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New methods for preservation of genetic diversity of black grouse, *Tetrao tetrix*: preliminary results

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A b s t r a c t . In this paper we present new approach to the problem of conservation the small and endangered populations of black grouse. We elaborated and checked in the field non-invasive methods enabling to obtain the genetic material without removing birds from natal populations. The main idea is to mate the tame, especially accustomed hen from the aviary with wild cock. After mating the hen lays the eggs in the aviary and we obtain the first generation which has a half of the gene pool coming from the population of its father. Consecutive, repeated crossing the females from such broods with next wild males leads in few years to bring the genes from original population to the aviary in non-invasive manner. Another method is to collect semen from wild males using a stuffed female. We describe some technical methods of such manipulations with the birds and present some preliminary results. These methods enable obtaining birds bred in aviary for future program of reintroduction.

Key words: protection, gene pool, insular population, genetic material, inbreeding, non-invasive method

Introduction

At present in Poland there are only about 1 500–2 000 black grouse (*Tetrao tetrix*). This is about 5% of the number that existed in the 1970s (Kamieniarz & Szymkiewicz 2001, K a m i e n i a r z 2002). Black grouse live in several small, isolated populations separated by distances too long for natural dispersal and maintenance of genetic connectivity. This situation probably favours inbreeding and genetic drift, as shown in the western European populations (H ö g l u n d et al. 2007).

Reintroduction into the wild of birds bred in captivity could be one approach for the conservation of genetic diversity in these insular populations threatened by extinction. Another approach that can be of significance for the preservation of the wild gene pool of insular populations is the collection of semen from wild males, as done in mammals (K r z y w i n s k i & B o b e k 1984, K r z y w i n s k i 1987).

The description of these new and non-invasive methods for obtaining genetic material from endangered wild populations is the object of the paper. In our opinion it is very important to create an adequate gene bank, which could be used in a future reintroduction programme (K r z y w i n s k i 2007a).

Methods

Mating a tame female with a wild cock

The first method was to place captive females near a lek where wild males could mate with them (Fig. 1). One or several females were put in a large enclosure (about 50 m^2). The enclosure was open from above but the females could not fly out since some primaries were cut off. In the same enclosure, but in a separate cage with small mesh, a captive, tame male was placed to aid in attracting wild males. The wild males, when hearing the displaying tame cock, come flying from distance up to 1 km, but the last some ten meters they passed on feet. They entered the enclosure by walking through a one-way door.

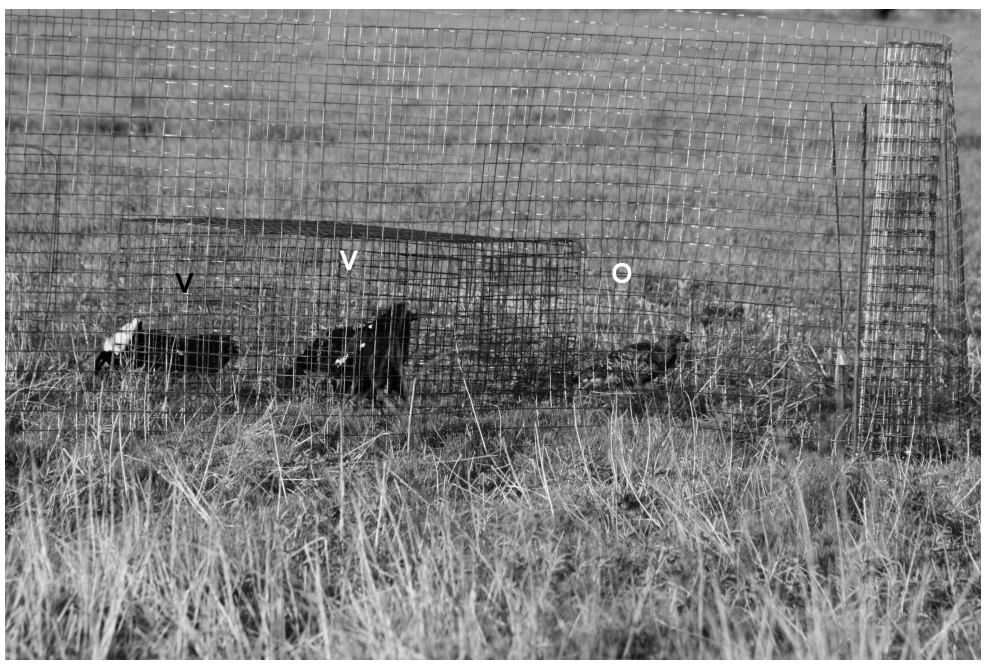

Fig. 1. Mating the tame female with a wild male (Biebrza National Park). Black arrow - wild cock; white arrow - tame male; white circle - tame female.

The birds used for experiments originated from captive German materials. According to Dr H. Aschenbrenner's information, these birds were bred in captivity for many generations. In addition, one female used for mating with the wild male was received from the central Poland.

For experiments the captive females and males should both be especially trained (Fig. 2, 3). The methods of training are similar to those used in falconry, including the night sessions. It is known phenomenon, that in darkness it is relatively easy for birds to tolerate close contact to human. If we restrict the importance of visual stimuli, the birds faster accept human's voice and touch. The best results were obtained when young birds had become accustomed to being handled. However, proper training was also regularly repeated with adults. The training of both females and males was similar. The birds must accept different kinds of cages (large and small ones, with large and small mesh), including those placed in

Fig. 2. Black grouses trained with a method similar to used in falconry.

the wild. They should sit quietly and also accept travel by car. During training, the birds were fed by preferred foods, e.g. cranberries (*Oxycoccus quadropetalus*). Such training is very important, because stress could perturb reproductive behaviour or even inhibit egg being laid.

Fig. 3. The especially accustomed black grouse male displaying in the natural habitat.

Fig. 4. The black grouse male accustomed for provoking.

After mating a wild male might continue lekking or copulate with another female. As a variant, we placed a tame single female in a small (1 x 1 m) cage-trap (Fig. 4).

Collecting semen from captive males and wild males in natural conditions

Based on our previous experience with different deer species (Krzywinski & Bobek 1984, Krzywinski 1987), we attempted collecting semen from captive black grouse and capercaillie (*Tetrao urogallus*) using a stuffed female. We prepared the stuffed hen in the crouched posture of a female, on the basis of photos of mating behaviour. Like in deer, the stuffed female was attached with a special socle to the ground. It is extremely important that a stuffed female is attractive both for captive and wild males. In the latter case it would be necessary to use a live, tame male to provoke the wild ones and attract them towards the stuffed collector hen. Otherwise there would be no possibility for wild males to find out the stuffed female. It should be mentioned that this method is poorly known in avian breeding, because in poultry the masturbation method is rather used.

Results

Mating tame female with wild cocks

Wild males, upon hearing the displaying tame male, rapidly approached, probably to drive away the new rival (Fig. 5). They started to display and fight among themselves. In such situations, the tame male should be protected with a double-net wall, against attacks by wild males. Otherwise the tame male can be beaten and will not display in the future. Using this

Fig. 5. Mating the tame female with a wild male (The Mazurian Lake District, Forest Inspectorate Drygały). Black arrow - wild cock; white arrow - tame cock provoking; white circle - tame female.

method it is also possible to lure males that do not display but are present nearby. So offspring can be obtained even from single males who do not normally display.

In May 2006, two females were mated by the variant method on a lek in the Forest District Drygały, a military range, in which one of the biggest Mazurian populations of black grouse resides. Both females laid fertile eggs. Because the female was vocally active, a wild male approached, entered the cage and copulated (Fig. 4). After mating, the male was caught, marked with a coloured ring and released. Apparently the male was not too disturbed by the handling, because he was observed on the same lek the next day. The coloured ring enables individual identification with binoculars. Handling males allow collecting feather sample for DNA extraction. Individual information is needed to hold a breeding book establishing the breeding centre as a genetic reserve for reintroduction programmes.

In May 2007, two females were mated with a wild male on a lek in the Biebrza National Park, after we had obtained permission from the Ministry of Environment. These females produced offspring.

Interestingly, during our experiments, a wild male near a group of tame birds also began to show tame behaviour toward a man. When the man had approached to only about 10 m the wild male flew off for about 50–100 m. When the observer left, this male continued to display and immediately came back to the enclosure.

Collecting semen from the tame and wild males

The especially accustomed males showed the sexual behaviour towards females not only of its own species but also other species with similar appearance. We observed that even partly tame males showed sexual behaviour towards a live female held in the hand of an investigator

like happens in domestic animals (cattle, horses, sheep, etc.). In 2007 the stuffed female was used for the experiment. From 13 tested males, 11 ones showed strong sexual behaviour towards it. One observation was made with a wild black grouse cock in natural conditions. This cock was attracted by the provoking tame male placed in a cage near the lekking site. This male also displayed by this stuffed female and tried to mate it.

Discussion

In no west European country have numbers of black grouse remained stable. Habitat destruction and fragmentation of the former range has resulted in the species living in isolated patches. Small numbers of black grouse living in these patches implies a conflict between two ways for the conservation of this species: the protection *in situ* and captive breeding. Storch (2005) cites many examples of extinction of populations smaller than 100 individuals. Such small populations are in danger of extinction due to chance demographic or environmental events, and also reduced genetic variability. Examples of remaining threatened small populations are in Belgium, Germany and the Netherlands (Storch 2005). At present, there is also a similar dramatic situation in Poland.
As indicated by Storch (2005), Höglund (2009) and Rutkowski (2005), the native sub-populations should be protected, because genetic variation is a prerequisite for any population's ability to adapt to a changing environment. To safeguard genetic diversity in Poland, a "gene bank" of endangered tetraonids (black grouse and capercaillie) representing different populations is to be established (Krzywinski 2007a, b). The progeny bred in captivity from males with genetic material from these insular populations could be used for reintroduction programmes even after extinction of such groups. A better situation would be to mate a tame female coming from the local population. The reintroduction could maintain the right genetic diversity, as well as increasing the population. For some years, work on habitat management, including reduction of predators, has been performed in many places in Poland (Kaszuba 2007). It would be important to establish "bridge populations", using locally adapted genetic material, between these insular small populations. On the other hand, the birds from captivity could be used to enrich genetic diversity in these probably inbred small island populations.

Preliminary experiments with semen collection (Krzywiński & Ciereszko 2007) indicate the possibilities using artificial reproduction to protect for the future the genetic diversity existing in Polish populations. A bank of frozen semen could be created that would contain the semen from birds of even the smallest populations. According to papers on artificial reproduction in birds (Chełmońska & Dutko 1971, Chełmońska & Kassner 2007), the semen from poultry can be frozen. The same should be true for grouse. The artificial insemination and cryo-preservation of semen from non-domestic birds has been described by Gee (1995), and a cryo-bank has been recently created for some endangered pheasants (Jalme et al. 2003).

For many years to breed birds in captivity and to subsequently release them into the wild was considered a proper method of grouse protection. However, many such experiments in various countries did not give the expected results. It seems that aviary breeding of black grouse and capercaillie now is not a serious problem for many breeding centres in some European countries, including Poland: the problem is the reintroduction of birds from captivity to nature. The captive raised birds could not adapt to living in natural conditions. For example, up to 2000 in Germany, nine programmes of capercaillie restoration (during 20 years 3 000 birds were released) and four of black grouse were carried out (Storch

2007). All these programmes failed, because after release most birds were killed by predators in a short time. A large programme of breeding and recovery of capercaillie was performed in Czech Republic for many years (started in 1976). Till 2006, 914 birds had been released. Unfortunately, this programme also failed. Among 112 radio-tagged birds, the survival rate varied between 23 and 139 days. (B e j č e k et al. 2007). The sole successful case of capercaillie reintroduction was noted in Scotland, where this species became extinct at the end of 13th century. In 1830, 64 birds were imported from Sweden and released. During 15 years the number of capercaillie increased to 2 000, and this population is stable up till recently. Another successful project was undertaken in Harz mountains with hazel hen *Bonasa bonasia* (B e r g m a n n & N i k l a s c h 1995). However, these were translocations, and not release from captivity. Recently, also in Poland a black grouse reintroduction programme has been performed in the Poleski National Park, by translocation of wild birds from the Ukraine. The preliminary results are promising (D z i e d z i c et al. 2007).

During the last three years in the Wildlife Park Kadzidłowo a new rearing method was elaborated and named Born To Be Free (K r z y w i ń s k i & K e l l e r 2005). The method means that newly hatched chicks are allowed to spend time in their natural habitat (for example in the place of future reintroduction) as much as they want (Fig. 6). The hen is placed in a small aviary and her role is limited to brood and warn against raptors. The chicks can go outside their aviary through special small "doors". It was found that the young birds are very active almost all the time, looking for natural food, mainly insects in the weeks after hatching. These studies suggested that young birds reared that way showed much better ability to survive in natural habitats, because they learned to search for natural foods and developed anti-predator behaviour. Of course it depends also of the density of predators in their natural habitat and telemetric studies should be undertaken to establish their survival rate and ability to reproduce.

Fig. 6. About one-month-old nestling reared with the method "born to be free" while feeding.

Conclusions

We show that genetic material can be obtained from even the smallest, insular, decreasing populations of black grouse. Their gene pool can be used for reintroduction programmes. It seems that the captive birds with locally adapted genes could be used for reinforcing local, genetically endangered populations. Like in mammals, elaboration of semen freezing could protect genetic diversity and improve the chances of survival of black grouse. These methods of protecting the genetic diversity of native black grouse populations involve both mating and semen collection. The methods are non-invasive, an important point in endangered species.

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

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