Studies on the inheritance of vaginal septa in mice, a trait with low penetrance

J. G. M. Shire

Department of Biology, University of Essex, Colchester CO4 3SQ, U.K.

Summary. Longitudinal vaginal septa were found in 1–3% of females of the BALB/cBy and C57BL/6By inbred strains and their F1, F2 and backcross hybrids. In the CXBJ recombinant-inbred strain over half the females were affected, showing that the parental strains differed at a minimum of 2 loci that increased the risk of septa. Crosses involving CXBJ mice showed that susceptibility could be transmitted by the male parent and suggested that the different genotypes producing low penetrance could be identified. Subline differences in C57BL were marked, the incidence ranging from 1% in C57BL/6By to about 26% in two lines congenic with C57BL/10ScSn. No association was found between incidence and H-2 histocompatibility type. Imperforate vagina occurred at a low frequency in 4 stocks in which septa were regularly found. The minor anomaly was about 15 times more likely than the malformation.

Introduction

Whilst major malformations of the reproductive tract often have a clear genetic basis in man and other mammals, the patterns of inheritance of less drastic anomalies are often obscured by incomplete penetrance or by variability in expression. An additional problem can arise when the same morphological anomaly can occur both as part of a complex syndrome of malformation and in isolation. Occurrences of the minor anomaly in natural populations could represent either minimal expression of the major anomaly or the occurrence of a different developmental defect, whose consequences are restricted to the minor structure. Distinctions between these two possibilities can be made in genetically controlled populations and in some pedigreed populations.

A longitudinal septum in the vagina has been reported in some women with partial or complete duplication or atresia of the reproductive tract (Davis & Fell, 1950; Dougherty & Spencer, 1972; Perrin, 1978). Such malformations are often associated with massive abnormalities of other organ systems. A vaginal septum can be distinguished from a duplication of the vagina by the absence of muscle from the central structure. In some instances a partial vaginal septum represents a less severe and incomplete form of duplication of the vagina, as in the hand-foot-uterus syndrome (Stern et al., 1970) and in 24 of the 71 cases of partial or complete duplication of the reproductive tract described by Dougherty & Spencer (1972).

Longitudinal vaginal septa, without uterovaginal duplication, occur in about 40% of people affected by the dominant mutation camptobrachydactyly (Edwards & Gale, 1972; Pinsky, 1974), whose most obvious effects are upon the hands and feet. Similarly, mice homozygous for Dominant hemimelia (Dh) had skeletal defects and uterovaginal deficiencies, whilst in mice homozygous for postaxial hemimelia (px) skeletal defects were accompanied by a longitudinal vaginal septum in a significant number of females (Searle, 1964). Longitudinal vaginal septa have been frequently associated with vaginal and anorectal restriction in Jersey cattle, especially in inbred herds.
(Leipold & Saperstein, 1975). Segmental aplasia of the female reproductive tract is occasionally accompanied by longitudinal vaginal septa or by narrow transvaginal strings in Holstein and Shorthorn cattle (Fincher & Williams, 1926; Hanset, 1959, 1960; Ginther, 1965; Settergren & Galloway, 1965). Doubling of the cervix has been found in inbred herds of cattle (Spriggs, 1945; Settergren & Galloway, 1965) with individual bulls implicated in two herds. Imperforate anus is quite often accompanied by vaginal anomalies in children (Perrin, 1978). McKusick, Bauer, Koop & Soctt (1964) described two Amish families in which duplication of the vagina, associated with a transverse septum and imperforate anus, appeared to be recessively inherited. Imperforate vagina was found, at frequencies of 6·6% and 3·7% in two inbred strains of mice, Aka (Chase, 1954) and ‘silver’ (Marx, 1936; Gowen & Heidenthal, 1942), and in almost a third of females heterozygous for the Looptail mutation (Lp: Strong & Hollander, 1949; Grüneberg, 1952).

Longitudinal vaginal septa, unaccompanied by any other structural abnormalities, have been reported in mice (Cunliffe-Beamer & Feldman, 1976), cattle and buffalo (Sittman, Rollins & Kendrick, 1961), horses (Finocchio, Hales & Wolfe, 1968), pigs (Teige, 1957) and people (8 cases: Dougherty & Spencer, 1972). The frequency of affected females was as high as 1·4% for Danish pigs and 38% for the BALB/cJ inbred strain of mice.

The incidence of longitudinal vaginal septa ranged from effectively zero to 38% in different inbred strains of mice (Cunliffe-Beamer & Feldman, 1976), suggesting the existence of genetic variation in predisposition to this anomaly. The high frequency of affected females in the BALB/cJ strain was unaffected by selective breeding from affected or unaffected individuals, implying that the observed incidence reflected a consistent probability that an individual mouse with a susceptible, homozygous, genotype would be overtly affected. The likelihood of genetic differences underlying the observed differences in penetrance was increased by the observation of consistent differences in incidence between sublines of the same strain, 0% in BALB/cWt, 1·3% in BALB/cBy and 38% in BALB/cJ (Cunliffe-Beamer & Feldman, 1976). Such differentiation between sublines often reflects variation at a single locus rather than the involvement of large numbers of loci (Bailey, 1978; Ciarnello, Hoffman, Shire & Axelrod, 1974; Ciarnello, Lipsky & Axelrod, 1974).

This paper describes the use of hybrid crosses, recombinant-inbred lines and congenic strains to analyse the inheritance of the predisposition to longitudinal vaginal septa in mice and to investigate the relation between the occurrence of septate and imperforate vagina.

Materials and Methods

All mice were raised under standard environmental conditions (Crichton & Shire, 1982). The inbred strains were BALB/cBy, C57BL/6By and C57BL/10ScSn, and the 7 recombinant inbred lines were CXBD/By, CXBE/By, CXBG/By, CXBH/By, CXBI/By, CXBJ/By and CXBK/By (abbreviated to D, E, G, H, I, J and K) derived from the F2 of BALB/cBy × C57BL/6By (Bailey, 1971). Reciprocal F1, F2 and backcross to BALB/cBy hybrid mice were bred. Three inbred stocks of mice (B10.BR, B10.c and B10.p; see Crichton & Shire (1982) for details) congenic with C57BL/10ScSn (B.10) were also studied, as were mice from the second cycle of backcrosses of C57BL/Tb to C57BL/10ScSn. This latter stock was segregating for a mutation, rp, that affects melanosome and lysosome function (Gibb, Håkansson, Lundin & Shire, 1981).

All observations were made on unmated adult females aged between 8 and 18 weeks. Most observations were made on mice between 8 and 12 weeks of age. All cases of imperforate vagina were confirmed by dissection.

Results

Vaginal septa occurred at a low frequency in mice of the BALB/cBy and C57BL/6By strains and sporadically amongst their hybrid offspring (Table 1). No significant differences in incidence were
found amongst the stocks listed in Table 1. One F2 mouse had an imperforate vagina. The 7 recombinant inbred lines derived from crossing C57BL/6By and BALB/cBy were very heterogeneous ($\chi^2(6) = 217, P < 0.001$; Table 2). Five of the lines had incidences as low as, or lower than, those of the parental strains. Over half the CXBJ mice examined had vaginal septa and 3% of them had an imperforate vagina. At 4-6% the incidence of abnormalities in CXBK mice was significantly lower ($\chi^2(1) = 36.9, P < 0.001$) than that in CXBJ mice but not significantly different from that in either of the parental strains.

Crosses were set up between the CXBJ line, with a high incidence, and two lines, CXBG and CXB1, with low incidences. Vaginal septa occurred in 11 out of 83 (13.3%) of the G × J females, in 1 out of 38 (2.6%) of the I × J females and in 0 of 33 G × I females. The incidence in the G × J females was significantly higher than in the other intercrosses or in the CXBG line itself (probabilities of 0.02, 0.05 and 0.002, Fisher exact test) and significantly lower than in CXBJ mice ($\chi^2(1) = 18.2, P < 0.01$).

Significant differences were found between sublines of C57BL in the incidence of vaginal septa ($\chi^2(5) = 62, P < 0.001$; Table 3). Abnormalities occurred at low frequencies in C57BL/6By and in the stock in which the $r_p$ mutation was being transferred from C57BL/Tb to C57BL/10ScSn. Vaginal septa were found in 12–26% of the 4 stocks of mice whose genome was entirely, or almost entirely, derived from C57BL/10ScSn. There was some residual heterogeneity amongst these 4 stocks in the incidence of abnormalities ($\chi^2(3) = 12.5, P < 0.01$), implying minor differences between them. The vagina was imperforate in about 2% of the B10.c and B10.p stocks.

Vaginal septa were occasionally found when 6-week-old mice used in a study of early cleavage (Shire & Whitten, 1980) were scored for the presence of vaginal plugs. The incidences of vaginal septa in these mice, which were bred at the Jackson Laboratory at Bar Harbor in Maine, were 6/90 (7%) in C57BL/6By, 3/60 (5%) in BALB/cBy, 0/30 (0%) in their F1 and 29/53 (55%) in CXBJ/By.

**Table 1.** The incidence of vaginal abnormalities in two mouse strains and their hybrids

<table>
<thead>
<tr>
<th>Stock</th>
<th>Age (weeks)</th>
<th>Septate</th>
<th>Imperforate</th>
<th>Normal</th>
<th>% Septate</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/cBy</td>
<td>8–15</td>
<td>3</td>
<td>0</td>
<td>91</td>
<td>3.2</td>
</tr>
<tr>
<td>C57BL/6By</td>
<td>8–15</td>
<td>1</td>
<td>0</td>
<td>87</td>
<td>1.1</td>
</tr>
<tr>
<td>C57 × BALB F1</td>
<td>8–16</td>
<td>0</td>
<td>0</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>BALB × C57 F1</td>
<td>8–14</td>
<td>1</td>
<td>0</td>
<td>81</td>
<td>1.2</td>
</tr>
<tr>
<td>Backcross to BALB</td>
<td>10–13</td>
<td>4</td>
<td>0</td>
<td>168</td>
<td>2.3</td>
</tr>
<tr>
<td>F2</td>
<td>8–12</td>
<td>4</td>
<td>1</td>
<td>170</td>
<td>2.3</td>
</tr>
</tbody>
</table>

**Table 2.** The incidence of vaginal abnormalities in recombinant-inbred strains of mice (8–13 weeks of age)

<table>
<thead>
<tr>
<th>Strain</th>
<th>H-2 type</th>
<th>Septate</th>
<th>Imperforate</th>
<th>Normal</th>
<th>% Septate</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>d</td>
<td>0</td>
<td>0</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>b</td>
<td>0</td>
<td>0</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>b</td>
<td>0</td>
<td>0</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>d</td>
<td>1</td>
<td>0</td>
<td>74</td>
<td>1.3</td>
</tr>
<tr>
<td>I</td>
<td>b</td>
<td>0</td>
<td>0</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>b</td>
<td>32</td>
<td>2</td>
<td>28</td>
<td>51.6</td>
</tr>
<tr>
<td>K</td>
<td>b</td>
<td>3</td>
<td>0</td>
<td>63</td>
<td>4.6</td>
</tr>
</tbody>
</table>
Table 3. The incidence of vaginal abnormalities in C57BL sublines and congenic strains of mice

<table>
<thead>
<tr>
<th>Stock</th>
<th>Age (weeks)</th>
<th>Vagina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Septate</td>
</tr>
<tr>
<td>C57BL/6By</td>
<td>8–15</td>
<td>1</td>
</tr>
<tr>
<td>B10 × C57BL/Tb</td>
<td>8–13</td>
<td>1</td>
</tr>
<tr>
<td>B10</td>
<td>8–14</td>
<td>16</td>
</tr>
<tr>
<td>B10.BR</td>
<td>8–18</td>
<td>16</td>
</tr>
<tr>
<td>B10.p</td>
<td>8–16</td>
<td>23</td>
</tr>
<tr>
<td>B10.c</td>
<td>8–13</td>
<td>21</td>
</tr>
</tbody>
</table>

Discussion

The similar, high, incidence of septate CXBJ mice in two countries implies that the susceptibility of these mice has a strong inherited component. The CXBJ inbred strain, in which half the females have vaginal septa, was derived by crossing two inbred strains in which this anomaly occurs only rarely. The appearance of new phenotypes when inbred strains of apparently identical phenotype are intercrossed is a result of the meiotic recombination of the different, but balanced, sets of genes present in each of the progenitor strains. Such crosses have identified hidden, or epistatic, genetic variation underlying morphological (Spickett & Thoday, 1966), endocrinological and behavioural characters (Shire & Spickett, 1967; Doering, Shire, Kessler & Clayton, 1973; Peets & Pomeranz, 1978; Champlin, Beamer, Carter, Shire & Whitten, 1980). The occurrence of one such genetic rearrangement in a sample of only 7 recombinant-inbred strains suggests that only a few loci are involved whilst the low incidence in the F1 hybrids rules out a one-locus model. Variation at two unlinked interacting loci would be sufficient and would be expected to result in one quarter of the recombinant lines having the susceptible aa bb phenotype. The genotypes of the unaffected parental strains would be complementary, AA bb and aa BB. The F1 mice would be doubly heterozygous, Aa Bb. On this hypothesis 1 in 16 of the F2 would have the aa bb genotype and, with a penetrance of 50% (as found in CXBJ), one in 32 of the females would express the anomalous phenotype. Although 1 in 35 of the C57BL × BALB F2 was found to have vaginal septa, in agreement with prediction, this frequency was not significantly different from that found in either of the parental strains. Definite ascertainment of the proportion of the F2 that were genetically susceptible for a character that was not fully penetrant would require extensive progeny testing of many mice. However, recombinant-inbred lines, because of their homozygosity, can be crossed to produce many, genetically identical, individuals of each of a number of hybrid genotypes. This allows the phenotype of each of these genotypes to be defined, even for characters with low penetrance. The occurrence of significant numbers of septate mice in the G × J Fl showed that susceptibility is not maternally determined. The different incidences in the G × J and I × J F1 hybrids showed that CXBG and CXBl differ at one or more of the loci involved. These two lines are known to differ at 30 loci, with CXBG having the BALB allele at 21 of them (Green, 1981). The loci that differ do not include the H-2 histocompatibility locus or the Tfa and Sco loci that are linked to it.

The C57BL sublines had incidences ranging from < 1 to 27%, extending the observations on the subline variation found in BALB/c (Cunliffe-Beamer & Feldman, 1976). All the C57BL sublines, except B10.BR, carry the H-2b haplotype. Septate mice occur in all 4 sublines closely related to C57BL/10ScSn, but at differing frequencies. Bonner (1981) found the frequency of septate mice to vary from 4 to 22% in lines congenic with C57BL/10ScSn. B10 lines with the H-2d and H-2k haplotypes had 15–16% of septate females whilst only 4% of the B10.A females, homozygous for a k-d recombinant haplotype, were septate. These data were considered to demonstrate the existence of...
susceptibility loci on each side of the H-2 region. A more reasonable interpretation of the congenic data, taken together with the variation in frequency within haplotypes in both C57BL and BALB/c and with the evidence against H-2 association in the recombinant-inbreds, is that the B.10A strain differs from the other B.10 lines at a locus elsewhere as well as the H-2 locus. Such additional differences between congenic strains are well documented (Gregorova, Ivanyi, Simonova & Mickova, 1977; Festing, 1979; Falconer, 1981).

Mice of the CXBH and CXBJ strains have identical sets of pigmentation genes but differ markedly in the frequency of vaginal anomalies. Three of the C57BL stocks were segregating for coat-colour mutations (c, p and rp). No differences in incidence were found between albino, pink-eyed or reduced pigmentation homozygotes and their normal coloured litter mates. Thus the situation in mice is unlike that in cattle in which the association between abnormality and coat-colour is strong enough for one syndrome to be called ‘white heifer disease’. Uterovaginal defects were found in 12–39% of homozygous white cattle, in 3–4% of heterozygous roan cattle and in <1% of red cattle in studies in the U.K. (Rendel, 1952) and Belgium (Hanset, 1960). Similarly the extent of white was correlated with the degree of utero-ovarian hypoplasia and the incidence of double cervices in Swedish white cattle (Settergren & Galloway, 1965). It is not clear from the studies on cattle whether the association with coat-colour variants is due to pleiotropic effects of the variant or to a closely linked susceptibility locus. Differences in incidence between provinces suggested the existence of modifying genes or differences in the linkage disequilibrium with the coat-colour locus (Hanset, 1960). In both Belgian lowland Red herds and in two closely related herds of American Herefords, individual bulls were found to produce offspring with high frequencies of double cervices and, in the Herefords, associated abnormalities of the Müllerian ducts (Hanset, 1959; Sittman et al., 1961).

The penetrance is lower in heterozygous cattle than in white homozygotes. The G × J hybrid data suggest the same for one of the loci involved in the C57 × BALB crosses, but only when the other locus is homozygous for the permissive state. F1 hybrids between high and low frequency sublines of BALB also had intermediate frequencies while hybrids between a high BALB subline and a low C57BL subline had a low frequency of septate mice (Cunliffe-Beamer & Feldman, 1976). Crosses between two low sublines or between two low strains always produced very low incidences in the F1 hybrids: 1/79 (this study), 0/150 (Shire & Whitten, 1980) and 0/627 (Cunliffe-Beamer & Feldman, 1976).

Mice with an imperforate vagina were found in 4 stocks. In the 3 strains with the highest frequency of septa an imperforate vagina was found in 6–8% of the abnormal females. One of the 5 abnormal F2 animals had an imperforate vagina. These figures suggest that, at least in stocks of uniform genotype, the risk of the severe abnormality bears a relatively constant proportion to the chance of the minor anomaly occurring. Overall the risk of imperforate vagina ranged from 0-6% in the F2 to 3-2% in CXBJ. The only imperforate animal found during the investigation described by Shire & Whitten (1980) was a CXBJ mouse. An imperforate vagina was found in 3-6% of ‘silver’ mice and in 6% of Aka mice (Grüneberg, 1952), suggesting that as many as 85% of the females in these stocks may have had septa.

Despite the high frequency of septa the CXBJ strain breeds well. At least 9 out of 16 septate CXBJ females mated and ovulated on the 3rd night after pairing with a male (Shire & Whitten, 1980). Cunliffe-Beamer & Feldman (1976) found 10% fewer fertile mice amongst septate BALB/cJ females than amongst their normal littersmates but no significant difference in the average size or number of litters per female. CXBJ females do, however, differ from both their progenitor strains and from the other recombinant-inbred strains in that their eggs, when fertilized in vitro and cultured in vitro, began cleavage later than those of the other stocks. CXBJ eggs took 26-8 h before half of them had divided whereas BALB/cBy eggs took 24-8 h and C57BL/6By eggs only 22-1 h (Shire & Whitten, 1980). The observed anomalies of the reproductive tract, or other alterations associated with them, may predispose eggs to late fertilization and thus to late cleavage.

Analysis of crosses between CXBJ and the other strains will enable the genes responsible for the
differences in susceptibility to be identified and mapped. Crosses with B.10 strains will show whether the loci involved in their susceptibility are the same as those in CXBJ and should reveal whether the H-2 region of chromosome 17 contributes to variation in susceptibility. The occurrence of a related abnormality and anomaly in defined genotypes of a laboratory species will enable embryological and microanatomical studies to be made that should elucidate the ontogeny of the conditions. The development of a suitable threshold model (Falconer, 1981), combined with the developmental studies, should lead to a better understanding of why some, but not all, individuals with the same genotype are affected. Such studies will help the investigation of susceptibility to congenital defects and anomalies in domestic animals and in the human species.

References


Received 7 July 1983