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## RESEARCH REPORTS

### **A translocation experiment for improving the genetic diversity of an isolated population of Pyrenean rock ptarmigan (*Lagopus muta pyrenaica*)**

**Claude Novoa, Nicolas Bech, Jean Resseguier, Ramon Martinez-Vidal, Diego Garcia Ferré, Jordi Sola de la Torre and Jérôme Boissier.**

#### **Abstract**

A recent study of rock ptarmigan population genetics in Europe found that the Pyrenean ptarmigan had a very low genetic diversity compared with that found in the Alps and Scandinavia. This genetic impoverishment is particularly marked at the eastern limit of the Pyrenean range where the population is small and isolated from the main mountain chain. To improve the genetic diversity of this population at risk, an experimental translocation program has recently been carried out as part of the European project “Gallipyr”. From 2008 to 2011, 12 rock ptarmigan were transferred from the main chain to the isolated population and radio-monitored. Subsequently, we did not find any differences in either survival rates or dispersion distances between transferred and resident birds. Out of 9 reproductive attempts involving at least one transferred female or male, 5 were successful and resulted in a total of 23 fledged young. We are monitoring the allelic richness and heterozygosity of the ptarmigan in the isolated population to see if the translocation results in an increase in genetic diversity.

#### **Introduction**

Following the last glacial retreat (about 10,000–15,000 years ago), many cold-climate species shifted their ranges north or became ice age relics in mountaintop refugia, the only zones which meet their ecological requirements in temperate latitudes (McCarty 2001). Among tetraonids, the rock ptarmigan is likely the most relevant example of this postglacial distribution pattern, with most populations inhabiting subarctic or arctic lands above 60°N. Below this latitude rock ptarmigan occur high in the mountains of southern Europe, central Asia and Japan.

In Europe, this species has survived only in mountaintop refugia of the Alps and the Pyrenees, where suitable habitat became contracted and fragmented into “sky island” patches above 2,000 m a.s.l. For species with moderate dispersal ability, this “sky island” distribution may increase the vulnerability of small isolates by reducing gene flow and demographic rescue between patches. Without gene flow among them, small and isolated populations risk inbreeding depression and increased vulnerability to extinction (Frankham et al. 2002). Indeed, there is now good evidence that reduced genetic variability might be an additional problem for the survival of small grouse populations (Westemeier 1998).

A loss of genetic variability has been documented in Pyrenean ptarmigan *Lagopus muta pyrenaica* compared with ptarmigan found in the Alps and Scandinavia (Caizergues et al. 2003). This genetic impoverishment could result from a genetic bottleneck associated with the progressive habitat fragmentation that occurred during the Holocene (Bech et al. 2009, 2013). These studies showed that a valley only 18 km wide constituted a barrier to gene flow and split the Pyrenean rock ptarmigan range into two distinct units: the main chain (from the central Pyrenees to Andorra) and the eastern chain (Puigmal-Canigou massif) (Figure 1). Data also showed that the genetic impoverishment was higher in the easternmost part of the massif (Canigou), which therefore required special conservation attention.

In agreement with the recommendations of the Grouse Action Plan 2006-2010, which stated that “...in the future translocations should likely to be used more to increase genetic heterogeneity and fertility of small isolated populations...” (Storch 2007), Bech et al. (2009) proposed to improve the genetic diversity of the rock ptarmigan population of the eastern chain by transferring birds from the main chain.

This translocation program was carried out from 2008 to 2011 as part of the “Gallipyr” project, a European program involving Andorra, Spain and France, and dedicated to the conservation of Pyrenean mountain galliformes. In this paper, we present the preliminary results obtained during this experiment and discuss briefly the potential benefits and risks of this project.



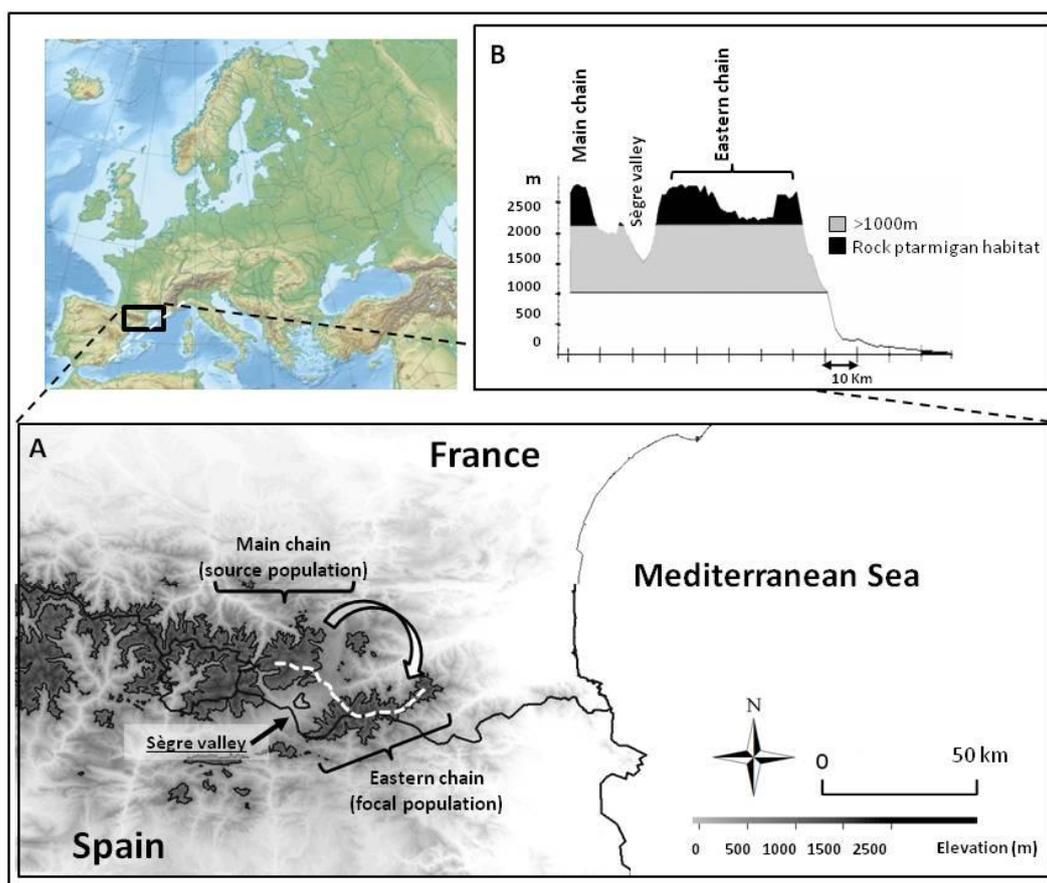


Figure 1. (A) Location of the rock ptarmigan translocations in the Pyrenees. The Sègre valley splits the species distribution range (darker areas) into two distinct units: the Main chain and the Eastern chain. (B) Altitudinal profile of the cross section linking the two chains (dashed line on map A). (Source of elevation map: SRTM 90 m Digital Elevation Model).

### Field work

In the initial project, we had planned to transfer 15 – 20 birds from the main chain (“source population”) to the eastern chain (“focal population”). To limit the impact on the “source population”, we chose to transfer juvenile birds caught just before the onset of their post-natal dispersion. Nevertheless, we also allowed the transfer of a few adult males given that the sex-ratio in rock ptarmigan populations is often skewed in their favour.

In a first step, we searched for brood-rearing females in July with the help of pointing dogs and caught the hens by luring them toward a net with a tape-recorded chick distress call (Brenot et al. 2002). Afterwards, the full-grown chicks were caught in September by driving the radio-monitored hens and their broods towards a long barrage of nets set above the brood locations. All birds were fitted with 7–9-g necklace radio tags (Holohil System Ltd.) with an expected lifespan of 24 months and including a mortality sensor. Some of the birds were immediately released at the capture site as “control” birds, the others were transferred by helicopter to the “focal” population and released in their new place after a 30-min helicopter flight.





*A rock ptarmigan has been driven towards a net barrage. The bird is hesitating in front of this unusual obstacle. Last and crucial instants before its capture. (photo Pere Ignasi Isern).*

Both transferred and control birds were located at least once every 15 days from the ground using a portable receiver and a handheld Yagi antenna. Aerial surveys by fixed-wing aircraft equipped with an antenna were undertaken if any transmitter signals were lost.

We collected feathers on each captured bird for genetic analysis. These samples were stored in absolute ethanol. To investigate changes in genetic variability after translocations on both populations, a total of 143 individuals were genotyped, 50 from source population (31 before and 19 after translocation), and 93 from the focal population (46 before and 47 after) using 11 microsatellites (see Bech et al. 2013 for laboratory methods).

## **Results**

### *Capture and translocation*

From 2008 to 2011, we captured 16 brood-rearing females in July-August from which we captured 16 full-grown chicks in September. In addition, 3 adult males roaming near the broods were also driven towards the net and captured. From these 19 birds, 12 (10 juveniles and 2 adult males) were transferred to the eastern chain and 7 (6 juveniles and 1 adult male) were released in the capture site. The birds transferred were released on sites where rock ptarmigan traditionally gather for moulting. Some juvenile birds radio-monitored in the focal population before translocation were also considered as “control” birds in survival and dispersion analysis.

### *Survival and dispersion*

Among the 12 birds transferred, one was predated within 15 days following the translocation, one was predated two months after translocation, one was censored (radio-tag failure) 5 months after and the 9 others survived at least until the next breeding season. The two adult males survived respectively 17 and 30 months after translocation. Survival rates of juvenile birds were estimated by modelling individual encounter histories from September of year  $n$  to July of year  $n+1$  with program Mark (known fate procedure). Estimations of survival rates were 0.78 [95% CI = 0.37 – 0.94] for transferred birds and 0.54 [95% CI = 0.27 – 0.74] for control birds ( $n=15$ ). Obviously, the small sample size prevents concluding that the survival of transferred birds was greater than that of control birds, but at a first glance it seems at least that translocation did not affect survival.

The dispersal distance was defined as the straight-line distance between release site in September (year  $n$ ) and the reproductive site in June-July (year  $n+1$ ). For females, the reproductive site was defined as the nest and for males as the median value of radio-locations recorded in June-July. For transferred birds, dispersal distances averaged 4.8 km [2.7-6.2] for 4 juvenile hens and 1.1 km [0.5-1.8] for 3 juvenile males. These distances may be compared with the natal dispersal distances observed in the eastern chain for 20 juveniles, 6.4 km [0.7-17.7] for 8 hens and 4.5 km [0.2-18.5] for 12 cocks. For the 2 adult males, the dispersal distances were 5.1 and 4.5 km. Hence, we may conclude that, at least for the juveniles,



translocation did not result in an over-dispersion of birds, which may sometimes be a cause of translocation failure (Dickens et al. 2009).



*A young rock ptarmigan male in early November, two months after his translocation. His winter plumage is almost complete but snow cover is only patchy (photo Pere Ignasi Isern).*

#### *Breeding*

Nine of the 12 birds transferred in autumn reached the following breeding season. Seven of these 9 birds paired with a native mate (2 did not pair) and a total of 9 reproductive attempts were observed from 2009 through 2011. One point to emphasize is the good mating success of the transferred juvenile males. Two of the 3 juvenile males found a mate the spring following their transfer whereas only 3 out of 14 resident juvenile males radio-monitored in the Canigou massif succeeded in pairing in their first breeding season (Novoa, unpublished data).

Among the 9 reproductive attempts, 5 were successful resulting in one brood of 2 full-grown chicks in 2010, 4 broods of respectively 3, 5, 5 and 8 full-grown chicks in 2011. In short, we could ascertain that the 7 “mixed” pairs (source male or female x focal male or female) produced a minimum of 23 full-grown chicks to the age of dispersal. This last result is likely the most unexpected outcome of this translocation experiment.

#### *Genetics*

No statistically significant changes in the genetic variability of the focal population were found after translocation (table 1), likely because the number of birds transferred was small and the duration of this experiment was short. As the translocation allowed us to add only 5 new alleles to the 47 pre-existing in the “focal” population, we would not expect a dramatic improvement in the genetic variability of the eastern chain. Nevertheless, genetic analysis revealed that a part of the transferred gene pool has been already incorporated into the “focal” population. Indeed, while all birds analysed on the Canigou massif before translocation were homozygous (fixed allele) regarding the microsatellite loci “BG15” (see table 1 in Bech et al. 2009), in 2012 and 2013, 6 birds caught in the same massif were heterozygote for this loci, suggesting that a new allele was added to the gene pool of the “focal” population.



Table 1. Genetic diversity of two populations of Pyrenean rock ptarmigan before (< 2008) and after (2008-2013) translocation. The Allelic richness (*Ar*) and heterozygosity (*He*) of the focal population were calculated including or not the 12 transferred birds. (*n* = number of birds genotyped and used for calculating *Ar* and *He*).

Populations	Genetic diversity <sup>1</sup>	Before translocation	After translocation	P value (Wilcoxon test)	
“Source”	Ar	5.088 (n = 31)	4.944 (n = 19)	0.575	
	He	0.650	0.656	0.328	
“Focal”	With transferred birds	Ar	4.503 (n = 46)	4.768 (n = 47)	0.424
		He	0.623	0.623	0.894
	Without transferred birds	Ar	4.503 (n = 46)	4.293 (n = 35)	0.424
		He	0.623	0.589	0.286

### Discussion

In this study we tried to improve the genetic diversity of an isolated rock ptarmigan population by translocating birds from a neighbouring population. A total of 12 birds were transferred from a “source” population to a “focal” population located at the easternmost part of the Pyrenean rock ptarmigan range. Both populations are separated by the Segre valley whose low altitude and the 18-km width represent a barrier to gene flow between the two massifs (Bech et al. 2009). By forcing the birds to cross the Sègre valley, this translocation attempt may be viewed as a kind of assisted dispersion.

Birds transferred survived well, mated with native birds and these “mixed” pairs produced a minimum of 23 full-grown chicks. The good survivorship and low dispersal rate of transferred birds was perhaps associated with the timing of the translocation on the one hand and with the choice of release sites on the other hand.



A view of the rock ptarmigan habitat in the eastern part of the Pyrenees (Canigou massif) (photo Pere Ignasi Isern).



Birds were captured in mid-September, i.e. just a few days before the breakup of broods and dispersion of juveniles. Hence, our translocations took place at an appropriate time in the rock ptarmigan life cycle and birds were transported quickly to the release site, two common features of successful translocations (Reese and Connelly 1997). Furthermore, as aforementioned birds were released near traditional moulting sites where rock ptarmigan gather from August to October, the presence of conspecifics near the release sites likely favoured the establishment of transferred birds on their new sites and reduced their dispersion. In addition, birds did not suffer any change in environmental conditions because these were very similar between main and eastern chains.

Despite these encouraging results, the number of transferred birds involved in this experiment was likely too small to achieve a significant change in the genetic diversity of the isolated population. So, at this stage, the translocation attempt reported here must be viewed as a feasibility study which provided valuable returns in terms of rock ptarmigan translocation practices. Indeed, this kind of project is rather scarce or nonexistent in European countries and more often limited to forest grouse (Unger and Klaus 2008, Ewen et al. 2009). While in North America translocations of wild-caught animals have been widely used to augment or reintroduce populations of ptarmigan (Hoffman and Giesen 1983, Kaler et al. 2010, Braun et al. 2011) or prairie grouse (Connelly 1997, Reese and Connelly 1997, Baxter et al., 2008, Schroeder et al., 2008), this management option has been rarely used in Europe where the release of captive bred birds has been more frequent for restoring or enhancing grouse populations (Ludwig and Storch 2011). Furthermore, projects dedicated to rock ptarmigan translocation are even rarer worldwide. The best documented example is the reestablishment of the species in Agattu island (Aleutian Archipelago, Alaska) by the translocation of 75 birds caught in the neighbouring Attu island (Kaler et al. 2010). In Italy, 16 hand-reared rock ptarmigan from the Alpenzoo d'Innsbruck were released in Monte Baldo (Trento province) in 2002-2003, but this reintroduction attempt failed (Brugnoli et al. 2012).

Although the innovative nature of our project yielded many technical achievements, it has also raised several questions. A fundamental assumption underlying translocation projects is that loss of genetic variability and inbreeding increase the extinction risks of small populations (Frankham 1995, Storch 2007). As an example, the low fertility observed in a decreasing population of greater prairie chickens (*Tympanuchus cupido pinnatus*) in Illinois, USA was associated with its reduced genetic diversity. Afterwards, the egg viability was restored in this remnant population by transferring birds from more genetically diverse populations. However, effects of reduced genetic variation on the viability of wild animal populations remain controversial. Indeed, other authors consider that the effects of demographic or environmental stochasticity may be more detrimental than the genetic issues for the persistence of small populations (Shaffer 1981, Lande 1988). Some populations may persist at least in the short term with a high level of homozygosity by purging the population of deleterious alleles.

Furthermore, genetic mixture of populations that are adapted to different local conditions can result in outbreeding depression. Indeed, translocations may reduce the fitness of the resident population due to introgression of poorly adapted gene complexes (Storfer 1999). In our study, given that environmental conditions were very similar between source and focal populations, it is unlikely that rock ptarmigan of the eastern chain developed specific local adaptations that were absent in the translocated birds.

To conclude, this rock ptarmigan translocation experiment was carried out according to the assumption that sufficient genetic resources appear to be critical for maintaining small and isolated populations of grouse. The preliminary results of this experiment are encouraging since transferred rock ptarmigan found their place in the focal population and participated in the reproduction. The monitoring of both genetic diversity and demography of the focal population will be continued to track the possible changes due to the translocation. In the mid-term, a next step could be also to renew this experiment with birds coming from other Pyrenean source populations.

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- Claude Novoa, Office national de la Chasse et de la Faune Sauvage, Espace Alfred Sauvy, 66500 Prades, France. [claudenovoa@oncfs.gouv.fr](mailto:claudenovoa@oncfs.gouv.fr)
- Nicolas Bech, Université de Poitiers, Laboratoire Ecologie et Biologie des Interactions, Rue Albert Turpin, 86022 Poitiers, France. [nicolas.bech@univ-poitiers.fr](mailto:nicolas.bech@univ-poitiers.fr)
- Jean Resseguier, Office national de la Chasse et de la Faune Sauvage, Espace Alfred Sauvy, 66500 Prades, France. [jeanedith@orange.fr](mailto:jeanedith@orange.fr)
- Ramon Martinez-Vidal, Generalitat de Catalunya, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural, Doctor Roux, 80; 08017 Barcelona, España, [rmartinezv@gencat.cat](mailto:rmartinezv@gencat.cat)
- Diego Garcia Ferré, Generalitat de Catalunya, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural, Doctor Roux, 80; 08017 Barcelona, España, [adgarfe@gencat.cat](mailto:adgarfe@gencat.cat)
- Jordi Sola de la Torre, Govern d'Andorra, C. Prat de la creu, 62-64, AD500 Andorra la Vella, Andorra, [Jordi.Sola@govern.ad](mailto:Jordi.Sola@govern.ad)
- Jérôme Boissier, Université de Perpignan Via Domitia, Labo Ecologie et Evolution des Interactions, 52 Avenue Paul Alduy, 66860 Perpignan, France [boissier@univ-perp.fr](mailto:boissier@univ-perp.fr)

## Aggressive encounters of Chinese Grouse *Tetrastes sewerzowi* in autumn at Lianhuashan natural reserve, Gansu, China

Siegfried Klaus<sup>1</sup>, Yingqiang Lou, Yun Fang, Wolfgang Scherzinger & Yue-Hua Sun

Key words: Chinese grouse, *Tetrastes sewerzowi*, aggressive behaviour, Lianhuashan reserve.

Running title: Encounters in Chinese Grouse

<sup>1</sup>) corresponding author

### Introduction

The Chinese grouse *Tetrastes sewerzowi* inhabits the coniferous forests mixed with willow (*Salix* spec.), the dominant food, and other deciduous trees in the high mountains of Gansu, Qinghai, Sichuan, Yunnan and Eastern Tibet. The territorial and mating behaviour of the Chinese grouse in spring has been described earlier (Klaus et al. 1996, Sun & Fang 1997, Scherzinger et al. 2003). Both hazel grouse *Tetrastes bonasia* and Chinese grouse defend territories in spring and autumn (Swenson 1991, Bergmann et al. 1996 for a review). The aggressive behaviour in autumn as a part of the territorial activity has been studied in October 2000, 2006 and 2013. The territorial behaviour during the mating time in spring was studied yearly (1995 -2013) allowing comparison between spring and autumn. Results of observations in only one season (in October 2000) have been described earlier (Klaus et al. 2009). From our telemetry studies (Sun et al. 2003), we have learned that both males and females establish territories in autumn and that both sexes enter flocks in winter (Sun & Fang 1997) similar to northern and eastern populations of hazel grouse (Swenson 1993, Swenson et al. 1995). Consequently, the territorial activities are interrupted while they lived in flocks. Studying a subpopulation of Chinese grouse partially colour-banded and equipped with transmitters, we describe

1. the aggressive behaviour in autumn
2. differences between autumn and spring.

### Study area and methods

#### Study area

The study of Chinese grouse was conducted in the Lianhuashan Natural Reserve in Gansu Province, central China (34°56' - 58°N, 103°44' -48' E). The highest peak is 3,578 m a.s.l. About 30% of the reserve (11,691 ha in total) is forested, but only 1,170 ha were mature coniferous forests growing on limestone-derived soils, mainly on northern slopes (Klaus et al. 2001, 2013). The tree canopy is dominated by *Abies fargesii*, *Picea asperata*, *P. purpurea*, *P. wilsonii*, three species of *Betula* and about 24 species of *Salix*. The optimal habitats of Chinese grouse are characterised by the close vicinity of coniferous forests with a shrub layer of different species (*Berberis*, *Lonicera*, *Rhododendron*, *Rosa*, *Viburnum*, *Crataegus*, *Spirea*, *Cotoneaster*, *Rubus* etc.) that provided cover, and groups of deciduous trees and shrubs (*Salix* spp., *Betula* spp., *Sorbus khoeniana*, *Hippophaea rhamnoides*) that provided food in winter. The dense ground cover of bamboo *Sinarundinaria nitida* disappeared after intensive flowering in 2007. The study area is described more thoroughly in Klaus et al. (1996, 2001, 2009a), Sun et al. (2003, 2006) and Wang et al. (2012).

