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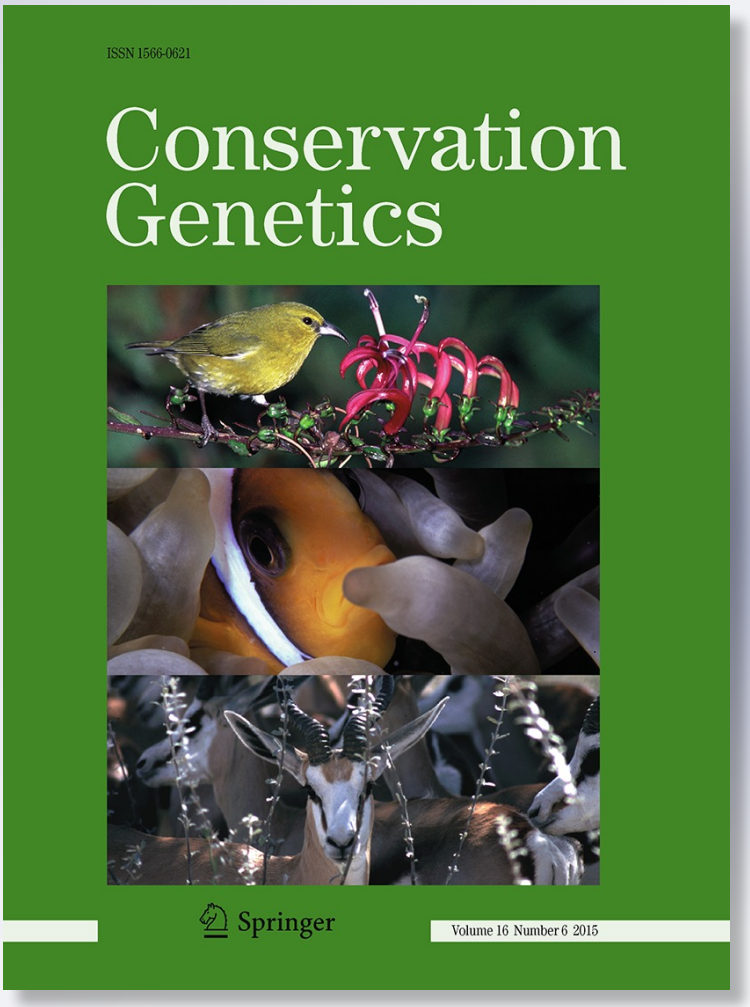
Conservation Genetics

ISSN 1566-0621
Volume 16
Number 6

Conserv Genet (2015) 16:1375-1394
DOI 10.1007/s10592-015-0747-8



Springer

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RESEARCH ARTICLE

Revealing the consequences of male-biased trophy hunting on the maintenance of genetic variation

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Received: 22 July 2014 / Accepted: 20 June 2015 / Published online: 30 June 2015

Abstract Demographic models accounting for operational sex ratio (OSR) show that male numbers can have a substantial influence on the dynamics of wild populations. We used the Cantabrian capercaillie, a forest bird, as a model to assess the effects of the reduction in the number of breeding males (increased OSR) associated to male-biased hunting, on the genetics of the population. We based our assessment in the comparison of the dynamics of neutral markers transmitted by both parents (microsatellites) versus markers transmitted only by females (mitochondrial DNA—mtDNA). Parallel to the analysis of field data, we ran computer simulations to explore how different levels of OSR and two other important demographic factors, population size and connectivity, might influence the dynamics of genetic variation of microsatellites and mtDNA. We found evidence of a genetic bottleneck and low genetic variability affecting microsatellites but not mtDNA early in our study period, when male-biased hunting was more intense. This was followed by a decline in mtDNA variation around 10–20 years later. Simulations suggested that changes in genetic variation associated with high OSR had the closest similarity to those observed at the beginning of our study, whereas a combination of reduced size and migration rate better resembled the patterns found later on. Our findings indicate that male-biased hunting might have triggered the ongoing decline of the Cantabrian capercaillie, on its own or in combination with habitat configuration, and support the need to incorporate OSR into decision making for the management and conservation of exploited populations.

Keywords Operational sex ratio · Population bottleneck · Demography · Male-biased hunting · Cantabrian capercaillie

Electronic supplementary material The online version of this article (doi:10.1007/s10592-015-0747-8) contains supplementary material, which is available to authorized users.

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Introduction

The operational sex ratio (OSR), i.e. the average ratio of fertilizable females to sexually active males at any given time (Emlen and Oring 1977), is a key parameter for population dynamics. Some human activities can have a strong effect on OSR. Among them, trophy hunting is probably the most common cause of non-natural sex-biased mortality (Rankin and Kokko 2007). In this modality, hunters target males carrying prominent sexually selected traits (e.g. striking colours, bigger body size or bigger

antler size) (Dickson et al. 2009) that are also attractive to females and/or beneficial in male–male contests, and so related to male mating success. Thus, trophy hunting removes males with high reproductive value, undermining the foundations of sexual selection, with the potential to modify the evolution, genetic diversity and demographic structure of harvested populations (Allendorf et al. 2008; Festa Bianchet and Lee 2009).

Immediate effects of trophy hunting on population dynamics are usually subtle. Normally only a few individuals are hunted, and because more males are supposed to be left than the number expected to reproduce, it is often perceived by hunters and managers as an activity with low impact on population growth and persistence (Rankin and Kokko 2007; Dickson et al. 2009; Festa Bianchet and Lee 2009). However this perception has been rarely evaluated, particularly in relation to the maintenance of genetic diversity. Here we explore this issue by assessing the dynamics of a game bird population subject to intensive male-biased hunting.

The capercaillie (*Tetrao urogallus*) is a polygynous forest grouse widely distributed in the Western Palearctic, and an important game species over most of its range. In some areas of Central and Southern Europe displaying males used to be hunted during the mating season. However, over the last decades this tradition has been halted in many countries, because it removes birds with high reproductive value at a key time for reproduction. Still, it remains in Bulgaria, Romania, Ukraine and Russia.

This hunting modality was already practised in the isolated capercaillie population of the Cantabrian Mountains early in the 20th century (Llano 1928). Due to a long history of deforestation, most the woodlands in this mountain range are distributed in small isolated forest patches (García et al. 2005), and this constrains the size and probably also the connectivity of the Cantabrian capercaillie population (Quevedo et al. 2006a). On its own, this landscape configuration might not be enough as to compromise the long-term persistence of the population, but it represents a background factor that can amplify the negative effects of any additional threat for population persistence. Hunting might have been one of those factors. Indeed, hunting was perceived as a critical problem in the past (Castroviejo et al. 1974), although no attempt to analyse its consequences had been carried out before.

Trophy hunting became very popular in these mountains during the 1940s (Castroviejo et al. 1974). Cantabrian capercaillie males were intensively hunted during the spring for over more than 30 years, i.e. not just the dominant males but most of the displaying males were removed from their lekking grounds (Castroviejo et al. 1974). Between the end of 1960s and early 1970s, the number of cocks shot yearly dropped sharply (Fig. 1), and

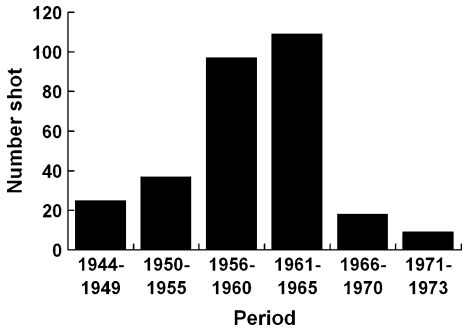


Fig. 1 Cocks shot in the spring in three areas of the western Cantabrian capercaillie subpopulation after Castroviejo et al. (1974). Data from the early fifties and before are incomplete

finding males became a difficult task in areas traditionally known to hold high bird densities (Castroviejo et al. 1974). This led game managers to think that the population was at risk. In 1979 a permanent ban was established but poaching of displaying males continued for no less than 20 years after the ban (Aedo et al. 1986). Since the first general survey of display sites, around three decades ago, subsequent surveys have uncovered a fast population decline whose starting point has remained unclear to date (Storch et al. 2006). Although a number of factors have been proposed to explain this decline, only habitat loss and fragmentation have been investigated so far (Quevedo et al. 2006a, b).

In this study, we investigate the potential impact of hunting on the ongoing decline of the Cantabrian capercaillie. We hypothesize that male-biased hunting caused two consecutive processes: (1) an initial reduction in the number of males contributing to reproduction that resulted in a loss of genetic variation; and (2) a subsequent shortage in male availability that decreased productivity and triggered the ongoing demographic decline. To explore this hypothesis, we searched for genetic signatures of population decline over several consecutive time periods. We used microsatellite and mitochondrial DNA extracted from preserved skins, stuffed birds and shed feathers collected over the last five decades. We based our approach on the comparison of both types of markers, bearing in mind the differences in ploidy and inheritance between them. Microsatellites are diploid and inherited from both parents, whereas mitochondrial DNA (mtDNA) is haploid and maternally inherited. Thus, for any given population, effective population size is about four times smaller for mtDNA than microsatellite markers, and so mtDNA markers are around four times more sensitive to population bottlenecks than microsatellites (Allendorf and Luikart 2007). Based on this, we infer that under a stable population of reproductive females, a decrease in the number of reproductive males, alike that associated to excessive male-

biased hunting, would reduce microsatellite variability but had limited or no effect on mtDNA variation. Conversely, any factor reducing the number of reproductive females would result in a sharper drop in mtDNA than microsatellite variation.

Methods

Studied population

The Cantabrian Mountains run ca. 500 km parallel to the Cantabrian Coast, in Northern Spain (Fig. 2). Over the last century, Cantabrian capercaillie known range covered around 3500 km^2 (Robles et al. 2006; Storch et al. 2006), with ca. 720 display sites within an altitude range of 700–1800 m (Robles et al. 2006) and distributed in two subpopulations (Vázquez et al. 2012). There are no data on population density, but the maximum recorded number of males per lek was 15 (Ena Álvarez et al. 1984). Our study focused in the western subpopulation, corresponding roughly to half of the total population range during last century (Quevedo et al. 2006a, Fig. 2). This subpopulation retains at present the best forest remnants and the last capercaillie strongholds in the range. Based on the population density reported for northern populations, we estimate that the carrying capacity of the western Cantabrian mountains in the last century (i.e., the maximum population size) must have been around 3500–7000 birds.

Sample collection

Capercaillie used to be a much appreciated trophy, and over most of the 20th century many birds were stuffed to be used as "ornaments" in homes, private and public buildings. We spent 6 years searching for stuffed specimens by asking individual hunters and hunting associations. We assigned spatial and temporal data to each specimen, and removed some feathers and a small piece of the footpads to extract DNA. We completed our sample by including feathers found in the field, as well as tissue taken from preserved skins and stuffed birds stored in museum collections (see Appendix for a list of the samples). Our study period starts in 1959, around the time when male-biased hunting was more intense (Fig. 1) and finishes in 2007.

DNA extraction and genotyping

We extracted DNA from footpads and feathers in a dedicated room, using aerosol resistant pipette tips and including negative controls in all reactions. For footpads, we followed a standard SDS-proteinase K-ClNa protocol (Miller et al. 1988) after a previous hydration of the samples for three consecutive days with 1 ml of TE solution.

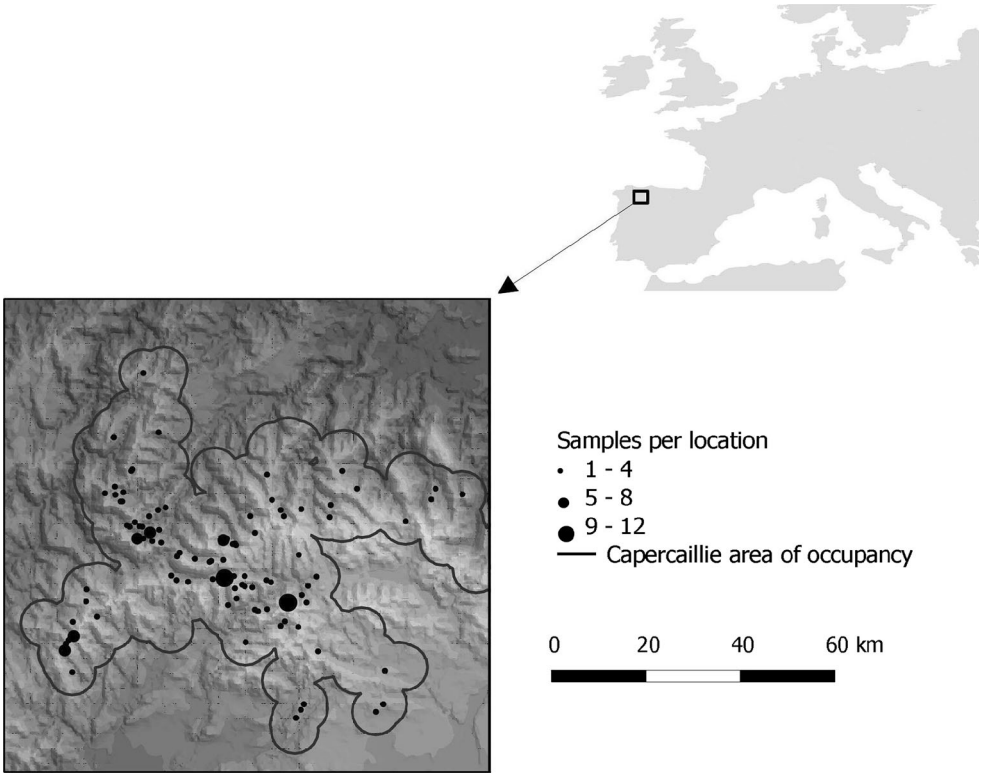


Fig. 2 Study area and location of sampling sites, covering the western portion of the Cantabrian capercaillie range. *Solid line* shows the limits of the species distribution in this subpopulation

To extract feathers DNA we used the method of Hórvath et al. (2005).

We amplified six microsatellite loci developed for capercaillie (TUT1, TUT3, TUD1, TUD2, TUD4 and TUD5, Segelbacher et al. 2000) and five developed for black grouse (TTD1, TTD2, TTD6, BG10 and BG15, Caizergues et al. 2001; Piertney and Höglund 2001). PCR conditions were as described elsewhere (Rodriguez-Muñoz et al. 2007). Genotypes were obtained with a MegaBace 1000 automated sequencer (GE Healthcare) and the patterns were scored and analyzed using the MegaBace Fragment Profiler 1.2 software (Amersham Biosciences). To obtain the consensus genotype for the DNA extracted from each feather sample, we followed the protocol of Frantz et al. (2003). For footpads, we performed a preliminary trial by genotyping each of 25 random samples three times to measure genotypic errors. Since no errors were scored the rest of the footpad samples were genotyped once. Null alleles and error rates were calculated using Micro-Cheker (Van Oosterhout et al. 2004), Gimlet 1.3.3 (Valière 2002) and Dropout (McKelvey and Schwartz 2005).

We amplified a fragment of approximately 400 bp of the mtDNA control region using primers PHDL (Fumihito et al. 1995) and PH-H521 (Randi and Lucchini 1998), as described elsewhere (Rodriguez-Muñoz et al. 2007). We obtained the sequences with an ABI3100 (MACROGEN Inc., Korea). To confirm new haplotypes, we amplified and sequenced them twice.

Demographic history

Changes in effective population size

There is nearly no information on the demography of this population before 1982; thereafter, data are mainly based on the presence/absence of birds at the display sites during the mating season. Therefore, we assessed the demographic history of the population during our study period using genetic data. Our main goal was to detect signatures of changes in population size and genetic variability, with a special emphasis on the detection of recent genetic bottlenecks. For the analyses, we grouped samples into four periods of 10–11 years: 1959–1968, 1969–1978, 1979–1988 and 1998–2007 (we excluded the 1989–1997 period due to small sample size). We ran Lamarc 2.1.6 (Kuhner 2006) to calculate most probable estimates (MPE) of θ. This is a population parameter that reflects effective population size and mutation rate and equals $4Ne\mu$ and $2Ne\mu$ (where Ne is the effective population size and μ the mutation rate) for microsatellites and mtDNA, respectively. Assuming a constant mutation rate across periods, θ can be compared among periods within the same type of marker, but not between markers with different mutation rates and sexes in polygynous species.

We ran Lamarc three times for each period and set of markers (5 initial and 2 final chains, 10^5 recorded genealogies sampled every 20 steps and burnin of 10^4). We also used Modeltest (Posada and Crandall 2001) to define the appropriate base-substitution model for sequences (HKY). For microsatellites we used the mixed K/stepwise model with a proportion of 0.9 of one-step mutations.

Population bottlenecks

We checked for microsatellite bottlenecks for each period described above using M-ratio (Garza and Williamson 2001) and Bottleneck (Cornuet and Luikart 1996). M-ratio is less powerful in small pre-bottleneck population sizes (Garza and Williamson 2001), thus it might be less suitable to be used here. Both methods require the setting up of mutation parameters. Mutation models range from the stepwise (SMM), where each mutation involves the change of a single tandem repeat, to the infinite alleles model (IAM), where any number of repeats can change for every new mutation. Intermediate to both is the two-phase mutation model (TPM), where the proportion of multi-step mutations (p_g) can be set at any value between 0 (equivalent to SMM) and 1 (equivalent to IAM). The assumed p_g has contrasting effects on the probability of Type I error (inferring the existence of a bottleneck in a stable population) between both programs. Regardless of the actual p_g, Bottleneck is quite robust against Type I error for p_g values ≥ 0.1, whereas robustness in M-ratio is high for $p_g \leq 0.1$ (Peery et al. 2012). Thus, to keep an overall Type I error probability low, we set $p_g = 0.1$ for M-Ratio, and covered the whole range (0, 0.1, 0.22 and 1) for Bottleneck (Piry et al. 1999; Peery et al. 2012), i.e. the three mutations models were tested with Bottleneck. Both programs require also the setting of the mean size for multi-step mutations (δ_g); we tried 2.8 and 3.5, covering the recommended range (Piry et al. 1999; Garza and Williamson 2001; Peery et al. 2012). M-ratio requires also the setting of θ. We tried four values that largely extended the results from our Lamarc analysis (see above). We removed TUD4 for the M-ratio test, because it had an allele that was farther from the locus average than any of the alleles in remaining loci (Garza and Williamson 2001).

We tested the existence of population decline in mtDNA using mismatch distributions and neutrality tests. Mismatch distributions of populations at demographic equilibrium or in decline should provide a multimodal distribution of pairwise differences, due to stochastic lineage loss, while an exponentially growing population has a smooth unimodal distribution (Slatkin and Hudson 1991). We employed the raggedness index (Rg) to compare the

observed mismatch distribution to that expected under the sudden expansion model (Harpending et al. 1993). Estimation and testing were done by bootstrap resampling (10,000 replicates) using ARLEQUIN 3.0 (Excoffier et al. 2005). We used Tajima's D (1989) and Fu's F's (1997) tests to analyze possible deviations from the expected neutrality under demographic expansion. Significance of these statistics was tested using 1000 permutations in ARLEQUIN 3.0 (Excoffier et al. 2005).

Genetic variability

We calculated several descriptors of genetic variability using GENEPOP (Raymond and Rousset 1995) and FSTAT (Goudet 2001). For microsatellite data we estimated allelic richness (*Ar*), an estimator that removes the effect of uneven sample sizes allowing the comparison between populations or periods, observed heterozygosity (*Ho*), the proportion of expected heterozygous genotypes (*He*) (FSTAT calculates un unbiased estimator of this parameter to be used as a measure of gene diversity) and the inbreeding coefficient (F_{IS}) as a measurement of the departure from Hardy–Weinberg equilibrium (Allendorf and Luikart 2007). For mtDNA, we calculated number of haplotypes (*H*), haplotype richness (*Hr*) and the haplotype diversity index (*h*) (Nei 1987). To explore the trends of microsatellites and mitochondrial genetic parameters (*Ar*, *Ho*, *He, FIS, Hr* and *h*), we used central moving averages of 11 years, centered on even years. We only included those averages where data were available for at least 3 years each side of the central year.

Exploring alternative scenarios through computer simulation

To get some insights about what would be the most plausible explanation for the genetic patterns found in the field for the Cantabrian capercaillie, we used EASYPOP 2.0.1 2001) to model the dynamics of *H*, *h*, number of alleles (*Ao*), *He, Ho* and F_{IS} under various levels of: (1) population size, (2) population fragmentation and (3) operational sex ratio. We run the simulations on two species, the Cantabrian capercaillie (using the local demographic and genetic parameters) and the closely related black grouse, *Tetrao tetrix* (using parameters from healthier populations available in the literature). Both simulations are thoroughly explained in the Appendix.

Results

Descriptive statistics of genetic markers

We successfully amplified DNA from 65 stuffed birds (either footpads or feathers) and 82 shed feathers, including 108 males, 32 females and seven of unknown sex (Appendix). We got 97 and 74 % positive PCRs for footpads and feathers respectively. Mean genotyping errors per locus across the 11 loci were 0.011–0.019 and 0.054–0.059 respectively. MICROCHECKER and DROPOUT indicated that null alleles and allelic dropout were non-significant. In 141 samples we amplified ≥5 microsatellites and in 127 we amplified for ≥8. Mean number of alleles per locus was 5.91 (range 3–8) and overall *Ho* and *He* were 0.54 and 0.62 respectively. Based on a minimum sample size of five, *Ar* varied from 3.0 (TUT3) to 6.9 (TUD5), with a mean value across loci of 5.12. We found no evidence of null alleles or linkage disequilibrium. Overall F_{IS} was 0.137. Since the spatial distribution of samples somewhat differed among periods, we checked that there was no correlation between genetic diversity and the area covered by the samples across different periods ($P = 0.490$).

D-loop sequences of 401 base pairs were successfully amplified for 126 individuals. The efficiency of mitochondrial amplification was similar for footpads (83 %) and feathers (87 %). A total of nine variable nucleotides, including seven transitions and two transversions, defined nine haplotypes. Four of them were new and have increased the list of 20 haplotypes recorded so far for the southern capercaillie lineage using the 351 homologous base pairs fragment of the control region that we sequenced (Bajc et al. 2011). Haplotypes C01–C04 (in concordance to Rodriguez-Muñoz et al. 2007) were the most common (Table 1). Newly described haplotypes C06–C09 were rare and found only in samples dated before 1980, despite the fact that the number of samples was much larger for the last decade (Table 1).

Table 1 Frequency distribution of the nine mtDNA haplotypes found in Cantabrian capercaillie samples from the western subpopulation, dated between 1959 and 2007

Period	*N*	*H*	C01	C02	C03	C04	C05	C06*	C07*	C08*	C09*
1959–1968	22	7	0.125	0.500	0.125	0.042	0.042	0.083	0.083		
1969–1978	24	6	0.136	0.455	0.227	0.045		0.091			0.045
1979–1988	15	5	0.067	0.400	0.267	0.200				0.067	
1998–1907	65	5	0.092	0.446	0.369	0.077	0.015				

* New haplotypes, reported in here for the first time

Demographic history

Changes in effective population size

Sample sizes were well within the recommendations by Kuhner (2006). We found no evidence of changes in effective population size across periods for the microsatellite markers (Table 2). However, mtDNA showed a sharp decline after the second period. Population decrease was around 70 % when comparing the maximum value obtained in 1969–1978 with that estimated for the last decade, 1998-2007 (Table 2).

Population bottlenecks

We found evidence of a recent population contraction from the analyses of both types of markers, although with a difference in the timing. Tests based on microsatellites suggest a population bottleneck happening before the start of our study, whereas mismatch distributions pointed to a later decrease in mtDNA population size, around one or two decades after the study was initiated.

Combining the results of M-RATIO (Table 3) and BOTTLENECK (Table 4) the evidence of a microsatellite bottleneck was more robust for the first period (1960s), when both tests were positive for θ values closer to those obtained for the studied population ($\leq$2, Table 2), particularly for delta values estimated from empirical mutation data in the literature (i.e. 2.8, Garza and Williamson 2001).

Neutrality tests (Tajima's D and Fu's F) were not significantly different from zero in any of the sampling periods, as would be expected for a stable or expanding population (Table 5). However, raggedness index was high and significant for the second and especially for the last

Table 2 Values of θ (proxy of effective population size and mutation rate) for the western Cantabrian capercaillie subpopulation in 10 years periods between 1959 and 2007

Period	Microsatellites				mtDNA (values $\times 10^2$)			
	N	MPE (b)	5 % (c)	95 % (d)	N	MPE	5 %	95 %
1959–1968	22	1.16 (0.03)	0.84 (0.01)	1.58 (0.02)	24	0.55 (1.13)	0.23 (0.42)	1.20 (1.22)
1969–1978	27	1.22 (0.05)	0.90 (0.01)	1.65 (0.02)	22	0.64 (1.62)	0.27 (0.38)	1.23 (0.59)
1979–1988	21	1.03 (0.01)	0.72 (0.01)	1.41 (0.02)	15	0.43 (3.01)	0.15 (0.22)	1.01 (1.22)
1998–2007	71	1.17 (0.09)	0.88 (0.04)	1.52 (0.03)	65	0.19 (1.00)	0.06 (0.52)	0.44 (1.63)

No data are available for 1989–1997

$\theta = 4Ne\mu$ for microsatellites and $2Ne\mu$ for mtDNA, where *Ne* is the effective population size and μ the mutation rate per generation

MPE most probable estimate (SD in brackets)

5 % percentile 5, *95 %* percentile 95

Table 3 Results of the population bottleneck analyses of the western Cantabrian capercaillie subpopulation in 10 years periods, between 1959 and 2007

Period	*N*	*M*	Mc							
			$\delta g = 2.8$				$\delta g = 3.5$			
			θ				θ			
			0.5	1	2	5	0.5	1	2	5
1959–1968	22	0.783	***0.842***	***0.817***	***0.795***	0.757	***0.814***	***0.781***	0.740	0.695
1969–1978	27	0.832	***0.843***	0.822	0.796	0.767	0.812	0.782	0.747	0.702
1979–1988	21	0.733	***0.836***	***0.819***	***0.792***	***0.754***	***0.812***	***0.778***	***0.742***	0.695
1998–2007	71	0.876	0.848	0.832	0.814	0.801	0.816	0.787	0.757	0.731

No data are available for 1989–1997), based on the MRATIO test (Garza and Williamson 2001) with a proportion of multi-step mutations (p_g) = 0.1

we have marked as significant the sixth value of first period where there is only a small difference in the third decimal

P values in bold-italics

N sample size m, *M* ratio of the number of alleles to range in allele size

Mc critical *M* values (i.e., those below which bottlenecks are inferred) for different combinations of parameter settings. *Mc* higher than measured *M* (i.e., those for which *M* is different from expected) are shown in bold-italics

δg mean size of multi-step mutations

$\theta = 4Ne\mu$ for microsatellites and $2Ne\mu$ for mtDNA, where *Ne* is the effective population size and μ the mutation rate per generation

Table 4 Results of the population bottleneck analyses of the western Cantabrian capercaillie subpopulation in 10 years periods between 1959 and 2007

Period	N	P value (Wilcoxon sign rank test)							
		$\delta g = 2.8$				$\delta g = 3.5$			
		TPM (p_g)				TPM (p_g)			
		0.00	0.10	0.22	1.00	0.00	0.10	0.22	1.00
1959–1968	22	0.139	*0.041*	*0.034*	*0.027*	0.160	*0.041*	*0.034*	*0.027*
1969–1978	27	0.840	0.551	0.289	*>0.001*	0.880	0.517	0.232	*>0.001*
1979–1988	21	0.483	0.160	0.074	*>0.001*	0.483	0.139	0.062	*>0.001*
1998–2007	71	0.966	0.740	0.416	*0.003*	0.966	0.584	0.298	*0.002*

No data are available for 1989–1997, based on the heterozygote excess test performed with BOTTLENECK (Cornuet and Luikart 1996); figures correspond to P values (Wilcoxon sign rank test—significant values in italics)

Values are shown for the three models available in BOTTLENECK: SMM (when TPM (p_g) = 0), TPM and IAM (when TPM (p_g) = 1)

N = Sample size

δg mean size of multi-step mutations

p_g proportion of multi-step mutations for the TPM

Table 5 Mismatch distributions (raggedness index) and neutrality tests (Tajima's D and Fu's F) for the western subpopulation of Cantabrian capercaillie over four historical periods

Period	N	Raggedness index	P	Tajima's D	P	Fu's F	P
1959–1968	24	0.054	0.650	−0.069	0.526	−2.322	0.061
1969–1978	22	0.190	*0.050*	0.786	0.789	0.403	0.620
1979–1988	15	0.065	0.650	−0.281	0.432	−0.669	0.299
1998–2007	65	0.320	*<0.001*	1.220	0.889	1.988	0.857

P values in italics

period (Table 5). These results suggest a shift in female population size from expansion to demographic equilibrium or population bottleneck (Harpending et al. 1993; Johnson et al. 2007).

Our sample for BOTTLENECK was smaller than ideal (>30), but it is within the range (20–30) recommended according to Piry et al. (1999). For the MRATIO, Garza and Williamson (2001) recommend a minimum sample size of 25 diploid individuals and, in addition to that, the sample should double the maximum allele size range found among the polymorphic loci used for the analysis. All our samples exceed the second criteria, as our maximum range in allele is nine, but two of them are slightly under the general minimum of 25. Thus, there is a possibility that the existence of a bottleneck based on MRATIO is affected by sample size. However, we think that the fact that both tests indicated the existence of a bottleneck early in our study period for a wide range of potential genetic parameters, combined with the low heterozygosity values, are indicative that microsatellite genetic variation was smaller than would be expected for a population with a high haplotypic variability.

Changes in genetic variability

We found a low initial *Ho* and a significant and positive *Fis* as compared to other populations in better demographic conditions (Segelbacher et al. 2003, Isomursu et al. 2012)(see "Descriptive statistics of genetic markers" section and Fig. 3). Overall, none of the three microsatellite parameters (*Ar*, *He* and *Ho*) showed significant changes between the start and the end of our study period, suggesting that any loss in genetic variation should have happened before. However, visual inspection of the trends shows an inflection point happening somewhere during the 1985–1997 gap (Fig. 3). Values before and after the gap were significantly different for the four parameters: *Ho* decreased, whereas *He*, *Ar* and *Fis* increased (Repeated Measures ANOVA; *Ar*: $F_{1,10} = 7.451$, $P = 0.021$; *He*: $F_{1,10} = 5.426$, $P = 0.042$; *Ho*: $F_{1,10} = 5.160$, $P = 0.046$; *FIS*: $F_{1,10} = 10.832$, $P = 0.008$).

Variation in mtDNA showed a different pattern: *Hr* and *h* remained stable until the second half of the 1970s, when they started to decrease. By the end of our study, more than one-third of the initial *H* had been lost (Fig. 3).

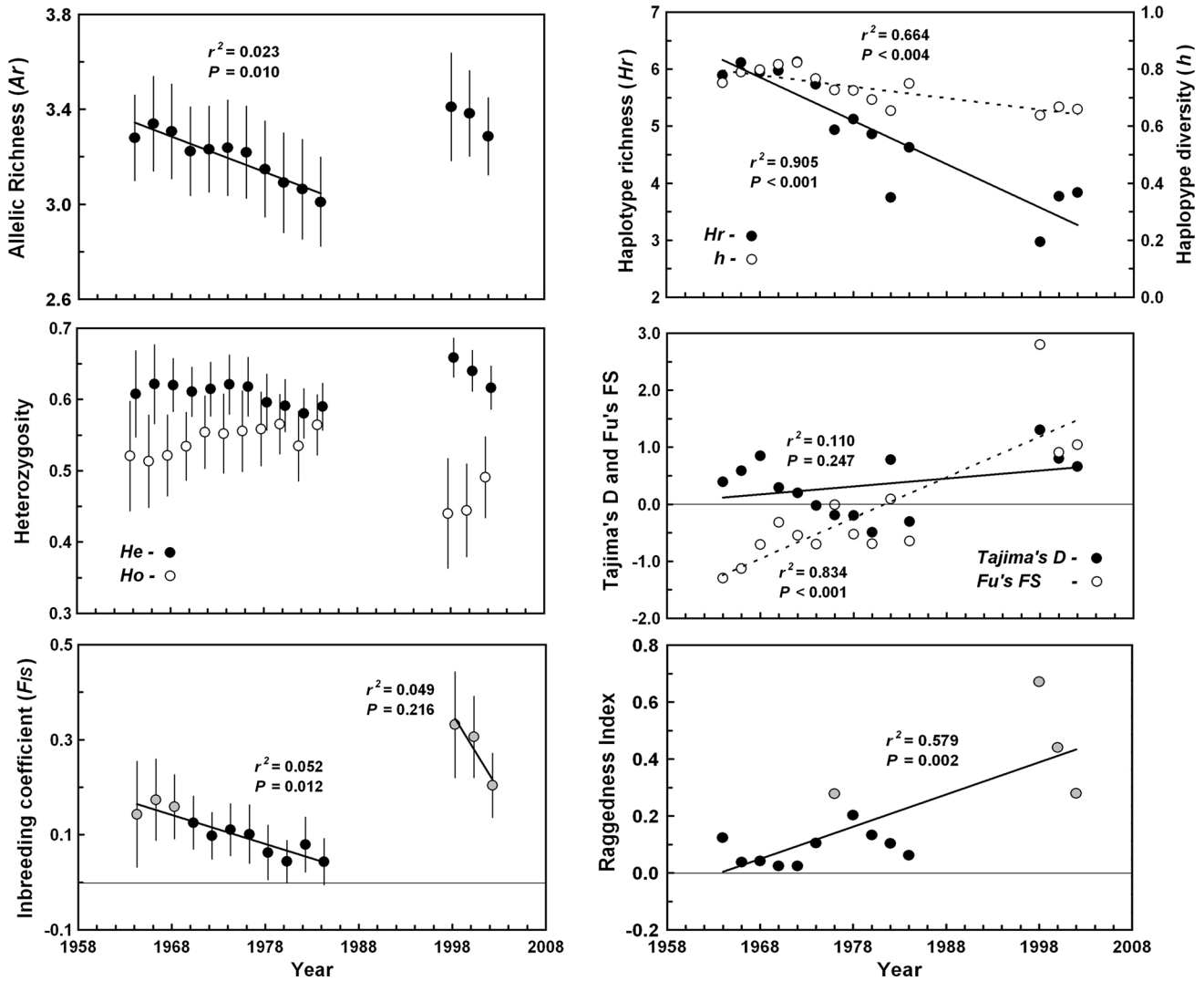


Fig. 3 Trends in genetic parameters based on central moving averages covering a time span of 11 years. Values for the microsatellites correspond to means over all loci ± SE. Allelic richness is based on a minimum sample size of five. *Grey dots* show significant deviations from Hardy–Weinberg equilibrium. We have included two *y*-axis for the *upper right corner* graph to accommodate the different scale ranges of the represented parameters

Simulations for alternative scenarios

Simulations showed similar patterns for both Cantabrian capercaillie and black grouse. The differences in the strength of the effect between both metapopulations were attributable to the different starting values of the genetic parameters (initial number of haplotypes and alleles). Higher initial values allow for a stronger initial loss of variability. For the capercaillie those values were taken from the Cantabrian bottlenecked population, whereas for the black grouse they corresponded to healthy Scandinavian populations (see Appendix).

Discussion

Our results support the hypothesis that removing reproductive males can have a significant effect on population demography, regardless of the stability of the female population. We suggest that the intensive and sustained removal of breeding males caused two consecutive processes in the Cantabrian capercaillie: an early reduction in the number of males contributing to reproduction that resulted in a loss of genetic variation; and a subsequent lack of males that became a limiting factor for female reproduction and caused a drop in productivity and a

Table 6 Haplotypy diversity (*h*) and richness (*Hr*) for five "populations" from shouth and north Europe and the Western Cantabrian capercaillie subpopulation (period 1958–1969)

Population	*h*	*Hr*				
		Mean	95 % C.I.	Range	N	Source
Cantabrian mountains	0.732	7.0	–	–	22	(1)
Sout-eastern Alps	0.877	9.0	8.5–9.6	3–11	102	(2)
Dinarides	0.504	4.7	4.3–5.1	3–7	150	(2)
Finland						
T. u. uralensis	0.674	10.1	9.5–10.8	7–14	100	(3)
Hybrid zone	0.824	7.1	6.7–7.5	6–10	88	(3)
T. u. urogallus	0.768	9.9	9.1–10.8	5–14	97	(3)

All *Hr* values have been standardized to the minimum sample size of 22 iavailable for the Cantabrians by taking the mean and 95 % C.I. of 30 random samples of 22 birds from the sample available for each population (N). Data from (1) present study, (2) Bajc et al. (2011) and (3) Liukkonen-Anttila et al. (2004)

subsequent decrease in overall population size. A similar process was found in the saiga antelope, where overhunting of adult males caused the collapse of the population (Milner-Gulland et al. 2003). Our results show that the ongoing decline of the Cantabrian capercaillie population (Storch et al. 2006) started before or around the mid of the 20th century, and point to the hunting of displaying males as a likely trigger of that decline.

Analysing microsatellite DNA extracted from birds shot in the early 1960s, we found evidence of a recent population bottleneck and a loss of genetic variability, happening before the first sampling period of our study. Observed heterozygosity (*Ho*) was lower than that reported for other bigger European populations (Segelbacher et al. 2003; Isomursu et al. 2012). However, mtDNA extracted from the same birds showed a different pattern, indicating a stable or expanding population in the 1960s. Haplotype richness (*Hr*) and haplotype diversity (*h*) were within the range of values found for other five European populations (Table 6). Because mtDNA is four times more sensitive to bottlenecks than microsatellites, any factor that had caused a decrease in population size should have left a stronger genetic signature in mitochondrial than microsatellite DNA. Our simulations for populations of different sizes and two different species were consistent with that, showing that as population size decreased, the loss in mtDNA variation was considerably faster and stronger than it was for microsatellites (Figs. 5a, 6 for capercaillie, and 8a, 9, 10 for black grouse). This suggests that the patterns we found in the field are unlikely to be a consequence of an overall population decline.

We also considered the possibility that a decrease in migration rate could cause a loss of microsatellite genetic variation with no effect on mtDNA, and carried out simulations for different migration rates. Indeed, we found that due to genetic drift, a decrease in migration rate contributed to the maintenance of mtDNA variation but this applied also to microsatellites. If our study population had suffered from a reduction in connectivity, we should have found a higher *He*, a lower *Ho* and, as a consequence an increase in inbreeding coefficient (*Fis*, Figs. 5b and 6). Black grouse simulations showed the same pattern (Figs. 8b, 9 and 10).

Among the simulations of the three factors, the increase in operational sex ratio showed the trends in genetic variation that better resembled what we found in the field. OSR does not affect mtDNA variation, but both *He* and *Ho* decreased as OSR increased. Again, simulations for both species produced similar results (Figs. 5c, 6, 8c, 9 and 10). This led us to conclude that the bottleneck detected for the microsatellites was the consequence of a decrease in the number of males contributing to reproduction. Regardless of whether any other unknown factor could have caused male-biased mortality, the intensive hunting of display males during the mating season is on itself a factor of male-biased mortality.

The changes that happened in the decades following that early bottleneck suggest that hunting had more consequences than the loss of genetic variation. In the early 1970s, after more than 20 years of hunting without planned quotas, gamekeepers warned about a decrease in the number of displaying males and the hunting bag decreased (Fig. 1). Initially this resulted in restrictions in some areas and finally into a permanent ban for the whole population in 1979. The ban had little effect on poaching though, and many displaying males were shot over the following 10–20 years. Genetic data fit into these historical records. From the 1960s to early 1980s, allelic richness (*Ar*) decreased and *He* remained low. A slight non-significant increase in *Ho* led the parameter to converge with *He*, and consequently *Fis* decreased significantly. We speculate that an increased dispersal of females in search for males as these became scarcer, lead to an increased admixture in the population, although at this point this is just a new open hypothesis in light of our results.

Analysis of mtDNA indicated that both, genetic variation and female effective population size experienced the first signs of decline in the 1970s, and one of the potential

factors to explain this would be the dearth of adult males detected by gamekeepers. Removal of adult males constrains mate choice and can reduce the number of males contributing to reproduction. At some point it can even limit the chances for females to find a mate (Allee effect, Kramer et al. 2009; Lee et al. 2011). These effects can reduce breeding success directly or as a consequence of inbreeding, as reported in other grouse (Hoglund et al. 2002; Westemeier et al. 1998), resulting in smaller population size.

In our study population, the trends in genetic variation showed an inflection point during the period 1989–1997, when we could not get any sample (legal hunting was forbidden and non-invasive samples were not available for these years). The situation resembled that shown in the simulations for an overall population decline. Four low frequency haplotypes disappeared, and *Ho* decreased significantly as opposed to *He* and *Ar*, pointing to the existence of genetic drift. *Fis* increased about four times as compared to the figures that it had before the data gap. It reached higher values than those found in other areas with different population sizes (Segelbacher et al. 2003), but closer to values recorded for the highly endangered Polish populations (Rutkowski et al. 2005). In the absence of null alleles, inbreeding and population substructure are the two main factors leading to heterozygote deficit (Allendorf and Luikart 2007). We found no evidence of null alleles and there is no evidence of population subdivision either (Vázquez et al. 2012), suggesting that inbreeding was the most likely cause of heterozygote deficit. Inbreeding might have been caused by a strong decrease in population size of both sexes. We speculate that during the data gap, the male population might have crossed a critical threshold, limiting female mating success and increasing inbreeding due to low number of males contributing reproduction. Unfortunately no information on reproductive success is available before 1997, but summer counts of females with broods carried out between 1997 and 2004, showed that only 26 % of the females had juveniles with them (Bañuelos et al. 2008). Whether this was due to inbreeding depression, females failing to mate or any other reason is unknown. During and after the data gap, capercaillie disappeared from most of the territories of the central and eastern Cantabrian Mountains.

Conclusions

Selective extraction of males can decrease genetic variation and population productivity. This supports the importance to incorporate OSR into population genetics modelling. Managers involved in the planning of trophy hunting quota should bear in mind that their targets are important for the health of the population. As previously stated (Caswell and Weeks 1986; Rankin and Kokko 2007; Lee et al. 2011), we recommend that game managers incorporate population genetic data into the information used to make hunting quota decisions, in order to foresee and prevent demographic declines. A comparative analysis of the changes in genetic variation between microsatellites and mtDNA, could be useful for the detection of excessive trophy hunting. We suggest that the hunting of male capercaillies during the mating season in areas affected by habitat loss and fragmentation should be stopped to avoid the demise of their populations.

Acknowledgments We are very grateful to K. Pérez, D. Ramos, J.R. Jiménez, E. González, E. de la Calzada, an anonymous taxidermist, and several hunters and small public and private collections for providing access to many of the preserved birds and old feather samples. Many people collaborated in the collection of shed feathers, we are especially grateful to the Environmental Administration of Asturias and their rangers, as well as B. Blanco, A. Fernández, M. González, M. Quevedo and L. Robles. J. Cabot, helped us with the specimens from the Estación Biológica de Doñana, and J. Barrerio with those from the Museo de Ciencias Naturales de Madrid. Thanks also to the people of the Museo de Ciencias de Santiago de Compostela, to J.C. Illera, who made possible all Mac based analyses and to M. Quevedo, who made useful comments on the final version of the manuscript. This work has been supported by the Natural Environment Research Council (Grant: 244 NE/E005403/1), FICYT (IB08-158 and POST10-41), MICINN (CGL2010-15990) and Agencia Nacional de Promoción Científica y Tecnológica (PME 2151).

Appendix: Computer simulations

Background

Starting around 3000 years ago, the Cantabrian Mountains have undergone intensive human driven deforestation and forest fragmentation (Muñoz Sobrino et al. 1997), a process that intensified over the last 1000 years. Woodlands lost and fragmentation are prevalent factors associated to the decline of forest fauna by reducing population size and connectivity (Allendorf and Luikart 2007). Therefore, together with male hunting, habitat loss and fragmentation are potential factors that could explain the ongoing population decline in this area, leading to a reduction in effective (male and female) population size and/or a reduction in population connectivity. We used EASYPOP 2.0.1 (Balloux 2001) to model the dynamics of number of haplotypes (*H*), haplotype diversity (*h*), number of alleles (*Ao*), expected and observed heterozygosity (*He* and *Ho*) and inbreeding coefficient (F_{IS}) under various levels of: (1) population size, (2) population fragmentation (i.e. variation in genetic flow by using migration rate as a proxy) and (3) operational sex ratio. We did not intend to accurately recreate the patterns of genetic variation that we found in the field through time, but to get some insights on how

different levels of each of the three parameters could influence genetic variation in mitochondrial versus nuclear DNA.

Although our aim is to get insights about what would be the most plausible explanation for the genetic patterns found in the field for the Cantabrian capercaillie, our hypotheses should apply to a more general context. Thus, we hypothesize that in any similar species (in terms of social structure and reproductive system), a decrease in the number of reproductive males, would reduce microsatellite variability but had limited or no effect on mtDNA variation; conversely, any factor reducing both the number of reproductive females and males (overall drop in population size), should result in a sharper drop in mtDNA than microsatellite variation. A severe limitation in connectivity, as a result of habitat loss and fragmentation, would also have limited or no effect in mtDNA, but would lead to a more inbred population, when compared to the effects of a decrease in the number of reproductive males.

There is a possibility that the simulation results obtained for our study population are constrained in some way, due to the use of genetic parameters coming from a declining population. In order to check the generality of our hypotheses, we have also explored these alternative scenarios for the black grouse (*Tetrao tetrix*), a polygynous lekking bird and a close relative of capercaillie. To run the simulations, we used demographic parameters extracted from the available literature on the large black grouse populations of Scandinavia. We did not aim to develop a detailed demographic model, but to explore how different levels of population size, population connectivity or operational sex-ratio (OSR), might affect nuclear verus mitochondrial genetic variation in a demographically healthy population of a lekking bird.

Cantabrian capercaillie

Simulation settings

All the simulations are based in a metapopulation made by 16 identical populations. The setting of most of the genetic and demographic parameters required by EASYPOP, are shared by all the simulations regardless of what factor is being modeled. These "fixed" parameters include the proportion of copulations by subordinate males, the migration model, the number of loci, the mutation scheme, rate and model and the number of possible allelic states. All other parameters varied according to the factor being modeled.

For the shared parameters, we started the simulation with the maximal variability achievable with the actual overall values of number of alleles and haplotypes that we found in our study population (average of 6 alleles per microsatellite for 11 locus and 9 haplotypes for the mtDNA control region). We set basic simulation parameters (population density and breeding success) according to figures reported for large capercaillie populations living in Finland (Helle et al. 1999), Norway and Russia (Rolstad et al. 2009). There are no data available about the proportion of males that contributes to mating in capercaillie, although a strong skew in male mating success in lekking species has been repeatedly reported, so that 10–20 % of the males get 70–80 % of the matings of a lek (Wiley 1991; Mackenzie et al. 1995). We set this parameter to a conservative 0.5, i.e. one male in each population gets half of the copulations. We followed a 2-dimension stepping stone migration model, with 4×4 populations (see Fig. 4). These 'populations' would resemble the groups of neighbouring lekking sites located in the same forest or group of nearby forests as they are found in the field. We set mutation rate to 5×10^{-4} for mtDNA (Brown et al. 1979) and 10^{-3} for microsatellites (Goldstein and Pollock 1997) and adopted the same mutation model we used for the estimation of effective population size from our field data, a SMM mutation model with a 0.1 proportion of KAM (K-Allele model) for the microsatellites (this is similar to the TPM model used in the bottleneck analysis), and a pure SMM mutation model for the mtDNA. We run five replicates per simulation.

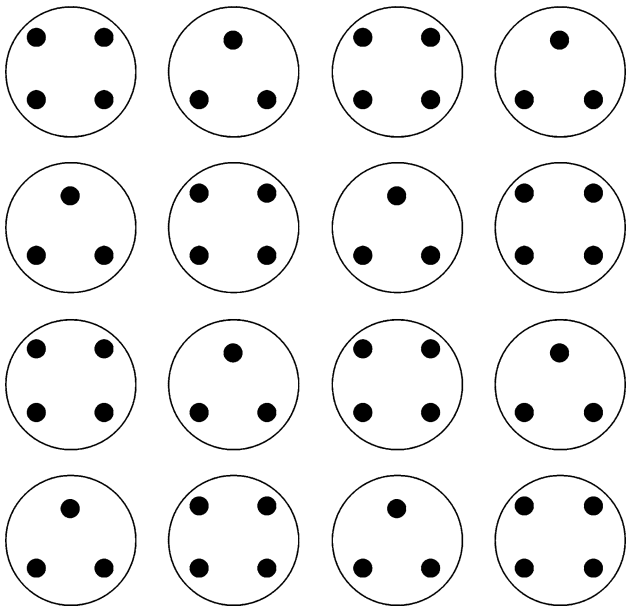


Fig. 4 Diagram representing the two-dimension stepping stone migration model used in Easypop. Each *black dot* represents a lek. Leks are grouped in batches of 3–4 (*encircled*) that correspond to the populations in the simulation. Migration rates correspond to movements among circles, each circle being connected to the four closest circles around it, with the exception of the borders, where *circles* are connected to two (*circles at the corners*) or three (*circles at the sides*) other *circles*

Table 7 Parameter values used to set up the 12 EASYPOP simulations for a model metapopulation including 16 populations of Cantabrian capercaillie, each one containing 3–4 leks. Simulations include three levels of population size and four of migration rate and operational sex ratio (OSR). The table includes only those parameters having some difference among simulations

	Population size	Migration rate	Operation sex ratio (OSR)
Female population	Changing factor	400	400
Male population	Changing factor	400	Changing factor
Female migration rate	0.5	Changing factor	0.5
Male migration rate	0.5	Changing factor	0.5
Changing factors	Female/male	Female/male	Female/male
	400/400	0.5/0.5	400/192
	192/192	0.2/0.2	400/96
	96/96	0.1/0.1	400/32
		0.0/0.0	400/16

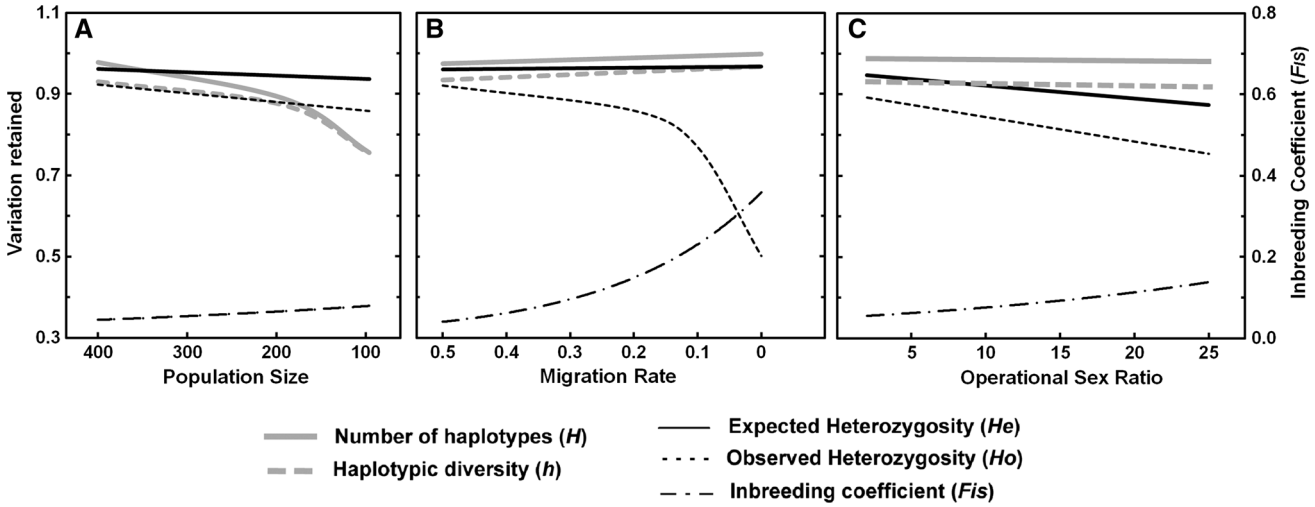
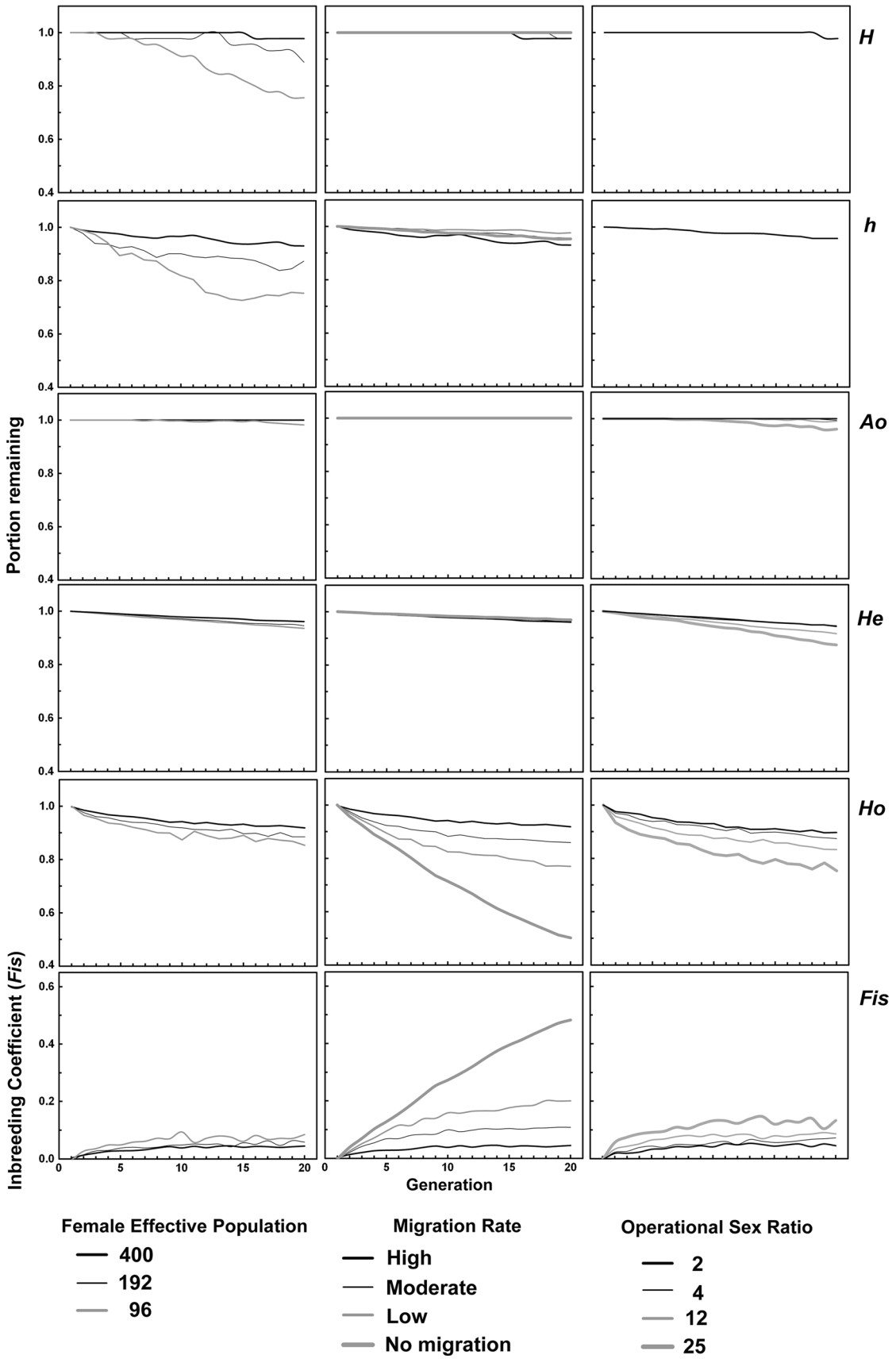


Fig. 5 Effect of different levels of population size (**a**), migration rate (**b**) and operational sex ratio (**c**) on inbreeding coefficient and the proportion of genetic variation (number of haplotypes, haplotypic diversity and expected and observed heterozygosities) retained after 20 generations of computer simulation using EasyPop (Balloux 2001). Simulations started with maximum diversity for a sequence of mtDNA with nine haplotypes and 11 microsatellite loci with six alleles each (microsatellites). Number of locus and alleles resemble values found in the Cantabrian capercaillie. *Lines* represent trends based on the mean of five independent simulation replicates. Simulations of population size were performed with a constant migration rate of 0.5 and an even sex-ratio. For migration rate, we used a constant population size of 800 and an even sex-ratio. For operational sex ratio, we used a constant migration rate of 0.5 and a female population size of 400. Sex ratio was increased by changing male population size

The "variable" parameters, i.e., those that were different depending on what factor was being modeled, included male and female population size and the proportion of male and female migration. To explore the effect of population size, we run three simulations with three levels of population size and a 1:1 sex-ratio. We set the highest level to 800 individuals (400 females), the maximum effective population size that we estimate could be supported bearing in mind the area occupied by the western Cantabrian capercaillie subpopulation and the densities found in the healthy populations living in Finland. For the lower levels we halved the preceding one each time, so we set 384 and 192 (we did not use 400 and 200 because we needed numbers that could be divided by the number of

Fig. 6 Effect of different levels of population size, migration rate and ▶ operational sex ratio (OSR), on the proportion of variation retained for the number of haplotypes (H), haplotypic diversity (h), number of alleles (Ao), genetic diversity (expected heterozygosity, He), observed heterozygosity (Ho) and inbreeding coefficient (Fis), over 20 generations. *Graphs* are based on the means of five replicates for each of 16 computer simulations using Easypop (see Supplementary Table S1 for parameter setting). Simulations for different population sizes (*left column*) were calculated for an even OSR, an equal migration rate of 0.5 for both sexes. Simulations for different migration rates were calculated for a female effective population of 400 and an even OSR. Simulations of OSR were calculated for a female population of 400 and an equal migration rate of 0.5 for both sexes. Increasing values of OSR are based on reducing male population size. All simulations started with maximum variability based on the genetic parameters found for the Cantabrian capercaillie (9 haplotypes and 11 loci with 6 alleles each)



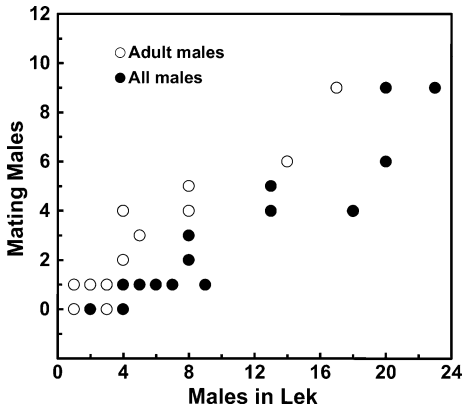


Fig. 7 Relationship between number of males getting copulations in a lek and total number of males present at the same lek. Each *dot* represents a lek-year after Alatalo et al. (1992)

Table 8 Values used to set up the 52 EASYPOP simulations run for a metapopulation including 16 populations of black grouse, each one containing 3–4 leks. Simulations include all possible interactions among four levels of migration rate, four of population size and four of operational sex ratio (OSR), with the only exceptions of those where the number of males per population is smaller than 1 (EASYPOP does not allow populations with 0 individuals of either sex). Each cell shows the OSR value corresponding to each of the simulations for the different combinations of total female population size and male and female migration rates. Number of males per simulation can be calculated dividing the number of females by OSR (numbers have been rounded to the nearest integer for female numbers of 80 and 160)

Male migration rate	0			0.3
Female migration rate	0	0.4	0.75	0.75
Female effective metapopulation size				
80	2	2	2	2
	4	4	4	4
160	2	2	2	2
	4	4	4	4
	12	12	12	12
400	2	2	2	2
	4	4	4	4
	12	12	12	12
	24	24	24	24
800	2	2	2	2
	4	4	4	4
	12	12	12	12
	23	23	23	23

populations—16). These simulations were run with a migration proportion of 0.5 for each sex. To model the effect of migration rate, we set population size to the highest level just described (800) and 1:1 sex-ratio, and run four simulations with one of four levels of proportion of migration each (identical values for each sex), no migration (0), low migration (0.1), moderate migration (0.2) and high migration (0.5). Finally, to explore the effect of operational sex-ratio, we set the same proportion of migration for each sex to 0.5, and the female population size to 400, and then run four levels of OSR, 2, 4, 12 and 25, by setting different male numbers (see Table 7).

Results

Reducing population size decreased the amount of variation retained for nuclear and mitochondrial markers. This decrease was much stronger and faster on mitochondrial than on microsatellite variability regardless of the parameter used to assess genetic variation (Figs. 5a and 6). The difference between observed and expected heterozygosities increased as population size decreased, resulting in an increase in inbreeding coefficient due to heterozygote deficit. The loss of haplotypes (H) and haplotype diversity (h) was particularly strong for the smallest population size, with a reduction in the number of haplotypes of 30 % after 20 generations (Figs. 5a and 6). In turn, the reduction in the number of alleles (Ao) for the smallest population size was around 2 % (Fig. 6).

Genetic drift associated to low migration rates, contributed to retain overall genetic variation for mitochondrial and microsatellites at the cost of a decrease in observed (actual) heterozygosity. Drift favours the fixation of different alleles at different populations, increasing the genetic diversity when pooling all the populations together. Thus, the number of haplotypes, haplotype diversity and expected heterozygosity remained nearly unchanged in simulations with no migration after 20 generations. However, the effect was opposite for observed heterozygosity, for which an increase in population subdivision favoured inbreeding and so reduced sharply the actual proportion of heterozygotes. This divergence between observed and expected heterozygosities led to a rapid increase of the inbreeding coefficient due to heterozygote deficit, particularly when migration rate was set to zero (Fig. 5b and 6).

Because mitochondrial DNA is only inherited from females, haplotype number and diversity are insensitive to increasing levels of OSR associated to a reduction in the number of reproductive males (the simulations assume no effect of OSR on a female's chances to mate). However, both heterozygosities (expected and observed) decreased with the reduction of male effective population (Figs. 5c and 6). The faster decrease of observed versus expected heterozygosity caused an increase of the inbreeding coefficient over the first 20 generations, although not as strong as the increase caused by severe population fragmentation.

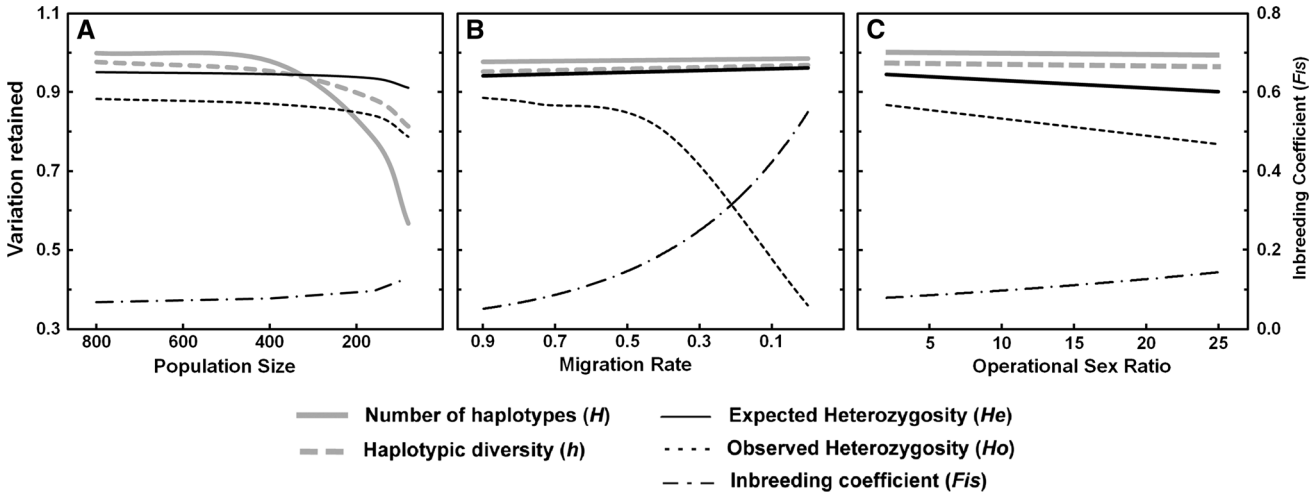


Fig. 8 Effect of different levels of population size (**a**), migration rate (**b**) and operational sex ratio (**c**) on inbreeding coefficient and the proportion of genetic variation retained after 20 generations of computer simulation using EasyPop (Balloux 2001) for several genetic parameters of a black grouse metapopulation. Simulations started with maximum diversity for a sequence of mtDNA with 10 haplotypes and 11 microsatellite loci with 12 alleles each. Number of locus and alleles were taken from the literature about the Scandinavian populations. *Lines* represent trends based on the mean of 100 simulation replicates. Simulations of population size were performed with a constant female migration rate of 0.75, no male dispersal and an OSR of 2. For migration rate, we used a constant female population size of 400 and an OSR of 2. For operational sex ratio, we used a constant female migration rate of 0.75 with no male dispersal and a female population size of 400. Sex ratio was increased by changing male population size

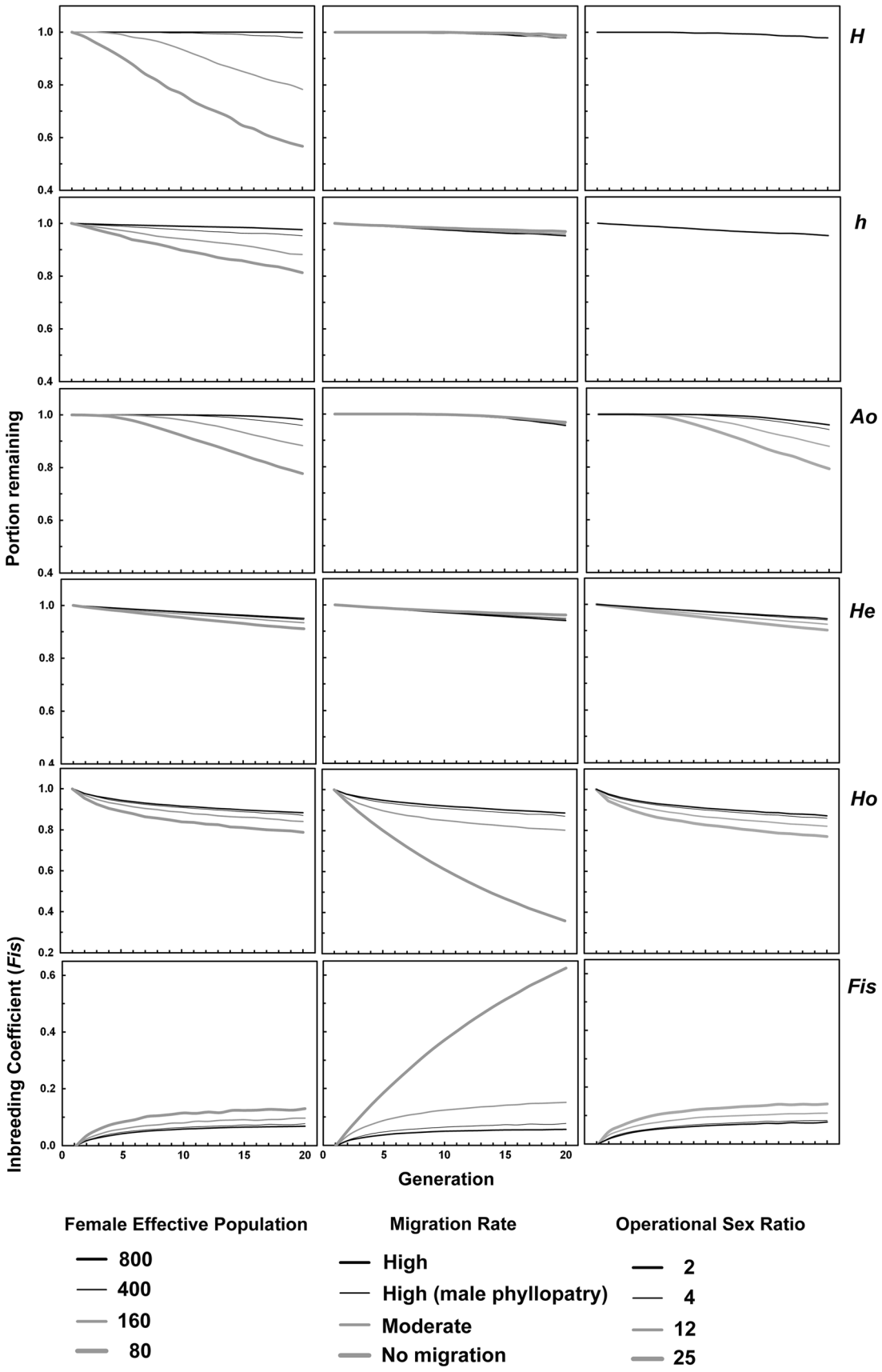
Black grouse

Parameter settings

According to Alatalo et al. (1992), the mean distance between neighboring black grouse leks is 2.1 km, and the average number of males per lek is 7.1. The operational sex ratio is quite high due the strong skew in male mating success. Around 55 % of the males in a lek are 2 years old or older, and these males get over 90 % of the copulations. At each lek a single dominant male gets between 50 and 100 % of the copulations, with an average of around 60 %. There is a strong relationship between the number of males that get copulations in a lek and the number of males present at that lek (Fig. 7). In most of the leks with less than 10 males, only 1–2 males mate, and only leks with more than 16 males have more than 6 mating males. Dispersal has a strong sex-skew. Males are philopatric, moving mainly around neighboring leks, whereas the median dispersal distance of yearling females is 9.2 km, and 75 % of them move farther than 4 km (Marjakangas and Kiviniemi 2005). Ten different haplotypes have been described for the mtDNA Control Region of the Scandinavian black grouse populations (Corrales et al. 2014), and the average number of alleles found for 14 microsatellite loci was 12 (Caizergues et al. 2003).

We run 52 simulations combining different population sizes, dispersal rates and operational sex-ratios as shown in Table 8. All simulations were run using a 2-dimension stepping stone migration model with 16 populations. This would be equivalent to a metapopulation of 56 leks grouped in 16 blocks of 3–4 neighboring leks (each block being one of the populations in the simulation, see Fig. 4). We started the simulation with the maximal variability achievable with the values of allele and haplotype richness described in the literature, an average of 12 alleles per microsatellite locus (14 loci) and 10 haplotypes for the mtDNA control region. We set mutation rate to 5×10^{-4} for mtDNA (Brown et al. 1979) and 10^{-3} for microsatellites (Goldstein and Pollock 1997) and adopted the same mutation model used for the capercaillie simulations, a SMM mutation model with a 0.1 proportion of KAM (K-Allele model) for the microsatellites, and a pure SMM mutation model for the mtDNA. We run 100 replicates of each simulation for a total of 20 generations.

The simulations covered all combinations among the different levels of each factor (see Table 8). They included four levels of metapopulation size, with a female effective population of: 80, 160, 400 and 800 birds (these numbers correspond to 5, 10, 25 and 50 females per population including 3–4 leks). We used also four levels for migration rates (female-male): 0–0 (no dispersal), 0.4–0 (moderate female dispersal), 0.75–0 (high female dispersal) and 0.75–0.3 (high female dispersal with moderate-low male dispersal). Finally, we tested four levels of OSR (by decreasing the number of males in relation to the number of females), using values of 2, 4, 12 and 25 females per male. Assuming an even sex-ratio, values of OSR range in nature



◀**Fig. 9** Effect of different levels of population size, migration rate and operational sex ratio, on the proportion of variation retained for the number of haplotypes (H), haplotypic diversity (h), number of alleles (Ao), genetic diversity (expected heterozygosity, He) and observed heterozygosity (Ho) and inbreeding coefficient (Fis), after 20 generations. *Graphs* represent the mean of 100 replicates per each of 12 computer simulations using Easypop (see Supplementary Table S2 above). Simulations for different population sizes (*left column*) were calculated for an OSR of two, a female migration rate of 0.75 and no male dispersal. Simulations for different migration rates were calculated for a female effective population of 400 and an OSR of 2. Simulations of OSR were calculated for a female population of 400, a female migration rate of 0.75 and no male dispersal. Settings are based on demographic parameters reported for the black grouse in Scandinavia

from 2 to 9, with an average of 4. Values of 1–4 mating males per lek are found for leks sizes of up to 17 males (Fig. 7), and there are leks with up to four males that get no copulations.

Results and discussion

The patterns obtained were very similar to those found for the Cantabrian capercaillie, with the differences being attributable to variation found in a bottlenecked versus a healthy population. The relationship between population

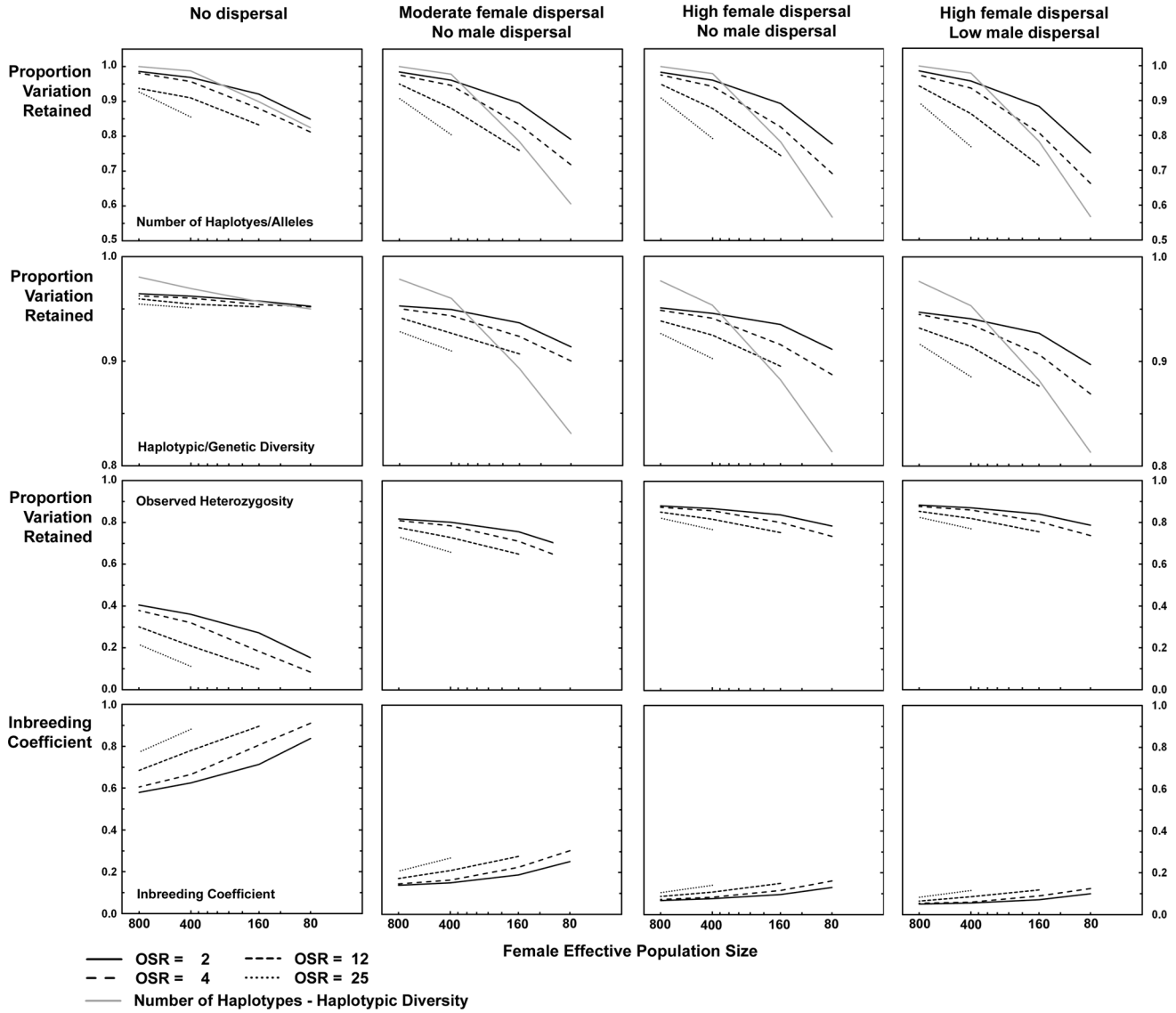


Fig. 10 Effect of different levels of population size, migration rate and operational sex ratio, on the proportion of variation retained for the number of haplotypes (H), haplotypic diversity (h), number of alleles (Ao), genetic diversity (expected heterozygosity, He) and observed heterozygosity (Ho) and inbreeding coefficient (Fis), after 20 generations (different parameters represented on different *series* or *rows*). Graphs represent the mean of 100 replicates per each of 52 computer simulations using Easypop. Simulations include all combinations among four different population sizes (*x*-axis), four levels of migration rate (*columns*) and four levels of OSR (*series within each graph*) (see Table S2 above). General parameter settings are based on demographic parameters reported for the black grouse in Scandinavia. Note that different to Fig. S5 above, the *x*-axis does not represent generations but population sizes

size and genetic variation was positive for both nuclear and mitochondrial DNA, i.e. a decrease in population size caused a loss of genetic variation. With population sizes of 400 and over, the loss of variation after 20 generations was very small for both types of markers, although smaller for microsatellites than mtDNA. However, with less than 400 females breeding females, genetic variation started to decrease more quickly, and this decrease was more pronounced for mtDNA than microsatellites (Figs. 8a and 9). The faster decrease of mtDNA variation for populations smaller than 400 females, did happen regardless of migration rate and OSR, with the only exception of zero migration, when trends were very similar to those found for microsatellites (Fig. 10). This shows that any relevant decrease in genetic variation associated to a reduction in effective population size would have a stronger effect for mtDNA than microsatellites.

Connectivity among populations had a similar effect for the two types of genetic markers. Decreasing migration rates increased metapopulation genetic diversity and decreased observed heterozygosity as a consequence of genetic drift among populations. The effect was small for moderate to high migration rates, but strong when populations were completely isolated. Thus, under zero dispersal, variability within populations was very small but remained high for the metapopulation as a whole (Figs. 4B and 5). For all the different combinations of population size and migration rate where a relevant decrease in genetic variation was observed, this was always much stronger for mtDNA than microsatellites (Fig. 10).

Due to maternal inheritance, mtDNA variation is not sensitive to changes in OSR, so this factor had no effect on it. For microsatellites, genetic diversity decreased with increasing OSR values (Figs. 8c and 9). This decrease did happen regardless of the levels of population size and migration rate (Fig. 6). This decrease was relevant in all cases except for genetic diversity when migration rate was set to zero. This exception was a consequence of the fixation of different alleles at different populations due to genetic drift. The effect was particularly strong for OSR levels of 12 or higher.

Conclusion

Population size and fragmentation are the two most influential factors reported in the literature in relation to the maintenance of genetic variation. Mithocondrial DNA is four times more sensitive than nuclear DNA to a decrease in population size and both are similarly sensitive to changes in migration rate. Our simulations for Cantabrian capercaillie and black grouse are consistent with that, showing also that for any combination of size and migration rate, relevant decreases in genetic variation are always stronger for mitochondrial than microsatellite DNA. We simulated a third factor, the operational sex ratio, and found that it has also a relevant effect on the maintenance of genetic variation, although because of its maternal inheritance, mtDNA is insensitive to OSR.

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