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Clinal analysis of a chromosomal hybrid zone in the house mouse

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Summary

These studies centre on the 'Barcelona' karyotypic race of the western house mouse (*Mus musculus domesticus*), first described by Adolph & Klein (1981). This is one of many races within *M. m. domesticus* characterized by metacentric chromosomes that have originated by repeated Robertsonian fusions, with perhaps further modification by whole-arm reciprocal translocations. Data on 111 mice from 20 sites show that the race is centred 24 km to the west of Barcelona city and has a homozygous metacentric karyotype of 2n = 28 (3·8, 4·14, 5·15, 6·10, 9·11, 12·13). The race has a small range, and mice with the standard 40-acrocentric karyotype were caught only 30 km from the race centre. Throughout the area of occurrence of metacentrics there is polymorphism (i.e. presence of acrocentrics in the population), although all six metacentrics approach fixation close to the race centre. Thus, there is a hybrid zone between the Barcelona and standard races. The centres and widths of all clines (except 3·8) were determined. Likelihood ratio tests showed that most of the cline centres differed significantly in position (i.e. the clines were staggered) and the clines for metacentrics 6·10 and 9·11 were significantly narrower than those for 4·14, 5·15 and 12·13. Overall, the clines tended to be wider the further they were from the race centre. There are various possible explanations for this hybrid zone structure and further data are needed to distinguish between them.

1. Introduction

The west European subspecies of house mouse, *Mus musculus domesticus*, displays a 40-chromosome karyotype over most of its range. However, there are numerous local karyotypic races, particularly found in the vicinity of the Alps and Apennines and in coastal regions, characterized by reduced chromosome numbers (2n = 22–38) (reviews: Boursot *et al.*, 1993; Sage *et al.*, 1993; Nachman & Searle, 1995). The standard karyotype which consists of only acrocentric (single-armed) chromosomes is modified by the presence of metacentric (bi-armed) chromosomes. The metacentric condition and reduced diploid number is attained by Robertsonian (centric) fusions, but the metacentrics so generated may evolve into new metacentrics by whole-arm reciprocal translocations (Hauffe & Piálek, 1997). The karyotypic races of the house mouse are thought generally to have formed *in situ* within the last 10 000 years (Britton-Davidian *et al.*, 1989).

The house mouse is a primary model system for studies of 'chromosomal speciation': reproductive isolation promoted by the presence of chromosomal rearrangements (King, 1993). This model status has come about not only because of the multitude of recently-formed karyotypic races available to study in the house mouse and the role of the species as a genetical and laboratory model, but also because the chromosomal rearrangements that occur in the house mouse have long been known to be associated with reduced fertility when in the heterozygous state

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(review: Searle, 1993). Thus, hybrids between certain karyotypic races in the house mouse may be highly infertile or sterile. The importance of chromosomal rearrangements in this infertility is emphasized by the close correspondence between degree of karyotypic difference and degree of hybrid infertility, with little apparent genic differentiation (Britton-Davidian *et al*., 1989; Searle, 1993). The karyotypically similar subspecies of house mouse *M. m. domesticus* and *M. m. musculus* are genically very distinct but hybrids show normal fertility (Vanlerberghe *et al*., 1986).

One approach to the study of chromosomal speciation in the house mouse is the analysis of hybrid zones between karyotypic races. Hybrid zones are potential sites of speciation, particularly by the process of 'reinforcement' (Noor, 1999), and the analysis of clinal variation within zones can provide much insight into hybrid unfitness (Barton & Gale, 1993). In the house mouse, there have been detailed studies of chromosomal hybrid zones in Belgium (Bauchau *et al*., 1990; Hübner & Koulischer, 1990), Denmark (Fel-Clair *et al*., 1996), central Italy (Spirito *et al*., 1980; Castiglia & Capanna, 1999), northern Italy (Hauffe & Searle, 1993), northern Scotland (Searle *et al*., 1993) and Tunisia (Saïd *et al*., 1999). In the case of the north Italian and Tunisian hybrid zones, there is evidence of strong hybrid unfitness (Saïd *et al*., 1993; Hauffe & Searle, 1998), and a possibility that this unfitness has promoted reinforcement (Saïd *et al*., 1999; Hauffe & Searle, 1992). The narrowness of the hybrid zone in central Italy may also reflect hybrid unfitness (Castiglia & Capanna, 1999). In the Belgian, Danish and Scottish hybrid zones, non-coincidence of the chromosomal clines ('staggering') means that the most unfit multiple chromosomal heterozygotes are either more rarely produced or not produced at all (Bauchau *et al*., 1990; Searle *et al*., 1993; Fel-Clair *et al*., 1996). It is clearly of value to have further studies on hybrid zones in the house mouse to expand on these initial findings, in order to fully understand the conditions under which speciation may be promoted within chromosomal hybrid zones and conditions when the hybrid zones actually weaken as genetic barriers rather than strengthen.

With this in mind, we initiated a detailed chromosomal survey of a previously described but poorly studied chromosomal hybrid zone in the house mouse, in the vicinity of Barcelona, along the north-eastern coast of Spain. In general, south-western Europe is occupied by standard all-acrocentric house mice (Klein *et al*., 1987) but S. Adolph (unpublished data), Adolph & Klein (1981) and Nachman *et al*. (1994) found metacentric mice in the vicinity of Barcelona. They described five metacentrics in this area (4·14, 5·15, 6·10, 9·11 and 12·13; where x.y refers to a metacentric composed of the ancestral acrocentrics x and y joined together); and their data suggested presence of a hybrid zone between a 30-chromosome race and the standard 40-chromosome race. In this paper, we describe our further characterization of this hybrid zone.

2. Materials and methods

A total of 20 farm sites were visited over two field trips (4–28 March 1996 and 14 May–7 June 1997) in the vicinity of Barcelona. From each site, 1–10 mice were live-trapped and karyotyped. At one site (Vilanova i la Geltrú) a mother and her four offspring (born in captivity) were karyotyped. Chromosome preparations were made from bone marrow (Ford, 1966). The slides were kept in the dark for 5 days before G-banding was conducted according to Evans (1987). Chromosome identification was made directly under the microscope according to the Committee on Standardized Genetic Nomenclature for Mice (1972). At least 10 cells were scored for chromosome number and at least five were fully analysed for precise chromosome composition.

Once the data on metacentric frequency was collated for each collection site, characteristics of the hybrid zone were determined. The Analyse 1.10 package developed by Barton & Baird (1998) was used to draw the best-fit one-dimensional clines for each of the metacentric chromosomes and to establish the widths and centres of these clines (with confidence intervals). The clines were fitted by maximum likelihood using the Metropolis algorithm (Szymura & Barton, 1986); the cline width represents the inverse of the maximum slope (Endler, 1977) and the cline centre is the position of maximum frequency change. The cline analysis package C-fit (devised by T. Lenormand) was used to test for similarity in cline widths (concordance) and cline centres (coincidence) between the different metacentrics by likelihood ratio tests, following the approach of Fel-Clair *et al*. (1996).

3. Results

Table 1 and Fig. 1 summarize the chromosomal data available for house mice from the vicinity of Barcelona. The work carried out previous to our study was based on 19 mice from seven sites and it was found that mice collected to the west of Barcelona had substantially metacentric karyotypes (sites C, F and 1) while mice from the north of Barcelona had standard karyotypes or close to the standard karyotype (sites 2–5) (S. Adolph, unpublished data; Adolph & Klein, 1981; Nachman *et al*., 1994). We therefore concentrated our study to the west of Barcelona. Of the 111 mice that we karyotyped from 20 sites (18 previously unsampled), we obtained full data from 94 individuals; the remaining 17 mice provided us with diploid chromosome numbers only.

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Table 1. *The locations and chromosomal characteristics of all collection sites in the Barcelona hybrid zone*

Site[a]		km	Latitude/Longitude	n^b	Mean 2n	Mean **I**[c]	**H**[d]	Frequency of metacentric chromosome 3·8	4·14	5·15	6·10	9·11	12·13
A	Garraf	7·4	41° 17′ N 1° 50′ E	7	30·0	+8·0	1·71	0·64	0·93	1	0·71	0·86	0·86
B	Viladecans	7·4	41° 19′ N 2° 01′ E	4(1)	30·2	+7·6	0·75	0·13	1	0·75	1	1	1
C	Avinyonet del Penedès	13·9	41° 22′ N 1° 46′ E	2(1), (*4*), **2**	31·2	+5·6	0·25	0	1	0·88	0·50	1	1
D	Sant Sadurní d'Anoia	17·6	41° 25′ N 1° 47′ E	7	34·7	−1·4	1·57	0	0·71	0·64	0·07	0·71	0·50
E	Vilanova i La Geltrú	18·6	41° 14′ N 1° 43′ E	7(5)[e]	31·8	+4·4	0·86	0	0·93	0·79	0·21	0·93	1
F	Sant Martí de Sarroca	28·5	41° 23′ N 1° 36′ E	4, (*1*)	32·2	+3·6	0·50	0	1	1	0	0·75	1
G	Vallbona d'Anoia	30·0	41° 31′ N 1° 42′ E	5(1)	39·3	−10·6	0·20	0	0	0	0	0	0·10
H	Sabadell	31·0	41° 34′ N 2° 06′ E	1	39	−10·0	1	0	0	0	0	0	0·50
I	Sant Llorenç del Penedès	31·8	41° 17′ N 1° 33′ E	5	36·6	−5·2	2·20	0	0·50	0·50	0	0·30	0·40
J	Calafell	32·0	41° 13′ N 1° 34′ E	5	35·8	−3·6	0·60	0	1	0·20	0	0	0·90
K	La Llacuna	37·2	41° 28′ N 1° 32′ E	2(3)	35·8	−3·6	0·33	0	0·25	0·25	0	0·25	1
L	Les Pobles	44·5	41° 21′ N 1° 24′ E	8(2)	38·2	−8·4	1·88	0	0·38	0·31	0	0	0·25
M	Les Ordes	45·0	41° 23′ N 1° 24′ E	1	38	−8·0	1	0	1	0	0	0	0
N	La Riera	49·0	41° 11′ N 1° 22′ E	8	40	−12·0	0	0	0	0	0	0	0
O	Santa Coloma de Queralt	51·8	41° 32′ N 1° 23′ E	7(2)	38·3	−8·6	1·14	0	0·50	0·07	0	0	0·14
P	Els Prats de Rei (Calaf)	58·3	41° 44′ N 1° 31′ E	6(2)	39·5	−11·0	0·17	0	0·08	0	0	0	0
Q	Fulleda	77·0	41° 27′ N 1° 01′ E	1	40	−12·0	0	0	0	0	0	0	0
R	Les Borges del Camp	78·2	41° 10′ N 1° 01′ E	5	40	−12·0	0	0	0	0	0	0	0
S	L'Espluga Calba	80·1	41° 29′ N 1° 00′ E	4	40	−12·0	0	0	0	0	0	0	0
T	Anglesola	80·8	41° 40′ N 1° 05′ E	5	39·6	−11·2	0·40	0	0·20	0	0	0	0
1	Barcelona West	15·8	41° 23′ N 2° 04′ E	(*3*)	35·3	−2·6	—	—	—	—	—	—	—
2	Valldoreix	17·1	41° 27′ N 2° 02′ E	(*1*)	38	−8·0	—	—	—	—	—	—	—
3	Badalona	30·7	41° 27′ N 2° 15′ E	(*2*)	39·5	−11·0	—	—	—	—	—	—	—
4	La Roca	45·6	41° 35′ N 2° 20′ E	*2*, **2**	40	−12·0	0	0	0	0	0	0	0
5	Moya	57·9	41° 49′ N 2° 06′ E	*2*	40	−12·0	0	0	0	0	0	0	0

[a] Sites are ordered according to distance (km) of each site (A–T, 1–5) from the mid-point between Garraf and Viladecans, the two populations with the lowest diploid number (considered the Barcelona race centre: see Fig. 1). Sites 1–5 were studied by previous workers (see footnote *b*).

[b] *n* refers to numbers of individuals for which the full karyotype (diploid number and metacentric identification) has been determined. Those in italics were mice karyotyped by S. Adolph (unpublished data) and Adolph & Klein (1981) and those in bold were karyotyped by Nachman *et al.* (1994). Numbers in parentheses refer to additional mice for which only diploid numbers are available; these were used in the calculations of mean 2n only.

[c] **I** is the hybrid index, where +12 indicates an individual with a fully metacentric Barcelona race karyotype (2n = 28) and −12 an individual with a fully acrocentric standard race karyotype (2n = 40).

[d] **H** is the mean number of heterozygous metacentrics per individual.

[e] These include a mother and her four offspring. The mother and one of the offspring were karyotyped with G-banding; for three of the offspring only a diploid number was obtained.

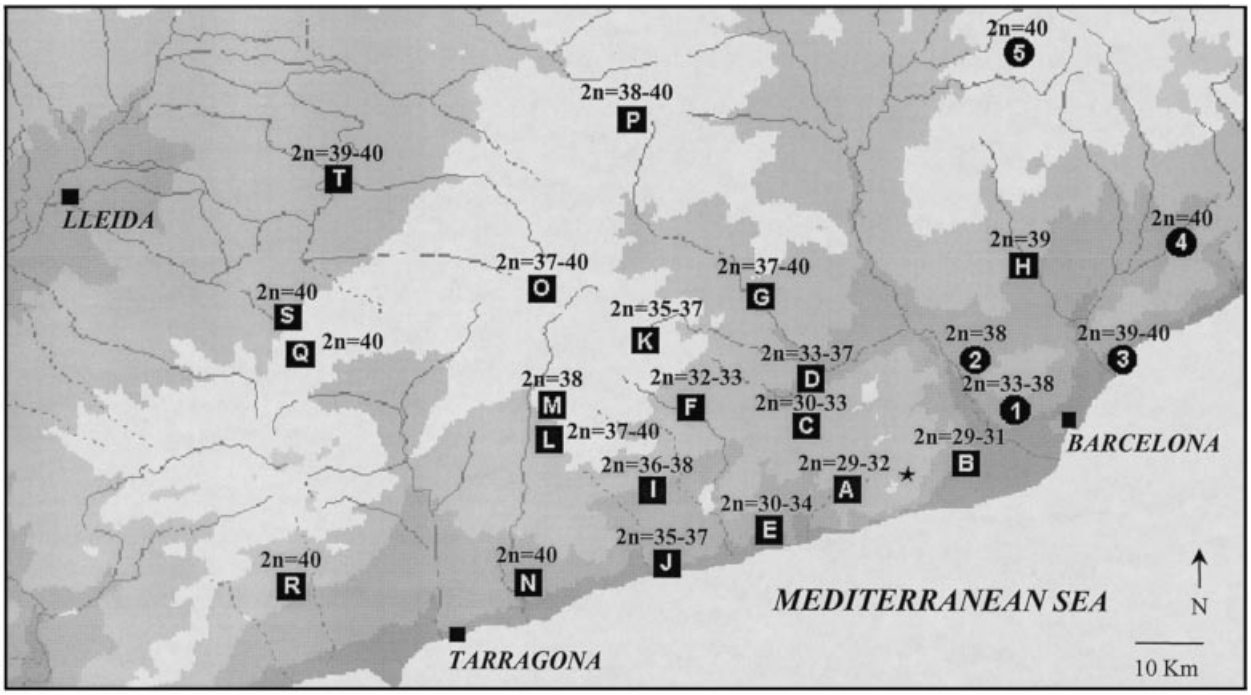

Fig. 1. Map showing the collection sites in the vicinity of the Barcelona hybrid zone and the range of diploid numbers found at each. Sites are labelled by a letter (sampled by us) or a number (sampled by previous workers only), as detailed in Table 1. A star indicates the presumed centre of the distribution of the 'Barcelona' karyotypic race. Shading indicates altitude, with the unshaded zone on land in the range 500–1000 m.

Diploid numbers ranged from 29 to 40 and a total of 36 different karyotypes were found on the basis of metacentric complement. A homogeneously staining region (HSR) has also been described on chromosome 1 in mice from the Barcelona area (specifically Sant Martí de Sarroca (site F): Traut *et al*., 1984; Agulnik *et al*., 1993), but this type of chromosomal variation was not analysed in the present study. The five metacentrics that were previously described by Adolph & Klein (1981) were also recorded by us, along with one new metacentric – 3·8 – which also occurs in Denmark (Nance *et al*., 1990), southern Germany (Adolph & Klein, 1983), central Italy (Capanna *et al*., 1976), northern Italy (Gropp *et al*., 1982) and Madeira (Britton-Davidian *et al*., 2000).

There is an expectation then, that close to Barcelona there are mice with 28 chromosomes and a homozygous karyotype including metacentrics 3·8, 4·14, 5·15, 6·10, 9·11 and 12·13. No individuals with this chromosomal composition were found, presumably because the sample sizes were small and the geographic coverage incomplete. At both Garraf (site A) and Viladecans (site B) individuals with $2n = 29$ were collected. Because of this and the low mean 2n found at these sites, it is considered that the Barcelona karyotypic race of house mice, characterized by the six metacentrics listed above, is centred half-way between Garraf and Viladecans, i.e. 24 km from the city centre of Barcelona. However, while we assume that this 'race centre' is the place with the highest frequency of all six metacentrics and the lowest mean diploid number, even here the 28-chromosome 'Barcelona race' karyotype may be at a frequency far short of fixation.

Moving away from the race centre along the coast or inland, chromosome numbers increase and, in tandem, metacentric frequencies decrease (Table 1, Fig. 1). All mice collected less than 30 km from the race centre had metacentric chromosomes within their karyotypes, but at distances of 30 km and over, mice with a standard karyotype appear and at distances over 45 km from the race centre, the standard karyotype is predominant. Thus, metacentrics are commonly found in house mice over an area of approximately 2000 km² to the west of Barcelona, though individuals with metacentrics may be found at considerable distances beyond that area. For example, two mice from Anglesola (site T), over 80 km from the race centre, were heterozygous for metacentric 4·14.

The karyotypic data can be expressed in terms of an index of hybridization, as used for other hybrid zones (e.g. Prager *et al*., 1993). Each individual can be given a hybrid index score calculated by summing the number of Barcelona-race-specific chromosomes (each assigned $+1$) and standard-race-specific chromosomes (each assigned -1). Thus a 28-chromosome Barcelona race individual would have a score of $+12$ and a 40-chromosome standard race individual would have a score of -12. Table 1 gives the mean hybrid index scores for each site, which clearly show that all

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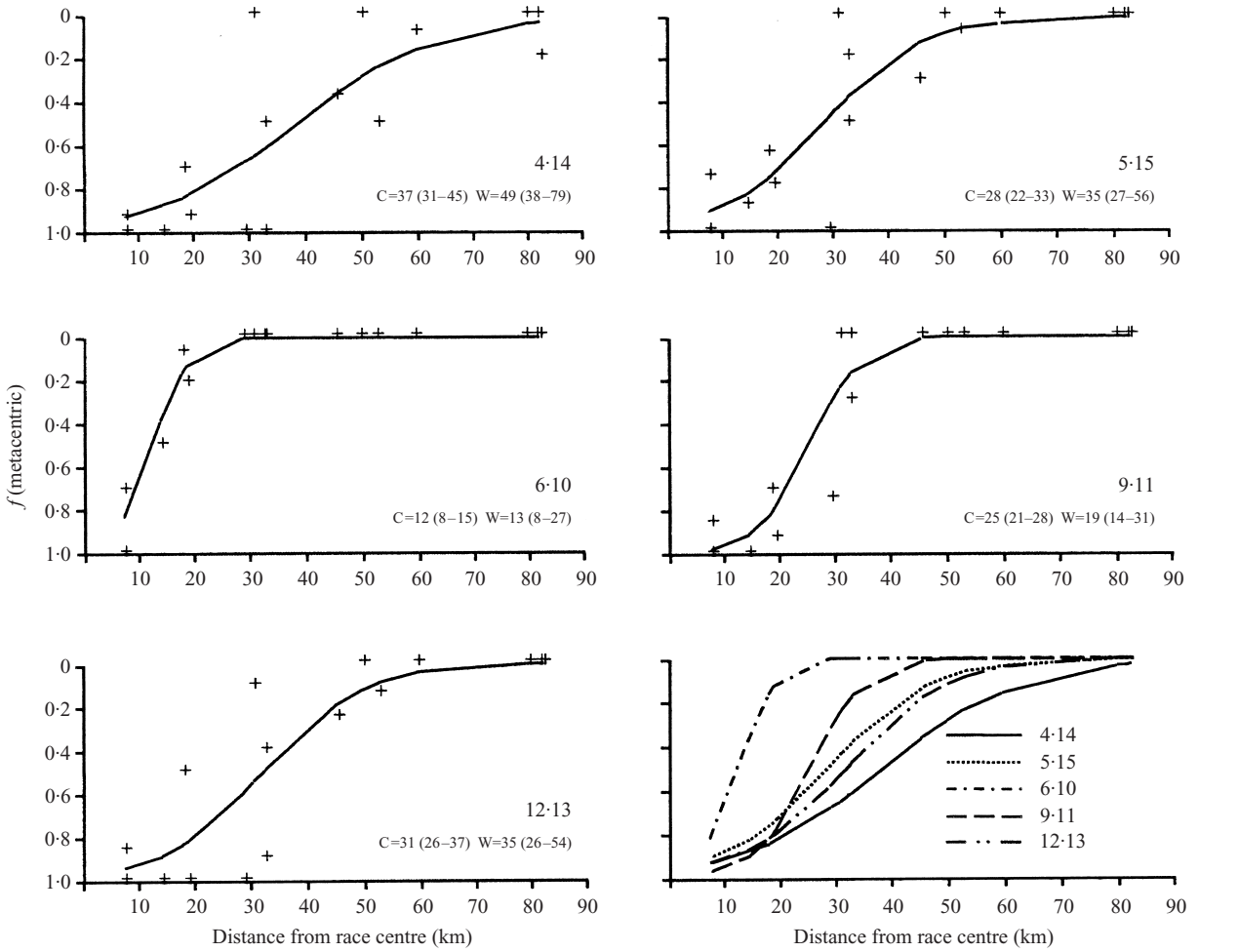

Fig. 2. The variation in frequency of each metacentric across the Barcelona hybrid zone, except 3·8 (for which there is insufficient information). The data for each site are given in terms of distance from the presumed Barcelona race centre (see Fig. 1). Only the sites to the west of Barcelona with complete data on four or more individuals are included; there is a lack of information on sites around Barcelona itself and to the north and east. For each metacentric a best-fit cline is shown. Also provided are the cline centre (C, distance from the Barcelona race centre) and cline width (W, defined according to Endler, 1977), with 95% confidence intervals. All the best-fit clines are shown together in the final diagram.

sites with Barcelona-race-like characteristics (i.e. a positive mean hybrid index score) are within a mere 30 km of the race centre, and that the majority of sites that we sampled are standard-race-like.

Inspection of Table 1 shows that 4·14 is the most widely distributed of the metacentrics that defines the Barcelona karyotypic race. Metacentrics 5·15 and 12·13 are next most widespread, then 9·11. Metacentric 6·10 and particularly 3·8 are much more restricted to the race centre. The frequency change in the metacentrics (excluding 3·8) was examined more quantitatively using Analyse 1.10. All westerly sites with G-band data from four or more individuals were used to generate a best-fit cline for each metacentric, from which the cline centre and cline width were calculated (Fig. 2). The dataset consisted of relatively small sample sizes collected in a broad westerly direction from the race centre, so it is not surprising that there is sometimes a considerable scatter of data

	6·10	**9·11**	**5·15**	**12·13**	**4·14**
6·10	—	2·2	13·5	13·5	22·4
9·11	41·3	—	8·0	8·0	18·7
5·15	31·8	1·6	—	0·0	2·5
12·13	47·2	7·9	1·9	—	2·7
4·14	50·7	20·4	10·3	4·1	—

Fig. 3. Tests of concordance (above diagonal) and coincidence of the metacentric clines. These are χ^2 values with 1 d.f. and represent the output of likelihood ratio tests comparing maximum likelihood models with and without constraint on cline slope (for tests of concordance) or cline centre (for tests of coincidence) (see table 3 in Fel-Clair *et al.*, 1996). A significance level of $P < 0{\cdot}005$ was used for each set of tests following the Bonferroni correction (Sokal & Rohlf, 1995); significant values are within the shaded cells.

points around the best-fit clines. Larger and more regular samples along a particular transect may have generated better supported clines; but we believe our

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Table 2. *The individual karyotypes of metacentric mice in the Barcelona hybrid zone*[a]

Site		*n*[b]	2n	**I**[c]	3·8	4·14	5·15	6·10	9·11	12·13
A	Garraf	1	29	+10	M	M	M	H	M	M
		1	29	+10	M	M	M	M	M	H
		2	30	+8	H	M	M	H	M	M
		1	30	+8	H	M	M	M	H	M
		1	30	+8	M	M	M	M	H	H
		1	32	+4	A	H	M	H	M	M
B	Viladecans	1	29	+10	H	M	M	M	M	M
		1	30	+8	A	M	M	M	M	M
		2	31	+6	A	M	H	M	M	M
C	Avinyonet del Penedès	2	30	+8	A	M	M	M	M	M
		1	32	+4	A	M	M	A	M	M
		1	33	+2	A	M	H	A	M	M
D	Sant Sadurní d'Anoia	1	33	+2	A	H	M	A	M	M
		1	33	+2	A	M	H	H	H	M
		1	34	0	A	M	H	A	M	H
		1	34	0	A	M	H	A	H	M
		1	35	−2	A	H	M	A	M	A
		1	37	−6	A	H	M	A	A	A
		1	37	−6	A	H	A	A	M	A
E	Vilanova i La Geltrú	1	31	+6	A	M	H	M	M	M
		3	32	+4	A	M	M	A	M	M
		1[d]	32	+4	A	M	H	H	M	M
		1	33	+2	A	H	M	A	M	M
		1	34	0	A	M	H	A	H	M
F	Sant Martí de Sarroca	2	32	+4	A	M	M	A	M	M
		2	33	+2	A	M	M	A	H	M
G	Vallbona d'Anoia	1	39	−10	A	A	A	A	A	H
H	Sabadell	1	39	−10	A	A	A	A	A	H
I	Sant Llorenç del Penedès	2	36	−4	A	H	M	A	A	H
		1	36	−4	A	M	A	A	H	H
		1	37	−6	A	A	H	A	H	H
		1	38	−8	A	H	A	A	H	A
J	Calafell	2	35	−2	A	M	H	A	A	M
		2	36	−4	A	M	A	A	A	M
		1	37	−6	A	M	A	A	A	H
K	La Llacuna	1	36	−4	A	H	A	A	H	M
		1	37	−6	A	A	H	A	A	M
L	Les Pobles	3	37	−6	A	H	H	A	A	H
		2	38	−8	A	H	H	A	A	A
		1	39	−10	A	H	A	A	A	A
		1	39	−10	A	A	A	A	A	H
M	Les Ordes	1	38	−8	A	M	A	A	A	A
O	Santa Coloma de Queralt	1	38	−8	A	M	A	A	A	A
		2	38	−8	A	H	A	A	A	H
		1	38	−8	A	H	H	A	A	A
		2	39	−10	A	H	A	A	A	A
P	Els Prats de Rei (Calaf)	1	39	−10	A	H	A	A	A	A
T	Anglesola	2	39	−10	A	H	A	A	A	A

[a] This table includes only metacentric-carrying individuals for whom metacentrics have been identified by G-banding, and sites where such individuals have been collected; further karyotypic data from the hybrid zone are given in Table 1. M, homozygous metacentric, H, heterozygous, A, homozygous acrocentric.
[b] Numbers of individuals with a particular karyotype.
[c] Hybrid index score for the individual, where +12 indicates an individual with a fully metacentric Barcelona race karyotype (2n = 28) and −12 an individual with a fully acrocentric standard race karyotype (2n = 40).
[d] This is an offspring of the individual with 31 chromosomes.

data provide a reasonable reflection of the widths and locations of clines on the western side of the race centre.

Tests of concordance and coincidence of the metacentric clines (excluding 3·8) were made with C-fit and the results are shown in Fig. 3. The clines for metacentrics 6·10 and 9·11 are significantly narrower than those of the other three metacentrics tested. Most of the clines were significantly non-coincident, except that 5·15 was not significantly staggered from

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9·11 or 12·13 and 12·13 was not significantly staggered from 4·14. There is a tendency for metacentric cline widths to increase the further the clines are located from the race centre ($r_s = 0{\cdot}975, 0{\cdot}05 < P < 0{\cdot}10$ based on the best estimates of cline widths and centres given in Fig. 2).

Among the mice collected from the Barcelona hybrid zone, the karyotypes of those carrying metacentric chromosomes are listed in Table 2. It has already been pointed out that no individuals with a fully metacentric Barcelona race karyotype (2n = 28) were found. Indeed, among the metacentric-carrying mice, very few had a homozygous karyotype (13 of 64 studied by us and Nachman *et al*. (1994): 20%). A 32-chromosome complement with metacentrics 4·14, 5·15, 9·11 and 12·13 was the most common homozygous karyotype; this was found in six individuals from three sites close to the race centre (sites C, E, F: Fig. 1). The 30-chromosome karyotype (4·14, 5·15, 6·10, 9·11, 12·13) has been found by us at site B (one individual) and Nachman *et al*. (1994) at site C (two individuals). Otherwise, the only homozygous metacentric karyotypes recorded were two individuals with 2n = 36 (4·14, 12·13) at site J and two individuals with 2n = 38 (4·14)(sites M and O).

The variation in chromosomal heterozygosity across the Barcelona hybrid zone is evident from Table 1. Clearly, the mean number of heterozygous chromosomes per individual (**H**) varies erratically between sites, which probably at least partially reflects the small sample sizes. Altogether there were 27 individuals heterozygous for a single metacentric (i.e. 42% of metacentric-carrying mice), 19 double heterozygotes (30%) and 5 triple heterozygotes (8%) (see Table 2).

The numbers of individuals per site was considered too small for a detailed statistical comparison between the observed karyotype data and the Hardy–Weinberg expectation. However, the broad findings provide no evidence of disruption of equilibrium due to selection, assortative mating or migration. On a chromosome-by-chromosome basis (i.e. equivalent to single-locus genotypes), there are no major departures from expectation (data in Tables 1 and 2). In terms of the complete karyotypes (i.e. equivalent to multilocus genotypes), diploid numbers varied little for any locality (Fig. 1), such that there were no individuals with extremely different karyotypes within the same site. The Hardy–Weinberg expectation of **H** was calculated for the five sites where seven or more mice (including metacentric-carrying individuals) were karyotyped (A, D, E, L, O); for three sites the observed **H** was higher than expected, while for the remaining two sites the opposite was the case. There were some surprises with regard to the range of multilocus genotypes within sites, but these may reflect the sampling procedures. For instance, the three individuals with the same triple heterozygous karyotype at Les Pobles (Table 2) were all collected from a single farm at the same time, and could easily have been litter mates.

4. Discussion

(i) *Nature and origin of the Barcelona karyotypic race*

Although we did not find any mice with the definitive 28-chromosome Barcelona race karyotype, there is a clear focus for all the Barcelona race metacentrics in the vicinity of Garraf and Viladecans (sites A and B: Fig. 1). Therefore, we considered half-way between these villages as the centre of the race and the area of Robertsonian polymorphism around this centre as a hybrid zone between the Barcelona race and the standard (2n = 40, all acrocentric) race.

The Barcelona race metacentrics could either have originated *in situ* or have been brought in passively with humans. On the basis of molecular analysis, it has been argued by Britton-Davidian *et al*. (1989) and Nachman *et al*. (1994) that, in general, the metacentric races of house mouse originated *in situ*. Certainly, the particular combination of metacentrics that characterize the Barcelona race has not been described elsewhere. However, there is the possibility that only one of the metacentrics was introduced and that initiated the formation of the race. From data on laboratory stocks, it appears that the presence of a metacentric may somehow trigger further Robertsonian fusion mutations in house mice (Nachman & Searle, 1995). The centre of the Barcelona karyotypic race is only 24 km from the hugely important Mediterranean port of Barcelona city, and so there is the potential for the metacentrics to have been brought in with mice from a distant source; Tichy & Vucak (1987) and Winking *et al*. (1988) have particularly argued in favour of such movement of metacentrics along major Mediterranean trade routes. The metacentric that is most likely to have been imported in this fashion is 5·15, which is found in northern and central Italy, Switzerland, southern Germany and Croatia as well as near Barcelona. However, by analysing microsatellite markers on this chromosome, Riginos & Nachman (1999) found no evidence of common identity of the Barcelona and Italian 5·15. Nor does 5·15 have the widest distribution among the metacentrics within the Barcelona hybrid zone, as might have been expected if it were the founding element.

Not only is the origin of the Barcelona race of interest, but also its possible fate, particularly in relation to the possibility of chromosomal speciation. However, any imminent speciation event involving

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this race is extremely unlikely given the data we have obtained from the Barcelona hybrid zone. Hybrids between the Barcelona race and the standard race are found over a very large area (over 5000 km²) and have a huge variety of different karyotypes (35 recorded) but have never been found to be heterozygous for more than three metacentrics. The Barcelona hybrid zone is not like the narrow zones involving house mice in central and northern Italy and Tunisia, which are characterized by highly unfit hybrids (Hauffe & Searle, 1993; Castiglia & Capanna, 1999; Saïd *et al*., 1999). It is the occurrence of such highly unfit hybrids which may lead to speciation by the reinforcement process. Therefore, there is no expectation that the races in the Barcelona hybrid zone are going to become reproductively isolated from each other in the near future.

(ii) *The staggered structure of the Barcelona hybrid zone*

At the time of the influential 1985 review of hybrid zones by Barton & Hewitt, it appeared that the overwhelming norm was that multilocus hybrid zones consisted solely of coincident clines. However, since then there have been an increasing number of examples where the clines for particular characters in hybrid zones have been found to be separated from each other, and this may be a rather frequent phenomenon (Barton, 1993; Parsons *et al*., 1993; Searle, 1993; Jaarola *et al*., 1997; Butlin, 1998). In contacts between well-differentiated races, such non-coincidence may be missed if too few characters are examined. For example, many of the clines identified in the well-studied Pyrenean hybrid zone between the grasshoppers *Chorthippus parallelus parallelus* and *C. p. erythropus* are coincident, but others are slightly separated from the majority and some are staggered by a very large distance (Butlin, 1998). Clearly, sampling only a subset of the characters may have failed to reveal the occurrence of non-coincident clines. Typically, several-to-many characters are scored simultaneously in the analysis of chromosomal hybrid zones, and there are now a substantial number of examples where such zones are known to have a staggered structure (Searle, 1993). The Barcelona hybrid zone represents another case with seven out of ten pairwise comparisons between clines showing significant non-coincidence.

There is a range of possible explanations to explain the non-coincidence of clines in a hybrid zone (Barton, 1993). As we will detail below, a number of these could apply to the Barcelona hybrid zone, and further studies are needed to distinguish between them.

As indicated above, the Barcelona race is likely to have been formed *in situ*, which raises the possibility that the contact with the standard race is primary, i.e. the metacentrics that define the race became fixed within part of the continuous distribution of the standard race, without the need for geographic isolation. White (1978) proposed this as the general mode of formation of karyotypic races of house mice, and envisaged that metacentrics have a selective advantage which promotes their spread from their site of origin. In this case, the staggering of clines could represent the sequence of formation of the metacentrics, with the metacentric that has the largest distribution (and cline centre furthest from the race centre) being the earliest formed metacentric (i.e. 4·14) and the metacentric with the smallest distribution (and cline centre closest to the race centre) being the most recently formed metacentric (i.e. 3·8). However in consideration of this model there are some unexplained details, such as the precise selective factor that promotes this rather slow and limited spread. Also, it is not clear why the metacentric clines should widen the further they are from the race centre.

As an alternative to the model of primary contact, the *in situ* origin of the Barcelona race could have occurred in allopatry at the local level, with subsequent secondary contact with the standard race. The current single centre to the distribution of all the metacentrics can be presumed to have been the location of the allopatric population. Clearly, historical records and studies with other genetic markers may help to confirm the existence of this past geographic isolate. On this model, when the fully formed Barcelona race came into secondary contact with the standard race, six coincident metacentric clines would have been formed. It is important to understand why the forces that are expected to hold coincident clines of unfitness together (Barton & Hewitt, 1985) might not have been sufficiently strong to prevent staggering in this case. It is interesting that among the well-studied hybrid zones in the house mouse between a metacentric race and the standard race, those (including the Barcelona zone) with up to six metacentric clines have a staggered structure, while those with nine clines do not display staggering (Bauchau *et al*., 1990; Searle *et al*., 1993; Fel-Clair *et al*., 1996; Castiglia & Capanna, 1999; Saïd *et al*., 1999; present study); it may be that when nine clines are present the levels of hybrid unfitness are sufficiently large to keep the clines together.

There are two general explanations why originally coincident metacentric clines in the Barcelona hybrid zone may have become separated: epistasis and genetic drift. These will be considered in turn.

On the epistatic model, individuals that are heterozygous for several metacentrics have an unfitness greater than the sum of unfitnesses associated with heterozygosity for each of the metacentrics on its own. This accords reasonably well with data from the house mouse, which show that single heterozygotes have

fertility that is virtually indistinguishable from homozygotes and highly heterozygous individuals have distinctly reduced fertility (Searle, 1993). Under epistatic selection, a recombinant homozygous karyotype (i.e. homozygous for some but not all the Barcelona race metacentrics) may have been favoured when the Barcelona and standard races first made contact, because individuals with this karyotype cannot produce the most highly heterozygous (and unfit) hybrids in crosses with either of the parental races. An increase in frequency of such a recombinant homozygote in the centre of the zone would inevitably have caused the separation of some of the clines. This process could have separated the six coincident clines of the original Barcelona-standard hybrid zone into two groups of clines (e.g. 3·8 and 6·10 from all the others); epistatic selection could further separate these clines into smaller groups or single clines (e.g. separation of the clines for 3·8 from 6·10).

Epistatic selection has been suggested as a cause of cline displacement in hybrid zones of several species including mice (e.g. Searle, 1993; Jaarola *et al*., 1997; Butlin, 1998). Applying the model to the Barcelona zone, there are, as expected, very few of the most highly heterozygous (and therefore most unfit) mice in the zone (even mice heterozygous for three metacentrics are unusual). However, novel homozygous forms are not at high frequency within the zone; in contrast to other staggered hybrid zones (e.g. the John o'Groats-standard mouse zone: Searle *et al*., 1993). To properly test the epistatic model, there is clearly a need to measure accurately the fitnesses of the different types of single and multiple heterozygotes, not only to provide an explanation of the non-coincidence of clines in the Barcelona hybrid zone but also the observed variation in their width. Previous modelling on non-coincidence in a shrew zone provides some indication that clines under epistatic selection may steepen as they move away from the site of original contact (Hatfield *et al*., 1992).

Rather than evoking selection to explain the separation of the metacentric clines in the Barcelona hybrid zone, particular population structures and dynamics may have created the conditions for cline separation by genetic drift. For example if, after initial contact, a gradient in population density caused movement of the zone, certain clines might have been left behind during the movement (as suggested for the Australian grasshopper *Caledia captiva*: Marchant *et al*., 1988). Alternatively, the bulk of the clines may have stayed put and only some moved. Another situation that might have caused cline separation relates to the tendency for the commensal habitat occupied by house mice to be patchy in distribution (e.g. Hauffe & Searle, 1993). If the contact between the Barcelona and standard races was initially limited to one of these habitat patches, a particular recombinant homozygous karyotype may have become fixed by genetic drift. This could have initiated the process of cline shift. Habitat patchiness within the hybrid zone could have promoted clinal movement subsequent to initial contact as well. Action of genetic drift within a patchy contact area has been suggested as a cause of staggering of morphological clines in the *Chorthippus* hybrid zone (Butlin *et al*., 1991). It should be noted, however, that epistatic selection may also be particularly effective in generating recombinant homozygotes within habitat patches (Piálek *et al*., in press). The degree of habitat patchiness is not only relevant to clinal displacement, it could also relate to the variation in width of the different clines in the Barcelona hybrid zone. Within a hybrid zone, the more patchy the habitat the wider the clines are likely to be (Nichols, 1989; Butlin *et al*., 1991). So, it would be of interest to compare habitat features with the variation in cline width and position for the Barcelona zone. The clines furthest from the race centre (4·14, 5·15 and 12·13) are particularly wide ($>$ 30 km) compared with other measured metacentric clines in the house mouse (Fel-Clair *et al*., 1996), and thus may be centred in the most patchy habitat.

As pointed out by Endler (1977), it is difficult to establish the history of a hybrid zone. This is certainly true of the Barcelona zone, although there are a number of clearly distinct explanations for the non-coincidence of clines in the zone. Further data on the ecology and population genetics of mice from the vicinity of the zone are needed to distinguish between them.

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