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Non-invasive genetic monitoring of capercaillie in the wild: individual tracking and breeding success

Francesco Foletti, Arnaud Hurstel & Gwenaël Jacob

Abstract

Reliable data on species dynamics are of major importance to design conservation areas and implement management measures, yet this data is difficult to obtain for elusive species like capercaillie (*Tetrao urogallus*). In this master project we present preliminary results on the monitoring of the capercaillie



population in the Vosges Mountains (France). From 2010 to 2012, a network of volunteers collected faecal samples in the field, mostly during early spring, when birds aggregate around displaying grounds (leks). Samples were genotyped at 21 microsatellite loci to allow for individual identification. A large majority of the individuals observed more than once during the three years of the study stayed within a restricted area. Most movements were detected between neighbouring sub-populations, with the exception of one female visiting three sub-populations within a month for a total distance of nearly 50 km. Parentage analyses conducted on a subset of sub-populations allowed us to identify parents–offspring trios with 80 % confidence. The amplification of additional markers will allow us to reliably assess (> 95 % confidence) individual breeding success in this population.

Context

As quoted in the IUCN Grouse action plan 2006-2010 (Storch 2007): “Although genetic studies have confirmed that central and western European capercaillie show metapopulation patterns (e.g. Segelbacher et al. 2003), information is almost completely lacking on juvenile dispersal rates and dispersal distances (Storch and Segelbacher 2000), and their roles in population genetics, dynamics and persistence.”

The present communication summarises the results of the Master thesis of Francesco Foletti (contact information gwenael.jacob@unifr.ch). Our aim was to develop and test the performance of genetic tools required to identify juveniles and track juvenile movements.

Study site and sampling

The study area was situated in the southern part of the Vosges Mountains (Regions Alsace and Lorraine, France). The capercaillie population in the Vosges Mountains declined from more than 2500 individuals in the 1930's to less than 100 in 2005 (Lefranc and Preiss 2008). The demographic decline of this population was accompanied by its fragmentation into seven geographically isolated sub-populations (Hurstel, unpublished). Faecal samples were collected at lek sites during the breeding season. A total of 266 samples were collected in 2010, 177 in 2011 and 221 in 2012.

Genotyping

We extracted DNA using the DNA Stool Mini Kit (Qiagen) or the PSP Spin Stool DNA Kit (Stratec) following a modified protocol, including negative controls to test for cross-sample contaminations (Jacob et al. 2010). Extracted DNAs were amplified at 21 microsatellite loci and a modified primer pair to determine the sex of the individuals (genotyping details in the Master thesis). All the samples have been replicated at least four times to control for genotyping errors (Taberlet et al. 1999). For each sample, we identified a consensus genotype, i.e. the most probable genotype, extrapolated from the analysis of the PCR replicates. Samples showing a maximum of three missing loci (no consensus genotype at a locus) were re-analysed. The aim was to amplify the missing markers and, if necessary, to correct these genotypes.

We found 237 genotypes with a maximum of two missing loci in 2010 (genotyping success = 89.1%), 141 (79.7%) in 2011 and 160 (72%) in 2012. After re-analysing samples showing missing loci, the number of samples with a maximum of two missing loci (those used in the subsequent analyses) increased from 498 samples (74.8%) to 538 (80.8%). This step also allowed us to correct genotyping errors. Of the 132 unique genotypes first identified, 128 unique genotypes remained after the second step of amplification, the number of genotypes identified only once decreased from 53 to 49 after and the number of genotypes identified twice from 19 to 14. This step allowed us to increase the reliability of the genotypes identified.

Among the 128 unique genotypes (= individuals), 73 were observed only once: 38 individuals (19 males and 19 females) in 2010, 21 individuals (18 male and 3 females) only in 2011 and 14 individuals (6 males and 8 females) only in 2012. A total of 49 individuals were recaptured: 17 individuals (11 males and 6 females) were observed in 2010 and 2011, 9 individual (1 male and 8 females) in 2010 and 2012, 11 individuals (5 males and 6 females) in 2011 and 2012, and 12 individuals (10 males and 2 females) were detected in 2010, 2011 and 2012. The date of sampling was uncertain for 6 individuals.

Individual movements

The distance moved by individuals was estimated as the Euclidean distance between the locations of samples assigned to a particular individual. Most of the individuals stayed in the sub-population where they were first identified. Eleven individuals (8.6 %) moved across larger distances (2–33 km) and visited one or more other sub-populations. Most long-distance movements were due to females (figure 1). The large majority of dispersal events are concentrated along the southern and eastern ridge of the Vosges Mountains, which can be seen as a dispersal corridor (figure 2).



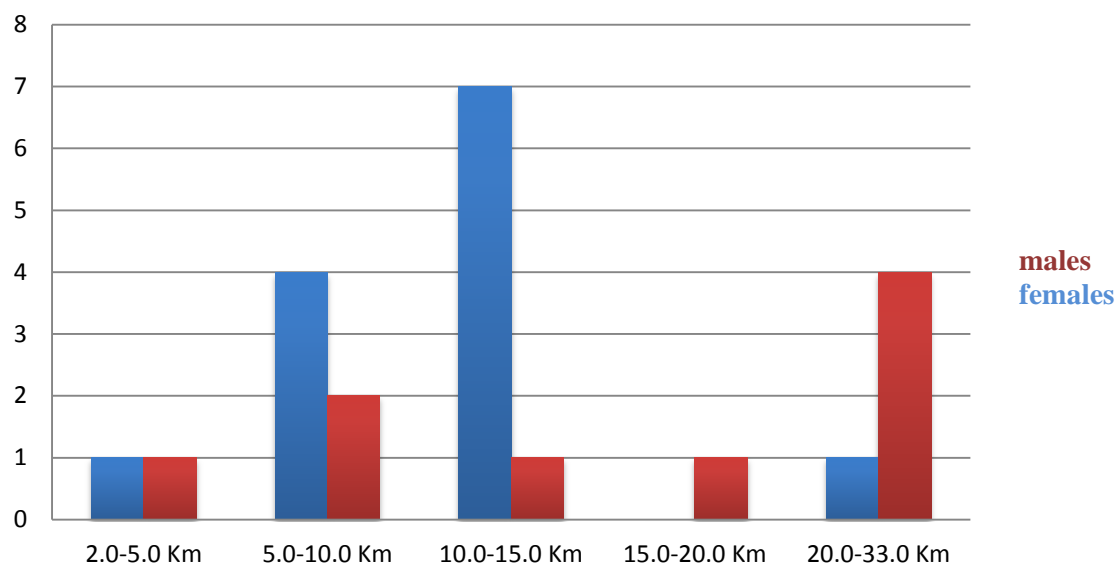


Figure 1. Individual movements among sub-populations by four males and seven females.

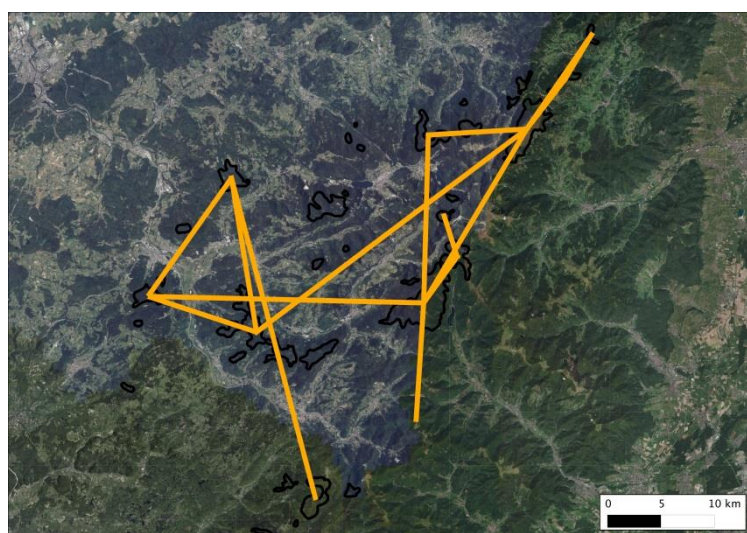


Figure 2. Illustration of individual movements among sub-populations.

Genetic variability in the population

We observed low levels of genetic variability in the capercaillie population in the Vosges Mountains, with a mean of 3.4 alleles per locus, compared to 6.1 alleles per locus in the Swiss Alps (Jacob et al., 2010). We estimated the proportion of the genetic variance among populations, among individuals within sub-populations and within individuals using an analysis of variance (AMOVA). Most of the molecular variance is found within individuals (71% for females and 87% for males; figure 3). Within sub-populations, genetic variation among individuals is 23% for females ($FIS = 0.240$) and 0% for males ($FIS = -0.006$). Genetic differentiation among sub-populations is lower for females (6%, $FST = 0.063$) than for males (13%, $FST = 0.132$). This result suggests that males are philopatric and females are the dispersing sex, as already described in capercaillie by Regnaut et al. (Regnaut et al. 2006). Indeed, few individuals, mostly females, visited more than one sub-population. Thus, female dispersal along dispersal corridors is essential to maintain the genetic connectivity among sub-populations.



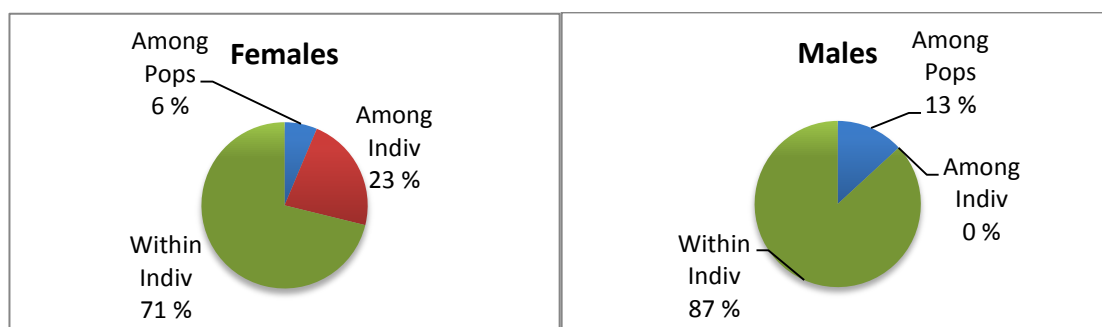


Figure 3. Partition of the molecular variance for females and males in the Vosges population

Parentage analysis

We conducted parentage analyses to reconstruct parent–offspring trios and to assess individual breeding success in the population. We used Cervus 3.0 (Field Genetics; Marshall et al 1998) to estimate allele frequencies and other genetic parameters and to run simulations to estimate the power of our set of microsatellite loci to identify parent–offspring trios. The simulation step allowed us to identify a critical LOD score (the likelihood of a potential father–mother duo being the true parents of an offspring) at which we can be 80 % or 95 % certain to identify the true parents of an offspring. Any candidate parent pair with a LOD score exceeding the critical LOD score for 95% confidence can be assigned with 95% confidence (it is the true parent at 95%). We ran simulations using different genotyping error and proportions of candidate parents sampled to assess the impact of these factors on the LOD score. Due to the low genetic variance in the population, assignment of parents was only possible with 80 % confidence. As a consequence there is a risk that some parent–offspring triplets may be incorrect. We currently work at increasing the number of markers to avoid this risk.

In our study, it was not possible to determine the year of birth of the individuals and we therefore considered all the 128 individuals as candidate offspring. We selected as potential parents 88 individuals from the sub-populations where sampling effort was highest (Gazon du Faing, $n = 39$, Ventron, $n = 24$, Saint-Antoine, $n = 8$, Haute-Meurthe, $n = 7$ and four satellite sub-populations). We ran simulations (estimation of the threshold LOD scores) and conducted parentage analyses assuming that 60% of the mothers and 80% of the fathers were sampled (more females than males are not detected at lek sites). A total of 30 parents–offspring trios were identified with a confidence of 80%. We present the results for one of the largest sub-population, Ventron, where all but six individuals were identified as potential parent or offspring, and two females observed in a neighbouring sub-population (but never in Ventron) reproduced (figure 4).

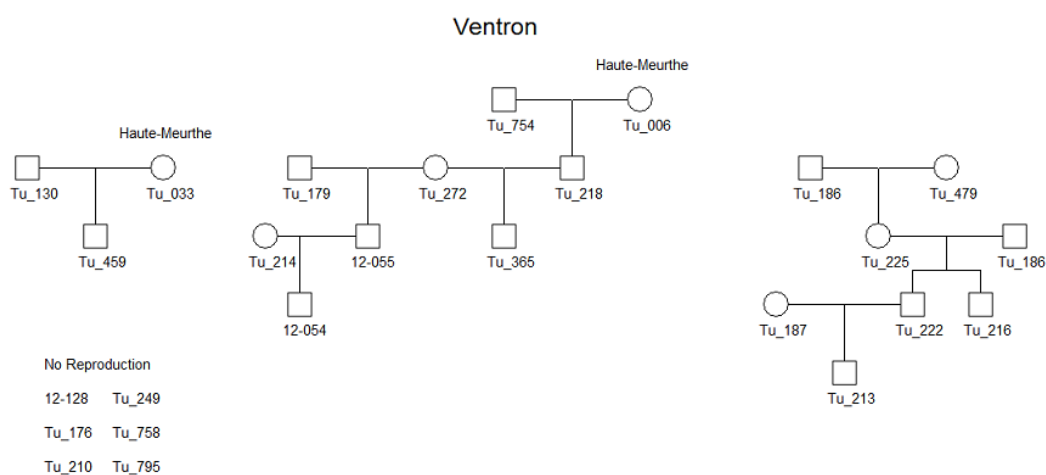


Figure 4. Pedigree of the Ventron sub-population as inferred from parentage analysis. The six individuals not involved in reproduction are indicated on the lower left corner. The two immigrants from the sub-population of Haute-Meurthe are also indicated.



Discussion

In the present study we showed that the genetic monitoring of a population based on non-invasive samples is suitable to identify dispersal corridors among sub-populations and, potentially, to assess breeding success of individuals in some sub-populations.

The sampling effort at lek sites, done by volunteers in Vosges Mountains, seems suitable for the monitoring of this population. Our results confirmed that at least four replicates are needed to control for genotyping errors (Taberlet et al. 1999). We detected 128 single individuals during these three years, which is most probably an underestimation of the true census size. Indeed, sampling was mostly restricted to lekking grounds and may have missed individuals not attending the leks. In addition, sampling effort was lower in the central part of the study area.

We could reconstruct parent–offspring trios in the sub-populations of Saint-Antoine, Ventron and Gazon du Faing. We observed evidence of direct inbreeding (two events of reproduction of a father with its daughter), which increases the risk of inbreeding depression in these sub-populations. Five females and two males reproduced after dispersal, thus promoting gene flow among sub-populations (effective dispersal). This observation further demonstrates the importance of dispersal in the dynamics of the population. Offspring of parents from the large sub-populations were observed in satellite sub-populations.

The present study demonstrates that the genetic monitoring of a wild population based on non-invasive samples is suitable to study the role of juvenile dispersal in the dynamics of the capercaillie population in the Vosges Mountains, and in other grouse population worldwide.

Acknowledgements

This project was financed in the frame of the LIFE+ project “Des forêts pour le Grand Tétrás” (2010 and 2011), and in the frame of Natura 2000 network (2012). This study would not have been feasible without the contribution of all the volunteers who collected samples during these three years.

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Successful semen collection from wild capercaillie

Ewa Łukaszewicz, Artur Kowalczyk and Zenon Rzońca

In Grouse News 43 (Rzońca et al. 2012) we presented shortly the history of capercaillie *Tetrao urogallus* population in Poland and the initial results of collaborative efforts of Wisla Forest District at Silesian

