

A 7-bp insertion in the 3' untranslated region suggests the duplication and concerted evolution of the rabbit *SRY* gene

Armando GERALDES^{a,b}, Nuno FERRAND^{a*}

^a CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, e Departamento de Zoologia e Antropologia, Faculdade de Ciências da Universidade do Porto, 4099-002 Porto, Portugal

^b IREC, Instituto de Investigación en Recursos Cinegéticos (CSIC/UCLM/JCCLM), Spain

(Received 12 August 2005; accepted 14 December 2005)

Abstract – In this work we report the genetic polymorphism of a 7-bp insertion in the 3' untranslated region of the rabbit *SRY* gene. The polymorphic GAATTAA motif was found exclusively in one of the two divergent rabbit Y-chromosomal lineages, suggesting that its origin is more recent than the separation of the *O. c. algirus* and *O. c. cuniculus* Y-chromosomes. In addition, the remarkable observation of haplotypes exhibiting 0, 1 and 2 7-bp inserts in essentially all *algirus* populations suggests that the rabbit *SRY* gene is duplicated and evolving under concerted evolution.

rabbit / *SRY* gene / polymorphic insertion / duplication / concerted evolution

1. INTRODUCTION

The recent sequencing of the human male-specific region of the Y chromosome (MSY) revealed a mosaic of X-transposed, X-degenerate and ampliconic sequence classes [15]. While the first two classes showed a total of 18 single-copy genes, the latter exhibited multiple copies of an additional nine genes that were mostly associated with palindromic regions. All these multi-copy genes are testis-specific and their evolution is shaped by abundant gene conversion as demonstrated by very high intra-palindromic sequence identity in humans and apes [14]. A notable exception to this pattern is the sex-determining gene, *SRY*, which is predominantly expressed in testes but is X-degenerate and represented by a single copy. Whereas a similar observation has been made in the house mouse, *Mus musculus*, evidence for two or more copies of the *SRY* gene in various rodent species is accumulating [5, 7, 9, 11].

* Corresponding author: nferrand@mail.icav.up.pt

The European rabbit (*Oryctolagus cuniculus*) is a mammalian species native to the Iberian Peninsula that successfully colonized many regions of the world by a combination of natural and human-mediated processes [10, 12] and whose domestication was achieved during the Middle Ages [4]. Biju-Duval *et al.* [1] first showed the occurrence of two highly divergent mtDNA lineages in Iberian wild rabbit populations by using RFLP of the whole molecule and tentatively dated their divergence at 1–2 million years ago. Later, Branco *et al.* [2, 3] described phylogeographical evidence for a recent contact zone bisecting the Iberian Peninsula from the Northwest to the Southeast and suggested that post-glacial population expansions from two different refugia could explain the observed patterns. Recently, we reported high levels of nucleotide diversity in the rabbit *SRY* gene and suggested that this result could be explained by strong population subdivision [6]. In fact, the subsequent analysis of a comprehensive sample of wild rabbits from the Iberian Peninsula showed the existence of a relatively sharp contact zone between two divergent Y-chromosome lineages, in complete concordance with mtDNA data (Geraldes *et al.*, unpublished data).

In this study, we describe the polymorphism and geographical distribution of a 7-bp insertion in the 3' untranslated region of the rabbit *SRY* that we previously identified in only one of the divergent Y-chromosome lineages [6] and use the strong population structure of Iberian wild rabbits to better understand the evolution of this sex-determining gene in Lagomorphs.

2. MATERIALS AND METHODS

Ear tissue was obtained from a total of 335 field-collected wild rabbits originating from 30 different populations in the whole Iberian Peninsula and southern France. In addition, a sample of 37 domestic rabbits from various breeds was also studied. Genomic DNA was isolated following standard protocols.

Initial PCR amplifications of the rabbit *SRY* gene followed the protocols described in Geraldes *et al.* [6] and confirmed the occurrence of a GAATTAA insertion between nucleotide positions 1490 and 1496, in the 3' untranslated region. Subsequently, the forward (5' to 3': CGGTGATGTGAAACACACAA) and reverse primers (5' to 3': TACAGGGAGATGCACAAACG) were developed and amplified fragments of 125-bp (absence of insertion) and 132-bp (presence of insertion). The amplification protocol included an initial denaturation at 94 °C for 5 min, 35 cycles, with denaturation at 94 °C for 20 s, annealing at 54 °C for 20 s, and elongation at 72 °C for 20 s, and a final extension step at 72 °C for 5 min. PCR products were visualized on 4.5% Metaphor agarose gels.

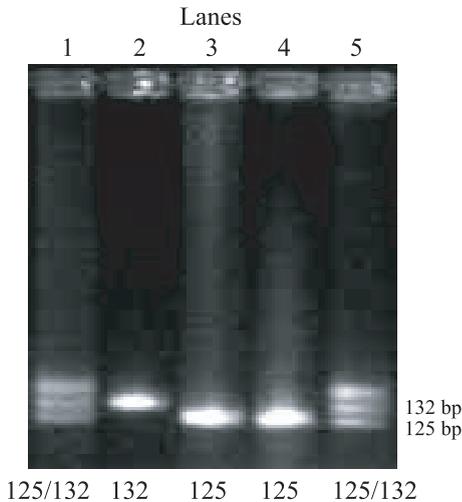


Figure 1. Agarose gel electrophoresis (Metaphor 4.5% w/v) showing the three banded patterns observed in the PCR amplification of the 3' region of the *SRY* gene. Lanes 1 and 5 exhibit PCR products of 125 and 132 bp and an additional heteroduplex band (corresponding to individuals with haplotype 125/132), lane 2 shows a single 132 bp band (corresponding to individuals with haplotype 132/132), and lanes 3 and 4 display a single 125 bp band (corresponding to individuals with haplotype 125/125).

3. RESULTS AND DISCUSSION

Routine separation of PCR products designed to amplify the GAATTAA insertion showed clear band patterns: one-banded patterns were easily interpreted and corresponded to individuals with or without the 7-bp insertion, but three-banded patterns were unexpected and more difficult to explain (Fig. 1). These patterns were formed by both the 125-bp and 132-bp bands, as well as by an additional third band with lower mobility that was interpreted as a heteroduplex. Sequencing of PCR products displaying all three patterns confirmed this interpretation and overlapped sequences comparable to those observed in heterozygotes for autosomal markers were obtained for individuals showing three-banded patterns (results not shown). The most likely explanation for this observation is the presence of two *SRY* copies in the rabbit Y chromosome with a polymorphic 7-bp insertion.

The large scale application of our PCR protocol to screen the polymorphic 7-bp insertion in a comprehensive sample of Iberian wild rabbits showed notably that this polymorphism is restricted to rabbits possessing the Y chromosome lineage A (Fig. 2). These populations, which also exhibit mtDNA lineage A [3] and correspond to the subspecies *Oryctolagus cuniculus algirus*, are confined to Southwest Iberia and show relatively similar amounts of haplotypes 125/125 (no insertion), 125/132 (insertion in only one *SRY* copy) and 132/132 (insertion in both *SRY* copies) (Tab. I). The distribution of these haplotypes does not show any obvious geographical pattern. In contrast, rabbit populations from Northeastern Iberia, which exhibit mtDNA lineage B and correspond to the subspecies *Oryctolagus cuniculus cuniculus*, do not show

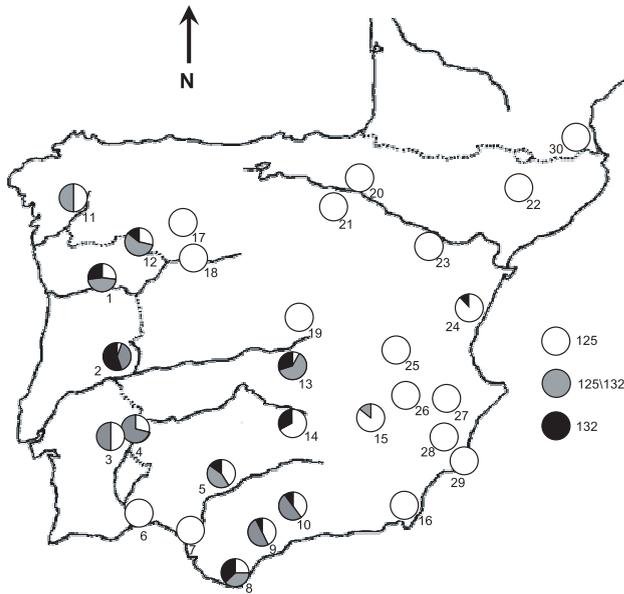


Figure 2. Geographical distribution of the 7-bp polymorphic insertion in wild rabbit populations from the Iberian Peninsula and southern France. Haplotypes 125/125, 125/132 and 132/132 are represented in white, grey and black, respectively. Partitions in the pie charts represent the haplotype frequencies for each population. 1 – Vila Real; 2 – Idanha; 3 – Vila Viçosa; 4 – Elvas; 5 – Sevilla; 6 – Huelva; 7 – Doñana; 8 – Las Lomas; 9 – Fuente Piedra; 10 – Córdoba; 11 – Verin; 12 – Bragança; 13 – Toledo; 14 – Ciudad Real; 15 – Albacete SW; 16 – Las Amoladeras; 17 – Benavente; 18 – Zamora; 19 – Madrid; 20 – Tudela; 21 – La Rioja; 22 – Lleida; 23 – Zaragoza; 24 – Rosell; 25 – Cuenca; 26 – Albacete N; 27 – Valencia; 28 – Alicante; 29 – Cartagena; 30 – Perpignan.

the occurrence of the 7-bp insertion. In addition, all domestic rabbits originated from various breeds also lacked the insertion, confirming their recent derivation from the single *O. c. cuniculus* subspecies. These data suggest that the 7-bp insertion arose exclusively in a rabbit population from Southwestern Iberia, after the divergence of the *algius* and *cuniculus* Y chromosomes. An alternative hypothesis would imply a recent *SRY* duplication occurring only in *algius* Y chromosomes. However, the observation that some individuals from both subspecies exhibit “heterozygosity” at a few nucleotide positions as well as at linked microsatellites [6] while bearing haplotypes 125/125 or 132/132 clearly favors the first hypothesis (Geraldes *et al.*, unpublished data).

A remarkable pattern within *algius* Y-chromosomes is the observation in almost all populations of haplotypes 125/125, 125/132 and 132/132. There

Table I. Geographical distribution of the 7-bp polymorphic insertion in the rabbit *SRY* gene observed in wild and domestic rabbits from the Iberian Peninsula and Southern France.

No.*	Population	<i>algius</i> individuals			<i>cuniculus</i> individuals		
		125/125	125/132	132/132	125/125	125/132	132/132
1	Vila Real	3	5	3			
2	Idanha	1	7	10			
3	Vila Viçosa	5	5				
4	Elvas	2	5				
5	Sevilla	11	12	4			
6	Huelva	6					
7	Doñana	8					
8	Las Lomas	2	3	3			
9	Fuente Piedra	6	7	1			
10	Córdoba	4	5	1			
11	Verín	3	3				
12	Bragança	1	4	1	1		
13	Toledo	1	16	8	1		
14	Ciudad Real	12		6			
15	Albacete SW	6	1		1		
16	Amoladeras				4		
17	Benavente				21		
18	Zamora				14		
19	Madrid				17		
20	Tudela				8		
21	La Rioja				4		
22	Lleida				5		
23	Zaragoza				9		
24	Rosell	1		1	6		
25	Cuenca	1			4		
26	Albacete N				8		
27	Valencia	4			5		
28	Alicante				18		
29	Cartagena				10		
30	Perpignan				11		
	Domestic				37		

* Population numbers as in Figure 2.

are at least two possible explanations for this observation: (i) the 7-bp insertion arose independently in the two *SRY* copies, or (ii) the insertion originated in a single *SRY* copy and gene conversion events have subsequently been responsible for its transference between copies. While the first hypothesis seems highly unlikely because the independent insertion of two identical

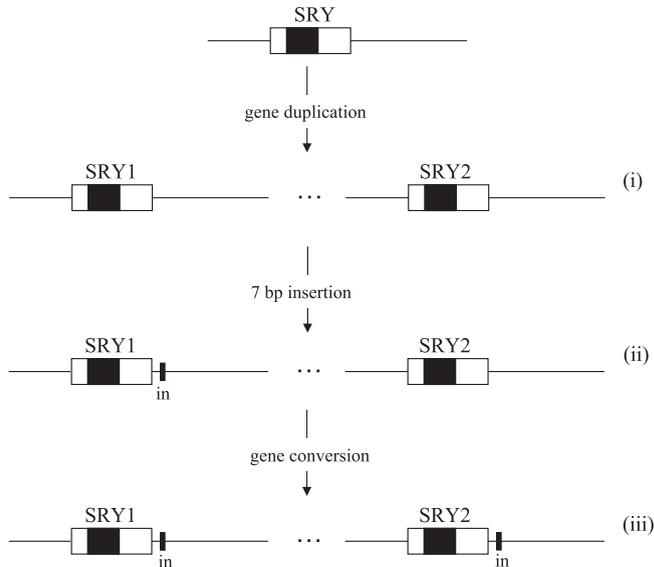


Figure 3. A schematic hypothesis proposed to explain the 7-bp polymorphism at the rabbit *SRY* locus. The coding region of *SRY* is represented by a white box, and the HMG domain is represented by a black box. The 7 bp insertion is located downstream from the coding region and is represented by a black bar and the letters “in”. (i) The two *SRY* copies resulted from an ancestral gene duplication; (ii) the GAATTAA motif is inserted in the 3' untranslated region of one *SRY* gene exclusively in *algiurus* Y-chromosomes; (iii) this 7-bp motif is transferred to the second *SRY* copy by a mechanism of gene conversion. Note that subsequently (i) can also be derived from (ii) depending on the converted *SRY* copy.

motifs is necessarily an extremely rare event, abundant gene conversion between paralogous sequences has recently been described in the Y chromosome of humans and the great apes [14, 15]. We thus hypothesize that the 7-bp insertion occurred only once in an *algiurus* Y chromosome, 3' to the HMG box of one *SRY* copy (giving rise to the 125/132 haplotype), and was afterwards copied by gene conversion to the second *SRY* gene (giving rise to the 132/132 haplotype) (Fig. 3). Given the non-equilibrium status of wild rabbit populations in Iberia due to recent demographic expansions in response to post-glacial climatic amelioration [3], we further suggest that the patterns we observe today for the 7-bp insertion polymorphism in *algiurus* populations are the likely result of a balance between drift (which tends to increase differences between populations) and migration and gene conversion (which tend to homogenize differences between populations).

The recent availability of the human Y chromosome sequence revealed a number of notable features of which the occurrence of multi-copy testis genes in palindromic sequences that may promote gene conversion and maintain sequence identity is probably the most remarkable. In the near future, comparisons with other Y chromosome sequences (*e.g.* mice as well as other species) are expected to reveal unprecedented biological insights due to the dramatic differences known to occur in the organization of this chromosome in mammals. The *SRY* gene may be an example of this because (i) it is a single copy gene surrounded by a unique sequence in humans, (ii) it is a single copy gene flanked by long inverted repeats in mice [7], and (iii) it is a multi-copy gene in several African [9] and European rodents [5]. Our data on the rabbit *SRY* suggests that this sex-determining gene is probably duplicated and evolving under concerted evolution in Lagomorphs. The recent identification of a clone that contains this gene [8] in a rabbit BAC library [13] will hopefully allow its complete sequencing in our laboratories and the examination of the hypotheses described above.

ACKNOWLEDGEMENTS

This work was partly supported by Fundação para a Ciência e a Tecnologia (SFRH/BD/4621/2001 Ph.D. grant to Armando Geraldes and Research Project POCTI/BSE/40280/2001). We also thank Rafael Villafuerte, Christian Gortazar and José Dávila (IREC, Instituto de Investigación en Recursos Cinegéticos, CSIC/UCLM, España) for their help in Spanish sampling campaigns. Hélène Hayes and two anonymous referees provided constructive criticisms that improved a previous version of this paper.

REFERENCES

- [1] Biju-Duval C., Ennafaa H., Dennebouy N., Monnerot M., Mignotte F., Soriguer R.C., Gaaied A., Hili A., Mounolou J.-C., Mitochondrial DNA evolution in lagomorphs: origin of systematic heteroplasmy and organization of diversity in European rabbits, *J. Mol. Evol.* 33 (1991) 92–102.
- [2] Branco M., Ferrand N., Monnerot M., Phylogeography of the European rabbit (*Oryctolagus cuniculus*) in the Iberian Peninsula inferred from RFLP analysis of the cytochrome *b* gene, *Heredity* 85 (2000) 307–317.
- [3] Branco M., Monnerot M., Ferrand N., Templeton A., Postglacial dispersal of the European rabbit (*Oryctolagus cuniculus*) on the Iberian Peninsula reconstructed from nested clade and mismatch analyses of mitochondrial DNA genetic variation, *Evolution* 56 (2002) 792–803.

- [4] Callou C., De la garenne au clapier. Étude archéozoologique du lapin en Europe occidentale, Mémoires du Muséum national d'Histoire naturelle, Paris, 2003.
- [5] Fernández R., Barragán M.J.L., Bullejos M., Marchal J.A., Martínez S., Guardia R.D., Sánchez A., Mapping the *SRY* gene in *Microtus cabreræ*: a vole species with multiple *SRY* copies in males and females, *Genome* 45 (2002) 600–603.
- [6] Geraldes A., Rogel-Gaillard C., Ferrand N., High levels of nucleotide diversity in the European rabbit (*Oryctolagus cuniculus*) *SRY* gene, *Anim. Genet.* 36 (2005) 349–351.
- [7] Gubbay J., Vivian N., Economou A., Jackson D., Goodfellow P., Lovell-Badge R., Inverted repeat structure of the *Sry* locus in mice, *Proc. Natl. Acad. Sci. USA* 89 (1992) 7953–7957.
- [8] Hayes H., Rogel-Gaillard C., Zijlstra C., de Haan N.A., Urien C., Bourgeaux N., Bertaud M., Bosma A.A., Establishment of an R-banded rabbit karyotype nomenclature by FISH localization of 23 chromosome-specific genes on both G- and R-banded chromosomes, *Cytogenet. Genome Res.* 98 (2002) 199–205.
- [9] Lundrigan B.L., Tucker P.K., Evidence for multiple functional copies of the male sex-determining locus, *SRY*, in African murine rodents, *J. Mol. Evol.* 45 (1997) 60–65.
- [10] Monnerot M., Vigne J.-D., Biju-Duval C., Casane D., Callou C., Hardy C., Mougél F., Soriguer R., Dennebouy N., Mounolou J.-C., Rabbit and man: genetic and historic approach, *Genet. Sel. Evol.* 26 (Suppl. 1) (1994) S167–S182.
- [11] Nagamine C.M., The testis-determining gene, *SRY*, exists in multiple copies in Old World rodents, *Genet. Res. Camb.* 64 (1994) 151–159.
- [12] Queney G., Ferrand N., Weiss S., Mougél F., Monnerot M., Stationary distributions of microsatellite loci between divergent population groups of the European rabbit (*Oryctolagus cuniculus*), *Mol. Biol. Evol.* 18 (2001) 2169–2178.
- [13] Rogel-Gaillard C., Piumi F., Billault A., Bourgeaux N., Save J.C., Urien C., Salmon J., Chardon P., Construction of a rabbit bacterial artificial chromosome (BAC) library: application to the mapping of the major histocompatibility complex to position 12q.1.1., *Mamm. Genome* 12 (2001) 253–255.
- [14] Rozen S., Skaletsky H., Marszalek J.D., Minx P.J., Cordum H.S., Waterston R.H., Wilson R.K., Page D.P., Abundant gene conversion between arms of palindromes in human and ape Y chromosomes, *Nature* 423 (2003) 873–876.
- [15] Skaletsky H., Kuroda-Kawaguchi T., Minx P.J., Cordum H.S., Hiller L., Brown L.G., Repping S., Pyntikova T., Ali J., Bieri T., Chinwalla A., Delehaunty A., Delehaunty K., Du H., Fewell G., Fulton L., Fulton R., Graves T., Hou S.-F., Latrielle P., Leonard S., Mardis E., Maupin R., McPherson J., Miner T., Nash W., Nguyen C., Ozersky P., Pepin K., Rock S., Rohlfig T., Scott K., Schultz B., Strong C., Tin-Wollam A., Yang S.-P., Waterston R.H., Wilson R.K., Rozen S., Page D.P., The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes, *Nature* 423 (2003) 825–837.