Low Levels of Sequence Divergence in Rock Wallabies (Petrogale) Suggest a Lack of Positive Directional Selection in Sry


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The rate of nucleotide mutation traditionally has been considered uniform over the entire genome, and any variations in substitution rate are deemed functions of differential selective constraints (Wolfe, Sharp, and Li 1989). Previous studies of sequence divergence of SRY, the mammalian male sex determining gene (Sinclair et al. 1990), in two eutherian groups of mammals suggested that the rapid evolution of the gene may indicate positive directional selection. Among pairwise comparisons between human and seven primate species (Whitfield, Lovell-Badge, and Goodfellow 1993), and between Mus musculus and six species of Old World mice and rats (Tucker and Lundrigan 1993), low amino acid identity led the authors to conclude that SRY was evolving rapidly. To examine the relative rates of evolution within these pairwise comparisons, $K_s/K_a$ values were determined for SRY, where $K_a$ is the rate of non-synonymous substitutions per nonsynonymous site and $K_s$ is the rate of synonymous substitutions per synonymous site. $K_s/K_a$ values in the primate study ranged from 0.47 to 1.88 and values in the mouse study ranged from 0.33 to 0.45. A comparison of 363 genes between mouse and rat estimated an average $K_s/K_a$ value of 0.14 within a range of 0.05-0.2, although Y-linked genes were not involved in this study (Wolfe and Sharp 1993). Neutral, or unconstrained, evolution would predict a $K_s/K_a$ value of 1 (Lee, Ota, and Vacquier 1995; Rosenberg et al. 1995), yet a value of 1.88 found in primates indicated a deviation from this and evidence of selective drive on the sequence. This drive has been suggested to be positive directional selection, which may promote reproductive isolation by causing disruption of sexual development in hybrids (Hale, Washburn, and Either 1993; Tucker and Lundrigan 1993; Whitfield, Lovell-Badge, and Goodfellow 1993; Hurst 1994).

While these studies suggest a plausible model for the directional selection of SRY, these species diverged several million years ago and any selective constraint, or lack thereof, on the sequence may have been masked by other nonselective or episodic events. To test for any directional selection or functional constraint on SRY, we have examined the nucleic acid and amino acid sequences and compared the substitution rates in a recently diverged and rapidly speciating group of mammals. The results of studies of sequence evolution over relatively short time scales should improve understanding of the longer-term dynamics of the gene.

This study focuses on the evolution of SRY in rock wallabies (Petrogale) (Marsupialia: Macropodidae), which recently diverged and hybridized readily in nature, producing sterile hybrid males, following Haldane’s Rule (Eldridge and Close 1993). The 15 species of rock wallaby are found in dispersed species clusters, often parapatrically and occasionally sympatrically, across the continent of Australia and on many offshore islands (Eldridge and Close 1993). Petrogale mareeba, P. coenensis, P. godmani, P. inornata, P. herberti, P. sharmani, P. assimilis, and P. penicillata are distributed in a continuous parapatric chain down the mesic eastern coastal ranges of Australia, while the allopatric populations of P. lateralis purpureicollis, P. xanthopus xanthopus, and P. xanthopus celeris are dispersed across xeric eastern and inland Australia (Eldridge and Close 1993). The only sympatric species cluster existing today occurs in tropical northern Australia, where P. brachyotis is sympatric with both P. burbidgei and P. concinna (Eldridge and Close 1993).

The entire coding region of SRY was PCR amplified from genomic DNA using primers from the Macropus eugenii (tammar wallaby) sequence (5’-GCTATGT-ATGGCTTCTTGATG, 5’-AATCAGTCTCGTATT-CAGGT) for 13 taxa of Petrogale. The ~600 bp product was sequenced using direct cycle sequencing. Two individuals of each taxon were sequenced to account for any intrapopulation polymorphism. $K_s/K_a$ values were calculated using Li’s method (Li, Wu, and Luo 1985; Li 1993) with a program supplied by F? Pamilo (personal communication). The results of sequence divergence over relatively short time scales should improve understanding of the longer-term dynamics of the gene.

Key words: SRY, rock wallaby, speciation, positive directional selection.

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Fig. 1.—Alignment of SRY amino acid sequences using Clustal V (GenBank accession numbers: U76510–U76522). Only variable sites are shown (positions: 17, 50, 110, 112, 121, 127, 139, 145, 146, 148, 150, 152, 159, 162, 164–171, 182). The only variable site within the HMG box has been outlined. Asterisks indicate symatric species, plus signs indicate parapatric species, and dashes indicate allopatric taxa.

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Fig. 2.—a. Plot of $K_A$ and $K_S$ values in pairwise comparisons (Petrogale vs. Petrogale). b. Plot of $d = K_A - K_S$ against $K_S$ with the regression line shown. The neutral line of evolution ($K_A = K_S$) is shown as $K_S = 0$. The expected range of values has been highlighted.
communication). Taxa used in this study are: Petrogale assimilis, Petrogale brachyotis, Petrogale burbidgei, Petrogale coenensis, Petrogale godmani, Petrogale herberti, Petrogale inornata, Petrogale lateralis purpureicollis, Petrogale marreeba, Petrogale penicillata, Petrogale sharmani, Petrogale xanthopus celeris, and Petrogale xanthopus.

While rock wallabies are found across the Australian continent and in an array of parapatric, sympatric, and allopatric clusters, their SRY sequences are not correspondingly diverse. Neither the nucleic acid sequence with 30/564 variable sites nor the amino acid sequence with 16/188 variable sites is species-specific (fig. 1). Among the 13 Petrogale taxa studied, there are only seven different SRY protein sequences present. Pairwise distance comparisons among the Petrogale (data not shown) of the nucleic acid sequence show extremely low divergence between taxa, with values ranging from 0.000 to 0.037. These results suggest a recent and rapid radiation of these taxa irrespective of SRY and render phylogenetic analysis based on this sequence uninformative.

These sequences do not follow any trend based on geography, nor do they form monophyletic groups with respect to chromosomal phylogenies (Eldridge and Close 1993). There is also no relation of these variable sites within the amino acid sequence to population condition (i.e., parapatric), with the exception of Petrogale burbidgei and Petrogale brachyotis, two sympatric species which are closely related chromosomally (Eldridge and Close 1993) and share a conservative amino acid change.

If positive directional selection is acting on a gene within a closely related group of organisms, the distribution of pairwise comparisons between $K_A$ and $K_S$ would fall above the neutral line of evolution ($K_A = K_S$) (Lee, Ota, and Vacquier 1995). $K_A$ and $K_S$ in pairwise comparisons were plotted against one another and compared to the neutral rate of evolution (fig. 2). The majority of the values fall below this line. To test for a trend away from neutral evolution, $d = K_A - K_S$ was plotted against $K_S$ and the deviation of the regression line below zero ($K_A = K_S$) was shown to be significant, ($F_{1,77} = 1,696.885; P = 0.0001$) (the intercept was not significantly different from zero). This indicates a lack of overall positive directional selection within the SRY sequence among closely related Petrogale taxa.

Previous studies show that if a gene undergoes positive directional selection (e.g., SRY through effects on reproductive isolation), recently diverged species, especially those residing parapatrically and/or sympatrically, have $K_A/K_S$ values significantly above the neutral line of evolution ($K_A/K_S = 1$) (Lee, Ota, and Vacquier 1995). $K_A/K_S$ values were calculated (data not shown) for pairwise comparisons of Petrogale taxa and 75.9% are <1.00, falling within a range similar to that found by Tucker and Lundrigan (1993). Values greater than 1.00 or undefined are most likely anomalies, for they do not constitute a trend based on geographic distribution or phylogeny. These values may indicate episodic directional selection of SRY. Yet the values of this study as a whole further support a conclusion of no overall positive directional selection over short evolutionary time scales.

The above data suggest that Petrogale SRY sequences are not directly involved in reproductive isolation and that SRY sequences in this species complex are not subject to positive directional selection. The rapid sequence evolution of SRY found in previous studies may indicate a lack of functional constraint on regions surrounding the HMG box and/or may be a result of past episodic selection events or Y-specific events such as chromosomal sweeps (Birky and Walsh 1988).

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LITERATURE CITED


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