Chromosomal and genic mechanisms of reproductive isolation: the case of *Suncus murinus*

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In this paper we review the results of our studies on the relative contribution of chromosomal and genic divergence in the formation of reproductive isolation in the house musk shrew *Suncus murinus* (Linnaeus, 1766). We crossed two laboratory strains of *S. murinus*: KAT (derived from a wild population of Kathmandu, Nepal) and SRI (Sri Lanka). The strains differed from each other by five Robertsonian fusions and variant chromosomes 7, X and Y. Despite their difference in karyotype, SRI and KAT shrews were able to hybridise in the laboratory and some of the offspring produced were fertile. However, many sterile males were found among the hybrids and the litter size of fertile hybrids was low. At the first glance this appears to be a good example of chromosome-mediated reproductive isolation. However, further analysis clearly demonstrated that chromosome divergence played a negligible (if any) role in either male sterility or the low fertility among the hybrid females.

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Introduction

When a species becomes subdivided into geographically isolated populations, each of them may accumulate different gene mutations and chromosome rearrangements. The accumulated divergence may be sufficient to cause reproductive isolation of the populations if they come into contact again. The contribution of chromosomal and genic divergence in formation of reproductive isolation and hence

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in speciation is a matter of discussion (Coyne *et al.* 1993, 1997, Searle 1993, King 1995).

If two reproductively isolated populations have identical karyotypes it seems obvious that their isolation is determined by genic factors. Reduced fertility of F1 hybrids between karyotypically diverged populations is usually interpreted as evidence of the chromosomal mechanism of reproductive isolation. However, the large body of data recently accumulated in studies of interracial hybrids of mammals contradicts this simple approach and demands the consideration of the divergence in genetic background even in cases of substantial chromosome divergence (Nachman and Myers 1989, Mercer *et al.* 1992, Nachman 1992, Wallace *et al.* 1992, Said *et al.* 1993, Searle 1993, 1998, Fraguedakis-Tsolis *et al.* 1997, Hauffe and Searle 1998, Narain and Fredga 1998).

The main issue lies in the difficulty in specifying the genetic background. That is, how many genes are involved in the control of hybrid sterility? Is the probability of hybrid sterility proportional to the divergence of the populations as defined by supposedly neutral markers such as mtDNA, allozymes, microsatellites, etc.? And finally, in cases when the populations are both karyotypically and genetically diverged, how should we distinguish the effects of heterozygosity for chromosome rearrangements from the effects of genic incompatibility?

These questions hardly have a simple and universal solution and an answer which is correct for one species, may not necessarily be so for another. However, the greater the number of "species-specific answers" that are found, the clearer the general picture becomes.

In this paper we review published data on genetic and chromosomal variability of the house musk shrew *Suncus murinus* (Linnaeus, 1766), and results of our studies on inter-racial hybrids of this species.

Geographic variation in *S. murinus*

The house musk shrew is a widespread species which is found from East Africa to East Asia (Hutterer 1993). It occurs in natural vegetation in the Indian subcontinent, but elsewhere it is almost exclusively found in association with humans, feeding on insects in houses, grain stores etc. *S. murinus* was transported by people to coastal Africa (Egypt to Tanzania and Madagascar), coastal Arabia, Afghanistan, the whole of Southeast Asia northwards to China and Japan, and several small oceanic islands of both the Indian and Pacific Oceans (Corbet and Hill 1992, Hutterer 1993).

Analysis of variation in mtDNA, allozymes and morphological traits revealed a high genetic diversity of local populations of *S. murinus*. Yamagata *et al.* (1990, 1995) analysed mtDNA variation in *S. murinus* from 31 local populations in 9 Asian countries and Mauritius. Fourteen and 9 mtDNA haplotypes were found from Bangladesh and Nepal, respectively, 3 mtDNA types were present in Sri Lanka, 1 to 4 haplotypes were detected in each southeast Asian country. A single haplotype was

present in Japan, Philippines, Vietnam, Thailand and Indonesia. High genetic diversity on the Indian subcontinent indicates that the species originated there and its population size is large. The presence of the same mtDNA haplotype on the coast and islands of eastern Asia suggests a recent and rapid spread of these animals throughout that region, presumably from southern India. The mtDNA differentiation between shrews from northern India-Bangladesh-Nepal and those from southern India-Sri Lanka is comparable with genetic distances between different subspecies of the house mouse (Yamagata *et al.* 1990, 1995, Boursot *et al.* 1993).

Subsequent allozyme studies by Ruedi *et al.* (1996) have shown that shrews of southern India-Sri Lanka and Southeast Asia-Philippines-Japan are genetically similar to each other and very distinct from the shrews of northern India--Bangladesh-Nepal.

The mtDNA and allozyme differentiation patterns are concordant with the differentiation of local populations of *S. murinus* in body size. Ishikawa *et al.* (1995) examined skull and external measurements in one Bangladeshi, one Sri Lankan and four Japanese strains of musk shrews. These six strains were clearly grouped by principal component analysis into three body-size types: large shrews from Bangladesh, intermediate shrews from Sri Lanka and small Japanese shrews.

The most common karyotype of *S. murinus*, found in central and northern India, Nepal, Bangladesh, Vietnam, Malaysia, Indonesia, Taiwan and Japan, consists of 40 chromosomes (Yosida 1982, Rogatcheva *et al.* 1996). Variation in the chromosome number, due to fixation of metacentrics formed by Robertsonian fusion (Rb), has been reported from Malaysia (2n = 40–35; Sam *et al.* 1979), Sri Lanka (2n = 30; Ishikawa *et al.* 1989), and southern India (2n = 30–32, 37, 40; Aswatharanayana and Krishna 1979). Sex chromosome variation has been recorded in *S. murinus*, with Y-chromosome polymorphism found to be very extensive (Yosida 1982). The Rb polymorphism in Malaya observed along of the west coast of country was interpreted by Yosida (1982) as the result of the human-mediated transport of shrews with Rb metacentrics from southern India/Sri Lanka to Malaya by sea and the hybridisation of these immigrants with the 40-chromosome individuals that already occupied Malaya. The common identity of two Malayan Rbs with those from Sri Lanka population (Rogatcheva *et al.* 1997) provides strong support for Yosida's suggestion.

Interracial hybridisation in the laboratory

In a series of papers we have analysed the relative contribution of chromosomal and genetic divergence in the formation of reproductive isolation using two laboratory strains of *S. murinus*, KAT and SRI, as a model system.

The KAT strain was derived from a wild population in Katmandu, Nepal (Oda *et al.* 1992) and was shown to have 2n = 40 (Rogatcheva *et al.* 1996). The SRI strain was derived from animals captured on the western coast of Sri Lanka (Ishikawa *et al.* 1989). The SRI strain differed from the KAT strain by five Robertsonian fusions
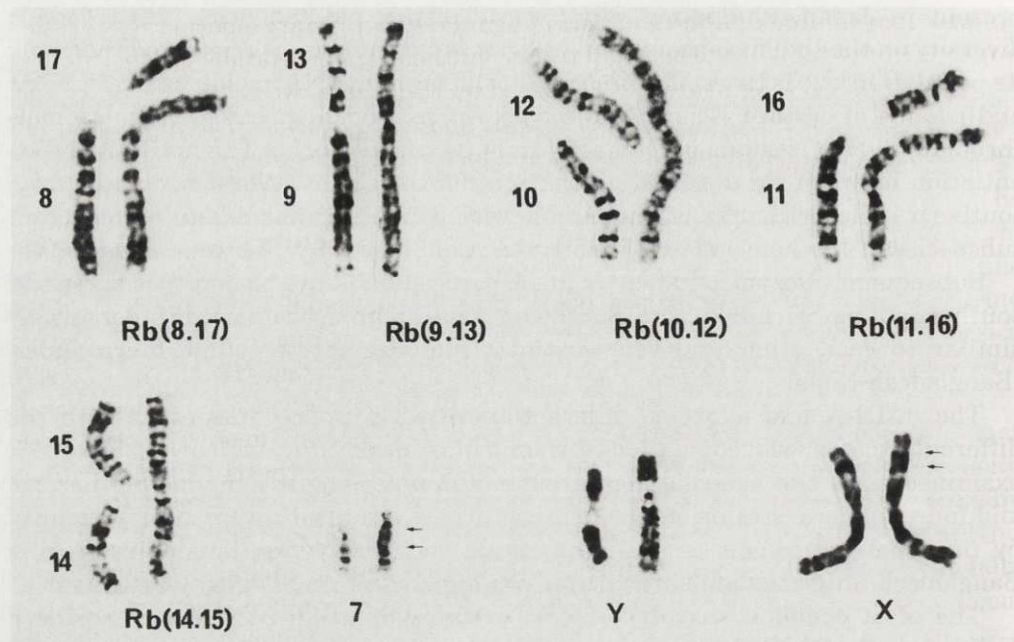
Fig 1. G-banded variable chromosomes of *S. murinus*, distinguishing the KAT and SRI strains. SRI chromosome variants are shown on the left, KAT variants – on the right.

and variant versions of chromosomes 7, X and Y (Fig. 1). The SRI chromosome 7 was twice as long as the KAT chromosome 7, apparently as a result of an insertion or amplification of heterochromatin. The SRI X chromosome had an additional C-band relative to the KAT X chromosome. The SRI Y chromosome was larger than the KAT Y chromosome and differed substantially in both the G- and C-banding pattern.

Despite being so different in karyotype, SRI and KAT shrews were able to hybridise in the laboratory and produce fertile offspring. A hybrid SK stock was set up as described earlier (Rogatcheva *et al.* 1997, 1998a). In general, the hybrid SK shrews demonstrated a reasonable reproductive performance. However, many sterile males were found among them.

Data on the sterility of the SK males was taken from two sources: from breeding records (when available) and from measured testes weights. It has been shown that testes weight is significantly reduced in 'genetically' and 'chromosomally' sterile males and can be used to classify sterility (Forejt and Ivanyi 1977, Forejt 1996). Among the SK males there were quite clear groups: those with testes lighter than 100 mg and others with testes heavier than 200 mg. The 'normal' testes of greater than 200 mg contained cells at all stages of spermatogenesis, including mature sperm, while the 'small' testes of less than 100 mg had very narrow and almost

empty seminiferous tubules. Spermatogonia and primary spermatocytes were present in the tubules of the small testes, but neither spermatids nor spermatozoa were detected (Borodin *et al.* 1998). Based on these data, we arbitrarily scored the males which produced at least one offspring and/or had normal testes as fertile. The males which did not produce offspring and had small testes were scored as sterile.

Chromosomal versus genic control of sterility in hybrids

It has been shown that heterozygosity for chromosomal rearrangements might lead to complete sterility or reduced fertility in hybrids due to meiotic difficulties. Meiosis in sterile SK males did not progress beyond pachytene (Borodin *et al.* 1998). It appeared to be a very attractive suggestion that heterozygosity for chromosome rearrangements was the cause of sterility. However, an extensive cytogenetic analysis of sterile and fertile males disproved this hypothesis (Axenovich *et al.* 1998, Borodin *et al.* 1998).

Electron-microscopic analysis of synaptonemal complexes (SC) in fertile hybrids demonstrated an orderly pairing of all chromosomes, including heterozygous combinations. In the sterile males chromosome pairing was distorted not only in heterozygous pairs, but also in homozygous ones: the number of aberrant SC per cell was much higher than the number of heterozygous pairs. Heterozygotes for each of the rearrangements were found among both sterile and fertile males (Fig. 2). There was no correlation between the number of heterozygous rearrangements per individual and its fertility. Sterile males had all possible combinations of the sex chromosomes X^K-Y^K, X^S-Y^S, X^K-Y^S, X^S-Y^K, hence compatibility of the sex chromosomes does not appear to play any role in the control of fertility. Therefore, we
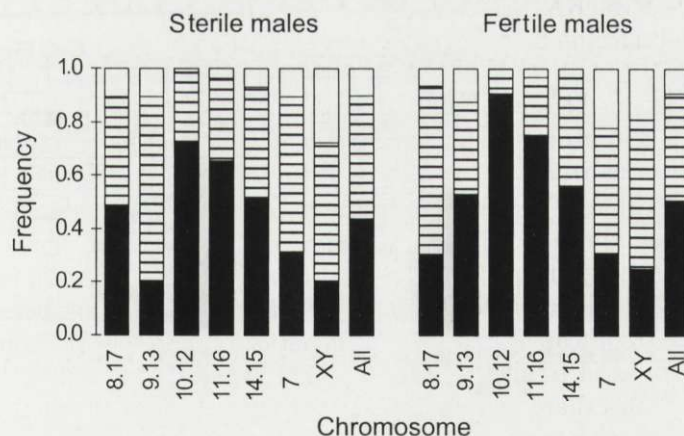

Fig. 2. Frequency of homozygotes and heterozygotes for SRI- and KAT variant chromosomes among sterile and fertile hybrid SK males of *Suncus murinus*. Open bars – SRI-variant chromosome homozygotes, filled bars – KAT-variant chromosome homozygotes, striped bars – heterozygotes.

concluded that meiotic arrest and sterility of interracial hybrids was not determined by structural heterozygosity (Borodin *et al.* 1998).

Analysis of the pedigree of the SK hybrid stock (Fig. 3) showed that the cases of male sterility were non-randomly distributed. Often the sterile males had sterile brothers, uncles or nephews. This finding prompted the search for a genic cause of hybrid sterility (Axenovich *et al.* 1998). There were at least three conceivable explanations of male sterility: cytoplasmic inheritance, sex-linked inheritance and autosomal sex-restricted control. The pedigree clearly demonstrated that some sterile males carried KAT cytoplasm and some had SRI cytoplasm. Thus, it was concluded that sterility factors were not located in the cytoplasm. Also sex-linked inheritance was ruled out because cytogenetic analysis showed that sterile males were as heterogeneous for the sex chromosome combinations as the fertile males (Borodin *et al.* 1998).

Several hypotheses of autosomal genetic control of male sterility were tested by segregation analysis (Axenovich *et al.* 1998). The first hypothesis assumed a simple 'one gene – two allele' model of inheritance of this trait. We supposed that one strain was homozygous for *A1* (*A1A1*) and another one for *A2* (*A2A2*), the hybrid *A1A2* genotype was male sterile. We found that the pedigree data fit to this model given incomplete penetrance of the hybrid *A1A2* genotype, ie that some but not all *A1A2* males were sterile. Incomplete penetrance may have trivial explanations like environmental variability and/or the effects of polygenes. However, it might also indicate that our model was incorrect.
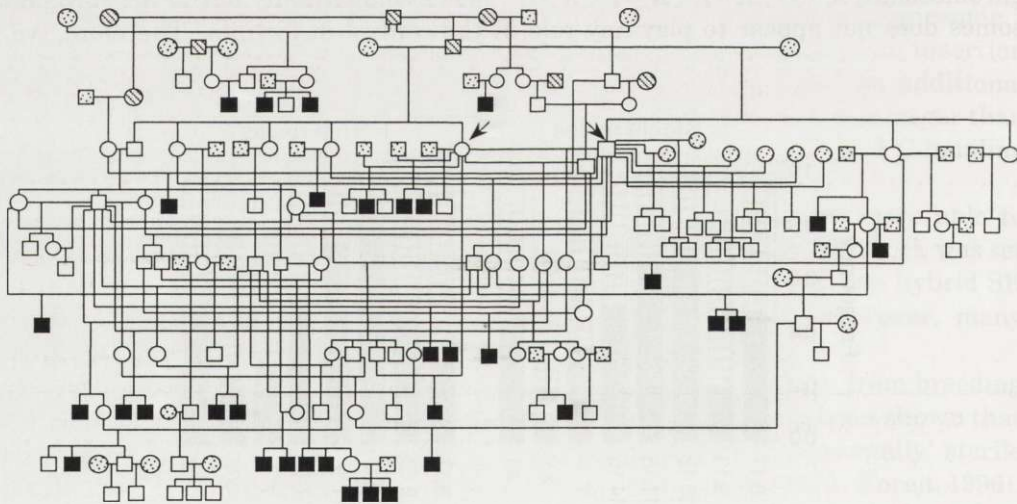

Fig. 3. Fragment of the hybrid pedigree. Uninformative individuals (unscored males and females, which did not produce offspring) are not included. Circles indicate females, squares – males; empty figures indicate SK individuals, dotted – KAT, dashed – SRI, sterile SK males – black squares. Arrows indicate crosses discussed in the text.

Results of some crosses in the pedigree can be interpreted as an indication of a polymorphism among F1 hybrid males (they are indicated by arrows at the Fig. 3). For example one F1 hybrid female was crossed with three KAT males and produced three sterile and three fertile males. Its half-brother was crossed with six KAT females and produced twelve males. All of them were fertile. This may indicate that the SRI father of both probands was heterozygous and transferred one allele to the son and the other to the daughter. Therefore we assumed that there might have been more than two alleles in the locus and that the parental populations were genetically heterogeneous

Taking these data into account, we considered a three-allele model, assuming that the KAT strain was *A2A2* homozygous, the SRI was polymorphic for allele *A1* and *A3*, and the hybrids (*A2A3*) were sterile. The results of segregation analysis indicated that this model fitted the pedigree data better than the simple monogene diallele one (Axenovich *et al.* 1998). This model seems to be rather close to the digene diallele models suggested by Dobzhansky (1936) and Muller (1939, 1940) and to a chromosomal modification of the later models proposed by Baker and Bickham (1986).

An evolutionary scenario of appearance of this mode of inheritance seems rather simple. An ancestral population was homozygous for the wild type allele, *A1A1*. Then, this population was geographically subdivided and two derived populations evolved independently. A new allele *A2* appeared in one derived population and the *A1A2* genotypes were fertile. In the other derived population, a mutation *A3* appeared, and the *A1A3* genotypes were fertile. Since the populations were geographically isolated the *A2* allele did not come into contact with the *A3* allele. When these two populations were crossed, the new allele combination *A2A3* appeared and males of such a genotype were sterile. It is important that this model does not involve a maladaptive step. Within the framework of this model one may deduce the sequence of events which could have taken place in the two geographically isolated populations of *S. murinus* in Nepal and Sri Lanka. They inherited from their common ancestor an identical genotype *A1A1*. Then in these two populations two different mutations (*A2* and *A3*) appeared at the locus controlling male meiosis. In heterozygotes with the wild type allele (*A1*) none of them produced sterility. Individuals from the Nepalese and Sri Lankan populations were used to set-up the KAT and SRI laboratory strains. The number of founders of these strains was very small: 2 females and 1 male in KAT and 2 females and 3 males in SRI. Therefore it is not surprising that the common allele could be subsequently lost in KAT. More surprising is that SRI remained polymorphic, and not only for these alleles, but also for two of five Rbs (Rogatcheva *et al.* 1997). When we crossed the SRI and the KAT strains the non-compatible combination of alleles *A2A3* appeared and manifested itself in male sterility.

The system of hybrid sterility, which was found in the *S. murinus*, appears to be somewhat similar to that discovered by Forejt and Ivanyi (1977) in the house mouse. They isolated the allele $Hst1^{ws}$ from wild *Mus musculus*. This allele in

combination with the allele $Hst1^s$ derived from the C57BL/10 strain of the laboratory mouse determined sterility of heterozygous males $Hst1^{ws}/Hst1^s$, while homozygotes and heterozygotes with another allele $Hst1^f$, derived from the C3H/Di strain $(Hst1^{ws}/Hst1^f)$ were fertile. It has been shown that the combination $Hst1^{ws}/Hst1^s$ affected spermatogenesis at meiotic prophase (Forejt and Ivanyi 1977). Several other monogene systems of hybrid sterility have been found in the house mouse (Forejt 1996).

The peculiarity of our system is that it affects hybrids between different populations of the same species. Therefore, we may consider this system as an initial step in the formation of postzygotic reproductive isolation due to divergence of the alleles of a gene controlling male meiosis.

Chromosomal versus genic control of litter size in hybrids

The hybrid shrews of the SK strain were heterozygous for up to 7 chromosome rearrangements and we expected them to have reduced fertility relative to homozygotes (parental strains), because of malsegregation at anaphase I, resulting in aneuploidy and consequent embryonic losses. Indeed, the F1 × F1 intercrosses demonstrated low fertility (Fig. 4). However, the SRI strain had on average a small

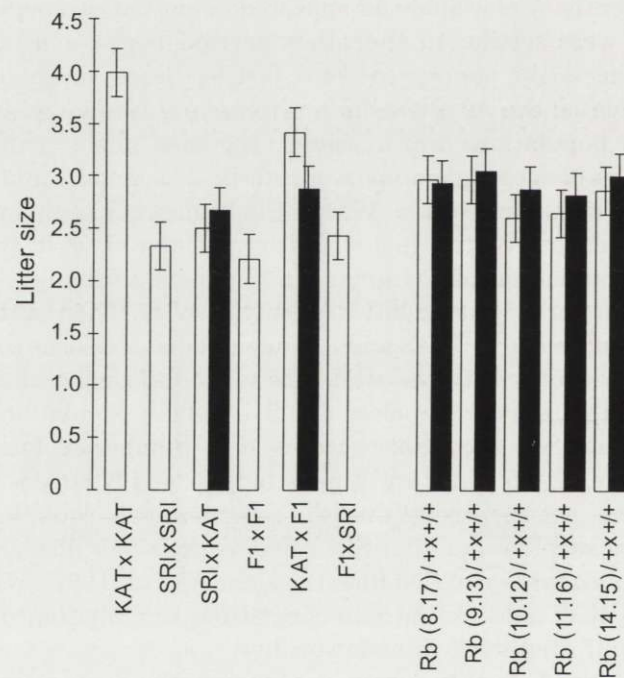Fig. 4. Litter size (± SE) in different crosses of SRI, KAT strains and SK Rb heterozygotes of *Suncus murinus*. Open bars – male genotype × female genotype; filled bars – reciprocal crosses.

litter size too, almost half that of the KAT strain. It is remarkable that the litter sizes in KAT × SRI and SRI × KAT crosses were significantly smaller than in pure-bred KAT and did not differ from that of the less fertile SRI parent. In other words, mating with SRI males reduced the fertility of KAT females down to that of the SRI female level. We thought that a low viability of the embryos heterozygous for several chromosome rearrangements might reduce the fertility of KAT females.

We tried to find a correlation between the karyotypes of the parents and their litter size, and to estimate the viability of heterozygous embryos (Rogatcheva *et al.* 1998a). However we did not detect any effect of either heterozygosity for any of the Rbs, or heterozygosity for variant chromosome 7, or the combined heterozygosity of both parents on the litter size. The litter size even in heterozygotes for five Rbs was no smaller than that in the less fertile parental strain (SRI) (Fig. 4). The viability of heterozygous embryos also appears to be similar to homozygotes: the patterns of segregation of each of the Rbs and the variant chromosome 7 in heterozygotes do not differ from Mendelian expectations (Fig. 5) (Rogatcheva *et al.* 1998a). Hetero-zygosity for Rbs did not lead to a substantial production of aneuploid progeny. Only one case of aneuploidy – an XXY male was detected. Paradoxically this male received an additional X chromosome from its mother, which was homozygous for all standard chromosomes, but not from its father, which was heterozygous for as many as three Robertsonian translocations (Rogatcheva *et al.* 1998b).
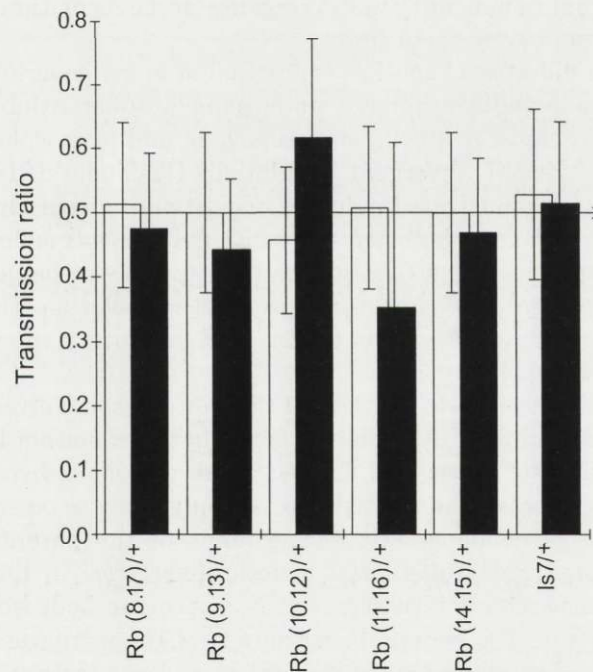

Fig. 5. Ratio of transmission (± SE) of SRI-variant chromosomes in crosses of heterozygous males with homozygous females of *Suncus murinus* (open bars) and reciprocal crosses (filled bars).

Thus our data clearly demonstrated that even if Rb heterozygosity did affect litter size, its effect was not as strong as the effect of genetic background of the less fertile SRI parents. This finding prompted our search for genetic factors controlling the litter size in the hybrid population (Aulchenko *et al.* 1998).

Segregation analysis of the hybrid pedigree data was performed on the basis of a mixed polygene and major-gene model. We suggested that the litter size depended of genotypes of both breeding partners; ie we considered the litter size as the combined phenotype of the breeding couple rather than the individual phenotype of the female. The result of the segregation analysis indicated that both major gene and polygenic components are necessary for a correct description of litter size inheritance in interracial hybrids. However, the relative contribution of the major gene component was much more substantial than that of the polygene one. The parental populations differ in the allele frequency of the major gene (the SRI population is homozygous, while the KAT population contains two alleles in approximately equal proportion) and in the values of polygenic effects (Aulchenko *et al.* 1998).

The lesson from *S. murinus*

Do the results of the study of *S. murinus* give us a clue to determine the relative contribution of chromosomal and genic divergence in the formation of reproductive isolation? Let us summarise them first.

S. murinus is a widespread species, comprised of many geographically isolated populations. These populations have undergone a substantial genetic differentiation in chromosomes, neutral genetic markers and in morphological traits.

When two karyotypically divergent populations (KAT and SRI) were mated in the laboratory, they demonstrated a degree of postmating reproductive isolation: many male hybrids were sterile, litter size in F1 hybrids was as low as in the low fertility parental strain. At the first glance this appears to be good evidence in favour of a chromosomal mechanism of reproductive isolation. Had we met this situation in nature and not been able to test it in laboratory situation, we would have come to this conclusion.

However, our data clearly demonstrated that chromosome divergence played a negligible (if any) role in fertility problems of the hybrids: neither in male sterility nor in the low fertility of the hybrid females. If chromosome divergence does not explain the fertility problems in the hybrids, we may apply to our case a universal explanation – divergence in genetic backgrounds of the parental populations. Indeed, they demonstrated substantial genetic divergence in mtDNA, morphological and quantitative characteristics, such as coat colour, body weight, litter size.

Does this mean that the genetic backgrounds of the introgressing population must be necessarily very divergent to prevent or at least restrict gene flow? Our data show that divergence for a single gene controlling early stages of male meiosis may be sufficient to create male hybrid sterility. The case of *S. murinus* indicates

that for two populations to be reproductively isolated they may, but need not be substantially genetically divergent.

All our conclusions came from laboratory crosses. The Sri Lankan and Nepalese shrews never come into contact in nature. In fact, they are half way to reproductive isolation. If these or other populations with such differences in their genetic backgrounds would meet in nature then the gene flow between them could be rather restricted. This might lead either to speciation or to formation of a hybrid population where selection would work *pro* or *contra* the 'isolating' alleles.

Thus, three main conclusions can be drawn:

(1) If two populations (races, species) differ from each other in karyotypes and their hybrids have fertility problems, it does not necessarily mean that chromosome heterozygosity is the cause of reduced fertility.

(2) A substantial genetic divergence increases the chance of reproductive isolation, although sometimes a small difference in the set of genes controlling reproductive processes might be sufficient for hybrid sterility.

(3) Therefore, if two populations (races, species) differ from each other in their karyotypes and their hybrids have fertility problems, but overall genetic distance between them measured by divergence of neutral genetic markers is not very high, we still cannot assume hybrid sterility by chromosome divergence, unless it is proved in laboratory experiments.

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