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 subspecie 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.

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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 heteroplasmmy, which means that the same individual can have diverse sequence lengths due to the different number of tandem repeats. Heteroplasmmy 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 heteroplasmmy, 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’-ATGCACCTTTACCCATTCATG-GTGG-3’) and FTPH2 (Phe tRNA: 5’-CCATCCTGACATCTTCACTGAGCCATGC-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 ºC, 3 min; 40 x 94 ºC, 30 s, 60 ºC, 60 s, 72 ºC, 90 s; 1 x 72 ºC, 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-
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.

Table 1

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

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 χ² 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): \( H' = -\Sigma pi \ln pi \), where \( pi \) 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...
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.]

<table>
<thead>
<tr>
<th></th>
<th>Tenerife</th>
<th>Gran Canaria</th>
<th>Fuerteventura</th>
<th>Lanzarote</th>
<th>Islets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenerife</td>
<td>0.0000 ± 0.0000</td>
<td>0.0000 ± 0.0000</td>
<td>0.0000 ± 0.0000</td>
<td>0.0000 ± 0.0000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Gran Canaria</td>
<td>0.43403</td>
<td>0.0270 ± 0.0139 NS</td>
<td>0.8558 ± 0.0246 NS</td>
<td>0.0090 ± 0.0091 P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Fuerteventura</td>
<td>0.16354</td>
<td>0.07704</td>
<td>0.0000 ± 0.0000 P &lt; 0.01</td>
<td>0.0000 ± 0.0000 P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Lanzarote</td>
<td>0.44409</td>
<td>-0.02595</td>
<td>0.09441</td>
<td>0.0180 ± 0.0121 NS</td>
<td></td>
</tr>
<tr>
<td>Islets</td>
<td>0.70736</td>
<td>0.17103</td>
<td>0.31802</td>
<td>0.13694</td>
<td></td>
</tr>
</tbody>
</table>

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.
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BIBLIOGRAPHY


