

TANDEM REPEATS IN THE mtDNA CONTROL REGION OF THE SOUTHERN GREY SHRIKE ENDEMIC TO THE CANARY ISLANDS

REPETICIONES EN TÁNDEM EN LA REGIÓN CONTROL DEL ADN MITOCONDRIAL DEL ALCAUDÓN REAL EN LAS ISLAS CANARIAS

M. Ángeles HERNÁNDEZ¹ *, Francisco CAMPOS²
and David P. PADILLA³

SUMMARY.—The endemic subspecies of the southern grey shrike *Lanius meridionalis koenigi* in the Canary Islands is restricted to four islands (Tenerife, Gran Canaria, Fuerteventura and Lanzarote) and two islets (Alegranza and La Graciosa). A total of 174 shrikes was captured from these areas. In the Control Region of the mtDNA, 56.3% of the birds presented two tandem repeats, 31.6% three, 10.4% 2+3 repeats and finally 1.7% 2+3+4 tandem repeats. The frequency of tandem repeats was significantly different among the shrike populations analysed. The greatest genetic diversity, in relation to the tandem repeats, appeared on Fuerteventura. The proportion of tandem repeats in *L. m. koenigi* from the Canary Islands was clearly different in relation to the *L. m. meridionalis* from the Iberian Peninsula.

RESUMEN.—Se han analizado las repeticiones en tándem de la región control del ADN mitocondrial en 174 ejemplares de la subespecie endémica del alcaudón real *Lanius meridionalis koenigi* de cuatro islas (Tenerife, Gran Canaria, Fuerteventura y Lanzarote) y dos islotes (Alegranza y La Graciosa) de las islas Canarias. El 56,3% de las aves tenían dos repeticiones en tándem, 31,6% con tres repeticiones, 10,4% con 2+3 repeticiones y 1,7% con 2+3+4 repeticiones. Los porcentajes difirieron significativamente entre islas. La mayor diversidad apareció en Fuerteventura (aves con 2, 3, 2+3 y 2+3+4 repeticiones) y la menor en los islotes (sólo dos repeticiones). Los porcentajes de *L. m. koenigi* difirieron claramente también de *L. m. meridionalis* de la península Ibérica.

¹ Departamento de Zoología y Ecología, Facultad de Ciencias, Universidad de Navarra.
E-31080 Pamplona, Spain.

² Universidad Europea Miguel de Cervantes. C/ Padre Julio Chevalier 2,
E-47012 Valladolid, Spain.

³ Island Ecology and Evolution Research Group (IPNA-CSIC).
C/ Astrofísico Francisco Sánchez 3, E-38206 La Laguna, Tenerife, Canary Islands, Spain.

* Corresponding author: mahermin@unav.es

Oceanic islands are ideal places to study evolutionary processes due to the lower number of species in comparison with mainland environments (Carlquist, 1974). The Canary Islands are a volcanic archipelago located in the North-eastern Atlantic Ocean, nearly 100 km west of mainland Africa. It comprises seven main islands and several islets that are ideal places to study some genetic diversification aspects within islands.

Tandem repeats in the mitochondrial DNA control region were found a long time ago in several species (Solignac *et al.*, 1986; Moritz *et al.*, 1987; Rand, 1993; Townsend and Rand, 2004). Some species present heteroplasmy, which means that the same individual can have diverse sequence lengths due to the different number of tandem repeats. Heteroplasmy is caused by mutation in the mitochondria during development and growth of the individual (Casane *et al.*, 1997; Nesbo *et al.*, 1998), and this character varies among species and populations (Berg *et al.*, 1995).

Some shrike species of genus *Lanius* present tandem repeats (Mundy *et al.*, 1996; Hernández *et al.*, 2004), which permit the study of phylogenetic relationships (Mundy and Helbig, 2004), species differentiation (Hernández *et al.*, 2004), or population variations, as in the case of the southern grey shrike *Lanius meridionalis* of the Iberian Peninsula (Gutiérrez-Corchero *et al.*, 2006).

Recently, Klassert *et al.* (2008) and González *et al.* (2008) demonstrated how the endemic southern grey shrike subspecies inhabiting the Canary Islands (*L. m. koenigi*), was phylogenetically more related to the subspecies of Northern Africa *L. m. algeriensis* than to the nominal subspecies *L. m. meridionalis* distributed in the Iberian Peninsula and Southern France. The southern grey shrike in the archipelago is restricted to four islands (Tenerife, Gran Canaria, Fuerteventura and Lanzarote) and some small islets such as La Graciosa and Alegranza, hereafter referred to as islets (Martín and Lorenzo, 2001).

This study assesses and compares the number of tandem repeats in the mitochondrial DNA control region, and degree of heteroplasmy, among the different southern grey shrike populations in the Canary Islands.

In the Canary Islands the southern grey shrike inhabits open shrub environments that extend from coastal xeric areas to high mountains (Martín and Lorenzo, 2001). A total of 174 shrikes were captured in the four islands and islets where they are present using a modified potter trap (table 1) in 2001 and 2007. We obtained a small blood sample (50-100 µL) of each bird by puncturing the brachial vein and storing on FTA Classic Cards®. DNA was extracted according to Gutiérrez-Corchero *et al.* (2002).

The mitochondrial DNA fragment was amplified using primers DLL2 (control region: 5'-ATGCACTTTTACCCCATTCATG-TGG-3') and FTPH2 (Phe tRNA: 5'-CC-ATCTTGACATCTTCAGTGCCATGC-3'), designed by Mundy *et al.* (1996). The PCR amplification was performed on a GeneAmp PCR system 2400 (Applied-Biosystems). The volume of the sample was 40 µl, including 0.5 units of *Taq* polymerase (Bioline), 10 x PCR buffer, dNTPs 10 mM, 1.5 mM MgCl₂ and 20 µM of each primer. The parameters of the thermocycler were 1 x 94 °C, 3 min; 40 x 94 °C, 30 s, 60 °C, 60 s, 72 °C, 90 s; 1 x 72 °C, 10 min. The PCR product was run on a 1.5% agarose gel and stained with ethidium bromide. The DNA marker was Φ -phage DNA digested with Hae III, which has 11 fragments from 72 to 1353 pb (IX174 marker HaeIII, Sigma). Each gel was photographed with a camera linked to a computer and an image analyzer (Molecular Analysis software, BIO-RAD).

First we calculated the king and frequency of tandem repeats from individuals from the different islands. Then we calculated the dissimilarity index between pairs of populations, similar to an *Fst* statistic, which was transformed to pairwise *Fst* that can be use to es-

TABLE 1

Characteristics of the Canary Islands and islets inhabited by the southern grey shrike and percentage of shrikes with two (T2), three (T3), 2 + 3 (T2 + 3), and 2 + 3 + 4 (T2 + 3 + 4) tandem repeats. N: sample size. H': Shannon diversity index.

[*Características de las islas e islotes del archipiélago canario ocupadas por el alcaudón real, y porcentaje de alcaudones con dos (T2), tres (T3), 2 + 3 (T2 + 3) y 2 + 3 + 4 (T2 + 3 + 4) repeticiones en tándem. N: tamaño muestral. H': índice de diversidad de Shannon.*]

	Tenerife	Gran Canaria	Fuerteventura	Lanzarote	Islets
Coordinates [Coordenadas]	28° 05' N 16° 35' W	27° 50' N 15° 30' W	28° 30' N 13° 53' W	29° 00' N 13° 30' W	29° 14' N 13° 30' W
Surface area (km²) [Superficie (km ²)]	2,034	1,560	1,659	846	39
T2	6.67	75.00	45.90	75.00	100
T3	66.67	21.43	39.34	15.63	0
T2+3	26.66	3.57	9.84	9.37	0
T2+3+4	0	0	4.92	0	0
N	30	28	61	32	23
H'	0.80	0.66	1.10	0.72	0

establish genetic distances between populations (Reynolds *et al.*, 1983; Slatkin, 1995). This index was calculated using the software Arlequin 3.1 (Excoffier *et al.*, 2005). Different types of tandem repeats in the Canary Islands and the Iberian Peninsula were also compared by χ^2 test. Because of the small sample size, all heteroplasmic individuals were grouped into a single class.

Finally, diversity of tandem repeats among islands was assessed using the Shannon diversity index (Magurran, 1988): $H' = -\sum p_i \ln p_i$, where p_i is the proportional abundance of the i th tandem repeats.

Out of 174 shrikes captured from the different islands, the 56.3% of the birds appeared with two tandem repeats, 31.6% with three repeats, 10.4% with 2 + 3 repeats and finally 1.7% with 2 + 3 + 4 repeats (table 1).

The frequency of tandem repeats differed significantly among the shrike populations analysed (table 2). On Fuerteventura, Lanzarote and the islets, the proportion of birds with two tandem repeats showed a clear South-North gradient, significant differences appearing between Fuerteventura and the other two areas. The shrikes on Gran Canaria showed a similar proportion of tandem repeats than those from Lanzarote and Fuerteventura. However, on Tenerife the repeats clearly differed between all the islands, the lowest and highest percentages were with two and three repetitions, respectively. All the birds captured on the islets presented two tandem repeats, which was significantly different from all the islands except Lanzarote (table 2).

In general, the Canary Island shrikes showed a large proportion of heteroplasmic

TABLE 2

Pairwise comparisons of *Fst* values of tandem repeats are shown below the diagonal, and *P* probability values are above the diagonal. NS: not significant.

[Valores *Fst* de las comparaciones de repeticiones en tándem dos a dos debajo de la diagonal. Valores de la probabilidad *P* encima de la diagonal. NS: no significativo.]

	Tenerife	Gran Canaria	Fuerteventura	Lanzarote	Islets
Tenerife		0.0000 ± 0.0000 <i>P</i> < 0.01	0.0000 ± 0.0000 <i>P</i> < 0.01	0.0000 ± 0.0000 <i>P</i> < 0.01	0.0000 ± 0.0000 <i>P</i> < 0.01
Gran Canaria	0.43403		0.0270 ± 0.0139 NS	0.8558 ± 0.0246 NS	0.0090 ± 0.0091 <i>P</i> < 0.01
Fuerteventura	0.16354	0.07704		0.0000 ± 0.0000 <i>P</i> < 0.01	0.0000 ± 0.0000 <i>P</i> < 0.01
Lanzarote	0.44409	-0.02595	0.09441		0.0180 ± 0.0121 NS
Islets	0.70736	0.17103	0.31802	0.13694	

individuals, with great differences between islands (range: 0-30.2 on islets and Tenerife, respectively). Fuerteventura was the only island where 2+3+4 heteroplasmic individuals appeared (4.9% of the total birds captured on this island).

Our data showed how the greatest genetic diversity, in relation to the tandem repeats, appeared on Fuerteventura with birds with two, three, 2+3 and 2+3+4 repeats. In Tenerife the percentages of shrikes with three and 2+3 repetitions (66.7% and 26.7%, respectively) were the greatest of the Canary Islands, while in Gran Canaria only 21.4% and 3.6% of the total individuals appeared with three and 2+3 repeats, respectively.

On the other hand, the proportion of shrikes with two and three repeats, and heteroplasmic individuals, was clearly different in relation to the shrikes from the Iberian Peninsula *L. m. meridionalis* ($\chi^2_2 = 23.144$, *P* < 0.01). This latter subspecies appeared with 50.4%,

47.5% and 2.1%, for two and three tandem repeats, and heteroplasmic individuals, respectively (*n* = 242, Gutiérrez-Corchero *et al.*, 2006). These data are in accordance with the studies of Klassert *et al.* (2008) and González *et al.* (2008), which showed high phylogenetic differences between both subspecies. However, Hernández *et al.* (2004) did not find differences in the proportion of tandem repeats between the shrikes from the Islands and the Iberian Peninsula, probably because they only took into account birds from one island (Fuerteventura). Additionally, new studies of tandem repeats including the subspecies *L. m. algeriensis* will be interesting to confirm the closer relationship of *L. m. koenigi* with this subspecies rather than with *L. m. meridionalis*, since the phylogeny results focused on cytochrome *b* (Klassert *et al.*, 2008) could not be related with the results found in the control region gene.

ACKNOWLEDGEMENTS.—Our thanks to F. Gutiérrez-Corcheró for providing blood samples from Fuerteventura, A. Amezcua for analyses performed in the laboratory, and Dr. M. Hernández (University of La Laguna) for his help and comments on the manuscript. This contribution was part of a project financed by the Canary Government (PIO42004/037), partially supported by the FEDER funds from the European Union. The Canary Government kindly provided sampling permits.

BIBLIOGRAPHY

- BERG, T., MOUM, T. and JOHANSEN, S. 1995. Variable numbers of simple tandem repeats make birds of the Ciconiiformes heteroplasmic in their mitochondrial genomes *Current Genetics*, 27: 257-262.
- CASANE, D., DENNEBOUY, N., DE ROCHAMBEAU, H., MOUNOLOU, J. C. and MONNEROT, M. 1997. Non-neutral evolution of tandem repeats in the mitochondrial DNA control regions of Lagomorphs. *Molecular Biology and Evolution*, 14: 779-789.
- CARLQUIST, S. 1974. *Island Biology*. Columbia University Press. New York.
- GONZÁLEZ, J., WINK, M., GARCÍA DEL REY, E. and DELGADO-CASTRO, G. 2008. Evidence from DNA nucleotide sequences and ISSR profiles indicates paraphyly in subspecies of the Southern Grey Shrike (*Lanius meridionalis*). *Journal of Ornithology*, 149: 495-506.
- GUTIÉRREZ-CORCHERO, F., ARRUGA, M. V., SANZ, L., GARCÍA, C., HERNÁNDEZ, M. A. and CAMPOS, F. 2002. Using FTA cards to store avian blood samples for genetic studies. Their application in sex determination. *Molecular Ecology Notes*, 2: 75-77.
- GUTIÉRREZ-CORCHERO, F., HERNÁNDEZ, M. A. and CAMPOS, F. 2006. Differentiation among Spanish southern grey shrike *Lanius m. meridionalis* population using tandem repeats in mtDNA control region. *Ardeola*, 53: 107-113.
- EXCOFFIER, L., LAVAL G. and SCHNEIDER, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1: 47-50.
- HERNÁNDEZ, M., CAMPOS, F., GUTIÉRREZ-CORCHERO, F. and AMEZCUA, A. 2004. Identification of *Lanius* species and subspecies using tandem repeats in the mitochondrial DNA control region. *Ibis*, 146: 227-230.
- KLASSERT, T. E., HERNÁNDEZ, M. A., CAMPOS, F., INFANTE, O., ALMEIDA, T., SUÁREZ, N. M., PESTANO, J. and HERNÁNDEZ, M. 2008. Mitochondrial DNA points to *Lanius meridionalis* as a polyphyletic species. *Molecular Phylogenetics and Evolution*, 47: 1227-1231.
- MAGURRAN, A. E. 1988. *Ecological diversity and its measurements*. Princeton University Press. Princeton.
- MARTÍN, A. and LORENZO, J. A. 2001. *Aves del archipiélago canario*. Francisco Lemus Editor. La Laguna.
- MORITZ, C., DOWLING, T. E. and BROWN, W. M. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics*, 18: 269-292.
- MUNDY, N. I., WINCHELL, C. S. and WOODRUFF, D. S. 1996. Tandem repeats and heteroplasmy in the mitochondrial DNA control region of the Loggerhead Shrike (*Lanius ludovicianus*). *Journal of Heredity*, 87: 21-26.
- MUNDY, N. I. and HELBIG, A. J. 2004. Origin and evolution of tandem repeats in the mitochondrial DNA control region of shrikes (*Lanius* spp.). *Journal of Molecular Evolution*, 59: 250-257.
- NESBO, C. L., ARAB, M. O. and JAKOBSEN, K. S. 1998. Heteroplasmy, length and sequence variation in the mtDNA Control Regions of three Percid Fish species (*Perca fluviatilis*, *Acerina cernua*, *Stizostedion lucioperca*). *Genetics*, 148: 1907-1919.
- RAND, D. M. 1993. Endoterms, ectoterms and mitochondrial genome size variation. *Journal of Molecular Evolution*, 37: 281-295.
- REYNOLDS, J., WEIR, B. S. and COCKERHAM, C. C. 1983. Estimation for the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, 105: 767-779.
- SOLIGNAC, M., MONNEROT, M. and MOUNOLOU, J. C. 1986. Mitochondrial DNA evolution in the *melanogaster* species subgroup of *Drosophila*. *Journal of Molecular Evolution*, 23: 31-40.
- SLATKIN, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, 139: 457-462.
- TOWNSEND, J. P. and RAND, D. M. 2004. Mitochondrial genome size variation in New World and Old World populations of *Drosophila melanogaster*. *Heredity*, 93: 98-103.

[Recibido: 09-07-2009]

[Aceptado: 15-06-2010]

