THE ANALYSIS OF MULTIPLE LINKAGE DATA

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IN these notes I indicate methods applicable to the interpretation of multiple linkage tests involving three or more linked loci. The methods advocated are based on two extensions of Kosambi's simple and extremely useful theory of linkage relations, the purpose of the extensions being to widen the range of usefulness without losing the powerful simplicity of that theory.

In the first place Kosambi's theory (1) may be supplemented by the introduction of a quantity K whose value varies with position on the chromosome arm. I have shown elsewhere that we may expect high values of K to be realised for sets of loci in the neighbourhood of the centromere, and low values to occur near the termini of the chromosome, K taking the value unity at some point related to the middle of the arm. In a survey (2) of data on Chromosomes II and III of *Drosophila melanogaster* (not yet fully published), I found excellent agreement with these predictions. Thus it seems likely that in other organisms the value of K as derived from experiments on multiple crossing-over can be of some use in suggesting the location on the chromosome arm of the loci concerned. In Part I, I deal with the statistical problems involved in the estimation of K and of the trend of K in the cases of three and four linked loci, and discuss some actual data obtained for the Agouti-pallid region of Chromosome V in the mouse.

Part II is of more abstract interest and extends Kosambi's theory (without introducing K) to multiple recombination with as many as six linked loci. Here the mathematical method used is not identical with that of Kosambi but proceeds from the general theory of linkage relations developed by Sir Ronald Fisher and myself (2-7).

Part III introduces K into Kosambi's theory and gives an elementary way of generalising Kosambi's formulæ to the special but useful cases of several fairly closely linked loci such as are treated in I. 4.

I. ESTIMATION OF THE KOSAMBI COEFFICIENT

I. INTRODUCTION

The Kosambi coefficient, denoted by K , is defined (5) in terms of the recombination frequencies of 3 linked loci, A, B and D by means of the equations

$$K = \frac{y_1 + y_2 - y_{1+2}}{4y_1y_2y_{1+2}} = \frac{y_{12}}{2y_1y_2y_{1+2}}.$$

Here the suffixes 1 and 2 refer to the segments AB and BD, while the

suffix 1+2 refers to the combined segment AD. Thus any three loci are characterised by a value of K . It will be seen that K is equal to $C/2y_{1+2}$, where C is the genetical coincidence for the two segments, defined by Stevens (8) as y_{12}/y_1y_2 . Kosambi (1) obtained the addition formula

$$y_{1+2} = \frac{y_1 + y_2}{1 + 4y_1y_2}$$

by an assumption equivalent to setting C equal to $2y_{1+2}$; i.e. to taking the value of K as unity. Regions of the chromosome in which K is not significantly different from unity have been called Kosambi regions (5), and in such regions interference is said to be at the Kosambi level (5). I have given (2, 5) theoretical reasons for supposing that in regions near the centromere K has a high value greater than unity, and diminishes steadily with increasing distance from the centromere, becoming relatively small near the terminus of an arm. Consequently there is a Kosambi region, where $K = 1$, situated medianly on the arm.

These expectations have been well fulfilled in the case of Chromosomes II and III in *Drosophila melanogaster* (2). Therefore, in the absence of evidence to the contrary, it seems reasonable in other organisms to use the observed K values as providing some indication of the distance of the loci concerned from the centromere, and of the orientation of segments relative to the centromere. This note is concerned with estimation of K values when data on 3 or 4 loci are available.

2. THE USE OF THREE POINT DATA

Backcross experiments provide the best determinations of the recombination fractions, and therefore of C and K . In such an experiment involving two segments AB and BD, suppose that out of a total of n organisms bred the observed class frequencies are

0	1	2	12
a_0	a_1	a_2	a_{12}

Here a_0 show recombination in neither 1 nor 2, a_1 show recombination in 1 but not in 2, a_2 show recombination in 2 but not in 1, while a_{12} show simultaneous recombination in 1 and 2. Thus

$$n_1 = a_1 + a_{12} \text{ show recombination in 1,}$$

$$n_2 = a_2 + a_{12} \text{ show recombination in 2,}$$

$$n_{1+2} = a_1 + a_2 \text{ show recombination in the combined segment 1+2 (AD),}$$

$$n_{12} = a_{12} \text{ show simultaneous recombination in 1 and 2.}$$

The estimators of $y_1, y_2, y_{1+2}, y_{12}$ are

$$\hat{y}_1 = n_1/n, \hat{y}_2 = n_2/n, \hat{y}_{1+2} = n_{1+2}/n, \hat{y}_{12} = n_{12}/n.$$

The sampling variance of any such function is $V(y) = y(1-y)/n$.

The estimator of C is $\hat{C} = nn_{12}/n_1n_2$, and Stevens (*loc. cit.*) has shown that its sampling variance is

$$V(\hat{C}) = C(1 - Cy_1 - Cy_2 - Cy_1y_2 + 2C^2y_1y_2)/ny_1y_2.$$

This may be estimated by inserting the estimates $\hat{y}_1, \dots, \hat{C}$, and a crude test of the significance of the difference of C from unity (*i.e.* a test for the presence of genetical interference) may be based on the estimated standard error or on the variance. *E.g.* with the data

0	1	2	12	n
1116	182	375	24	1697

we have the percentage values

$$\hat{y}_1 = 12.139, \quad \hat{y}_2 = 23.512, \quad \hat{C} = 49.551$$

Estimated $V(\hat{C}) = 84.2176$ (per cent.)² = 0.008,421,76

Estimated $s(\hat{C}) = 9.1770$ per cent.

Thus $\hat{C} = 49.55 \pm 9.18$ per cent. which is significantly less than unity and indicative of genetical interference.

The maximum likelihood estimator of K is simply

$$\hat{K} = \hat{C}/2\hat{y}_{1+2} = n^2n_{12}/2n_1n_2n_{1+2}.$$

This statistic is biased, the bias being given approximately by

$$\beta = \hat{K} \left(\frac{3}{n_{1+2}} - \frac{3}{n} - \frac{4\hat{K}n_{12}}{n^2} \right).$$

The estimated sampling variance is given by

$$V(K) = K^2 \left\{ \frac{1}{n_{12}} + \frac{5}{n_{1+2}} + \frac{2n_{12}}{n_1n_2} - \frac{1}{n_1} - \frac{1}{n_2} - \frac{4}{n} - \frac{4(n_1+n_2)\hat{K}}{n^2} \right\}$$

or may be got by inserting the estimates in

$$\frac{K^2}{n} \left\{ \frac{1}{y_{12}} + \frac{5}{y_{1+2}} + 2C - \frac{1}{y_1} - \frac{1}{y_2} - 4 - 4K(y_1 + y_2) \right\}.$$

In the example we have

$$\hat{K} = 75.48 \pm 15.26 \text{ per cent.}, \quad V(\hat{K}) = 232.8934 \text{ (per cent.)}^2.$$

The bias is not seriously large, since we have

$$\beta = 0.004,041 = 0.40 \text{ per cent.}$$

To test departure of K from unity we calculate

$$\chi^2_{[1]} = (\hat{K} - 1)^2/V(K) = 4.5317,$$

where $V(K)$ is now calculated from equation (1) with K put equal to unity.

K therefore deviates just significantly from unity.

In cases such as the present example when K is not very significantly different from unity a more refined procedure ought next to be used. We assume y_1, y_2 as free parameters and take y_{1+2}, y_{12} as given as functions of y_1, y_2 by Kosambi's formulæ. A maximum likelihood scoring technique gives amended estimates which are then used to calculate expected class frequencies for a $\chi^2_{(1)}$ fit to the observations. If the fit is good, *i.e.* if χ^2 is not significantly large then we may retain the hypothesis $K = 1$. If the fit is bad we may then estimate K as above, together with its estimated sampling variance and standard error.

3. THE USE OF FOUR POINT DATA

When data on four loci A, B, D, E are available, the appropriate treatment is determined by the closeness of the observed linkage between them. When the segments are sufficiently long for doubles to be at least moderately frequent, the best use of the data requires that K be calculated separately for the two triads ABD, BDE , the data being reduced to three point data in respect of each triad. If the values K_1, K_2 are significantly different then the direction of the trend may be taken as presumptive evidence of the orientation of the segment AE relative to the centromere O . *E.g.* if $K_1 > K_2$ the preferred order is OAE .

To test the significance of the difference $(K_1 - K_2)$ we may compare it with its sampling standard deviation using either a χ^2 , χ^2 - or t -test. The variance of $(K_1 - K_2)$ is

$$V(K_1) - 2C(K_1, K_2) + V(K_2),$$

where $C(K_1, K_2)$ is the sampling covariance of K_1 and K_2 , given approximately by

$$\begin{aligned} \hat{K}_1 \hat{K}_2 \left(\frac{n_{12}}{n_1 n_2} + \frac{n_{23}}{n_2 n_3} + \frac{n_{13}}{n_1 n_3} + \frac{a_2}{n_2 n_{1+2}} + \frac{a_2}{n_2 n_{2+3}} + \frac{a_2}{n_{1+2} n_{2+3}} \right. \\ \left. + \frac{n_{123}}{n_{12} n_{23}} - \frac{n_{123}}{n_1 n_{23}} - \frac{n_{123}}{n_3 n_{12}} - \frac{1}{n_2} - \frac{4}{n} \right) \end{aligned}$$

This expression has been derived by means of a formula cognate to that given by Sir Ronald Fisher for the approximate sampling variance of a statistic (9). If $m_1, m_2, \dots, m_r$ (totalling n) are the frequencies observed in r classes subject to a multinomial distribution, and if T_1, T_2 are homogeneous functions of degree 0 or 1 in $m_1, m_2, \dots, m_r$ and n , then we have as the approximate sampling covariance of T_1 and T_2 the expression

$$C(T_1, T_2) = \sum_{i=1}^r m_i \frac{\partial T_1}{\partial m_i} \frac{\partial T_2}{\partial m_i} - n \frac{\partial T_1}{\partial n} \frac{\partial T_2}{\partial n}.$$

When $T_1 = T_2$, this becomes Fisher's formula

$$V(T) = \Sigma m \left(\frac{\partial T}{\partial m} \right)^2 - n \left(\frac{\partial T}{\partial n} \right)^2$$

Each formula holds exactly when T_1 and T_2 are linear functions of $m_1/n, \dots, m_r/n$.

4. THE USE OF FOUR POINT DATA FOR SHORT SEGMENTS

When A and E are close together K_1 and K_2 will differ significantly. Double recombination classes will occur with small frequencies and triples will be absent. For if p, q, r are the three recombination fractions the expected frequencies of doubles and triples will be given to good approximation by the expressions given below (part III), namely :—

$$\begin{aligned} \gamma_{12} &= 2Kpq(p+q), & \gamma_{23} &= 2Kqr(q+r), & \gamma_{13} &= 2Kpr(p+q+r), \\ \gamma_{123} &= 4K^3pqr\{(p+q)(q+r) - \frac{1}{3}q^2\}. \end{aligned}$$

If the observations are

0	1	2	3	23	13	12	123	Total
a_0	a_1	a_2	a_3	a_{23}	a_{13}	a_{12}	0	n

then it may be shown by inspection of the maximum likelihood equations for the four unknowns p, q, r, K subject to joint estimation, that the estimators approximate closely to the "intuitive" ones

$$\hat{p} = (a_1 + a_{12} + a_{13})/n, \text{ etc.}$$

$$\hat{K} = (a_{23} + a_{13} + a_{12})/D = (\text{observed doubles})/(\text{expected doubles}).$$

Here D is the expected total frequency of doubles calculated as

$$\begin{aligned} D &= n(\gamma_{12} + \gamma_{23} + \gamma_{13}) \\ &= \frac{2}{3}(p+q+r)^3 - \frac{2}{3}(p^3 + q^3 + r^3) \end{aligned}$$

estimated by inserting $\hat{p}, \hat{q}, \hat{r}$. Without serious error we may for this purpose take for $\hat{p}, \hat{q}, \hat{r}$ the quantities $a_1/n, a_2/n, a_3/n$. Thus we get the approximate estimator

$$\hat{K} = 3n^2(a_{23} + a_{13} + a_{12})/2(s_1^3 - s_3)$$

where $s_r = a_1^r + a_2^r + a_3^r$, ($r = 1, 2, 3, \dots$).

For the variance of K , we get by the approximate method of the last section

$$V(\hat{K}) = \hat{K}^2 \left\{ \frac{1}{a_{23} + a_{13} + a_{12}} + \frac{9(s_1^5 - 2s_1^2s_3 + s_5)}{(s_1^3 - s_3)^2} - \frac{4}{n} \right\}$$

The first term is usually dominant so that to a rough approximation

$$V(\hat{K}) = \hat{K}^2/(\text{observed doubles}).$$

Example.—In a four point backcross experiment recently completed, of which a full account is not yet published, the four loci *Agouti*, *undulated*, *wellhaarig*, and *pallid* in the house mouse were employed. The following class frequencies were obtained.

	1	2	3	23	13	12	123	n
From female heterozygotes .	117	185	104	1	0	0	0	2503
From male heterozygotes .	115	111	56	1	0	1	0	2499
Total . . .	232	296	160	2	0	1	0	5002

On the combined data for males and females we have

$$\hat{p} = 4.66 \text{ per cent.}, \quad \hat{q} = 5.98 \text{ per cent.}, \quad \hat{r} = 3.24 \text{ per cent.}$$

$$\begin{aligned} \text{Observed doubles} &= 3. & s_1 &= 688, & s_1^2 &= 47.3344 \times 10^4 \\ s_3 &= 42.5175 \times 10^6, & s_1^3 &= 325.6607 \times 10^6, \\ s_5 &= 304.9229 \times 10^{10}, & s_1^5 &= 1.5415 \times 10^{14} \end{aligned}$$

Thus $\hat{K} = 39.76 \pm 23.1105$ per cent., $V(\hat{K}) = 0.055,263$, and $\hat{K}$ is significantly less than unity, its value being suggestive of a region towards the chromosome terminus or at least between mid-arm and the terminus. The segment *Agouti-pallid* is thus between 30-100 cM from the centromere according as to what is assumed for the map length of the arm.

The treatment is however rather inaccurate on account of the marked sex-difference of two of the recombination fractions, and is best carried out separately for the two sexes. We obtain

	K per cent.	Information
Females . . .	16.24 ± 16.41	37.15
Males . . .	96.63 ± 68.29	2.30
Combined . . .	20.92 ± 15.09	39.45

Thus in the females K is significantly low, but in the males does not differ significantly from 0 or unity. On the basis of the male and female data considered jointly we might legitimately assume the segment to be moderately distant from the centromere, since the difference between male and female K values is not itself significant.

5. SUMMARY

Methods of estimation are given for K and its sampling variance in the case of triads of loci, and for the covariance of K values in overlapping triads. It is shown how to estimate K in the case of three short segments with special reference to the *Agouti-pallid* region of Chromosome V in the house mouse. It is suggested that *Agouti* is moderately far from the centromere.

II. THE FREQUENCIES OF SIMULTANEOUS RECOMBINATION OF SEVERAL LINKED LOCI

I. INTRODUCTION

Kosambi (1) proposed the formula

$$y = \frac{1}{2} \tanh 2x$$

as an approximate representation of the relation between the genetical map-length x of the segment joining two loci and y the amount of genetical recombination between those loci. For 3 loci defining adjacent segments x_1 and x_2 this formula implies that

$$y_{12} = 2y_1y_2y_{1+2} = 2y_1y_2 \frac{y_1+y_2}{1+4y_1y_2},$$

where y_{12} is the frequency of recombination in both simultaneously, and y_{1+2} is the frequency of recombination over the combined segment x_1+x_2 . I have indicated (5, 7) the conditions under which these approximations are valid. If, in particular, the interference is represented by the function $4u \exp(-2u)$ employed in previous work (4-7), then, for a segment near the (metrical) mid-point of a long chromosome arm, we obtain the relation

$$y = \frac{1}{2}(1 - e^{-2x} \cos 2x).$$

For segments less than 35 cM in map-length this function differs from $\frac{1}{2} \tanh 2x$ to an extent which in practice is quite inappreciable, the values of y given by the two formulæ being discrepant by at most 8 parts in 302. Thus Kosambi's formula receives a simple theoretical justification as an approximation likely to apply in segments median to long chromosome arms.

Using the methods indicated by previous work it has been shown (7) that there are simple expressions giving all recombination frequencies for 4 loci (3 segments) which also are likely to apply when all 4 loci are reasonably close to metrical mid arm. I gave the new relations

$$y_{13} = y_1y_3 - z_1z_3e^{-4x_1}, \quad w_{12} = w_1w_2,$$

and

$$y_{123} = R(w_1w_2w_3) + \frac{1}{2}z_1z_3(1 - e^{-4x_1}).$$

Here

$$z = \frac{1}{2}e^{-2x} \sin 2x, \quad w = y + iz, \quad i = \sqrt{-1},$$

and

$$R(w_1w_2w_3) = y_1y_2y_3 - y_1z_2z_3 - y_2z_1z_3 - y_3z_1z_2.$$

I showed further that these relations approximate to

$$y_{13} = y_1y_3 \left\{ 1 - \frac{1-2y_2}{1+2y_2} \frac{(1-2y_1)(1-2y_3)}{1+4y_1y_3} \right\}$$

and

$$y_{123} = y_1y_{23} - y_2y_{13} + y_3y_{12}.$$

These equations may be regarded as natural generalisations of Kosambi's formulæ. While receiving their justification for short

segments only, like Kosambi's original formulæ they may often be found to apply in good approximation for larger segments. It will be seen that their form is consistent with the known disappearance of interference between long segments or widely separated segments.

It may be remarked in passing that the formulæ for 4 loci are adequate for the derivation of all formulæ required for 5 loci (4 segments) in virtue of identities of the type

$$\mathcal{Y}_{12,3+4} = \mathcal{Y}_{123} + \mathcal{Y}_{124} - 2\mathcal{Y}_{1234}.$$

In the present note I extend the results to give formulæ in $\mathcal{Y}$, z and x adequate to deal with as many as 6 loci (5 segments), and show how the formulæ may be adapted to apply to cases in which any or all of the pairs of segments are non-adjacent.

2. FORMULÆ

(a) 4 loci (3 adjacent segments).—

$$\mathcal{Y}_{12} = \mathcal{Y}_1\mathcal{Y}_2 - z_1z_2, \quad \mathcal{Y}_{23} = \mathcal{Y}_2\mathcal{Y}_3 - z_2z_3,$$

$$\mathcal{Y}_{13} = \mathcal{Y}_1\mathcal{Y}_3 - z_1z_3e^{-4x_2},$$

$$\mathcal{Y}_{123} = \mathcal{Y}_1\mathcal{Y}_2\mathcal{Y}_3 - \mathcal{Y}_1z_2z_3 - \mathcal{Y}_2z_1z_3 - \mathcal{Y}_3z_1z_2 + \frac{1}{2}z_1z_3(1 - e^{-4x_2}).$$

(b) 5 loci (4 adjacent segments).—Only one formula is essentially new, namely that for $\mathcal{Y}_{1234}$, all the others resulting either directly or by mere change of variables from those for 4 loci. However, it is instructive to write out the full set.

$$\mathcal{Y}_{12} = \mathcal{Y}_1\mathcal{Y}_2 - z_1z_2, \quad \mathcal{Y}_{14} = \mathcal{Y}_1\mathcal{Y}_4 - z_1z_4e^{-4(x_2+x_3)}$$

$$\mathcal{Y}_{23} = \mathcal{Y}_2\mathcal{Y}_3 - z_2z_3, \quad \mathcal{Y}_{24} = \mathcal{Y}_2\mathcal{Y}_4 - z_2z_4e^{-4x_3}$$

$$\mathcal{Y}_{13} = \mathcal{Y}_1\mathcal{Y}_3 - z_1z_3e^{-4x_2}, \quad \mathcal{Y}_{34} = \mathcal{Y}_3\mathcal{Y}_4 - z_3z_4,$$

$$\mathcal{Y}_{123} = \mathcal{Y}_1\mathcal{Y}_2\mathcal{Y}_3 - \mathcal{Y}_1z_2z_3 - \mathcal{Y}_2z_1z_3 - \mathcal{Y}_3z_1z_2 + \frac{1}{2}z_1z_3(1 - e^{-4x_2}),$$

$$\mathcal{Y}_{124} = \mathcal{Y}_1\mathcal{Y}_2\mathcal{Y}_4 - \mathcal{Y}_1z_2z_4e^{-2x_3} - \mathcal{Y}_1z_1z_4e^{-2x_2} - \mathcal{Y}_4z_1z_2 + \frac{1}{2}z_1z_4(1 - e^{-4x_2})e^{-4x_3},$$

$$\mathcal{Y}_{134} = \mathcal{Y}_1\mathcal{Y}_3\mathcal{Y}_4 - \mathcal{Y}_1z_3z_4 - \mathcal{Y}_3z_1z_4e^{-2x_2} - \mathcal{Y}_4z_1z_3e^{-2x_2} + \frac{1}{2}z_1z_4(1 - e^{-4x_2})e^{-4x_3},$$

$$\mathcal{Y}_{234} = \mathcal{Y}_2\mathcal{Y}_3\mathcal{Y}_4 - \mathcal{Y}_2z_3z_4 - \mathcal{Y}_3z_2z_4 - \mathcal{Y}_4z_2z_3 + \frac{1}{2}z_2z_4(1 - e^{-4x_2}),$$

$$\mathcal{Y}_{1234} = \mathcal{Y}_1\mathcal{Y}_2\mathcal{Y}_3\mathcal{Y}_4 - \mathcal{Y}_1\mathcal{Y}_2z_3z_4 - \mathcal{Y}_2\mathcal{Y}_3z_1z_4 - \mathcal{Y}_1\mathcal{Y}_3z_2z_4$$

$$- \mathcal{Y}_1\mathcal{Y}_4z_2z_3 - \mathcal{Y}_2\mathcal{Y}_4z_1z_3 - \mathcal{Y}_2\mathcal{Y}_4z_1z_2 + z_1z_2z_3z_4$$

$$+ \frac{1}{2}\mathcal{Y}_1z_2z_4(1 - e^{-4x_3}) + \frac{1}{2}\mathcal{Y}_2z_1z_4(1 - e^{-4x_3})$$

$$+ \frac{1}{2}\mathcal{Y}_3z_1z_4(1 - e^{-4x_2}) + \frac{1}{2}\mathcal{Y}_4z_1z_3(1 - e^{-4x_2})$$

$$- \frac{1}{2}z_1z_4(1 - e^{-4x_2})(1 - e^{-4x_3}).$$

(c) 6 loci (5 segments).—Only two essentially new formulæ are required, namely :

$$\mathcal{Y}_{135} = \mathcal{Y}_1\mathcal{Y}_3\mathcal{Y}_5 - \mathcal{Y}_1z_3z_5e^{-4x_4} - \mathcal{Y}_3z_1z_5e^{-4(x_2+x_3)}$$

$$- \mathcal{Y}_5z_1z_3e^{-4x_4} + \frac{1}{2}z_1z_5(1 - e^{-4x_2})e^{-2(x_2+x_3)}$$

$$\begin{aligned}
\mathcal{Y}_{12345} = & \mathcal{Y}_1 \mathcal{Y}_2 \mathcal{Y}_3 \mathcal{Y}_4 \mathcal{Y}_5 - \Sigma z_1 z_2 \mathcal{Y}_3 \mathcal{Y}_4 \mathcal{Y}_5 \\
& - \frac{1}{2} \{ z_1 z_5 \mathcal{Y}_3 \mathcal{Y}_4 (1 - e^{-4x_2}) + z_1 z_5 \mathcal{Y}_2 \mathcal{Y}_4 (1 - e^{-4x_3}) \\
& + z_1 z_5 \mathcal{Y}_2 \mathcal{Y}_3 (1 - e^{-4x_4}) + z_1 z_3 \mathcal{Y}_4 \mathcal{Y}_5 (1 - e^{-4x_2}) \\
& + z_1 z_2 \mathcal{Y}_4 \mathcal{Y}_5 (1 - e^{-4x_3}) + z_1 z_4 \mathcal{Y}_3 \mathcal{Y}_5 (1 - e^{-4x_2}) \\
& + z_1 z_4 \mathcal{Y}_2 \mathcal{Y}_5 (1 - e^{-4x_3}) + z_2 z_5 \mathcal{Y}_1 \mathcal{Y}_3 (1 - e^{-4x_4}) \\
& + z_2 z_5 \mathcal{Y}_1 \mathcal{Y}_4 (1 - e^{-4x_3}) + z_3 z_5 \mathcal{Y}_1 \mathcal{Y}_2 (1 - e^{-4x_4}) \} \\
& - \frac{1}{2} \{ z_1 z_3 z_4 z_5 (1 - e^{-2x_2}) + z_1 z_2 z_4 z_5 (1 - e^{-2x_3}) \\
& + z_1 z_2 z_3 z_5 (1 - e^{-2x_4}) \} \\
& + \frac{1}{4} (z_1 z_5 \mathcal{Y}_2 + z_2 z_5 \mathcal{Y}_1) (1 - e^{-4x_2}) (1 - e^{-4x_3}) \\
& + \frac{1}{4} (z_1 z_5 \mathcal{Y}_3 + z_3 z_5 \mathcal{Y}_1) (1 - e^{-4x_4}) (1 - e^{-4x_2}) \\
& + \frac{1}{4} (z_1 z_5 \mathcal{Y}_4 + z_4 z_5 \mathcal{Y}_1) (1 - e^{-4x_2}) (1 - e^{-4x_3}) \\
& - \frac{1}{8} z_1 z_5 (1 - e^{-4x_2}) (1 - e^{-4x_3}) (1 - e^{-4x_4}).
\end{aligned}$$

(d) *Extension of formulae to separated segments.*—It is easy to generalise any formula relating to contiguous segments so that it applies to non-adjacent ones. For example, the expression for $\mathcal{Y}_{1357}$ relating to intervals x_1, x_3, x_5, x_7 out of the contiguous set $x_1, x_2, x_3, x_4, x_5, x_6, x_7$, can be derived as follows :

If x_2, x_4, x_6 were all zero so that 1, 3, 5 and 7 were contiguous we would have

$$\begin{aligned}
\mathcal{Y}_{1357} = & \mathcal{Y}_1 \mathcal{Y}_3 \mathcal{Y}_5 \mathcal{Y}_7 - z_1 z_3 \mathcal{Y}_5 \mathcal{Y}_7 - z_1 z_5 \mathcal{Y}_3 \mathcal{Y}_7 - z_1 z_7 \mathcal{Y}_3 \mathcal{Y}_5 \\
& - z_3 z_5 \mathcal{Y}_1 \mathcal{Y}_7 - z_3 z_7 \mathcal{Y}_1 \mathcal{Y}_5 - z_5 z_7 \mathcal{Y}_1 \mathcal{Y}_3 \\
& + z_1 z_3 z_5 z_7 + \frac{1}{2} z_1 z_7 \mathcal{Y}_5 (1 - e^{-4x_3}) + \frac{1}{2} z_1 z_7 \mathcal{Y}_3 (1 - e^{-4x_5}) \\
& + \frac{1}{2} z_1 z_5 \mathcal{Y}_7 (1 - e^{-4x_2}) + \frac{1}{2} z_3 z_7 \mathcal{Y}_1 (1 - e^{-4x_6}) \\
& - \frac{1}{4} z_1 z_7 (1 - e^{-4x_3}) (1 - e^{-4x_5}).
\end{aligned}$$

When x_2, x_4, x_6 are not zero this formula becomes

$$\begin{aligned}
\mathcal{Y}_{1357} = & \mathcal{Y}_1 \mathcal{Y}_3 \mathcal{Y}_5 \mathcal{Y}_7 - z_1 z_3 \mathcal{Y}_5 \mathcal{Y}_7 e^{-4x_2} - z_1 z_5 \mathcal{Y}_3 \mathcal{Y}_7 e^{-4(x_2 + x_4)} \\
& - z_1 z_7 \mathcal{Y}_3 \mathcal{Y}_5 e^{-4(x_2 + x_4 + x_6)} - z_3 z_5 \mathcal{Y}_1 \mathcal{Y}_7 e^{-4x_4} \\
& - z_3 z_7 \mathcal{Y}_1 \mathcal{Y}_5 e^{-4(x_4 + x_6)} - z_5 z_7 \mathcal{Y}_1 \mathcal{Y}_3 e^{-4x_6} \\
& + z_1 z_3 z_5 z_7 e^{-4(x_2 + x_6)} \\
& + \frac{1}{2} z_1 z_7 \mathcal{Y}_5 (1 - e^{-4x_3}) e^{-4(x_2 + x_4 + x_6)} + \frac{1}{2} z_1 z_7 \mathcal{Y}_3 (1 - e^{-4x_5}) e^{-4(x_2 + x_4 + x_6)} \\
& + \frac{1}{2} z_1 z_5 \mathcal{Y}_7 (1 - e^{-4x_2}) e^{-4(x_2 + x_4)} + \frac{1}{2} z_3 z_7 \mathcal{Y}_1 (1 - e^{-4x_6}) e^{-4(x_4 + x_6)} \\
& - \frac{1}{4} z_1 z_7 (1 - e^{-4x_3}) (1 - e^{-4x_5}) e^{-4(x_2 + x_4 + x_6)}
\end{aligned}$$

The rule of derivation consists merely of inserting an attenuation factor corresponding to each term $z_j z_k$ as written and determined by the total length of "gap intervals" between the segments j and k .

3. METHOD OF PROOF OF THE FORMULÆ

For $(k+1)$ loci (k contiguous segments $x_1, x_2, \dots, x_k$) infinitely distant both from the centromere and the terminus of the chromosome arm we have a generating function (5) which takes the value

$$P(\lambda_1, \lambda_2, \dots, \lambda_k) = \frac{1}{2} Q \exp\left(-\sum_{j=1}^k D_j\right),$$

where

$$D_j = 2x_j \quad (j = 1, 2, \dots, k),$$

$$\text{and } Q = 2C_1C_2 \dots C_k + \Sigma S_1C_2 \dots C_kL_1 + \Sigma S_1S_2C_3 \dots C_kL_{12} + \dots \\ \dots + S_1S_2 \dots S_kL_{12} \dots k$$

with

$$C_j = \cosh 2\sqrt{\lambda_j}, \quad S_j = \sinh 2\sqrt{\lambda_j}.$$

In a typical item such as $S_1S_2C_3S_4C_5C_6S_7C_8L_{1247}$ the L symbol L_{1247} is to be read as the function

$$\left(\frac{\sqrt{\lambda_1} \sqrt{\lambda_4}}{\sqrt{\lambda_2} \sqrt{\lambda_7}} + \frac{\sqrt{\lambda_2} \sqrt{\lambda_7}}{\sqrt{\lambda_1} \sqrt{\lambda_4}} \right).$$

Similarly L_{34568} in $C_1C_2S_3S_4S_5S_6C_7S_8C_9C_{10}L_{34568}$ is

$$\left(\frac{\sqrt{\lambda_3} \sqrt{\lambda_5} \sqrt{\lambda_8}}{\sqrt{\lambda_4} \sqrt{\lambda_6}} + \frac{\sqrt{\lambda_4} \sqrt{\lambda_6}}{\sqrt{\lambda_3} \sqrt{\lambda_5} \sqrt{\lambda_8}} \right).$$

The multiple segmental recombination fractions can be calculated from the 2^n quantities obtained from P by substituting values $+1$ or -1 for the λ 's (5). For example, if we set

$$\lambda_{n_1} = \lambda_{n_2} = \dots = \lambda_{n_r} = -1$$

and all other λ 's equal to $+1$, the resulting value of P is equal to

$$P_{n_1n_2 \dots n_r} = 1 - 2\gamma_{n_1+n_2+\dots+n_r}$$

where $\gamma_{n_1+n_2+\dots+n_r}$ is the probability that the sum of the numbers of exchange points in the segments $x_{n_1}, x_{n_2}, \dots, x_{n_r}$ will be odd.

It is easy to verify the elementary identity

$$1 - 2\gamma_{n_1+n_2+\dots+n_r} = 1 - 2\Sigma \gamma_{n_1} + 4\Sigma \gamma_{n_1n_2} - \dots + (-1)^r 2^r \gamma_{n_1n_2 \dots n_r},$$

where $\gamma_{n_1n_2}$ is the probability that segments x_{n_1} and x_{n_2} will show simultaneous recombination, and so on. Thus the segmental recombination fractions $\gamma_{n_1n_2}, \gamma_{n_1n_2n_3}, \dots, \gamma_{12 \dots k}$ can be calculated inductively.

For example, with 3 adjacent segments we have

$$\begin{aligned} 1 - 2(\gamma_1 + \gamma_3) + 4\gamma_{13} &= 1 - 2\gamma_{1+3} = P_{13} = P(-1, 1, -1) \\ &= e^{-(D_1 + D_3)} (\cos D_1 \cos D_3 - e^{-2D_2} \sin D_1 \sin D_3) \\ &= (1 - 2\gamma_1)(1 - 2\gamma_3) - 4z_1z_3e^{-4x_2}. \end{aligned}$$

Thus

$$\gamma_{13} = \gamma_1\gamma_3 - z_1z_3e^{-4x_2}.$$

Similarly

$$\begin{aligned} & 1 - 2(\gamma_1 + \gamma_2 + \gamma_3) + 4(\gamma_{12} + \gamma_{23} + \gamma_{13}) - 8\gamma_{123} \\ &= 1 - 2\gamma_{1+2+3} = P_{123} = P(-1, -1, -1) \\ &= e^{-(D_1+D_2+D_3)}(\cos D_1 \cos D_2 \cos D_3 - \sin D_1 \sin D_2 \cos D_3 \\ &\quad - e^{-2D_1} \sin D_1 \cos D_2 \sin D_3 - \cos D_1 \sin D_2 \sin D_3) \end{aligned}$$

which ultimately gives the value of γ_{123} .

4. FORMULÆ IN TERMS OF RECOMBINATION FRACTIONS ONLY

The substitutions

$$\begin{aligned} e^{-4x_r} &\triangleq (1 - 2\gamma_r)/(1 + 2\gamma_r) \\ z_r z_s &\triangleq (1 - 2\gamma_r)(1 - 2\gamma_s)/(1 + 4\gamma_r \gamma_s) \end{aligned}$$

give formulæ generating those of Kosambi (1) and Owen (7). With $(k+1)$ linked loci they could be used for fitting the k parameters $\gamma_1, \gamma_2, \dots, \gamma_k$ to observed recombination data by maximum likelihood scoring.

5. THE AUXILIARY QUANTITY z

$$z = \frac{1}{2}e^{-2x} \sin 2x$$

is the imaginary part of w where

$$1 - 2w = e^{-2x(1+i)},$$

so that $w = y + iz$ satisfies Trow's formula for adjacent segments at mid-arm.

i.e.

$$w_{1+2} = w_1 + w_2 - 2w_1 w_2$$

Thus the effect of interference is formally removed for adjacent segments by the use of w , which may be called the *complex recombination fraction* or "*complexit*", its components y and z being named respectively the "*recombit*" and the "*cocombit*".

For short segments we have the expansions

$$y = \frac{1}{2}(1 - e^{-2x} \cos 2x) = x - \frac{4}{3}x^3 + \dots$$

as in Kosambi's formula.

$$z = \frac{1}{2}e^{-2x} \sin 2x = x - 2x^2 + \frac{4}{3}x^3 + \dots$$

$$x = y + \frac{4}{3}y^3 + \dots$$

Therefore

$$z = y - 2y^2 + \frac{8}{3}y^3 + \dots$$

$$= y(1 - 2y)/(1 - \frac{8}{3}y^2) + o(y^4).$$

The second order approximation to the cocombit is therefore $y(1 - 2y)$, but its use is rather less effective than that of the approximation for $z_r z_s$. It may be noted, however, that a good second order approxima-

tion is provided by $y(1 - 1.56y)$. The following table gives percentage values of x (cM), of y and of $\frac{1}{2} \tanh 2x$ and also of z and of $y(1 - 1.56y)$.

x cM	$\frac{1}{2} \tanh 2x$	y	z	$y(1 - 1.56y)$
0	0	0	0	0
5	4.984	4.984	4.517	4.597
10	9.869	9.879	8.133	8.357
15	14.566	14.614	10.946	11.282
20	18.998	19.130	13.052	13.421
25	23.106	23.386	14.540	14.854
30	26.852	27.352	15.494	15.681
35	30.218	31.010	15.995	16.009
40	33.202	34.348	16.116	15.943
45	35.815	37.364	15.924	15.585
50	38.080	40.062	15.478	15.025
55	40.025	42.451	14.833	14.339
60	41.683	44.543	14.036	13.591
65	43.086	46.355	13.130	12.834
70	44.268	47.904	12.150	12.105
75	45.257	49.211	11.129	11.432
80	46.084	50.029	10.091	10.983
85	46.770	51.175	9.058	10.320
90	47.340	51.894	8.049	9.883
95	47.812	52.462	7.077	9.527
100	48.201	52.904	6.153	9.242

6. LOCI PROXIMAL TO CENTROMERE ON A LONG ARM

It is easy to derive results applying to segments on a long arm which are at finite distances from the centromere. We replace the map distance x , wherever it appears in the existing formulæ, by the interference metric t and treat the centromere as having the properties of a localised exchange point (4, 5).

Consider 3 adjacent segments 0, 1, 2 which have the metrical specification $(0, t_0)$, (t_0, t_1) , (t_1, t_2) . We have

$$y_{02} = y_0 \eta_2 - z_0 \zeta_2 e^{-4D_1}$$

where

$$\eta = \frac{1}{2}(1 - e^{-2x} \cos 2D), \quad \zeta = \frac{1}{2}e^{-2D} \sin 2D$$

and D is now metrical length, *i.e.* $D_1 = (t_1 - t_0)$.

The probability of recombination in 2 conditional on recombination in 0 is

$$y_{02}/y_0 = \eta_2 - e^{-4D_1} \zeta_2 (z_0/y_0).$$

The limit of this probability as $t_0 \rightarrow 0$ is clearly the probability of recombination in 2 conditional on there being an exchange point at $t = 0$, and therefore gives the recombination over a segment (t_1, t_2) at metrical distance t_1 from the centromere as

$$y_2 = \eta_2 - e^{-4t_1} \zeta_2$$

as given by Owen (4).

Similarly for simultaneous recombination in separated intervals we have

$$\mathcal{Y}_{13} = \eta_1\eta_3 - \zeta_1\zeta_3 e^{-4D_1} - e^{-4t_1} \{ \zeta_1\eta_3 + \zeta_3\eta_1 e^{-4D_1} - \frac{1}{2}\zeta_3(1 - e^{-4D_1})e^{-4D_3} \}.$$

Also

$$\begin{aligned} \mathcal{Y}_{123} = & \eta_1\eta_2\eta_3 - (\eta_1\zeta_2\zeta_3 + \eta_2\zeta_1\zeta_3 + \eta_3\zeta_1\zeta_2) + \frac{1}{2}\zeta_1\zeta_3(1 - e^{-4D_1}) \\ & - e^{-4t_1} \{ \eta_1\eta_2\zeta_3 + \eta_2\eta_3\zeta_1 + \eta_1\eta_3\zeta_2 - \zeta_1\zeta_2\zeta_3 \\ & - \frac{1}{2}\eta_1\zeta_3(1 - e^{-4D_1}) - \frac{1}{2}\eta_2\zeta_3(1 - e^{-4D_1}) \\ & - \frac{1}{2}\zeta_2\eta_3(1 - e^{-4D_1}) - \frac{1}{4}\zeta_3(1 - e^{-4D_1})(1 - e^{-4D_3}) \}. \end{aligned}$$

7. SUMMARY

Generalisations of Kosambi's formulæ are obtained serving to give the frequencies of multiple recombination in as many as 5 adjacent or separated segments. The formulæ are expressed in terms of recombination fractions for single segments and auxiliary quantities z . It is shown how to obtain complete generalisations of Kosambi's formulæ, expressed purely in terms of the recombination fractions.

III. ELEMENTARY FORMULÆ FOR MULTIPLE RECOMBINATION OF CLOSELY LINKED LOCI

I. INTRODUCTION

It has been seen that for contiguous segments 1 and 2 Kosambi (1) gives the useful addition formula

$$\mathcal{Y}_{1+2} = (\mathcal{Y}_1 + \mathcal{Y}_2) / (1 + 4\mathcal{Y}_1\mathcal{Y}_2)$$

connecting the recombination fractions $\mathcal{Y}_1$, $\mathcal{Y}_2$ and $\mathcal{Y}_{1+2}$ in the segments 1 and 2 and the joint segment 1+2. This has been found to be substantially correct for long segments in the mouse (10), in rice (11), and in the sweet pea (12) and to hold for loci situated medianly in the arms of chromosomes VI and III in *Drosophila melanogaster* (2). Theoretical reasons have been advanced why this should be so (5, 7).

Since the frequency of simultaneous recombination is given always by

$$\mathcal{Y}_{12} = \frac{1}{2}(\mathcal{Y}_1 + \mathcal{Y}_2 - \mathcal{Y}_{1+2})$$

it follows from Kosambi's formula that the frequency of doubles is given by

$$\mathcal{Y}_{12} = 2\mathcal{Y}_1\mathcal{Y}_2(\mathcal{Y}_1 + \mathcal{Y}_2) / (1 + 4\mathcal{Y}_1\mathcal{Y}_2)$$

It was shown above that for non-contiguous segments an appropriate generalisation of this result is

$$\mathcal{Y}_{13} = \mathcal{Y}_1\mathcal{Y}_3 \left\{ 1 - \frac{1 - 2\mathcal{Y}_2}{1 + 2\mathcal{Y}_2} \frac{(1 - 2\mathcal{Y}_1)(1 - 2\mathcal{Y}_3)}{1 + 4\mathcal{Y}_1\mathcal{Y}_3} \right\}.$$

For short segments when y_1, y_2, y_3 are small, if we retain only terms of the third order we get the formulæ

$$y_{12} = 2y_1y_2(y_1 + y_2), \quad y_{13} = 2y_1y_3(y_1 + 2y_2 + y_3).$$

Similar formulæ could be got for the frequencies of triple, quadruple crossing over, etc. using the previous results. However, Professor Sir Ronald Fisher suggested to me that they could be derived rather simply by a completely elementary method, which may be illustrated by the case of 4 linked loci (3 contiguous segments).

We note that if segment 3 is so short that y_3 may be replaced by the differential dy then the probability of simultaneous recombination in segment 1 and the segment dy is

$$y_{13} = 2y_1(y_1 + 2y_2)dy \quad . \quad . \quad . \quad (1)$$

Equation (1) also implies that the probability of recombination in 1 conditional on recombination in dy is

$$y_{13}/dy = 2y_1(y_1 + 2y_2) \quad . \quad . \quad . \quad (2)$$

Results (1) and (2) enable the frequencies of simultaneous recombination in any number of short contiguous segments to be calculated inductively provided that a further simple assumption is made. This assumption is in accordance with recombination theory as previously developed. We suppose that a cross-over point once established effectively divides the chromosome into non-interfering regions.

Let now p, q, r be the frequencies of recombination in contiguous segments AB, BC, CD supposed short but not infinitesimal. Consider an elementary segment dy located at a point P interior to BC. Then to the present approximation the recombination over BP is y and over PC is $(q - y)$. By (1) the probability of simultaneous recombination in AB and dy is

$$2p(p + 2y)dy.$$

But by (2) the probability of recombination in CD conditional on recombination in dy is

$$2r(r + 2q - y).$$

Thus the probability of recombination simultaneously in AB, dy and CD is

$$4pr(p + 2y)(r + 2q - y)dy.$$

It is now easy to see that, on account of the assumed smallness of p, q and r , the probability of simultaneous recombination y_{12} in AB, BC and CD is merely

$$\int_0^q 4pr(p + 2y)(r + 2q - y)dy$$

which is

$$4pr\{(p + q)(q + r) - \frac{1}{3}q^2\}.$$

The results of this method are assembled for 4, 5 and 6 loci in section 3. It will be seen that a parameter K has been introduced. This will be discussed below.

2. THE KOSAMBI COEFFICIENT

The Kosambi coefficient K for three linked loci is defined as

$$K = \frac{y_1 + y_2 - y_{1+2}}{4y_1y_2y_{1+2}} = \frac{y_{12}}{2y_1y_2y_{1+2}}.$$

The value of K is in general different from unity. K is equal to unity, if and only if, Kosambi's formula holds exactly. The value of K as estimated from 3 point data may be used to test agreement with Kosambi's formula. When K is significantly less than unity then the genetical interference which is operating must be concluded to be more intense than that predicted by Kosambi's formula. The opposite conclusion is indicated by an observed K significantly exceeding unity. Reasons have been given (6) for supposing that in regions near the centromere K is large, and thereafter diminishes steadily becoming less than unity in regions near the terminus of a chromosome arm. At some intermediate region (actually near metrical mid-arm) K is approximately unity and Kosambi's formula will apply there. These expectations are confirmed by the data on Chromosomes II and III of *Drosophila melanogaster* (2). Thus an observed value of K is some indication of the location of the triplet of loci on the chromosome arm.

Inclusion of the parameter K modifies the formulæ for short segments in a simple way and confers generality upon them.

If K is defined as above then we have identically for the frequency of doubles

$$y_{12} = 2Ky_1y_2y_{1+2} = 2Ky_1y_2(y_1+y_2)/(1+4Ky_1y_2),$$

which for short segments is effectively

$$y_{12} = 2Ky_1y_2(y_1+y_2) = 2Ky_1(y_1y_2+y_2^2)$$

Now suppose that the segment y_2 is increased to be y_2+dy by the addition of an elementary segment dy . Then the resulting increase in y_{12} is given by

$$dy_{12} = 2Ky_1(y_1+2y_2)dy + 2y_1y_2(y_1+y_2)\frac{\partial K}{\partial y_2}dy$$

The second term is of the third degree in y_1, y_2 and consequently may be neglected in the present approximation. Thus

$$dy_{12} = 2Ky_1(y_1+2y_2)dy$$

and the probability of simultaneous recombination in AB and dy becomes

$$2Kp(p+2y)dy,$$

K appearing merely as a multiplier.

To the present order of accuracy therefore, K is to be regarded as a constant over the total region occupied by the segments and the formulæ are merely multiplied by powers of K .

3. FORMULÆ

(a) *Notation.*— p, q, r, s, t , denote contiguous segments taken in order. It is convenient to define the following symbols.

$$(p, q, r) = (p+q)(q+r) - \frac{1}{3}q^2$$

$$(p, q, r, s) = (p+q)(q+r)(r+s) - \frac{1}{3}q^2(r+s) - \frac{1}{3}r^2(p+q)$$

$$(p, q, r, s, t) = (p, q, r)(r, s, t) - \frac{1}{3}r^2(p+q)(r+t)$$

(b) *4 loci (3 segments).*—

$$y_{12} = 2Kpq(p+q), \quad y_{23} = 2Kqr(q+r)$$

$$y_{13} = 2Kpr(p+2q+r)$$

$$y_{123} = 4K^2pqr(p, q, r)$$

(c) *5 loci (4 segments).*—

$$y_{12} = 2Kpq(p+q), \quad y_{13} = 2Kpr(p+2q+r)$$

$$y_{14} = 2Kps(p+2q+r+s), \quad y_{23} = 2Kqr(q+r),$$

$$y_{24} = 2Kqs(q+s), \quad y_{34} = 2Krs(r+s),$$

$$y_{234} = 4K^2qrs(q, r, s), \quad y_{123} = 4K^2pqr(p, q, r),$$

$$y_{124} = 4K^2pqs\{(p+q)(q+2r+s) - \frac{1}{3}q^2\},$$

$$y_{134} = 4K^2prs\{(r+s)(p+2q+r) - \frac{1}{3}r^2\},$$

$$y_{1234} = 8K^3pqrs(p, q, r, s).$$

It will be seen that with the exception of the last three, these expressions result *mutatis mutandi* from the appropriate 3-point formulæ. For instance y_{14} is derived from $2Kpr(p+2q+r)$ by the substitutions s for r , $q+r$ for q ($q+r$ being to the present degree of approximation the recombination in the separating segment $(2+3)$). The formulæ for y_{124} and y_{134} contain nothing essentially new being calculated from the relations

$$y_{124} = y_{123+4} - y_{123}, \quad y_{134} = y_{1+234} - y_{234},$$

which hold in the present approximation. Their form shows them however to be derivable by a simple rule. We may write

$$y_{124} = 4K^2pqs(p, q, s)^1$$

where $(p, q, s)^1$ results from (p, q, s) by inserting in the item $(q+s)$ the term $2r$ corresponding to the separating segment r .

(d) *6 loci (5 segments).*—Formulæ for two segments such as

$$y_{15} = 2Kpt(p+2q+2r+2s+t)$$

follow trivially from the previous results.

The formulæ for three or four segments follow either directly or by application of the rule for separating segments, *e.g.*

$$\begin{aligned} \mathcal{Y}_{125} &= 4K^2 pqt(p, q, t)^1 = 4K^2 pqt\{(p+q)(q+2r+2s+t) - \frac{1}{3}q^2\}, \\ \mathcal{Y}_{135} &= 4K^2 prt(p, r, t)^1 = 4K^2 prt\{(p+2q+r)(r+2s+t) - \frac{1}{3}r^2\}, \\ \mathcal{Y}_{1235} &= 8K^3 pqrt(p, q, r, t)^1 \\ &= 8K^3 pqrt\{(p+q)(q+r)(r+2s+t) - \frac{1}{3}q^2(r+2s+t) - \frac{1}{3}r^2(p+q)\}, \\ \mathcal{Y}_{1245} &= 8K^3 pqs(p, q, s, t)^1 \\ &= 8K^3 pqs\{(p+q)(q+2r+s)(s+t) - \frac{1}{3}q^2(s+t) - \frac{1}{3}s^2(p+q)\}. \end{aligned}$$

The formula for 5 segments is essentially new and can be found only by integration.

$$\mathcal{Y}_{12345} = 16K^4 pqrst(p, q, r, s, t)$$

4. VERIFICATION

The expression

$$\mathcal{Y}_{123} = \mathcal{Y}_1 \mathcal{Y}_2 \mathcal{Y}_3 - \mathcal{Y}_1 \mathcal{Z}_2 \mathcal{Z}_3 - \mathcal{Y}_2 \mathcal{Z}_1 \mathcal{Z}_3 - \mathcal{Y}_3 \mathcal{Z}_1 \mathcal{Z}_2 + \frac{1}{2} \mathcal{Z}_1 \mathcal{Z}_2 \mathcal{Z}_3 (1 - e^{-4x_1}),$$

given previously (loc. cit.) reduces to the form $4pqr(p, q, r)$ on putting

$$\begin{aligned} z &= y - 2y^2 + \frac{8}{3}y^3, \\ e^{-4x} &= 1 - 4y + 8y^2 - 16y^3 + \dots = (1 - 2y)(1 + 2y) \\ &= \exp\left\{-4\left(y + \frac{8}{3}y^3\right)\right\} + o(y^4), \end{aligned}$$

and retaining only terms of the fifth degree. This serves as a mutual check of both formulæ.

A similar comparison may be made with the generalised Kosambi formula given by the writer (loc. cit.) namely

$$\mathcal{Y}_{123} = p\mathcal{Y}_{23} - q\mathcal{Y}_{13} + r\mathcal{Y}_{12}$$

with $\mathcal{Y}_{12}, \mathcal{Y}_{13}$ given by Kosambi's formula and

$$\mathcal{Y}_{13} = pr \left\{ 1 - \frac{1-2q}{1+2q} \cdot \frac{(1-2p)(1-2r)}{1+4pr} \right\}.$$

With small segments we have correct to the 5th degree

$$\mathcal{Y}_{123} = 4pqr \cdot 2q(p+q+r).$$

Consequently the generalised Kosambi formula implies rather a higher frequency of triples (about 50 per cent. more). This is because it is essentially a quadratic approximation and not a cubic one.

5. SUMMARY

An elementary method is developed by which the frequencies of simultaneous recombination of several closely linked loci can be specified, and formulæ are given for as many as 6 such loci.

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