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Is C-*mos* phylogenetically informative at lower taxonomic levels in reptiles? An assessment of variation within *Lacerta* (*Teira*) *dugesii* MILNE-EDWARDS, 1829

(Squamata: Sauria: Lacertidae)

Ist C-*mos* in der Reptilientaxonomie auf niedrigem Niveau phylogenetisch informativ? Eine Beurteilung der Variabilität innerhalb *Lacerta* (*Teira*) *dugesii* MILNE-EDWARDS, 1829
(Squamata: Sauria: Lacertidae)

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KURZFASSUNG

Die Schlußfolgerungen der meisten phylogeographischen Untersuchungen beruhen auf mitochondrialen DNS-Sequenz-Daten. Wir untersuchen hier den Wert des Kerngens C-*mos*, das typischerweise bei phylogenetischen Analysen verwendet wird und berichten über die intraspezifische Variabilität dieses Gens bei *Lacerta* (*Teira*) *dugesii* vom Madeira-Archipel. Das Vorhandensein eines fixierten anzestralen Zustandes bei den Tieren auf den Porto Santo Inseln deutet darauf hin, daß diese Inseln wahrscheinlich als erste besiedelt worden waren. Die vom Menschen auf den Azoren eingeführten Populationen stammen von Madeira. Beim Vergleich des relativen Anteils von Substitutionen in C-*mos* und mitochondrialer DNS finden sich zwischen taxonomischen Gruppen beträchtliche Unterschiede.

ABSTRACT

Most phylogeographic studies rely solely on mitochondrial DNA sequence data for making inferences. We assessed the value of a region of the nuclear gene, C-*mos*, that has typically been used in phylogenetic analyses. We report intra-specific variation of this gene within *Lacerta* (*Teira*) *dugesii* from the Madeira archipelago. The presence of a fixed ancestral state on Porto Santo islands indicates this was probably the first island colonized. Anthropogenically introduced populations on the Azores originated from Madeira. The relative rate of substitutions in C-*mos* compared to mitochondrial DNA varies considerably between taxonomic groups.

KEY WORDS

Reptilia: Squamata: Sauria: Lacertidae; *Lacerta dugesii*, *Teira*, Madeira, Selvagens, C-*mos*, Azores, introduction, phylogeography

INTRODUCTION

C-*mos* is a proto-oncogene encoding a serine/threonine kinase that is a regulator of meiotic maturation (YEW et al. 1993). Single copy and without introns, a fragment of this gene is now the most widely used of any nuclear gene for assessing relationships within reptiles. It has been used across squamates (SAINT et al. 1998; HARRIS et al. 1999, 2001), across snakes (SLOWINSKI & LAWSON 2002), within families (DONNELLAN et al. 1999; JOGER et al. 2001; PELEGRINO et al. 2001), and within genera (BREHM et al. 2001; CARRANZA et al. 2002; JESUS et al. 2002). Since none of these studies reported sequencing heterozygotes it might be assumed that intraspecific variation was very low, although variation has been reported within *Lacerta schreiberi* BEDRIAGA, 1878 (DOMINGUES et al. 2001), and HARRIS et al. (2004) have also reported intraspecific variation within *Tarentola* geckos. Using nuclear genes in phylogenetic studies is not as straightforward as using mtDNA sequence data. There are theoretical arguments why nuclear gene trees are less likely to be congruent with the species tree than mitochondrial DNA (mtDNA) gene trees, because of lineage sorting (MOORE 1995). The branching pattern of a gene tree will correspond to the species tree if coalescence occurs be-

tween bifurcations, or speciation events. The probability of coalescence increases as effective population size decreases, thus mtDNA with an effective population size of one quarter of a nuclear autosomal gene has an increased likelihood of accurately tracking the species tree. This is likely to be especially important for island populations, which are characterised by having extremely small founding populations. It is therefore important to assess the expected coalescent time of variation within C-*mos*, and the degree of variation within species if it is used in phylogenetic studies. We examined these questions by sequencing C-*mos* from island populations of the lizard *Lacerta* (*Teira*) *dugesii* MILNE-EDWARDS, 1829 (following HARRIS & CARRETERO 2003). *Lacerta dugesii* is an endemic lizard from Madeira, the Desertas Islands, Porto Santo and the Selvagens and was recently introduced to the Azores. Because of the well-known geological history of these islands (GELDMACHER et al. 2000) and the high population densities of the lizards it is a model organism for studying genetic variation across a fragmented habitat (BREHM et al. 2003a, 2003b). At the same time C-*mos* variation might give additional insights into colonization events that cannot be completely determined using the mtDNA sequence data.

MATERIALS AND METHODS

Twenty-nine specimens of *L. dugesii* were collected across the known range – nine from Madeira, eight from Desertas, five from Porto Santo and five from the Selvagens. These represented the four distinct monophyletic lineages previously identified using mitochondrial DNA sequences (BREHM et al. 2003a). In addition two individuals were included from the introduced populations on the Azores islands. Total genomic DNA was extracted from small pieces of tail using standard methods. Polymerase Chain Reaction primers used in both amplification and sequencing G73 and G74 from SAINT et al. (1998). Amplification conditions were the same as described by SAINT et al. (1998). Amplified fragments were sequenced from both strands on a 373 Applied Biosystem DNA Sequencing Apparatus. We sequenced both strands and repeated any sequence that was not completely clean so that we could accurately identify heterozygotes, which showed single positions with two clear peaks in the electropherograms. Sequences were aligned by eye to two published sequences of *Podarcis* (HARRIS et al. 2001) which were included as a closely related outgroup. Since this is a coding region and there are no indels, alignment is facile. All analysed sequences were 375 base pairs long. GenBank accession numbers are AF211198 - AF211202. All sequences were translated to check for changes in the amino acid code.

RESULTS AND DISCUSSION

Three positions within the C-*mos* fragment analyzed vary within the *L. dugesii* included in this study. Frequencies of the identified mutations are shown in table 1. This can be compared to the five fixed differences between *L. dugesii* and *Podarcis*. All three involve transition changes and occurred in the first, second and third coding positions respectively. Despite the fact that each island group has monophyletic lineages for mtDNA, in general the C-*mos* variation is not fixed within each island. Eight of the twenty-nine individuals sequenced were heterozygous for one or other of the first two variable positions. The first two of the three variable positions caused amino acid replacements in some individuals (GTA-ATA and ATT-ACT respectively).

Intra specific variation within C-*mos* has previously been demonstrated within *L. schreiberi*, and in this study in *L. dugesii*. While no variation was reported between subspecies of *Gallotia* (CARRANZA 2002) this could be due to sample size. This has important implications for the use of C-*mos*

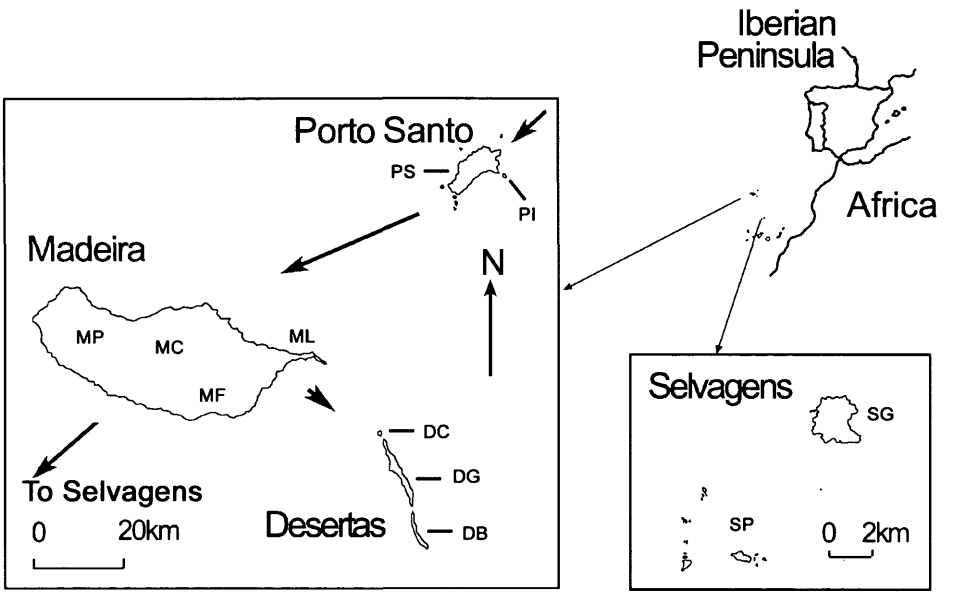

Fig. 1. Map of the islands from which *Lacerta dugesii* MILNE-EDWARDS, 1829 was sampled. Arrows indicate the presumed direction of colonization.

Abb. 1: Karte der Inseln, von denen die untersuchten *Lacerta dugesii* MILNE-EDWARDS, 1829 stammen. Pfeile bezeichnen die vermuteten Besiedelungsrichtungen.

for phylogenetic studies between closely related organisms. Similarly heterozygotes can be expected when direct sequencing C-*mos* and this should be reported and taken into account in any analyses.

For *L. dugesii* variation with mtDNA within island groups is at most 1% (BREHM et al. 2003a). A rough molecular clock estimate of 2% divergence per million years would thus imply that this section of mtDNA would require 0.5 MY years to coalesce. The major mtDNA lineages on Madeira, Desertas and Porto Santo differ from each other by 3-4%, while the Selvagens differ from the Madeira lineage by about half this, implying lizards have been separated for time periods longer than that required to reach coalescence; around 1.5-2 MY for most island groups, and 0.75-1 MY separating Madeiran lizards from those on the Selvagens. Thus, as would be predicted, the mtDNA lineages are monophyletic on each island group. The C-*mos* sequences have generally not coalesced, despite the presumed small founding populations, and thus studies using C-*mos* to determine patterns of relationships between forms that are separated by similar levels of mtDNA variation should examine intraspecific variation. However the C-*mos* data does give additional information regarding colonization patterns of these islands. In the estimate of relationships based on mtDNA sequences, the lineage leading to haplotypes on Porto Santo Island is sister taxon to all other *L. dugesii* lineages. This does not, however, mean that it is most parsimonious to predict that Porto Santo was the first island colonized (see EMERSON 2002). Using the C-*mos* data the Porto Santo population for mutation 1 is fixed for the ancestral state. If Porto Santo was colonized first, this mutation to the derived state could have occurred in the second colonizing population, either in Madeira or the Desertas. This therefore is evidence that Porto Santo was the first island colonized, and matches with its greater geological age than the other islands. Since the individuals from the Azores have both states in the first mutation, this would rule out a recent introduction from either Porto Santo or the

Table 1: Proportions of each nucleotide for the three variable positions in the C-*mos* fragment analysed. Ancestral states were predicted based on the sequences from *Podarcis*. Numbers in parentheses indicate the number of individuals that were heterozygous. Positions 1 and 2 cause amino acid changes.

Tab. 1: Anteile der Nukleotide an den drei variablen Positionen im untersuchten C-*mos*-Fragment. Aussagen über den ursprünglichen Zustand (Ancestral State) erfolgten auf Grundlage von *Podarcis*-Sequenzen. Anzahl heterozygoter Individuen in Klammern. Die Positionen 1 und 2 bewirken Aminosäureaustausch.

	Position 1 (A/G)	Position 2 (T/C)	Position 3 (C/T)
Ancestral State	A	T	C
Madeira	6/12 (4)	12/6	4/14
Porto Santo	10/0	7/3 (1)	6/4
Desertas	2/14	9/7 (1)	2/14
Selvagens	0/10	1/9(1)	0/10
Azores	2/2	1/3(1)	0/4

Selvagens (which is fixed for the derived state). As indicated by the mtDNA evidence, it thus seems likely that the source population for this introduction was Madeira.

In passerine birds, C-*mos* nucleotide substitutions accumulate at a rate similar to that of mitochondrial transversion substitutions (LOVETTE & BERMINGHAM 2000). Within *L. dugesii* the 0-3 variable positions within C-*mos* is the same as the number of transversions in the 12S rDNA sequences (also 0-3) but much less than the number of transversions in the combined 12S rDNA and cytochrome *b* sequence data. Previous phylogenetic analyses have indicated the possibility of a non-linear relationship of genetic divergence between C-*mos* and mtDNA across divergent taxa. *Tarentola* from the Cape Verde islands show lower levels of variation within C-*mos* between clades separated by high mtDNA divergence when compared to *Mabuya* (JESUS et al. 2002). Comparisons of cytochrome *b* to C-*mos* divergence also showed that the relative rates of divergence differed between snakes, teiids and lacertid lizards (HARRIS 2003).

In conclusion, C-*mos* sequence data can be an extremely valuable marker for phylogenetic studies, and also can clearly be used as a nuclear marker at the intraspecific levels. However, just as codon bias should be accounted for in phylogenetic studies at deeper taxonomic levels (HARRIS 2003), so intraspecific variation needs to be assessed when C-*mos* sequences are used at lower phylogenetic levels.

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REFERENCES

BREHM, A. & JESUS, J. & PINHEIRO, M. & HARRIS, D. J. (2001): Relationships of scincid lizards *Mabuya* (Reptilia: Scincidae) from the Cape Verde Islands based on mitochondrial and nuclear DNA sequences.- Molecular Phylogenetics and Evolution, Chicago; 19: 311-316.

BREHM, A. & JESUS, J. & SPÍNOLA, H. & ALVES, C. & VICENTE, L. & HARRIS, D. J. (2003a): Phylogeography of the Madeiran endemic lizard *Lacerta dugesii* inferred from mtDNA sequences.- Molecular Phylogenetics and Evolution, Chicago; 26: 222-230.

BREHM, A. & HARRIS, D. J. & ALVES, C. & JESUS, J. & THOMARAT, F. & VICENTE, L. (2003b): Structure and evolution of the mitochondrial DNA complete control region in the lizard *Lacerta dugesii* (Lacertidae, Sauria).- Journal of Molecular Evolution, New York; 56: 46-53.

CARRANZA, S. (2002): Los métodos moleculares en el estudio de las sistemática y filogenia de los anfibios y reptiles ibéricos; pp. 547-577. In: Atlas y libro rojo de los anfibios y reptiles de España. Madrid (Ministerio de medio ambiente).

CARRANZA, S. & ARNOLD, E. N. & MATEO, J. A. & GENIEZ, P. (2002): Relationships and evolution of the North African geckos *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and

nuclear DNA sequences.- Molecular Phylogenetics and Evolution, Chicago; 23: 244-256.

DOMINGUES, V. & GODINHO, R. & FERRAND, N. & CRESPO, E. G. (2001): SSCP of a partial fragment of the C-*mos* gene offers a useful nuclear marker in the phylogeographic study of the endemic Iberian Schreiber's green lizard *Lacerta schreiberi*. pp. 46. In: PÉREZ-MELLADO, V. (Ed.): Abstracts of the fourth international symposium on the lacertids of the Mediterranean Basin. Menorca 2001.

DONNELLAN, S. C. & HUTCHINSON, M. N. & SAINT, K. M. (1999): Molecular evidence for the phylogeny of Australian gekkonoid lizards.- Biological Journal of the Linnean Society, London; 67: 97-118.

EMERSON, B. C. (2002): Evolution on oceanic islands: molecular phylogenetic approaches to understanding patterns and process.- Molecular Ecology, Edinburgh; 11: 951-966.

GELDMACHER, J. & VAN DER BOGAARD, P. & HOERNLE, K. & SCHMINCKE, H.-U. (2000): ^{40}Ar / ^{39}Ar age dating of the Madeira Archipelago and hotspot track (eastern North Atlantic).- Geochemistry, Geophysics, Geosystems [electronic Journal], Washington D.C.; 1:1999GC000018.

HARRIS, D. J. & SINCLAIR, E. O. & MERCADER, N. L. & MARSHALL, J. C. & CRANDALL, K. A. (1999): Squamate relationships based on C-*mos* nuclear DNA sequences.- Herpetological Journal, London; 9: 147-151.

HARRIS, D. J. & MARSHALL, J. C. & CRANDALL, K. A. (2001): Squamate relationships based on C-*mos* nuclear DNA sequences: increased taxon sampling improves bootstrap support.- Amphibia-Reptilia, Leiden; 22: 235-242.

HARRIS, D. J. (2003): Codon bias variation in C-*mos* between squamate families might distort phylogenetic inferences.- Molecular Phylogenetics and Evolution, Chicago; 27: 540-544.

HARRIS, D. J. & CARRETERO, M. A. (2003): Comments on the taxonomic value of (sub)genera within the family Lacertidae (Reptilia).- Amphibia-Reptilia, Leiden; 24: 119-122.

HARRIS, D. J. & BATISTA, V. & CARRETERO, M. A. & FERRAND, N. (2004): Genetic variation in *Tarentola mauritanica* (Reptilia: Gekkonidae) across the Strait of Gibraltar derived from mitochondrial and nuclear DNA sequences.- Amphibia-Reptilia, Leiden; 25 (4): 451-459.

JESUS, J. & BREHM, A. & HARRIS, D. J. (2002): Relationships of *Tarentola* (Reptilia: Gekkonidae) from the Cape Verde Islands estimated from DNA sequence data.- Amphibia-Reptilia, Leiden; 23: 47-54.

JOGER, U. & NAGY, Z. & VENCES, M. & WINK, M. (2001): Biogeography of colubrid snakes of Socotra and Madagascar: multiple colonization events demonstrated by analyses of nuclear and mitochondrial DNA.- Biota, Race; 2 (Supplement): 32.

LOVETTE, I. J. & BERMINGHAM, E. (2000): C-*mos* variation in songbirds: molecular evolution, phylogenetic implications and comparisons with mitochondrial differentiation.- Molecular Biology and Evolution, Oxford; 17: 1569-1577.

MOORE, W. S. (1995): Inferring phylogenies from mtDNA variation: Mitochondrial-Gene trees versus nuclear-gene trees.- Evolution, Lawrence; 49: 718-726.

PELLEGRINO, K. C. M. & RODRIGUES, M. T. & YONENAGA-YASSUDA, Y. & SITES, J. W. Jr (2001): A molecular perspective on the evolution of microteiid lizards (Squamata, Gymnophthalmidae), and a new classification for the family.- Biological Journal of the Linnean Society, London; 74: 315-338.

SAINT, K. M. & AUSTIN, C. C. & DONNELLAN, S. C. & HUTCHINSON, M. N. (1998): C-*mos*, a nuclear marker useful for squamate phylogenetic analysis.- Molecular Phylogenetics and Evolution, Chicago; 10: 259-263.

SLOWINSKI, J. B. & LAWSON, R. (2002): Snake phylogeny: evidence from nuclear and mitochondrial genes.- Molecular Phylogenetics and Evolution, Chicago; 24: 194-202.

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