviour of wild cattle: implications for conservation (ed. M. Meletti, J. Burton) Cambridge University Press: 115–173.
- Nowak Z., Olech W., Bukowczyk I., Perzanowski K., Krzewska L. 2009. Zmienność genetyczna wschodniej i zachodniej populacji żubrów bieszczadzkich. Mat. Międzynarodowej Konferencji: 80 lat restytucji żubra w Puszczy Białowieskiej. Białowieża 28–29.09, Stowarzyszenie Miłośników Żubrów: 39.
- Olech W., Perzanowski K. 2002. A genetic background for reintroduction program of the European bison *Bison bonasus* in the Carpathians. Biological Conservation, 108, 2:221–228.
- Olech W., Perzanowski K., Nowak Z. 2008. Genetic enrichment of the European bison population in the Carpathians. Proc. Conf. Protection of free – living mammal populations in Central and Eastern Europe. 26–28.09, Poznań: 18–19.
- Olech W., Perzanowski K. 2013. Cele i efekty wzbogacania genetycznego populacji żubra w Karpatach. Mat. CEPL Rogów, 36, 3:13–18.
- Perzanowski K., Olech W. 2004. Recommendations for the reintroduction program of the European bison population in Bieszczady Mountains, Poland. Biosphere Conservation 6, 1: 19–23.
- Perzanowski K., Olech W. 2014. The case study restitution of the wisent *Bison bonasus* to the Carpathians. [In:] Ecology, evolution and behaviour of wild cattle: implications for conservation (ed. M. Meletti, J. Burton) Cambridge University Press: 385–392.
- Soorae P.S. (ed.) 2013. Global re-introduction perspectives. IUCN/SSC Re-introduction Specialist Group, Gland, 282 pp.
- Tokarska M., Bunevich A. N., Demontis D., Sipko T., Perzanowski K., Baryshnikov G., Kowalczyk R., Voitukhovskaya Y., Wójcik J. M., Marczuk B., Ruczyńska I., Pertoldi C. 2015. Genes of the extinct Caucasian bison still roam the Białowieża Forest and are the source of genetic discrepancies between Polish and Belarusian populations of the European bison, *Bison bonasus*. Biological Journal of the Linnean Society 114, 4: 752–763.

Poprawa struktury genetycznej poprzez wsiedlenie wybranych osobników ex situ do wolnościowej populacji żubrów w Bieszczadach

Streszczenie: Podsumowane zostały dane dotyczące 37 żubrów przewiezionych do Polski w latach 2001–2014, obejmujące ich rodowód, wiek, płeć, miejsce urodzenia oraz losy po introdukcji w Bieszczadach. Analizie poddano efekty wcześniejszych reintrodukcji dla struktury genetycznej tej populacji i wysunięto sugestie możliwych konsekwencji przywozu genetycznie wyselekcjonowanych osobników dla poprawy zmienności genetycznej oraz lepszej reprezentacji niedostatecznie reprezentowanych przodków założycieli w jej strukturze.