GENETIC STUDIES OF *ALECTORIS RUFA* AND *A. GRAECA* IN SPAIN

ESTUDIOS GENÉTICOS EN *ALECTORIS RUFA* Y *A. GRAECA* EN ESPAÑA

Arruga, M.V., M.T. Tejedor, M.R.Villarroel, A. Heriz, E. Ferreira and F.J. Abenia

Laboratory of Cytogenetics and Molecular Genetics. Faculty of Veterinary Sciences. C/ Miguel Servet, 177. E-50013, Zaragoza. Spain.

Additional keywords

Cytogenetics. Population genetics. Partridges.

Palabras clave adicionales

Citogenética. Genética de poblaciones. Perdices.

SUMMARY

The gene pool of the Spanish red-legged partridge (*A. rufa*) has recently been polluted by unregulated hybridization with the non-native rock partridge (*A. graeca*). The genetic characteristics of both species are poorly known although their use in alimentation and hunting is very important in Spain. We have analysed both species (but primarily *A. rufa*) in terms of cytogenetics, morphometrics, protein electrophoresis, DNA fingerprinting and PCR. Red-legged partridges have 2n= 18 macrochromosomes and 30 pairs of microchromosomes.

The number of microchromosomes does not appear to be a reliable indicator of species type. Sixteen morphological measurements were made of each individual and later compared to genetic results. Results of protein analyses show that many enzymes are conserved between species but several are highly variable and informative. DNA fingerprints, using pV47 and *Alu* I, were made of 17 animals. Average band number per individual was 11. Bandsharing was high in both species; unrelated individuals shared, on average, 19 p.cent of their bands. Finally, preliminary analyses by PCR using ADL176 primers (GenBank # GO1598) provided polymorphic fragments near 600kb between *A. rufa* and *A. graeca*.

RESUMEN

El conjunto de genes de la perdiz roja española (*A. rufa*) se ha contaminado recientemente por hibrización clandestina con la perdiz griega foránea (*A. graeca*). Las características genéticas de ambas especies son poco conocidas aunque su uso en alimentación y caza es muy importante en España. Hemos analizado ambas especies, (pero principalmente *A. rufa*) en términos de citogenética, morfometría, electroforesis de proteínas, huella dactilar de ADN y PCR. Las perdices rojas tienen 2n= 18 macrocromosomas y 30 pares de microcromosomas. El número de microcromosomas no parece ser un indicador real del tipo de especie. Se han tomado dieciséis medidas morfológicas de cada individuo y posteriormente se han comparado con los resultados genéticos. Los resultados de los análisis de proteínas muestran que se han conservado muchas enzimas entre especies pero algunas son altamente variables e informativas. Se han obtenido las huellas dactilares de ADN de 17 individuos, usando pV47 y *Alu* I. El promedio de bandas por individuo fue de 11. El número de bandas compartidas fue alto en ambas especies: los individuos no relacionados compartían, en promedio, un 19 p. 100 de sus bandas. Finalmente, los análisis preliminares de PCR usando cebadores

ADL176 (GenBank # GO1598) proporcionan fragmentos polimórficos de unas 600 kb entre *A. rufa* y *A. graeca*.

INTRODUCTION

The red-legged partridge (*Alectoris rufa*) is one of the most important small game species in Spain. This autochthonous bird (Galliformes, Phasianidae) is present in Portugal, Spain, south and central France, northwestern Italy and Corsica. It was introduced to the Balearic Islands in the XIIIth century. The Canary Islands' population seems to be the result of freeing some pairs in Gran Canaria, the only island where acclimatization was possible. It was also introduced in the South of England (XVIIth and XVIIIth centuries), Germany, Hungary, Norway and Sweden.

Recent ecological modifications have greatly influenced Spanish partridge populations. Continuous hunting and adverse environmental conditions (persistent drought, use of pesticides) have also contributed to population decrease.

The gene pool of the Spanish red-legged partridge has also recently been polluted by unregulated hybridization with the non-native rock partridge (*A. graeca*). The genetic characteristics of both species are poorly known; so, we have analysed both species (but primarily

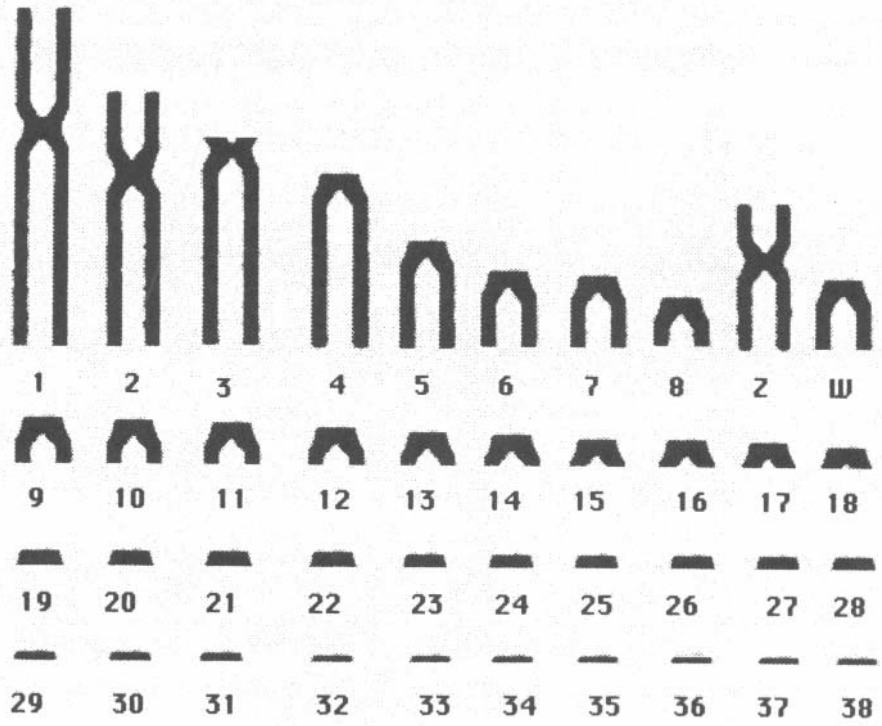

Figure 1. *Idiogram of the red-legged partridge.* (Idiograma de la perdiz roja).

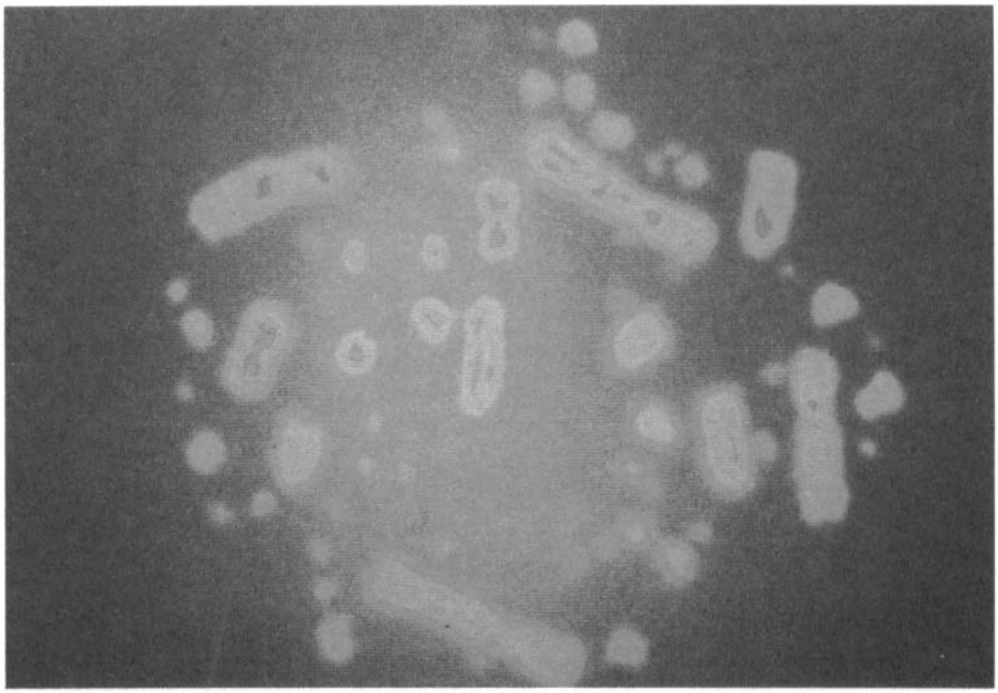

***Figure 2**. Digitized image of a chromosome spread.* (Imagen digitalizada de una extensión cromosómica).

A. rufa) in terms of cytogenetics, morphometrics, enzyme electrophoresis, DNA fingerprinting and PCR.

MATERIALS AND METHODS

These studies were based on an overall population composed by 137 wild birds captured in different Spanish regions and a small sample of 6 rock partridges from one farm.

Cytogenetic studies were performed from white blood cells by usual techniques of macro and micro culture.

Morphometric studies included 8 body measures, analysed by using Statview program for MacIntosh computers.

Individual liver samples were analysed by electrophoretical methods on cellulose acetate gels in order to study the genetic variability of several enzymatic *loci*

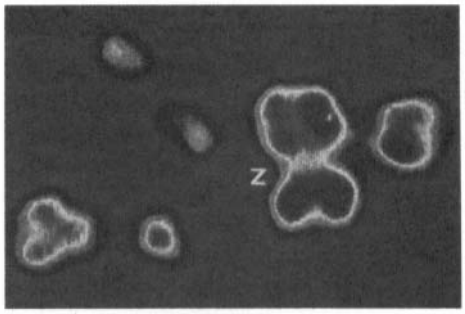

***Figure 3**. Digitized image of the Z chromosome.* (Imagen digitalizada del cromosoma Z).

Table I. *Allele frequencies with standard error (s.e.) for 6 enzymatic* loci. (Frecuencias alélicas con su error estándar (s.e.) para 6 *loci* enzimáticos).

		A. rufa (n=137)	*A. graeca* (n=6)
Locus	Allele	Frequency ± s.e.	Frequency ± s.e.
PGD	PGD*A	0.007 ± 0.005	-
	PGD*B	0.942 ± 0.014	0.833 ± 0.108
	PGD*C	0.051 ± 0.013	0.167 ± 0.108
GPI	GPI*A	0.004 ± 0.004	-
	GPI*B	0.996 ± 0.004	1.000
GOT1	GOT1*A	0.036 ± 0.011	-
	GOT1*B	0.945 ± 0.014	0.583 ± 0.142
	GOT1*C	-	0.167 ± 0.108
	GOT1*D	0.015 ± 0.007	0.250 ± 0.125
	GOT1*E	0.004 ± 0.004	-
IDH1	IDH1*A	0.500 ± 0.030	-
	IDH1*B	0.500 ± 0.030	1.000
ME1	ME1*A	0.073 ± 0.016	-
	ME1*B	0.350 ± 0.029	-
	ME1*C	0.551 ± 0.030	0.167 ± 0.108
	ME1*D	0.026 ± 0.009	0.833 ± 0.108
MPI	MPI*A	0.007 ± 0.005	-
	MPI*B	0.905 ± 0.018	1.000
	MPI*C	0.088 ± 0.018	-

(Meera Khan, 1971; Van Someren *et al.*, 1974; Womack and Moll, 1986).

DNA studies were carried out from blood or individual liver samples.

Fingerprints were obtained using Alu I and pV47 probe, according to Longmire *et al.* (1990). The labelling of the probe was made using biotin (Feinberg and Vogelstein, 1983, modified by Hodgson and Fisk, 1987).

PCR analysis used the following primers: GenBank # L23883 and L23884, GenBank #GO1547, 1561, 1593, 1594, 1598, 1630 and 1687.

RESULTS

I. CYTOGENETIC STUDIES

For the first time, Spanish red-legged partridge caryotype is described. Spanish red-legged partridges have 2n= 18 macrochromosomes and 30 pairs of microchromosomes. The number of microchromosomes does not appear to be a reliable indicator of species type **(figure 1, 2** and **3)**.

II. MORPHOMETRIC STUDIES

The following individual parameters were analysed: wing length, tail length,

Table II. *Results from fingerprinting techniques.* (Resultados de las técnicas de huella dactilar genética).

Parameter	*A. rufa* (n =12)	*A. graeca* (n =3)	Overall (n=15)
$\bar{f}$±s.e.	12.42±1.63	7.67±1.45	11.47±1.41
$\bar{x}$	0.23	0.57	0.20
$\bar{m}$±s.e	2.86±0.20	4.37±0.29	2.29±0.16

$\bar{f}$ = mean number of resolvable bands; $\bar{x}$ = mean probability that a band present in one individual is also present in another randomly chosen individual; $\bar{m}$ = mean number of bands shared by random pairs of individuals; s.e. = standard error.

alula length, weight, bill length, head length, head width and length from bill to tail. Sex and age were also considered.

Significant differences were found for every measure between *A. rufa* and *A. graeca*.

III. ENZYME ELECTROPHORESIS

Results are shown in **table I**. Hardy-Weinberg equilibrium was found in every case.

IV. DNA FINGERPRINTING

Table II shows obtained results. For *A. rufa v.s. A. graeca*, individual x values ranged from 0.00 to 0.14 for 9 *A. rufa* individuals v.s. the 3 considered *A. graeca* individuals, with $\bar{x}$=0.05 and $\bar{m}$±s.d.= 0.67±0.16. But for the 3 remaining *A. rufa* individuals v.s. the 3 studied *A. graeca* individuals, x values were 0.14 - 0.29 with $\bar{x}$=0.18 and $\bar{m}$±s.d.=2.81±0.47. Significant differences ($p<0.01$) were found between the parameters from these two groups. Therefore, the last 3 birds, previously supposed to be purebred *A. rufa*, might be considered as hybrids *A. rufa x A. graeca.*

V. PCR ANALYSES. AMPLIFICATION OF MICROSATELLITE DNA *LOCI* OF SPANISH PARTRIDGES (*A. RUFA* AND *A. GRAECA*) BY CHICKENSPECIFIC PRIMERS

Nine primer pairs were used in the polymerase chain reaction (PCR) to amplify red-legged patridge (*A. rufa*) and rock partridge (*A. graeca*) genomic DNA *loci* in an attempt to distinguish these two species genetically. Two pairs of primers, GenBank # L23883 and L23884, that are known to amplify (TG)n rich sites in turkey (genus *Meleagris*), generated amplification products in both partridge species as did seven other pairs: GenBank # GO1547, 1561, 1593, 1594, 1598, 1630, 1687, kindly provided by the National Animal Genome Research Program of the USDA (Population Tester Kit).

In an effort to analyze polymorphisms in fragment lengths between *A. rufa* and *A. graeca*, that can be useful in assigning species type, we are presently verifying PCR products for over 300 individual partridges. Our preliminary results indicate that a significant proportion of chicken microsatellites markers can be used for genomic mapping and linkage analysis in partridges (genus *Alectoris*).

REFERENCES

Feinberg, A.P. and B. Vogelstein. 1983. *Analytical Biochemistry.* 132: 6-13.

Hodgson, C.P. and R.Z. Fisk. 1987. *Nucleic Acids Research.* 15: 6295.

Table II. *Results from fingerprinting techniques.* (Resultados de las técnicas de huella dactilar genética).

Parameter	*A. rufa* (n =12)	*A. graeca* (n =3)	Overall (n=15)
$\bar{f}$±s.e.	12.42±1.63	7.67±1.45	11.47±1.41
$\bar{x}$	0.23	0.57	0.20
$\bar{m}$±s.e	2.86±0.20	4.37±0.29	2.29±0.16

$\bar{f}$ = mean number of resolvable bands; $\bar{x}$ = mean probability that a band present in one individual is also present in another randomly chosen individual; $\bar{m}$ = mean number of bands shared by random pairs of individuals; s.e. = standard error.

alula length, weight, bill length, head length, head width and length from bill to tail. Sex and age were also considered.

Significant differences were found for every measure between *A. rufa* and *A. graeca*.

III. ENZYME ELECTROPHORESIS

Results are shown in **table I**. Hardy-Weinberg equilibrium was found in every case.

IV. DNA FINGERPRINTING

Table II shows obtained results. For *A. rufa v.s. A. graeca*, individual x values ranged from 0.00 to 0.14 for 9 *A. rufa* individuals v.s. the 3 considered *A. graeca* individuals, with $\bar{x}$=0.05 and $\bar{m}$±s.d.= 0.67±0.16. But for the 3 remaining *A. rufa* individuals v.s. the 3 studied *A. graeca* individuals, x values were 0.14 - 0.29 with $\bar{x}$=0.18 and $\bar{m}$±s.d.=2.81±0.47. Significant differences ($p<0.01$) were found between the parameters from these two groups. Therefore, the last 3 birds, previously supposed to be purebred *A. rufa*, might be considered as hybrids *A. rufa x A. graeca.*

V. PCR ANALYSES. AMPLIFICATION OF MICROSATELLITE DNA *LOCI* OF SPANISH PARTRIDGES (*A. RUFA* AND *A. GRAECA*) BY CHICKENSPECIFIC PRIMERS

Nine primer pairs were used in the polymerase chain reaction (PCR) to amplify red-legged patridge (*A. rufa*) and rock partridge (*A. graeca*) genomic DNA *loci* in an attempt to distinguish these two species genetically. Two pairs of primers, GenBank # L23883 and L23884, that are known to amplify (TG)n rich sites in turkey (genus *Meleagris*), generated amplification products in both partridge species as did seven other pairs: GenBank # GO1547, 1561, 1593, 1594, 1598, 1630, 1687, kindly provided by the National Animal Genome Research Program of the USDA (Population Tester Kit).

In an effort to analyze polymorphisms in fragment lengths between *A. rufa* and *A. graeca*, that can be useful in assigning species type, we are presently verifying PCR products for over 300 individual partridges. Our preliminary results indicate that a significant proportion of chicken microsatellites markers can be used for genomic mapping and linkage analysis in partridges (genus *Alectoris*).

REFERENCES

Feinberg, A.P. and B. Vogelstein. 1983. *Analytical Biochemistry.* 132: 6-13.

Hodgson, C.P. and R.Z. Fisk. 1987. *Nucleic Acids Research.* 15: 6295.