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Hst-3: an X-linked hybrid sterility gene

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Summary

A gene, *Hst-3*, responsible for sterility in F1 males from crosses between *Mus spretus* and laboratory strains of mice such as C57BL/6, has been localized on the distal part of the X chromosome, using both DNA probes and biochemical markers on a panel of F1(C57BL/6 × SEG) × C57BL/6 backcross males. This gene may be a model for studying mammalian hybrid sterility.

Introduction

Analysis of the genetic variation found among classical laboratory strains of mice indicate that they are all derived from ancestors of the *Mus musculus* species complex with a major component from the *Mus musculus domesticus* species (Bonhomme *et al.* 1987). In other words these strains represent a set of man-made interspecific recombinant inbred genotypes of polyphyletic origin which are more or less related to one another.

For the geneticists, this has both advantages and drawbacks. It is an advantage if one considers the high degree of genetic purity and the high level of standardization inherent in each inbred strain. It is a drawback for those who are looking for high levels of genetic divergence between any two strains. In this respect, as a consequence of intense genetic drift, the laboratory mouse is at great disadvantage compared to man.

In order to by-pass this drawback, mouse geneticists have developed strategies involving interspecific crosses between unrelated species of the genus *Mus*. The most popular crosses nowadays are those involving *Mus spretus* males and females of various laboratory strains, because considerable genetic polymorphism segregates as a consequence of the more than one million years of divergence existing between these species (Bonhomme *et al.* 1978). Whilst the F1 females from crosses of *Mus spretus* males with females of the laboratory inbred strains BALB/c, C57BL/6 or DBA/2 are fertile, the F1 males are invariably sterile as a result of the Haldane's effect (Haldane, 1922). In most instances this male hybrid sterility does not cause major hardship since it is always possible to use the F1 females as heterozygous progenitors in a backcross. It does however prevent the establishment of interspecific recombinant inbred strains and forbids the computation of recombination percentages in the male.

Here we report on additional data concerning the genetic localization of a hybrid sterility factor, which we designate *Hst-3*, on the X chromosome.

Animals and Methods

The *Mus spretus* progenitors which have been used in the present studies were all derived from wild animals trapped near Granada (Spain). They were first established as a laboratory strain in 1977 (Bonhomme *et al.* 1978) and have been propagated by inbreeding from that time onwards. The laboratory strains used were of the C57BL/6-Pas classical inbred strain. The F1s were obtained either by natural mating or by artificial insemination with the male parent being *Mus spretus* in all cases.

Although F1 females are able to produce a viable backcross progeny with both parental species, we have only studied the offspring bred from the C57BL/6 laboratory strain. In order to test their fertility, the male offspring of this backcross progeny were individually mated to several females of a general-

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purpose random-bred laboratory strain selected for its high breeding performances (OF1 from the IFFA/CREDO breeding laboratories, Saint Germain sur l'Arbresle – France). Vaginal plugs have not been checked on a regular basis and males classified as fertile were those producing at least one viable progeny over a mating period of two months. This procedure obviously introduces a certain bias and our estimation of the proportion of fertile males must therefore be considered as minimal. High molecular weight DNA samples were prepared from the spleen of a set of 75 (C57BL/6 × *Mus spretus*) F1 × C57BL/6 backcross males out of which 17 were classified as fertile. The DNA samples were individually digested for the detection of RFLPs with several restriction endonucleases and separated by electrophoresis through a 0·6 % agarose gel at 75 V for about 15 h. The gels were then acid depurinated and transferred to Hybond™ membrane by the technique of Southern (Southern, 1975). Fragments of the plasmid clones containing mouse genomic DNA probes were labelled with [^{32}P]dCTP and [^{32}P]dGTP using the random hexamer priming method (Feinberg & Vogelstein, 1983).

The hybridization and washing procedures were performed according to the method of Church & Gilbert (1984) with modifications adapted to the probe characteristics. The filters were exposed to X-ray film with intensifying screens at −70 °C for various periods. Several molecular probes known to localize to different chromosomes were analysed in order to detect possible linkage between the fertility phenotype and specific chormosomal region identified by the molecular probes.

Results

(i) *Number of segregating hybrid sterility genes*

Out of a total of 140 males tested for fertility only 23 (proportion ± standard deviation: 16·4 ± 3·1 %) produced viable progeny. Considering the probable underestimation of the proportion of fertile males mentioned above we conclude that such a proportion, in a backcross progeny, indicates that probably no more than two *Hst* genes are segregating in our cross. In a previous paper we have designated these genes *Hst-2* and *Hst-3* respectively in compliance with previously established rules for gene nomenclature (Forejt & Ivanyi, 1975; Forejt, 1985).

(ii) *Localisation of the hybrid sterility genes on mouse chromosomes*

Using more than 130 DNA probes or biochemical markers covering at least 85 per cent of the total genetic map we found independent segregation with the fertility phenotype in all instances except for two X-linked probes namely DXPas11 (see Fig. 1) and DXPas18. For these two probes, which are located on the distal half of chromosome X, we found absolute cosegregation of fertility with the C57BL/6 X chromosome segment. With another X linked probe (DXPas5) we found at least three recombinant genotypes thus indicating that the entire C57BL/6-X chromosome is not essential for fertility to occur.

In 1982 (Bonhomme *et al.* 1982) we reported on the localization of one of the sterility genes (*Hst-2*), segregating in a *Mus musculus* × *Mus spretus* cross, to mouse chromosome 9, close to the *Mod-1* locus (no recombinant on 13 fertile backcross tested). We have not however been able to confirm this result in the present study although we used six different probes covering the whole length of chromosome 9. This may be due to the fact that, in the 1982 experiment, the laboratory strain we used was BALB/c and not as in the present study C57BL/6. It may also result from a segregation distortion phenomenon, which is quite common in this sort of cross, which could have resulted in overproduction of mice homozygous for a

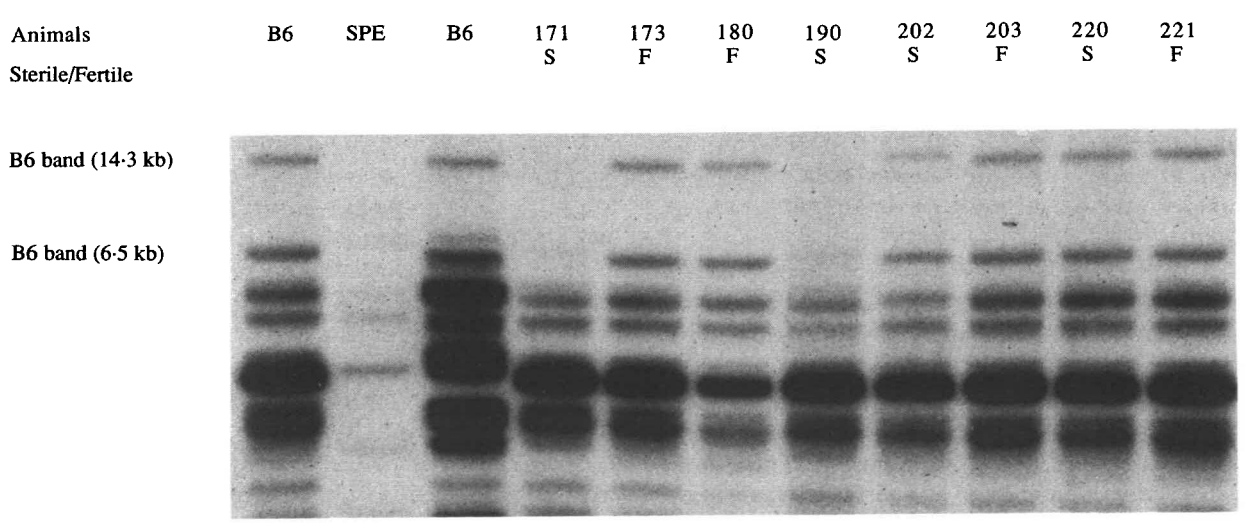

Fig. 1. Southern blot of TaqI-digested DNA from C57BL/6 (B6), *Mus spretus* (SPE) and 8 backcross males (171, 173, 180, 190, 202, 203, 220, 221) hybridized with DXPas11 probe. Two bands (14·3 kb and 6·5 kb) are specific for C57BL/6. Males were classified as being fertile (F) and sterile (S). All of the fertile males show the two bands derived from C57BL/6.

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given chromosomal segment in the sample of fertile backcross male thus introducing a bias in the genetic analysis. Although we checked concurrently a set of nine sterile males for the same chromosome 9 markers (*Mod-1* and *Mpi-1*) and did not find evidence of any segregation distortion this explanation is not unlikely as we have found several highly significant deviations from the classical 1 to 1 ratio for chromosome 11 in the present set (F1 × (C57BL/6 × *Mus spretus*) × C57BL/6) of backcross mice.

Since we have no way of clarifying this discrepancy as we no longer have samples left from the 1982 studies we consider that the location of *Hst-2* on chromosome 9 requires reassessment with another set of BALB/c mice.

Discussion

The production of interspecific hybrids, involving domestic animals, has been undertaken or at least attempted for centuries, mainly for zootechnical purposes, in order to combine, in the same individuals, various interesting or desirable traits. Mules and Hinnies are the best known examples of such interspecific hybrids but other examples have also been reported: domestic cattle (*Bos taurus*) × Bison (*Bison bison*) (the so-called Cattalos) and chicken (*Gallus domesticus*) × pheasant (*Phasianus colchicus*) for example (Basrur, 1969). In the case of mules both sexes are sterile as a rule and these F1 are of no interest to the agricultural geneticist. This is not the case, however, for the Cattle × Bison or Chicken × Pheasant hybrids and interesting genetic characteristics can, at least theoretically, be transmitted from one species to the other.

Except in the case of *Hst-1* in the mouse, which has been relatively well documented (Forejt & Ivanyi, 1975; Forejt, 1985), many of the problems associated with mammalian hybrid sterility have received only superficial considerations and have remained poorly understood because of the time and expense involved in conducting such studies on the species concerned. Since the mechanisms responsible for hybrid sterility are supposed to be basically the same in all higher vertebrates and possibly in other animals, the *Mus musculus* × *Mus spretus* model seems to be of interest. It is even conceivable to try to genetically isolate these factors in the form of congenic–sterile strains and eventually to isolate the genes involved.

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