$P < 0.01$). Parameter estimates obtained for the best model are $h = 0.64$, $c = 0.53$, $\mu = 0.86$, $p = 0.19$, $r = 0.84$ *and* $b = 0.20$, so that 41% of the variance in educational attainment is attributable to additive genetic factors (h^2), 28% to the environmental effects of family background (c^2), and 19% to genotype/environmental covariation ($2hac$), the remaining 13% of the variance being attributable to environmental effects not shared by relatives. Our estimate of the importance of genotype/environmental covariation depends critically on the assumptions made about the mechanisms of environmental transmission and mate selection, which cannot be tested with these data. If we were to make certain alternative assumptions, no genotype/environmental covariation would be predicted[5] and the contribution of the family enviroment would be estimated at 47%.

For twins born after 1939, we find significant heterogeneity of gene expression and environmental effects across sexes. Purely environmental models are rejected with a high level of significance (1940-49: $\chi^2_{50} = 89.44$, $P < 0.001$; 1950-60: $\chi^2_{53} = 169.55$, $P < 0.001$). Models which allow for both additive gene action and environmental transmission from parent to offspring yield negative estimates for the environmental transmission parameter p, which suggests that any environmental effects of parents on their offspring are being masked by genetic dominance in this design. Models which allow for sex-dependent additive gene action, dominance and environmental effects shared by twins, but no environmental transmission from parent to offspring, give the best fits to these data (1940-49: $\chi^2_{48} = 52.61$, d.f. = 48; $\chi^2_{51} = 56.59$, d.f. = 51). Parameter estimates obtained for this model are, for twins born between 1940 and 1949: $h = 0.70$, $h' = 0.67$, $d = 0.50$, $d' = 0$, $c = 0.29$, $c' = 0.64$, $\mu = 0.72$, $r = 0.82$, $b = 0.31$; and, for twins born between 1950 and 1960: $h = 0.52$, $h' = 0.53$, $d = 0.63$, $d' = 0.32$, $c = 0.45$, $c' = 0.71$, $\mu = 0.73$, $r = 0.82$, $b = 0.40$. These estimates imply that genetic factors (including genetic dominance) account for 74% of the variance in educational attainment in male twins born in 1940-49, and 67% in male twins born in 1950-60, but account for only 45% of the variance in female twins born in 1940-49, and 38% in female twins born after 1949. Conversely, environmental effects shared by twins account for only 8% (1940-49) or 20% (1950-60) of the variance in males, but for 41% (1940-49) or 50% (1950-60) in females. As genetic dominance is a major source of variation in IQ[1-3,22], the absence of evidence for dominance in the pre-1940 sample might be interpreted as evidence against a major influence of IQ on educational attainment in individuals born before 1940. However, dominance may be masked by strong environmental transmission in these data.

The results presented here are clearly consistent with the hypothesis that the importance of genetic influences on educational attainment is subject to secular change. Other explanations of our findings seem implausible. Perhaps more pre-war monozygotic male twin pairs have been misclassified as dizygotic, thus inflating the pre-war dizygotic male correlation? The proportion of pre-war male twins who are dizygotic (45.3%) differs little from the proportion of dizygotic twins in the other twin cohorts (44.4-48.8%). Perhaps a 'special twin environment' effect[23] is involved? As there has been a progressive decrease in the influence of parental educational levels on offspring educational attainment (see Table 1), we would expect the difference between male MZ and DZ twin correlations to have declined rather than increased in recent years. The most likely explanation, confirming the hypothesis of Scarr-Salapatek[8], is that increased educational opportunity has led to an increased dependence of educational attainment on innate ability.

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The genetic basis of Haldane's rule

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'Haldane's rule', formulated by J. B. S. Haldane in 1922, states that: "When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex" (ref. 1). His rule is now known to apply in mammals, lepidopterans, birds, orthopterans and dipterans[1-5]. In *Drosophila*, for example, Bock[6] cites 142 cases of interspecific hybridizations that produce one sterile and one fertile sex in the offspring, all but one of these crosses yielding sterile XY males and fertile XX females. Despite much speculation, however, the genetic basis of Haldane's rule remains unknown. Haldane himself rejected the simple explanation that males are innately more sensitive than females to the effects of hybridization because groups with heterogametic females (such as birds and butterflies) usually show female sterility in hybrids, so that heterogamety itself is the critical feature. He and others[1,2,4,7] suggested that heterogametic infertility or inviability in hybrids arises by a genetic imbalance between X chromosomes and autosomes. An alternative explanation[5,8,9] is that this syndrome is caused by a mismatch of X and Y chromosomes. Here I show that in the *Drosophila melanogaster* subgroup, Haldane's rule for fertility apparently arises from a genetic interaction between X and Y chromosomes and not from an imbalance between sex chromosomes and autosomes. This finding has important implications for understanding the evolution of interspecific reproductive isolation.

Haldane's original explanation for asymmetrical sterility or viability rests on the fact that homogametic hybrids have a complete haploid set of chromosomes from each parent, but heterogametic hybrids lack an X chromosome from one of the parental species. The latter hybrids may lack the gene products from both autosomes and sex chromosomes of a complete haploid set which are thought to be necessary for hybrid fertility or viability. The genetic implications of this model are that epistatic interactions between conspecific X chromosomes and autosomes are necessary for proper gene action[7,10]. Alternatively, different species may differ in their linkage relationships; some loci present on the X chromosome of one might be carried on the autosomes of the other. Homogametic hybrids would then have the normal dose of each locus, while heterogametic hybrids would suffer from duplication and deficiencies[11].

One way to test this X-autosome imbalance theory is to produce interspecific hybrid females having both X chromosomes from a single parental species. Because such females will have the same degree of X-autosome mismatch as that of sterile males, they would be expected to be sterile. Only in three species of *Drosophila* (*D. simulans*, *D. mauritiana* and *D. sechellia*) is it possible to carry out such a test, as the three species will hybridize to produce fertile females and sterile males[12-15] and there exists in *D. simulans* a stock with attached X chromosomes. *D. simulans* is cosmopolitan, and *D. mauritiana* and *D. sechellia*

are found on the island of Mauritius and on Cousin and Praslin (Seychelles), respectively. The two island species probably arose by independent colonization of the islands by a *simulans*-like ancestor[13,14].

I used the attached-X *D. simulans* stock to test the fertility of interspecific female hybrids having both of their X chromosomes from *D. simulans* but two species-specific sets of autosomes, one from *D. simulans* and one from another species. The sector of Fig. 1 enclosed by dotted lines shows the cross producing such hybrids. Virgin hybrid females and control intraspecific females were tested for fertility by mass mating them for 72 h to males from both parental species, and then placing individual females in vials containing males from both parental species. Vials were inspected for female survival after 48 h, and for larvae and pupae after 1 week.

Table 1 shows that female hybrids with two *D. simulans* X chromosomes suffer no loss of fertility compared with the controls, as the control cross shows the highest proportion of sterile females (0.044) and the three interspecific crosses are not heterogeneous for fertility ($G_2 = 5.32$, $P > 0.05$). The control cross also has the highest proportion of attached-X females not surviving the 48-h egg-laying period. Female hybrids therefore suffer no decrease in fertility when both of their X chromosomes come from a single species, and the X-autosome imbalance theory cannot be a correct explanation of hybrid sterility in this group.

Table 1 Fertility of attached-X females in interspecific and control crosses

Cross	Fertile	Non-fertile	Dead	Total
Control (*D. simulans*)	171	8	34	213
Interspecific hybrids				
simulans × *sechellia*	193	5	5	203
simulans × *mauritiana* (Bowling Green)	137	0	21	158
simulans × *mauritiana* (mixed stock)	124	2	19	145

For a description of the crosses, see text and Fig. 1 (cross enclosed by dotted lines).

An alternative (and largely ignored) explanation for Haldane's rule is that fertility is only possible when both X and Y chromosomes come from the same species[5,8,9]. In *Drosophila*, nearly all loci on the Y chromosome affect spermatogenesis, and genetic interactions affecting spermatogenesis have been found between homologous loci on the X and Y chromosomes[16,17]. This suggests that male fertility in *Drosophila* may depend strongly on interactions between the X and Y chromosomes. I investigated the validity of the X/Y interaction hypothesis by constructing two genotypes differing (as far as possible) only in the species identity of the Y chromosome. If 'deleterious' interactions between X and Y chromosomes from different species are the cause of Haldane's rule, then males with heterospecific sex chromosomes should be sterile.

Figure 1 shows the two crosses yielding males with different species combinations of X and Y chromosomes. Cross *a* produces males with a complete *D. simulans* X chromosome, a *D. mauritiana* Y chromosome, hybrid cytoplasm and an average of three-quarters of the autosomes from *D. simulans*. Cross *b* produces males with hybrid cytoplasm and with autosomes identical to those of cross *a*, but with a Y chromosome from *D. simulans* and a mixture of X chromosomal material from both species. A complicating variable in this comparison is that male *mauritiana*/*simulans* hybrids bearing the base of the *D. mauritiana* X chromosome in cross *b* produce no motile sperm[18]. The reason for this sterility is not known, but segregation of this region in this backcross leads to the expectation of sterility in at least half the male progeny. If X/Y chromosomal mismatch has no effect on sterility, males from cross *b* should have at most half the fertility of males from cross *a*. However, if X/Y mismatch makes a large contribution to sterility, male fertility in cross *a* should be lessened, and might perhaps be lower than that from cross *b*. Male fertility was measured as the proportion of 4-day-old virgin males with motile sperm[18].

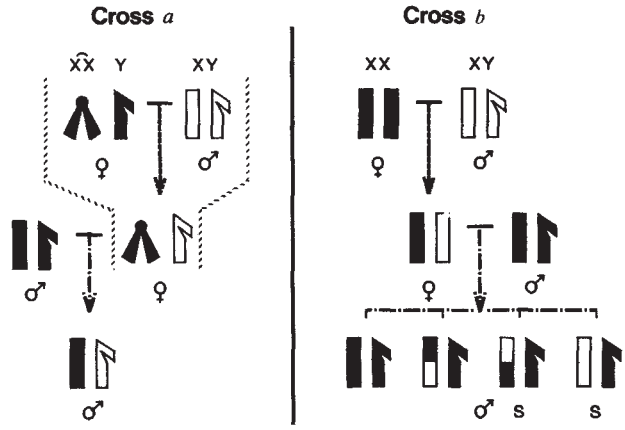

Fig. 1 Test of autosome–sex chromosome 'matching' hypothesis (cross enclosed by dotted lines): Attached-X *D. simulans* females [C(1) *y w*, Y], with sex chromosomes shown in black, are crossed to *D. mauritiana* or *D. sechellia* males, with sex chromosomes shown in white. Two strains of *D. mauritiana* were used, one from the Bowling Green Stock Center and the other a 'mixed' line of six isofemale strains provided by J. David. The resulting female interspecific hybrids have a haploid complement of autosomes from each species but both X chromosomes from *D. simulans*. They also contain a Y chromosome from their father, but this chromosome has no detectable phenotypic effects in such females and in particular does not affect fertility[19,20]. Thus, this cross is not a test of the X/Y interaction theory. A control cross to assess the degree of intraspecific sterility was made between the attached-X *D. simulans* females and males from a conspecific strain collected in Oxnard, California. Test of the X-Y 'interaction' hypothesis (complete figure): Two genotypes were produced by backcrossing *D. simulans*/*D. mauritiana* hybrid females to *D. simulans* males from the Oxnard strain. One genotype (cross *a*) used as the original *D. simulans* female parent the attached-X stock of *D simulans* (sex chromosomes in black); this was crossed to *D. mauritiana* males (sex chromosomes in white). The resulting F_1 hybrid females, which have free recombination among the autosomes[21] and possess both X chromosomes from *D. simulans* and a Y chromosome from *D. mauritiana*, were backcrossed to *D. simulans* males. The resulting male offspring (shown at bottom left) have hybrid cytoplasm, a *D. simulans* X chromosome, a *D. mauritiana* Y chromosome and an average of three-quarters of the autosomes from *D. simulans*. The second cross (cross *b*) used as the original *D. simulans* female parent the wild-type Oxnard strain (sex chromosomes in black); these were crossed to *D. mauritiana* males (sex chromosomes in white). The F_1 hybrid females, which show free recombination, were backcrossed to *D. simulans* males, giving male offspring shown at bottom right. These males have hybrid cytoplasm, half of their X chromosomal material from *D. simulans*, half from *D. mauritiana* and a Y chromosome from *D. simulans* (various mixtures of X chromosomes are shown in the figure). The autosomes are identical in constitution to males from cross *a*. Genotypes with the base of the *D. mauritiana* X chromosome (shown by 'S') are known to have no motile sperm[18].

The results of these crosses are unambiguous. Males from cross *a* (with completely discordant X and Y chromosomes) had motile sperm in 5 of 615 individuals tested. The cross *b* males, however, who had a mixture of *D. simulans* and *D. mauritiana* X-chromosomal material and a *D. simulans* Y chromosome, showed motile sperm in 65 of 682 individuals tested, a value close to that obtained by other workers[12] for fertility in this backcross, and reflecting the segregation of at least five loci affecting sperm motility[18]. The difference in fertility between the crosses is highly significant ($G_1 = 57.7$, $P < 0.001$) and is in the direction predicted by the X/Y interaction theory. Given that at least half of the males from cross *b* are known from the outset to lack motile sperm, the substitution of a Y chromosome from *D. mauritiana* for one from *D. simulans* must reduce by at least 96% the incidence of individuals with motile sperm.

These two experiments, then, militate against the X-autosome imbalance theory for Haldane's rule and support the hypothesis that interactions between X and Y chromosomes from different species are largely responsible for heterogametic sterility in species hybrids. An example of such interactions may be the recent demonstration that the *Stellate* locus, which affects spermatogenesis in *D. melanogaster*, is found in multiple copies on both the X and Y chromosomes, and that Y-linked copies may regulate the transcription of those on the X. The sibling species *D. simulans*, however, lacks the *Stellate* gene on its X chromosome and has a reduced number of copies on the Y[17].

Other possible explanations for hybrid male sterility are not supported by the crossing relationships of these species. For example, theories of deleterious interspecific interactions between the cytoplasm and X or Y chromosomes are not supported by the sterility of males from both reciprocal crosses between *D. mauritiana* and *D. simulans*[12]. A theory of Y-autosome imbalance is not supported by the fact that genotypes unbalanced in this way can have motile sperm in up to 20% of the individuals, while no individual with heterospecific X and Y chromosomes in the same cross has motile sperm[18].

If X/Y interactions as a cause of heterogametic sterility prove to be widespread, theories of speciation must explain why X and Y chromosomes diverge genetically to produce reproductive barriers between geographically isolated populations. Sterility of the heterogametic sex often precedes all other reproductive and developmental disharmonies in hybrids, and as a first symptom of speciation deserves more attention from evolutionary biologists.

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Genetic mapping and diagnosis of haemophilia A achieved through a *Bcl*I polymorphism in the factor VIII gene

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Haemophilia A is the most common inherited bleeding disorder in man, affecting approximately 1 male in 10,000 (ref. 1). The disease is caused by a deficiency in the gene for factor VIII, a component of the intrinsic coagulation pathway. Due to the broad range of clotting activity in normal and heterozygous females, it is often difficult to confirm the status of women at risk for carrying the disease[2]. A genetic marker in the form of a restriction fragment length polymorphism (RFLP) within or tightly linked to the factor VIII gene would serve as a tag for the haemophilia gene, thus allowing both accurate carrier detection and improved, earlier prenatal diagnosis by chorionic villi sampling[3,4]. The recent isolation of the factor VIII gene[5,6] has allowed a search for RFLPs within the gene, and we report here the identification of a common polymorphism within the factor VIII gene, revealed by the restriction enzyme *Bcl*I, which can be used diagnostically in about 42% of all families. Although the disease haemophilia A has been mapped to the distal portion of Xq (ref. 7), the *Bcl*I RFLP makes possible higher-resolution genetic linkage mapping with respect to other polymorphic markers on this portion of the X chromosome. We have established close linkage of the factor VIII gene to several useful RFLP markers, including the highly informative marker St14 (ref. 8). These markers should also be useful for prenatal diagnosis of haemophilia A and for detection of its carriers.

Table 1 Summary of the search for polymorphisms in the factor VIII gene

Enzyme	No. of bands	No. of chromosomes	Enzyme	No. of bands	No. of chromosomes
*Aha*III	8	12	*Msp*I	7	22
*Apa*I	8	12	*Nci*I	5	6
*Ava*II	14	6	*Nco*I	9	9
*Bam*HI	8	34	*Nsi*I	14	11
*Ban*II	7	12	*Pst*I	13	12
*Bcl*I	12	12	*Pvu*II	11	12
*Bgl*I	6	10	*Rsa*I	14	12
*Bgl*II	11	36	*Sau*3AI	7	16
*Bst*EII	6	12	*Sau*96I	13	10
*Bst*XI	12	12	*Sca*I	13	10
*Eco*RI	15	45	*Scr*FI	10	10
*Eco*RII	15	6	*Sph*I	10	12
*Eco*RV	8	12	*Sst*I	10	20
*Hae*III	7	6	*Stu*I	9	12
*Hin*cII	9	12	*Taq*I	11	12
*Hin*dIII	9	18	*Tth*111I	3	12
*Hin*fI	3	6	*Xba*I	5	12
*Hpa*I	9	12	*Xmn*I	10	10
*Kpn*I	7	10			

Southern blots of human DNA (5 μg per lane) digested with each of the enzymes listed were hybridized to a full-length (9.0-kb) probe[5] which included the complete factor VIII coding sequences and 3′-untranslated region. Male DNA, having one X chromosome, was generally used to eliminate ambiguity in the analysis of the band patterns. DNA samples were derived mainly from the blood of haemophiliacs at the Royal Free Hospital, London, although some samples were contributed by Utah families and employees of Genentech. The table lists the number of hybridizing bands and the number of independent chromosomes screened for each enzyme. Of these enzymes tested, only *Bcl*I revealed a factor VIII RFLP.

DNA samples from numerous individuals were examined for the presence of polymorphisms in the factor VIII gene. For each of 37 restriction enzymes, DNA samples were analysed by Southern blot[9] hybridization to a full-length factor VIII DNA probe[5]. Table 1 gives, for each enzyme, the number of hybridizing bands and the number of chromosomes tested. For the enzymes with which the factor VIII gene has been completely mapped[6] (*Bam*HI, *Eco*RI, *Kpn*I, *Sst*I, *Taq*I), almost every exon