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SHORT COMMUNICATION

Backcrossing of a capercaillie × black grouse hybrid male in the wild revealed with molecular markers

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Abstract

The black grouse *Lyrurus tetrix* and the capercaillie *Tetrao urogallus* are known to produce hybrids in wild populations. In general, these hybrids are regarded as infertile; however, conclusive evidence that F1-hybrids are infertile and unable to backcross in the wild are lacking. Using a molecular approach, we examined the ancestry of a bird assumed to be a male hybrid based on phenotypic characters. The specimen was legally shot during the hunting season in northern Norway in an area where the black grouse is common and the capercaillie is relatively rare. Analysis of the maternally inherited mitochondrial DNA revealed that the mother of the specimen was of capercaillie origin, while a diagnostic sex-chromosome (Z) linked microsatellite marker showed that the father had a black grouse allele. Diagnostic autosomal microsatellite markers revealed that the specimen was a backcross and not a first-generation hybrid. As galliform birds follow Haldane's rule, i.e., that hybrid sterility is common in the heterogametic sex (female in birds), the hybrid parent of the backcross was, thus, likely a male. Our findings provide molecular evidence that capercaillie × black grouse F1-hybrid males can be fertile and successfully mate and backcross in a wild population.

Keywords Hybridization · MtDNA · Microsatellites · *Lyrurus tetrix* · *Tetrao urogallus*

Introduction

Hybridization between some grouse species is rather common (Johnsgard 1983; McCarthy 2006), especially the combination of the black grouse *Lyrurus tetrix* and the capercaillie *Tetrao urogallus* which has been well known in Scandinavia as early as 1744 (Johnsgard 1983). Male hybrids between a male black grouse and a female capercaillie are the most common hybrids and they have even been given a specific name, "rakkelhane" in Norwegian. These male hybrids are observed most often on black grouse leks during spring, where they dominate and sometimes kill black grouse males (Andersen 2015), and are reproductively active (Porkert et al. 1997). Black grouse females do not solicit copulations with hybrid males, however, female capercaillie do so (Porkert et al. 1997; Flor 2009). In captivity, it has been shown that first-generation (F1) hybrid males have viable spermatozoa despite reduced semen quality (Ciereszko et al. 2009), and that they are indeed fertile and able to backcross with capercaillie females (Johnsgard 1983; Höglund and Porkert 1989). However, female F1-hybrids were sterile (Höglund and Porkert 1989). In general, hybrid sterility is common in the heterogametic sex (Haldane 1922), and it has been shown that galliform birds follow Haldane's rule (Arrieta et al. 2013). Both skin material and description of lekking behavior of wild birds exist for probable F2- and F3-hybrid birds (Porkert et al. 1996; Porkert et al. 1997). However, conclusive evidence for such backcrossing in wild populations is currently lacking.

In this study, we used species-specific genetic markers to analyse a grouse specimen assumed to be a capercaillie × black grouse hybrid male based on phenotypic characters. The main aim was to test if the specimen was a hybrid and, if so, identify the maternal and paternal species origin.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10344-020-01377-y) contains supplementary material, which is available to authorized users.

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Materials and methods

On 2 November 2013 a grouse, assumed to be a male capercaillie, was legally shot during the hunting season in Bodø municipality in northern Norway (67° N, 14° E). The bird had several phenotypical characteristics of both the black grouse and the capercaillie. The bird was sent to the NTNU University Museum in Trondheim, where the specimen was mounted and are kept, and a tissue sample was obtained for DNA analysis. Additionally, body mass, total wing length (flattened and stretched), and length of central tail feathers were measured. As reference material for the molecular analyses, we used DNA from 15 capercaillie and 15 black grouse individuals from central Norway, collected as part of a previous project on power-line induced mortality (Bevanger et al. 2014).

DNA analysis was carried out at the DNA laboratory at the Norwegian Institute for Nature Research in Trondheim. Genomic DNA was extracted with the DNeasy blood and tissue kit (Qiagen) following the manufacturer's protocol. To determine the maternal origin of the specimen, we sequenced a standard barcoding region of the cytochrome oxidase I (COI) gene (Hebert et al. 2004), which is located on the mitochondria and, thus, maternally inherited. DNA was amplified and sequenced using the primers BirdF1 and BirdR2 (Hebert et al. 2004; Kerr et al. 2007). To determine the paternal origin of the specimen, we analysed five autosomal loci and one sex-linked (Z-chromosome) locus (Table 1) that all had been shown to differ in fragment sizes between the capercaillie and the black grouse (Jacob et al. 2010; O. Kleven unpublished data).

Table 1 Microsatellite genotypes of the hybrid specimen and reference samples from the black grouse *Lyrurus tetrix* and capercaillie *Tetrao urogallus*

Species	Sex	BG15[1]	BG18[1]	Clock[2]	sTuD5[3]	sTuT2[3]	Z-054[4*]
Hybrid specimen	Male	*140*/*140*	**160**/*200*	**271**/*276*	*139*/*147*	*160*/*164*	*244*/**254**
Black grouse	Female	190/206	160/164	271/271	91/91	118/118	254
Black grouse	Female	190/190	156/160	271/271	91/91	118/118	254
Black grouse	Female	198/198	160/168	271/271	91/91	118/118	250
Black grouse	Female	198/198	168/172	271/271	91/91	118/118	250
Black grouse	Female	186/190	156/168	271/271	91/91	118/118	254
Black grouse	Female	178/186	168/168	271/271	91/91	118/118	250
Black grouse	Female	190/194	168/172	271/271	91/91	118/118	254
Black grouse	Female	186/190	160/160	271/271	91/91	118/118	254
Black grouse	Female	186/190	160/168	271/271	91/91	118/118	254
Black grouse	Male	190/190	164/168	271/271	91/91	118/118	250/254
Black grouse	Male	186/206	156/160	271/271	91/91	118/118	250/250
Black grouse	Male	182/190	156/160	271/271	91/91	118/118	254/258
Black grouse	Male	186/194	160/160	271/271	91/91	118/118	254/254
Black grouse	Male	182/194	156/160	271/271	91/91	118/118	250/254
Black grouse	Male	186/190	156/160	271/271	91/91	118/118	254/254
Capercaillie	Female	136/144	192/208	276/276	129/137	152/160	244
Capercaillie	Female	136/144	196/200	276/276	135/143	144/152	244
Capercaillie	Female	136/144	192/196	276/276	137/141	144/152	246
Capercaillie	Female	136/136	200/204	276/276	135/143	144/168	244
Capercaillie	Female	136/140	196/200	276/276	135/141	160/164	244
Capercaillie	Female	136/140	196/200	276/276	143/153	144/152	244
Capercaillie	Female	136/136	192/200	276/276	145/153	152/152	244
Capercaillie	Male	140/140	188/204	276/276	135/145	144/160	244/244
Capercaillie	Male	136/140	200/200	276/276	137/143	152/156	244/244
Capercaillie	Male	136/136	196/200	276/276	133/135	144/156	244/246
Capercaillie	Male	136/136	192/196	276/276	141/143	144/144	244/244
Capercaillie	Male	136/136	200/208	276/276	135/137	144/156	244/244
Capercaillie	Male	132/144	200/204	276/276	135/143	152/164	244/244
Capercaillie	Male	132/140	196/204	276/276	137/143	144/156	244/244
Capercaillie	Male	140/140	192/204	276/276	143/145	152/156	244/244

Primers from [1] Piertney and Höglund (2001); [2] Johnsen et al. (2007); [3] Jacob et al. (2010); [4] Dawson et al. (2015).
*In birds, females are the heterogametic sex (ZW) and thus carry only one copy of the Z chromosome

For the hybrid specimen, alleles in bold indicate black grouse alleles and italics indicate capercaillie alleles

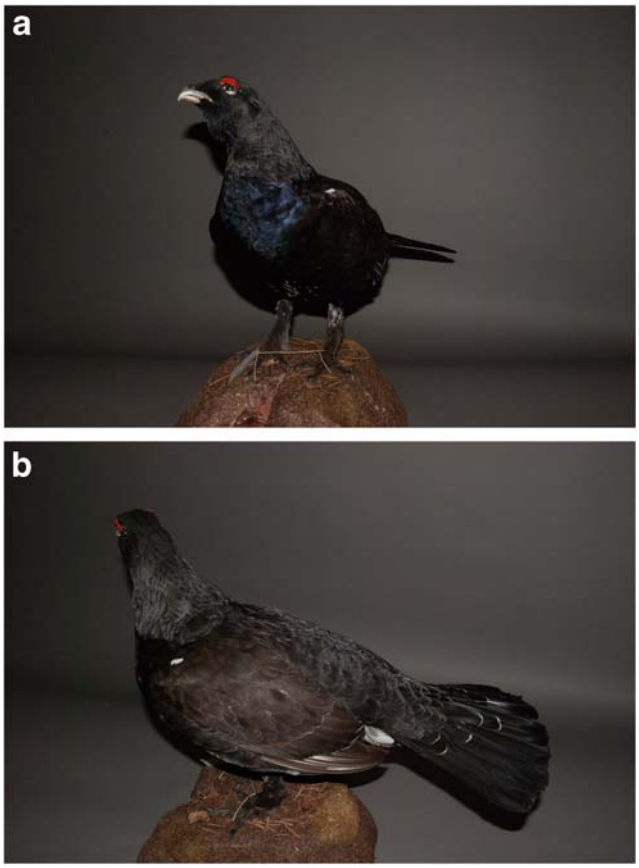

Fig. 1 Photos taken 5 February 2020 showing the investigated bird. The specimen was mounted by taxidermist Per Gätzschmann and is stored at the NTNU University Museum in Trondheim, Norway

To determine the maternal origin of the investigated specimen, the amplified mitochondrial sequence was compared with reference sequences in the Barcode of Life Database (Ratnasingham and Hebert 2007).

The 30 reference samples (autosomal microsatellite genotypes) from the black grouse and the capercaillie were used to generate 100 simulated genotypes for each of five classes, i.e., pure black grouse, pure capercaillie, first generation hybrids (F1), backcross (male F1 x female capercaillie), and second generation hybrids (F2) using HybridLab (Nielsen et al. 2006). The simulated genotypes and the genotype of the investigated specimen was then analysed using NewHybrids (Anderson and Thompson 2002) to calculate the probability that the investigated specimen belong to one of the five genotype frequency classes. NewHybrids was run with an initial burn-in of 1×10^{-5} iterations followed by 1×10^{-6} Monte Carlo Markov Chain generations.

Results and discussion

The investigated specimen had several phenotypic characteristics of a capercaillie, like horn-white beak, brownish wings, greyish-black back, and a rounded tail with straight outer tail feathers. However, the breast feathers had a metallic blue/violet colour more similar to black grouse, not greenish as in capercaillie (see photos in Fig. 1). The bird weighed 3403 g, total wing length was 351 mm and length of central tail feathers was 230 mm. Biometric data from the literature showed that male capercaillie weigh 3720–4800 g, wing length range from 383 to 400 mm and central tail feathers range from 291 to 322 mm, while male black grouse weigh 1050–1750 g, wing length range from 247 to 266 mm and central tail feathers range from 98 to 108 mm (Cramp 1980). Both the phenotypic characteristics and the biometric measurements of the focal bird, thus, indicated that it was a hybrid.

A 440-base-pair fragment was amplified from the maternally inherited mitochondrial COI region of the investigated specimen (GenBank accession number: MT037872). An identification search in the BOLD data base revealed that the sequence matched (99.8% similarity) to the capercaillie (Online Resource 1). The mother of the investigated specimen was, thus, of capercaillie origin.

At the Z-linked microsatellite locus (Z-054), the investigated specimen was heterozygous (Table 1). Thus, confirming the sex as male, because males are the homogametic (ZZ) sex and females the heterogametic (ZW) sex in birds. One of the two alleles (244 bp) was identical to an allele found in the capercaillie only, while the other allele (254 bp) was identical to an allele found in the black grouse only (Table 1). The two different and species diagnostic alleles on the Z-linked locus showed that the investigated specimen was a hybrid. As the mtDNA revealed that the mother was a capercaillie, the Z-linked locus indicated that the father was of black grouse origin.

An examination of the microsatellite genotype of the investigated specimen showed that of ten autosomal alleles, eight were identical to alleles found in the capercaillie only, while two were identical to alleles found in the black grouse only (Table 1). As a F1-hybrid will share half of the genome with each of the two parental species, the father (as female hybrids are sterile; Höglund and Porkert 1989) of the investigated specimen, thus, also had to be a hybrid. This was further supported by the Bayesian model-based clustering analysis in NewHybrids (Anderson and Thompson 2002), which revealed that the investigated specimen most likely was a backcross (posterior probability 0.81) of a male F1-hybrid and a female capercaillie. As some simulated F2 genotypes had similar posterior probability values of being a backcross (Online Resource 2), we cannot exclude the possibility that the investigated specimen was a F2-hybrid. However, given the rarity of F1-hybrids, this seem less likely. A schematic illustration of the most likely pedigree of the investigated specimen is shown in Online Resource 3.

In general, it has been shown that hybridization occurs more frequently when one of the two hybridizing species is rare (Randler 2002). In the area where the investigated hybrid

specimen was shot, the population size of the capercaillie was very low, while the black grouse were abundant (KJ pers. obs.). A female capercaillie in this area would, thus, more likely encounter a male black grouse than a male capercaillie, and produce a F1-hybrid. Behavioural observations in the wild have revealed that capercaillie × black grouse F1-hybrid males attend and display on both black grouse and capercaillie leks (Porkert et al. 1997). While female capercaillie solicit copulations with hybrid males, black grouse females do not (Porkert et al. 1997). Backcrossing will, therefore, most likely involve hybrid males and capercaillie females.

In conclusion, we have in this study molecularly determined the parental origin of a wild capercaillie × black grouse hybrid specimen. Our findings provide molecular evidence that capercaillie × black grouse hybrid males can be fertile and successfully mate to produce backcrosses in wild populations under natural conditions.

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