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Chromosomal Heterozygosity and Fertility in House Mice (*Mus musculus domesticus*) From Northern Italy

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ABSTRACT

Following the discovery of over 40 Robertsonian (Rb) races of *Mus musculus domesticus* in Europe and North Africa, the house mouse has been studied extensively as an ideal model to determine the chromosomal changes that may cause or accompany speciation. Current models of chromosomal speciation are based on the assumption that heterozygous individuals have a particularly low fertility, although recent studies indicate otherwise. Despite their importance, fertility estimates for the house mouse are incomplete because traditional measurements, such as anaphase I nondisjunction and germ cell death, are rarely estimated in conjunction with litter size. In an attempt to bridge this gap, we have taken advantage of the house mouse hybrid zone in Upper Valtellina (Lombardy, Italy) in which five Rb races interbreed. We present data on the fertility of naturally occurring ("wild-caught") hybrids and of offspring from laboratory crosses of wild-caught mice ("laboratory-reared"), using various measurements. Wild-caught mice heterozygous for one fusion were more infertile than predicted from past studies, possibly due to genic hybridity; laboratory-reared heterozygotes carrying seven or eight trivalents at meiosis I and heterozygotes carrying one pentavalent also had low fertilities. These low fertilities are especially significant given the probable occurrence of a reinforcement event in Upper Valtellina.

TO discover how speciation occurs, we need to understand which genetic differences between species are responsible for causing reproductive isolation. Many closely related species differ in chromosome number: since Robertsonian (Rb) translocations (the fusion of two acrocentric chromosomes to form one metacentric, or the fission of a metacentric to form two acrocentrics) result in a change of diploid number, these mutations have frequently been implicated in the process of speciation (White 1978a; King 1993). This is because the contact of two populations, one homozygous for no rearrangements and one homozygous for at least one rearrangement, will produce hybrids that are expected to have a reduced fertility (likewise for the contact of two populations characterized by different combinations of Rb rearrangements). Heterozygous disadvantage has the potential to restrict gene flow between two such chromosomally differentiated populations, either completely, if hybrids are sterile (*i.e.*, speciation in allopatry), or perhaps enough to promote selection for intraracial matings, resulting in the cessation of hybridization and the creation of two species (*i.e.*, speciation by reinforcement; Dobzhansky 1970; White 1978b; Butlin 1987; Sites and Moritz 1987; Howard 1993; Searle 1993). One of the best ways to understand the role of Rb rearrangements in speciation, therefore, is to study the fertility of heterozygotes found in hybrid zones between populations of a species fixed for different Rb rearrangements.

Although polytypy due to Rb differences occurs in a number of animals, notably small mammals (*e.g.*, shrews, Searle 1984, 1988; spiny mice, Wahrman and Goitein 1972; gerbils, Wahrman and Gourewitz 1973; mole rats, Nevo 1991; mole-voles, Lyapunova *et al.* 1980), an extreme example is found in the "West" European house mouse, *Mus musculus domesticus.* This subspecies has a standard karyotype of 40 acrocentric chromosomes (Boursot *et al.* 1993), but many populations across Europe and North Africa have been found with lower diploid numbers due to Rb fusions (Gropp *et al.* 1970; Bauchau 1990; Nachman and Searle 1995), possibly modified by whole-arm reciprocal translocations (Winking 1986; Searle 1993; Capanna and Redi 1995; Hauffe and Piálek 1997). Groups of contiguous populations that share the same homozygous set of Rb fusions are referred to as "races" (Hausser *et al.* 1994). More than 40 different races of the house mouse have been described thus far (Nachman and Searle 1995). Hybrid zones are formed where an Rb race comes into contact with the standard race (40AA), or where two Rb races meet (see Searle 1993 for review).

Hybrids between different chromosomal races are expected to be heterozygous for Rb rearrangements. These heterozygotes display nonbivalent configurations at metaphase I (MI), either trivalent (chain-of-three)

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configurations if they are heterozygous for one or more fusions without homology (called "single" or "multiple simple heterozygotes"; often formed on hybridization of 40AA and a Rb race), or longer chains and rings if the heterozygous fusions have monobrachial homology (called "complex heterozygotes"; often the result of hybridization of two Rb races; Gropp and Winking 1981; Redi and Capanna 1988; Searle 1993). Individuals heterozygous for one or more Rb fusions may suffer a reduced fertility for a number of reasons, the most important of which are: (1) the incomplete pairing of chromosomal axes at pachytene, as seen in trivalent or longer chain configurations, which may lead to germ cell death (GCD), and (2) the malsegregation of such configurations, called "nondisjunction" (NDJ), generating aneuploid gametes, which lead to inviable embryos (Gropp *et al.* 1982a; Searle 1993; Everett *et al.* 1996). Meiotic anomalies in hybrids between chromosomal races in the house mouse are expected to lead to fewer and smaller litters and a lower lifetime reproductive success or fitness.

Comprehensive studies of the fertility of these hybrids began in the 1970s. Although single simple heterozygotes were shown to be relatively fertile, most multiple simple and complex heterozygotes were believed to be almost totally sterile (Gropp and Winking 1981). These early studies supported the idea that Rb fusions were involved in speciation because single Rb fusions could spread and accumulate in different races without excessive heterozygous disadvantage, and yet it appeared that an accumulation of different fusions in two races would almost certainly result in reproductive isolation between them (especially those involving monobrachial homology; Baker and Bickham 1986).

The relevance of these early studies to natural situations was difficult to interpret because many of the hybrids under investigation were produced from crosses involving the introduction of wild-type fusions into the genetic background of the laboratory mouse or from crosses of wild mice collected from widely separated sites and maintained in captivity for many generations. Many studies have suggested that this genic hybridity might exaggerate the effects of Rb heterozygosity (Everett *et al.* 1996 and references therein). Instead, most hybrid zones involve genetically similar races and, over many generations in such zones, selection may reduce heterozygous disadvantage by eliminating alleles that cause meiotic problems (Searle 1993).

More recently, investigators have looked to wild populations to test theories of chromosomal speciation, not only in mice but also in other species (*e.g.*, Searle 1990; Nachman 1992; Reed *et al.* 1995; Sites *et al.* 1995). Table 1 lists the results of several studies of the fertility of house mice that were Rb heterozygotes, either naturally occurring ("wild-caught" hybrids), or produced in the laboratory from wild mice caught in the field using crosses that could reasonably occur in nature ("laboratory-reared" hybrids). These results indicate that one to three heterozygous fusions may have little effect on fertility, whereas many heterozygous fusions lower fertility dramatically (but not necessarily resulting in sterility).

Although the studies in Table 1 are suggestive, only two investigated the fertility of male wild-caught mice, and only one looked at the fertility of male and female laboratory-reared multiple simple heterozygotes; no studies have been published on the fertility of wild-caught or laboratory-reared complex heterozygotes. Since reproductive isolation is most likely to occur when the contact of two races results in the production of multiple simple and complex heterozygotes, fertility measures of these types of hybrids are essential for testing theories of chromosomal speciation. In addition, because each of the studies in Table 1 concentrates on only one or two fertility measures, they are far from comprehensive; moreover, very little has been published about female hybrids.

In the present article, we report fertility estimates of

TABLE 1

Previous fertility studies on wild-caught or laboratory-reared house mice heterozygous[a] for Rb fusions

Category of mouse	Sex tested	Geographic origin	Type of heterozygote[a] (*n* trivalents produced at MI)	Effects of heterozygosity	Reference[b]
W	m	Croatia	Single simple (1)	NDJ negligible	1
W	m	Scotland	Single simple (1)	Low NDJ and GCD	2
L	m, f	N. Italy	Single simple (1)	Normal litter sizes	3
L	m, f	Belgium	Single simple (1)	Normal litter sizes	4
L	m	Scotland	Multiple simple (3)	NDJ fairly low (13%)	5
L	m, f	Tunisia	Multiple simple (9)	Sometimes arrested spermatogenesis; small litter sizes	6

[a] See Introduction for definitions.

[b] 1, Winking *et al.* 1988; 2, Wallace *et al.* 1992; 3, Britton-Davidian *et al.* 1990; 4, Viroux and Bauchau 1992; 5, Scriven 1992; 6, Saïd *et al.* 1993.

W, wild caught; L, laboratory reared.

Rb heterozygotes using mice from Upper Valtellina, Northern Italy, which contains one of the better-documented house mouse hybrid zones (Hauffe and Searle 1993). In this narrow alpine valley, five chromosomal races of the house mouse make contact and hybridize (Figure 1); interracial hybrids, including those with backcross karyotypes, are common (56/214 mice, or 26%). For this study, laboratory-reared hybrids were produced by crossing three of the races that occur in Upper Valtellina: the standard race (40AA), the Poschiavo race (26POS; previously considered a separate species, *Mus poschiavinus*; see Gropp *et al.* 1970; Capanna and Corti 1982), and the Upper Valtellina race (24UV; Figure 1). As these hybrids are some of the most complicated heterozygotes expected in the field, they provide an estimate of the maximum possible fertility reduction in this hybrid zone. The same methods for measuring fertility were applied to wild-caught heterozygotes.

To our knowledge, this is the first study of the fertility of several types of heterozygous mice with wild-caught parents from the same chromosomal hybrid zone that combines several indirect estimates of fertility with an estimate of litter size for both male and female hybrids.

MATERIALS AND METHODS

Laboratory-reared homozygotes and heterozygotes: *Animals and breeding:* House mice were live-trapped in Upper Valtellina in September 1989 from the following villages (Figure 1): Migiondo (26POS), Mazzo (40AA), and Villa di Tirano (24UV). Four pairs of each of the laboratory crosses listed in Table 2 were set up using F_1 individuals. The karyotype and the number and type of configuration expected at MI of meiosis in the offspring of each of these crosses are also shown in Table 2. Offspring were killed by cervical dislocation. The following procedures were completed immediately following death.

Mitotic chromosome preparations: Direct chromosome preparations were made from a suspension of bone marrow cells by the method of Ford (1966) and stained by the G-banding protocol of Evans (1987). At least five, clear, G-banded spreads were scored per animal to identify karyotype. Individual chromosome arms were described according to the Committee on Standardized Genetic Nomenclature for Mice (1972).

Fertility estimates in males: The body (with all organs intact), the seminal vesicles, and the left testis of each male offspring were weighed. While body mass is a rough guide to sexual maturity in house mice, the mass of the seminal vesicles is used routinely as a more sensitive index in males (Grocock and Clarke 1974). Testis mass is believed to be correlated with the number of germ cells (Searle and Beechey 1974; Searle *et al.* 1978). A one-way ANOVA and SS-STP tests were used to compare the mean values between particular karyotypic groups for each of the above measurements (Sokal and Rohlf 1981). Correlation analysis suggested that seminal vesicle mass varied with body mass ($r^2 = 0.36$, $P = 0.0001$), so seminal vesicle mass was compared relative to body mass. A high F_{max} value indicated that a square-root transformation of the ratios of seminal vesicle mass to body mass was appropriate before an ANOVA and SS-STP tests were performed.

For each male offspring, the right caput epididymus was cut out and macerated in 2 ml of 34 mm (1%) trisodium citrate solution, and number of sperm per caput was estimated using an Improved Neubauer Haemocytometer. Mean sperm counts were also square-root transformed before an ANOVA was applied.

Meiotic chromosome preparations of the left testis were made using the air-drying method of Evans *et al.* (1964) with minor modifications as suggested by M. W. Nachman (personal communication), and slides were stained by the C-banding method of Sumner (1972). For each male offspring, 50 to 150 clear, C-banded late MI spreads (a total of 200 spreads per karyotypic group) were scored for the number and type of configurations and frequency of univalency (recorded as either XY or autosomal). Fifty to 150 metaphase II (MII) spreads per male were also scored (a total of 200 spreads per karyotypic group). The NDJ frequency has traditionally been calculated as the number of hyperploid plus the number of hypoploid cells $(n < 20 + n > 20)/T$, where T is the total number of cells counted, estimated as two times the number of hyperploid cells divided by T because hypoploid cells can be caused by cell breakage. However, this calculation does not take into account cells in which NDJ occurs such that $n = 20$ (for example, NDJ of two trivalents in multiple simple heterozygotes). The proportion of the total number of $n = 20$ cells that could potentially be "false" increases from two thirds for double simple heterozygotes to almost one for hybrids carrying nine trivalents. However, the true proportion of false $n = 20$ cells is expected to be closer to the proportion of $[(n + 2) + (n - 2)]$ cells. Therefore, we have calculated NDJ to be $\{2[(n + 1) + (n + 2)] + 2(n + 2)\}/T$. This estimate still does not take into account aneuploid cells missed due to

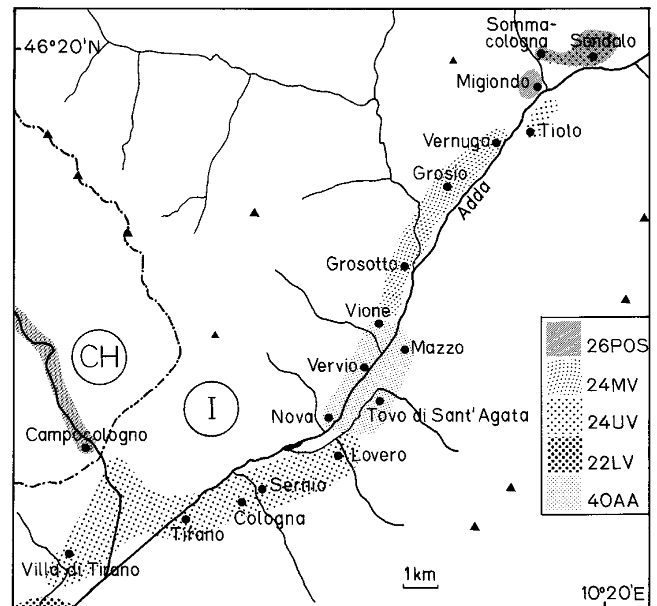

Figure 1.—Map of Upper Valtellina (Lombardy, Italy). Center of villages marked by closed circles. Mountain peaks marked by closed triangles. Chromosomal races that dominate each village indicated by shading (see legend): 26POS, Poschiavo race [$2n = 26$: carrying Rb fusions Rb (1.3), Rb (4.6), Rb (5.15), Rb (8.12), Rb (9.14), Rb (11.13), Rb (16.17)]; 24MV, Mid Valtellina race [$2n = 24$: Rb (1.3), Rb (4.6), Rb (5.15), Rb (7.18), Rb (8.12), Rb (9.14), Rb (11.13), Rb (16.17)], 24UV, Upper Valtellina race [$2n = 24$: Rb (1.3), Rb (2.8), Rb (4.6), Rb (5.15), Rb (9.14), Rb (10.12), Rb (11.13), Rb (16.17)]; 22LV, Lower Valtellina race [$2n = 22$: Rb (1.3), Rb (2.8), Rb (4.6), Rb (5.15), Rb (7.18), Rb (9.14), Rb (10.12), Rb (11.13), Rb (16.17)]; 40AA, all-acrocentric race (no fusions).

TABLE 2

Laboratory crosses and number and type of configuration expected at MI in offspring

Cross[a]	2*n* of offspring	Abbrev. used in text for F_1 offspring	No. (type) configurations expected at MI in offspring[b]
40AA × 40AA	40	40AA	20 (20 abiv)
26POS × 26POS	26	26POS	13 (7 mbiv, 6 abiv)
24UV × 24UV	24	24UV	12 (8 mbiv, 4 abiv)
40AA × 26POS	33	33(AA × POS)	13 (7 CIII, 6 abiv)
40AA × 24UV	32	32(AA × UV)	12 (8 CIII, 4 abiv)
24UV × 26POS	25	25(UV × POS)	11 (1 CV, 6 mbiv, 4 abiv)

[a] See Figure 1 for list of abbreviations.

[b] abiv, acrocentric bivalents; mbiv, metacentric bivalents; CIII, trivalent (chain-of-three); CV, pentavalent (chain-of-five); number preceding these abbreviations indicates number of the particular configuration.

cell breakage; however, we have calculated that inclusion of cell breakage would change our estimates by only 1–5% and is, therefore, fairly unimportant to the overall pattern of NDJ.

The right testis of each male was fixed in Bouin's solution and subsequently embedded in paraffin wax. A standard histological protocol was used to obtain 7-μm serial sections that were stained using the periodic acid-Schiff reaction and counter-stained with haemotoxylin (LeBlond and Clermont 1952). For each specimen, five truly transverse cross-sections of tubules for each of stages I, VI, and XI of the 12-stage seminiferous epithilium cycle were examined to calculate the ratio of primary spermatocytes (∂) to round spermatids (β) using the protocol of Wallace *et al.* (1992). Percentage GCD for each individual was calculated as $100[1 - (\partial/4\beta)]$. These values were compared between karyotypes using a Mann-Whitney U test.

Fertility estimates in females: Total body mass and the mass of the smallest ovary were recorded for each female offspring. These measures were compared statistically the same way as those for males. Ovary mass was subjected to a log transformation before an ANOVA and SS-STP tests were performed.

The largest ovary of each female was dissected out in prewarmed Flow 1X (Modified) TC199 and transferred to M2 cell culture medium. Oocytes showing a clear germinal vesicle were incubated at 37° (5% CO_2) for 20 hr in equilibrated M16 cell culture medium, which brought the majority of the oocytes to MII (Whittington 1971; Quinn *et al.* 1982). The oocytes were fixed according to Tarkowski (1966). Preparations were C-banded following Sumner (1972). Spreads were scored if the polar body could be easily identified. One to 10 spreads were counted per female (for a total of 40–48 spreads per karyotypic group). The small number of spreads scored per female does not reflect an inefficient preparation technique but a low number of mature oocytes per ovary. That genetically wild mice have low numbers of mature oocytes, even compared to considerably younger mice of laboratory strains, has already been noted by Garagna *et al.* (1990). The frequency of NDJ was calculated as it had been for males (see above).

The smallest ovary from each female specimen was fixed in Bouin's solution, embedded in paraffin wax, and stained conventionally. For four females of each cross, 50 consecutive cross-sections from the center of each ovary were scored for the number of growing follicles (after Wallace *et al.* 1991). Primordial oocytes could not readily be discerned from interstitial cell types and were not recorded. Corrected cell counts (Abercrombie 1946) were used to calculate the total number of each type of growing follicle per ovary (very early growing, early growing, preantral, small antral, and large antral follicles) by assuming that the ovary was spherical. (Volume was calculated using the diameter of the largest cross-section.) For statistical analysis, preantral and antral follicles were pooled, as it was difficult to decide whether small "holes" reflected a technical artifact or a developing antral cavity. The total number of corpora lutea per ovary was counted by tracing individual corpora lutea through the entire set of ovarian sections under ×40 magnification. Mann-Whitney U tests were used to compare the mean number of each type of follicle between karyotypic groups.

Litter sizes of male and female heterozygotes: Four male and four female offspring of the 40AA × 26POS, 40AA × 24UV, and 24UV × 26POS laboratory crosses were backcrossed to 40AA individuals to estimate litter sizes of these three kinds of heterozygote. Four 40AA × 40AA crosses were used as controls. Weanlings were killed by cervical dislocation at the age of 28–30 days and weighed. Litter sizes of each type of backcross were compared using a Kruskal-Wallis test.

Wild-caught homozygotes and heterozygotes: *Specimens:* From September to November 1991, mice were trapped in various villages in Upper Valtellina, Northern Italy, on farms known from a previous study to contain a high number of karyotypically heterozygous house mice (Sondalo, Sommacologna, Grosotto, Nova, Tovo di Sant' Agata; see Figure 1 and Hauffe and Searle 1993). Individuals were taken the same day of capture to the Laboratorio Centrale of the Enrico Moretti Hospital, Sondalo, where they were killed by cervical dislocation, and chromosome preparations and fertility estimates were made. Each mouse was categorized as a young adult (4–6 mo), adult (6–12 mo), or old adult (>12 mo) on the basis of its general appearance and the experimenters' past observations of laboratory-reared mice of all ages. (Older mice tend to be larger, with many fighting scars, dull fur, and/or damaged ears.)

Mitotic chromosome preparations and fertility estimates: For the study of wild-caught house mice to be as directly comparable as possible to that of laboratory-reared heterozygotes, methods of chromosome preparation and fertility estimates were almost identical to those mentioned previously. Nine heterozygous males with diploid numbers ranging from $2n = 24$–39 were captured (Table 3). For each of these nine males, whole body mass, seminal vesicle mass, and left testes mass were recorded; the number of sperm per caput and GCD was calculated, 100 MI cells were scored for number of configurations and any univalency, and the number of hyperhaploid cells out of 100 MII spreads was noted.

Thirteen female house mice with diploid numbers ranging from 25 to 40 were studied (Table 3). Meiotic chromosome

preparations were made and the smallest ovary of each female was weighed, but follicular counts were not made because both ovaries were needed to harvest oocytes for MII counts.

RESULTS

Incidence of univalence: In total, 1200 MI spreads from male laboratory-reared house mice (six karyotypic groups) were scored for univalence (Table 4). Acrocentric and metacentric chromosomes normally expected to form bivalents at MI were found separate in only 4 out of 1200 MI spreads; however, it was not possible to determine which autosomes were involved. Autosomal univalence was absent in heterozygotes. The *X* and *Y* chromosomes appeared unpaired with a low frequency in many homozygotes and heterozygotes (0–1.5% of cells per karyotypic group); however, 40AA animals showed by far the highest incidence with 19% (38/200) of spreads showing *XY* univalence.

In wild-caught males, in 939/1000 spreads, the expected configurations also formed regularly (Table 5), including all trivalents and pentavalents (chains-of-five) observed in heterozygotes. Autosomal bivalents were found to be unpaired in 25 cells, or 0–15 cells for each wild-caught individual. The highest frequency of autosomal univalence was 15%, observed in a mouse carrying one trivalent, T102. The *X* and *Y* chromosomes were seen in close association in 964/1000 cells, and *XY* univalence seemed to occur at a fairly constant frequency of 0–7% in all wild-caught heterozygotes.

Frequency of NDJ: *Males:* No spreads with more than the expected number of 20 arms were observed in the MII cells of laboratory-reared 40AA, 26POS, and 24UV males (Table 4). In contrast, NDJ frequencies of 36 and 44% were recorded for multiple simple heterozygotes, 33(AA × POS) and 32(AA × UV), respectively. The NDJ frequency for the complex hybrids, 25(UV × POS), was estimated to be somewhat lower (18.5%).

For wild-caught males carrying one trivalent with high diploid numbers ($2n = 35$–39), NDJ frequencies ranged from 12–22% with a mean of 15.2% (Table 5). The highest number of hyperhaploid cells was observed in T102, heterozygous for the fusion Rb(16.17) (NDJ: 22%); however, two heterozygotes (SD104 and SD105) with low diploid numbers ($2n = 25$) had only 6% aneuploid cells. Among the four symmetrical Rb fusions present in a heterozygous state in the Upper Valtellina mice

TABLE 3

Description of male and female wild-caught house mice used in this study

Mouse[a]	Karyotype[b]	No. (type) configurations expected at MI[c]	Fusion(s) involved in MI chains
		Males	
T122	39a	19 (1 × CIII, 18 abiv)	Rb (1.3)
T102	39g	19 (1 × CIII, 18 abiv)	Rb (16.17)
T120	39g	19 (1 × CIII, 18 abiv)	Rb (16.17)
NV7	37c	18 (1 × CIII, 2 mbiv, 15 abiv)	Rb (4.6)
NV5	35a	17 (1 × CIII, 4 mbiv, 12 abiv)	Rb (11.13)
SD104	25	12 (1 × CIII, 7 mbiv, 4 abiv)	Unknown
SD105	25(UV − 2.8)	12 (1 × CIII, 7 mbiv, 4 abiv)	Rb (2.8)
T113	37a	17 (3 × CIII, 14 abiv)	Rb (1.3), Rb (8.12), Rb (11.13)
GS102	24(LV × POS)	10 (1 × CV, 1 × CIII, 4 mbiv, 4 abiv)	Rb (2.8), Rb (8.12), Rb (10.12), Rb (7.18)
		Females	
T113	40AA	20 (all abiv)	No fusions
T117	40AA	20 (all abiv)	No fusions
T118	40AA	20 (all abiv)	No fusions
T114	39f	19 (1 × CIII, 18 abiv)	Rb (11.13)
T107	39g	19 (1 × CIII, 18 abiv)	Rb (16.17)
T108	39g	19 (1 × CIII, 18 abiv)	Rb (16.17)
T109	39g	19 (1 × CIII, 18 abiv)	Rb (16.17)
T119	39g	19 (1 × CIII, 18 abiv)	Rb (16.17)
NV4	39g	19 (1 × CIII, 18 abiv)	Rb (16.17)
NV3	37c	18 (1 × CIII, 2 mbiv, 15 abiv)	Rb (4.6)
SC108	25(UV − 2.8)	12 (1 × CIII, 7 mbiv, 4 abiv)	Rb (2.8)
SD101	25(UV − 2.8)	12 (1 × CIII, 7 mbiv, 4 abiv)	Rb (2.8)
GS106	25(MV × POS)	12 (1 × CIII, 7 mbiv, 4 abiv)	Rb (7.18)

[a] Abbreviations for villages in Upper Valtellina where mice were trapped are as follows: T, Tovo di Sant' Agata; NV, Nova; SD, Sondalo; GS, Grosotto; SC, Sommacologna.

[b] After Hauffe and Searle 1993; see Figure 1 for abbreviations.

[c] See Table 2, footnote *b*.

TABLE 4

Incidence of univalence at MI, NDJ frequency and aspects of GCD in laboratory-reared males

Karyotype[a]	No. (type) configurations expected at MI[a]	Origin of univalent chromosomes: Autosomal	XY	No. of MII spreads with: n	n + 1	n + 2	% NDJ[b]	Body mass (g)	Seminal vesicles: body mass ($\times 10^3$)	Left testes mass (mg)	Sperm per caput 10^6	Mean ratio spermatocytes: spermatids	% germ cell death[c]
40AA	20 (20 abiv)	3	38	189	0	0	0	18.9 ± 0.5 (10)	11.25 ± 0.82 (9)	101.4 ± 2.2 (9)	6.52 ± 0.37 (10)	1:2.97 (4)	25.8 ± 2.5 (4)
26POS	13 (7 mbiv, 6 abiv)	0	1	193	0	0	0	17.0 ± 0.7 (10)	6.39 ± 0.70 (8)	72.4 ± 2.8 (8)	5.51 ± 0.90 (10)	1:3.10 (4)	22.6 ± 2.2 (4)
24UV[d]	12 (8 mbiv, 4 abiv)	1	3	177	0	0	0	19.6 ± 0.6 (10)	7.39 ± 0.99 (10)	88.7 ± 2.5 (10)	6.15 ± 0.56 (10)	1:3.04 (4)	24.0 ± 1.8 (4)
33(AA × POS)	13 (7 CIII, 6 abiv)	0	0	117	28	4	36	19.7 ± 0.8 (10)	9.08 ± 1.06 (10)	69.5 ± 3.6 (10)	3.10 ± 0.61 (10)	1:1.80 (4)	55.0 ± 3.2 (4)
32(AA × UV)	12 (8 CIII, 4 abiv)	0	2	97	26	9	44	21.3 ± 0.5 (10)	9.64 ± 0.96 (10)	74.1 ± 3.2 (10)	2.64 ± 0.49 (9)	1:1.94 (4)	51.5 ± 4.8 (4)
25(UV × POS)[e]	11 (1 CV, 6 mbiv, 4 abiv)	0	2	72	10	0	18.5	17.8 ± 0.7 (10)	4.47 ± 0.40 (10)	51.3 ± 3.2 (10)	1.23 ± 0.29 (10)	1:1.78 (3)	55.5 ± 3.2 (3)

For each karyotypic group, 200 MI cells were counted. A total of 50 MI and 50 MII cells were counted for each of four males unless otherwise indicated. The number of individuals measured is in parentheses.

[a] See Table 2.

[b] Percentage NDJ is calculated as $\{2[(n+1)+(n+2)]+2(n+2)\}/T \times 100$, where T is the total number of cells counted.

[c] Percentage germ cell death for each individual is calculated as $100[1-(\partial/4\beta)]$, where ∂ is the number of primary spermatocytes, and β is the number of round spermatids (see materials and methods).

[d] 150 MI and 150 MII counts from one male and 50 MI and 50 MII counts from another.

[e] 50 MI counts from two males and 100 from the third; 29, 33 and 46 MII counts (total 108) for each of three males.

examined [Rb(1.3), Rb(4.6), Rb(11.13), and Rb(16.17); Table 3], there was no indication that NDJ varied according to metacentric size.

The multiple simple hybrid, T113 (heterozygous for three fusions), had NDJ frequency of 22%. The complex hybrid, GS102 (carrying one trivalent and one pentavalent), had the highest frequency of hyperhaploid cells among these wild-caught males with a NDJ frequency of 38%.

Females: Oocyte spreads were prepared from 67 laboratory-reared females for a total of 252 MII cells (Table 6). The frequency of NDJ in female house mice ranged from 5–15% for pure-race groups (6 hyperhaploid cells out of a total of 124), but NDJ in laboratory-reared females heterozygous for eight or seven fusions was estimated at 100% from the formula that we used. In contrast, NDJ frequency in 25(UV × POS) hybrids was much lower (37.8%).

Given the small sample size, the following NDJ frequencies for wild-caught females should only be taken as a rough estimate (Table 7): No hyperhaploid cells were evident among 12 oocytes produced by three 40AA females. Four out of 21 oocytes were hyperhaploid in simple Rb heterozygotes, with diploid numbers ranging from $2n = 37$–39 (NDJ: 38%); the frequency in simple hybrids with $2n = 25$ was slightly lower (NDJ: 33%).

Body size, gonad size and numbers of germ cells: *Males:* Although no karyotypic group of males had individuals that were significantly heavier than those of other groups, 26POS males weighed less than males from all other karyotypic groups ($SS_{crit} = 35.91$, $SS_{sample} = 56.05$, $P < 0.05$; Table 4). Significant sexual dimorphism existed in all karyotypic groups except 40AA, with males being heavier than females in all cases {one-way ANOVA, $F = 19.59$ (26POS), $F = 8.43$ (24UV), $F = 9.45$ [33 (AA × POS)], $F = 31.45$ [32(AA × UV)], $F = 24.28$ [25(UV × POS)]; $P < 0.05$ for all groups; Tables 4 and 6}. The 40AA males had the heaviest testis of any karyotypic group ($SS_{crit} = 940.74$, $SS_{sample} = 4887.66$, $P < 0.05$; Table 4), whereas 25(UV × POS) hybrids had the lightest ($SS_{crit} = 940.74$, $SS_{sample} = 4641.71$, $P < 0.05$). These 40AA males also had larger seminal vesicles than other pure-race males ($SS_{crit} = 2.49$, $SS_{sample} = 3.49$, $P < 0.05$; Table 4), whereas 25(UV × POS) had the smallest seminal vesicles among the heterozygous groups ($SS_{crit} = 2.49$, $SS_{sample} = 7.84$, $P < 0.05$). The sperm count per caput of all hybrid types was substantially lower than that of pure-race males ($SS_{crit} = 2.23$, $SS_{sample} = 2.98$, $P < 0.05$; Table 4); in addition, complex hybrids had significantly less sperm than multiple simple hybrids ($SS_{crit} = 2.23$, $SS_{sample} = 2.39$, $P < 0.05$). The 40AA, 26POS, and 24UV males appeared to suffer less GCD than the heterozygotes observed in this study (Mann-Whitney U, $Z = -4.157$, $P < 0.0001$; Table 4).

With regard to the wild-caught males, the low seminal vesicle mass, testes mass, and sperm count of SD105 indicated that this was an immature animal, and thus

TABLE 5

Incidence of univalence at MI, NDJ frequency, and aspects of GCD in wild-caught males

Male[a]	No. (type) configurations expected at MI[b]	Origin of univalent chromosomes: Autosomal	XY	No. of MII spreads with: n	n + 1	n + 2	% NDJ[c]	Body mass (g)	Sem. ves.: body mass ($\times 10^3$)	Testes mass (mg)	Sperm per caput (10^6)	Ratio s'cytes: s'tids	% germ cell death[d]
T122(Y)	19 (1 × CIII, 18 abiv)	0	5	89	7	0	14	14.5	7.8	72.8	3.36	1:3.38	15.5
T102(Y)	19 (1 × CIII, 18, abiv)	15	5	76	11	0	22	17.7	8.1	70.5	3.64	1:3.14	21.5
T120(Y)	19 (1 × CIII, 18 abiv)	4	5	87	6	0	12	15.7	4.9	74.9	4.04	1:3.29	17.8
NV7(Y)	18 (1 × CIII, 2mbiv, 15 abiv)	2	4	80	6	0	12	13.5	3.4	52.5	1.30	1:2.44	39.0
NV5(A)	17 (1 × CIII, 4 mbiv, 12 abiv)	0	2	83	8	0	16	18.0	3.5	69.6	2.10	1:2.57	35.8
SD104(A)	12 (1 × CIII, 7 mbiv, 4 abiv)	1	3	87	3	0	6	14.6	10.0	48.2	3.20	1:3.13	21.8
SD105(I)	12 (1 × CIII, 7 mbiv, 4 abiv)	1	7	88	3	0	6	15.4	1.6	43.8	0.56	1:2.91	27.2
T113(Y)	17 (3 × CIII, 14 abiv)	2	0	71	11	0	22	21.7	9.4	93.8	4.44	1:3.14	21.5
GS102(O)[f]	10 (1 × CV, 1 × CIII, 4 mbiv, 4 abiv)	0	5	53	15	2	38	20.8	9.2	71.1	1.24	1:2.34	41.5

100MI and 100MII spreads were counted for each individual, except GS102 (see footnote e). All MI spreads contained the expected number of chromosomes.
[a] See Table 3, footnote a; age categories: I, immature (less than 4 mo); Y, young adult (4–6 mo); A, adult (6–12 mo); O, old adult (> 12 mo).
[b] See Table 2, footnote b.
[c] See Table 4, footnote b.
[d] See Table 4, footnote c.
[e] 200 MI spreads were counted; therefore, for comparison with other individuals, the number of univalent chromosomes is divided by two.

TABLE 6

NDJ frequency and aspects of GCD in laboratory-reared females

Karyotype[a]	No. (type) configurations expected at MI[a]	No. of females/MII cells counted	No. of spreads with: n	n + 1	n + 2	% NDJ[b]	Body mass (g)[c]	Total number of follicles per ovary[d,e]: VEG	EG	SA	LA	Total no. of corpora lutea[e]	Volume of smallest ovary (mm^3)	Mass of smallest ovary (mg)[f]
40AA	20 (20 abiv)	10/40	35	1	0	5	19.0 ± 0.4 (9)	277.1 ± 32.8	35.6 ± 8.3	9.9 ± 2.7	3.2 ± 1.4	16.2 ± 5.8	2.76 ± 0.89	3.19 ± 0.52
26POS	13 (7 mbiv, 6 abiv)	9/44	39	2	0	9	13.4 ± 0.5 (9)	202.3 ± 46.6	28.9 ± 7.5	7.5 ± 2.4	6.6 ± 2.0	0	0.43 ± 0.12	0.99 ± 0.12
24UV	12 (8 mbiv, 4 abiv)	11/40	31	1	2	15	17.2 ± 0.5 (10)	146.9 ± 39.5	17.6 ± 5.5	10.2 ± 1.2	9.8 ± 2.2	4.5 ± 4.2	2.30 ± 0.70	1.19 ± 0.16
33(AA × POS)	13 (7 CIII, 6 abiv)	11/40	7	10	10	100	16.7 ± 0.6 (10)	272.9 ± 65.3	42.8 ± 6.8	2.0 ± 1.0	2.9 ± 1.4	6.3 ± 1.2	1.57 ± 0.28	2.11 ± 0.32
32(AA × UV)	12 (8 CIII, 4 abiv)	13/40	7	9	8	100	17.0 ± 0.5 (10)	270.9 ± 63.4	43.1 ± 19.6	9.1 ± 3.4	7.3 ± 1.6	5.2 ± 3.7	0.73 ± 0.10	1.62 ± 0.17
25(UV × POS)	11 (1 CV, 6 mbiv, 4 abiv)	13/48	28	7	1	37.8	14.2 ± 0.5 (9)	247.6 ± 63.9	23.1 ± 8.1	6.6 ± 2.7	5.5 ± 1.9	0	0.59 ± 0.15	0.99 ± 0.12

[a] See Table 2.
[b] See Table 4, footnote b.
[c] Number of individuals measured shown in brackets.
[d] The total number of each type of follicle per ovary was calculated as 1/(proportion of total ovary volume included in 50 7-μm sections) × (total number of particular follicle in 50 sections) after Abercrombie's correction. VEG, very early growing; EG, early growing; SA, small antral; LA, large antral.
[e] 4 females measured per karyotypic group.
[f] 10 females measured per karyotypic group.

TABLE 7

Body mass, NDJ frequency, and mass of the smallest ovary in wild-caught females

Female mouse[a]	No. (type) configurations expected at MI[b]	Body mass (g)	No. of spreads with $n-1$	n	$n+1$	% NDJ[c]	Ovary mass (mg)
T113 (Y)	20 (all abiv)	14.7	0	4	0		2.35
T117 (Y)	20 (all abiv)	10.7	0	3	0	0	0.82
T118 (A)	20 (all abiv)	20.6	0	5	0		—
T114 (O)	19 (1 × CIII, 18 abiv)	24.8 (P)[d]	2	2	1		3.51
T107 (A)	19 (1 × CIII, 18 abiv)	21.0	0	2	1		2.10
T108 (Y)	19 (1 × CIII, 18 abiv)	16.5	0	2	0		1.23
T109 (A)	19 (1 × CIII, 18 abiv)	26.0 (P)	0	1	0	38	2.01
T119 (A)	19 (1 × CIII, 18 abiv)	22.0	2	3	2		2.33
NV4 (Y)	19 (1 × CIII, 18 abiv)	18.3	0	2	0		3.07
NV3 (Y)	18 (1 × CIII, 2 mbiv, 15 abiv)	15.7	0	1	0		1.22
SC108 (Y)	12 (1 × CIII, 7 mbiv, 4 abiv)	14.7	0	3	0		—
SD101 (A)	12 (1 × CIII, 7 mbiv, 4 abiv)	15.7	1	1	0	33	2.27
GS106 (Y)	12 (1 × CIII, 7 mbiv, 4 abiv)	11.1	1	4	2		2.94

[a] See Table 3, footnote *a*; Table 5, footnote *a*.
[b] See Table 2, footnote *b*.
[c] See Table 4, footnote *b*.
[d] P, pregnant.

it has been left out of the following calculations. Body mass of the remaining male wild-caught heterozygotes ranged from 13.5 to 21.7 g (mean, 17.1 ± 1.07; Table 5). There was also substantial individual variation in testes mass (48.2–93.8 mg; mean, 69.2 ± 5.0), relative seminal vesicle mass (3.4–10.0; mean, 7.0 ± 1.0), numbers of sperm per caput (1.24–4.44 × 10^6; mean, 2.92 ± 0.43) and incidence of GCD (15.5–41.5%; mean, 26.8 ± 3.6).

Females: Among laboratory-reared females, 40AA females were heavier than females from all other karyotypic groups ($SS_{crit} = 26.99$, $SS_{sample} = 79.10$, $P < 0.05$; Table 6), whereas 26POS and 25(UV × POS) females weighed significantly less than other females ($SS_{crit} = 26.99$, $SS_{sample} = 92.27$ and 74.52, $P < 0.05$). The mean number of growing follicles was not significantly different between any of the karyotypic groups at any stage of folliculogenesis (Table 6), although a high degree of individual variation coupled with a low sample size may have made differences difficult to detect. Despite the limitations of the data, it appears that oogenesis proceeded quite normally even in multiple simple and complex heterozygotes. Qualitatively, however, ovary sections of pure-race individuals looked healthier than those of the hybrids, and, although there were clearly good numbers of normal oocytes, many antral follicles in heterozygous females showed signs of atresia. The number of corpora lutea per ovary varied widely (Table 6). The 40AA females appeared to have more corpora lutea than any other karyotypic group, but this difference is not significant (again due to high levels of variation). The smallest ovaries of 40AA, 33(AA × POS) and 32(AA × UV) were significantly heavier than those of 26POS, 24UV, and 25(UV × POS) animals ($SS_{crit} = 0.5$, $SS_{sample} = 1.37$, $P < 0.05$; Table 6); however, small ovaries do not appear to be a detriment to oogenesis as all mice had good numbers of growing follicles. Wild-caught females had a large range of body mass (10.7–26.0 mg; mean 17.8 ± 1.3; Table 7), probably because wild-caught females were from different age groups, and two were pregnant. Ovary mass also varied greatly between wild-caught females (0.82–3.51 mg; mean 2.17 ± 0.2; Table 7).

Litter sizes: Details of laboratory-reared backcrosses and their litters are presented in Table 8. Six pairs (18.8%) failed to produce litters: one pair involving a male heterozygote [m33(AA × POS) × f40AA] and five pairs involving female heterozygotes. The litters of male heterozygotes were much smaller than those of 40AA males (H = 21.56, $P = 0.0001$). Laboratory-reared female heterozygotes also produced far smaller litters than control 40AA animals (H = 23.10, $P < 0.0001$). The mean mass of male and female weanlings at 28–30 days after birth was not significantly different.

DISCUSSION

The fertility of wild-caught and laboratory-reared homozygotes and heterozygotes from Upper Valtellina: The range of GCD estimates for male simple wild-caught hybrids from Upper Valtellina (15.5–39.0%; Table 5) was similar to that found previously in other single simple wild-caught heterozygotes (22.2–40.0%; Wallace *et al.* 1992), and the mean value for these Upper Valtellina

hybrids (25.5%) was even very close to that of laboratory-reared homozygous mice (24.1%; Table 4). However, the measures of NDJ in male hybrids from Upper Valtellina were rather high (6–22%; Table 5) compared to those previously recorded for wild-caught (2.7%; Wallace *et al.* 1992) and laboratory-reared hybrids (up to 2.2%; Winking 1986). Some of our high NDJ values may simply be due to individual variation, but genic hybridity may also be an important factor. The two mice captured where two Rb races dominate, SD104 and SD105, had low frequencies of NDJ (6%; Hauffe and Searle 1993), whereas the males T122, T102, T120, NV7, and NV5 (NDJ = 12–22%) were trapped in a zone where Rb races and the 40AA race hybridize. The Rb races in Upper Valtellina have been shown to be genetically very similar, whereas several lines of evidence indicate that the 40AA race is genetically distinct from the Rb races in the valley (morphology, allozymes, mitochondrial DNA; H. C. Hauffe, S. Fraguedakis-Tsolis, and J. B. Searle, unpublished results). Because most wild-caught 40AA × Rb race hybrids are heterozygous for one to three fusions (Hauffe and Searle 1993), we can assume that most of these hybrids were formed by repeated backcrossing with 40AA, thereby introducing the Rb fusions into a genetic background that is almost exclusively 40AA. Searle (1988) and Everett *et al.* (1996) also suggested genic hybridity as an explanation for high NDJ in male single simple heterozygotes.

Female single simple heterozygotes also showed relatively high frequencies of NDJ (Table 7). No other values for wild-caught mice are available, but these NDJ frequencies are high compared to those of a previous study of laboratory-reared hybrids (12–16%; Harris *et al.* 1986); however, they are low compared to female laboratory stock hybrids (generated in the laboratory from crosses between standard laboratory strain mice and laboratory stocks characterized by a wild-derived Rb fusion introduced into a laboratory mouse genetic background; Searle 1988; NDJ up to 61%).

Male multiple simple heterozygotes showed moderate levels of GCD, as indicated by histological studies, testes masses (lower than the mean testes mass of the two parental races), and low number of sperm per caput (51.1 and 43.6% of normal, if the mean sperm per caput of the three homozygous races, 6.06×10^6, is taken as normal; Table 4). These mice can be considered "subfertile" according to the definition of Redi *et al.* (1985). However, many of these sperm are expected to be aneuploid because NDJ frequencies in these males were high (Table 4); this probably accounts for the small litter sizes recorded in Table 8. Our results agree with work by Saïd *et al.* (1993) on laboratory-reared mice but also with many studies involving laboratory stock hybrids (*e.g.*, Tettenborn and Gropp 1970; Winking and Gropp 1976; Redi *et al.* 1979; Gropp and Winking 1981; NDJ up to 52%).

We did not detect differences in GCD between different karyotypic groups of females (Table 6). However, we only counted growing follicles: Young females of all karyotypes are expected to ovulate a similar number of oocytes, and any oocytes entering folliculogenesis are liable to complete all stages of differentiation (Garagna *et al.* 1990); therefore, in any case, sterility in females is unlikely to be due to GCD (Chandley 1984; deBoer 1986; but see Searle 1993) but to the loss of inviable embryos. Female multiple simple heterozygotes were expected to display very low fertility because almost all oocytes were aneuploid (NDJ estimated at 100%; Table 6). This value of NDJ is high even compared to studies of laboratory stock hybrids (Gropp and Winking 1981; NDJ up to 77%). In fact, these females produced small, infrequent litters (Table 8; see also similar data of Saïd *et al.* 1993). In addition, we cannot rule out the possibility that substantial numbers of primordial follicles die in these heterozygotes, leading to a reduced oocyte pool and, therefore, a shortened reproductive lifespan (see Burgoyne and Baker 1984; Garagna *et al.* 1990; Searle 1993).

TABLE 8

Litter sizes produced by male (m) and female (f) heterozygotes when crossed with 40AA individuals

Cross	No. (type) configurations expected at MI of backcross mouse	No. pairs with no litters[b]	No. litters	Litter size	Mass of male weanling (g)	Mass of female weanling (g)
m40AA × f40AA (control)	20 (20 abiv)	0	17	6.7 ± 0.8	12.8 ± 0.4 (12)	11.2 ± 0.9 (12)
m33(AA × POS) × f40AA	13 (7 CIII, 6 abiv)	1	16	4.1 ± 0.4	13.3 ± 0.8 (12)	11.3 ± 0.5 (13)
m32(AA × UV) × f40AA	12 (8 CIII, 4 abiv)	0	18	2.6 ± 0.3	13.2 ± 0.6 (12)	11.9 ± 0.8 (10)
m25(UV × POS) × f40AA	11 (1 CV, 6 mbiv, 4 abiv)	0	19	3.8 ± 0.3	12.6 ± 0.5 (12)	11.8 ± 0.5 (14)
f40AA × m40AA (control)	20 (20 abiv)	0	18	6.8 ± 0.4	12.2 ± 0.5 (32)	10.8 ± 0.4 (35)
f33(AA × POS) × m40AA	13 (7 CIII, 6 abiv)	1	7	1.0 ± 0	14.8 ± 0.7 (2)	11.8 ± 1.1 (4)
f32(AA × UV) × m40AA	12 (8 CIII, 4 abiv)	2	10	3.1 ± 0.6	11.9 ± 0.9 (16)	10.7 ± 0.6 (11)
f25(UV × POS) × m40AA	11 (1 CV, 6 mbiv, 4 abiv)	2	11	4.0 ± 0.5	12.4 ± 0.6 (21)	10.9 ± 0.3 (13)

[a] See Table 2, footnote *b*.
[b] Four pairs per cross were observed.

It was previously believed that chain-forming hybrids would be sterile. Our studies of GCD in 25(UV × POS) male mice indicate that these mice can be considered subfertile (Table 4), in agreement with Gropp *et al.* (1982b) and Searle (1993). In addition, these hybrids showed low NDJ frequencies compared to the multiple simple heterozygotes observed in this study (Table 4), indicating that the pentavalent interferes less with spindle function than seven or eight trivalents. But, strangely, although 25(UV × POS) mice were therefore expected to be more fertile than multiple simple hybrids, their litter sizes were not the largest of all the male laboratory-reared hybrids in this study (Table 8). Instead, female laboratory-reared complex hybrids did, as expected, produce the largest litters of all the female laboratory-reared hybrids (Table 8).

Our studies show that even a hybrid expected to form a pentavalent and a trivalent, like the wild-caught male GS102, may not be sterile (Table 5). This is the first report of the subfertility, rather than sterility, of such a hybrid.

Overall, NDJ frequencies for females were twice to three times as high as those for males with similar heterozygous configurations (Tables 4–7). Although it has been shown previously that some female hybrids tend to have a higher NDJ frequency than similar male hybrids (Winking and Gropp 1976; Redi *et al.* 1979; Gropp and Winking 1981), the reason a heterozygous oocyte should be more prone to NDJ has not been elucidated (Gropp and Winking 1981). In addition, Gropp and Winking (1981) also predicted that male complex hybrids may suffer higher NDJ frequencies than female complex hybrids; however, laboratory-reared 25(UV × POS) females had twice the frequency of male complex hybrids in this study (37.8% compared to 18.5%; Tables 4 and 6).

Male and female heterozygous individuals producing such small litters, such as the 33(AA × POS) and 32(UV × AA) mice studied here, may have been expected to compensate for their lack of progeny by increasing mass at weaning for each offspring (Bengtsson 1980); however, we found no differences between weanling mass for litters produced by homozygous and heterozygous males and females in the laboratory (Table 8).

The high frequency of *XY* univalence in male 40AA mice is somewhat mysterious (Table 4). In the past, *XY* univalence was taken to be an indication of genic hybridity (deBoer 1986). While 40AA mice from Valtellina may be assumed to have a mixed genetic background due to backcrossing in the part of the hybrid zone where they are found (Hauffe and Searle 1993), it is peculiar that *XY* univalence was virtually absent in laboratory-reared hybrids (Table 4) and equally low in all other wild-caught hybrids regardless of origin within the hybrid zone. In part, the high value among 40AA mice can be attributed to individual variation: One individual accounts for 22 out of the 38 observations; however, if the 40AA population has recently been introduced to Valtellina, it is possible that high *XY* univalence is characteristic of the population of origin. More 40AA individuals need to be examined to resolve this issue.

In summary, our results suggest that wild-caught and laboratory-reared hybrids from Upper Valtellina have a fairly low fertility, whether compared to homozygous mice from the valley or to similar hybrids from previous studies. We showed unexpected differences in the effect of certain heterozygosities between males and females. In addition, although many previous authors have based their conclusions on one or two traditional measures of GCD and NDJ, in our study, we found that these did not always corroborate each other or correspond with expected differences in litter sizes. Ideally, future studies should be based on complete fertility measurements of wild-caught males and females. However, modeling of such hybrid zones would be made even more complete by measures of lifetime reproductive success.

The consequences of low fertility for evolution in the house mouse: Previous studies on single simple heterozygotes in both mice and other mammals have suggested that where there is a single segregating Rb fusion in nature, it can be viewed as a neutral or only marginally underdominant marker (see Introduction and Searle 1993 for review). However, our results from Upper Valtellina suggest that, at least in some situations, infertility (and therefore underdominance) in these hybrids may be quite substantial. If infertility is caused by the heterozygous fusion (*i.e.*, not by genic hybridity), this has implications for models of fixation of Rb fusions in the house mouse (see Nachman and Searle 1995 for review). Further studies are necessary to assess whether the findings for Upper Valtellina are repeated elsewhere.

This study also indicates that speciation by reinforcement (see Introduction) could play an important role in the evolution of the house mouse because many hybrids were shown to be highly infertile but not sterile. For the house mouse, there is clear evidence for only one such reinforcement event, that between the 24UV race and the 26POS race in the village of Migiondo in Upper Valtellina (Capanna and Corti 1982; Hauffe and Searle 1992, 1993). We show here that the 25(UV × POS) hybrids do suffer substantial infertility, in terms of high NDJ frequencies and GCD, and reduced litter sizes but are not sterile. However, because these two races continue to interbreed in villages other than Migiondo, heterozygous disadvantage alone is obviously not enough to cause speciation between the 24UV and 26POS races; in fact, probably only very special circumstances allowed the first speciation event to happen (Hauffe and Searle 1993; Fraguedakis-Tsolis *et al.* 1997). We suspect that the races in Upper Valtellina have been in contact for less than 200 yr; consequently, while the present abundance and fertility of various heterozygous karyotypes in the valley certainly indicate

that the races have not become completely reproductively isolated in allopatry, it may be that there has not been sufficient time for slight differences in fitness to result in selection against interbreeding of other races. In addition, stochastic forces, such as the frequent extinction and recolonization of habitats and genetic drift, may prevent these processes of reinforcement (Hauffe and Searle 1993). We are currently looking into these various possibilities.

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