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Chromosomal phylogeny of Robertsonian races of the house mouse on the island of Madeira: testing between alternative mutational processes

JANICE BRITTON-DAVIDIAN[1]*, JOSETTE CATALAN[1], MARIA DA GRAÇA RAMALHINHO[2], JEAN-CHRISTOPHE AUFFRAY[1], ANA CLAUDIA NUNES[3], ELODIE GAZAVE[1], JEREMY B. SEARLE[4] AND MARIA DA LUZ MATHIAS[3]

[1] *Institut des Sciences de l'Evolution, UMR 5554, Laboratoire Génétique et Environnement, CC65, Université Montpellier II, 34095 Montpellier Cedex 5, France*
[2] *Centro de Biologia Ambiental, Museu Bocage, Universidade de Lisboa, Rua da Escola Politécnica, 1268-102 Lisbon, Portugal*
[3] *Centro de Biologia Ambiental, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Edifício C 2, 3° Piso, Campo Grande, 1749-016 Lisbon, Portugal*
[4] *Department of Biology, University of York, PO Box 373, York YO10 5YW, UK*

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Summary

The ancestral karyotype of the house mouse (*Mus musculus*) consists of 40 acrocentric chromosomes, but numerous races exist within the *domesticus* subspecies characterized by different metacentric chromosomes formed by the joining at the centromere of two acrocentrics. An exemplary case is present on the island of Madeira where six highly divergent chromosomal races have accumulated different combinations of 20 metacentrics in 500–1000 years. Chromosomal cladistic phylogenies were performed to test the relative performance of Robertsonian (Rb) fusions, Rb fissions and whole-arm reciprocal translocations (WARTs) in resolving relationships between the chromosomal races. The different trees yielded roughly similar topologies, but varied in the number of steps and branch support. The analyses using Rb fusions/fissions as characters resulted in poorly supported trees requiring six to eight homoplasious events. Allowance for WARTs considerably increased nodal support and yielded the most parsimonious trees since homoplasy was reduced to a single event. The WART-based trees required five to nine WARTs and 12 to 16 Rb fusions. These analyses provide support for the role of WARTs in generating the extensive chromosomal diversification observed in house mice. The repeated occurrence of Rb fusions and WARTs highlights the contribution of centromere-related rearrangements to accelerated rates of chromosomal change in the house mouse.

1. Introduction

Rates of chromosomal evolution are known to vary widely among mammals and are particularly enhanced in murid rodents (Bush *et al.*, 1977; White, 1978; Searle, 1993). Recent advances in comparative genome mapping confirm these differential rates and further indicate that the lineage leading to the house mouse, *Mus musculus*, has accumulated twice as many rearrangements as that of the human (Burt *et al.*, 1999). The house mouse belongs to the subgenus *Mus* along with 10 other species, almost all of which share the same 40-acrocentric G-band karyotype (Boursot *et al.*, 1993; Suzuki *et al.*, 2004). The two exceptions are the Indian pygmy field mouse, *M. terricolor*, in which fusions, inversions and heterochromatin additions are documented (Bardhan & Sharma, 2000), and populations belonging to one subspecies of the house mouse, *M. m. domesticus*. In the latter, chromosomal change has proceeded by the accumulation of one to nine pairs of metacentrics produced by the joining of two chromosomes at the centromere by the Robertsonian (Rb) fusion process, thus leading to a gradual reduction in diploid number from 2n = 40 to 2n = 22 (Gropp & Winking, 1981; Nachman & Searle, 1995). Although *M. m. domesticus* has a worldwide distribution, most chromosomal races have been described from Western Europe, where it was

* Corresponding author. Tel: +33 4 67143910. Fax: +33 4 67143622. e-mail: britton@isem.univ-montp2.fr

introduced from the Middle East as a commensal of humans within the last 5000 years (Auffray & Britton-Davidian, 1992; Auffray *et al*., 1990), although a recent archaeozoological study provides an even later date (i.e. 3000 years in Cucchi *et al*., 2005). These colonization dates are consistent with the distribution of allozyme diversity and mitochondrial DNA haplotypes of house mouse populations in this region (Britton-Davidian *et al*., 1989; Nachman *et al*., 1994). Such a timeframe thus indicates that *M. m. domesticus* has a rate of fixation of Rb fusions at least two orders of magnitude higher than other mammals (Nachman & Searle, 1995). Also important is the diversity of metacentrics. In *M. m. domesticus*, all autosomes contribute to metacentrics, albeit at different frequencies (Gazave *et al*., 2003), and 103 of the 171 possible pairwise combinations of autosomes have so far been observed in wild populations (Piálek *et al*., 2005).

Numerous studies based on different approaches have contributed to our understanding of the processes of metacentric formation in the house mouse. Molecular analyses have shown that Rb fusions in this subspecies are formed by breakage in the centromeric satellite DNA sequences of two non-homologous acrocentric chromosomes, and their subsequent reunion (Garagna *et al*., 2001, 2002). The mechanism thus consists of an interchromosomal exchange between satellite blocks of two acrocentrics, leading to the loss of proximal telomeres and part of the minor satellite sequences on both chromosomes (Nanda *et al*., 1995). As a consequence, the fission of Rb fusion products is considered as highly unlikely in the house mouse, since telomere sequences as well as centromeric material essential for chromosome integrity and segregation would be missing in the neo-acrocentrics produced. This contrasts with other species in which relict telomeric sequences persist in the centromeric regions of Rb fusions suggesting a different mechanism of formation (Metcalfe *et al*., 1997, 1998; Fagundes & Yonenaga-Yassuda, 1998; Pellegrino *et al*., 1999; Go *et al*., 2000; Finato *et al*., 2000; Kasai *et al*., 2000; Castiglia *et al*., 2002; Ruiz-Herrera *et al*., 2002; Viera *et al*., 2004). Although Rb fusions are essential to generate the metacentric condition, another process leading to chromosome diversification has been described. This is known as a WART (whole-arm reciprocal translocation), in which an exchange of chromosomal arms occurs either between two metacentrics or between one metacentric and an acrocentric chromosome, thereby generating new metacentrics (Winking, 1986; Searle *et al*., 1990; Capanna & Redi, 1995; Hauffe & Piálek, 1997; Castiglia & Capanna, 1999; Catalan *et al*., 2000; see Fig. 1). This type of rearrangement is compatible with the observed centromeric structure of Rb fusion products (Garagna *et al*., 2001).

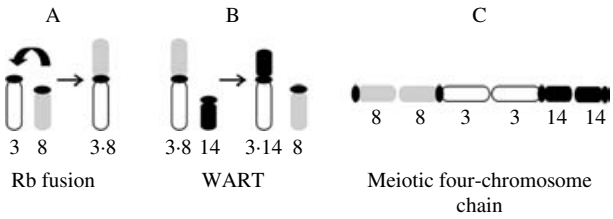

Fig. 1. Schematic diagram showing an Rb fusion between acrocentrics 3 and 8 (*A*), a WART between metacentric 3·8 and the acrocentric chromosome 14 (*B*), and the resulting meiotic pairing configuration of an individual heterozygous for this type of WART (*C*).

The accumulation of different numbers and combinations of metacentrics in populations has led to the formation of a large diversity of chromosomal races distributed in geographically separate systems (Piálek *et al*., 2005). Each system comprises one to several related parapatric races carrying one or more metacentrics in common. In the simplest cases, differentiation of races within a system conforms to the model of sequential accumulation of Rb fusions (Capanna *et al*., 1977; Capanna, 1982). Examples of such systems are present in Denmark, Croatia, the Peloponnese and Central-Southern Italy (Piálek *et al*., 2005). In other cases, the systems are much more complex, suggesting that additional processes are involved. Attempts to reconstruct chromosomal phylogenies in these highly diverse systems have led several authors to suggest that WART events and/or processes of zonal raciation and introgression have most likely contributed to the diversification of these races (Capanna, 1982; Corti *et al*., 1986; Searle, 1991, 1993; Hauffe & Piálek, 1997; Piálek *et al*., 2001, 2005).

The aim of the present study was to assess the relative importance of Rb fusions and WARTs in metacentric formation in the house mouse. This analysis was performed on the system of six chromosomal races on the Portuguese island of Madeira (Britton-Davidian *et al*., 2000). In common with other island systems used in evolutionary biology, the Madeira island system offers many advantages as a model. First, the six races show an impressive degree of karyotypic variation: chromosome numbers vary between 2n = 22 and 2n = 28, and 20 different metacentrics are distributed among the races. The extent of this differentiation suggests that at least some of the races are reproductively isolated from each other. Second, the Madeira system is clearly defined geographically, because it is present in one small island. Finally, it offers a unique advantage over other systems of chromosomal races in the house mouse in that the timing of colonization is relatively well known. Historical documents date the first human occupation of Madeira at the end of the fifteenth century AD by Portuguese settlers, suggesting that mice could have been introduced into the island at that time. However,

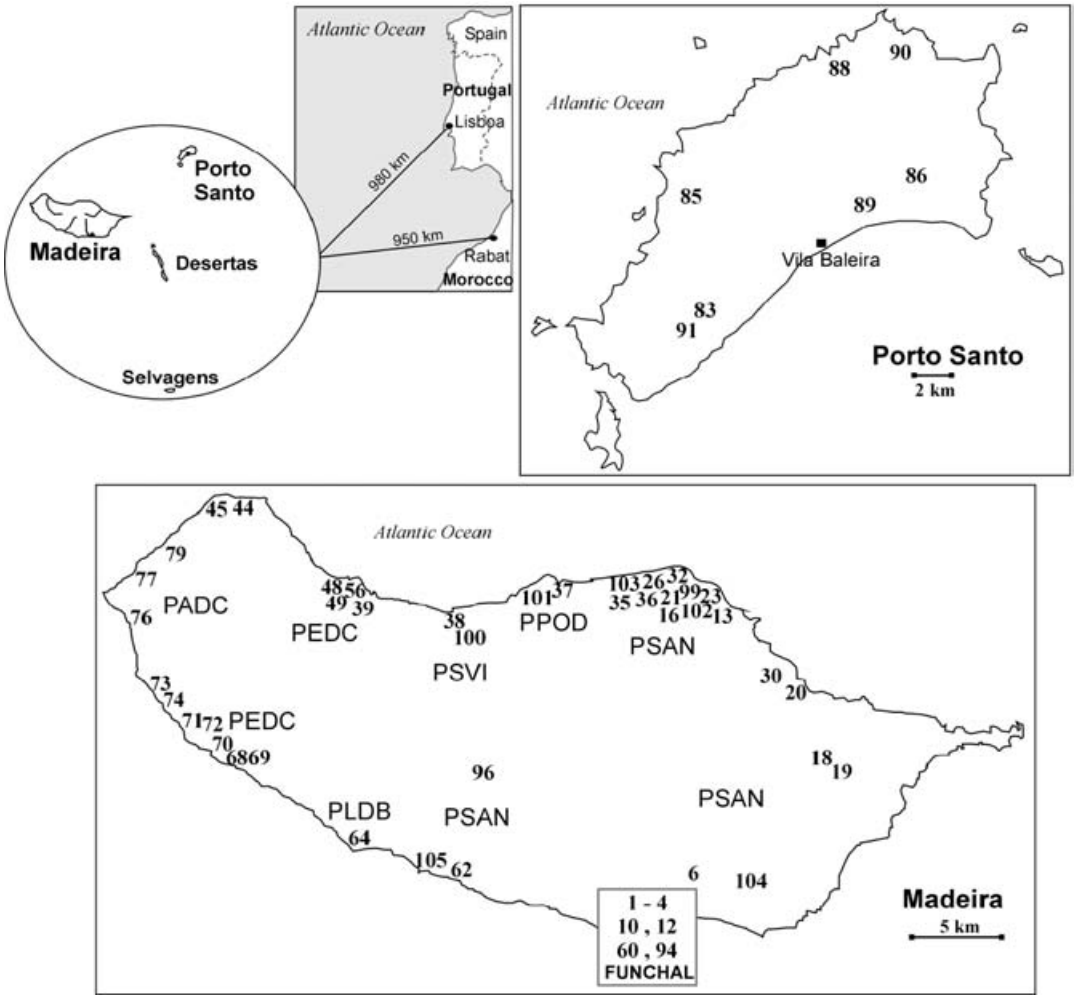

Fig. 2. Maps showing the geographic location of the Madeira archipelago, as well as that of the sampled localities in Porto Santo and Madeira (site numbers are provided). The distribution of the six chromosomal races is indicated: PSAN, Santana; PADC, Achadas da Cruz; PEDC, Estreito da Calheta; PPOD, Ponta Delgada; PSVI, São Vicente; PLDB, Lugar de Baixo. Data for Funchal are from Gazave *et al.* (2003).

recent mitochondrial (mt) DNA data indicate that these mice may have a northern European origin related to Viking expeditions during the ninth century AD (Gündüz *et al.*, 2001). These molecular analyses further indicate that mice from Madeira and the neighbouring island of Porto Santo have a common single origin (Gündüz *et al.*, 2001), suggesting that the colonizing mice most likely carried the standard karyotype which is present in Porto Santo (Mathias & Ramalhinho, 1992), with subsequent chromosomal divergence occurring only in Madeira. This allows us to provide a rough fixation rate in Madeiran mice of nine metacentrics (the maximum in any one chromosomal race) in 500–1000 years or 10^{-3} per generation (see Nachman & Searle, 1995). So, not only do Madeiran mice show an impressive variety of chromosomal variants, but also these variants evolved remarkably quickly.

To reconstruct the sequence and type of events leading to the formation of the chromosomal races on Madeira, phylogenetic analyses were performed using Rb fusions/fissions and WARTs as possible characters. The relative performance of these rearrangements in resolving the relationships between races is tested and their role in generating the accelerated rate of chromosomal differentiation in the house mouse from Madeira is discussed.

2. Materials and methods

(i) *Mouse samples and chromosomal analysis*

The karyotypes of 209 of the mice have previously been published (Britton-Davidian *et al.*, 2000; Gazave *et al.*, 2003). An additional 49 individuals are included in this analysis to more accurately estimate within-race chromosomal variability. The complete set of 258 mice were live-trapped in a total of 56 sites in commensal habitats (houses, buildings, gardens, farms, cultivated and fallow fields) between May 1998 and June 1999 on the islands of Madeira and Porto Santo (Fig. 2). In addition, three specimens were collected

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on two other islands of the Madeiran archipelago: the Desertas (1 male, 1 female) and Selvagem Grande (1 male).

Karyotypes were prepared from bone marrow cells after yeast stimulation using the air-drying method (Lee & Elder, 1980). Metacentrics were identified following the G-banding method of Seabright (1971), with reference to the karyotypes in Cowell (1984). Two to five G-banded metaphases were analysed per individual. Chromosomal analyses were performed using a Zeiss Axiophot photomicroscope equipped with an image analyser (Genevision and Cytovision, Applied Imaging) and a Leitz Dialux 20 microscope with an attached Leica DC 350F camera equipped with a Leica Chantal V4.1 image analysis system. Metacentrics were designated by their chromosome arm number combination, i.e. 3·8 derives from the joining of the acrocentric chromosomes 3 and 8 (see Fig. 1). The names of the chromosomal races follow the nomenclature of Ramalhinho *et al.* (2005) and Piálek *et al.* (2005). The latter authors considered a seventh race (Arco di San Jorge, PASJ; localities nos. 35, 36, 103) which carried all the fusions present in the Santana race (PSAN) except (2·19). However, since this fusion was subsequently found in additional mice captured from these localities, we chose to attribute these samples to PSAN. The frequencies of the different metacentrics were calculated per sites or group of sites pooled according to geographic proximity (see Fig. 2) and/or chromosomal similarity (i.e. shared metacentrics and/or polymorphisms; see Table 1).

(ii) *Cladistic analysis*

Phylogenetic reconstruction using chromosomal characters was performed following a cladistic approach using PAUP4b10 (Swofford, 1999). Several step matrices and weighting regimes were applied and subjected to analysis by parsimony. In all cases, chromosomal rearrangements were coded as characters and their presence/absence as the state for each character (Dobigny *et al.*, 2004). These characters were polarized by the outgroup which consisted of the 2n = 40 all-acrocentric karyotype. In the first analysis, the metacentrics present in Madeiran mice were considered as the product of 20 independent Rb fusions/fissions (see Table 2A). No weighting constraints were applied, i.e. Rb fusions and fissions were equally probable. In addition, the Lundberg rooting option was used in order to constrain the ancestral states. The second analysis involved the same 20-character matrix, but included a weighting regime in which Rb fissions were arbitrarily assigned a weight of 100 and Rb fusions a weight of 1. In the last series of analyses, the step matrix allowed for the occurrence of WARTs between a metacentric and an acrocentric chromosome (i.e. type-b WART in Hauffe & Piálek, 1997; see Fig. 1). This was achieved by grouping pairs or triplets of metacentrics with one arm in common into multistate characters and allowing them to derive from one another (see for example Table 2B, C). Thus, the metacentrics included in one multistate character may be formed either by a Rb fusion or a WART event. Four step matrices including five to 10 multistate characters were explored to test the performance of WARTs versus Rb fusions in resolving the relationships between races. In these analyses, three weighting regimes were applied in addition to that for Rb fissions: (i) Rb fusions and WARTs had an equal weight of 1, (ii) Rb fusions were favoured over WARTs (respective weights 1:10), and the reciprocal situation in which WARTs were favoured over Rb fusions (1:10). In all analyses, two options were applied to favour the early (ACCtran) or delayed (DELtran) occurrence of events, leading to lower and higher numbers of convergence events respectively. Branch node support was estimated by bootstrap percentage values over 1000 iterations, as well as by the Bremer index (Decay Index, DI; Bremer, 1988).

3. Results

(i) *Within-race chromosomal polymorphism*

The chromosomal analysis of house mice from the Madeiran archipelago showed that chromosomal variation was entirely restricted to the island of Madeira, the mice from the Desertas and Selvagem Grande carrying the standard diploid number (2n = 40). The exception to this pattern involved the mice from Porto Santo where two individuals carried one metacentric each, 5·14 or 11·12, that also occurred in Madeira (Table 1). In this chromosomal survey, no additional metacentrics or chromosomal races were observed on Madeira compared with previous data (Britton-Davidian *et al.*, 2000; Gazave *et al.*, 2003). Thus, six chromosomal races were present carrying a total of 20 different metacentrics, each of which shared a monobrachial (one-arm) homology with one or more other metacentrics on the island (Table 1). Although no interracial hybrid karyotypes were observed, in one locality (site 70 in Fig. 2 and Table 1) two mice were trapped each carrying metacentrics that discriminate the two westernmost races (PADC and PEDC). In contrast, within-race polymorphism involving metacentrics and their homologous acrocentrics was found. This polymorphism involved 10 of the 20 metacentrics present in Madeira; half of them concerned metacentrics unique to one race, while the remainder consisted of metacentrics common to several races. Heterozygosity was present in all races except Lugar de Baixo (PLDB), with levels varying between 0·04 and 0·50 per metacentric, and involving from one up to

Table 1. *Frequency and distribution of metacentrics in mice from Madeira*

				Metacentric																		
Race	Locality	*N*	2n	2·4	2·19	3·8	3·14	4·5	4·16	5·14	5·18	6·7	7·15	8·11	9·10	9·12	9·18	10·16	11·12	11·19	13·17	14·17	15·18
Funchal	1–4, 10, 12, 60, 94	86	22–40	0	0·76	0·52	0	0	0·74	0·34	0	0·23	0	0	0·62	0	0	0	0·53	0	0·59	0	0·51
PSAN	62, 96, 105	5	22	0	1	1	0	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	1
	6	31	22–23	0	1	1	0	0	1	1	0	1	0	0	0·98	0	0	0	1	0	1	0	1
	18, 19, 104	5	22–23	0	1	1	0	0	1	1	0	1	0	0	1	0	0	0	1	0	0·70	0	1
	20, 30	3	22	0	1	1	0	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	1
	13, 16, 21, 23, 99, 102	12	22	0	1	1	0	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	1
	26, 32	5	22	0	1	1	0	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	1
	35, 36, 103	7	23–24	0	0·07	1	0	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	1
PPOD	37, 101	9	28–30	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0·50	0
PSVI	38, 100	23	25–27	1	0	1	0	0	0	0	1	0·98	0	0	0	1	0	1	0	0·07	1	0	0
PEDC-N	39, 48, 49, 56	5	24–26	1	0	0	1	0	0	0	1	0·80	0	1	0	1	0	1	0	0	1	0	0
PEDC-S	71, 72	3	24–26	1	0	0	1	0	0	0	1	0·33	0	1	0	1	0	1	0	0	1	0	0
	68, 69	6	24–25	1	0	0	1	0	0	0	1	0·75	0	1	0	1	0	1	0	0	1	0	0
PEDC-S/ PADC	70	1	24	1	0	0	1	0	0	0	1	1	0	1	0	1	0	1	0	0	1	0	0
	70	1	26	1	0	0	1	0	0	0	1	0	0·50	1	0	0·50	0	1	0	0	1	0	0
PADC	44,45	3	26–27	1	0	0	1	0	0	0	0·33	0	0·50	1	0	1	0	1	0	0	1	0	0
	76, 77, 79	6	24–25	1	0	0	0·92	0	0	0	1	0	0·83	1	0	1	0	1	0	0	1	0	0
	73, 74	11	25–27	1	0	0	1	0	0	0	1	0	0·27	1	0	0·95	0	1	0	0	1	0	0
PLDB	64	8	24	1	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	0	1	0	1
Porto Santo	85	8	39–40	0	0	0	0	0	0	0·06	0	0	0	0	0	0	0	0	0·13	0	0	0	0
	83–91	20	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Values are calculated per sampled site or group of pooled sites among races. Numbers of sites refer to those indicated in Fig. 1.
N, number of karyotyped specimens; 2n, range of diploid number; PSAN, Santana; PPOD, Ponda Delgada; PSVI, São Vicente; PEDC, Estreito da Calheta (northern (N) and southern (S) localities; see Fig. 2); PADC, Achadas da Cruz; PLDB, Lugar de Baixo. Pooled metacentric frequencies for the city Funchal were calculated from data in Gazave *et al.* (2003).

Table 2. *Character matrices of the parsimony analyses*

A	Character	B	Character		C	Character		
1	Rb(2·4)	1	Rb(2·4)		1	Rb/W(2·4)	Rb/W(4·5)	Rb/W(2·19)
2	Rb(2·19)	2	Rb(2·19)		2	Rb/W(3·8)	Rb/W(3·14)	
3	Rb(3·8)	3	Rb/W(3·8)	Rb/W(3·14)	3	Rb/W(4·16)	Rb/W(10·16)	
4	Rb(3·14)	4	Rb(4·5)		4	Rb(5·14)		
5	Rb(4·5)	5	Rb/W(4·16)	Rb/W(10·16)	5	Rb/W(5·18)	Rb/W(15·18)	
6	Rb(4·16)	6	Rb(5·14)		6	Rb/W(6·7)	Rb/W(7·15)	
7	Rb(5·14)	7	Rb/W(5·18)	Rb/W(15·18)	7	Rb(8·11)		
8	Rb(5·18)	8	Rb/W(6·7)	Rb/W(7·15)	8	Rb(9·10)		
9	Rb(6·7)	9	Rb(8·11)		9	Rb/W(9·12)	Rb/W(11·12)	
10	Rb(7·15)	10	Rb(9·10)		10	Rb(9·18)		
11	Rb(8·11)	11	Rb/W(9·12)	Rb/W(11·12)	11	Rb(11·19)		
12	Rb(9·10)	12	Rb(9·18)		12	Rb/W(13·17)	Rb/W(14·17)	
13	Rb(9·12)	13	Rb(11·19)					
14	Rb(9·18)	14	Rb(13·17)					
15	Rb(10·16)	15	Rb(14·17)					
16	Rb(11·12)							
17	Rb(11·19)							
18	Rb(13·17)							
19	Rb(14·17)							
20	Rb(15·18)							

The characters are the rearrangements affecting the metacentrics: Rb, Rb fusion/fission; Rb/W, Rb fusion/fission or WART.
A: Each rearrangement is an independent event.
B: 15-character matrix with five multistate characters involving metacentrics having one arm in common.
C: 12-character coding scheme with seven multistate characters involving metacentrics having one arm in common.

four independently segregating metacentrics (i.e. Achadas da Cruz race, PADC; see Table 1).

(ii) *Phylogenetic analysis*

The analyses using Rb fusions/fissions as independent characters yielded three most parsimonious trees (26 steps) when no constraints were considered, i.e. Rb fusions and fissions were equally probable. All trees showed six homoplasies consisting of multiple Rb fusions and fissions, and involving the same chromosomes: 3·8, 5·18, 6·7, 10·16, 11·12 and 15·18. The only difference between the trees lay in the number of each type of event, i.e. two fissions/four multiple fusions (Fig. 3A), three of each (tree not shown) and five fissions/one fusion (Fig. 3B). Note that in all the branches along which fissions occur, a related monobrachial homologous Rb fusion appears. The topologies of the three trees were almost identical: the PPOD race was the first to branch off followed by PSAN, then PSVI, and finally the three most derived races (PEDC, PADC and PLDB), the relationships between which were either unresolved (Fig. 3B) or clustered PEDC with PADC (Fig. 3A). The phylogenetic reconstructions were poorly supported as the topologies were generally associated with low bootstrap (33–77%) and DI values (0–2).

A second analysis was performed by heavily weighting Rb fissions to bias chromosomal evolution in favour of Rb fusions (Fig. 4). The two most parsimonious trees produced (28 steps) no longer involved Rb fissions, but required eight homoplasies consisting of convergent fusion events, involving five of the homoplasious Rb fusions of the previous analysis, with the addition of 13·17. The topologies of the trees differed from the preceding ones, in that two independent clusters were present grouping PSAN and PPOD together on the one hand, and the four other races on the other. Additionally, the trees varied in the topology of one cluster, PLDB occurring as a sister group to PEDC and PADC (Fig. 4A), or PADC as a sister race to the other two (Fig. 4B). These reconstructions required two convergent events for each homoplasious Rb fusion except 5·18 and 6·7, which appeared three and up to four times, respectively. The nodes in these trees had similar low levels of bootstrap support (35–75%) and DI values (0–2) as those in the previous ones.

In the last series of analyses, the characters were recoded to include whole-arm reciprocal translocations (WARTs) between a metacentric and an acrocentric chromosome. This type of WART was postulated (Fig. 1) since none of the metacentrics observed in the different races could be derived directly from WARTs between two metacentrics such as described in other studies (see Table 1; Winking, 1986; Capanna & Redi, 1995; Garagna *et al.*, 1997; Castiglia & Capanna, 1999). The metacentrics coded as multistate characters were the five involved in fissions in the unconstrained tree, to which were

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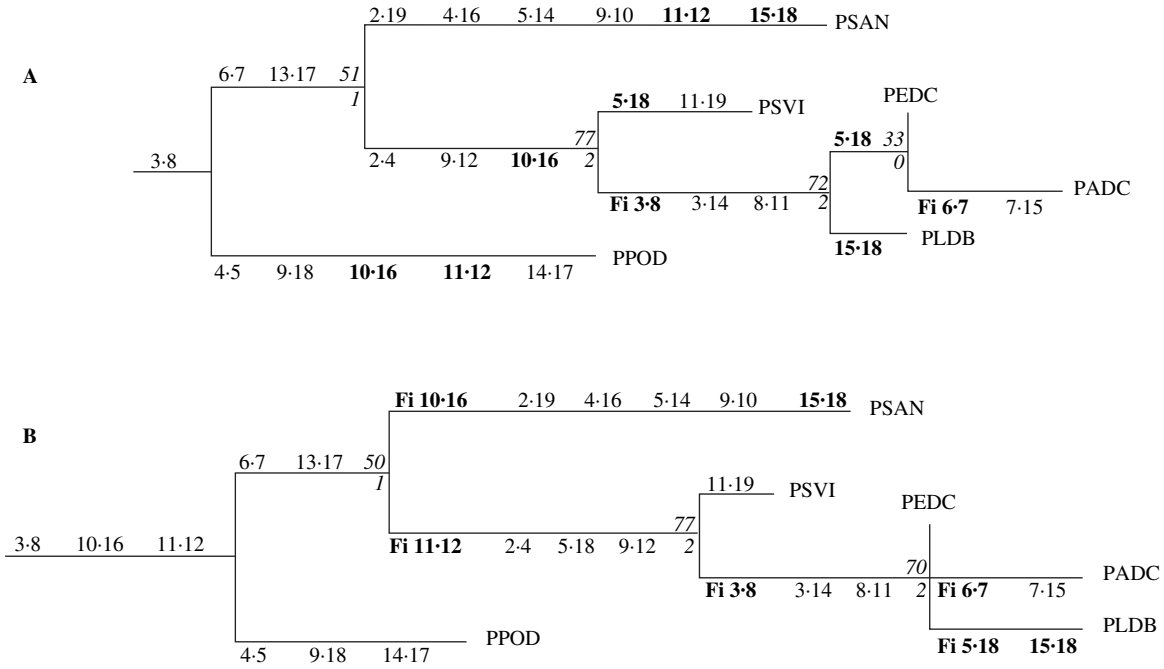

Fig. 3. Two of the 26-step phylogenetic trees obtained using the no-constraint 20-character matrix. All events are marked along the branches with multiple Rb fusions and fissions (Fi) highlighted in bold. Node support is indicated in italics by bootstrap and DI values respectively above and below the branches. (*A*) Tree with three multiple Rb fusions and two fission events (option DELtran), and (*B*) Tree with one multiple Rb fusion and five fission events (option ACCtran). Note the different topology for PEDC, PADC and PLDB. PSAN, Santana; PADC, Achadas da Cruz; PEDC, Estreito da Calheta; PPOD, Ponta Delgada; PSVI, São Vicente; PLDB, Lugar de Baixo.

added one of the monobrachially homologous metacentrics occurring along the same branches (see Fig. 3B). Increasing the number of multistate characters was achieved by including additional monobrachially homologous metacentrics that were compatible with the sequence of events as shown in the unconstrained tree. These different step matrices were explored, but all except the 15- and 12-character schemes (see Table 2B, C) were discarded, since they resulted in the persistence of incompatible metacentrics (i.e. showing monobrachial homology) along several branches. Likewise, the alternate weighting regimes between Rb fusions and WARTs produced either the same trees as in Fig. 4 (when Rb fusions were favoured over WARTs), or showed no difference from those involving a 1:1 weighting (when WARTs were favoured over Rb fusions).

All the analyses using multistate characters yielded 21-step trees showing the same topology as the unconstrained tree in which the relationship between the PEDC, PADC and PLDB was unresolved. In the WART scenario with five multistate characters, two trees were produced, both with nodes showing moderate to high bootstrap support (70–96%; only one tree is shown in Fig. 5A). The difference between the two trees was the order of appearance of 5·18, and the homoplasious event involving 15·18, which in one tree appeared once by fusion and once by WART (Fig. 5A), and in the second, twice by WART (tree not shown). Thus, this coding regime involved the occurrence of 15 or 16 metacentrics generated by Rb fusion and a WART origin for five or six of them. In the alternative scenario using the 12-character code, one of the two trees produced was identical to one from the previous analysis (Fig. 5A). The other involved nine WARTs and 12 Rb fusions as well as the multiple occurrence of 15·18 by WART (Fig. 5B). The difference between the low- and high-WART scenarios lay in the order of occurrence of events along the branches, particularly the basal one on which three to five Rb fusions respectively were present. These results showed that the allowance of WARTs as a mechanism of formation of metacentrics resolved all but one of the homoplasies present in the unconstrained tree, and produced trees with a higher overall bootstrap support (68–98%) and DI values (1–2) than those only involving Rb fusions and/or fissions.

Furthermore, the order of events in the WART-based trees suggested that an alternate type of WART involving three chromosomes may have occurred. For example, in the branch leading to PSAN (Fig. 5) an exchange between metacentric 10·16 and both acrocentrics 4 and 9 could have simultaneously generated both 4·16 and 9·10. Two other similar cases involve metacentric 3·8 and chromosomes 11 and 14 resulting in 3·14 and 8·11, as well as metacentric 5·18 and chromosomes 14 and 15 yielding 5·14 and 15·18. Although such double WARTs would reduce the

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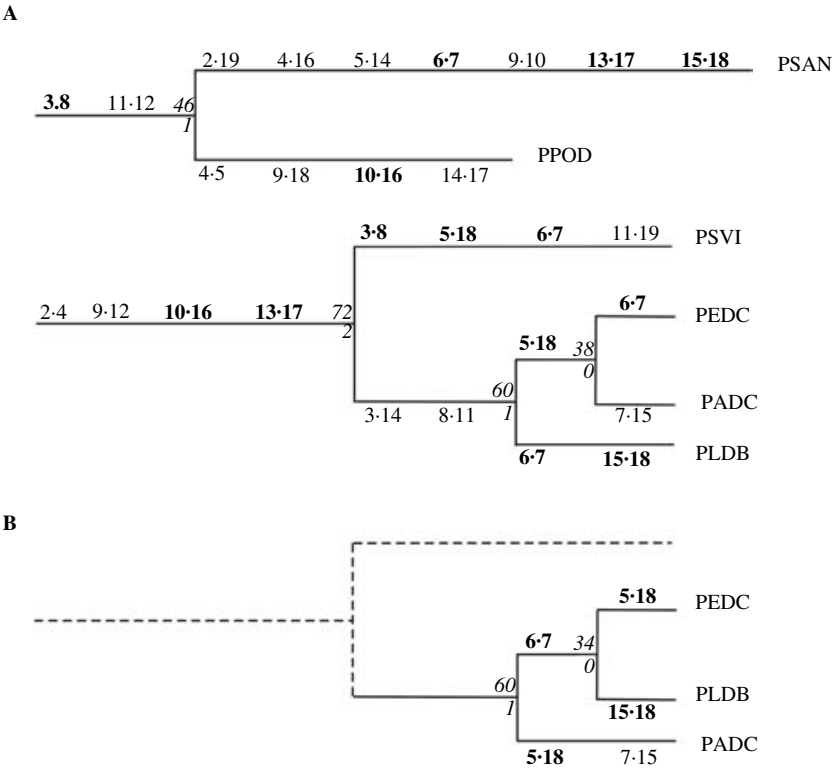

Fig. 4. (*A*) A 28-step tree obtained by heavily weighting fissions versus Rb fusions (100:1). All events are marked along the branches with multiple Rb fusions highlighted in bold. Node support is indicated in italics by bootstrap and DI values respectively above and below the branches. The homoplasies involve only multiple Rb fusions. Options ACCtran and DELtran yielded the same two identical trees. PSAN, Santana; PADC, Achadas da Cruz; PEDC, Estreito da Calheta; PPOD, Ponta Delgada; PSVI, São Vicente; PLDB, Lugar de Baixo. (*B*) Part of an alternative 28-step tree, which differs only in the sister group relationship for PEDC, PADC and PLDB.

number of steps in the tree to 18, they do not resolve the homoplasy involving metacentric 15·18.

4. Discussion

(i) *Phylogenetic relationships and modes of Rb fusion formation*

The chromosomal analysis of house mice from the Madeira archipelago confirmed that karyotypic change was restricted to Madeira itself. On that island, there were six chromosomal races characterized by a large diversity of metacentrics, the fixation of which may have been enhanced by geographic isolation (Britton-Davidian *et al*., 2000). The only exception to this pattern were the two metacentric mice from Porto Santo, which most likely correspond to introgression events following passive transport between these two islands. Additional indications of immigration were provided by the mice from Funchal in which all metacentrics of the easternmost race (PSAN) were segregating with their homologous acrocentrics, suggesting continued hybridization with standard karyotype mice (Gazave *et al*., 2003). As mice from Porto Santo and Madeira have a common and unique origin (Gündüz *et al*., 2001), the colonizing mice most likely carried the standard karyotype, with subsequent chromosomal differentiation occurring on Madeira but not Porto Santo.

The chromosomal phylogenetic analyses allowed us to infer the sequence and type of events leading to this diversification. The analyses in which each metacentric was scored as the result of an Rb fusion yielded poorly supported trees requiring six to eight homoplasious events, and involving Rb fissions and/or multiple fusions. However, coding rearrangements as multistate characters by including WARTs reduced homoplasy to a single event and considerably increased nodal support. A similar conclusion was arrived at in a previous phylogenetic reconstruction of races within the North Italy System, which included the occurrence of WARTs and hybridization events (Hauffe & Piálek, 1997). In the present study, several phylogenies combining reticulate evolution with WARTs were also explored (data not shown), but were discarded as they required a large number of migration and introgression events, and were thus less parsimonious than those using the cladistic approach. As such, the step-by-step procedure used in the present phylogenetic analysis may provide a useful means

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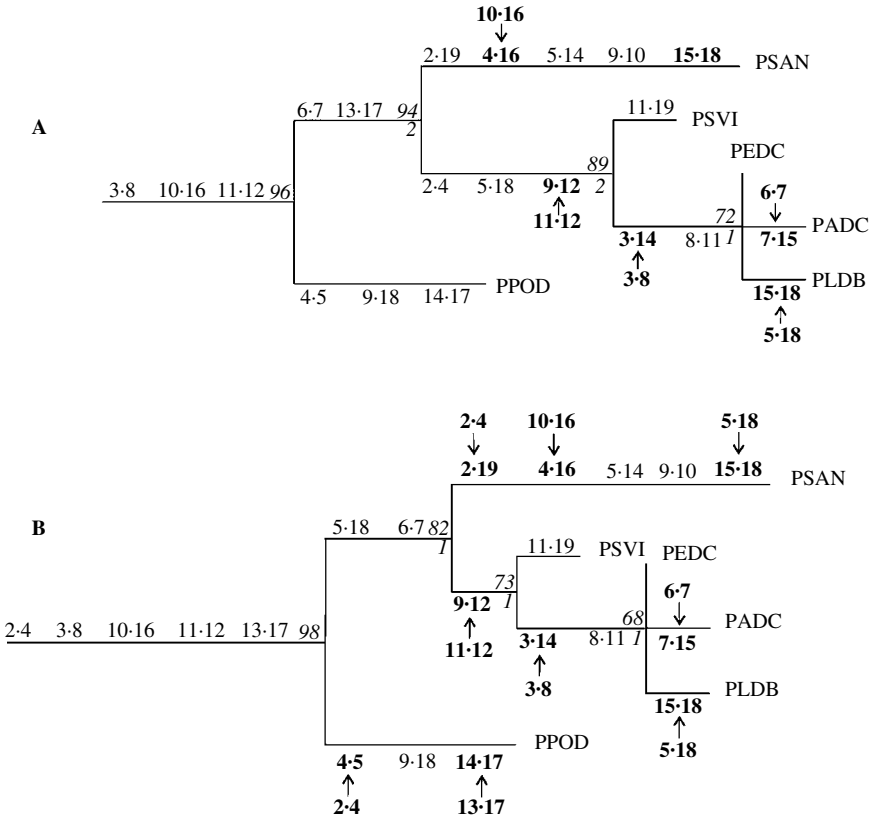

Fig. 5. Two 21-step trees obtained using the multistate character matrices allowing for WARTs and a similar weight for Rb fusions vs WARTs (1:1). All events are marked along the branches with the multiple Rb fusion and WARTs (arrows) highlighted in bold. Node support is indicated in italics by bootstrap and DI values respectively above and below the branches. Both trees show only one homoplasy (15·18), and involve five WARTs (*A*; option DELtran; see matrix in Table 2B), or nine WARTs (*B*; option ACCtran; see matrix in Table 2C). PSAN, Santana; PADC, Achadas da Cruz; PEDC, Estreito da Calheta; PPOD, Ponta Delgada; PSVI, São Vicente; PLDB, Lugar de Baixo.

of testing alternative hypotheses of chromosomal evolution in other complex systems of the house mouse, as well as in other taxa characterized by similar accumulations of diverse metacentrics (Piálek *et al*., 2005; Rumpler *et al*., 2005).

The results of the phylogenetic analyses clearly indicate that the formation of the Rb races in Madeira is complex, as it requires that either Rb fissions, multiple Rb fusions or WARTs have occurred. Allowance for the latter yielded the best supported and most parsimonious trees. Two additional arguments favour the occurrence of WARTs versus the other two scenarios. First, molecular analyses have indicated that Rb fissions are highly unlikely in house mice, as such an event would require the *de novo* acquisition of telomeric and centromeric sequences (Nanda *et al*., 1995). However, recent studies are accumulating molecular evidence for neo-centromere formation, chromosome healing by telomere sequence seeding, as well as for a fission model involving prior duplication of pericentromeric sequences (Melek & Shuippen, 1996; Sprung *et al*., 1999; Godfrey & Masters, 2000; Kolnicki, 2000; Ventura *et al*., 2001, 2004; Eder *et al*., 2003; Amor *et al*., 2004; Nergadze *et al*., 2004). Notwithstanding, given the structure of metacentrics formed by Rb fusion in house mice, fissions would require the simultaneous occurrence of two very rare events, i.e. centromere pre-duplication and the *de novo* addition of telomere repeats onto non-telomeric DNA, thus considerably reducing the probability of this event. Additional support for the rarity of fissions in mice from Madeira will need sequence and *in situ* hybridization analyses of the pericentromeric regions of chromosomes. Second, although the convergent generation of metacentrics is known to occur in the house mouse (Riginos & Nachman, 1999), the number of such events required in the multiple Rb fusion scenario considerably reduces the likelihood of this process. This is further supported by the fact that some of the recurrent metacentrics are not known elsewhere, and that in one case chromosome 7 is involved which has been shown to be less fusion prone than other similarly sized chromosomes (Gazave *et al*., 2003).

All WART-based trees shared a similar topology, with three to five Rb fusions occurring on the basal branch from which the PPOD and PSAN races branched off first, followed by PSVI. In all trees, the

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PEDC, PADC and PLDB races formed a poorly resolved cluster; these races differ from one another by only two metacentrics. The results are compatible with the within-race polymorphism of unique metacentrics, as they may be considered as the last ones to appear and would be in the process of fixation (i.e. 2·19, 7·15, 9·10, 11·19, 14·17). However, several of the polymorphic metacentrics do not conform to this pattern, and this is particularly true for two of the four metacentrics segregating with homologous acrocentrics in PADC which occur early in the tree (5·18 and 9·12; see Table 1 and Fig. 5A). In addition, as some of these metacentrics are caused by WARTs, one of the chromosome arms of the pair is not available as a free acrocentric, i.e. in the arm exchange between 6·7 and chromosome 15 yielding 7·15, chromosome 7 does not occur in an acrocentric form. The existence of such acrocentric chromosomes can only be accounted for by Rb fissions or introgression following immigration of acrocentric-bearing mice. The latter event has been postulated as a source of acrocentrics in other metacentric populations (Hauffe & Searle, 1993; Piálek *et al*., 2005).

(ii) *Chromosomal underdominance and rates of chromosomal change*

High rates of chromosomal evolution such as observed in house mice on Madeira must be a reflection of the mutation rate and the processes involved in fixation of these mutations. Traditionally, chromosomal rearrangements are considered to be associated with high levels of underdominance in mammals, leading to the expectation of low rates of fixation. In the case of the house mouse, levels of underdominance are now known to be low for heterozygotes involving single metacentrics, providing theoretical support for the plausibility of the successive accumulation of Rb fusions (Harris *et al*., 1986; Winking, 1986; Wallace *et al*., 1992; Hauffe & Searle, 1998; Castiglia & Capanna, 2000; Wallace, 2003). Even so, such a factor alone will not lead to accelerated rates of fixation. The only convincing selective advantage stems from recent studies demonstrating the existence of transmission distortion in meioses of female heterozygotes for Rb fusions (Pardo-Manuel de Villena & Sapienza, 2001). However, experimental data in the house mouse indicate that acrocentrics are favoured over metacentrics; this process will in fact tend to reduce fixation probabilities, unless a reversal in meiotic preference occurs as metacentrics accumulate in the genome as postulated in the model of Pardo-Manuel de Villena & Sapienza (2001). This has yet to be demonstrated in the house mouse. Values of underdominance for WARTs vary according to their type. The phylogenetic reconstruction involves whole-arm exchanges leading to chain-of-four heterozygotes (see Fig. 1), the fertility of which has been shown to vary from normal to complete sterility depending on the sex of the individuals and the metacentrics involved (Forejt, 1979; Gropp *et al*., 1982; Redi *et al*., 1984; Mahadevaiah *et al*., 1990). However, these data were measured in laboratory-bred hybrids between house mouse strains in which one or several metacentrics had been introgressed from wild populations. As interaction between metacentrics and the genetic background is known to inflate infertility scores (Winking, 1986; Wallace *et al*., 2002), such estimates need to be re-evaluated in wild genomes. This can be performed by analysing hybrids between the contiguous western races in Madeira (PEDC-PADC, and PEDC-PLDB; see Fig. 2) which would carry a chain-of-four meiotic configuration. The fertility data of such hybrids will provide an accurate assessment of the unfitness of this type of configuration in wild mice, and thus allow us to indirectly approximate the fixation probabilities of WARTs. If fixation of WARTs occurs repeatedly in the chromosomal evolutionary history of the house mouse, one must assume that the fertility of these heterozygotes, although presumably lower than for Rb fusions, is not drastically reduced for fixation to occur with a reasonable probability.

On the basis of the above discussion, there is no clear evidence that elevated fixation rates explain the high rates of chromosomal evolution on Madeira. However, there are grounds to believe that elevated mutation rates may be an important factor. Rough estimates inferred from the spontaneous occurrence of Rb fusions in laboratory strains reach 10^{-4} per generation, but increase to 10^{-2}–10^{-3} in mouse genomes that have already fixed one Rb fusion (Winking, 1986), a value that would be compatible with the fixation rate observed in the mice from Madeira (Nachman & Searle, 1995). These results lead to the suggestion that a predisposition to this type of rearrangement may exist in some lineages of *M. m. domesticus*. As WARTs can only occur in genomes in which at least one Rb fusion is already present, both of these rearrangements may have similar frequencies as those suspected for fusion-prone lineages. Recent studies on rearrangement breakpoint comparative mapping indicate that the standard house mouse karyotype results from a large number of interchromosomal changes often involving segmental duplications in pericentromeric regions (Thomas *et al*., 2003; Bailey *et al*., 2004; Friedman & Hughes, 2004; Zhao *et al*., 2004). These data suggest that the genome of *M. m. domesticus* may exhibit a sensitivity to rearrangements involving centromeric regions canalizing subsequent chromosomal evolution toward Rb fusion and WART types of events. Molecular investigations of relevant centromeric sequences (Garagna *et al*., 2002; Chaves *et al*., 2003) or markers

(Riginos & Nachman, 1999) are required to determine the existence of a molecular signature of the occurrence of or predisposition for these rearrangements, and to assess the contribution of such events to the accelerated rates of chromosomal change in the house mouse both on Madeira and in the house mouse in general.

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