An Introduction to Mammalian Interspecific Hybrids

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Haldane's law states that in interspecific hybrids, it is the heterogametic sex that is likely to be absent, rare, or sterile. In mammals, there is increasing evidence to suggest that this may be due to the high mutation rate of male sex-determining genes on the Y chromosome. The mule, humanity's first successful attempt at genetic engineering, provides some support for this concept. Interspecific hybrids may also shed new light on the importance of the maternal transmission of mitochondrial DNA and the phenomenon of genomic imprinting.

An interest in mammalian hybrids is as old as history; the mule is mentioned in Genesis, and whole civilizations from the Roman Empire to present-day China have depended on its hybrid vigor, its surefootedness, and its stubborn reliability as a beast of burden in peace and war (Short 1975). It represents humantity's first and most successful attempt at genetic engineering.

In more recent times, animal breeders and zoos have created, either by accident or design, a spectacular range of mammalian hybrids, from Ligers and Tigons to Zebrorses and Zebronkeys, Cattalos and Yakows, Shoats and Geep, and all these are listed in Annie Gray's masterful bibliography (Gray 1971). But in addition to their curiosity value, these hybrids are now providing some exciting new scientific challenges for today's molecular and developmental biologists.

The starting point for this resurgence of scientific interest in mammalian hybrids was Haldane's (1922) classic paper "Sex ratio and unisexual sterility in hybrid animals." In the opening paragraph, Haldane made the following bold statement: "I believe . . . that the following rule applies to all cases so far observed. . . . When in the F_1 offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous sex." In the concluding paragraph, Haldane went on to speculate that "If these facts are anything more than a coincidence they may be due to a greater difficulty of fusion of chromosome pairs in the heterozygous sex, and this in time may be a contributory cause of its sterility."

Geneticists have puzzled over the mechanisms responsible for Haldane's law, and a recent review (Davies and Pomiankowski 1995) concluded that it must be due to mutations on the X chromosome. The homozygous female would be protected by dominant alleles on the second chromosome, whereas in the heterozygous male, all X-linked genes would act as dominants in the homozygous state.

However, there is another even more important mechanism that may be at work, which involves the Y chromosome. The recent identification, mapping, and sequencing of the sex-determining gene on the mammalian Y chromosome, SRY, has shown that it shows great interspecific variability, especially in the C-terminal region that flanks the central 78 amino acid homeobox region, which is the DNA binding domain and the presumed functional region of the gene. Although the homeobox region itself is more conserved across species, it also shows considerable variability (Tucker and Lundrigan 1993; Whitfield et al. 1993). The molecular biologists were surprised by this high degree of variability in SRY between species, and were unable to suggest an explanation for it.

Perhaps the reason is that the testis is a much more important site for germ cell mutations than the ovary (Ketterling et al. 1993; Montandon et al. 1992). Most germline mutations are thought to be due to endogenous factors rather than to mutagens in the external environment (Sommer 1992), and oxygen-free radicals or tissue metabolites are probably more mutagenic than copying errors during DNA replication. Since the male germ cells are so

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metabolically active, it is not surprising that they are so mutation-prone; indeed, scrotal descent and the associated cooling of the testis may be a way of keeping the mutation rate in check by lowering the metabolic activity of the testis.

Since the Y chromosome, unlike the X, never escapes from the testis as far as the germ line is concerned, it is likely to be particularly mutation-prone. Furthermore, it is unable to repair any defects in the DNA of its sex-determining region by crossing over with the X chromosome, since there is no homologous paring in this region (Graves 1995). Indeed, the genes that have survived on the Y chromosome appear to be only life-or-death genes, solely concerned with the formation and gametogenic function of the testis. Perhaps any somatic genes that the Y might once have carried mutated into oblivion in the "hot" testicular environment if they were not involved in the transmission of life, thus accounting for the large amount of repeated DNA sequences in the Y chromosome, representing dead or dying genes.

In contrast to the Y chromosome, the X chromosome of mammals spends much of its evolutionary life "at rest" in the less mutagenic environment of the ovary. Unlike the Y, the X is also able to repair any defects in its DNA by crossing over with the homologous X during female meiosis. Thus, although X-linked genes might have a somewhat higher mutation rate than autosomal genes because of their haploid existence in the testis where recombination is not possible, it would not be as high as the mutation rate of Y-linked genes. Indeed, the X chromosome contains many somatic housekeeping genes, and Ohno's law states that if a gene is found to be X-linked in one mammal, it is likely to be X-linked in all. One X chromosome is essential for life, but the Y chromosome is superfluous; XO individuals can survive (Turner's syndrome), but OY is lethal.

So perhaps it is mutations on the Y chromosome, not the X, that are responsible for Haldane's law, at least in mammals. In the case of the mule (male donkey × female horse), it seems highly probable that the genes on the donkey's Y chromosome will have diverged significantly from those on the horse's Y chromosome. Donkey SRY may therefore be only partially successful in inducing testicular development in XY mules; the occasional failures may explain the female-skewed sex ratio of 44 male:56 female mules observed at birth (Craft 1938), which is significantly different from the normal horse sex ratio of 52.5 males:47.5 females. This could be verified experimentally by looking for the existence of XY female mules, which might be expected to be anestrous, with "streak" gonads because of the failure of XY germ cells to be transformed into oocytes.

Many XY mules obviously do form testes and develop as phenotypic males, although spermatogenesis is usually absent or abnormal. One reason for this, and for the corresponding lack of oocytes in the ovaries of female mules, is the gross dissimilarity in chromosome number and shape between the two parental species, making pairing of homologous chromosomes at meiosis extremely difficult (Chandley et al. 1974), just as Haldane predicted. But in addition, when spermatogenesis does occur in male mules, the spermatozoa are morphologically abnormal and aneuploid. This could be due to mutations in the donkey Y-linked spermatogenesis gene(s). Thus there are several reasons why there is not a single documented report of a fertile male mule, but several fertile females are known to exist (see Allen and Short, 1997). Y chromosome mutations would seem to be much more likely than postulated mutations of the X chromosome as a cause of this male sterility.

The mule and its reciprocal hybrid, the hinny (male horse × female donkey), pose a number of other challenging questions. Is there any consistent phenotypic or behavioral difference between these hybrids? If there were, it could be explained by the exclusively maternal inheritance of mitochondrial DNA or by genomic imprinting (Villar and Pedersen 1997), such as when the maternal genome is preferentially expressed in the embryo and the paternal genome in the placenta. In either case, we might expect mules to be more horselike and hinnies more donkeylike. Reliable information on this point is still lacking.

There could also be interesting behavioral differences between mules and hinnies that await discovery. Horses and donkeys obviously show major differences in their vocalizations, and in their estrous behavior. It would be fascinating to discover to what extent any differences in the behavior of mules and hinnies are genetically determined, and to what extent they are maternally imprinted after birth. Another puzzling and hitherto unexplained feature of equine hybrids is why matings to produce mules, where the jack donkey is the sire, should be so much more fertile than when the stallion is the sire. Since this is true regardless of the sex of the offspring, Haldane's law is not involved.

Enough has been said to illustrate some of the potential scientific interest of interspecific mammalian hybrids for today's geneticists, molecular and developmental biologists, and behaviorists, but hybrids also have much to tell us about the origin of species. Granted that geographical isolation is likely to have been the most important cause of species divergence, it is interesting to know how the species are kept apart if the geographical barriers are subsequently removed. Behavioral mechanisms are obviously important—it requires human ingenuity to persuade a jack donkey to mate with a mare—but behavioral barriers can also be broken down in other ways. We have the recent example of a female blue whale mating with a male fin whale to produce a female hybrid, which subsequently became pregnant from a male blue whale (Spilliaert et al. 1991). With the excessive harvesting of blue whales, perhaps a frustrated lone female blue, deprived of a male in the immediate vicinity, was forced to mate with a male fin. The resultant hybrid, reared by the blue whale mother, would presumably have behaved more like a blue whale than a fin, and hence it is not surprising that when it reached puberty it sought out a male blue whale for its mate, producing a hybrid fetus of unknown sex. We know that the baleen whales all have the same chromosome number, $2n = 44$, and the karyotypes are also very similar, although the species are thought to have diverged some 7–9 million years ago. Has selective culling of one species by whaling inadvertently forced the introgression of baleen whale species by breaking down the fragile behavioral barriers that normally keep the species apart, or will Haldane's law come to their rescue?

The study of mammalian hybrids has at last come of age, and in addition to its considerable intellectual appeal, surely with all our modern technology we could produce some new hybrids of economic importance that will rival our first successful attempt, the mule, created all those thousands of years ago? The most promising candidates appear to be the deer hybrids recently created in large numbers in New Zealand, and several articles in this symposium describe exciting new findings in this area. How Haldane would have reveled in all this!

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