An Analysis Procedure Illustrated on a Triple Linkage of Use for Prenatal Diagnosis of Myotonic Dystrophy

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Diagnosis of a number of simple genetic conditions in man is now feasible prenatally (Nadler, 1971 and others) hence, for the first time, parents sometimes have the opportunity of requesting a selective abortion. For those simple genetic conditions that cannot be so diagnosed by direct means, indirect diagnosis through the prenatal phenotype for a marker locus known to be linked to the disease locus (Edwards, 1956; Renwick, 1969), is a useful substitute with potentially wide applicability. A special case is the use, in certain families, of the chromosomal sex of the embryo to give a 50% or 0% prognosis of haemophilia for male and female respectively. Attention is now drawn to an autosomal linkage between the myotonic dystrophy locus (*Dm*) and the ABH secretor locus (*Se*) that can be used in favourable circumstances to predict fairly accurately the outcome of a particular pregnancy in a family with this dystrophy.

A method of three-locus analysis was required in this work because an additional locus, *Lu* (for the Lutheran blood group), was involved. Much of the present report concerns an extension—very suitable for this purpose—of a standard Bayesian two-locus analysis (Renwick, 1969, based on Smith, 1959).

Data

On sib-pair analysis of linkage data on the pedigrees of Thomasen (1948), Mohr (1954) found indications of linkage of *Dm* with *Se* as scored from the Lewis red-cell phenotype. He found a similar indication with the Lutheran blood group locus (*Lu*), which, in turn, he knew to be linked to *Se* (Mohr, 1954; Cook, 1965).

A comprehensive, computer-based analysis has now clarified the real strength of the evidence and has encouraged collection of a further sample. The

confirmation afforded by this is the subject of a separate report (Renwick *et al*, 1971), which also deals with certain problems of scoring the dystrophy and ABH secretion genotypes.

Genetic Model Underlying the Analysis

Some of the assumptions and approximations underlying linkage analysis in man are discussed by Renwick (1971). Only a few of these points require mention here.

(1) Intervals between loci are customarily measured as map-lengths because these are additive, whereas recombination fractions cannot be combined in a simple fashion over neighbouring intervals, unless these are short.

(2) A morgan is the map-length of any segment of a recovered chromosome strand that has experienced, on average, one crossover event in one meiosis.

(3) Chromatid interference is believed to be at a high level in man, leading to the mapping function, $4w = \tan^{-1} 2\theta + \tanh^{-1} 2\theta$, where w morgans is the map-length of an interval that gives a recombination fraction, θ .

(4) In women, the susceptibility to crossing-over is, for at least one and perhaps for most autosomal regions, higher than in men (Renwick, 1968). If consistency is provisionally assumed, the current estimate of the ratio, female:male, is 1.40 (95% probability limits 1.04 and 1.98) from unpublished calculations of J. H. Renwick and D. R. Bolling on data from many laboratories.

(5) The total autosomal map-length, T , based on chiasma counts will be taken to be $27\frac{1}{2}$ morgans in men. (This is an overestimate, hence it errs slightly on the side of safety in reducing the posterior odds on syntenic hypotheses.) The susceptibility-ratio estimate, 1.4, implies a map-length of about $38\frac{1}{2}$ morgans in women.

(6) Meiotic studies in men (M. Hultén, personal communication; P. L. Pearson, personal communication) show more chiasmata on long autosomes than on short ones. The approximation is here made that the relationship is a linear one. The physical lengths given by the Chicago Conference (1966) then offer a basis for partitioning the total autosomal map-length among the autosomal pairs of chromosomes. The resulting estimate of the map-length of the i th chromosome pair will be denoted by A_i and will be used in calculating prior probabilities as if it were the true value.

Bayesian Analysis for Three Loci

An analysis appropriate for multiple loci has been presented by Bolling (1970). Only the three-locus analysis will be given here but the same computer program embraces also the four-locus analysis. A brief outline of the Bayesian framework will be followed by a description of the procedure in more detail.

Prior Probabilities. The prior probability of three autosomal loci being on the same chromosome (3-syteny) is $\Sigma(A_i/T)^3$ or about 3/900. The prior probability of 2-on, 1-off (2-syteny; 1-asyteny) is $3\Sigma(A_i/T)^2(T-A_i)/T$ or $3\Sigma(A_i/T)^2 - 3\Sigma(A_i/T)^3$. (This happens to be three times the difference between the probabilities for 2-syteny and for 3-syteny.) Its numerical value is about 138/900. In Tables I and II this is partitioned equally between three options, in each of which a different locus is left out of the syntenic group. The probability for 3-syteny is likewise partitioned equally between the three possible orderings of the loci (ignoring orientation throughout). The remaining hypothesis (asyteny), under which all three loci are on different autosomes, carries the remaining probability, 759/900.

Contributions of the Data. The theorem of Bayes (1763) can be stated in terms of odds as follows: the odds on a hypothesis in the light of observations are proportional to the probability of those observations given the hypothesis multiplied by the prior odds on this hypothesis. In the present problem, the asytenic hypothesis remains simple but the syntenic ones must be considered as composite comprising a multitude of sub-hypotheses. There is one of these sub-hypotheses for each pair of values of the map-lengths of adjacent intervals when a given order of the three loci is considered.

From the pedigree data, we calculate on a simple Mendelian model the relative probability of finding these data if there is a true recombination fraction

θ_a between Dm and Se ; θ_b between Se and Lu ; θ_c between Dm and Lu . To have a constant standard for comparison, we standardize these odds (to antilods) by relating them to the probability when $\theta_a = \theta_b = \theta_c = \frac{1}{2}$ —ie, when the loci are on different chromosomes, for example. We shall write the map-lengths corresponding to $\theta = \frac{1}{2}$ arbitrarily as $w = 1000M$: they are more correctly infinite—the loci are asytenic and there is, at least notionally, an infinite amount of crossing-over between them.

We suppose that w_1 (the length of the $Dm:Se$ interval) yields the recombination fraction θ_a ; w_2 (the $Se:Lu$ map-length) yields θ_b ; w_3 (the $Dm:Lu$ map-length) yields θ_c . As already mentioned, the map-length and recombination fraction are taken to be related by the Carter-Falconer mapping function of the form: $4w = \tan^{-1} 2\theta + \tanh^{-1} 2\theta$. For asyteny, $\theta_a = \theta_b = \theta_c = \frac{1}{2}$; the antilod is then 1 by the standardization procedure.

Fortuitously, with one exception in Mohr's data (1954) to be considered later, no mating segregates fruitfully for more than two of the loci. The antilods from them are therefore largely indifferent to the lengths of the other intervals. Thus, for a given ordering, say $Dm:Se:Lu$, the antilod (denoted by α_{12}) for any pair of values of w_1 and w_2 (implying $w_3 = w_1 + w_2$) is given by

$$\alpha_{12}(D|w_1, w_2, w_3) = \alpha(D_1|w_1) \cdot \alpha(D_2|w_2) \cdot \alpha(D_3|w_3)$$

where D_1 , D_2 and D_3 are non-overlapping parts that constitute the total data, D .

The lods (denoted by z) are obtained by taking logarithms with base 10:

$$z_{12}(D|w_1, w_2, w_3) = z(D_1|w_1) + z(D_2|w_2) + z(D_3|w_3).$$

Non-independence. The exceptional mating is in Pedigree 4 of Mohr's data. The children of I.34 can be scored for recombination between Dm and Se ; Se and Lu ; Lu and Dm simultaneously, as she is probably heterozygous. The data, D' , cannot here be split into three non-overlapping parts, each relevant to a single interval. Fortunately, to achieve an approximate adjustment, we can use the following argument:

For a given ordering, say $Dm:Se:Lu$ (when w_1 and w_2 are the primary variables)

$$\alpha_{12}(D'|w_1, w_2, w_3) = \alpha(D'|w_1) \cdot \alpha(D'|w_2).$$

To gain a degree of symmetry and hence independence of the ordering we write this as follows:

$$\alpha_{12}(D'|w_1, w_2, w_3) = [\alpha(D'|w_1) \cdot \alpha(D'|w_2) \cdot \alpha(D'|w_3)] \div \alpha(D'|w_3)$$

or, in logarithmic terms,

$$z_{12}(D'|w_1, w_2, w_3) = [z(D'|w_1) + z(D'|w_2) + z(D'|w_3)] - z(D'|w_3).$$

That part of the expression that is enclosed in square brackets is symmetrical in w_1, w_2, w_3 . It is therefore independent of order and is the same in z_{13} and z_{23} as in z_{12} . In this particular sibship, the remaining term also happens to have approximately the same numerical value whether it is $z(D'|w_3)$ for z_{12} or $z(D'|w_2)$ for z_{13} or $z(D'|w_1)$ for z_{23} . (The interdependence of the coupling phases of the three loci has been ignored but the resulting error is probably trivial in this instance.) It is convenient to rewrite each expression in terms of an adjustment, c , to each lod where $c \simeq -(1/3)z(D'|w_3) \simeq -z_1(3,0)/3$; eg, $z_{12}(D'|w_1, w_2, w_3) = [z(D'|w_1) + c] + [z(D'|w_2) + c] + [z(D'|w_3) + c]$.

We may paraphrase this argument for events in a single meiosis as follows: For any ordering, the data concerning the two intervals that are contiguous are independent of each other; but the data on the joint interval are entirely determined by the data (from this meiosis) on the other two and must be discounted. In the raw analysis, the offspring of I.34 comprise roughly three recombinants or three non-recombinants—the lods are $z_1(3,0)$ —for each of the three pairs of loci. As the three orders are taken to be equally likely, the appropriate adjustment involves the discounting of one third of these lods for each of the three pairs. This is approximately achieved by addition of the adjustment, c .

Posterior Probabilities. For each ordering of the loci, an appropriate prior density, as discussed later, can be combined with the three antilods for trial values of the true map-lengths, to yield points on a posterior unnormalized probability surface. Integration over all possible values of these map-lengths gives the volume under the surface and this represents the posterior unnormalized probability of the corresponding syntenic hypothesis. Estimates of map-lengths and their limits, conditional on a given sequence of loci, are readily obtained from the appropriate marginal distribution. This procedure of partitioning a hypothesis into a multitude of exhaustive and non-overlapping sub-hypotheses, each with its characteristic pair of map-length values, and then applying Bayes' argument, is essentially that introduced into the genetic field by Smith (1959).

In order to try to isolate the various components of the calculations, we calculate the 'change in the odds' or an 'averaged antilod', A' , by dividing the posterior integral of a specified part of the distribution by the corresponding prior integral. This averaged antilod is not free of prior elements since we have, in effect, used them as weights in the averaging procedure.

Detailed Procedure

Joint Prior Probability Distribution of Interval Lengths. Let the total autosomal map length be T morgans.

Let the length of the i th autosome be $A_i > A_{i+1}$ morgans, where $0 < i < 22$.

3-syteny. Let the map-lengths FG, GH, FH, between three loci be w_1, w_2 , and w_3 morgans respectively. The ordering FGH is called 12 (because the component lengths are w_1, w_2); GHF is 31. From equation 4 of Irwin (1955), it follows that that part of the joint probability density for w_1, w_2, w_3 , that concerns the syteny of the loci in a specific ordering on a known autosome, i , is:

$$f_i(w_1, w_2, w_3) = 2[A_i - \max(w_1, w_2, w_3)]T^{-3} \\ \text{if } 0 \leq \max(w_1, w_2, w_3) \leq A_i \\ = 0 \quad \text{if } A_i \leq \max(w_1, w_2, w_3) \leq A_{i+1}.$$

The combined limits, $0 - A_1$, demarcate the syntenic part of a distribution. (Over all values that lie within these limits and over all three orderings, this part integrates to $[A_i/T]^3$ —the prior probability that three autosomal loci, chosen effectively at random, are on this i th autosome.)

When the loci are syntenic in the specified ordering but the autosome is *not* specified, the prior density is:

$$f(w_1, w_2, w_3) = \sum_{i=1}^{22} f_i(w_1, w_2, w_3) \\ 0 \leq \max(w_1, w_2, w_3) \leq A_1.$$

Over all values that lie within the limits $0 - A_1$, the partial distribution described by this density function integrates, appropriately, to $\frac{1}{3} \sum_{i=1}^{22} (A_i/T)^3$, the prior probability of autosomal 3-syteny with ordering specified—about 1/900.

For each ordering, $\max(w_1, w_2, w_3)$ is always the sum of the other two lengths, hence $f(w_1, w_2, w_3)$ may be presented graphically in a bivariate form, with these two shorter lengths as the primary variables. It constitutes a surface of 22 planes of progressively decreasing tilt as shown in Fig. 1 for the ordering 12. The line of mutual intersection of any two of these is parallel to the line, $w_1 + w_2 = A_1$, in which the final plane meets the base. This diagonal line in the base is the limit beyond which the conjunct interval would exceed the length, A_1 , of the longest chromosome (No. 1). As already mentioned, the proportional autosomal map-lengths (A_i/T) have been taken to be equal to the proportional physical lengths at mitotic metaphase.

We now consider the only other parts of the joint

Fig.1. The prior probability surface for the ordering *Dm:Se:Lu* for lengths of two adjacent intervals, w_1 and w_2 , on an unknown autosome. The surface consists of 22 planes of decreasing tilt intersecting each other in lines parallel to the line $w_1 + w_2 = A_1$ where A_1 is the map length of No. 1 chromosome (the longest). The surface represents only the 3-syntenic part of the distribution of map-interval lengths.

distribution of map-lengths that have non-zero prior densities. These parts involve asyntenies but we may avoid the introduction of a two-valued 'tenic' variable by adopting the convention that an interval between asyntenic loci is ascribed a length of 1000 morgans except when the additivity rule of map-lengths requires it to be longer. We may partially justify this device. In a formal sense, the independent assortment of loci on different pairs of autosomes produces the same proportion of gametes that are recombinant for these loci as would be produced by an infinite amount of crossing-over between syntenic loci. The interval between chromosome pairs might indeed be regarded as one of infinite susceptibility to 'crossing-over' so that physical continuity was constantly being disrupted. Formally, then, the map-length between asyntenic loci is infinite but there are important advantages in substituting an arbitrarily large value such as 1000 morgans: addition of map-lengths is, for one thing, still meaningful.

2-syteny and its part of the distribution. There

are again three possible orderings of the loci, here depending on which one is asyntenic. This one will be written as the last locus: for example, the ordering 12 (FGH or GFH) then implies that F and G are syntenic, H is not. $\text{Min}(w_1, w_2, w_3)$ denotes the map distance between the two syntenic loci. For a specific ordering, the joint prior distribution of map-lengths has a contribution, $f_i(w_1, w_2, w_3)$, that arises when the syntenic loci are on the i th autosome:

$$\begin{aligned} f_i(w_1, w_2, w_3) &= 2[A_i - \text{min}(w_1, w_2, w_3)]T^{-2}(T - A_i)T^{-1} \\ &\quad \text{if } 1000 \leq \text{max}(w_1, w_2, w_3) \leq 1000 + A_i \\ &= 0 \\ &\quad \text{if } 1000 + A_i \leq \text{max}(w_1, w_2, w_3) \leq 1000 + A_1. \end{aligned}$$

(For this specific ordering and for this i th chromosome and over all values that lie within either of these sets of limits, the corresponding part of the distribution that is described by this density function integrates appropriately to $[A_i/T]^2 - [A_i/T]^3$. This is the probability that two specific loci lie on the i th autosome and that the third lies elsewhere.)

When the autosome on which the two loci are syntenic is *not* specified (the usual case), the prior density is:

$$f(w_1, w_2, w_3) = \sum_{i=1}^{22} f_i(w_1, w_2, w_3).$$

That part of the distribution for which this density is appropriate integrates to $\sum_{i=1}^{22} [(A_i/T)^2 - (A_i/T)^3]$. Its numerical value is about 46/900. The full distribution includes of course three such parts, one for each 'ordering' specifying which locus is asyntenic.

Asyteny of all three loci. The probability for asyteny is about 759/900 as already mentioned and is not distributed.

Averaged Antilod and Posterior Distribution. For a specific ordering of the loci, one interval length is completely defined by the sum of the other two, hence it does not make any non-redundant contribution to the prior densities. The data D may, however, refer to all three intervals largely in an independent and non-overlapping manner and, accordingly, may be considered in three parts— D_1 , D_2 , and D_3 . The posterior probability density (unnormalized) for interval lengths, w_1 , w_2 , w_3 , in ordering 12 (where $w_3 = w_1 + w_2$) is therefore the algebraic product of the three likeli-

hood ratios, $\alpha(D_1|w_1)$, $\alpha(D_2|w_2)$, $\alpha(D_3|w_3)$, and the prior joint density $f_{12}(w_1, w_2, w_3)$.

The above presentation differs superficially from that of Bolling (1970) which is in terms of *conditional* distributions.

Se Linkage Group: An Illustration of the Method. The lod scores were computed for the *Dm:Se*, *Dm:Lu*, and *Se:Lu* intervals from all the available published and unpublished pedigree data by the program of Renwick and Schulze (1961). They are given in Table I of Renwick *et al* (1971)—for the equal-susceptibility situation, ie, when male and female map-lengths are taken to be equal.

For a specific ordering of the loci, the volume of the 3-syntenic part of the posterior probability distribution (unnormalized) was determined from these three sets of total lods by the MAPIN computer program (Bolling, 1970). MAPIN is written in Fortran IV and uses two numerical integration methods which usually show good agreement with each other. The ratio of this volume to the prior volume is the averaged antilod, A' , or the change in the odds, for this particular ordering of the loci. The procedure was repeated for the other possible orderings.

In Table I, these values of A' obtained from Mohr's data (1954) are set out for each syntenic hypothesis. Those for the 2-syntenic hypotheses

TABLE I

BAYESIAN ANALYSIS OF MOHR'S DATA (1954) ON LINKAGE RELATIONSHIPS OF *Dm*, *Se*, *Lu*, LOCI (A colon separates the symbols of syntenic loci; an oblique stroke (virgule) separates the symbols of asyntenic loci.)

Hypothesis	Loci	Prior Odds	Change in Odds	Posterior Odds	Posterior Probability
3-syteny	<i>Dm:Se:Lu</i>	1	3.0×10^{19}	3.0×10^{19}	6.8×10^{19}
	<i>Dm:Lu:Se</i>	1	2.2×10^{19}	2.2×10^{19}	
	<i>Lu:Dm:Se</i>	1	1.6×10^{19}	1.6×10^{19}	
2-syteny	<i>(Lu:Se)/Dm</i>	46	1.1×10^{17}	0.5×10^{19}	0.5×10^{19}
	<i>(Dm:Se)/Lu</i>	46	6	276 only	
	<i>(Dm:Lu)/Se</i>	46	14	644 only	
asyteny	<i>Lu/Se/Dm</i>	759	1	759 only	
Any of these . . .		900	. . .	7.3×10^{19} 7.3×10^{19}	1.00

TABLE II

BAYESIAN ANALYSIS OF MOHR'S DATA SUPPLEMENTED BY THOSE OF RENWICK *ET AL* (1971)

Hypothesis	Loci	Prior Odds	Change in Odds	Posterior Odds	Posterior Probability
3-syteny	<i>Dm:Se:Lu</i>	1	30.6×10^{19}	30.6×10^{19}	55.4×10^{19}
	<i>Lu:Dm:Se</i>	1	20.4×10^{19}	20.4×10^{19}	
	<i>Dm:Lu:Se</i>	1	4.4×10^{19}	4.4×10^{19}	
2-syteny	<i>(Lu:Se)/Dm</i>	46	1.2×10^{17}	0.6×10^{19}	0.6×10^{19}
	<i>(Dm:Se)/Lu</i>	46	23	1058 only	
	<i>(Dm:Lu)/Se</i>	46	14	644 only	
asyteny	<i>Lu/Se/Dm</i>	759	1	759 only	
Any of these . . .		900	. . .	56.0×10^{19} 56.0×10^{19}	1.00

The odds on the 3-syntenic orderings, *Dm:Se:Lu*, *Lu:Dm:Se*, *Dm:Lu:Se*, are seen to be left at 30:20:4 or about 6:4:1.

were computed, for strict comparability, on the 3-synteny model with the invention of a dummy as an extra syntenic locus on which the data do not bear. (All orderings were, of course, again considered.) Mohr's data alone yielded final odds on 3-synteny of 12:1 over all other hypotheses. With the inclusion of the data of Renwick *et al* (1971), these odds became 92:1 (Table II), corresponding to a posterior probability of 0.99 that the dystrophina

Fig. 2. Posterior distribution, $p(\theta) d\theta$, of the recombination fraction, θ , between *Dm* and *Se*, summed over all three orders of the loci, *Dm*, *Se*, *Lu*. The scale is unnormed. This distribution is derived from the marginal distribution of the corresponding map interval.

The graph of $\theta p(\theta)$ is also given (on a 20-fold scale shown on the right). The ratio of the areas under these two graphs, corrected for scale, is the overall probability of a recombinant,

$$P(R) = \frac{\int_0^{0.5} \theta \cdot p(\theta) \cdot d\theta}{\int_0^{0.5} p(\theta) \cdot d\theta} = 0.073.$$

locus belongs to the *Se:Lu* linkage group. Given 3-synteny, the current odds on the alternative sequences, *Dm:Se:Lu*, *Dm:Lu:Se*, *Se:Dm:Lu*, are found to be 6:4:1.

The marginal distributions were also computed by MAPIN. That for the longest interval was constructed by integrating the distribution along lines parallel to $w_1 + w_2 = A_1$ for the ordering 12; or along equivalent lines otherwise. As expected, the shape of the marginal distribution for one of the intervals—the *Lu:Se* interval—is so dominated by the data bearing directly on it that it remains almost invariant whatever the ordering of the loci, whereas the marginal distributions for the *Dm:Se* and *Dm:Lu* interval lengths reflect fewer data and therefore vary considerably among the three possible orderings.

The aggregate of the three marginal distributions for the *Dm:Se* interval gives a most probable map-length of 0.04 morgans between the two loci. The

distribution of the corresponding recombination fraction, θ (see Fig. 2) is critical when we apply these data in prenatal diagnosis as explained later.

A joint analysis (such as that outlined in this paper) is required not only to estimate each interval-length using all the data but to assess too the probability that *Dm* does indeed belong to the *Se:Lu* linkage group. This follows from the mutuality of the lengths of the three intervals, one being the sum of the other two. The powerful influences of the prior probabilities are, in themselves, good reasons for this joint analysis being of Bayesian type.

Antenatal Prediction of Myotonic Dystrophy.

It was mentioned in the introduction that linkages can theoretically be used for indirect diagnosis *in utero*. The dystrophina:secretor linkage is one of the first autosomal examples. An important requirement has been met by the recent finding by Harper and Hutchinson (1970) that the amniotic fluid does reflect the secretor genotype of the fetus. (That study was stimulated by our analysis of Mohr's data.) In certain suitable families in which the affected parent is heterozygous for both loci, it should be possible to infer what allele was transmitted from the dystrophy locus from knowledge of what allele was transmitted from the secretor locus. The security of the inference would, of course, depend on the risk that the gamete was recombinant. This risk $P(R)$ would be equal to the true recombination fraction θ . This remains unknown but we do possess in the light of the linkage data a probability distribution for the values it might take. We cannot justifiably take the modal estimate of θ in place of its true value because the distribution is skewed markedly (towards high values of θ). Pursuing such reasoning, we take the posterior probability, $P(R)$, that a particular gamete will be recombinant for alleles at the dystrophy and secretor loci to be the sum of elements of conditional probability, $p(R|\theta)d\theta$, each weighted by the posterior probability density, $p(\theta)$, appropriate for recombination fractions lying between θ and $\theta + d\theta$. For sufficiently small increment, $d\theta$,

$$P(R) = \int_0^{0.5} p(R|\theta) \cdot p(\theta) \cdot d\theta = \int_0^{0.5} \theta p(\theta) \cdot d\theta.$$

We require, therefore, the centre of gravity (ie, the mean) of the posterior distribution of θ . We can obtain it graphically by plotting the $\theta p(\theta) \cdot d\theta$ function against θ and integrating it. To do this, we measure the area under it and the area under the distribution itself. After allowance for scaling (such as the 20-fold scaling used in Fig. 2) the ratio

of the areas which is found to be about 0.073 is the required probability if, on the one hand, there is synteny of *Dm* and *Se* (probability 0.99). If, on the other hand, this condition of synteny does not hold (probability 0.01), the recombination fraction is exactly 0.5. Using the combined data of the present work and of Mohr (1954), we therefore assess the probability that any specified gamete is a recombinant as $0.99(0.073) + 0.01(0.5)$ or 0.078. The probability that it is a non-recombinant is thus about 92%—so a prediction, that could be given with 100% security but for recombination, has about 92% security when we take recombination into account.

It might seem, at first sight, that the probability of a recombinant, contrary to the above, should be equal to the probability of recombination, as estimated modally from the data. If the estimate of θ were associated with only a trivial bias or only a trivial variance this would indeed be approximately true. In more real situations, all possible values of θ must be considered with weights proportional to their posterior probability densities. This can perhaps be better understood by means of an artificial oversimplification:

Consider that only three values, 0.05, 0.1, 0.2 are possible with posterior probabilities 0.7, 0.2, 0.1 respectively. The most probable recombination fraction would then be 0.05 but the probability, $P(R)$, of a particular gamete being a recombinant would be

$$P(R) = (0.7)(0.05) + (0.2)(0.1) + (0.1)(0.2) = 0.075.$$

Influence of Sex. The *Se:Lu* region is known to be one of those that have an excess of crossing-over in females. If this region and its neighbours are average in this respect, we could perhaps use, for its female:male ratio of susceptibility to crossing-over, the overall estimate, 1.40, that we obtained recently from an unpublished analysis of available data on a number of autosomal linkages. To a first approximation, we can then derive map-length and recombination estimates for males by multiplying the above raw estimates by 5/6 and for females by multiplying by 7/6. The security of prediction is then, for a father, $100 - (7.8)(5/6)$ or 93% and, for a mother, $100 - 7.8(7/6)$ or 91%.

The main approximation involved here lies in the fact that the raw estimates are based partly on data relevant to male meiosis and partly on data relevant to female meiosis. These two sets of data can be expected in general to be unequal in size and moment, so that raw estimates obtained by pooling them are only approximations to the required unweighted average of male and female estimates

separately derived. A more sophisticated analysis may be envisaged. It would be possible to compute these estimates subject to the current estimate of the overall susceptibility ratio if this ratio were deemed approximately uniform and therefore appropriate to this chromosomal region.

Summary

Re-analysis of data of Thomasen (1948) and Mohr (1954) that was undertaken as part of a wider computer-based linkage analysis gave evidence that the loci of myotonic dystrophy (*Dm*), ABH secretion (*Se*), and the Lutheran blood group system (*Lu*) might be on the same chromosome (syntenic). Like Mohr, who made the same observation by a different method, we should have found the evidence difficult to evaluate but for a fortuitous concurrence with the designing of a Bayesian computer analysis for linkage data involving 3 or 4 loci. Extension to 5 loci appears feasible but extension to 6 without further approximation does not. That method is published for the first time here.

The posterior probability, 0.93, that the 3 loci are syntenic stimulated collection of further data that now raise it to 0.99 (Renwick *et al*, 1971). The method when applied to these two sets of data, gives a preferred map *Dm:Se:Lu* with consecutive intervals of lengths 0.04:0.13 morgans, though other orderings are also possible.

The *Dm:Se* linkage is important since it can be used for an indirect and novel form of prenatal diagnosis. In suitable pedigrees, the dystrophina can be predicted with 92% security on the basis of the secretor phenotype of the fetus as determined on its amniotic fluid (Harper and Hutchinson, 1970).

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