Contact zones between chromosomal races of *Mus musculus domesticus*. 1. Temporal analysis of a hybrid zone between the CD chromosomal race (2*n*=22) and populations with the standard karyotype

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A temporal analysis of a hybrid zone between chromosomal races of *Mus musculus domesticus* was performed to evaluate its dynamics over a 20-year period. The transect chosen was one previously analysed by Spirito *et al.* (1980) across the hybrid zone between the chromosomal race CD 2*n*=22 (central Italy) and the surrounding populations with standard karyotype (2*n*=40). The results show that 20 years of hybridization have not had any significant effect on the position of this hybrid zone, nor on the location of its extreme margin, except for one site for which passive transport of mice may have been important. The absence of a significant shift of the tension zone excludes a possible imbalance between the two cytotypes; a fitness superiority of one homozygote greater than 0.01 would have produced an appreciable shift of the zone. The internal chromosomal structure of the zone shows a constant gradual increase in metacentric chromosome frequency towards the localities nearest the area inhabited by the 22-CD homozygote population. This contrasts with the previously reported data which indicated an irregular trend, particularly evident for the Rb(3.8), Rb(5.17) and Rb(6.13) chromosomes. For each Rb metacentric, we observed greater values of F_{ST} in the past sample than in the present one. These differences reveal a tendency towards the homogenization of adjacent populations, perhaps because of greater migration between adjacent demes resulting from increased commercial traffic. The Mantel test was performed for both temporal samples. A positive correlation between the geographical and chromosomal differentiation was demonstrated.

Keywords: hybrid zones, *Mus musculus domesticus*, Robertsonian races.

Introduction

The dynamics of hybrid zones and their evolutionary significance are topics of great interest (Barton & Hewitt, 1985; Harrison, 1990). The notion that hybrid zones are ephemeral, leading to introgressive fusion or reinforcement, is based on the assumption that they are unstable (Harrison, 1990). By contrast, if hybrid individuals are better adapted to local ecological conditions, hybrid zones should be considered stable both spatially and temporally (Moore & Buchanan, 1985). Moreover, mathematical models describing the dynamics of tension zones (hybrid zones that persist in time in dynamic equilibrium between selection against heterozygotes and

migration) allow the possibility of changes in their position and width (Barton, 1979; Barton & Hewitt, 1985).

The best approach to test these different hypotheses is to perform direct long-term observations of hybrid zones. However, few such attempts have been made (Harrison, 1990 for a review). With few exceptions, all the temporal analyses concentrate on the morphological characters of the hybridizing taxa, owing to the scarcity of genetic data in the literature. In fact, only taxa with a long history of genetic studies can be used (Polyakov *et al.*, 1997). One of these is the house mouse *Mus musculus domesticus*, which has been the subject of cytogenetic studies since the 1960s (Gropp *et al.*, 1969; Capanna *et al.*, 1976); its high chromosomal variability leads to a large number of chromosomal races with a diploid number ranging from 2*n* = 40, all telocentric

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chromosomes, to $2n = 22$ with nine Robertsonian (Rb) fusions.

We re-analysed an 18-km transect, located in central Italy, crossing the hybrid zone between the chromosomal race CD $2n = 22$ and the surrounding populations with standard karyotype ($2n = 40$) of *M. m. domesticus*. This transect was previously examined by Spirito *et al.* (1980) using a cytogenetic approach. Their study was based on about 40 animals collected in 1976 (M. Cristaldi, pers. comm.). Hoechst 33258 staining allowed identification of the chromosomal arms of Rb metacentrics for 20 animals only. Sampling for the present study was carried out between December 1996 and April 1998. Therefore, more than 20 years had elapsed since the analysis by Spirito *et al.* (1980). This period of time in the house mouse, in a temperate Mediterranean region, corresponds to around one hundred generations because of its continuous reproductive activity and precocious sexual maturity. We have found pregnant or lactating females from March to November and, according to the data of Berry (1981) and Pelikán (1981), this means four or five generations per year.

This new analysis is interesting because it can provide evidence of some factors affecting the dynamics of the hybrid zone. The supposed selective superiority of the chromosomal rearrangements (Sage *et al.*, 1993) could be verified if we were to reveal a shift in the position of the zone. The importance of the commensalism with man, which promotes intense passive transport, can be seen by the evidence of long-distance spread of mice characterized by different cytotypes. We will be able to detect any temporal changes in the chromosomal structure of the populations, and a comparison between the features of the area presently investigated and that of an adjacent transect of the same hybrid zone (Corti & Ciabatti, 1988), with different geographical and human activity patterns, will elucidate the role played by geographical factors in the process.

We have planned an extensive analysis of this hybrid zone which will comprise all aspects related to the fertility of the hybrids and the efficiency of the hybrid zone as a barrier to genetic and chromosomal flow, as well as a careful analysis of the genetic and chromosomal structure of each deme. In this paper we report on the evaluation of the changes that have occurred in the hybrid zone after 20 years.

Materials and methods

Seventy-five animals were trapped between 1996 and 1998 along the transect in 10 localities between 1.3 and 4.4 km apart (Fig. 1 and Table 1). The distance between localities was measured along the valley road that connects them. Six localities are the same as those
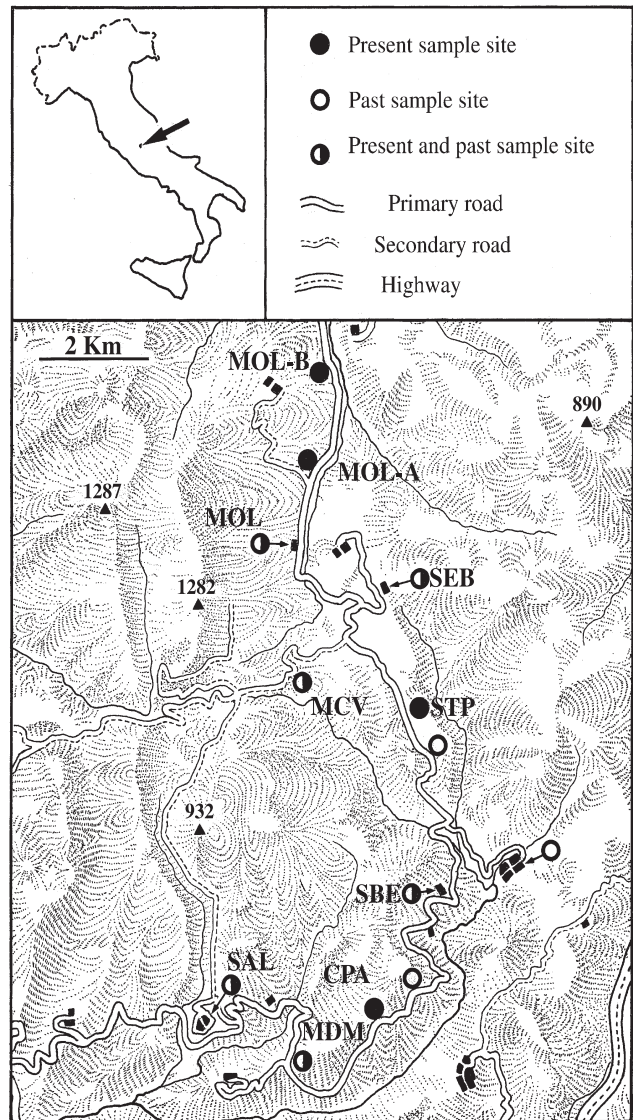

Fig. 1 Location of sample sites along the Sabina transect.

studied by Spirito *et al.* (1980): Salisano (SAL), Madonna del Mattone (MDM), San Benedetto (SBE), Montecavallo (MCV), San Sebastiano (SEB; called Monte San Giovanni in Spirito *et al.*, 1980) and Le Mole (MOL). At the Colle Paradiso (CPA) and Santo Pietro (STP) villages, the animals were collected 1 km and 400 m, respectively, from the sites of the previous sample (see Fig. 1). Animals were also trapped at two new sites near Le Mole at the end of the transect in the area of the homozygous 22 chromosome population (Mole-A and Mole-B).

Karyotype analyses were performed on somatic metaphase plates from both bone marrow preparations, following the standard air-drying procedure suggested by Hsu & Patton (1969), and cultured cells from ear

Table 1 Sample site, sample size, location, mean diploid chromosome number, mean structural heterozygosity (*Ko*), number of heterozygous chromosomes per individual (mean value and range) and frequencies of metacentric chromosomes in the sample sites along the transect of house mice (see also Fig. 1)

Site	N	Km from SAL	$2n$ (mean)	Ko	Mean number of het. chromosomes per individual (min–max)	Freq. of metacentric chromosomes								
						1.7	2.18	3.8	4.15	5.17	6.13	10.11	9.16	12.14
SAL	6	0	37.5	0.24	2.17 (2–3)	0	0	0	0.08	0.42	0	0	0.5	0.25
MDM	16	1.3	39.8	0.02	0.19 (0–1)	0.03	0	0	0	0	0	0.03	0.03	0
CPA	15	2.9	38.8	0.07	0.67 (0–2)	0.1	0	0	0	0.07	0.33	0	0.1	0
SBE	12	5.6	35	0.39	3.5 (0–7)	0.29	0.17	0.42	0.21	0.42	0.21	0.08	0.46	0.25
STP	7	10	26.3	0.32	2.86 (1–5)	0.79	0.71	1	0.93	0.57	0.93	0.57	1	0.36
MCV	4	11.3	24.75	0.14	1.25 (0–2)	1	0.87	1	0.75	0.75	0.75	1	1	0.5
SEB	1	12.6	22	0	0	1	1	1	1	1	1	1	1	1
MOL	2	14.3	22.5	0.05	0.5 (0–1)	1	1	0.75	1	1	1	1	1	1
MOL-A	4	16.3	22	0	0	1	1	1	1	1	1	1	1	1
MOL-B	8	18.3	22	0	0	1	1	1	1	1	1	1	1	1
Total	75													

biopsy (Stanyon & Galleni, 1991). G-bands induced according to Seabright's routine (1971) allowed a correct assessment of the Rb metacentrics. An unbiased estimate of the *P*-value of Fisher's exact test was computed with the GENEPOP 3.1B program, an updated version of GENEPOP 1.2 described in Raymond & Rousset (1995), to detect any temporal differences in the chromosomal frequencies in those polymorphic populations for which direct comparison was possible: Salisano, Madonna del Mattone, San Benedetto and Montecavallo. To test for population differentiation between the spatially and temporally separated samples, we calculated F_{ST} values using the GENETIX 3.3 program (Belkhir *et al.*, 1996–98) and the *P*-value for $F_{ST} > 0$ according to Weir & Cockerham (1984). The significance of the F_{ST} values was tested using 5000 permutations. The Mantel test (Mantel, 1967) was performed for the present and past samples to test the isolation by distance model with the program ISOLDE of the GENEPOP package (Raymond & Rousset, 1995) using 10 000 permutations. As input for the two matrices we used pairwise F_{ST} values and geographical distances. Relationships were plotted according to Slatkin (1993) as $\log_{10}(M) = (1/F_{ST} - 1)/4$ against $\log_{10}$ distance (*d*).

Results

Table 1 shows the locality, the number of specimens studied, the mean diploid number, the average structural heterozygosity (*Ko*), the mean number of heterozygous chromosomes per individual and the frequencies of each metacentric chromosome for each locality. The same data have been inferred from the paper of Spirito *et al.*

(1980) and partly compared with the present data in Figs 2 and 3. The graph showing the mean diploid chromosome numbers (Fig. 2a) indicates a close correspondence between the two sets of data. For the average heterozygosity in the population (Fig. 2b), the comparison shows the same pattern of variation in the two samples: higher in the centre of the transect than at the extremities. In both samples, the maximum values were found in the village of San Benedetto. These values are very similar, i.e. $Ko = 0.37$ in 1976 and $Ko = 0.39$ in the current sample. Minor variations were found in both diploid number and structural heterozygosity at a local level. The mean number of chromosomes in the heterozygous condition per individual is high in the populations at the centre of the transect, i.e. SBE (mean 3.5) and STP (mean 2.9) in which extreme individuals were found with seven and five chromosomes in heterozygosity, respectively. Spirito *et al.* (1980) found similar mean values but the maximum number of metacentrics in the heterozygous condition was four in the SBE population. The approximate cline width of the zone can be estimated using the definition of Barton & Gale (1993) (inverse of maximum slope) on a graph plotting the average frequencies of the Rb metacentrics in each population against the distances along the transect. The graph (Fig. 2c) representing the past and present situations shows a close correspondence, with a width equal to 9100 m.

In spite of the correspondence in diploid numbers, the chromosomal structure of the zone, as revealed by the frequencies of each Rb fusion, appear to have changed (Fig. 3). In the present sample, the trends of the frequencies of the metacentrics show a constant gradual
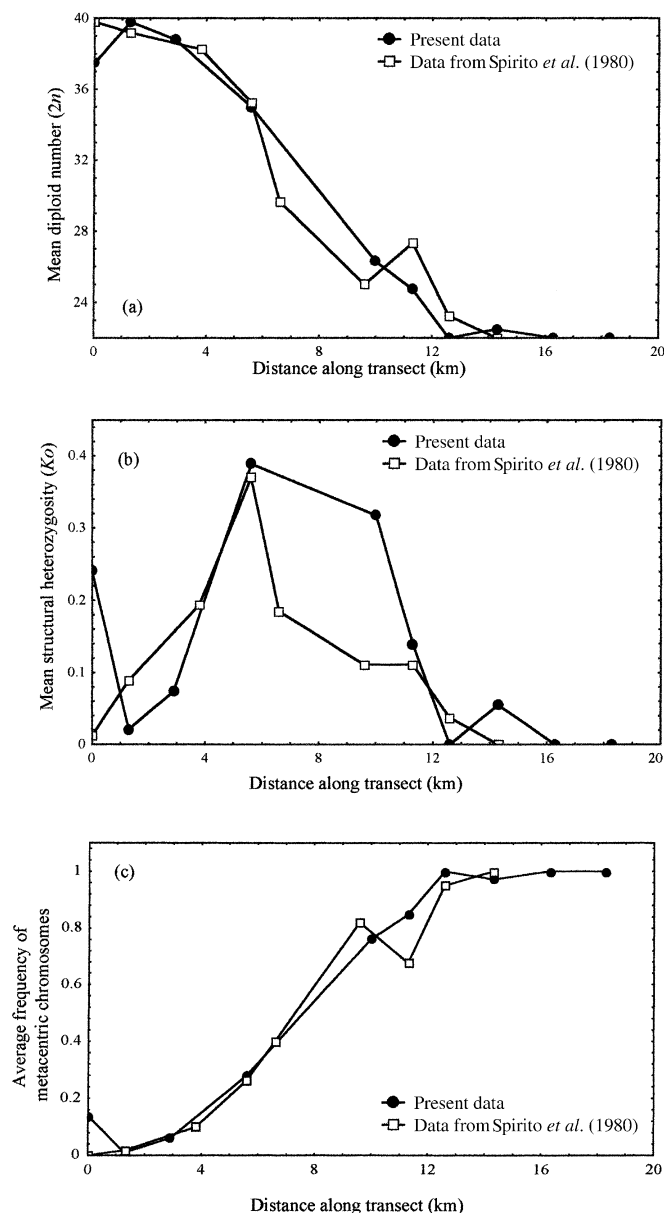
Fig. 2 The variation in (a) mean diploid chromosome number (2n), (b) mean structural heterozygosity (Ko) and (c) average frequency of metacentrics in house mice along the transect, according to Spirito *et al.* (1980) (□) and the present observations (●). The distance between localities was measured along the valley road that connects them.

increase towards the locality nearest the area inhabited by the 22-CD homozygote population. These findings contrast with the previously reported data (Spirito *et al.*, 1980) which indicated an irregular trend for the distribution of chromosomal frequency even in nearby populations.

This is evident for Rb(3.8), Rb(5.17) and Rb(6.13). Significant differences in the chromosomal frequencies

of two of these chromosomes have been detected in two populations: in Montecavallo Rb(3.8) is now present (Fisher's exact test, $P = 0.002$) (double arrow in Fig. 3c) and in San Benedetto the frequency of Rb(6.13) has decreased (Fisher's exact test $P = 0.009$) (double arrow in Fig. 3f).

Other differences emerged from a comparison of the chromosomal frequencies (Fisher exact test). In Salisano four different metacentrics are now present, i.e. Rb(4.15), Rb(5.17), Rb(9.16), Rb(12.14), whereas only one (not identified) was found with a low frequency in the previous sample. The frequencies are statistically different for Rb(5.17) ($P = 0.007$) and Rb(9.16) ($P = 0.002$) (double arrows in Fig. 3e,g).

Figure 3(h) shows a clinal trend of two Robertsonian metacentrics, i.e. Rb (10.11) and Rb (12.14), which were not distinguished by Spirito *et al.* (1980) because of the low definition of the banding pattern obtained by the Hoechst method.

We computed the interlocality karyotypic heterogeneity (F_{ST}) as a measure of the among-subdivision variance in chromosomal frequencies. Overall F_{ST} (Table 2) values calculated for each Rb metacentric for all polymorphic populations were very high in both samples, indicating strong differentiation between subpopulations. In the present sample, the F_{ST} values range from 0.22 to 0.78, all values being significantly greater than zero ($P < 0.001$). For all Rb metacentrics, $F_{ST} = 0.55$ ($P < 0.001$). For the Spirito *et al.* (1980) sample, the F_{ST} values are higher for each metacentric (range 0.67–0.94), all values being significantly greater than zero ($P < 0.001$). Overall $F_{ST} = 0.80$ ($P < 0.001$). These values demonstrate that the populations were more differentiated in the earlier sample than in the present one. A lower level of migration between localities in the past might account for this pattern. Chromosomal differences between the temporal samples were found to be significant (pairwise F_{ST} values) in Salisano $F_{ST} = 0.44$ ($P < 0.01$), San Benedetto $F_{ST} = 0.17$ ($P < 0.02$) and Montecavallo $F_{ST} = 0.48$ ($P < 0.01$). The Mantel test performed for both temporal samples revealed a positive correlation between the geographical and chromosomal differentiation. The probability of the association being random was, respectively, 0.017 and 0.0005. Pairwise values related to the present (circles) and the past samples (squares) are plotted in Fig. 4.

Discussion

Our results show that 20 years of hybridization have not had significant effects on the position of this hybrid zone, nor on the location of its extreme margin. Thus the hybrid zone does not change its position or width
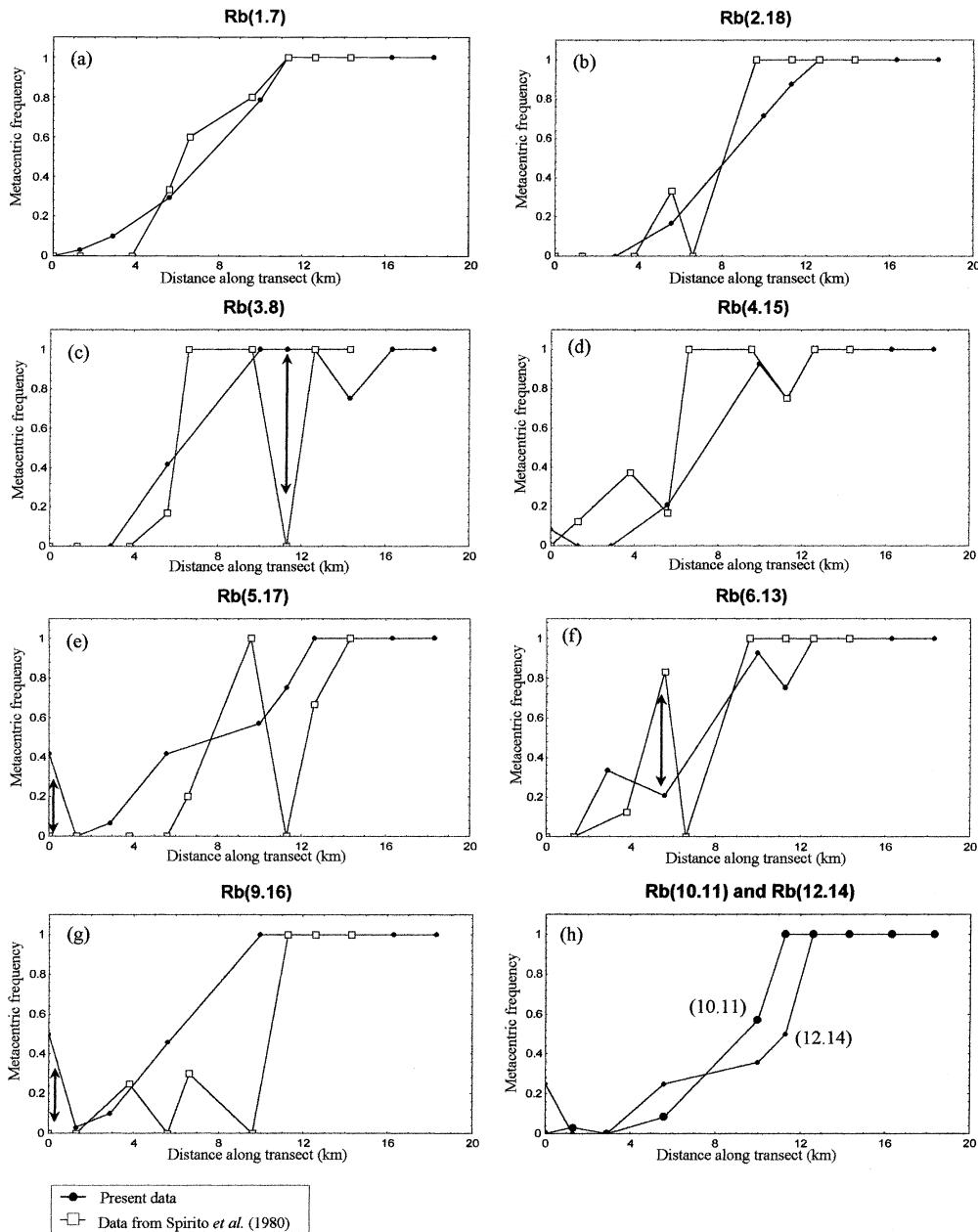
Fig. 3 (a–g) The variation in metacentric frequencies across the house mouse hybrid zone transect in 1976 (□) and in 1997–98 (●). (h) The variation in the frequencies of Rb(10.11) and Rb(12.14) in the present sample. The distance between localities was measured along the valley road that connects them. Double arrows indicate significant differences in frequencies between the two temporal samples.

rapidly. Nonetheless, the consistent presence of metacentric chromosomes in Salisano, described by Spirito *et al.* (1980) as a border deme with standard karyotype (only one $2n = 39$, out of nine mice, was found) could be considered of some interest. It may be the result of a recent passive introgression. Indeed the animals in this site were trapped in an olive oil mill where numerous trucks stop during the olive harvest period. Mice could

thus easily be transported from neighbouring areas. This hypothesis is supported by the high structural heterozygosity ($Ko = 0.24$) and by the high number of metacentrics in heterozygosity (range 2–3) in SAL with respect to the marginal position of this sample on the transect. These characters can be considered as arising from crossings of local all-telocentric mice with mice bearing metacentric chromosomes in homozygosity.

Table 2 Interdeme karyotype heterogeneity (F_{ST}) of house mice for each Rb metacentric and for all the metacentrics computed for the present data and for the data of Spirito *et al.* (1980)

Metacentric	F_{ST} Present data	F_{ST} Spirito *et al.* (1980)
Rb(1.7)	0.541	0.701
Rb(2.18)	0.630	0.903
Rb(3.8)	0.779	0.944
Rb(4.15)	0.658	0.733
Rb(5.17)	0.342	0.672
Rb(6.13)	0.495	0.883
Rb(10.11)	0.639	—
Rb(9.16)	0.575	0.691
Rb(12.14)	0.223	—
All Rb	0.553	0.798

We found animals with high chromosomal heterozygosity in the locality at the centre of the transect; the maximum number observed was seven (found in SBE). Because such high heterozygosity has a detrimental effect on fertility (for reviews see Redi & Capanna, 1988 and Hauffe & Searle, 1998), we assume that these chromosomal clines are maintained by a selection–dispersal balance (Barton & Hewitt, 1985). The first step is to verify the supposed selective imbalance between the two homozygote chromosomal races. Barton (1979) suggested a mathematical model showing that a tension zone, involving a single-locus cline, can move in response to a selective imbalance between the two homozygotes. The speed of the movement is $(S/2)\sqrt{2m}/\sqrt{s}$, where $2S$ is the superiority of one homozygote over the other and
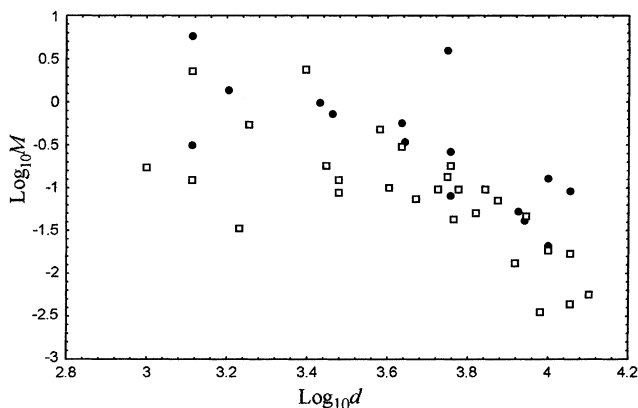

Fig. 4 Pairwise F_{ST} values for karyotypic heterogeneity in house mice and distance between sample sites: present data (●) and data from Spirito *et al.*, 1980 (□). Relationships were plotted according to Slatkin (1993) as $\log_{10}(M) = (1/F_{ST} - 1)/4$ against $\log_{10}$ distance (d).

Table 3 Expected speed of the zone as a result of the action of different values of selective superiority ($2S$) of one homozygote over the other, according to the model of Barton (1979) (see text)

Homozygote superiority $2S$ (Barton, 1979)	Expected speed (m/gen.)
0.01	22.75
0.02	45.5
0.04	91
0.08	182

$\sqrt{2m}/\sqrt{s}$ is the width (w) of the zone in a tension zone model, m being the variance in progeny position per generation and s the fitness deficit for the hybrid. With the width of the zone assumed to be 9100 m, Table 3 shows, for different values of $2S$, the expected shift per generation of the zone according to the Barton (1979) model. A selective imbalance between the two homozygotes produces a sensible shift of the zone, into the less fit race. For values of $2S$ between 0.01 and 0.02, the estimated shift over 100 generations is between 2275 and 4550 m, respectively. For higher values of $2S$ (0.04 and 0.08) the shift becomes quite marked (9100 m and 18 200 m, respectively). Therefore the positional stability of this transect does not agree with the hypothesis of a selective superiority of one chromosomal race over the other, at least in the range of the selective disadvantage values used in this analysis.

However, the position of hybrid zones can be maintained if different parental types are favoured in different ecological conditions (Howard & Warings, 1991). The area of hybridization we analysed is situated on the border between the Apennine zone (mesophilic) and the alluvial plain of the Tiber River (thermoxerophilic) (Tomaselli *et al.*, 1973). Ecological constraints could thus contribute to the separation of the two chromosomal races, as has been hypothesized for other hybridization areas of *M. m. domesticus* (Corti & Thorpe, 1989; Said & Britton-Davidian, 1991; Searle *et al.*, 1993). In support of this view, significant differences have been found in litter size between the CD race and the standard one (mean litter size CD = 6.2, standard = 4.5; see Castiglia, unpubl. obs.). This difference in reproductive output may represent a different demographic strategy related to the habitat segregation (Said *et al.*, 1993).

Capanna & Redi (1994) suggested a model in which the chromosomal configuration can be a selective factor. It is based on the possibility that Rb fusion can alter the spatial organization of the interphase nucleus, changing the gene cluster locations into nuclear domains programmed for regulatory functions. Hence the different expression of the genes could affect relative fitnesses under different environmental conditions.

The spatial stability we detected in this hybrid zone might also be explained by the tension zone model, which shows how natural barriers or regions of low population density can trap tension zones (Barton, 1979). In this area, no true geographical barriers are present, although human settlements can play a role in spacing the microdemes of mice that are commensal with man. Villages, or small groups of houses, are present in a continuous pattern throughout the transect, although a thinning of buildings can be observed between San Benedetto and Santo Pietro (Fig. 1). The Mantel test showed a significant correlation between interdeme distance and pairwise F_{ST} values in both temporal samples. Hence the greater distances between human settlements in the centre of the transect than at the margins can influence (i.e. lessen) the migration between populations, acting as a partial barrier to genetic flow. In this regard, it is important to underline the geographical character of this transect: all the human activities are concentrated along this single valley route and it determines the pattern of spreading of the mouse populations. An alternative route between SAL and MCV exists (Fig. 1) but it runs through mountain forests and lacks stable human settlements. Consequently it is not suitable for house mouse populations.

Corti & Ciabatti (1988) found no evidence of clinal variation in diploid chromosome number nor in the frequencies of any one Rb metacentric in another transect in the same hybrid zone located 60 km north of the area investigated by us. The geographical characters of the two transects are quite different, which can account for the different chromosomal structures. The transect studied by Corti & Ciabatti (1988) is located in a hilly, intensively cultivated area crossed by a network of country roads. Consequently the hybrid zone assumes a greater width and an unorientated structure as far as the distribution of the diploid number is concerned. A thorough analysis of the chromosomal structure of the two areas is in progress and will be presented in a further paper.

Although the hybrid zone was found to be stable, the chromosomal structure of its populations has been altered, leading to a smoother gradient in Rb frequency change than previously observed. This change is marked by significant differences in chromosomal frequencies and in pairwise F_{ST} values (significantly > 0) in three of the four localities for which direct comparison was possible. We observed greater values of F_{ST} for each Rb metacentric in the earlier sample than in the present one. These differences reveal a homogenizing of adjacent populations. Nonetheless, the underlying reason for this pattern is not easy to explain. A break in the local isolation could have occurred as a result of increasing migration between adjacent populations, caused by increases in commercial traffic or by recent improvements of the mountain agriculture in this district.

The present study has demonstrated the stability of this hybrid zone, at least over the short term. Several factors can account for this stability: the action of a partial barrier to genetic flow, represented by the central area of the transect where the populations are more spaced out; an absence of selective differences between the two different cytotypes; and adaptation to the different ecological conditions by the parental populations. Nevertheless, the spatial stability of a hybrid zone is not unequivocal evidence in support of a given hypothesis, as was stated by Moore & Buchanan (1985). In fact, 20 years of hybridization may not be long enough for slow movement of this hybrid zone. However, we demonstrate that within the hypothesized values, a selective imbalance between the two races would be revealed. The changes we observed in the internal structure of the demes of the hybrid zone, concerning chromosomal frequencies and F_{ST} values, can been explained in terms of a decrease of interdeme isolation and/or a more careful data analysis performed on a larger sample than that of Spirito *et al.* (1980). Fertility estimates and analysis of gene flow are presently in progress, in order to evaluate selection against heterozygotes and to investigate some aspects of this tension zone model.

Acknowledgements

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