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THE HOUSE MOUSE CHROMOSOMAL HYBRID ZONE IN VALTELLINA (SO): A SUMMARY. OF PAST AND PRESENT RESEARCH

HEIDI C. HAUFFE*°+, JAROSLAV PIÁLEK** AND JEREMY B. SEARLE°

* *Via Retta 22, 23030 Tovo S. Agata (SO), Italy.*
° *Department of Biology, University of York, PO Box 373, York, YO10 5YW, UK.*
***Academy of Sciences of the Czech Republic, Institute of Vertebrate Biology, CZ-675 02 Studenec 122, Czech Republic.*
+ *Centro di Ecologia Alpina, 38040 Viote del Monte Bondone* ***(TN),*** *Italy*

ABSTRACT - Karyotypic variation due to the centric (Robertsonian: Rb) fusion of chromosomes is a widespread phenomenon among small mammal species. In 1993, we described a house mouse chromosomal hybrid zone in Upper Valtellina (SO). Here, we found mice with 32 different karyotypes, including the standard, or all-acrocentric race (2n=40), four Rb races (2n=22-26) and 27 hybrid types (2n=23-39). This hybrid zone presents a unique opportunity to study the role of Rb fusions and races in speciation. We have been studying this dynamic hybrid system using a wide variety of techniques: karyology, histology, breeding, mark-recapture and DNA sequencing. All four Rb races appear to be closely related, but 40AA has probably been introduced recently into the valley. However, the fertility of laboratory-reared hybrids between several of these races (24UV, 26POS, 40AA) are lower than expected compared to homozygotes and previous studies. Effective subpopulation size and migration rates within and between villages are also relatively low. We discuss the use of these parameters to study the process of speciation in ongoing computer simulations.

Key *words: Mus musculus domesticus,* Robertsonian race, speciation, fertility, mark-recapture.

INTRODUCTION

Throughout most of its range, the house mouse, *Mus musculus domesticus*, has a karyotype consisting of 40 acrocentric chromosomes (19 autosomal pairs and XY sex chromosomes). However, over 50 'chromosomal races' (sensu Hausser *et al.*, 1994) of this species have been described in Europe and North Africa, which have diploid numbers ranging from 22 to 38 (Nachman and Searle, 1995). The reduction of 2n has been achieved by a series of centric, or Robertsonian (Rb), fusions. Further variation may also have been introduced by whole-arm reciprocal translocations and hybridization (Searle *et al.*, 1990; Hauffe and Piálek, 1997).

Where there are no geographical barriers, the distributions of pairs of Rb races, or of an Rb race and an adjacent all-acrocentric population, may overlap, forming a hybrid zone. Ten such hybrid zones of the house mouse have been studied in some detail (Searle, 1993). Offspring of two races differing in chromosome number and/or type of fusion are expected to have a reduced fertility because they will carry fusions in the heterozygous state (see Searle, 1993 for review). Because reinforcement could occur in such areas, it was recognized that these hybrid zones are an important source of information for understanding the process of speciation (Sage *et al.*, 1993 and references therein).

Since Gropp described the first Rb race from Val Poschiavo (Switzerland) in 1970, the distribution of Rb races in Italy has been

documented in detail (e.g. Capanna ***et al.***, 1977, 1989 and references therein; Capanna, 1982; Gropp ***et al.***, 1982). Rb races are particularly common in the Alps, Apennines and on some Italian islands.

In 1982, Capanna and Corti reported that in the tiny village of Migiondo, Valtellina (SO) two Rb races of the house mouse existed in close sympatry (the 24UV and 26POS races; see Table 1). Despite examining over 150 mice, they failed to find one hybrid, although the two races were known to interbreed in a neighbouring village. They concluded that speciation of these two races had occurred in Migiondo. Unfortunately, this fascinating case can no longer be studied, since one of the races in Migiondo (24UV) has disappeared (Hauffe and Searle, 1992).

In 1993, we described a house mouse chromosomal hybrid zone in Valtellina involving four Rb races and the all-acrocentric race (Hauffe and Searle, 1993; Table 1). This hybrid zone is unique because more than two races make contact along a 20 km-stretch of valley. A large number of hybrids with varying diploid numbers made this zone an ideal model with which to study the effect of heterozygous Rb fusions on fertility of wild mice (Hauffe and Searle, 1998).

Since 1989, we have been studying various aspects of the Valtellina hybrid zone using a wide range of techniques. The distribution of the different karyotypes was mapped, the genetic relatedness of the five chromosomal races was investigated, the fertility of laboratory-reared and wildcaught hybrids was estimated, and population parameters in one small village were calculated. The ultimate aim of these investigations is to use these estimates in computer simulations to make predictions about speciation. This paper summarizes our results and conclusions to date.

Material and Methods

Distribution of karyotypes

In the autumns of 1989, 1990 and 1991, mice were trapped on suitable farms from Villa di Tirano to Sondalo using Longworth live traps (Fig. 1). For each individual, direct chromosome preparations were made following Ford (1966) and G-banded after Evans (1987). Individual chromosome arms were described according to the Committee on Standardized Genetic Nomenclature for Mice (1972).

Genetic relatedness of races

DNA was phenol-chloroform extracted from 2mm of 34 tail samples of wildcaught mice from Valtellina. Part of the control region of the mitochondria1 DNA (mtDNA) was amplified using primers L15774 and H16498 (Shields and Kocher, 1991; P. Taberlet, pers. comm.) on a Perkin-Elmer Cetus thermal cycler with hot bonnet in 30 cycles (50C for 1', 72C for 2', 93C for 50"). 125ng of double-stranded PCR product was directly sequenced on an Applied Biosystems, Inc. automated sequencer according to the manufacturer's directions to obtain a total sequence of 352 base pairs. Sequences were aligned using the Genetics Computer Group package (Devereux ***et al.***, 1984)..

Fertility of homozygotes and heterozygotes

House mice from the villages of Migiondo (26POS; Table 1, Fig. 1), Mazzo (40AA) and Villa di Tirano (24UV) were bred in the laboratory from 1989-1992 to produce male and female hybrids with 32 (40AAx24UV; heterozygous for eight fusions, producing eight trivalents at meiosis), 33 (40AAx26POS; heterozygous for seven fusions, seven trivalents at meiosis) and 25 (26POSx24UV; one pentivalent at meiosis). Wildcaught hybrids were trapped in various villages in the autumn of 1991. Mitotic chromosome preparations of each individual were made as described above (for complete details, see Hauffe and Searle, 1998).

For each male laboratory-reared offspring (and nine wildcaught hybrid males), the body, the seminal vesicles and the left testis were weighed. The number of sperm per

Table 1 - Chromosomal races of the house mouse found in Upper Valtellina (SO).

Race	Abbreviated title[a]	Robertsonian (centric) fusions[b]									
Poschiavo	26POS	1.3		4.6	5.15		8.12	9.14		11.13	16.17
Mid Valtellina	24MV	1.3		4.6	5.15	7.18	8.12	9.14		11.13	16.17
Upper Valtellina	24UV	1.3	2.8	4.6	5.15			9.14	10.12	11.13	16.17
Lower Valtellina	22LV	1.3	2.8	4.6	5.15	7.18		9.14	10.12	11.13	16.17
All-acrocentric	40AA	no fusions									

[a]number indicates 2n.
[b]Rb fusions are named x.y according to the acrocentric chromosomes which formed them, so that, for example, the metacentric formed from the acrocentrics 1 and 3 is called 1.3.

Table 2 - Summary of fertility measures of various karyotypes of wildcaught and laboratory-reared house mice. N=10 unless otherwise indicated in brackets.

Karyotype	Body mass (g)		% nondisjunction		Left testes	Seminal vesicle:	Sperm	% germ cell	Litter size of	
	males	females	males	females	mass (mg)	body mass ($x10^3$)	per caput ($x10^6$)	death	males	females
Wildcaught mice"										
AAxRb	14.2 (6)	18.7 *(5)*	16.3 (6)	38.0 (6)	72.4 (6)	6.2 (6)	3.15 (6)	25.2 (6)		5.0 (3)
RbxRb	16.9 (3)	13.8 *(3)*	38.0 (1)	33.0 (1)	71.1 **(1)**	9.2 (1)	1.24 (1)	41.5 (1)		6.0 (3)
Laboratory-reared mice										
40AA	18.9	19.0	0	5.0	101.4 (9)	11.2 (9)	6.52	25.8 (4)	6.7 (4)	6.8 (4)
26POS	17.0	13.4	0	9.0	72.4 (8)	6.4 (8)	5.51	22.6 (4)		
24UV	19.6	17.2	**0**	25.0	88.7	7.4	6.15	24.0 (4)		
33(AAxPOS)	19.7	16.7	36.0	100.0	69.5	9.1	3.10	55.0 (4)	4.1 (4)	1.0 (4)
32(AAxUV)	21.3	17.0	44.0	100.0	74.1	9.6	2.64 (9)	51.5 (4)	2.6 (4)	3.1 (4)
25(UVxPOS)	17.8	14.2	18.5	37.5	51.3	4.5	1.23	55.5 (3)	3.8 (4)	4.0 (4)

[a]AAxRb: hybrids produced by a cross between 40AA and a Rb race; RbxRb: hybrids resulting from the interbreeding of mice from two Rb races.

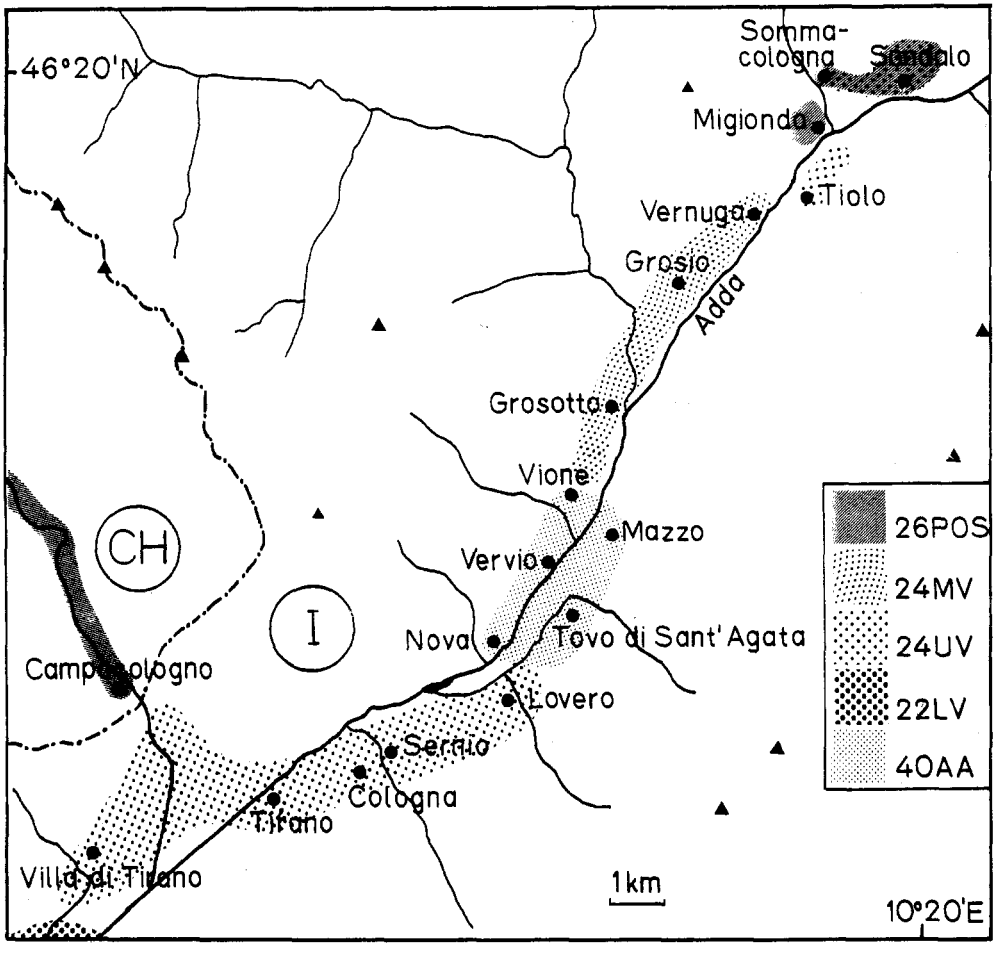

Figure 1 - Map of Upper Valtellina (Lombardy, Italy). Centre of villages marked by closed circles. Mountain peaks marked by closed triangles. Chromosomal races which dominate each village indicated by shading (see Table 1). Reprinted, with permission, from Hauffe and Searle, 1998 (© 1998 the Genetics Society of America).

caput was estimated from the right caput epididymus. C-banded meiotic chromosome preparations were made from the left testis (Evans ***et al.***, 1964; Sumner, 1972) in order to score metaphase II (MIT) spreads for the number of chromosomes. Non-disjunction frequency (NDJ) was calculated as $\{2[(n+1)+(n+2)]+2(n+2)\}/T$, where n is the number of hyperploid cells and T is the total number of cells counted. Serial sections were made of the right testis and stained (LeBlond and Clermont, 1952). The ratio of the number of primary spermatocytes (∂) and round spermatids (13) was calculated using the protocol of Wallace ***et al.*** (1992). Percent germ cell death (GCD) for each individual was calculated as $100[1-(\partial/4ß)]$. The body mass for each laboratory-reared female (and 13 wildcaught females), was recorded. Oocytes from the largest ovary

were brought to MII, fixed and C-banded (Whittingham, 1971; Quinn *et al.*, 1982; Tarkowski, 1966). NDJ was calculated as above. The smallest ovary was sectioned and stained conventionally. Cross-sections from the center of each ovary were scored for the number of growing follicles (after Wallace *et al.*, 1991).

Four male and four female offspring of the laboratory crosses were backcrossed to 40AA individuals to estimate litter sizes of these three kinds of heterozygote. Four 40AA x 40AA crosses were used as controls.

Population parameters

Every 2.5 months (primary sampling period; Pollock *et al.*, 1990), from the autumn of 1993 to December 1995, Longworth traps were placed in all potential house mouse habitats in the village of Tovo S. Agata at a density of one per two m^2 floor space, and left for five nights (house mice are strictly commensal in this area). On first capture, each mouse was sexed, weighed, given a numbered ear-tag and released as close as possible to where it was captured; the date and place of capture were noted. Subpopulation size was considered the total number of distinct individuals caught in one primary sampling period (Otis *et al.*, 1978), where 'subpopulation' is defined as the mice in a group of rooms where less than 10% of the total number of mice moved between those rooms during a primary sampling period. Definitions of mean and effective population size can be found in Wright (1938), of extinction rate per population per year in McCauley (1989) and mean longevity of a subpopulation in Whitlock (1992). Generation time was considered as six months. A migration between subpopulations was said to have occurred if a mouse moved to another subpopulation and was found in this second subpopulation in the next primary sampling period.

RESULTS

Distribution of karyotypes

In total 214 house mice were trapped on 39 farms in 19 villages, with diploid numbers ranging from 22 to 40, including four Rb races and the all-acrocentric race (Hauffe and Searle, 1993; Table 1). The dominant race in each area of Valtellina is shown in Figure 1. Twenty seven hybrid karyotypes were found on 19 farms (60 individuals or 18%). In general, neighbouring farms tended to have the same races and hybrids, except those divided by the River Adda. One farm had eight hybrid types.

The Mid Valtellina race was reported for the first time by us. Considering its chromosome complement and position in the valley, we believe that this race may be the result of hybridization between the 22LV and 26POS races, an example of zonal raciation (Searle, 1991; Hauffe and Searle, 1993).

Genetic relatedness of races

Polymorphism at one site on the mtDNA (15597; Bibb *et al.*, 1981) divided the haplotypes into two main clades: one containing all the 40AA individuals and one containing individuals of the four Rb races (with three exceptions; Hauffe and Searle, unpubl. data).

Fertility of homozygotes and heterozygotes

Fertility measures are summarized in Table 2 (Hauffe and Searle, 1998). Our results suggest that laboratory-reared hybrids from Upper Valtellina suffer a fairly high infertility: NDJ rates for both males and females are high; the sperm count per caput of all laboratory-reared male hybrids was significantly lower, and GCD was higher, than that of pure-race males (NDJ: Analysis of variance and sum of squares simultaneous test procedure: $SS_{crit}=2.23$, $SS_{sample}=2.98$, $P<0.05$; GCD: Mann-Whitney U, $Z=-4.157$, $p<0.0001$). Wildcaught males also have high NDJ and GCD. There was no difference detected in the mean number of growing folli-

Table 3 - Summary of population parameters for house mice from Tovo S. Agata (SO). For further definitions, see Materials and Methods.

Parameter	Value	Reference
Number of subpopulations	34	
Range of subpopulation size	1-23	
Mean subpopulation size[a]	6.20 mice	Wright, 1938
Effective subpopulation size	4.72 mice	Wright, 1938
Extinction rate/subpopulation/year	0.56	McCauley, 1989
Recolonization rate	1-2 generations	
Mean longevity of a subpopulation	1.2 years	Whitlock, 1992
Maximum migration distance	320 m	
Mean migration distance	28.4 m (1993/4) 16.8 m (1995/6)	
Effective migration rate/population/generation	1-3 mice	
Mean number of migrants recolonizing an empty habitat patch	6 (2 males, 2 females, 2 juveniles)	
Mean stay of migrants moving to an empty patch	4 days	
Mean stay of migrants moving between existing populations	6-7 days	

[a]excluding populations of 1 or 2 individuals.

cles between any of the karyotypic groups at any stage of folliculogenesis in laboratory-reared females, but many more antral follicles in heterozygous females showed signs of atresia. The litters of all three types of male and female heterozygotes were much smaller than those of 40AA males and females (Kruskal-Wallis: males: $H=21.56$, $p=0.0001$; females: $H=23.10$, $p<0.0001$).

Population parameters

A summary of preliminary calculations from the mark-recapture data is given in Table 3. All 34 subpopulations were small, although some were more stable than others (associated with farm animals). Although 56% of subpopulations went extinct per year, they were recolonized within two generations (one year) indicating that turnover is very high. 15-20% of all mice moved between subpopulations, usually adjacent ones. Most mice moved only once, and most migrants also disappeared after a few days in their new subpopulations; therefore reproduction of most migrants is improbable and effective migration rate is estimated at 1-3 individuals per subpopulation per generation. Migrants moving to an empty habitat patch (mean stay: 4 days) were not more successful than migrants moving between existing subpopulations (mean stay: 6-7 days) at establishing themselves in their new subpopulations.

DISCUSSION

Long-term study (1989-present) of the Valtellina house mouse chromosomal hybrid zone has rendered a detailed picture of this complex system. We now know, for example, that hybrids in Valtellina have a relatively low fertility, especially compared to homozygous mice in the valley, but also with respect to previous studies of wild mice (Hauffe and Searle, 1998). The effective subpopulation size (as measured in one village), 4.72 individuals, is smaller than previously reported (even if these are considered 'demes' or family groups); turnover of

mice is high; and effective migration rate is low.

Figure 1 shows that despite obvious hybridization between all five chromosomal races in Valtellina, the races are not distributed at random throughout the valley, but are patchy. This distribution can probably be dated from 1807, when a landslide blocked the Adda, flooding Valtellina from Lovero to Grosio, likely killing all the mice in this region. The mtDNA study supports the view that the 40AA race is genetically divergent from Rb races in Valtellina; therefore, our hypothesis (Hauffe and Searle, 1992) that the 40AA race was introduced after the flooding into Valtellina may be valid, We are not able determine from this study, however, if and how the Rb races evolved from one another.

One approach to this problem is to use the values of the parameters we have measured in computer simulations. One such simulation has been done using a two-locus, two allele model based on a two-dimensional stepping-stone array (Piálek and Hauffe, 1998). Parameter values were based on the results of the fertility studies and the first year of the mark-recapture study: migration rate (m)= 0.35 within villages and 0.025 between villages; number of mice per deme (N)= 12 (the modal population size); selection (s)= 0 for parental races and 0.2-0.35 for hybrids (based on rates of NDJ and GCD). Three patches were used, the outer two occupied by 22LV or 26POS and the middle one left empty, The computer simulations showed that after some generations, the middle patch became occupied at random by 22LV or 26POS, and nearly no hybrids were formed. We aim to improve this model by including the possibility of long-distance migration and reassessing the m, N and s values used. For example, a more precise measure of m could be gained by calculating the actual reproductive success of migrants and gene flow between subpopulations using genetic markers.

We also intend to use the computer simulation approach to evaluate the cause of the patchiness of Rb races in Valtellina, and more, generally, whether these races are likely to diverge and become new species.

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