Microsatellite markers show distinctiveness of released and wild grey partridges in Finland

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Abstract

Microsatellite markers show distinctiveness of released and wild grey partridges in Finland.— The main aim of this study was to study whether the present game farm stocks used for releases to the wild in Finland are similar to wild populations in their genetic structure, and if not, whether the wild populations show any signs of hybridisation. A total of 301 feather samples and ten microsatellite loci were used. Samples were collected from France, Great Britain, Finland (wild and captive) and Greece. We estimated pairwise F_{ST}–values between study populations, examined population structure and identified possible first generation migrants. Pairwise F_{ST}–values indicated structuring among studied populations. Results indicate that the farm stock used for releases deviates from the wild populations. No signs of hybridisation between the released and native birds were detected.

Key words: Captive stock, Grey partridge, Microsatellites, Native stock, *Perdix perdix.*

Resumen

Los marcadores de microsatélites ponen de manifiesto las diferencias entre las perdices pardillas liberadas y silvestres en Finlandia.— El objetivo principal de este estudio consistió en estudiar si las poblaciones de las granjas cinegéticas utilizadas para las liberaciones en el medio natural en Finlandia son parecidas a las poblaciones silvestres en cuanto a su estructura genética y, en el caso de no serlo, si las poblaciones silvestres muestran signos de hibridación. Se utilizaron en total 301 muestras de pluma y 10 loci de microsatélite. Las muestras se recogieron en Francia, Gran Bretaña, Finlandia (silvestres y en cautividad) y Grecia. Calculamos los valores de F_{ST} entre pares de poblaciones del estudio, examinamos la estructura de la población y determinamos los posibles migrantes de primera generación. Los valores de F_{ST} entre pares indicaron la presencia de estructuración entre las poblaciones estudiadas. Los resultados indican que la población de granja utilizada para las liberaciones es distinta de las poblaciones silvestres. No se detectaron signos de hibridación entre las aves liberadas y las nativas.

Palabras clave: Población en cautividad, Perdiz pardilla, Microsatélites, Población nativa, *Perdix perdix.*

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Introduction

The distribution range of the grey partridge (*Perdix perdix*) covers large areas in Europe and Asia, all the way from Ireland to the Ural Mountains. The world-wide decline in the numbers of the grey partridge is well documented. A marked decline in the distribution range has occurred during the last century, mostly as a result of modern agricultural practices (for review, see Potts 1986).

In Finland, the grey partridge lives at the edge of its northernmost distribution range. According to Kivirikko (1948), the grey partridge arrived in Finland from the southeast at the beginning of the 1800s, although the earliest observations were reported in 1690 (Merikallio, 1958). In 2007 the population size in Finland was estimated *ca.* 4,000 individuals and the species was classified as near–threatened (Liukkonen, 2007), but in the latest Finnish Red–List the species is classified as 'Least Concern' resulting from population size increase (Rassi et al., 2010).

The first introductions were conducted in the middle of the 18th century (Merikallio, 1958) for hunting purposes with birds imported from Sweden (Kreuger, 1950). Captive–rearing and releasing of partridges has traditionally been carried out for game management purposes with the main aim to increase the size of the game bag. This kind of game management is and has been common for centuries. Early on, origin of stocks used for releases was rarely considered. At present, there are rules and recommendations for supplementing or replacing wild populations. In the IUCN Guidelines for the Re–introductions of Galliformes (WPA & IUCN/SSC Re–Introduction Specialist Group, 2009) it is stated that 'the sourcing of birds for re–introduction must not harm present populations and should be of appropriate (*i.e.* non–harmful) genetic stock. The taxonomic status of all remaining populations should be studied and, in most cases, the same subspecies or race should be used for reintroductions as those which were extirpated (unless adequate numbers are not available)'.

The above–mentioned harmful effects are related to outbreeding depression, a phenomenon when matings between individuals from distinct populations break up co–adapted gene complexes and result in lower fitness of hybrid offspring. Examples of outbreeding depression range from plants and invertebrates to vertebrates and include reduction for example in viability, fertility, reproductive success and immune resistance (reviewed in Edmans, 2007).

The European grey partridge is divided into two lineages by mitochondrial DNA (mtDNA). These lineages are assumed to refer to two different subspecies, *P. p. perdix* and *P. p. lucida*. After the last glaciation, colonisation of Europe occurred from two different glacial refugia, namely the Balkan Peninsula or Caucasus in the east and the Iberian Peninsula in the west (Liukkonen–Anttila et al., 2002). The western lineage, *perdix,* is widely found in Central Europe, for instance in France, Germany, Italy, Poland and the UK, whereas the eastern lineage, *lucida,* can be found in Finland, Greece, Bulgaria, Kazakhstan and Ireland. It is possible, that at least in Estonia, Russia and Ukraine, populations are mixed, that is, either human–induced or naturally occurring, because birds of unknown origin have been released into these areas.

Table 1. Collection locations, numbers and the assumed mtDNA–lineage of the grey partridge (*Perdix perdix*) feather samples used in this study.

*Tabla 1. Lugares y cifras de recolección y linaje esperado del ADNmt de las muestras de plumas de perdiz pardilla (*Perdix perdix*) utilizadas en este estudio.*

Location	mtDNA–lineage	n
France	Western	20
Great Britain	Western	46
Finland, sites with releases	Western+ eastern?	107
Finland, sites with no releases	Eastern	54
Game farm stock	Western+ eastern?	52
Eastern captive stock	Eastern	7
Greece	Eastern	15

In Finland, the native wild population represents the eastern mtDNA lineage, whereas most captive birds used for releases represent the western lineage (Liukkonen, 2006). This raises the question, have birds of wrong origin been released into the wild and have these releases had an impact on the wild population? Interest in managing grey partridge populations and willingness to conserve the native subspecies is not new in Finland. The Gene Bank Project has been going on for almost ten years. In this project the main aim has been to establish a general stock of eastern birds to be used in any possible releases to avoid mixing of these two lineages.

The aims of this study were: 1) to study whether the game farm stock is similar to wild population in genetic structure and, 2) if not, to study whether the Finnish wild population shows signs of hybridisation between released and native birds.

Materials and methods

Sampled birds and laboratory methods

Altogether, 301 feather samples of the grey partridge were used for this study (table 1). Samples were collected between 1998 and 2011 from Finland and, for comparisons, also from Great Britain, French Pyrenees and Greece (Liukkonen–Anttila et al., 2002; Liukkonen, 2006; this study; fig. 1). The Finnish wild

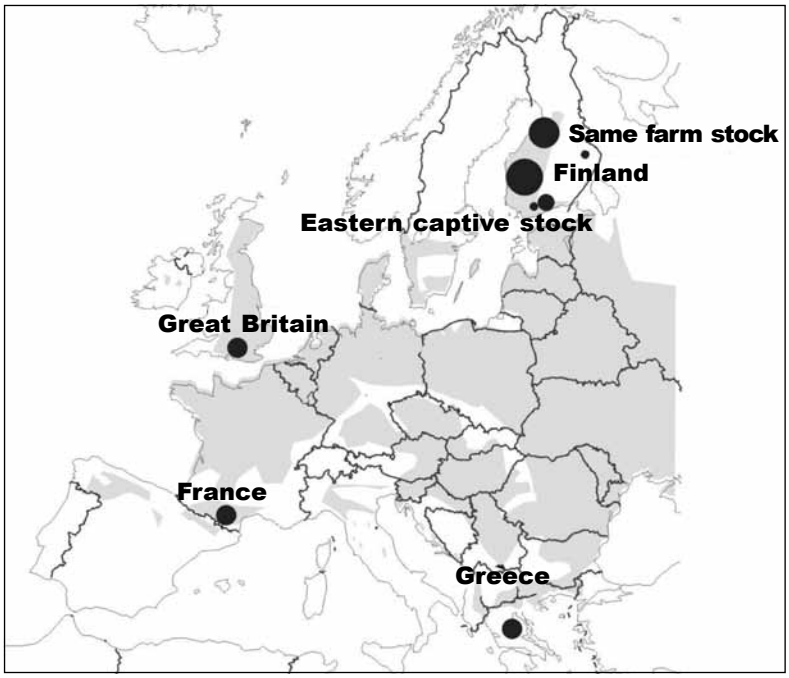

Fig. 1. Sampling locations of the grey partridges (*Perdix perdix*) used in this study.

*Fig. 1. Lugares de muestreo de las perdices pardilla (*Perdix perdix*) utilizadas en este estudio.*

samples were collected from two types of sites; sites where no introductions have been made and sites where active releases for sport hunting take place. Samples from two captive populations were also obtained, one (game farm stock) is used for releases and the other (eastern captive stock) represents birds originating from the wild but not yet actively used for releases. DNA was extracted from feather quills as described in Liukkonen–Anttila et al. (2002) or by using QuickExtract solution (Epicentre) following the manufacturer's protocol. Table 2 shows the microsatellite markers and modifications on PCR to amplify the loci used. The PCR products were run with ABI PRISM 3730 DNA Analyzer (Applied Biosystems) and scored using GeneMapper v. 4.0.

Genetic variation

Expected and observed heterozygosities were calculated with Arlequin v.3.11 (Excoffier et al., 2005). Allelic richness (corrected for the sample size bias with the rarefaction method) and inbreeding coefficients (F_{IS}) were estimated with FSTAT v.2.9.3 (Goudet, 2001) excluding locus MNT408, because too few individuals successfully scored for this locus. Values were estimated for France, Great Britain (western subspecies), Finnish sites with no releases, Finnish sites with releases, game farm stock, eastern captive stock, and Greece (eastern subspecies).

Genetic structure

Arlequin v.3.11 was used to estimate pairwise F_{ST}–values between the study populations. In addition, molecular variance analysis (AMOVA) was used to examine population structure using different groups defined *a priori*. Variation was estimated at three hierarchical levels; among groups (F_{CT}), among populations within groups (F_{SC}) and among populations (F_{ST}).

Programme Structure v.2.2 (Pritchard et al., 2000; see also Falush et al., 2003) was used to infer the number of populations (K) in the data using the Markov chain Monte Carlo (MCMC) approach. A model with population admixture and correlated allele frequencies within populations (Falush et al., 2003) without prior information of the sampling locations was assumed. Five runs for each value of K between 1 and 12 were conducted, with a burn–in period of 100,000 iterations, and data were collected for 500,000 iterations. The likelihood of the data and following log probabilities for the different numbers of subpopulations were calculated for each K , the K with the highest log probability should equal the number of populations in the data. The largest change in log probability of data between consecutive numbers of populations, ΔK, has been proposed to estimate the actual K, and it has been found to perform better than the log probability *per se* (Evanno et al., 2005). This method should detect the highest level of population structure, when

Table 2. Microsatellite markers used for the analysis of the grey partridge (*Perdix perdix*) population structure. $MgCL_2$ concentration (in mM) and annealing temperature (T, in ºC) are modifications of the original PCR protocols (Bech et al., 2010; Ferrero et al., 2007).

*Tabla 2. Marcadores de microsatélites utilizados para el análisis de la estructura de la población de perdiz pardilla (*Perdix perdix*). La concentración de $MgCl_2$ (en mM) y la temperatura de hibridación (T, en ºC) son modificaciones de los protocolos originales de la PCR (Bech et al., 2010; Ferrero et al., 2007).*

Marker	Reference	$MgCl_2$	T
Aru1A1	Ferrero et al., 2007	2.5	56
Aru1G4	Ferrero et al., 2007	2.5	56
Aru1E66	Ferrero et al., 2007	2.5	50
Aru1E102	Ferrero et al., 2007	2.0	50
Aru1F114	Ferrero et al., 2007	2.0	50
MNT12	Bech et al., 2010	2.0	53
MNT412	Bech et al., 2010	2.0	53
MNT477	Bech et al., 2010	2.0	55
MNT45	Bech et al., 2010	2.5	53
MNT408	Bech et al., 2010	2.5	53

several hierarchical levels exist, *i.e.* lower hierarchical structure may also be present. The results from Structure were used as input to this *ad hoc* method by Evanno et al. (2005).

Factorial correspondence analysis (FCA) in the programme Genetix v. 4.0 (Belkhir et al., 2004) was used to visualise the relative similarity among samples and possible genetic structure within each region in a multivariate space. FCA tries to find the best linear combination of variables (*i.e.* allele frequencies at different loci in this case), which describe variation between individual observations. The factorial axes are ordered by their eigenvalues and the location of individuals is defined according to the axis. The proximity of individuals along the axes expresses how genetically similar these individuals are.

An assignment analysis and identification of possible first generation migrants between the sites was performed using the programme Geneclass 2 (Piry et al., 2004). This programme includes a Bayesian individual assignment method by Rannala & Mountain (1997) to estimate the marginal probability of each given individual genotype compared with the distribution of marginal probabilities of randomly generated genotypes (1,000 replicates) using the resampling method of Paetkau et al. (2004). We chose individuals that scored for at least four loci for this analysis. The assignment threshold was set at 0.05 and alpha–level for the MCMC simulations was 0.01.

Results

The highest observed heterozygosities (table 3) were found in Greece and in the Finnish population with releases (0.748 and 0.710, respectively). The highest expected heterozygosities were found in the French population and again in the Finnish population with releases (0.759 and 0.763). These populations also harboured the highest allelic richness (2.905 and 2.811). The lowest observed heterozygosities were found in the British population and the Finnish game farm stock (0.537 and 0.610) and expected heterozygosities in the British and Greek populations (0.623 and 0.624). Inbreeding coefficients were significantly positive in the French population and in the Finnish sites with no releases (0.181 and 0.113; table 3).

The pairwise F_{ST} values (table 4) between the study populations were almost all significant, with the exception that the Finnish sites with releases and sites with no releases did not differ from each other. The Greek population did not differ from the French population or from Finnish sites with no releases. The AMOVA analysis with different groupings yielded the highest F_{ST}–values when the British and French populations and Finnish game farm stock were grouped into one group and all the other Finnish populations with the Greek population (table 5). This grouping also resulted in the highest F_{CT}–values (genetic difference among groups) and lowest F_{SC}–values (difference among populations within groups).

Results on the population structure suggested that the most likely number of populations would be five (mean Ln P(D) was –4373.7, for K = 4 mean Ln P(D) was –4382.86). By applying Evanno's ΔK, the most likely number of populations was reduced to two (fig. 2A). The bar plots showing the proportion of each individual to belong to clusters indicate distinctiveness of the Finnish game farm stock from other Finnish samples. In addition, the bar plot for K = 2 suggests that this captive stock genetically belongs to the same cluster with most of the individuals from Great Britain and France (representing *P. p. perdix*). The individuals from the Finnish sites of releases and no releases and eastern captive stock were similar to the Greek individuals (representing *P. p. lucida*) (fig. 2B). The factorial correspondence analysis did not group populations into clearly distinct clusters. The individuals representing the eastern subspecies *P. p. lucida* and the Finnish sites and farm stocks were located 'in a pocket' within the individuals representing the western subspecies *P. p. perdix* indicating larger genetic variation in the western subspecies than in the other subspecies. The samples from Finnish sites with and without introductions and eastern farm stock tended to cluster together and separately from the Finnish game farm stock (fig. 3).

The assignment test showed that no individual significantly deviated from the populations it was sampled from (table 5). However, in several cases

Table 3. Observed (H_o) and expected (H_e) heterozygosities, allelic richness (A) and inbreeding coefficient (F_{IS}) estimated from the studied populations. Standard deviations (SD) are given in the parentheses. Significant F_{IS}–values ($p < 0.05$) are shown in bold.

Tabla 3. Heterocigosis observada (H_o) y esperada (H_e), riqueza alélica (A) y coeficiente de endogamia (F_{IS}) estimados de las poblaciones estudiadas. Las desviaciones estándar (SD) se indican entre paréntesis. Los valores significativos de F_{IS} (p < 0,05) se muestran en negrita.

Population	N	H_o (SD)	H_e (SD)	A (SD)	F_{IS}
France	20	0.626 (0.262)	0.759 (0.170)	2.905 (0.585)	**0.181**
Great Britain	47	0.537 (0.251)	0.623 (0.212)	2.479 (0.663)	0.140
Finland, sites with no releases	99	0.685 (0.177)	0.730 (0.125)	2.697 (0.468)	**0.113**
Finland sites with releases	54	0.710 (0.178)	0.763 (0.103)	2.811 (0.416)	0.008
Game farm stock	52	0.610 (0.181)	0.643 (0.110)	2.395 (0.274)	0.080
Eastern captive stock	7	0.652 (0.145)	0.634 (0.176)	2.436 (0.573)	–0.029
Greece	15	0.748 (0.392)	0.624 (0.268)	2.430 (0.794)	–0.263

Table 4. Pairwise F_{ST}–values between the study populations: FR. Finnish sites with releases; FNR. Finnish sites with no releases; GFS. Game farm stock; ECS. Eastern captive stock. (Significant values with $p < 0.05$ are shown in bold.)

Tabla 4. Valores de F_{ST} entre pares de poblaciones estudiadas: FR. Lugares finlandeses con liberaciones; FNR. Lugares finlandeses sin liberaciones; GFS. Población de granja cinegética; ECS. Población oriental en cautividad. (Los valores significativos con p < 0,05 se muestran en negrita.)

	France	Great Britain	FR	FNR	GFS	ECS
Great Britain	**0.0525**					
FR	**0.0370**	**0.1162**				
FNR	**0.0320**	**0.0708**	–0.0168			
GFS	**0.0779**	**0.0933**	**0.1032**	**0.0853**		
ECS	**0.1524**	**0.2339**	**0.0353**	**0.0705**	**0.1526**	
Greece	–0.0279	**0.0420**	**0.0405**	–0.0121	**0.0536**	**0.2056**

the individuals yielded a higher assignment probability to belong to a population other than their own. These involved: Finnish sites with releases: one to Greece (N = 107); sites with no releases: nine to sites with releases (N = 54); game farm stock: seven to sites with releases, three to Great Britain, two to France and one to Finnish sites with no releases (N = 52); eastern captive stock: two to Finnish sites with releases, two to sites with no releases (N = 7). Only two possible first generation migrants were detected; one from Greece to the Finnish site with releases, and one from the site with releases to eastern captive stock. This observation merely reflects the affinities of these individuals to those populations and does not represent true migration events.

Discussion

Genetic variation

The results of genetic variation obtained by using microsatellites were congruent with those obtained by mtDNA control region 1 sequences (Liukkonen–Anttila et al., 2002; Liukkonen, 2006). Expected heterozygosity and allelic richness were highest in the French population, whereas observed heterozygosity was highest in the Finnish sites with releases and in Greece. The lowest estimates of expected heterozygosity and allelic richness were obtained from Great Britain, Greece and the game farm stock, and the lowest observed heterozygosities were obtained from Great Britain and game farm stock.

Table 5. AMOVA results using different groupings of the populations (GB. Great Britain; FR. Finnish sites with releases; FNR. Finnish sites with no releases; GFS. Game farm stock; ECS. Eastern captive stock): AG. Among groups; APG. Among populations within groups; WP. Within populations. (The grouping resulting to the highest F_{ST}– and F_{CT}– and the lowest F_{SC}–values is marked in italics and significant values with $p < 0.001$ are in bold.)

Tabla 5. Resultados de AMOVA utilizando distintas agrupaciones de poblaciones (GB. Gran Bretaña; FR. Lugares finlandeses con liberaciones; FNR. Lugares finlandeses sin liberaciones; GFS. Población de granja cinegética; ECS. Población oriental en cautividad); AG. Entre grupos; APG. Entre poblaciones dentro de los grupos; WP. Dentro de las poblaciones. (La agrupación que tiene como resultado los valores mayores de F_{ST} *y* F_{CT} *y los menores para* F_{SC} *está en cursiva y los valores significativos con* $p < 0{,}001$ *se muestran en negrita.)*

Grouping	AG	APG	WP	F_{ST}	F_{CT}	F_{SC}
GB+France/All Finnish+Greece	2.74	5.31	91.94	**0.0806**	0.0275	**0.0546**
GB+France+FR/FNR+GFS+ECS+Greece	–2.86	8.49	94.37	**0.0563**	–0.0286	**0.0825**
GB+France+FNR/FR+GFS+ECS+Greece	–1.59	7.64	93.94	**0.0606**	–0.0159	**0.0752**
GB+France+GFS/FR+FNR+ECS+Greece	*4.67*	*3.66*	*91.68*	***0.0832***	*0.0467*	***0.0383***
GB+France+ECS/FR+FNR+GFS+Greece	1.02	6.11	92.87	**0.0713**	0.0102	0.0617
GB+France+GFS+ECS/FR+FNR+Greece	3.18	4.57	92.25	**0.0775**	0.0318	**0.0472**
GB+France+GFS+ECS+FR/FNR+Greece	–3.34	8.26	95.08	**0.0492**	–0.0334	**0.0799**
GB+France+FR+FNR/GFS+ECS+Greece	1.96	5.63	92.41	**0.0760**	0.0196	**0.0574**
GB+France+FR+FNR+GFS+ECS/Greece	–4.44	7.42	97.01	**0.0299**	–0.0444	**0.0711**

The significantly positive inbreeding coefficients in the French and in the Finnish sites of no releases may result from small effective population sizes and isolation of these populations. French samples were collected from a small isolated area in the Pyrenees, indicating that the positive inbreeding coefficient may result from real inbreeding. However, the Finnish samples were collected from an area which is not geographically isolated. Thus, the positive inbreeding coefficient may also reflect the existence of an undetected population structure.

The lowest observed heterozygosities were found in the British population and the Finnish game farm stock. The British population samples were from birds collected from the wild. The origin of these birds, however, is in translocated partridges. Translocation could have created a founder effect and loss of variation. The game farm stock in Finland has lost a great amount of genetic variation (Liukkonen, 2006), also likely resulting from an original founder effect followed by successive bottlenecks during breeding in captivity. Similar results on reduced amount of genetic variation were also found in the captive bred Mediterranean chukar partridge (*Alectoris chukar*, Barbanera et al., 2009a).

Genetic structure

Almost all pairwise F_{ST}–values between study populations were significant, thus supporting the previous results on the mtDNA control region 1 sequences (Liukkonen–Anttila et al., 2002; Liukkonen, 2006). Samples from the wild in Finland grouped together with the Greek samples and the eastern captive birds, whereas Great Britain, France and the game farm stock grouped together. Finnish sites of releases and no releases did not differ from each other. As most introduced birds originate from the game farm stock, this indicates that released birds do not contribute to the wild population, but instead that their mortality after release might be high.

The Greek population did not differ from the Finnish site of no releases, which supports the earlier results. In previous mtDNA studies, the Greek partridges represented the eastern lineage together with Finnish partridges (Liukkonen–Anttila et al., 2002; Liukkonen, 2006). However, against expectations, the Greek population did not differ from the French population either, possibly due to low sample sizes. Interestingly, the game farm stock, which is also used for releases, was clearly different from the wild population in Finland as well from the eastern captive stock. This result supported the earlier studies (Liukkonen–Anttila et al., 2002; Liukkonen, 2006). Also in the chukar partridge, farm stock in Crete differs from that used for releases (Barbanera et al., 2009b).

The AMOVA analysis with different groupings yielded to the highest F_{ST}–values when the British and French populations and Finnish game farm stock were grouped into one group and all the rest of the Finnish populations with the Greek population. This grouping also resulted in the highest F_{CT}–values (genetic difference

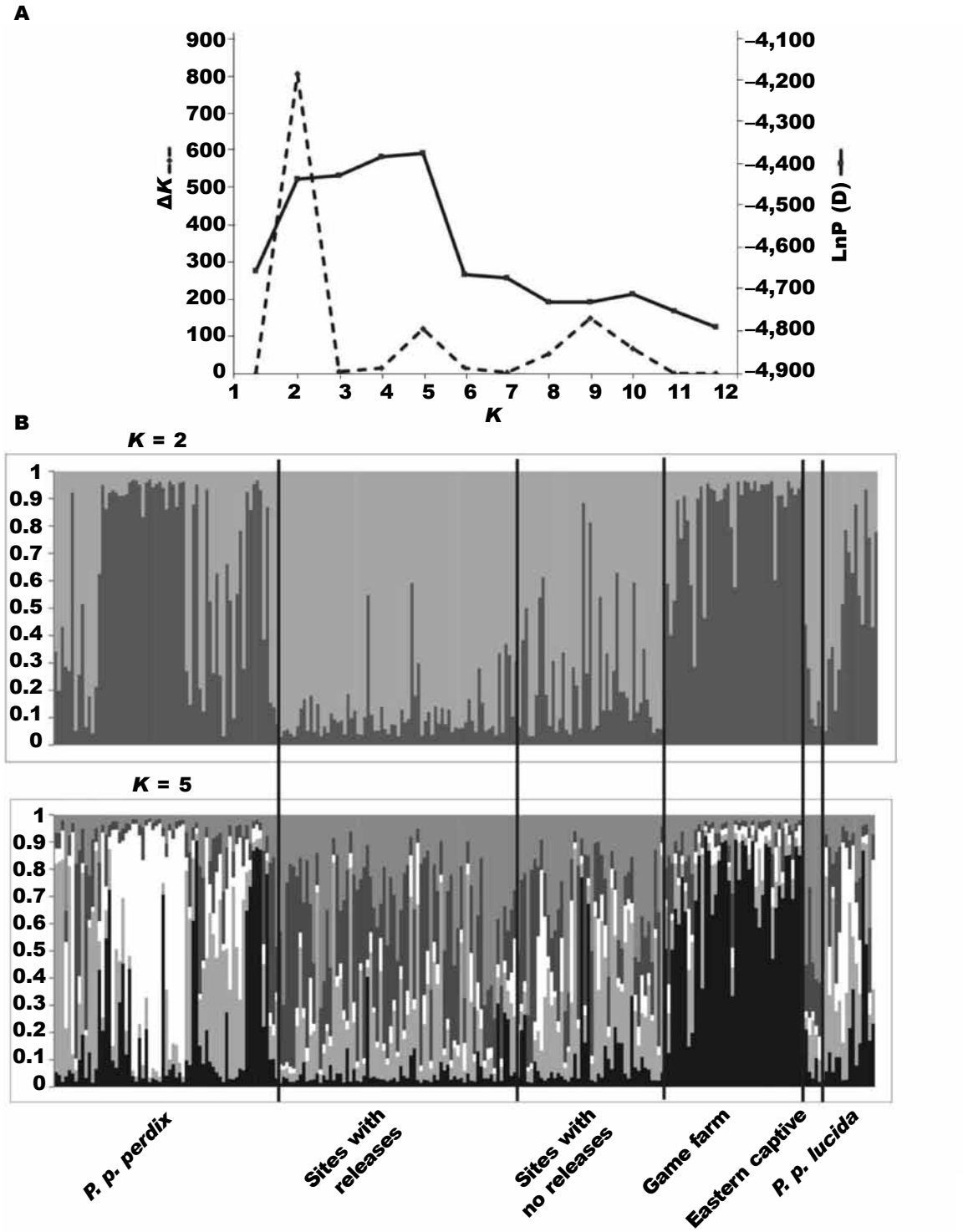

Fig. 2. Population structure of the grey partridge (*Perdix perdix*): A. The estimation suggested that the most likely number of populations would be five, but applying the Evanno's Δ*K*, the most likely number of populations was reduced to two; B. The bar plots on the grey partridge show the proportion of individuals belonging to different clusters.

*Fig. 2. Estructura de la población de perdiz pardilla (*Perdix perdix*): A. La estimación sugirió que el número más probable de poblaciones sería el de cinco, sin embargo al aplicar la ΔK de Evanno el número más probable de poblaciones se redujo a dos; B. los gráficos de barras de perdiz pardilla muestran la proporción de individuos pertenecientes a los diferentes grupos.*

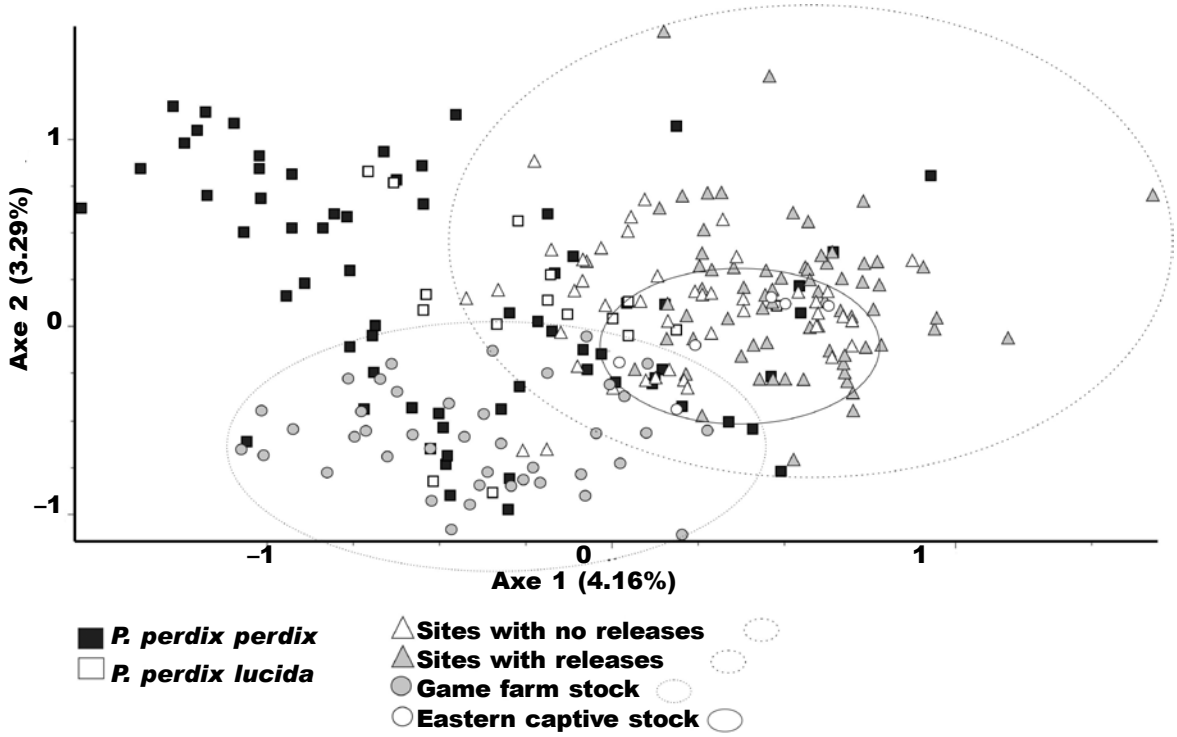

Fig. 3. The results of the factorial correspondence analysis on the grey partridge (*Perdix perdix*). The farm stock used for introductions shows distinctiveness from the wild populations.

*Fig. 3. Resultados del análisis factorial de correspondencia de perdiz pardilla (*Perdix perdix*). La población de granja utilizada para las introducciones muestra diferencias con las poblaciones silvestres.*

among groups) and lowest F_{SC}–values (difference among populations within groups). This, together with the results from structure analysis and factorial correspondence analysis, suggested that the game farm stock belongs to the same cluster with the western subspecies, whereas the native Finnish birds cluster with the eastern subspecies. If the game farm stock used for the releases into the wild is of the wrong origin, this is most likely detrimental to the natural population. It might lead to lowering fitness of the native population by breaking up adaptive gene complexes, especially if the released birds introduce traits that are adaptive in the environment they originate from but not in the environment they are released in. Releases of western subspecies of the grey partridge have been assumed to be one reason for the population crash, resulting from the differences in adaptation to cold environmental conditions in Finland (Siivonen, 1957). Captive–reared grey partridges and capercaillies are also known to clearly differ from their wild counterparts in several physiological and morphological traits (Putaala & Hissa, 1995; Pyörnilä et al., 1997; Liukkonen–Anttila et al., 2000), and this, too, may result in their low survival and contribution to the wild population.

So far, however, no signs of hybridisation between the released and native birds could be detected with microsatellite markers. It is possible, that these specific markers were not sensitive enough to reveal any hybrids. It is also possible that the native and the released partridges do not interbreed, that the released birds do not survive to breeding season (Puigcerver et al., 2007; Putaala et al., 2001), or that the possible hybrids have low fitness and disappear from the wild (Puigcerver et al., 2007).

Released captive–bred red–legged and rock partridges (*Alectoris graeca*) are known to reproduce and hybridise in the wild (Randi, 2008). Introgressive hybridisation between wild local and captive released stocks might be threatening native populations by raising risks of outbreeding depression and loss of local adaptations. Massive translocations and releases of nonindigenous populations have threatened worldwide indigenous game bird populations as in the Italian grey partridge (*P. p. italica*, Liukkonen–Anttila et al., 2002), the common quail (*Coturnix c. coturnix*, Barilani et al., 2005) and the red–legged partridge (Tejedor et al., 2007; Randi, 2008; Barbanera et al., 2009b).

Conclusions

The Finnish native population seems to harbour quite a lot of genetic variation and it clusters together with individuals of the eastern subspecies, *P. p. lucida*. It is

evident that the game farm stock, which has been used for releases, deviates from this wild population. The birds from the eastern captive stock, which is derived from the wild, clustered together with the individuals from the native population and eastern subspecies. Microsatellite markers used in this study did not reveal any hybridisation between captive and wild populations. This finding supports the idea that 1) the game farm stock used for releases was of wrong origin and 2) luckily, no signs of hybridisation have yet been found between captive and native populations.. Whether this results from the lack of sensitivity among the used markers or is due to poor contribution of released grey partridges to the native populations remains to be solved in future studies.

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