

Absence of *Y*-chromosome introgression across the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus*

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Summary

A cloned *Y*-specific sequence (Bishop *et al.* 1985) was used as a diagnostic probe to distinguish between *Mus musculus domesticus* and *Mus musculus musculus* *Y*-chromosomes. Analysis of the RFLPs obtained with genomic DNA isolated from wild mice caught along the contact zone between *M. m. domesticus* and *M. m. musculus* in Bulgaria and Denmark showed that the *Y*-chromosome flow between the two semi-species is very limited. The degree of *Y*-chromosome penetration was compared with that of seven diagnostic autosomal loci and the mitochondrial DNA. Breeding experiments showed that the lack of *Y*-chromosome introgression from one semispecies to the other was not due to a major hybrid breakdown. The results suggest that the disruption of differentiated co-adapted gene systems could play a role in limiting *Y*-introgression.

1. Introduction

When genetically differentiated populations come into contact they sometimes produce hybrid zones. Analysis of the genotypes found in hybrid populations can provide information on the factors which maintain these hybrid zones and by extrapolation, the evolutionary fate of the taxa involved. (For a review see Barton & Hewit, 1985).

The analysis of restriction fragment length polymorphism (RFLP) is a powerful tool for detecting genetic exchanges. In population studies most of the data obtained up to now by this technique concern the maternally transmitted mitochondrial genome. As a paternal counterpart, a marker on the haploid *Y*-chromosome is therefore of particular interest for the study of male-mediated gene exchanges between the two parapatric semispecies *Mus musculus domesticus* and *Mus musculus musculus*. One such sequence, PY353/B, was isolated from a *Y*-chromosome enriched library by Bishop *et al.* (1985). It has been estimated that 100–200 copies of this and related sequences are present on the *Y*-chromosome of BALB/c mice (Bishop *et al.*, in press). Although the repetitive nature of the sequence results in relatively complex patterns of bands when it is hybridized to genomic blots of mouse DNA, Bishop *et al.* (1985)

have shown that with certain restriction enzymes such as *Hind* III, *M. m. domesticus* and *M. m. musculus* individuals coming from wild-derived strains representative of each semispecies could be distinguished unambiguously on the basis of the RFLP obtained. The contact between *M. m. domesticus* and *M. m. musculus* occurs in a narrow hybridization zone which crosses Europe from Denmark to Bulgaria (Boursot *et al.* 1984) (Fig. 1). Genetic exchanges have been revealed in this contact zone (Hunt & Selander, 1973) and they occur both at the nuclear and the mitochondrial level. The width of the cline of introgression for autosomal genes, in which 80% of the changes in allele frequencies occur, is limited to 40 km both in Denmark (Hunt & Selander, 1973) and Bulgaria (Vanlerberghe, unpublished results) (Fig. 2). So even if these two parapatric semispecies are not reproductively isolated, they are genetically isolated. The introgression is asymmetrical and rapidly tends to zero in *domesticus* populations, while in *musculus* populations there are some traces of introgression that extend over a distance of at least 150 km from the contact point (Fig. 2). In Denmark there is, or has been, considerable mitochondrial gene flow north-eastwards from *M. m. domesticus* to *M. m. musculus*, so that Danish and Scandinavian *M. m. musculus* populations have now fixed *M. m. domesticus* mitochondrial DNA (Ferris *et al.* 1983). In Bulgaria however,

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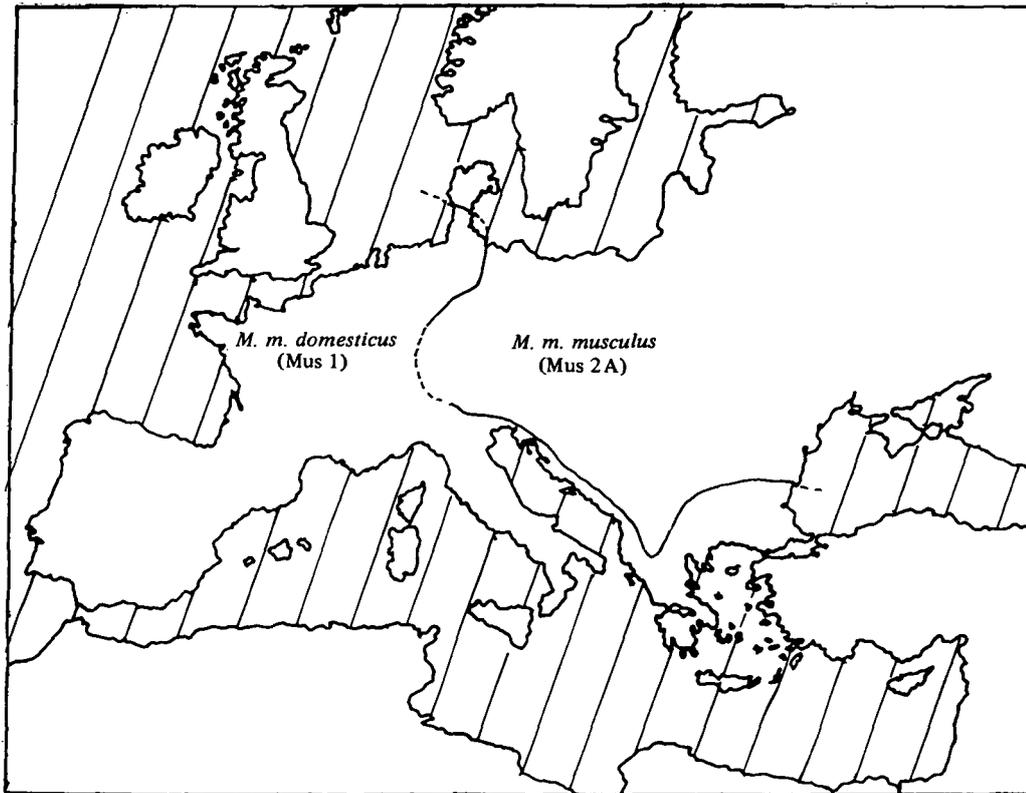


Fig. 1. Map of Europe showing the contact zone between *M. m. domesticus* (West side) and *M. m. musculus* (East side), indicated by a thick line from Denmark to the Black

Sea. (These semi-species have also been termed Mus 1 and Mus 2; Bonhomme *et al.* 1978, 1982).

the flow occurs in the opposite direction from *M. m. musculus* towards *M. m. domesticus* (Boursot *et al.* 1984, Vanlerberghe, unpublished results) but without fixation of either type. It therefore appeared important to assess the degree of Y-chromosome exchange. The extent to which the Y-chromosome of the semi-species can co-exist in a genome where the majority of the autosomal genes are assigned to the other semi-species is of considerable interest. We used the sequence described above as a probe to study the Y-introgression that occurs in wild-mice populations that occupy the contact zone. The results support the idea that the two Y-chromosomes are genetically

differentiated units that could play a leading part in achieving the reproductive isolation between the two semispecies.

2. Material and Methods

(i) Animals

Wild animals were trapped along a north-south transect across the *domesticus*-*musculus* contact zone, in Bulgaria between 1981 and 1984 (80 male mice from 15 sampling sites) and in Denmark in 1985 (35 male mice from 4 sampling sites). The assignment of each

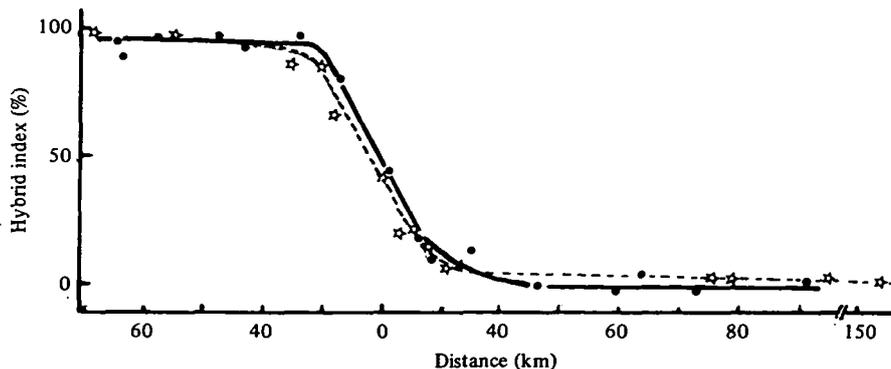


Fig. 2. Variation in the hybrid index value along two north-south transects through the hybridization zone. ●—●, Bulgarian transect. ☆---☆, Danish transect

(from Hunt & Selander, 1973). Distance is measured in kilometres from the point where the hybrid index is 50%.

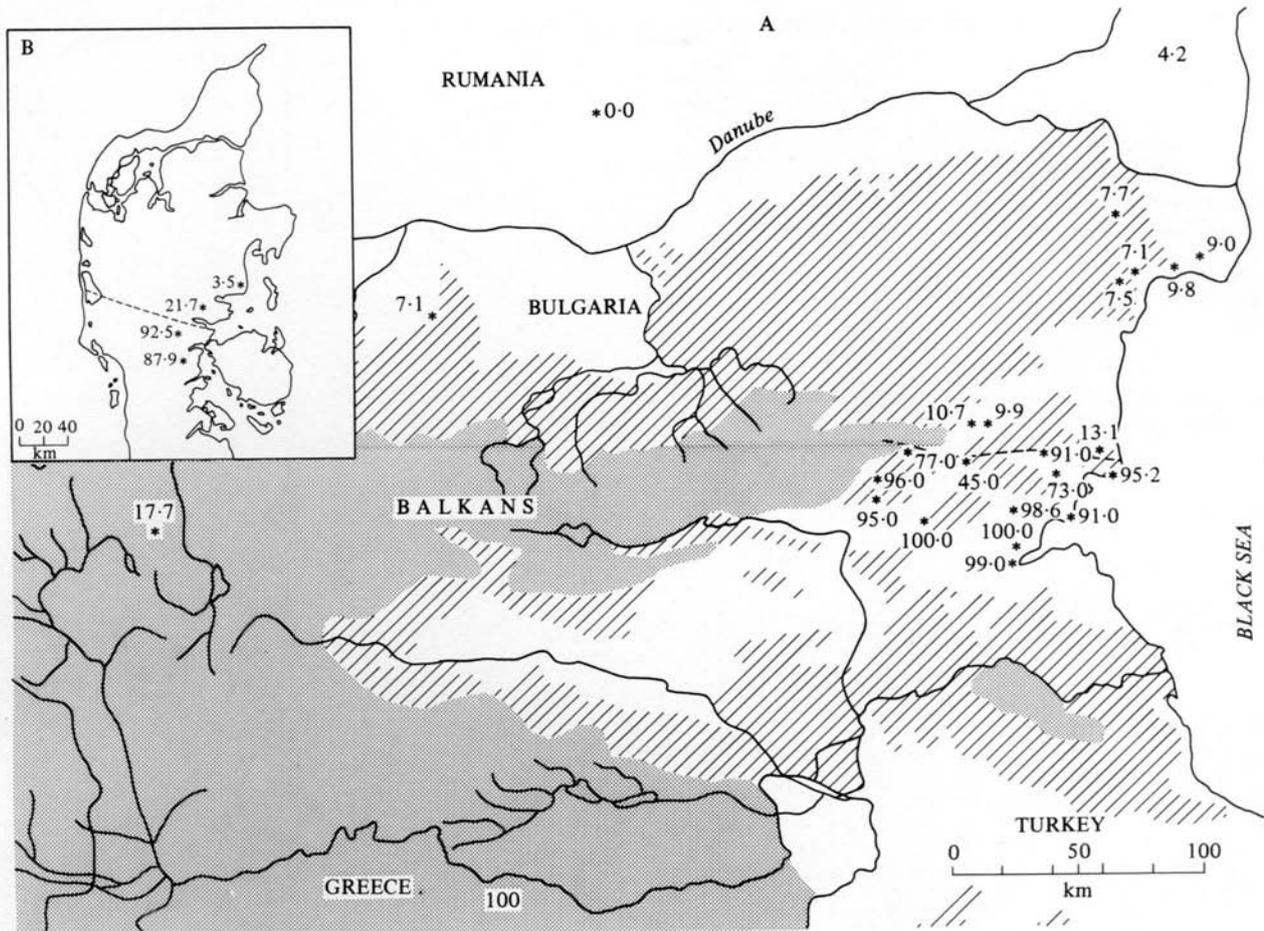


Fig. 3. Geographic variation in the percentage hybrid index score based on seven diagnostic enzyme loci. (A) In

Bulgaria. (B) In Denmark. Dashed line indicates the centre of the hybridization zone.

sample to one or other semispecies was made according to the alleles found at seven loci which are diagnostic between *M. m. domesticus* and *M. m. musculus* (Boursot *et al.* 1984, Vanlerberghe, unpublished results). For each sample we computed a hybrid index which is equal to the average frequency of *domesticus* alleles. This index ranges from 0% for a pure *M. m. musculus* sample to 100% for a pure *M. m. domesticus* sample (Fig. 3A, B). The geographical position of the contact point is defined arbitrarily by a 50% introgression index.

(ii) DNA transfer and hybridization

DNA was prepared by the method of Gross-Bellard *et al.* (1973) from nuclei that had been isolated from fresh liver and kidneys and then stored at -60°C . 10 μg samples of genomic DNA were digested with *Hind* III, electrophoresed on 0.6% agarose gels and transferred to DBM paper (Christophe *et al.* 1982) or Hybond-N membranes (Amersham). The blots were hybridized at 42°C in $6 \times \text{SSC}$, 50% formamide with the Y-specific sequence PY353/B (kindly provided by Bishop, Institut Pasteur) isolated and ^{32}P -labelled as described by Feinberg & Vogelstein (1983). They were then washed at 68°C twice for 30 min in $2 \times \text{SSC}$,

0.1% SDS, one to three times in $0.1 \times \text{SSC}$, 0.1% SDS and then exposed to Kodak X-AR5 film at -60°C . The presence of two major bands at 10 and 12 kb corresponds to the *M. m. musculus* variant while in *M. m. domesticus* the 10 kb band is replaced by one of about 14 kb (Bishop *et al.* 1985).

3. Results

Introgression of the Y chromosome in M. m. domesticus and M. m. musculus wild mice along their contact zone

Genomic blots of *Hind* III-digested genomic DNA isolated from wild mice caught in the contact zone show one or other of the patterns described by Bishop *et al.* (1985) when hybridized to the Y-specific probe PY353/B. This confirms that the RFLPs detected by this probe can be used as markers which can distinguish between the Y chromosomes of the two semispecies. The distribution of these two patterns in the populations studied is shown in Table 1. The percentage of introgression of autosomal alleles of these males was not significantly different from that of the females in the same population. We detected no sex reversal among the individuals we studied (115 males and 30 females), showing that there is a real equiv-

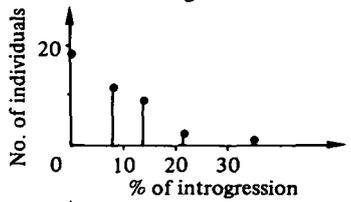
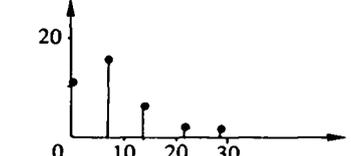
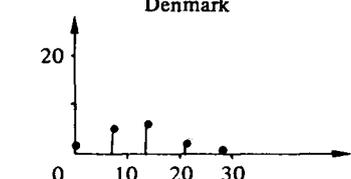
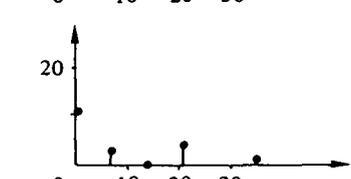
Semi species	Proteins	Y chromosome	
		<i>M. m. dom.</i>	<i>M. m. mus.</i>
Bulgaria			
<i>M. m. dom.</i> (39 indiv.)		39	0
<i>M. m. mus.</i> (39 indiv.)		2	37
Denmark			
<i>M. m. dom.</i> (16 indiv.)		15	1
<i>M. m. mus.</i> (19 indiv.)		0	19

Table 1. shows the autosomal introgression and Y-chromosome distribution in Bulgarian and Danish wild-mice populations caught along the hybrid zone.

alence between phenotypic sex and the genotypic sex diagnosed with this Y-probe.

The Y-chromosome flow between the *M. m. domesticus* and *M. m. musculus* semispecies is very limited. In a total sample of more than 100 animals there were only 3 cases where the Y variant did not correspond to the semispecies assignment of the majority of the alleles found at the seven diagnostic loci. We found one Danish *domesticus* population with a hybrid index of 92.5%, in which one animal had a *M. m. musculus* Y-chromosome, and two Bulgarian *musculus* populations with an index of 11%, in each of which one animal had a *M. m. domesticus* Y-chromosome. The level of autosomal introgression of these three males allows us to exclude the possibility that they are first-generation migrants.

The differences in relative penetration of the various genomic components from one semispecies into the other are shown in Fig. 4. We computed the weighted distance of penetration D using the formula $D = 1/n\sum$ (frequency of the foreign gene in a population \times distance from the contact point) for the mt DNA, the Y chromosome and each diagnostic autosomal locus, in n populations. If there was no bias in the sampling, the

histograms show that there is very little introgression of the Y chromosome in either semispecies in both Denmark and Bulgaria.

This low rate of introgression suggests that either there are little if any successful male-mediated migrations or that there is an incompatibility between the genomes of the two semispecies involving a hybrid breakdown. In order to test the last hypothesis we carried out some laboratory breeding experiments with Bulgarian individuals. The results shown in Table 2 indicate that the sex ratio and viability of the offspring of *M. m. domesticus* males and *M. m. musculus* females and from the reciprocal cross is normal. The F_2 are also viable. Backcrosses of the F_1 females with *M. m. domesticus* and *M. m. musculus* males were fertile as were the backcrosses between the F_1 males and *M. m. musculus* females. The backcrosses between the F_1 males and *M. m. domesticus* females appeared to be less fertile. However, this result will have to be reproduced in independent experiments before it can be considered as significant. Nevertheless on the basis of these experiments we can conclude that there is no major hybrid breakdown in the Bulgarian semispecies.

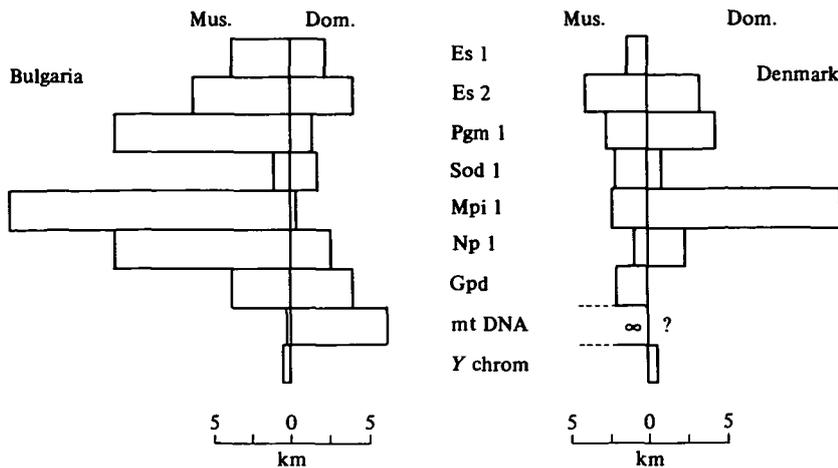


Fig. 4. Histograms showing the distance of penetration of the Y-chromosome, mitochondrial DNA and each autosomal allele in the two semispecies in Bulgaria and Denmark. Mus, *M. m. musculus*; Dom, *M. m. domesticus*.

4. Discussion

From the point of view of population genetics the Y-chromosome can be considered as the paternally inherited analogue of the maternally transmitted mitochondrion. However, they do not show similar patterns of exchange between the two semispecies, probably because they have very different functions and do not show the same degree of autonomy in the overall genetic background. In both Bulgarian and Danish populations of mice, Y-introgression is either non-existent or extremely limited, and was only detected close to the contact zone.

It can be seen from Fig. 4 that the Y chromosome

is one of the nuclear genomic components that introgresses the least. It means that the Y chromosome is bound to the genome by strong interactions which limit the diffusion of the Y-chromosome from one semispecies into the other.

This peculiar behaviour of the Y-chromosome could be the consequence of the role that this chromosome could play in sex determination and the development of functional gonads. Sage *et al.* (1986) and Winking (pers. comm.) have reported the occurrence of male sterility of some F₁ hybrids between *M. m. domesticus* and *M. m. musculus* from Northern Europe. We propose that the perturbation of the interactions between the Y-chromosome and the other

Parents		Offspring			
Crosses	Fertility		Sex ratio	Viability	Fertility
♀ dom. × ♂ mus.	☆☆☆	F ₁	balanced	☆☆☆	☆☆☆
♂ dom. × ♀ mus.	☆☆☆			☆☆☆	☆☆☆
♂ F ₁ × ♀ F ₁	☆☆☆	F ₂	balanced	☆☆☆	☆☆☆
♀ F ₁ × ♂ mus.	☆☆☆☆	B a c k c r o s s e s	balanced	☆☆☆	
♀ F ₁ × ♂ dom.	☆☆☆			☆☆☆	
♂ F ₁ × ♀ mus.	☆☆☆			☆☆☆	
♂ F ₁ × ♀ dom.	●●●			☆☆☆	

Table 2. The results of the breeding experiments giving the sex-ratio, viability and fertility of the offspring.

☆☆☆☆, excellent; ☆☆☆, good; ●●●, poor. Mus, *M. m. musculus*; Dom, *M. m. domesticus*.

chromosomes could have a panel of ontogenetic effects ranging from mild or severe male sterility to partial or complete sex reversal. Models have already been proposed to account for this latter phenomenon as those of Eicher *et al.* (1982) and Chandra (1985). In the model of Chandra, a multicopy non-coding DNA sequence (not a structural gene for a sex-determining protein) carried on the *Y*-chromosome would be responsible for the choice of sexual phenotype during development. Its function would be to bind with high affinity to a repressor-like molecule of autosomal origin which occurs in limiting concentration. The transcription product of a testis-determining gene (*Tdx*) on the *X*-chromosome is assumed to be essential for the determination of the male sex. In the absence of the *Y*-linked non-coding sequences, the repressor-like molecules would bind to and inactivate the *Tdx* gene for which they have less affinity. The copies of *Y*-linked sequences would be expected to cease to be male-determining when their capacity to bind all the repressor falls below a certain critical level so that the low-affinity *Tdx* sites are saturated by the repressor. As a consequence, development would lead to a female phenotype or at least to gonadal development breakdown.

Following Chandra's model, what could be the consequences of such a sex-determination system in the first hybrid generation? Suppose that the two semispecies each produce a specific autosomal repressor and that the repressor of one has low affinity for the *Y*-linked sequence of the other. Hybrid males are repressor-heterozygotes and all the repressor available could not be efficiently titrated out by the *Y* chromosome and could bind to the *Tdx* gene, giving gonadal development breakdown in the F_1 . While the lack of *Y*-introgression in Northern Europe could be explained by a model of testis determination supported by the F_1 male sterility suspected by Sage *et al.* (1986) and Winking (pers. comm.), such an explanation seems to be inadequate for the results in Bulgaria where no significant F_1 hybrid breakdown has been detected. In fact the introgression patterns including that of the *Y*-chromosome are very similar in both the geographical areas, which leads us to think that the differences in the interactions between the two genomes in Denmark and in Bulgaria, if confirmed, cannot be due to the *Y*-chromosome.

If F_1 male sterility is not responsible for this lack of *Y*-introgression, what can be? Besides the part it plays in sex determination, the *Y*-chromosome is very peculiar as compared to the autosomes because it cannot undergo meiotic recombination. We suggest a model that is consistent with the results on both Bulgarian *Y*-introgression and our breeding experiments and in which the genetic differentiation between *M. m. domesticus* and *M. m. musculus* is such that the introduction of a foreign *Y* chromosome may perturb specific co-adapted gene combinations that have evolved in each semispecies. We can assume that

while the specific *Y*-chromosome-autosome combinations are intact in the hybrid genomes, which is the case in the F_1 generation and in the great majority of the F_2 males, there would be no hybrid breakdown in the gonadal development. The eventual breakdown resulting in loss of fitness would depend on the segregation of the foreign *Y* chromosome and its complementary set of autosomes during hybridization. The probability of such systems being disrupted increases in the following generations and the less favourable gene complexes would be progressively selected against. Note that in an actual hybrid zone which is 40 km wide, no F_1 nor F_2 are ever found but only highly recombinant genomes. One would expect that only those elements which are neutral and not too tightly linked to specific co-adapted systems would have a significant chance of introgressing. For autosomal genes this would mean that they must be rapidly dissected out from selectively unfavourable regions by meiotic recombination in a series of backcrosses. However as only a small region of the *Y*-chromosome can undergo meiotic recombination this chromosome has to be eliminated as a whole if it is selectively unfavourable. While our results support the hypothesis of disrupted co-adapted gene systems, we cannot exclude the possibility that other factors, such as mate choice or semispecies differences in male mediated migrations participate in limiting *Y*-chromosome introgression.

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