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Using Microsatellites to Study the Conservation of Black Grouse (*)

by

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Key words : *Tetrao tetrix*, Black Grouse, *Tetrao urogallus*, Capercaillie, isolated populations, genetics, microsatellites inbreeding, non-invasive techniques, conservation.

SUMMARY

We outline the use of microsatellite markers to study the conservation biology and genetics of black grouse (*Tetrao tetrix*) and other grouse species. Microsatellites are useful in conservation genetics because they allow the detection of fine scale genetic structure, migration and possible negative effects of inbreeding in local populations while requiring minute samples of target DNA. Thus non-invasive techniques such as using shed feathers or faecal samples provide enough DNA for PCR-based genotyping. We have developed microsatellite libraries for both black grouse(*Tetrao tetrix*) and capercaillie (*Tetrao urogallus*). These markers contain a high number of alleles and allow us to address the questions outlined above. In this paper we show that the markers are variable, and allow genotypic assignment of black grouse to leks separated by only 5.5 km.

Introduction

Black grouse (*Tetrao tetrix*) populations in Western Europe have during the last decades become increasingly fragmented and numbers have declined (STORCH 2000). Remaining populations are in many areas of the distribution now so isolated that dispersal between population fragments probably has become impossible. Conservationists are thus facing several problems when managing black grouse populations. Among these are: what is the population genetic structure of the remaining black grouse populations ? Is dispersal between fragments still possible ? Have isolated populations lost genetic variation ? Are local populations suffering from inbreeding depression? In

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order to be able to answer such questions, conservationists are in need of genetic markers that allow the detection of fine scale genetic variation, that do not rely on destructive sampling and that are cost effective. The use of microsatellite markers meets many of these demands.

In small and isolated populations there is growing concern that inbreeding, mating between close kin, may have a detrimental effect on population persistence (CHARLESWORTH and CHARLESWORTH 1987, NEWMAN and PILSON 1997). Inbreeding, mating between closely related individuals, sometimes, but not always lead to inbreeding depression, i.e. a loss of fitness in comparison with outbred individuals (CHARLESWORTH and CHARLESWORTH 1987). This is well established in feral and captive populations (RALLS *et al.* 1988, LACY *et al.* 1993) and data from wild populations are accumulating (KELLER 1998, CRNOKRAK and ROFF 1999). From a population genetic point of view, inbreeding is manifested as a deficiency of heterozygotes in the population and there are two major explanations for inbreeding depression (CHARLESWORTH and CHARLESWORTH 1987). First, inbreeding depression may be due to the expression of recessive deleterious alleles (partial dominance hypothesis). Second, it could be due to a loss of a general heterozygote advantage affecting loci linked to fitness because inbred individuals tend to be homozygous (overdominance hypothesis). There is as yet no general consensus in favour of any of these two theses.

One example from a wild population where inbreeding depression may have contributed to local extinction is the case of a butterfly species, the Glanville fritillary (*Melitaea cinxia*). A metapopulation system of this species has been extensively studied on the Åland islands in the Baltic Sea between Sweden and Finland (SACCHERI *et al.* 1996, 1998, NIEMINEN *et al.* 2001). Here subpopulations signified by low levels of heterozygosity (possibly due to inbreeding) were more likely to go extinct than subpopulations that were more heterozygous (Saccheri *et al.* 1998.). This example thus fit the scenario that low levels of genetic variation and possibly inbreeding effects may contribute to local extinction. Other populations have obviously been severely inbred and yet no negative effects of this have been observed. The case of the Mauritius kestrel (*Falco punctatus*) may serve as example of this kind (GROOMBRIDGE *et al.* 2000). On the island of Mauritius in the Indian Ocean the population number were down to one breeding pair around 1975. Since then, and with the aid of a conservation program, numbers has increased to about 250. This population has thus faced considerable inbreeding without any observable negative effects on reproduction. How can these two contrasting observations be reconciled ?

One possible solution is that there are, at least, two aspects of genetic variation important for conservation. On one hand high genetic variation is good for a population. This means that when conditions change there is genetic variation to select from and the population may adapt to new conditions (LANDE and SCHEMSKE 1985). This can be referred to as the adaptability of a population, which is important for long-time persistence. On the other hand all genetic

variation comes from mutations some, if not most, which are detrimental to individual fitness. The accumulation of such mutations is referred to as the genetic load of the population (CROW 1993). One reason for why inbreeding depression may lead to reduced fitness is, as discussed above, that inbreeding leads to the phenotypic expression of such alleles.

Population dynamic processes may affect the genetic variation of populations. Severe reductions (bottlenecks) affect the genetic diversity in such a way that alleles are purged from the population. This effect obviously affects both good and bad alleles at each locus and thus reduces the genetic load (KIRKPATRICK and JARNE 2000). Applied to the butterfly and kestrel cases above, we may imagine the following scenarios. In the case of the kestrel population, the bottleneck may have been so severe that almost all deleterious alleles were lost. Thus breeding between close relatives following the bottleneck did not lead to inbreeding depression. In the case of the Glanville fritillary only some small populations were purged. However, the larger populations persisted and these are the ones that are the source for immigrants that re-colonise the patches that became extinct. Since the large populations are not purged genetic variation, including deleterious alleles, may persist in the population and inbreeding depression may be observed.

When applied to black grouse it is thus clear that it is needed to establish the patterns of genetic variation in threatened and isolated populations. Whether black grouse populations are facing a genetic load problem, suffer from inbreeding depression or lack genetic variability are all factors that may affect the persistence of populations. However, the conservation measures to counteract such problems are different. It is therefore important to study these in relation to possible conservation measures such as captive breeding programs and translocations of individuals between populations. Such measures cannot be undertaken until empirical knowledge has been gained.

Microsatellites

Microsatellites are short tandem sequences of DNA (e.g.(GATA)$_n$) scattered throughout the genome. Microsatellite loci usually are co-dominant markers, showing simple Mendelian inheritance and allele frequencies usually do not deviate from what is expected from Hardy-Weinberg equilibrium. Allele numbers at loci is sometimes large and thus genetic variation is easy to score. This is done by PCR (Polymerase Chain Reaction) which has the advantage that minute samples of DNA are enough to type any given individual. The DNA content of a moulted feather or even in faecal droppings is enough (see TABERLET *et al.* 1999). This allows non-invasive sampling and thus microsatellites are excellent tools for the study of genetic variation of endangered wild populations. A disadvantage with microsatellites is that loci are often species specific and the probability that any given locus will amplify a PCR product using a cross-specific primer-pair might be low and even if it does, shows a decreased level of polymorphism.

Microsatellites have been used successfully to estimate exchange rates between vertebrate populations (WASER & STROBECK 1998) and dispersing individuals can be assigned with great reliability to the population of origin (FAVRE *et al.* 1997). We have therefore developed microsatellite libraries for both *Tetrao* species (PIERTNEY & HÖGLUND unpublished, SEGELBACHER *et al.* 2000). High levels of variability could be detected with those markers, which makes them useful for fine-scaled population genetic studies.

Methods

Microsatellite markers were screened and developed as described in SEGELBACHER *et al.* 2000 and PIERTNEY and HÖGLUND (unpublished). Here we report on overall genetic diversity (heterozygosity, H, and mean squared distance of allele sizes, mean d^2) from a Finnish population of black grouse where we have blood sampled 132 males and genotyped these at 15 microsatellite loci (see below). H was calculated as the mean number of heterozygous loci of all loci screened for each individual. Mean d^2 was defined as the squared difference in repeat units between two alleles at a locus averaged over all loci at which an individual was scored (COULSON *et al.* 1998, PEMBERTON *et al.* 1999, SLATE *et al.* 2000) such that:

$$\text{mean } d^2 = 1/n \, \Sigma^{n}(i_a - i_b)^2$$

where i_a and i_b are the lengths in repeat units of alleles a and b at locus i, and n is the total number of loci.

We collected feathers from two different black grouse leks in Scotland (Abernethy Forest, Stathspey, Scotland, UK). We observed birds at lek 1 from the 6-7.5.2000, at lek 2 from 7-8.5.2000. After observations we collected all feathers, which could be found on the leks and stored them dry. We sampled 40 feathers at lek 1 and 14 feathers at lek 2. The distance between both sites is 5.46 km. DNA was extracted from the feather shaft using standard protocols. Laboratory procedures for the amplification and visualisation of allelic diversity at each locus follow that in the above cited studies. Briefly, alleles where amplified using PCR, separated using polyacrylamide electrophoresis and visualized using silver staining. Individuals were typed for 7 loci. Only samples where at least 4 loci could be typed have been included in the analysis (28 samples from lek 1, 11 samples from lek 2).

Results

Genotypic diversity proved to be substantial in the Finnish black grouse population. Average mean d^2 was about 5 and the average mean heterozygosity (H) was about 0.75 (**Fig. 1**). Thus these markers contain substantial genetic variation.

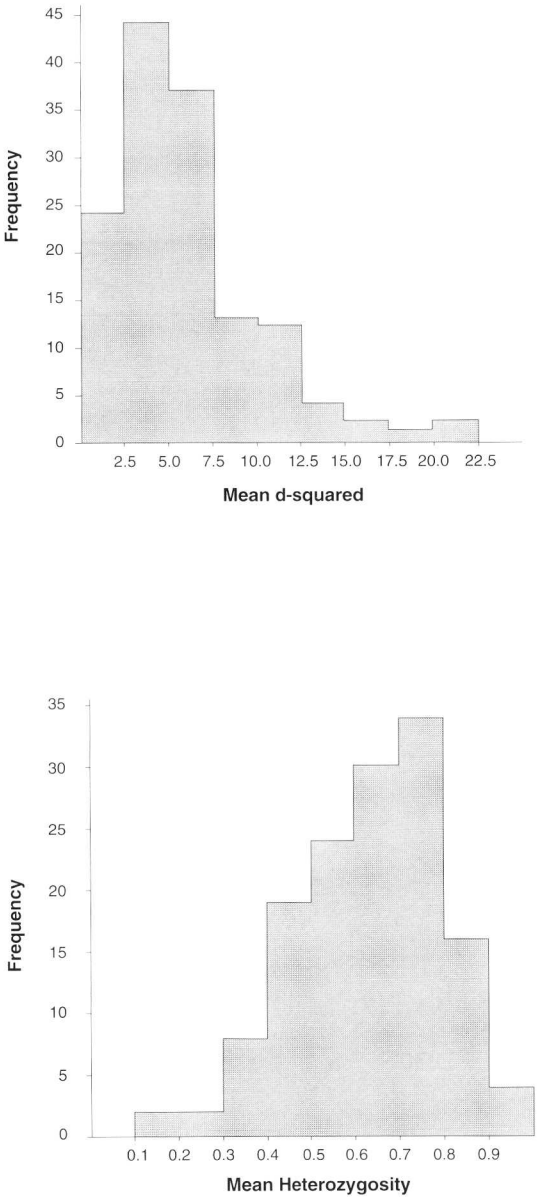

Fig. 1. Distribution of a) mean d^2 and b) mean heterozygosity for 132 male black grouse where at least seven of fifteen microsatellite loci were genotyped.
Verteilung von a) mittlerem d2 und b) mittlerem Heterozygotiegrad für 132 Birkhähne. Mindestens 7 von 15 Mikrosatelliten wurden genotypisiert.
Distribution de a) d^2 moyenne et b) hétérozygotie moyenne pour 132 tétras lyres mâles où au moins 7 loci de microsatellites sur 15 ont été génotypés.

Individuals could be typed and identified correctly using the microsatellite markers (**Fig. 2**).

At lek 1 the genetically identified number of individuals matches the number of observed males, at lek 2 we could only detect 7 individuals, where 8 males have been observed.

Using two different assignment tests (COURNET *et al.* 1999, PRITCHARD 2000) we could assign all genetically typed individuals except one to the lek where they have been sampled.

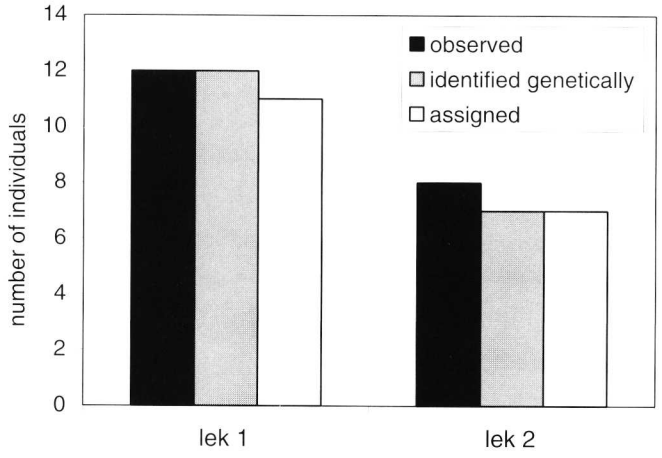

Fig. 2. Individuals observed, genetically typed and assigned on two different black grouse leks being 5 km apart.
Beobachtete, genetisch identifizierte und korrekt zugeordnete (assignment-test) Birkhähne an zwei verschiedenen, 5km entfernten, Balzplätzen.
Individus observés, typés génétiquement et correctement attribués sur deux arènes de parade distantes de 5 km.

Discussion

As shown by our Finnish data microsatellites are very variable genetic markers suitable for the detection of fine scale genetic structure. The level of genetic variation detectable is much higher than for other genetic techniques such as tm-DNA sequencing, restriction fragment analyses and RAPD variation. Using feathers for population analysis provides a non-invasive tool, which is especially valuable for endangered wild populations. We showed that we could identify 19 out of 20 birds correctly, although we sampled only once at each lek. With little effort the sample size could be increased and therefore pro-

blems of non-usable samples, due to DNA degradation could be avoided. To assign unknown samples to the population of origin however a reference database must be established first.

We conclude that with these genetic markers we are able to address the above outlined questions of population structure on a small scale. This will provide valuable information for conservationists managing declining and fragmented black grouse populations

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ZUSAMMENFASSUNG :

Wir zeigen die Möglichkeiten von genetischen Markern für den Naturschutz am Beispiel des Birkhuhns (Tetrao tetrix) auf. Mikrosatelliten Marker sind für den Artenschutz besonders geeignet. Nur geringe Mengen an DNA sind notwendig um Informationen über die genetische Struktur, den Austausch zwischen Populationen und mögliche negative Effekte von Inzucht in kleinen Populationen ermöglichen. Mittels PCR kann selbst DNA von Mauserfedern oder Kotproben für eine Genotypisierung verwendet werden. Wir haben für das Birkhuhn hochpolymorphe Mikrosatelliten entwickelt, die uns ermöglichen die ausgeführten Fragen zu beantworten. Wir zeigen, dass mit diesen hochvariablen Markern eine genoypische Zuordung von Birkhähnen zu Balzplätzen möglich ist, die nur 5.5. km von einander entfernt liegen.

Schlüsselwörter : Microsatellites, Birkhuhn, Auerhuhn, Naturschutz Inzucht.

RESUME : Intérêt des marqueurs microsatellites pour l'étude et la conservation du Tétras lyre.

Les microsatellites — marqueurs en tandem d'ADN — se révèlent du plus haut intérêt pour l'étude de la génétique et donc de la conservation des Tétras lyres et autres tétraonidés dont les populations d'Europe centrale et occidentale sont de plus en plus isolées et fragilisées. Au départ d'échantillons minimes de séquences cibles d'ADN, il est en effet possible de détecter des structures génétiques à très fine échelle, d'identifier des marqueurs populationnels, de mettre en évidence des déplacements et mouvements migratoires, de mettre au jour d'éventuels effets négatifs de l'endogamie, d'évaluer la diversité et donc le pouvoir d'adaptabilité des petites populations insularisées en danger d'extinction. Il est de toute première importance pour les conservationnistes que ces perspectives reposent sur des techniques non invasives respectant l'intégrité des oiseaux ; des plumes ou des fèces récoltées sur le terrain fournissent en effet suffisamment d'ADN pour réaliser des analyses fines des génotypes. Au départ d'un important échantillon finlandais, les auteurs ont d'abord constitué pour les deux espèces de tétras européens — Tétras lyre et Grand Tétras — une bibliothèque de marqueurs microsatellites de référence. Ils ont ensuite récolté des plumes sur deux arènes de parade en Ecosse. Les marqueurs considérés contiennent un nombre important d'allèles dont on peut déterminer la diversité. Celle-ci se révèle importante pour le vaste échantillon finlandais. Pour l'échantillon écossais, 19 coqs sur 20 ont pu être identifiés génétiquement et être assignés sur cette base à l'une ou l'autre de deux arènes distantes seulement de 5,5 km. La technique se révèle donc pleine de promesse pour fournir l'information nécessaire à une politique de conservation.

Mots-clés : *Tetrao tetrix*, Tétras lyre, *Tetrao urogallus*, grand Tétras, populations isolées, marqueurs génétiques, microsatellites, techniques non-invasives, conservation

Genetic approaches to conservation in black grouse

by
Gernot SEGELBACHER & Jacob HÖGLUND

In our talks we demonstrated the possible use of genetic information for conservation in black grouse. We have developed species-specific microsatellite markers and could demonstrate the use of feathers and droppings as non-invasive sampling techniques. Although feather and faeces samples require special precautions in sampling and analysising, we could show that these techniques provide information about connectivity and population structure. This allows us to gain information about gene flow, inbreeding and population size and leads finally to concrete conservation plans. In the workshop discussion we focused on questions concerning the application of molecular methods in black grouse. One aim was to build a network of people doing research on genetic topics and field workers interested in genetic analysis. We discussed questions of inbreeding and restocking especially in small vulnerable populations. One major problem of restocking programs is that local adaptation might be lost, when introducing birds from elsewhere. On the other hand possible inbreeding depression could be avoided by restocking. As we do not know much about the genetic composition of black grouse populations in Central Europe we emphasised the importance of screening the populations with genetic markers before making conservation decisions. All researchers, especially those that study small populations, might address genetic questions and send samples for analyses to the authors, which will coordinate the further genetic network on black grouse. A detailed description how to sample feathers is published on the webpage http://www.ulg.ac.be/museezoo/cccc .