

## Altitudinal distribution and outdoor occurrence in chromosomal races of the house mouse (*Mus musculus domesticus*) in central Italy

Riccardo CASTIGLIA<sup>1\*</sup> and Marco CAPORIONI<sup>2</sup>

<sup>1</sup> Department of Human and Animal Biology, University of Rome “La Sapienza”, Via A. Borelli 50, 00161 Rome, Italy; e-mail: castiglia@uniroma1.it

<sup>2</sup> Via Casoria 47, 00182 Rome, Italy

Received 16 November 2004; Accepted 27 July 2005

**Abstract.** We reviewed the distribution of Robertsonian (Rb) races of *Mus musculus domesticus* in central and southern Italy. This Rb system is called the Apennine system and includes four races (Cittaducale, ICDE, 2n=22; Ancarano, IACR, 2n=24; Campobasso, ICBO, 2n=22; Colfiorito, ICOL, 2n=33–34) surrounded by standard populations with karyotype 2n=40. Here we evaluate the relationships between the altitudinal distribution of races, and the indoor vs. outdoor behaviour of populations, inferred from literature data on the diet of the barn owl *Tyto alba*. We assume that a higher prevalence of mice in owl pellets reflects a higher outdoor occurrence of mice. The IACR and ICDE races were found at higher altitudes than the standard populations, while the ICBO race is present at lower altitudes like the standard race. The standard race has indoor and outdoor populations; in all the Rb races an indoor life has been suggested by our data. This behaviour is only partly due to altitude, since the ICBO race also lives at sea level. We speculated that indoor life is an intrinsic characteristic of the ICBO race irrespective of the environment. This pattern reinforces the idea that indoor life, through its population dynamics, has played a significant role in the evolutionary history of Rb races.

**Key words:** barn owl, evolutionary history, house mouse, Robertsonian race, chromosomal speciation, *Tyto alba*

### Introduction

Many animal and plant species exhibit chromosomal polytypism in natural populations (King 1993). The long-tailed house mouse, *Mus musculus domesticus*, is one of the best-studied cases of chromosomal variability in mammals, with many races scattered throughout Europe and North Africa (Nacchman & Searle 1995). 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; there are races with all the intermediate diploid numbers.

The evolutionary significance of these races has been debated since their discovery (Gropp et al. 1969). Previous studies have dealt with a wide spectrum of fields, including the molecular characterisation of the Rb fusion (Gargna et al. 2002), the fertility of structural hybrids (Redi & Capanna 1988), the structure of hybrid zones between chromosomal races (Searle 1993) and the behavioural interactions between races (Capanna et al. 1984, Ganem & Searle 1996, Carpineti & Castiglia 2004). There is very little information about the behavioural ecology of Rb vs. standard populations (Ganem et al. 1996, Chatti et al. 1999). From an ecological point of view, house mouse populations are traditionally divided into feral and commensal populations also referred as “outdoor” and “indoor” (Anderson 1961, Bronson 1979, Pocock et al. 2004). The

---

\*Corresponding author

former populations live in natural environments not closely related to the presence of humans; the latter include commensal populations closely associated with buildings.

This bimodality of environmental preferences is at least partly due to climate: populations living in continental areas are primarily commensal while Mediterranean populations show different patterns of ecological preferences (Auffray et al. 1990). However, the distinction between these two extreme habitat preferences is not sharp, and even in strict commensal conditions, short- or long-distance migration between demes can ensure genetic exchanges (Auffray et al. 1990). Moreover, areas with predominantly outdoor mouse populations may also include mice occupying indoor habitats.

It has been suggested that ecological preferences by house mouse populations have played an important role in the chromosomal diversification observed in the subspecies (Gane 1993); one consequence of living indoors is that populations are geographically isolated by inhospitable “outdoor” environments, with significant effects on the genetic structure of populations. Isolation is thought to be an important prerequisite for the formation of Rb populations because it enhances genetic drift, favouring the fixation of metacentric chromosomes (Fraguetsalis-Tsolis et al. 1997).

Indeed, almost all continental Rb populations live indoors in close association with humans, while the standard populations occur in a great variety of ecological conditions, both indoors and outdoors (Gane 1993). Nevertheless, the relationship between indoor life and the formation of Rb races is far from clear; the presence of standard populations living indoors and a few Rb populations living outdoors indicates that commensalism is not a necessary condition to become Robertsonian. Moreover, commensalism may only be a by-product of the demographic and ecological conditions that have led to the formation of Rb races, without any causal effect.

One approach to this subject is to compare the ecological characteristics of Rb and standard mouse populations living in the same Rb system, i.e. groups of metacentric populations with an apparently common evolutionary origin, grouped on the basis of shared metacentrics and geographical proximity (Pialek et al. 2005); this allows to minimize the influence of different environmental factors.

The Robertsonian system in central Italy consists of four metacentric races: Cittaducale ICDE (2n=22), Ancarano IACR (2n=24), Campobasso ICBO (2n=22) (Capanna et al. 1977) and Colfiorito ICOL (2n=33–34); no homozygote populations have been found for ICOL (Sadoyan et al. 2003). Although there have been several studies of wild Rb and standard populations of this system (Castiglia & Capanna 1999, Castiglia et al. 2002, 2005, Sadoyan et al. 2003), a complete overview of the distribution of the chromosomal races is lacking.

Here we review the distribution of all the localities in central and southern Italy where house mice have been karyotyped (including all literature and unpublished data) in order to depict the distribution of different metacentric races as accurately as possible, as well as the distribution of the standard 40-chromosome race. We use altitudinal data as a good indicator of ecological and bioclimatic conditions (Pignatti 1995). The outdoor vs. indoor characteristics of the populations were inferred from literature data on the diet of the barn owl in different localities. The presence of house mice in owl pellets reflects, albeit with some caution (see methods), the outdoor occurrence of the species (Auffray et al. 1990).

In this way, we provide an ecological characterisation, i.e. the pattern of altitudinal distribution and the outdoor vs. indoor occurrence in the different races, of the chromosomal races

of the Apennine system and discuss the results within the chromosomal diversification of the house mouse.

## Materials and Methods

All the chromosomal data from central and southern Italy were reviewed (Table 1, Fig. 1). The altitude and location were recorded for each site. Some sites were pooled on the map because of their close proximity. Chromosomal identifications from unpublished sources were also provided. In these cases, chromosome preparations were based on bone marrow (Hsu & Patton 1969) and cultured cells from ear biopsy (Stanyon & Galleni 1991); G-bands were enhanced with trypsin to identify the different telocentrics involved in chromosomal fusion (Seabright 1971).

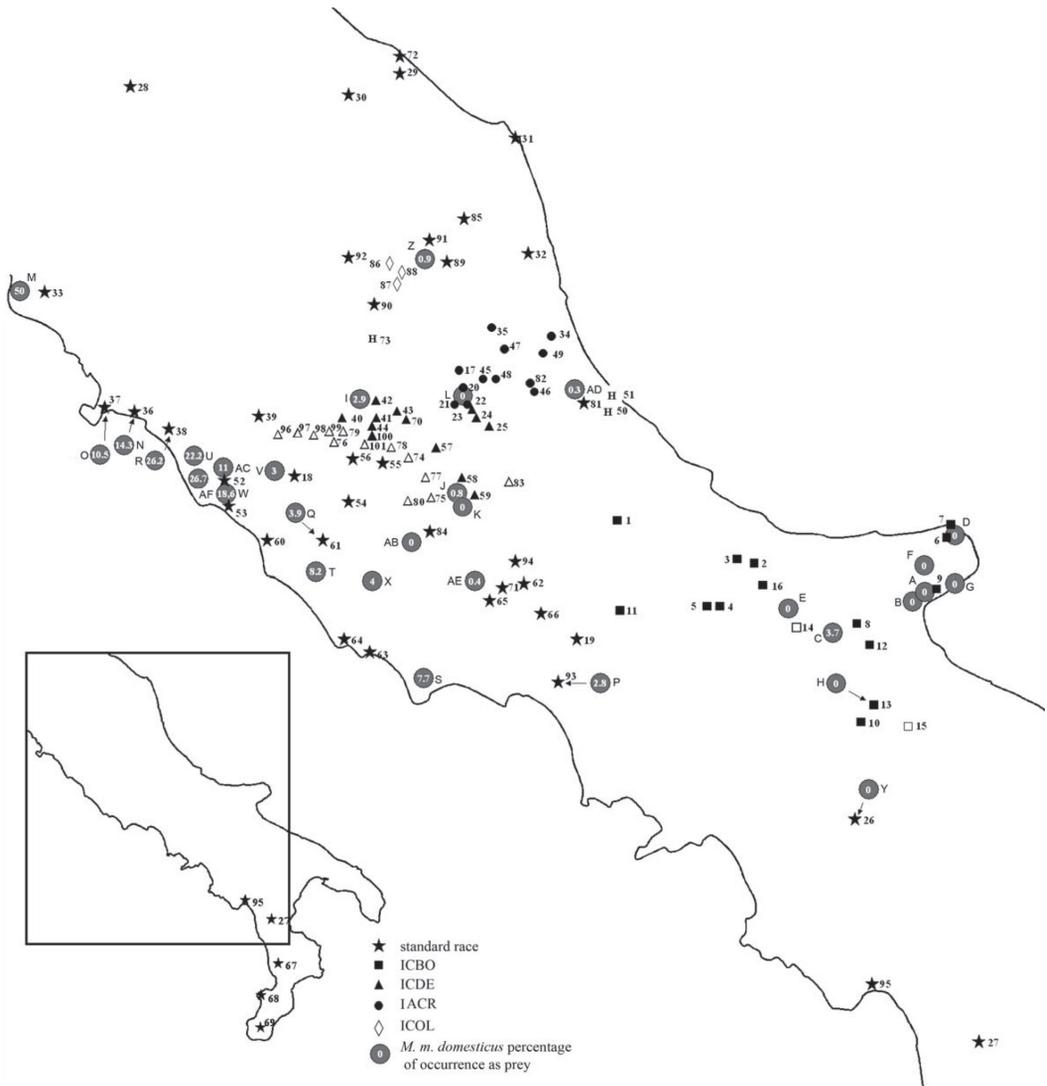
Data on the presence of *M. m. domesticus* were taken from the literature and are reported as the percentage of *M. m. domesticus* individuals with respect to the overall small mammal prey in barn owl pellets (Table 2). We selected the occurrence localities on the basis of their distance from chromosomal data sites. In a few cases, the chromosomal data site was the same as the site with owl pellet data; in other cases, the owl pellet site was very close to or clearly within the range of the determined race (Fig. 1). We used the percentage of occurrence of *M. m. domesticus* individuals to compare outdoor vs. indoor behaviour between different sites. This method has some limitations; for example, a population could live outdoors but not be very numerous and thus be under-represented in owl pellets. Moreover, in a few cases, the barn owl could also prey on indoor house mice, for example inside a barn or in a granary. Nevertheless, the advantage of this method is clear: the data are based on a very large sample and each owl pellet site can represent an area of approximately 7 km<sup>2</sup> (Mikkola 1983).

In the study area, the altitude ranges from sea level to 2912 m asl (Gran Sasso Mountain) in the central Apennines. This altitudinal gradient corresponds to wide temperature variation: the average annual temperature ranges from 8°C in the mountain subatlantic belt (from 1300 to 2000 m asl) to 18°C in dry Mediterranean areas (below 600 m asl). The annual rainfall is 500–1500 mm (Pignatti 1995). The proportion expressing the presence of mice in the pellets from each locality were regressed against altitude. Moreover, we describe the altitudinal distribution of the different races pooling sites in six different altitudinal belt: 0–199, 2) 200–399, 3) 400–599, 4) 600–799, 5) 800–999, 6) >1000 m. An ANOVA with “post-hoc” pairwise comparison based on Tukey’s HSD test was used to evaluate the differences in mean altitude between races.

## Results

### Distribution of the chromosomal races and hybrid zone

Chromosomal data are available for 118 localities (Table 1). However, some of them were very close together and were pooled. Therefore, 101 sites are reported on the map in Fig. 1. The standard race was found in 41 localities, the ICBO race in 14, IACR in 17 and ICDE in 16. Hybrid individuals were found in 30 localities, almost all in hybrid zones between the ICDE and standard races; detailed analyses of the contact zone are available for these areas (see Castiglia & Capanna 1999, Castiglia et al. 2002). Hybrids between the ICBO and standard races were found in 2 sites. Hybrids were also recorded in 3 other sites, but in these cases the interbreeding races are unknown.



**Fig. 1.** Distribution of chromosomal races in central and southern Italy. White symbols indicate hybrid populations. Grey circles indicate the localities for which barn owl predation data are available. The number inside the grey circle indicates the percentage of occurrence of *M. m. domestica* individuals with respect to the overall mammal prey. Numbers and letters identifying the localities are as in Table 1 and 2.

### Altitudinal distribution of races

The altitudinal distribution of the three Rb races and the standard populations is shown in Fig. 2 where, for each race, the percentage of the localities for each altitudinal belt is indicated. The standard race mainly inhabits areas up to 400 m asl, representing 70% of all standard mouse presences. The remaining 30% of observations fall between 400 and 1000 m. There are no records over 1000 m. The mean altitude is  $267 \pm 260$  m (mean  $\pm$  standard deviation).

**Table 1.** Site number on the map; name of the locality; altitude (m asl); chromosomal race (Cittaducale ICDE (2n=22), Ancarano IACR (2n=24), Campobasso ICBO (2n=22), Colfiorito ICOL (2n=33–34), standard (2n=40)); reference from which the chromosomal data were obtained. When hybrid populations are present in the population, the number corresponds to the mean diploid number in the population and the race in brackets is the Rb race interbreeding with the standard race (if known).

| Site number | Locality             | m (a.s.l.) | Race        | Ref. |
|-------------|----------------------|------------|-------------|------|
| 1           | Palena               | 868        | ICBO        | f    |
| 2           | Larino               | 366        | ICBO        | f    |
| 3           | Guardialfiera        | 169        | ICBO        | f    |
| 4           | Matrice              | 697        | ICBO        | f    |
| 5           | Ripamolisani         | 653        | ICBO        | f    |
| 6           | Vico del Gargano     | 399        | ICBO        | f    |
| 7           | Peschici             | 0          | ICBO        | f    |
| 8           | Palmori-Lucera       | 219        | ICBO        | f    |
| 9           | Monte Aquilone       | 101        | ICBO        | f    |
| 10          | Candela              | 332        | ICBO        | f    |
| 11          | Fornelli             | 460        | ICBO        | f    |
| 12          | Foggia               | 58         | ICBO        | f    |
| 13          | Ascoli Satriano      | 396        | ICBO        | f    |
| 14          | Pietra Montecorvino  | 509        | 24.7 (ICBO) | f    |
| 15          | San Carlo            | 300        | 22.2 (ICBO) | f    |
| 16          | Bonefro              | 620        | ICBO        | h    |
| 17          | Torrìta              | 1032       | IACR        | h    |
| 18          | Settevene            | 210        | standard    | h    |
| 19          | Cassino              | 989        | standard    | h    |
| 20          | Capitignano          | 948        | IACR        | e    |
| 20          | Marana               | 812        | IACR        | e    |
| 20          | Mopolino             | 948        | IACR        | e    |
| 20          | Piedicolle           | 948        | IACR        | e    |
| 20          | S. Giovanni Paganico | 948        | IACR        | e    |
| 21          | Cascina              | 800        | IACR        | e    |
| 22          | Barete               | 726        | IACR        | e    |
| 22          | Pizzoli              | 742        | IACR        | e    |
| 23          | Pizzoli 1            | 742        | ICDE        | e    |
| 23          | Pizzoli 2            | 742        | ICDE        | e    |
| 24          | San Vittorino        | 670        | ICDE        | e    |
| 25          | L'Aquila             | 637        | ICDE        | e    |
| 26          | Muro Lucano          | 421        | standard    | f    |
| 27          | Campotenesè          | 980        | standard    | j    |
| 28          | Compiobbi            | 203        | standard    | a    |
| 29          | Carignano            | 100        | standard    | a    |
| 30          | Urbino               | 277        | standard    | a    |
| 31          | Monte Conero         | 50         | standard    | a    |
| 32          | Petrìtoli            | 209        | standard    | a    |
| 33          | Tìrli                | 375        | standard    | a    |
| 34          | Ancarano             | 293        | IACR        | a    |
| 35          | Quintodecimo         | 787        | IACR        | a    |
| 36          | Lago di Burano       | 0          | standard    | a    |
| 37          | Orbetello Scalo      | 0          | standard    | a    |

|    |                       |      |              |   |
|----|-----------------------|------|--------------|---|
| 38 | Montalto di Castro    | 25   | standard     | a |
| 39 | Viterbo               | 359  | standard     | a |
| 40 | Calvi d'Umbria        | 400  | ICDE         | a |
| 41 | Collebeccaro          | 488  | ICDE         | a |
| 42 | Greccio               | 705  | ICDE         | a |
| 43 | Piana Reatina         | 388  | ICDE         | a |
| 44 | S. F. di Contigliano  | 548  | ICDE         | a |
| 45 | Campotosto            | 1313 | IACR         | a |
| 46 | Isola del Gran Sasso  | 559  | IACR         | a |
| 47 | Rocca S. Maria        | 1074 | IACR         | a |
| 48 | Senarica-Crognaleto   | 1100 | IACR         | a |
| 49 | Teramo                | 361  | IACR         | a |
| 50 | Moscufo               | 143  | 39.8         | a |
| 51 | Spoltore              | 109  | 39.8         | a |
| 52 | Monti Tolfa           | 449  | standard     | a |
| 53 | Santa Severa          | 30   | standard     | a |
| 54 | Monterotondo          | 151  | standard     | a |
| 55 | Poggio nativo         | 400  | standard     | a |
| 56 | Torrta Tiberina       | 35   | standard     | a |
| 57 | Fiamignano            | 834  | ICDE         | a |
| 58 | Torano                | 692  | ICDE         | a |
| 59 | Scurcola Marsicana    | 704  | ICDE         | a |
| 60 | Maccarese             | 4    | standard     | a |
| 61 | Roma                  | 15   | standard     | a |
| 62 | Sora                  | 308  | standard     | a |
| 63 | Foce verde            | 0    | standard     | a |
| 64 | Lavinio               | 0    | standard     | a |
| 65 | Torrice               | 206  | standard     | a |
| 66 | Roccasecca            | 255  | standard     | a |
| 67 | Aiello Calabro        | 535  | standard     | a |
| 68 | S. Domenico di Ricadi | 15   | standard     | a |
| 69 | Aspromonte            | ?    | standard     | a |
| 70 | Cittaducale           | 406  | ICDE         | b |
| 71 | Boville Ernica        | 190  | standard     | b |
| 72 | Pesaro                | 41   | standard     | b |
| 73 | Spoletto              | 500  | 37           | b |
| 74 | Castel di Tora        | 526  | 36.3 (ICDE)  | b |
| 75 | Pereto                | 861  | 38 (ICDE)    | b |
| 76 | Stimigliano           | 117  | 34 (ICDE)    | b |
| 77 | Nespolo               | 794  | 37.5 (ICDE)  | b |
| 78 | Ornaro                | 599  | 32 (ICDE)    | b |
| 79 | Cantalupo in Sabina   | 106  | 39.3 (ICDE)  | b |
| 80 | Riofreddo             | 593  | 39.5 (ICDE)  | b |
| 81 | Penne                 | 289  | standard     | l |
| 82 | Ornano                | 430  | IACR         | l |
| 83 | Ovindoli              | 1341 | 22.2 (ICDE)  | l |
| 84 | Subiaco               | 694  | standard     | l |
| 85 | Tolentino             | 378  | standard     | i |
| 86 | Colfiorito            | 760  | 36–39 (ICOL) | i |
| 87 | Forcella              | 1073 | 37 (ICOL)    | i |

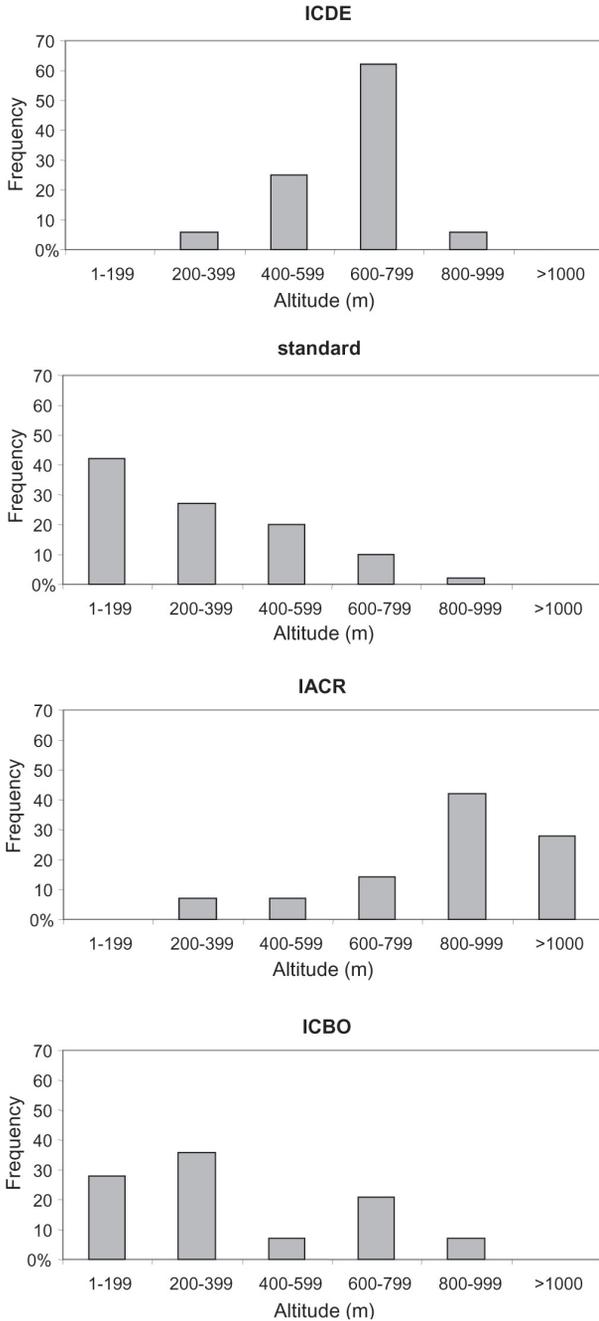
|     |                      |     |              |   |
|-----|----------------------|-----|--------------|---|
| 88  | Costa                | 640 | 38–39 (ICOL) | i |
| 89  | Acquacarina          | 700 | standard     | i |
| 90  | Trevi                | 425 | standard     | i |
| 91  | Camerino             | 459 | standard     | i |
| 92  | Assisi               | 422 | standard     | j |
| 93  | Piana del Garigliano | 8   | standard     | a |
| 94  | Balsorano            | 468 | standard     | b |
| 95  | Policastro           | 35  | standard     | h |
| 96  | Carbognano           | 400 | 28.1 (ICDE)  | g |
| 96  | Vignanello           | 369 | 37 (ICDE)    | g |
| 97  | Fabbrica             | 296 | 28.7 (ICDE)  | g |
| 97  | Gallese              | 150 | 33.3 (ICDE)  | g |
| 98  | Borghetto            | 230 | 31 (ICDE)    | g |
| 99  | Forano               | 208 | 38 (ICDE)    | g |
| 99  | Gavignano            | 207 | 39.6 (ICDE)  | g |
| 100 | Le mole A            | 749 | ICDE         | d |
| 100 | Le mole B            | 749 | ICDE         | d |
| 101 | Le mole              | 749 | 22.5 (ICDE)  | d |
| 101 | S. Sebastiano        | 749 | ICDE         | d |
| 101 | Montecavallo         | 749 | 24.7 (ICDE)  | d |
| 101 | Santo Pietro         | 700 | 26.3 (ICDE)  | d |
| 101 | San Benedetto        | 699 | 35 (ICDE)    | d |
| 101 | Colle Paradiso       | 400 | 38.8 (ICDE)  | d |
| 101 | Madonna del Mattone  | 209 | 39.8 (ICDE)  | d |
| 101 | Salisano             | 209 | 37.5 (ICDE)  | d |

References are as follow: a, Amori et al. (1984); b, Capanna et al. (1977); c, Capanna et al. (1994); d, Castiglia & Capanna (1999); e, Castiglia et al. (2002); f, Cerone & Aloise (1993); g, Corti & Ciabatti (1988); h, Nachmann et al. (1994); i, Sadoyan et al. (2003); j, Castiglia & Caporioni, pers. comm.; k, Corti, pers. comm.; l, Civitelli, pers. comm.

There is clear variability in the altitudinal distribution of the Rb races. The ICDE and IACR races were never recorded below 200 m, their presence beginning with a few observations between 200 and 399 metres. The IACR race was found at higher altitudes (70% of observations over 800 m), while the ICDE race occurred between 400 and 800 m (87% of all observations). The difference between the means between these two races is not significant (IACR =  $795 \pm 290$  m; ICDE =  $662 \pm 148$  m) ( $Q = 2.84$ ,  $P = 0.19$ ).

The altitudinal distribution of the ICBO race is different from that of the other Rb races, more closely resembling the distribution of the standard race (Fig. 2). Although ICBO mice were present from sea level to 800–999 m, 64% of the observations were between 0 and 400 m (mean  $381 \pm 259$  m). The difference in mean altitude between the ICBO and standard races is not significant ( $Q = 1.87$ ,  $P = 0.55$ ), while the differences between the ICBO race and the IACR and ICDE races are significant (respectively  $Q = 6.80$ ,  $P < 0.001$  and  $Q = 3.96$ ,  $P < 0.05$ ).

The hybrid zone for which most data are available is that between the ICDE and standard races. A negative correlation ( $N = 22$ , Pearson's  $r = -0.51$ ,  $P = 0.01$ ) between diploid number and altitude confirms previous observations made in a single-transect study (Spirito et al. 1980, Castiglia et al. 1999).



**Fig. 2.** Altitudinal distribution of the chromosomal races of *M. m. domesticus* of the Apennine system. The percentage of the localities where each race has been found (Y axis) in each altitudinal belt (X axis) is indicated. Sample sizes are as follow: ICDE: N = 14, standard: N = 40, IACR: N = 15, ICBO: N = 14. Sample size is slightly different from the number of the localities because very close localities were lumped.

**Table 2.** Presence of *Mus musculus* in barn owl pellets: data taken from the literature and reported as the percentage of *M. musculus* individuals with respect to the overall small mammal prey. The site is indicated with letters (see also the map in Fig. 1); also indicated are the altitude (m asl), total number of prey analysed, number and percentage (in brackets) of *Mus musculus domesticus*, the chromosomal race (“~” indicates that the chromosomal data site is very close to or clearly within the range of the determined race), the reference from which chromosomal data were obtained.

| Site | Locality             | m (asl) | N° tot. mammal prey | N° <i>Mus</i> (%) | Race      | Ref. |
|------|----------------------|---------|---------------------|-------------------|-----------|------|
| A    | Tratturo             | 50      | 45                  | 0 (0)             | ~ICBO     | d    |
| B    | San Leonardo         | 100     | 158                 | 0 (0)             | ~ICBO     | d    |
| C    | Lucera               | 186     | 54                  | 2 (3.7)           | ~ICBO     | d    |
| D    | Manacore             | 230     | 88                  | 0 (0)             | ~ICBO     | d    |
| E    | Casale Monter.       | 392     | 94                  | 0 (0)             | ~ICBO     | d    |
| F    | Valle Palombara      | 450     | 200                 | 0 (0)             | ~ICBO     | d    |
| G    | V. inferno           | 500     | 169                 | 0 (0)             | ~ICBO     | d    |
| H    | Ascoli Satriano      | 396     | 108                 | 0 (0)             | ICBO      | d    |
| I    | S. Pastore           | 500     | 341                 | 10 (2.9)          | ~ICDE     | b    |
| G    | Tagliacozzo          | 750     | 118                 | 1 (0.8)           | ~ICDE     | b    |
| K    | Monte Bove           | 1200    | 202                 | 0 (0)             | ~ICDE     | b    |
| L    | Marana di Montereale | 850     | 537                 | 0 (0)             | IACR      | b    |
| M    | Piombino             | 0       | 34                  | 17 (50)           | ~standard | f    |
| N    | Stagno di Burano     | 0       | 14                  | 2 (14.3)          | standard  | f    |
| O    | Argentario           | 0       | 256                 | 27 (10.5)         | standard  | f    |
| P    | Piana del Garigliano | 8       | 3626                | 101 (2.8)         | standard  | a    |
| Q    | Centocelle           | 15      | 255                 | 10 (3.9)          | standard  | f    |
| R    | Montalto di Castro   | 25      | 80                  | 21 (26.2)         | standard  | f    |
| S    | Circeo foresta       | 50      | 26                  | 2 (7.7)           | ~standard | f    |
| T    | Castel Porziano      | 50      | 290                 | 24 (8.2)          | ~standard | f    |
| U    | Tarquinia            | 150     | 45                  | 10 (22.2)         | ~standard | f    |
| V    | Sabatino             | 167     | 223                 | 7 (3)             | ~standard | c    |
| W    | Pian Sultano         | 200     | 86                  | 16 (18.6)         | ~standard | f    |
| X    | Albano               | 245     | 318                 | 14 (4)            | ~standard | c    |
| Y    | Muro lucano          | 300     | 289                 | 1 (0.3)           | standard  | e    |
| Z    | Pontelatrave         | 400     | 106                 | 1 (0.9)           | ~standard | b    |
| AB   | Bellegra             | 420     | 134                 | 0 (0)             | ~standard | b    |
| AC   | Tolfetano            | 445     | 6808                | 771 (11)          | ~standard | c    |
| AD   | Mallo-Penne          | 450     | 290                 | 1 (0.3)           | ~standard | b    |
| AE   | S. Sebastiano        | 500     | 237                 | 1 (0.4)           | ~standard | b    |
| AF   | Mole del Mignone     | 500     | 127                 | 34 (26.7)         | ~standard | f    |

References are as follow: a, A m a r e n a et al. (1993); b, A m o r i et al. (1991); c, A s t e & C o n t o l i (1987); d, B u x et al. (2000); e, C e r o n e & A l o i s e (1993); f, C o n t o l i (1975).

## Outdoor occurrence

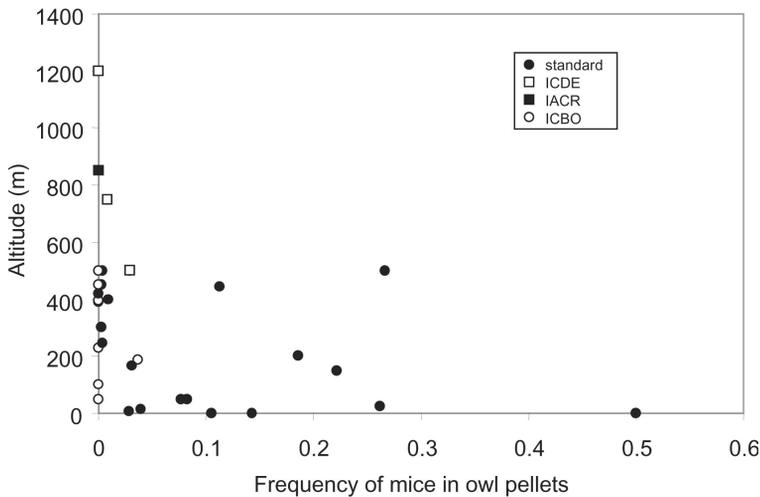
Fig. 1 shows the distribution of the sites for which data on presence of house mouse in the pellet are available. A high occurrence of mice in pellet (greater than 10%) has been observed only in the western part of the studied area (Fig. 1). Figure 3 shows the relationships between the proportion of house mouse in the pellets and altitude for each site. When considering the entire dataset, the outdoor occurrence is slightly related to the altitude ( $r = -0.37$ ,  $P = 0.04$ ).

For the standard race, we found 19 sites with barn owl predation data (Table 2). Therefore, the pellet observations cover an area of approximately 133 Km<sup>2</sup>. Six of these localities coincide exactly with chromosomal data sites. The locations of the other 13 sites make the presence of the standard race very likely. The presence of *M. m. domesticus* individuals with respect to the total number of mammal prey varies from 0 to 50%. The percentage of occurrence in pellets is not significantly correlated with altitude for this race ( $r = -0.35$ ,  $P = 0.14$ ) (Fig. 3). In five populations, the percentage is very high (18.6 – 50%), indicating that the house mouse uses both outdoor and indoor habitats in these sites (see introduction). All these localities are settled within 0–400 m and are present in coastal Mediterranean bioclimate. In five cases, the presence in pellets is sporadic (less than 1.0 %), suggesting that the house mouse is strictly commensal. These localities are settled all within 300–500 m in a more temperate bioclimate.

All the Rb races have very low level of presence in pellets irrespective with altitude that ranges from the sea level up to 1200 m (Fig. 3). Mice within the IACR and the ICDE races were only searched in four sites, and the low percentage presence (0 – 2.9%) suggests a strong indoor occurrence in these localities. They are all in high-altitude areas, i.e. from 500 to 1200 m, with low mean annual temperature. Therefore, the indoor behaviour may be related to the cold climate in these areas.

More information is available for the ICBO race. Seven of the owl pellet sites are in close proximity to chromosome sites or within the range of the race; one locality coincides with the chromosome site. The presence of ICBO house mice in owl pellets is very low, indicating the strict indoor behaviour of the populations of this race. These owl pellet sites are all at low altitude (50–450 m), comparable to the altitude of the standard race.

In Table 3 the percentage of house mice in owl pellets from different altitudinal belts, obtained by pooling all sites within the same belt, is shown for the different chromosomal races.



**Fig. 3.** Scatterplot indicating the relationships between proportion of house mice in the pellets and the altitude of the site. Standard: black circles; IACR: black square; ICDE: white square; ICBO: white circle.

The presence of house mice in owl pellets is very low for the Rb races (mean: ICBO 0.2%; IACR 0%; ICDE 1.7%; Table 3). The outdoor presence of the standard populations is instead much higher (8 %) ( $P < 0.001$  for all comparisons).

**Table 3.** Percentage of house mice in owl pellets from different altitudinal belts for the different chromosomal races, obtained by pooling all sites within the same belt. Literature is cited in the legend of Table 2.

| Alt. belt | Standard (N = 19) |            |      | ICBO (N = 8) |            |     | ICDE (N = 3) |            |     | IACR (N = 1) |            |   |
|-----------|-------------------|------------|------|--------------|------------|-----|--------------|------------|-----|--------------|------------|---|
|           | tot               | <i>Mus</i> | %    | tot          | <i>Mus</i> | %   | tot          | <i>Mus</i> | %   | tot          | <i>Mus</i> | % |
| 1         | 4626              | 214        | 5.6  | 257          | 2          | 0.8 | –            | –          | –   | –            | –          | – |
| 2         | 916               | 38         | 4.1  | 290          | 0          | 0   | –            | –          | –   | –            | –          | – |
| 3         | 7702              | 808        | 10.5 | 369          | 0          | 0   | 341          | 10         | 2.9 | –            | –          | – |
| 4         | –                 | –          | –    | –            | –          | –   | 118          | 1          | 0.8 | –            | –          | – |
| 5         | –                 | –          | –    | –            | –          | –   | –            | –          | –   | 537          | 0          | 0 |
| 6         | –                 | –          | –    | –            | –          | –   | 202          | 0          | 0   | –            | –          | – |
| Tot       | 13244             | 1060       | 8    | 916          | 2          | 0.2 | 661          | 11         | 1.7 | 537          | 0          | 0 |

## Discussion

We reviewed a total of 118 localities (88 from homozygotes and 30 from hybrid populations) so that this Rb system provides one of the best descriptions of the distribution of chromosomal races in the house mouse. Nonetheless, additional areas may reserve some surprises. For example, three new Rb populations, characterised by new metacentrics, were recently discovered at the border between the regions of Umbria and Marche, not far from the ranges of the ICDE and IACR races.

The geographic range of the chromosomal races allows us to characterise them ecologically through their altitudinal distribution and outdoor occurrence. The results show that the IACR and ICDE races occur at higher altitudes than the ICBO and standard races. The differences in altitudinal distribution among races, as observed in this system, do not seem to be a common pattern in other Rb systems of the house mouse. Although there are many cases of Rb races living in the mountains or at high altitude (C a p a n n a et al. 1977), on a small geographic scale we have often observed adjacent chromosomal races living at the same altitude with no evident ecological segregation as, for example, in the complex systems of alps (H a u f f e & S e a r l e 1993) and Madeira (B r i t t o n - D a v i d i a n et al. 2000).

Altitudinal segregation between two chromosomal races of the insectivorous *Sorex araneus* was recently shown on a similar geographic scale (P o l y a k o v et al. 2001). Among arthropods, altitudinal segregation has been found between different Rb races of the Opiliones *Gagrellopsis nodulifera* (G o r l o v & T s u r u s a k i 2000). In both cases, it was hypothesized that the two races are adapted to different ecological conditions.

The central Italian Rb races of the house mouse probably originated *in situ* from at least three distinct areas (C a s t i g l i a et al. 2005). It is possible, therefore, that these races originated not far from the actual range and the distribution at high altitude reflects the original position of their formation. Adaptation of the ICDE race to a mesophilic climate was suggested in a long-term study of a contact zone between ICDE and standard karyotype populations (C a s t i g l i a & C a p a n n a 1999). In this case, the hybrid zone remained in the same position for over 20 years, corresponding to about 100 mouse generations, and this area lay exactly at the border between the Apennine (mesophilic) and lowland (thermoxerophilic) climatic areas. Although other explanations may account for the stability of this zone (see C a s t i g l i a & C a p a n n a 1999 for a complete discussion), the correspondence between race distribution and climate suggests that the ICDE and standard races are adapted to different altitudes.

A high presence of house mouse bones in owl pellets may be informative to infer the outdoor occurrence of house mouse (see introduction). A high presence of mice on pellets has been only found near the coast in the west part of the studied area (Fig. 1). However there are not ecological differences between the west and east coasts both of them falling in the termoxerophilic climate. For these reasons the effect of position should not be involved in the observed pattern and other factors may explain this observation.

Altitude may be one of these factors. In fact a cold environment might prevent the mice from establishing outdoor populations, at least for many months of the year; this may be the case of the the ICDE and IACR races for which the raiation process could, in part, be directly related to the higher altitude. In fact, isolation of populations may be an important prerequisite for race formation, as it facilitates the fixation of Rb fusion in the sub-population and thus the raiation process (F r a g u e t s a l i s - T s o l i s et al. 1997).

The standard race shows a very variable percentage of presence in owl pellets throughout its range, indicating different levels of outdoor occurrence. This pattern could be partly related to altitude but the correlation fail to be significant. In the study area, the standard race is present in a narrow altitudinal range and the bioclimatic variation is probably not sufficient to produce such a variable pattern of outdoor occurrence among populations. Factors other than elevation might explain the differences in outdoor occurrence of the standard race. For example, micro-habitat factors in each site may play an important role in determining the ecological preferences of populations. In any case, higher levels of outdoor occurrence were found in coastal areas under 200 m, which have a dry Mediterranean climate.

The ICBO race has a different pattern of altitudinal distribution and outdoor occurrence. It is found at low altitudes in environments where standard populations have a high degree of outdoor presence. However, the ICBO race shows a low level of occurrence in pellets, suggesting a predisposition for indoor life. Even coastal populations of this race have a low degree of outdoor presence despite the favourable environmental conditions. Therefore, we can speculate that indoor behaviour is an intrinsic characteristic of the ICBO race irrespective of the external environment.

Different scenarios can be proposed to explain the pattern shown by the ICBO race. If it originated *in situ*, the indoor behaviour may represent a condition present during the formation of the race, even though the climatic conditions were favourable to outdoor living. Alternatively, it is possible that the ICBO race was once distributed further north, at higher altitudes in colder areas. Thus the origin does not correspond to the observed range, and this race may have spread by replacing the standard race. In this case, the indoor ecology would represent the maintenance of an adaptive behaviour that is no longer necessary. In both cases, commensalism seems to be a characteristic of this race.

In Tunisia, C h a t t i et al. (1999) found differences in ecological preferences between the Robertsonian races and the standard populations that were clearly unrelated to different climates. The Monastir race (2n=22) was more “indoor”, inhabiting only the older section of towns and the adjacent neighbourhoods, while the standard race seemed to be more plastic, inhabiting every habitat type, in town where the other race was absent, or only agricultural and industrial zones in sympatric conditions. This appears to be exactly the same situation found in our Apennine Rb system, with the standard race showing different levels of outdoor occurrence and the Rb races living indoors.

This relationship may not be a random finding, since the population dynamics determined by indoor life (small deme size, low gene flow) are the same that enhance fixation of meta-centrics in the population (B a r t o n & R o u h a n i , 1991, M i c h a l a k i s & O l i v i e r i

1993). Therefore, indoor life may favour chromosomal evolution in all kinds of “external” ecological conditions.

If the ICBO race maintains indoor behaviour even in ecological conditions favourable to outdoor living, it likely presents characteristics that permit indoor life. There are indications that indoor vs. outdoor life has some influence on house mouse social structure and behaviour. In fact, a strong hierarchical social organisation is expected in commensal mice due to the high population density (B r o n s o n 1979), while a territorial system has been found in feral populations (C a s s a i n g & C r o s e t 1985). Physiological correlates of commensalism were investigated by G a n e m (1991), who found that mice living in outdoor habitats were more responsive to stress than mice from commensal habitats: the outdoor mice had lower corticosterone levels and a higher amplitude of response to stress than commensal mice. Moreover, Rb mice showed a different response from standard mice: it was intermediate between outdoor and indoor standard populations, indicating that some Rb populations present peculiar physiological characteristics. The physiological distinctiveness of Rb races should be confirmed by broader studies, but it seems reasonable that if the populations that gave rise to the ICBO race were physiologically adapted to indoor life (with all the advantages of living in a more stable environment without predators), they would maintain these characters in all environmental conditions.

The results of the present study even if strictly descriptive indicate that there is a strong relationship between Rb races and commensalisms in the studied area; this relationship should be investigated further to clarify the ecological conditions that might trigger chromosomal diversification in the house mouse. However, it remains to be seen whether commensalism is a prerequisite for race formation or a by-product of the raiation process.

#### A c k n o w l e d g e m e n t s

We want to thank E. C a p a n n a , “father” of the Italian Rb mice, and G. A m o r i for discussion and comments on a previous version of the manuscript.

## L I T E R A T U R E

- AMARENA D., CONTOLI L. & CRISTALDI M. 1993: Coenotic structure skull asymmetries and other morphological anomalies in small mammals near an electronuclear power plant. *Hystrix* 5: 31–36.
- AMORI G., CRISTALDI M. & CONTOLI L. 1984: [Distribution of Rodents (Gliridae, Arvicolidae, Muridae) in peninsular and insular Italy with respect to the mediterranean bioclimate]. *Animalia* 11: 217–269 (in Italian).
- AMORI G., CIPOLLONI A. & DI RUSSO C. 1991: [Small mammals in the pellets of the barn owl in the central Apennines]. *Suppl. Ric. Biol. Selv.* 16: 533–537 (in Italian).
- ANDERSON P.K. 1961: Density, social structure and non social environment in house mouse populations and the implication for the regulation of numbers. *Transactions of the New York Academy of Science* 23: 447–451.
- ASTE F. & CONTOLI L. 1987: Diversity and similarity of trophic system “Barn owl-Terrestrial Mammals” in the volcanic districts of Latium (Italy). *Hystrix* 2: 15–25.
- AUFFRAY J.-C., BELKHIR K., CASSAING J., BRITTON-DAVIDIAN J. & CROSET H. 1990: Outdoor occurrence in Robertsonian and standard populations of the house mouse. *Vie Milieu* 40: 111–118.
- BARTON N.H. & ROUHANI S. 1991: The probability of fixation of a new karyotype in a continuous population. *Int. J. Org. Evolution* 45: 499–517.

- BRITTON-DAVIDIAN J., CATALAN J., DA GRACA RAMALHINHO M., GANEM G., AUFRAY J.C., CAPELA R., BISCOITO M., SEARLE J.B. & DA LUZ MATHIAS M. 2000: Rapid chromosomal evolution in island mice. *Nature* 403: 158.
- BRONSON F.H. 1979: The reproductive ecology of the house mouse. *The Quarterly Review Of Biology* 54: 265–299.
- BUX M., RIZZI V., COCUMAZZI B. & PAVONE A. 2000: An analysis of Apulian micromammal populations by studying owl's pellets. *Hystrix* 11: 55–59.
- CAPANNA E., CORTI M., MAINARDI D., PARMIGIANI S. & BRAIN P.F. 1984: Karyotype and intermale aggression in wild house mice: ecology and speciation. *Behaviour Genetics* 14: 195–208.
- CAPANNA E., REDI C.A., VALERI S. & GENTILI G. 1994: Robertsonian chromosomes with new arm combination in a natural hybrid zone between two chromosomal races. *Rend. Fis. Acc. Lincei* 5: 269–276.
- CAPANNA E., CIVITELLI M.V. & CRISTALDI M. 1977: Chromosomal rearrangement, reproductive isolation and speciation in mammals. The case of *Mus musculus*. *It. J. Zool.* 44: 213–246.
- CARPINETI M. & CASTIGLIA R. 2004: Analysis of behavioural discrimination mechanisms in a contact zone between two metacentric races of the house mouse, *Mus musculus domesticus*, in central Italy. *Rend. Fis. Acc. Lincei.* 9: 31–41.
- CASSAING J. & CROSET H. 1985: Organisation spatiale, competition et dynamique des populations sauvages de Souris (*Mus spretus* Lataste et *Mus musculus domesticus* Ruddy) du Midi de la France. *Z. Säugetierkd.* 50: 271–284.
- CASTIGLIA R. & CAPANNA E. 1999: 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., ANNESI F. & CAPANNA E. 2002: Contact zone 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). *Heredity* 89: 219–224.
- CASTIGLIA R., ANNESI F. & CAPANNA E. 2005: Geographical pattern of genetic variation in the Robertsonian system of *Mus musculus domesticus* in central Italy. *Biol. J. Linn. Soc.* 84: 395–405.
- CERONE G. & ALOISE G. 1993: Small mammals fauna of the Muro Lucano area (Potenza, Italy). *Hystrix* 15: 110–115.
- 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.* 266: 1561–1569.
- CONTOLI L. 1975: Micromammals and environment in central Italy: data from *Tyto alba*. *It. J. Zool.* 42:223–229.
- CORTI M. & CIABATTI C.M. 1988: The structure of a chromosomal hybrid zone of house mice (*Mus domesticus*) in central Italy: cytogenetic analysis. *Z. zool. Syst. Evol.* 28: 277–288.
- FRAGUEDAKIS-TSOLIS S., HAUFFE H.C. & SEARLE J.B. 1997: Genetic distinctiveness of a village population of house mice: relevance to speciation and chromosomal evolution. *Proc. R. Soc. Lond. B.* 264: 355–360.
- GANEM G. & SEARLE J.B. 1996: Behavioural discrimination among chromosomal races of the house mouse (*Mus musculus domesticus*). *J. Evol. Biol.* 9: 817–831.
- GANEM G. 1991: A comparative study of different populations of *Mus musculus domesticus*: emotivity as an index of adaptation to commensalisms. *Comp. Biochem. Physiol.* 99: 531–536.
- GANEM G. 1993: Ecological characteristics of the house mouse Robertsonian populations. Is their habitat relevant to their evolution? *Mammalia* 57: 349–357.
- GANEM G., ALIBERT P. & SEARLE J.B. 1996: An ecological comparison between standard and chromosomally variable house mice in northern Scotland. *Z. Säugetierkd.* 61: 176–188.
- GARAGNA S., ZUCCOTTI M., CAPANNA E. & REDI C.A. 2002: High-resolution organization of mouse telomeric and pericentromeric DNA. *Cytogenet. Genome Res.* 96: 125–129.
- GORLOV I.P. & TSURUSAKI N. 2000: Staggered clines in a hybrid zone between two chromosome races of the harvestman *Gagrellopsis nodulifera* (Arachnida: Opiliones). *Int. J. Org. Evolution* 54: 176–90.
- GROPP A., TETTENBORN U. & VON LEHMANN E. 1969: Chromosomenuntersuchungen bei der Tabakmaus (*M. poschiavinus*) und bei den Hybriden mit der Laboratoriumsmaus. *Experientia* 25: 875–876.
- HAUFFE H.C. & SEARLE J.B. 1993: Extreme karyotypic variation in a *Mus musculus domesticus* hybrid zone: the tobacco mouse story revisited. *Int. J. Org. Evolution* 47: 1374–1395.
- HSU T.C. & PATTON J.L. 1969: Bone marrow preparations for chromosome studies. In: Benirschke K. (ed.), Comparative Mammalian Cytogenetics. *Springer-Verlag, Berlin*: 454–460.

- KING M. 1993: Species evolution: the role of chromosome change. *Cambridge University Press, Cambridge, UK*
- MIKKOLA H. 1983: Owls of Europe. *T & AD Poyser, Calton.*
- MICHALAKIS Y. & OLIVIERI I. 1993: The influence of local extinctions on the probability of fixation of chromosomal rearrangements. *Journal of Evolutionary Biology* 6: 153–170.
- NACHMAN M.W., BOYER S.N., SEARLE J.B. & AQUADRO C.F. 1994: Mitochondrial DNA variation and the evolution of Robertsonian chromosomal races of house mice, *Mus domesticus*. *Genetics* 136: 1105–1120.
- NACHMAN M.W. & SEARLE J.B. 1995: Why is the house mouse karyotype so variable? *Trends Ecol. Evol.* 10: 397–402.
- PIÁLEK J., HAUFFE H.C. & SEARLE J.B. 2005: Chromosomal variation in the house mouse. *Biol. J. Linn. Soc.* 84: 535–563.
- PIGNATTI S. 1995: Ecologia vegetale. *UTET, Torino.*
- POCOCK M.J.O., SEARLE J.B. & WHITE P.C.L. 2004: Adaptations of animals to commensal habitats: population dynamics of house mice *Mus musculus domesticus* on farms. *J. Anim. Ecol.* 73: 878–888.
- POLYAKOV A.V., VOLOBOUEV V.T., ANISKIN V.M., ZIMA J., SEARLE J.B. & BORODIN P.M. 2001: Altitudinal partitioning of two chromosome races of the common shrew (*Sorex araneus*) in West Siberia. *Mammalia* 67: 201–207.
- REDI C.A. & 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: 315–359.*
- SADOYAN T., CASTIGLIA R., CAPANNA E. & SERVA L. 2003: Robertsonian polymorphism in house mouse *Mus musculus domesticus* from an area of intense seismic activity. *Acta Theriol.* 48: 189–195.
- SEABRIGHT M. A. 1971: A rapid banding technique for human chromosomes. *Lancet* 2: 971–972.
- SEARLE J.B. 1993: Chromosomal hybrid zones in Eutherian mammals. In: Harrison R.G. (ed.), *Hybrid zones and the evolutionary process. Oxford University Press, New York: 309–353.*
- SPIRITO F., MODESTI A., PERTICONE P., CRISTALDI M., FEDERICI R. & RIZZONI M. 1980: Mechanisms of fixation and accumulation of centric fusions in natural populations of *Mus musculus* L. I. Karyological analysis of an hybrid zone between two populations in the central Apennines. *Int. J. Org. Evolution* 34: 453–466.
- STANYON R. & GALLEN L. 1991: A rapid fibroblast culture technique for high resolution karyotypes. *Boll. Zool.* 58: 81–83.