

# Contact zones between chromosomal races of *Mus musculus domesticus*. 3. Molecular and chromosomal evidence of restricted gene flow between the CD race ( $2n = 22$ ) and the ACR race ( $2n = 24$ )

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The contact zone between the Ancarano (ACR;  $2n = 24$ ) and Cittaducale (CD;  $2n = 22$ ) races of *Mus musculus domesticus* was studied. We used chromosomes and mitochondrial DNA (mtDNA) sequences of the control region as genetic markers to detect introgression between races. In total, 76 mice were trapped at 17 localities. Cytogenetic analysis was performed on 73 mice. A segment of the control region (468 bp) was sequenced in 41 specimens. The two races are distributed parapatrically and the contact zone was identified inside a village (Pizzoli). No mixed population was found in the study area. The contact zone does not correspond to any geographical or ecological barrier but is located

in a zone of potentially high density of mice. The sequence analysis clearly demonstrates genetic differentiation between races (1.4% of sequence divergence). Hybridization is rare. Evidence of introgression was found in two individuals in the contact zone: one individual of the ACR race carries a metacentric belonging to the CD race, while another ACR individual carries a CD-like haplotype. In these ecological conditions, the observed distribution pattern and the very low level of hybridization suggest the presence of a premating mechanism of reproductive isolation. *Heredity* (2002) 89, 219–224. doi:10.1038/sj.hdy.6800129

**Keywords:** hybrid zone; house mouse; Robertsonian fusion; reproductive isolation

## Introduction

The long-tailed house mouse, *Mus musculus domesticus*, is one of the best studied cases of chromosomal variability, with many chromosomal races scattered throughout Europe and North Africa (Sage *et al.*, 1993). This variability is due to Robertsonian fusion between telocentric chromosomes which lowers the chromosomal number from the standard  $2n = 40$  all-telocentric karyotype to  $2n = 22$  characterised by nine pairs of metacentric chromosomes, with races of all the intermediate diploid numbers. One of the main questions raised by the discovery of such races is the amount of gene flow across the contact zones between chromosomal races (Capanna *et al.*, 1985). Since structural heterozygosity is expected to reduce the fitness of hybrid individuals, gene flow between races should be reduced. Moreover, gene flow can be affected by chiasma repatterning accompanying the chromosomal rearrangements (Bidau *et al.*, 2001; Rieseberg, 2001; Castiglia and Capanna, 2002). A number of chromosomal hybrid zones between chromosomal races, differing in the number and identity of metacentrics, have been investigated to try to answer the question. However, resolution of the problem is complicated by the lack of known genetic markers

between chromosomal races. In fact, the absence of genetic differentiation can be interpreted as the presence of free gene flow between races or it could be due to the recent formation of the races (Britton-Davidian *et al.*, 1989). Many studies have focused on the contact zones between Robertsonian races and the standard one (see Searle, 1993 for a review; Castiglia and Capanna, 1999a; Gundüz *et al.*, 2001a). All these studies have found hybrids showing trivalents at diakinesis, with hybrid zone widths from 0.5 km (Chatti *et al.*, 1999) to 30 km (Searle *et al.*, 1993). Fertility studies show that gene flow should occur freely between races differing in only a few independent metacentrics: heterozygotes for 1–3 metacentrics have normal levels of fertility (Winking *et al.*, 1988; Wallace *et al.*, 1992; Castiglia and Capanna, 2000). However, if the differences between races involve many independent metacentrics the fertility of structural heterozygotes is more severely impaired (Redi and Capanna, 1988; Said *et al.*, 1993; Castiglia and Capanna, 2000). In these extreme cases, both gene flow and the number of hybrid individuals is expected to be low, as in the Tunisian hybrid zone between the Monastir race  $2n = 22$  and the standard one (Chatti *et al.*, 1999).

Less well known are the contact zones between metacentric races (references in Searle, 1993). If the two races have metacentrics in the condition of monobrachial homology, heterozygotes show longer multivalent configurations at meiosis (chains or rings), resulting in more severe impairment of gametogenesis and even sterility

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(references in Searle, 1993; Hauffe and Searle, 1998). The only case of sympatry without hybridization was found by Capanna and Corti (1982) in Valtellina between the Poschiavo race (POS)  $2n = 26$  and the Upper Valtellina race (UV)  $2n = 24$  in the village of Migiondo. However, at present only the POS race lives in Migiondo, the other probably having been eliminated by competition due to aggressive interaction between males (Capanna *et al*, 1984; Hauffe and Searle, 1992).

In central Italy, three chromosomal races are present: the Ancarano race (ACR;  $2n = 24$ ), the Cittaducale race (CD;  $2n = 22$ ) and the Campobasso race (CB;  $2n = 22$ ). Notwithstanding the supposed parapatric distribution of these races, there have been no previous studies of their contact zones. In this study, we investigated the contact zone between the ACR ( $2n = 24$ ) and CD ( $2n = 22$ ) races, which are different for all the metacentrics. Thus they represent an extreme case of diversification between chromosomal races in *Mus*. At meiosis, first-generation hybrids show one long multivalent ring of 14 metacentrics and a shorter chain of five chromosomes. Laboratory-reared  $F_1$  males are completely sterile, based on electron microscopic observations of the testes (Malorni *et al*, 1982).

Many molecular studies indicate that, in *Mus musculus*, the mtDNA control region can be a very useful molecular marker to identify the phylogeographical pattern over wide geographical regions (Prager *et al*, 1993; Nachman *et al*, 1994) or to reconstruct the colonisation histories (Gundüz *et al*, 2001b). Phylogeographical analyses have shown that mtDNA control regions of mice from different Apennine metacentric races fall into different clades (Prager *et al*, 1993; Nachman *et al*, 1994). Thus we used control region sequences to detect the level of introgression in the contact zone between the CD and ACR races. The aims of this study were: (1) to find and characterize the zone of contact between these two extremely different races by cytogenetic analysis, and (2) to track the introgression of mitochondrial DNA between the different races in the zone of parapatry.

## Materials and methods

In total, 76 mice were trapped between June 1998 and March 2000 at 17 localities along the Aterno River (Figure 1, Table 1). In the Apennines, river valleys represent the main communication routes for humans; thus contact zones between chromosomal races of *Mus musculus* can easily be found along them. In the literature, the nearest localities known for the two races were Campotosto and L'Aquila, about 90 km away. The first step of the study was to locate the contact zone, followed by concentrated trapping inside it.

Karyotype analyses were performed on somatic metaphase plates from bone marrow preparations following the standard air-drying procedure suggested by Hsu and Patton (1969). G-bands induced according to Seabright's routine allowed correct identification of the Rb metacentrics (Seabright, 1971).

DNA sequences were obtained from 41 mice, representing a subset of the mice subjected to chromosomal analysis. Only three mice for which chromosomal data were not available were sequenced (one from site 10 and two from site 8). Total genomic DNA was obtained from muscle preserved at  $-70^\circ\text{C}$  or in 80% ethanol. DNA extraction was performed using the Qiagen method

(DNeasy tissue kit). Mitochondrial sequences containing a 1100 bp fragment encompassing the control region were isolated via the polymerase chain reaction (PCR). Amplified DNA was obtained by combining the primers L15320 (5'-ATAAACCATTACTCTGGTCTTGTAACCC-3') and H00072 (5'-ATTAATTATAAGGCCAGGACCAAACCT-3') (Nachman *et al*, 1994). Amplifications were performed in an MJ MiniCycler machine, using the following procedure: a first cycle of an initial denaturation at  $94^\circ\text{C}$  for 2 min, then 35 cycles with denaturation at  $94^\circ\text{C}$  for 1 min, annealing at  $55^\circ\text{C}$  for 1 min, and extension at  $72^\circ\text{C}$  for 3 min. These steps were followed by a 10 min extension at  $72^\circ\text{C}$ . Each PCR reaction was obtained using 50–500 ng of template DNA, 200 ng of each primer, 0.2 mM of each dNTP and 2 U of Taq polymerase (Promega) in a final volume of 50  $\mu\text{l}$ . Amplified products were purified using the Qiagen QIAquick purification kit and prepared for automated sequencing. A fragment of 468 bp was sequenced in 41 specimens (from position 15372 to 15840; numbering according Bibb *et al*, 1981) with primer L15320. This fragment encompasses the hypervariable region (Nachman *et al*, 1994).

Sequences were visualised with CHROMAS version 1.62, aligned with CLUSTAL X version 1.8. Genetic differences between races were assessed through nucleotide differences. mtDNA polymorphism indices were assessed through estimation of both haplotype (H; Nei, 1987) and nucleotide ( $\pi$ ; Tajima, 1983) diversity with ARLEQUIN version 2.0. A minimum spanning network between haplotypes was also constructed with ARLEQUIN version 2.0.

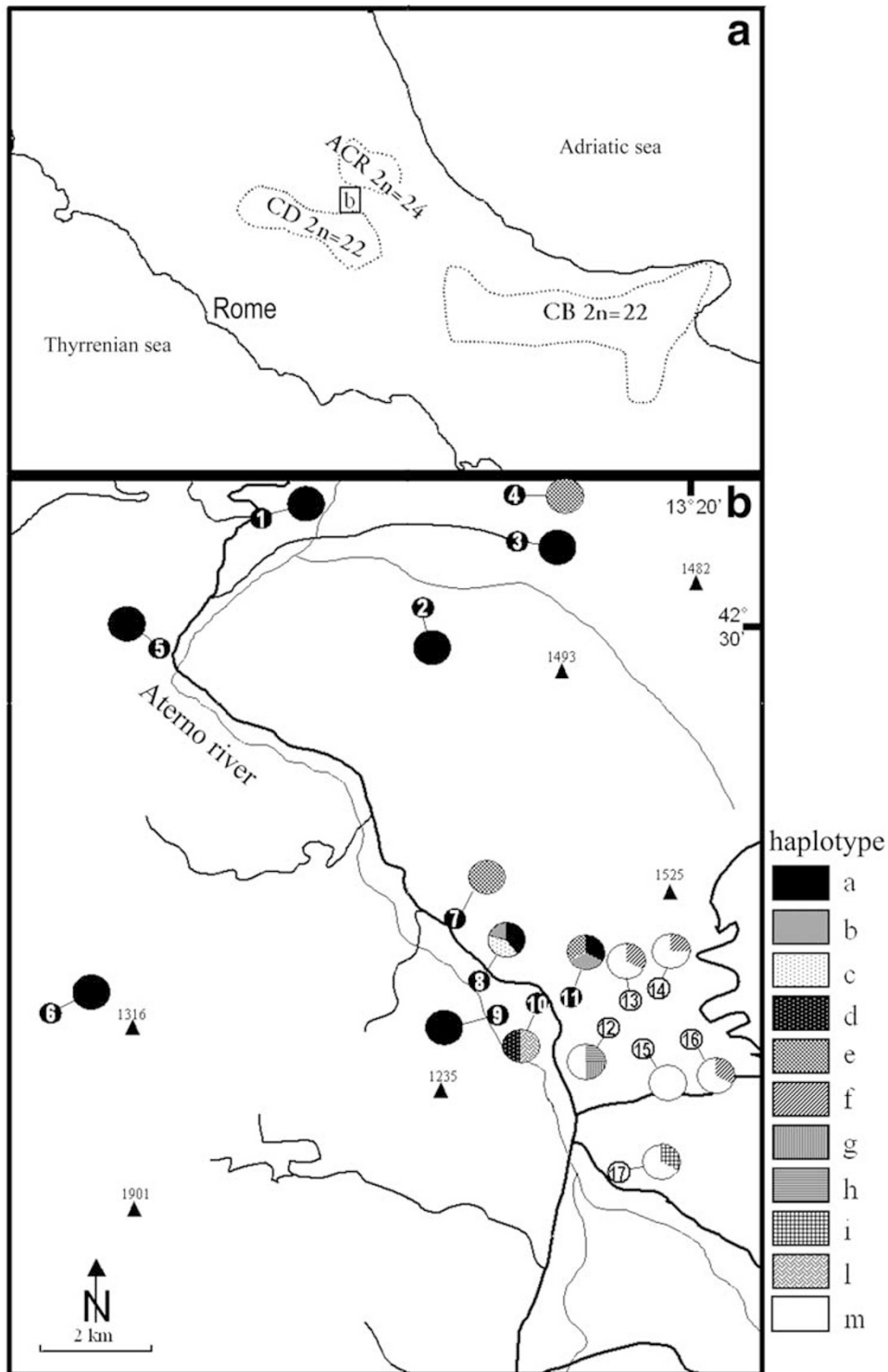
## Results

**Location and chromosomal structure of the contact zone**  
Figure 1 shows the location of the trapping sites of the analysed mice. Table 1 reports the number of mice analysed per site. All the mice except one (see below and Castiglia and Capanna, 1999b) have homozygous karyotypes conforming to the two races. The ACR race is distributed in the north-western part of the study area while the CD race is found in the south-eastern part. Both chromosomal races are found in the village of Pizzoli, extending along the main road for about 3 km. In this village, we trapped at nine sites (sites 8–16) and caught 39 mice. No mixed population was found in the study area. The nearest sites where different races were found are indicated in Figure 1 as 11 and 12; they are about 700 metres away. The contact zone does not correspond to any geographical or ecological barrier; to the contrary, it is located in a zone with a high density of human buildings, a habitat favourable to mice.

In population 11 (see Figure 1), we found a female with a karyotype not conforming to those of the two parental races. This mouse, described in Castiglia and Capanna (1999b), is characterised by a complex heterozygosity (a ring of four metacentrics) in an ACR chromosomal background. The differences consist in two metacentrics that are not present in the ACR complement: one derives from CD (6.13) and one is a new metacentric deriving by whole-arm reciprocal translocation (WART) (5.16).

### MtDNA analysis

Eleven haplotypes were identified (namely a–m) (Table 2) (GenBank accession number AF517776–AF517786): five



**Figure 1** (a) Distribution of Rb races in central Italy. (b) Distribution of karyotypes and control region haplotypes in the contact zone between the CD ( $2n = 22$ ) and ACR ( $2n = 24$ ) races. Closed and open small circles with locality numbers (Table 1) provide an accurate location of ACR and CD localities, respectively. Thin lines: rivers, thick lines: roads.

haplotypes are present in mice of the CD race and six haplotypes in mice of the ACR race. The number of polymorphic sites is 12 (nine transitions, three transversions). The sample size, frequencies of the observed haplotypes in the population and their geographical distribution are reported in Figure 1 and Table 2.

The two Robertsonian races are clearly differentiated

genetically. The mean number of differences is  $2.20 \pm 1.03$  among the CD haplotypes and  $2.40 \pm 0.97$  among the ACR haplotypes (excluding an introgressed specimen, see below). The mean number of differences between the CD and ACR haplotypes is  $7.04 \pm 0.84$  (1.4% of sequence divergence). Five differences (four transitions, one transversion) are fixed between the two races.

**Table 1** Number of mice subjected to cytogenetic and molecular analyses

Village	Site	Chromosomal analysis (no.)	Control region (no.)
Piedicolle	1	1	1
S. G. Paganico	2	1	1
Capitignano	3	6	1
Mopolino	4	2	1
Marana	5	3	2
Cascina	6	13	3
Barete	7	8	2
Pizzoli	8	10	5
	9	3	1
	10	1	2
	11	5	4
	12	4	4
	13	4	3
	14	5	4
	15	1	1
	16	3	3
S. Vittorino	17	3	3
<b>Total</b>		<b>73</b>	<b>41</b>

Figure 2 shows the minimum spanning network connecting the 11 haplotypes. Both races are characterised by one widespread haplotype with a high frequency: m-haplotype in the CD race (67% of CD mice) and a-haplotype in the ACR race (59% of ACR mice). The other haplotypes can derive from the most widespread ones through one or two substitutions (Figure 2). The distribution of the different haplotypes is not related to their geographical location. The only discordance between karyotype and mtDNA is present in one specimen (from site 10). Chromosomally, this mouse pertains to the ACR race but its haplotype (l-haplotype) clearly falls among those characterising the CD race (Figure 2; Table 2). The mouse with the ACR-like karyotype and carrying a WART (from population 11) has a haplotype conforming to those of the ACR race (a-haplotype).

Table 3 reports the molecular diversity indices in the ACR and CD samples. The ACR specimen with CD haplotype was omitted from this calculation. For the ACR sample, estimates were computed in two subsets of

the population (total population and populations 7–11) to permit comparison between samples belonging to comparable geographical areas. The haplotype and nucleotide diversity values do not differ significantly between the races (see Table 3 for details).

## Discussion

The ACR and the CD races differ for all their metacentrics; this indicates their independent origin from populations with standard karyotype. Thus the contact zone here reported easily represents an area of secondary contact between two races chromosomally differentiated in allopatry. The extent of hybridization and genetic diversity was assessed by cytogenetic and sequence analyses. The haplotype and nucleotide diversity values fall within the range of values reported in the literature (Gundüz *et al*, 2001b) and do not differ significantly between the races. This indicates that the two races might have had similar evolutionary histories in terms of random genetic drift, variation of effective population size or natural selection.

Hybridization is rare so that gene flow between the chromosomal races is drastically reduced. Since the nearest pure race sample are 700 m apart, it is possible that hybrids may be uncovered with a more intense sampling effort in the contact zone; anyway hybridization should be limited to the area between sites 10–11 and site 12 (Figure 1). Sporadic hybridization events have been detected by the presence of one individual of the ACR race carrying a metacentric that belongs to the CD race and one ACR individual carrying a CD-like haplotype. In the first case, as discussed in Castiglia and Capanna (1999b), the occurrence of such mouse with complex chromosomal heterozygosity may have derived from two sources: (1) fortuitous inter-racial hybridization, (2) chromosomal rearrangement occurring accidentally and unrelated to the hybridization event. The first hypothesis is supported by Catalan *et al* (2000) that recently found the occurrence of WART in mice chromosomal hybrids. Unfortunately, the mtDNA analysis cannot confirm one or the other of these alternatives because this mouse has an ACR-type haplotype.

The origin of this introgressed mouse is not easily identified because the presumptive F<sub>1</sub> males carrying a

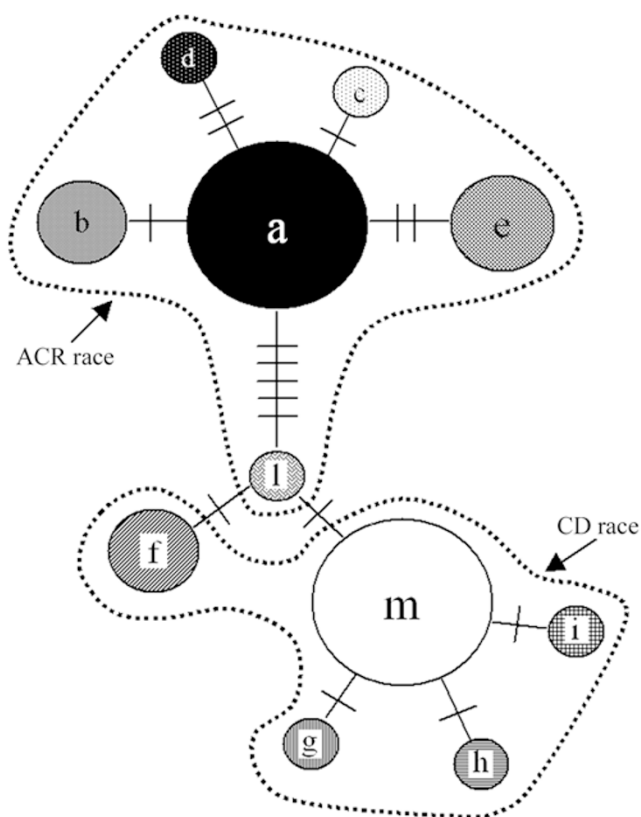
**Table 2** Alignment of all obtained haplotypes (468 bp), showing variable sites only. Dots indicates identity with the a-haplotype the dash at position 452 indicates a deletion relative to the sequence of Bibb *et al* (1981) (first line). Numbering starts at position 15372 of Bibb *et al* (1981)

Haplotype	No.	2	7	1	1	1	1	1	1	1	1	1	2	2	2	4	4
		7	8	2	3	9	4	3	4	6	7	7	1	1	6	9	2
Bibb <i>et al</i>		.	.	T	.	.	.	.	A	.	.	.	.	.	C	.	T
a	13	C	T	A	A	C	A	A	G	A	T	A	C	T	G	-	.
b	3	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
c	1	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
d	1	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.
e	4	.	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.
f	3	.	.	.	.	T	G	.	A	.	C	T	T	.	.	.	.
g	1	.	G	.	G	T	G	.	A	.	C	T	.	.	.	.	.
h	1	.	G	.	G	T	G	.	A	.	C	T	.	.	A	.	.
i	1	T	.	.	G	T	G	.	A	.	C	T	.	.	.	.	.
l	1	.	.	.	.	T	G	.	A	.	C	T	.	.	.	.	.
m	12	.	.	.	G	T	G	.	A	.	C	T	.	.	.	.	.



**Table 3** Genetic diversity in the mtDNA control region within the ACR and CD populations. Populations were pooled as indicated in the table. No. = number of individuals; NH = number of haplotypes; S = number of polymorphic sites; H = average haplotype diversity  $\pm$  SD;  $\pi$  = average nucleotide diversity  $\pm$  SD. Introgressed specimen (I-haplotype) is omitted from the calculation (see text for explanation)

	No.	NH	S	H	$\pi$
ACR (pop 1–11)	23	5	6	0.623 $\pm$ 0.099	0.0024 $\pm$ 0.0018
ACR (pop 7–11)	14	5	6	0.795 $\pm$ 0.076	0.0034 $\pm$ 0.0024
CD (pop 12–15)	18	5	5	0.549 $\pm$ 0.127	0.0021 $\pm$ 0.0017
Total	41	10	12	0.800 $\pm$ 0.041	0.0080 $\pm$ 0.0046



**Figure 2** Minimum spanning network connecting control region (mtDNA) haplotypes (468 bp). Filling in each circle correspond to the legend in Figure 1. Size of each circle is proportional to the frequency of the haplotype.

ring of 14 metacentrics and a chain of five chromosomes are sterile (Malorni *et al*, 1982). However Piálek *et al* (2001) and Garagna *et al* (1990) have shown that, in other cases of complex heterozygosities in house mouse, females with multivalent chains or rings show less impairment of gametogenesis than males; hence we cannot exclude the sporadic presence of  $F_1$  females with a certain degree of fertility.

Since the introgression of mitochondrial markers depends only on females, for the uniparentally inheritance of the mitochondrion, the presence of a CD haplotype in a complete ACR chromosomal background indicates that in some cases females  $F_1$  hybrids can reproduce successfully.

Such a level of genetic isolation has only been observed between the POS and UV races in Valtellina (northern Italy) (Capanna and Corti, 1982) and in some contact

areas in the hybrid zone between the Monastir race and the standard race in Tunisia (Said and Britton-Davidian, 1991; Chatti *et al*, 1999). However, no detailed molecular studies are available for these zones.

The observed level of reproductive isolation and the pattern of distribution seem similar to those observed for contact zones between chromosomal races of *Sorex araneus* in Poland (Fedyk *et al*, 1993), in the Alps (Hausser *et al*, 1991; Brunner and Hausser, 1996) and in Scotland (Mercer and Searle, 1991). In all these cases, the contact zones contained few or no hybrid karyotypes. A genetic analysis is available only for the contact zone between the Cordon and Valais races: a certain level of genetic introgression of mitochondrial and nuclear markers was detected (Balloux *et al*, 2000). In all these reported cases, the location of the contact zone corresponded to some geographical barrier (river or stream) or to an area of unsuitable habitat. This is because hybrid zones are most likely in areas of low population density (Barton, 1979). In contrast, in our case, the contact zone is in an area of potentially high population density, indirectly indicating that this contact zone should be shifting at present.

In these ecological conditions, the observed distribution pattern and the very low level of hybridization suggest the existence of a premating mechanism of isolation or at least some level of assortative mating. Aggressive behaviour between males seems to be the premating isolation mechanism responsible for the lack of hybridization in Migiondo (Capanna *et al*, 1984). In our case, however, the lack of geographical isolation excludes the possibility of extinction of one race, as observed in Migiondo (Hauffe and Searle, 1992). When one race is more aggressive than the other, the most likely outcome is a shift of the contact zone. It is likely that the chromosomal differences existing between the two races caused a strong post mating barrier during the first time of contact. Then behavioural differences, genetically determined, selected by reinforcement could have completed the process of speciation. On the other hand we cannot exclude that the behavioural differences were present before the contact of the two races.

Reinforcement is believed to be favoured if hybrid individuals are strongly selected against and if the contact between the two taxa occurs in conditions of isolation (Howard, 1993). In this way, gene flow deriving from the population outside the contact zone does not diminish the effect of selection inside the contact zone (Howard, 1993). It would be interesting to test the reinforcement hypothesis in this zone since only the first of the two conditions (hybrids strongly selected against) seems to be present in the contact zone while the continuity of human settlement prevents the condition of isolation predicted by this model.

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## References

- Balloux F, Brünnner H, Lugon-Moulin N, Hausser J, Goudet J (2000). Microsatellites can be misleading. An empirical and simulation study. *Evolution* **54**: 1414–1422.
- Barton NH (1979). The dynamics of hybrid zones. *Heredity* **43**: 341–359.
- Bibb MJ, Van Etten RA, Wright CT, Walberg MW, Clayton DA (1981). Sequence and gene organization of mouse mitochondrial DNA. *Cell* **26**: 167–180.
- Bidau CJ, Gimenez MD, Palmer CL, Searle JB (2001). The effects of Robertsonian fusions on chiasma frequency and distribution in the house mouse (*Mus musculus domesticus*) from a hybrid zone in northern Scotland. *Heredity* **87**: 305–313.
- Britton-Davidian J, Nadeau JH, Croset H, Thaler L (1989). Genic differentiation and origin of Robertsonian populations of the house mouse (*Mus musculus domesticus*, Ruddy). *Genet Res* **53**: 29–44.
- Brünnner H, Hausser J (1996). Genetic and karyotypic structure of a hybrid zone between the chromosomal races of the common shrew *Sorex araneus*. *Heredity* **125**: 147–158.
- Capanna E, Corti M (1982). Reproductive isolation between two chromosomal races of *Mus musculus domesticus* in the Rhaetian Alps (northern Italy). *Mammalia* **46**: 107–109.
- Capanna E, Corti M, Mainardi D, Parmigiani S, Brain PF (1984). Karyotype and intermale aggression in wild house mice: ecology and speciation. *Behav Genet* **14**: 195–208.
- Capanna E, Corti M, Nascetti G (1985). Role of contact areas in chromosomal speciation of the European long-tailed house mouse (*Mus musculus domesticus*). *Ital J Zool* **52**: 97–119.
- Castiglia R, Capanna E (1999a). Contact zones between chromosomal races of *Mus musculus domesticus*. 1. Temporal analysis of a hybrid zone between the CD chromosomal race ( $2n=22$ ) and populations with the standard karyotype. *Heredity* **83**: 319–326.
- Castiglia R, Capanna E (1999b). Whole-arm reciprocal translocation (WART) in a feral population of mice. *Chromosome Res* **7**: 493–495.
- Castiglia R, Capanna E (2000). Contact zones between chromosomal races of *Mus musculus domesticus*. 2. Fertility and segregation in laboratory-reared and wild mice multiple heterozygous for Robertsonian rearrangements. *Heredity* **85**: 147–157.
- Castiglia R, Capanna E (2002). Chiasma repatterning in a hybrid zone between chromosomal races of *Mus musculus domesticus*. *Genetica* **114**: 35–40.
- Catalan J, Auffray JC, Pellestor F, Britton-Davidian J (2000). Spontaneous occurrence of a Robertsonian fusion involving chromosome 19 by single whole-arm reciprocal translocation (WART) in wild-derived house mice. *Chromosome Res* **8**: 593–601.
- Chatti N, Ganem G, Benzekri K, Catalan J, Britton-Davidian J, Said K (1999). Microgeographical distribution of two chromosomal races of house mice in Tunisia: pattern and origin of habitat partitioning. *Proc R Soc Lond B Biol Sci* **266**: 1561–1569.
- Fedyk S, Zajkowska M, Chętnicki W (1993). Study of a contact between two chromosomally monomorphic races of *Sorex araneus* L. (common shrew). *Heredity* **71**: 221–226.
- Garagna S, Redi CA, Zuccotti M, Britton-Davidian J, Winking H (1990). Kinetics of oogenesis in mice heterozygous for Robertsonian translocation. *Differentiation* **42**: 167–171.
- Gundüz I, Lopez-Fuster MJ, Ventura J, Searle JB (2001a). Clinal analysis of a chromosomal hybrid zone in the house mouse. *Genet Res* **77**: 41–51.
- Gundüz I, Auffray JC, Britton-Davidian J, Catalan J, Ganem G, Ramalhinho MG *et al* (2001b). Molecular studies on the colonization of the Madeiran archipelago by house mice. *Mol Ecol* **10**: 2023–2029.
- Hauffe HC, Searle JB (1992). A disappearing speciation event? *Nature* **357**: 26.
- Hauffe HC, Searle JB (1998). Chromosomal heterozygosity and fertility in house mice (*Mus musculus domesticus*) from Northern Italy. *Genetics* **150**: 1143–1154.
- Hausser J, Bosshard F, Tamberlet P, Wójcik J (1991). Relationships between chromosome races and species of *Sorex* of the araneus group in the western Alps. *Mem Soc Vaud Sci Nat* **19**: 79–95.
- Howard DJ (1993). Reinforcement: the origin, dynamics, and the fate of an evolutionary hypothesis. In: Harrison RG (ed) *Hybrid Zones and the Evolutionary Process*, Oxford University Press, New York, pp 46–69.
- Hsu TC, Patton JL (1969). Bone marrow preparations for chromosome studies. In: Benirschke K (ed) *Comparative Mammalian Cytogenetics*, Springer-Verlag: Berlin, pp 454–460.
- Malorni W, Capanna E, Cristaldi M, De Martino E (1982). Changes of seminiferous epithelium in hybrids of mice carrying Robertsonian karyotype. *Arch Androl* **9**: 333–341.
- Mercer SJ, Searle JB (1991). Preliminary analysis of a contact zone between karyotypic races of the common shrew (*Sorex araneus*) in Scotland. *Mem Soc Vaud Sci Nat* **19**: 73–78.
- Nachman MW, Boyer SN, Searle JB, Aquadro CF (1994). Mitochondrial DNA variation and the evolution of Robertsonian chromosomal races of house mice, *Mus domesticus*. *Genetics* **136**: 1105–1120.
- Nei M (1987). *Molecular Evolutionary Genetics*. Columbia University Press: New York, NY, USA.
- Piálek J, Hauffe HC, Rodriguez-Clark KM, Searle JB (2001). Racialization and speciation in house mice from the Alps: the role of chromosomes. *Mol Ecol* **10**: 613–625.
- Prager EM, Sage RD, Gyllesten U, Thomas WK, Hübner R, Jones CS *et al* (1993). Mitochondrial DNA sequence diversity and the colonization of Scandinavia by house mice from East Holarctica. *Biol J Linn Soc* **50**: 85–92.
- Redi CA, Capanna E (1988). Robertsonian heterozygotes in the house mouse and the fate of their germ cells. In: Daniel A (ed) *The Cytogenetics of Mammalian Autosomal Rearrangements*, Alan R. Liss: New York, pp 315–359.
- Rieseberg LH (2001). Chromosomal rearrangements and speciation. *Trends Ecol Evol* **7**: 351–364.
- Sage RD, Atchley WR, Capanna E (1993). House mice as models in systematic biology. *Syst Biol* **42**: 523–561.
- Said K, Britton-Davidian J (1991). Genetic differentiation and habitat partition of Robertsonian house mouse populations (*Mus musculus domesticus*) of Tunisia. *J Evol Biol* **3**: 409–427.
- Said K, Saad A, Auffray J-C, Britton-Davidian J (1993). Fertility estimates in the Tunisian all-acrocentric and Robertsonian populations of the house mouse and their chromosomal hybrids. *Heredity* **71**: 532–538.
- Seabright MA (1971). A rapid banding technique for human chromosomes. *Lancet* **2**: 971–972.
- Searle JB (1993). Chromosomal hybrid zones in Eutherian mammals. In: Harrison RG (ed) *Hybrid Zones and the Evolutionary Process*, Oxford University Press: New York, pp 309–353.
- Searle JB, Navarro YN, Ganem G (1993). Further studies of a staggered hybrid zone in *Mus musculus domesticus*. *Heredity* **71**: 523–531.
- Tajima F (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics* **105**: 437–460.
- Wallace BM, Searle JB, Everett CA (1992). Male meiosis and gametogenesis in wild house mice (*Mus musculus domesticus*) from a chromosomal hybrid zone; a comparison between "simple" Robertsonian heterozygotes and homozygotes. *Cytogenet Cell Genet* **61**: 211–220.
- Winking HB, Dulic B, Bulfield G (1988). Robertsonian karyotype variation in the European house mouse, *Mus musculus*: survey of present knowledge and new observations. *Z Säugetierk* **53**: 148–161.