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The non-random occurrence of Robertsonian fusion in the house mouse

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Summary

Chromosomal rearrangements such as Robertsonian (Rb) fusions constitute a major phenomenon in the evolution of genome organization in a wide range of organisms. Although proximate mechanisms for the formation of Rb fusion are now well identified, the evolutionary forces that drive chromosomal evolution remain poorly understood. In the house mouse, numerous chromosomal races occur in nature, each defined by a unique combination of Rb fusions. Among the 106 different Rb fusions that were reported from natural populations, the low involvement of chromosome 19 in Rb fusions is striking, prompting the question of the randomness of chromosomal involvement in Rb fusions. We uncover a significant quadratic relationship between chromosome size and probability of fusing, which has never previously been in this species. It appears that fusions involving chromosome 19 are not particularly infrequent, given the expected low fusion probability associated with the chromosome's size. The results are discussed, assessing selective processes or constraints that may operate on chromosome size.

1. Introduction

A Robertsonian (Rb) fusion is a chromosomal rearrangement involving a centric fusion between two acrocentric chromosomes that results in a single metacentric chromosome. Because these rearrangements change chromosome size, shape and number, their study may provide important clues to the evolution of karyotypes. The house mouse *Mus musculus* presents a standard karyotype of 2n = 40 acrocentric chromosomes. However, a massive accumulation of Rb fusions has occurred within the past 10 000 years (Auffray, 1993) in various populations of the Western European subspecies *M. m. domesticus*. More than 40 Rb races have been identified by the number and type of fusions they have fixed. These races are spread over Western Europe and North Africa (for a review see Nachman & Searle, 1995). Among the 171 different fusions which can theoretically be formed by the 19 pairs of autosomes, 106 have been reported in wild populations (Table 1). The number of fusions recorded and the diversity of autosomes involved in these rearrangements led some authors to consider that the involvement of autosomes in Rb fusions was random (Capanna *et al.*, 1977; Gropp & Winking, 1981). Furthermore, the molecular process of Rb formation predicts that this process should be random (Redi *et al.*, 1990). However, these authors also underlined that some chromosomes seem more prone to be involved in Rb fusions than others. For example, until recently, fusions involving chromosome 19 had never been found in the wild. Bauchau (1990) noticed this peculiarity, and tried to estimate the probability of this fusion event. A selective process was invoked by Nachman & Searle (1995) to explain the absence of fusions involving chromosome 19 in nature.

The spontaneous occurrence of a Rb(4.19) fusion in laboratory-bred progeny of wild mice allowed the

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Table 1. *Inventory of all Rb fusions existing in natural populations*

Chromosome number	Chromosome number																	
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	×	×	×	×	×	×		×	×	×				×			×	
2			×	×			×				×		×	×	×	×	×	×
3			×		×		×	×	×		×	×	×			×		
4				×	×				×	×	×	×	×	×	×	×		
5					×	×			×		×	×	×	×	×	×	×	
6						×		×	×	×	×	×	×	×	×	×		
7							×			×				×			×	
8								×	×	×	×	×	×	×	×	×		
9									×	×	×	×	×	×	×		×	
10										×	×	×	×	×	×	×		
11											×	×	×	×	×	×	×	×
12												×	×					
13													×	×	×	×		
14																×		
15																×	×	
16																×		
17																		
18																		

Each cross indicates that the fusion has been found at least once in the wild.

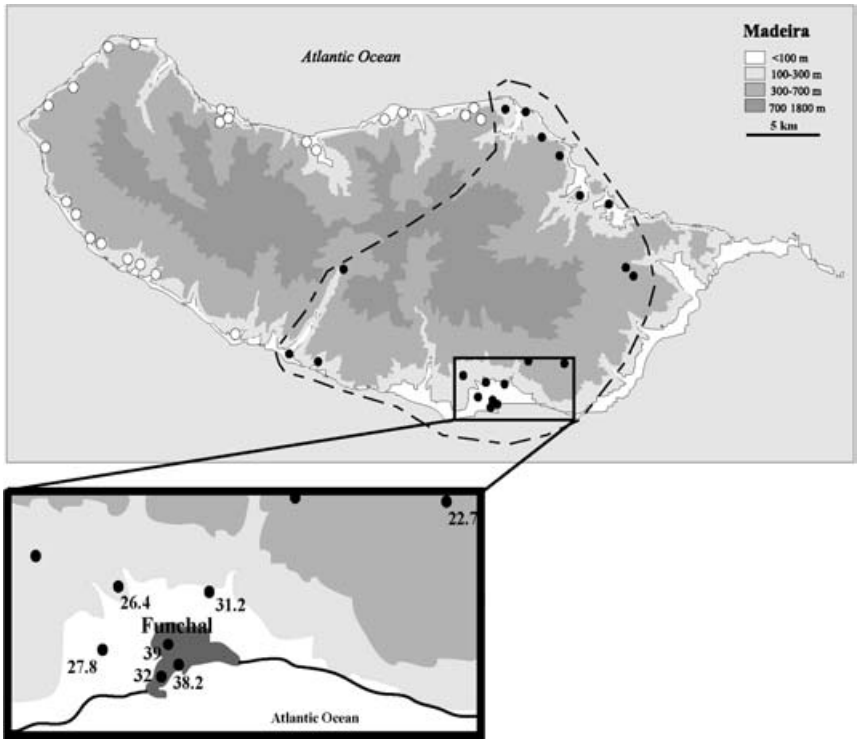

Fig. 1. Sampling locations on the island of Madeira and detail of the Funchal area. Black dots represent localities where the individuals belong either to the race which possesses the Rb(2.19) fusion or to hybrids between this race and standard mice (2n = 40). Inside the dashed line, mean diploid number for the locality is specified when it differs from 2n = 22. White dots represent sites where other chromosomal races were found. No mice were caught in the central part of the island, probably because the habitat is unfavourable for mice. (Map source: Atlas Digital do Ambiente-DGA.)

comparison of developmental stability between carriers and non-carriers of this fusion (Auffray *et al.*, 2001). The results failed to show any perturbations in development associated with the presence of Rb(4.19). Since then, two different Rb fusions involving chromosome 19 have recently been found in wild populations of the house mouse, on the island of Madeira (Britton-Davidian *et al.*, 2000). The most eastern race

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Table 2. *Details of samples involved in the clinical analysis*

Distances[a] (km)	Mean 2n	Sample size[b]	No. of copies for each metacentric								
			(2.19)	(3.8)	(4.16)	(5.14)	(6.7)	(9.10)	(11.12)	(13.17)	(15.18)
0	38·25	8	2	0	0	0	1	3	0	1	0
0·27	32	8	5	2	6	1	2	4	6	3	3
0·39	39	12	0	2	0	2	0	1	1	0	0
1·81	31·21	78	51	28	59	22	24	37	48	41	33
1·84	27·78	18	16	15	15	7	5	15	14	12	11
2·26	26·39	46	46	42	45	24	6	45	21	42	42
4·09	22	2	2	2	2	2	2	2	2	2	2
4·64	22·03	62	62	62	62	62	62	61	62	62	62
7·15	22·67	6	6	6	6	6	6	6	6	4	6
11·54	23	2	2	2	2	2	2	2	2	1	2
12·00	22	2	2	2	2	2	2	2	2	2	2
12·46	22	2	2	2	2	2	2	2	2	2	2
14·46	22	4	4	4	4	4	4	4	4	4	4
15·31	22	4	4	4	4	4	4	4	4	4	4
15·54	22	2	2	2	2	2	2	2	2	2	2
16·31	22	4	4	4	4	4	4	4	4	4	4
17·23	22	8	8	8	8	8	8	8	8	8	8
18·39	22	16	16	16	16	16	16	16	16	16	16
20·39	22	2	2	2	2	2	2	2	2	2	2
20·77	22	8	8	8	8	8	8	8	8	8	8

[a] Distances are given from Funchal harbour to the sampled populations.
[b] Number of haploid genomes.

(Fig. 1) has 9 fixed Rb fusions of which one is Rb(2.19), reducing its diploid number to 2n = 22. This race hybridizes with individuals from the standard race (2n = 40) in the city of Funchal. Hence, in this area, individuals with and without Rb(2.19) coexist, allowing us to estimate the selection differential under natural conditions.

In this study, we tested the randomness of Rb fusion formation in house mice, with a special emphasis on chromosome 19. We approached the question in two ways. First, using data from the hybrid zone in Funchal, we attempted to estimate the intensity of selection against Rb(2.19), using both an analysis of the frequency cline for each fusion and a population genetic analysis. Our second approach was based on the frequency of occurrence of Rb fusions in all chromosomal races of *M. m. domesticus* reported to date. We used generalized linear models to test the null hypothesis that all acrocentrics were equally likely to fuse – that is, that the variation in the number of Rb fusions observed per acrocentric is due to sampling. Chromosome 19 being the smallest autosome, the models tested included chromosome size as a variable.

2. Materials and methods

(i) *Samples and cytogenetic analysis*

Twenty populations were sampled in Funchal and surrounding areas (Fig. 1). All animals ($n = 147$) were karyotyped in order to determine their diploid number and the Rb fusions present. The karyotype was prepared from yeast-stimulated bone marrow cells, using the air-drying technique (Lee & Elder, 1980). For each individual, identification of the Rb fusions following the nomenclature of Cowell (1984) was performed using the G-banding method of Seabright (1971). Two to five metaphases per individual were observed under a Zeiss Axiophot microscope and karyotyped using Genevision software (Applied Imaging).

(ii) *Genetic and clinical analyses*

Rb fusions versus unfused chromosomes were considered as loci with two alleles, corresponding to the acrocentric and metacentric state, and fusion frequencies were calculated for each population. Genotypic associations between each pair of fusions were tested in each population using the probability test described by Raymond & Rousset (1995*a*). For each pair of fusions, global tests (Fisher's method) were performed across all populations. Conformity to Hardy–Weinberg (HW) expectations was globally tested for the proportions of genotypes across samples for each fusion, using the exact *U*-score against the alternative hypothesis of heterozygote deficiency (Rousset & Raymond, 1995). Departure from HW expectations was measured using the F_{IS} estimator proposed by Weir & Cockerham (1984). The level of heterozygote deficiency was measured in order to estimate selection

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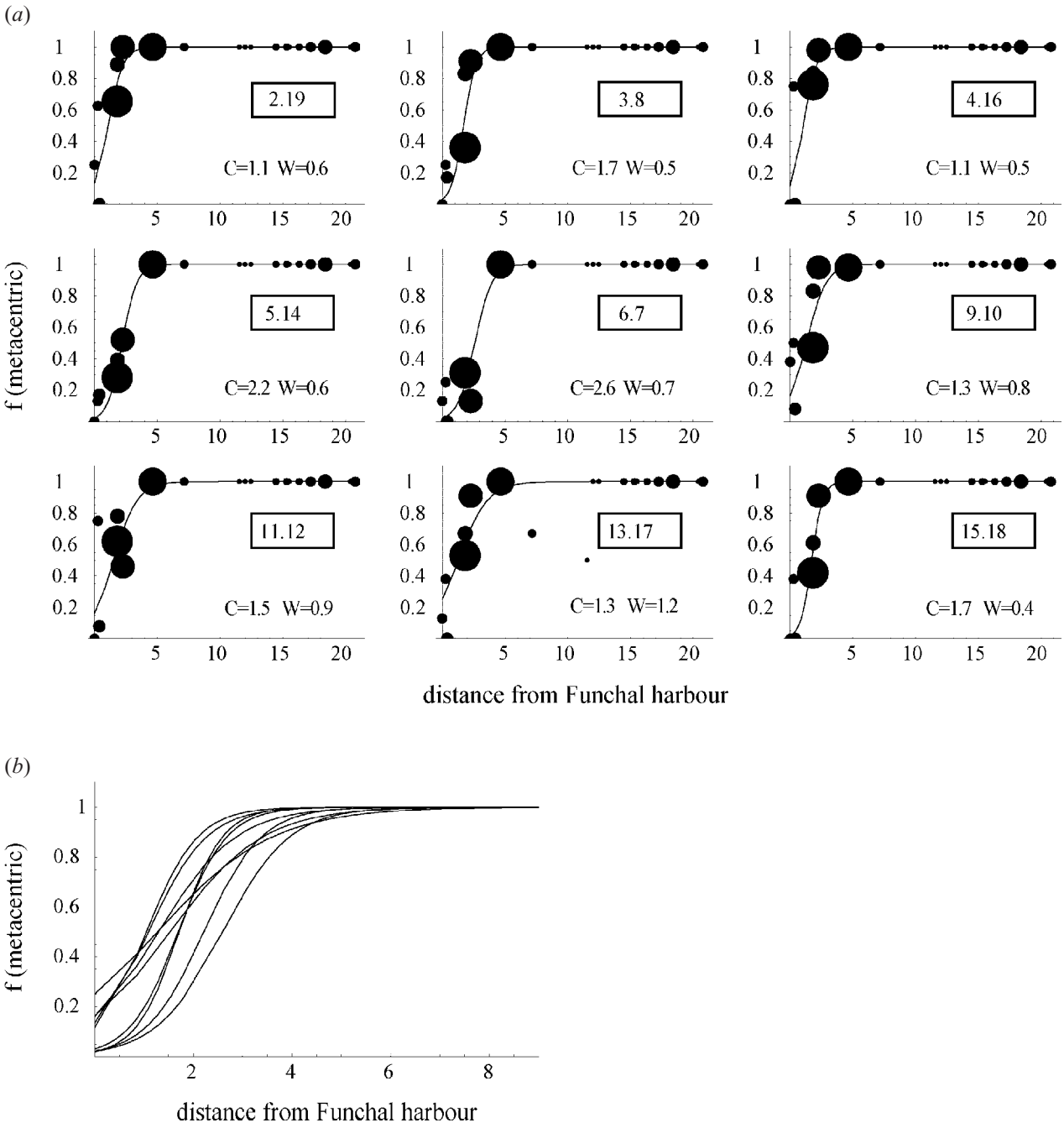

Fig. 2. Changes in frequencies of each Rb fusion as a function of distance in kilometres. (*a*) Detail for each fusion. Distance zero corresponds to Funchal harbour. For each cline, the centre (C) and the width (W) are provided in kilometres. The area of each point is proportional to sample size. (*b*) Comparison of the nine fitted clines, between 0 and 9 km only.

against hybrids. All computations were performed with Genepop software version 3.3 (Raymond & Rousset, 1995*b*).

In the clinal analysis, as the populations were not located on a transect, radial distances from the harbour of Funchal (0 km) to each population were used. A similar approach is commonly used in clinal studies of this species (e.g. Chatti *et al.*, 1999; Gündüz *et al.*, 2001). Details of sample sizes for each fusion and for each locality are given in Table 2. A logit function, $f(x) = e^{w(x-c)}/1 + e^{w(x-c)}$, was used to fit the clines, where x is the distance and w and c are respectively the width and the centre of the cline. The maximum likelihood of the fit for each fusion was estimated by a Metropolis algorithm (Szymura & Barton, 1986). A likelihood ratio test was performed to determine the significance of the differences in cline width (coincidence) and slope (concordance) between each pair of fusions. All analyses were computed using C-fit software (devised by T. Lenormand).

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Table 3. *Chi-square values of the coincidence* (*upper diagonal*) *and concordance* (*lower diagonal*) *tests of the fitted clines of the frequencies of metacentric chromosomes*

	Fusions								
Fusions	(2.19)	(3.8)	(4.16)	(5.14)	(6.7)	(9.10)	(11.12)	(13.17)	(15.18)
(2.19)	–	12·97	0·03	49·24	80·05	0·77	3·93	0·40	14·68
(3.8)	0·48	–	14·81	15·20	37·54	5·85	0·89	4·28	0·02
(4.16)	0·30	0·04	–	52·47	83·40	1·13	4·67	0·60	16·71
(5.14)	0·01	0·34	0·18	–	5·02	32·02	14·77	21·47	15·23
(6.7)	0·27	1·44	1·23	0·38	–	55·96	31·17	37·96	38·82
(9.10)	1·42	3·21	3·05	1·66	0·62	–	1·27	0·01	6·79
(11.12)	3·22	5·63	5·59	3·68	2·16	0·35	–	1·12	1·17
(13.17)	7·52	10·88	10·97	8·66	6·68	2·44	1·01	–	4·86
(15.18)	1·20	0·15	0·40	0·95	2·56	4·66	7·44	13·20	–

Shaded cells indicate significant χ^2 values (1 d.f.), at the level of $P<0{\cdot}05$, taking into account multiple testing. The slope of the Rb(2.19) cline is not significantly different from that of the others.

Table 4. *Details of the models fitted to the number of fusions per chromosome* (y)*, as functions of chromosome size* (x)

Fitted model		Residual deviance	χ^2	d.f.	P value	% of deviance explained
(*a*) First counting method[a]						
Null model						
	$y=c$	19·207				
Linear model						
	$y=bx+c$	17·153	2·054	1	0·13	10·7
Quadratic model						
	$y=ax^2+bx+c$	9·794	9·413	2	0·009	49·0
(*b*) Second counting method[a]						
Null model						
	$y=c$	23·596				
Linear model						
	$y=bx+c$	22·851	0·745	1	0·39	3·2
Quadratic model						
	$y=ax^2+bx+c$	9·581	14·015	2	0·0009	59·4

[a] For details of the counting methods see Section 2.

In case of multiple testing, the Bonferroni method (described in Sokal & Rohlf, 1995) was used to calculate the significance threshold, taking into account the number of tests sharing the same null hypothesis.

(iii) *Testing the equiprobability of fusing for each chromosome*

In house mice, chromosomal races are distributed in geographically disjoint Rb complexes which may share one to several Rb fusions. The origin of Rb fusions common to different races or complexes is debated, and two alternative views prevail. One of them considers that most Rb fusions have a unique origin, and have spread by migration between races and complexes. For example, in the Rhaetian Alps complex, the existence of the same fusion in nearby localities was interpreted as being mostly due to migration (e.g. Hauffe & Pialek, 1997). The other view favours an independent origin for most of these shared fusions. Riginos & Nachman (1999) succeeded in demonstrating two independent origins for the Rb(5.15) chromosome by the study of microsatellite markers.

Since the number of independent occurrences of each fusion cannot be precisely determined, two procedures were used to estimate the number of times a given autosome is involved in a Rb fusion from natural populations. In the first procedure, each fusion was counted only once, regardless of the number of times it occurred in different chromosomal races. This method assumes that each fusion has originated once and provides a minimum estimate of the number of fusions per chromosome. The second procedure took into account all fusions currently known in all races (e.g. a fusion found in two different chromosomal races was counted twice). Thus a maximum estimate of the

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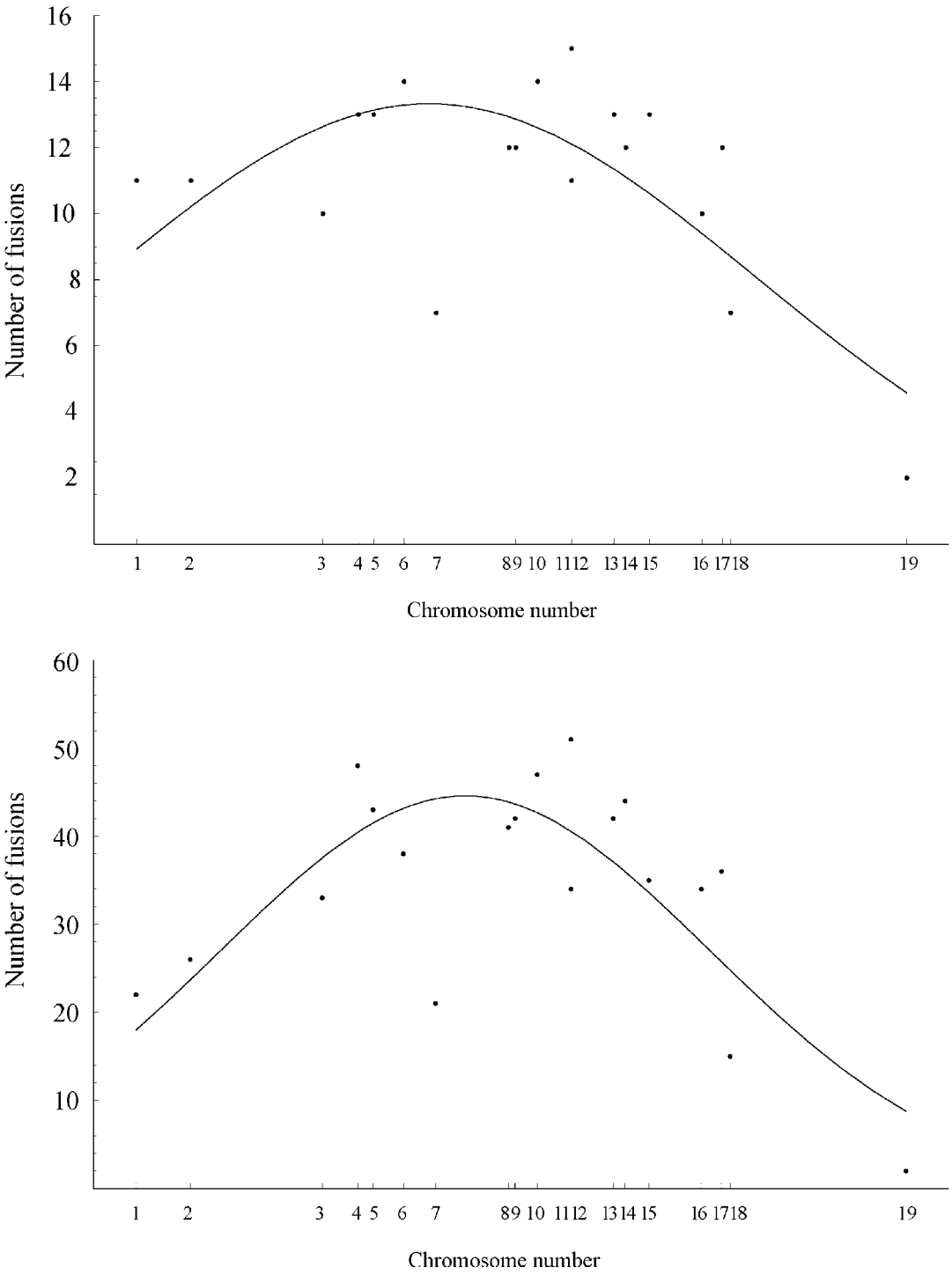

Fig. 3. Frequency of involvement of each of the 19 autosomes of Robertsonian fusions as a function of chromosome size. The line indicates the best fitting model, significantly different from a null model of equiprobability of fusing for each chromosome. The *x*-axis represents chromosome size in arbitrary units, presented from the largest to the smallest. Chromosome numbers are also indicated. According to the conventional cytogenetical nomenclature, the largest chromosome is numbered 1. Hence, sizes are represented in decreasing order. The upper graph corresponds to the first counting procedure, which considers each fusion only once. The lower graph corresponds to the second procedure, which counts the fusions as many times as they are found in different populations.

number of independent fusions was used. Data on the number of Rb fusions were collected from the literature (Gropp & Winking, 1972; Capanna *et al*., 1976; Gropp *et al*., 1982; Adolph & Klein, 1983; Winking *et al*., 1988; Bauchau, 1990; Hauffe & Searle, 1993; Hübner *et al*., 1994; Nachman *et al*., 1994; Nachman & Searle, 1995; Garagna *et al*., 1997; Britton-Davidian *et al*., 2000, 2002; Gündüz *et al*., 2000) and unpublished

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sources (Rb(1.15) and Rb(9.15): J. Britton-Davidian, unpublished data).

Generalized linear models were fitted to the data to explain the number of times each autosome is involved in a Rb fusion. The null hypothesis (equiprobability of fusing for all chromosomes) was tested against different alternative hypotheses, each one evaluating the possible influence of the chromosome size (independent variable) on the number of fusions (dependent variable). For this purpose, linear and quadratic functions were fitted to the data, to test whether these functions described the data better than chance alone. Among the various values of chromosome size available in the literature, we have chosen those published by Nesbitt & Francke (1973), as they are based on 325 karyotypes from both inbred and outbred animals. Because the data involved counts, Poisson error was used in the model. The significance of the terms of these models was tested according to the procedure of step-by-step simplification proposed by Crawley (1993), which compares the deviance of the fitted model before and after the withdrawal of a term. The change in deviance follows a chi-square with the appropriate degree of freedom. All these computations were performed with GLIM version 4 (Baker *et al.*, 1983).

3. Results

(i) *Clinal analysis of Rb fusion frequency*

Few standard individuals (2n = 40) were observed in Funchal harbour, i.e. in the area of the eastern Rb race of Madeira (2n = 22) on which we focused in this study. Together with the concentric pattern of hybrid diploid numbers around the port, this argues for passive immigration of standard mice by boat, and diffusion of acrocentric chromosomes outwards. For this reason, none of the nine fitted clines begins with a frequency of zero. The slopes of these clines (Fig. 2) were generally not significantly different from one another, except for Rb(13.17), for which the slope was shallower than those of Rb(3.8), Rb(4.16) and Rb(15.18) (Table 3). The slope of Rb(2.19) did not differ from the others ($P > 0{\cdot}20$, taking into account multiple testing). The centres of the different clines were often not coincident, but no particular pattern was evident (Fig. 2). These results suggest that, assuming a similar migration rate for all the fusions, Rb(2.19), heterozygotes are not more selected against than those for the other Rb fusions in the Funchal hybrids.

(ii) *Population genetic analysis*

No significant genotypic association was found ($\chi^2 > 20{\cdot}61$, d.f. = 8, all P values NS after a Bonferroni correction, $k = 36$ tests) between fusions across all populations. The absence of linkage disequilibrium suggests independence of all fusions. Consequently, the frequency of Rb(2.19) in the populations may be thought not to be driven by a strong association with another Rb fusion.

Heterozygote deficiency was not significant for any of the fusions (global test across populations for each fusion, $-0{\cdot}1033 < \hat{F}_{IS} < 0{\cdot}21$; all $P \gg 0{\cdot}05$, after a Bonferroni correction, $k = 9$ tests). Though the power of our test was moderate, it was sufficient to detect strong selection (Rousset & Raymond, 1995). We can therefore conclude that there is absence of strong selection against any of the fusions when heterozygous, including Rb(2.19) ($\hat{F}_{IS} = -0{\cdot}0109$; $P > 0{\cdot}6$).

(iii) *Testing the equiprobability of fusing for each chromosome*

The model that best predicts the fusion probability of a given chromosome is a quadratic function of its size, regardless of the counting method (Table 4). This model explains approximately 49·0% and 59·4% of the deviance with the first and the second counting method, respectively. In both cases, chromosomes with an intermediate size seem to be more often involved in Rb fusions than those of extreme sizes. Notice that the mode of the theoretical distribution is not centred on the mean size but shifted towards large chromosome sizes, suggesting that the nature or the intensity of the processes lowering the contribution of large and small autosomes in Rb fusions could be different. Differences between observed and fitted values (residuals) show that several chromosomes display stronger residuals than chromosome 19: chromosomes 3, 7 and 17 using the first counting procedure, and chromosomes 4, 7, 12, 14, 17 and 18 using the second procedure. This suggests that, once the size effect is taken into account, chromosome 19 has a normal chance of being involved in a fusion. Note that in both models the worst prediction corresponds to chromosome 7 (Fig. 3), for which the observed number of Rb fusions is markedly less than the expected value regardless of the counting procedure.

4. Discussion

(i) *Selection against Rb(2.19) heterozygous*

The analysis of the clines for the nine Rb fusions in Funchal indicated that significant differences were present between some pairs of centres and slopes. However, the nine clines taken together globally show a similar form, and no fusion displays a pattern significantly different from the eight others. The differences observed between some of the clines may reflect subtle differences in selection against fusions when heterozygous, but they also may be due to sampling or genetic drift. The most remarkable result is that

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Rb(2.19) does not appear to be more selected against than the other Rb fusions. Thus, a fusion involving chromosome 19 does not seem to differentially disfavour the individuals that carry it, compared with the others. All the results of the clines are interpreted under the hypothesis of identical migration rates for the nine fusions, because to our knowledge there is no reason to consider that individuals bearing these various fusions migrate differently.

The population genetic analyses revealed the absence of preferential associations between Rb fusions, indicating that all fusions behaved independently. Thus, the change in frequency of Rb(2.19) along the hybrid zone, which did not significantly differ from that of the other fusions (cf. results of the cline), may not be attributed to a strong association with another Rb fusion. Additionally, the absence of a heterozygote deficit for each fusion indicated that neither Rb(2.19), nor any other fusion in the eastern race of Madeira, seemed to be strongly selected against when heterozygous. Thus, there is no reason to consider the probability of fixation of Rb(2.19) to be different from that of the others. These results are consistent with those obtained by Auffray *et al.* (2001) for Rb(4.19).

(ii) *The effect of size on fusion probability*

In natural populations of *M. m. domesticus*, a significant relationship exists between the size of a chromosome and its probability of fusing. According to our model, between 49·0% and 59·4% of the variance in the number of fusions per chromosome can be predicted by chromosome size. The relation between the size and the number of fusions appears to be quadratic. This result suggests: (1) mechanisms that prevent some fusions from being formed (constraints), and/or (2) mechanisms acting once the fusions have appeared (selection). Contrary to constraint, selection would act once the fusion is formed, and so would act on metacentrics. Hence, the question is how the final size of a metacentric can be the factor that explains the greater involvement of acrocentrics of intermediate size in fusions. Selection favouring medium-sized metacentrics easily explains the higher involvement of medium-sized acrocentrics in the formation of Rb fusions.

Chromosome size may be adaptive and precisely regulated by selection within each species. For example, chromosome size is thought to affect recombination rate (Kaback *et al.*, 1992; Qumsiyeh, 1994). Furthermore, Rb fusions induce a chiasma repatterning on each arm (Castiglia & Capanna, 2002). Hence, selection for an optimal recombination rate may select for an optimal chromosome size (medium-sized), explaining the bell-shaped distribution. The trait under selection may be the variation in chromosome size among chromosomes of an individual. Advantages to lowering this variation may exist. Some authors have shown that synchronous chromosome behaviour is necessary for a correct functioning of mitosis and meiosis (Skibbens & Hieter, 1998). A great variance in chromosome size would lead to an important variation in the timing of chromatids division (long chromatids are linked more strongly and so divide more slowly than short ones), leading to severe perturbations of cellular divisions (Skibbens & Hieter, 1998). Such a hypothesis of equalizing selection is supported by Gorlova & Gorlov (2000). An advantage to lower variation of arm size within a chromosome may also exist. White (1973) proposed that non-disjunction rates during meiosis may be higher in metacentrics involving arms of quite different lengths, because of the asymmetric configurations formed. A selection against such metacentrics would explain the greater involvement of medium-sized acrocentrics in Rb fusions.

The mechanisms discussed above are those that may explain how the size of a chromosome may influence the involvement of this chromosome in a fusion. They are presumed to concern medium-sized chromosomes and extreme-sized ones in a different way but they are not expected to affect large and small chromosomes differently. However, in our model the mode of the distribution is not centred on the mean size, but shifted towards large chromosome sizes. This feature indicates that small acrocentrics are less involved in Rb fusions than large ones. No fusion is reported between the three smaller chromosomes, but there are such fusions among the three larger ones. A constraint may prevent tiny metacentrics from appearing. Such a possibility is supported by the data collected by Imai (1978) on 16 817 individuals in 723 mammalian species. He depicted, for each type of chromosome (acro-, telo- and metacentric), the distribution of their frequency as a function of their size. For each type, this author found a bell-shaped curve. The curve for the metacentrics is shifted towards larger sizes of chromosomes, and displays a striking absence of tiny metacentrics in mammals. This may reflect a constraint in mammals, preventing fusion of small acrocentrics. Concerning the house mouse, a deficit in small metacentrics as compared with large ones was proposed (Y. Kurihara, personal communication) to be related to the presence of nucleolar organiser regions (NORs), which are exclusively located in the centromeric regions of rather small chromosomes (12, 15, 16, 18 and 19) in the house mouse (Henderson *et al.*, 1976). However, the existence in Madeira of one fusion between NOR-bearing chromosomes (Rb(15.18)) indicates that there is no constraint that prevents this kind of fusion from appearing. This rather suggests that such fusions are more likely to be selectively disadvantageous.

Despite the strong effect of size to predict chromosome involvement in Rb fusions, not all chromosomes

fit neatly into this pattern. This suggests individual variations of fusion propensity for chromosomes, superimposed on the general fusion pattern. These differences may be related to molecular specificities of the centromeric region.

In summary, we have established that, although infrequent in the wild, fusions involving chromosome 19 are no more infrequent than expected, given the chromosome's size. Compared with other fusions of the same hybrid zone, Rb(2.19) does not display evidence of strong selection against it, supporting the fact that fusions involving chromosome 19 are not particularly different from those involving other chromosomes. The unexpected importance of chromosome size for predicting their involvement in Rb fusion was uncovered. However, more detailed knowledge of molecular mechanisms will be essential for a thorough understanding of the formation of Rb fusions in the house mouse.

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