INTERPRETATION OF DATA OBTAINED WITH RECOMBINATION-DEFECTIVE MEIOTIC MUTANTS

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ABSTRACT

According to probability laws, additive interaction between factors influencing recombination frequency suggests that the factors affect the same event. This analysis, applied to CARPENTER and SANDLER's (1974) data, permits separation of different sequential events during establishment of preconditions for exchange. The validity of another mathematical model for meiotic mutant characterization, suggested by those authors, is discussed.

A number of recessive meiotic mutants with altered recombination properties are now known in *D. melanogaster*. From these properties, inferences can be made about the normal processes governed by wild-type alleles of the mutants studied. SANDLER *et al.* (1968) suggested that a "flow-chart" illustrating the normal course of meiosis could be arranged in this way. They also formulated a most important model for meiotic mutant analysis. According to that model, meiotic mutants changing coincidence affect preconditions for exchange, while those where coincidence is unaltered should presumably affect the actual exchange.

CARPENTER and SANDLER (1974) discuss three further criteria for mutant characterization. These are (1) uniform or non-uniform alteration of recombination frequency along chromosome arms, where uniformity indicates an exchange mutant and non-uniformity a precondition mutant, (2) degree of dominance, which presumably provides no information on whether the mutant studied affects preconditions or exchange, and (3) susceptibility or non-susceptibility to interchromosomal effects of heterozygous, heterologous inversions (which is not interpreted by CARPENTER and SANDLER).

These criteria refer to the many mutants which affect recombination in all chromosomes (see SANDLER *et al.* 1968; and BAKER and CARPENTER 1972). If mutants affecting a limited region in the genome (VALENTIN 1973a) are also considered, two additional criteria appear relevant: (4) recombination affected in all chromosomes or in one of them only (the latter case suggests specific homologous pairing is defective), and (5) presence or absence of major segregation anomaly (the former case may involve "distributive" or completely random segregation).

Furthermore, the *amount* of manifestation of the interchromosomal effect, or any effect, can as discussed below provide considerable information regarding the place of action of a particular meiotic gene in a "flow-chart".

CARPENTER and SANDLER (1974) also suggest a more elaborate mathematical model of meiosis. This is aimed to explain (a) mutants that change recombination non-uniformly, suggesting preconditions are affected, but still do not change coincidence, and (b) the scarcity of mutants reducing coincidence. For reasons set out below, we believe that this new model is less useful than the general model of SANDLER *et al.* (1968).

INTERACTION BETWEEN FACTORS

The model of SANDLER *et al.* (1968) assumes that a chromatid exchange depends on (at least) two sequential events, *viz.* establishment of preconditions, and the actual exchange. Consider two adjacent, small chromosome regions. Probabilities of these two events in these regions are defined as $a = P$ (preconditions established in region A only), $b = P$ (ditto, region B only), $d = P$ (ditto, both regions) and $x = P$ (exchange), the conditional probability of exchange where preconditions are established. The differentiation of precondition probabilities into a , b and d is not directly necessary for the reasoning here, but will be used later in the paper.

Consider first recombination in one region, the A region, only. The recombination frequency in this region is the joint probability of preconditions and exchange, i.e. $RF_A = x(a+d)$.

Being a probability, this follows the elementary rules that alternative probabilities are additive, as a and d , while independent probabilities should be multiplied, as x and $(a+d)$.

If one factor, as a *mei-* locus, changes recombination, it may affect preconditions or exchange. According to probability theory, the effect of the factor is always *added* at the level where it *truly* acts. For instance, preconditions may become established at a lower (or higher) number of sites along the chromosome, leading to the new precondition probability for region A, $(a+d+f_1)$, where f_1 may be negative or positive.

If two factors, f_1 and f_2 , affect recombination simultaneously, two simple alternatives are conceivable. The addition outlined above may refer to the same event for both factors, or to one event for each factor. In the former case, RF_A becomes $x(a+d+f_1+f_2)$ or $(x+f_1+f_2)(a+d)$. In the latter case, $RF_A = (x+f_1)(a+d+f_2)$ or $(x+f_2)(a+d+f_1)$.

In other words, if the two factors affect the same event, additive interaction is expected, but if they affect different events, proportional interaction should occur.

Of course, the situation may be more complex in a living system. For instance, one factor could affect both preconditions and exchange simultaneously, or one factor could interfere with the action of another factor. However, if good additivity or proportionality is observed, the simplest possible interpretation is that this is due to the factors examined affecting (if additive) the same event, or, (if proportional), different events in the formation of a chromatid exchange.

Table 1 describes properties of some factors affecting recombination frequency. Table 2 shows a selection of data on interaction between these factors distally in the X chromosome of *D. melanogaster* that illustrates the reasoning.

TABLE 1

Some properties of certain factors influencing recombination frequency

Ref.	Factor	Recombination	Nondisjunction	Coincidence	Interpretation
Mutant genes:					
1	<i>abo</i>	Decreased non-uniformly, all chromosomes	High level	Unaltered	Preconditions affected
1	<i>mei-9</i>	Decreased uniformly, all chromosomes	High level	Unaltered (?)	Exchange affected
2	<i>mei-1</i>	Decreased non-uniformly, X chromosome only	Nearly absent	Unaltered (or decreased?)	Preconditions affected
1	<i>mei-218</i>	Decreased non-uniformly, all chromosomes	High level	Increased	Preconditions affected
3	<i>mei-S282</i>	Decreased non-uniformly, all chromosomes	High level	Increased	Preconditions affected
Other factors:					
4	Interchromosomal effects of inversions	Increased non-uniformly, all heterologs	Varies with conditions	Increased	Preconditions affected
5	X-rays	Decreased distally, increased proximally, all chromosomes	Some	Unknown	Preconditions affected distally, exchange affected proximally

References: 1: CARPENTER and SANDLER (1974); 2: VALENTIN (1973a); 3: PARRY (1973); 4: LUCCHESI and SUZUKI (1968); 5: BATEMAN and CHANDLEY (1965).

Using data from controls and from series with one factor present, expected values for two-factor series were calculated on assumptions of additivity or proportionality, and then compared with the actual observations. Unfortunately, no really suitable statistical method is available for distinction between the alternatives. As a first approximation, we have therefore obtained empirical variances for "observed" and "expected" two-factor series, using computer simulation. With the aid of these variances, t-tests of "observed" *vs.* the two "expected" alternatives were possible.

The interaction between the meiotic mutant, *abo*, and interchromosomal effects is complex (Table 2). Presumably, both factors affect preconditions, but in the simultaneous presence of *abo* and inversions, recombination frequency is much higher than expected either on an additive or a proportionality basis. In this complex situation the additivity/proportionality is of little help.

A different picture emerges for *mei-9*. This mutant shows a reasonably good proportionality in its interaction with inversions (the proximal regions, not

TABLE 2
Examples of joint effects of two factors on recombination frequency in Drosophila melanogaster

Ref.	Region	Factors		Recombination frequencies (%)				Expectations		Interpretation
		f_1	f_2	Control	f_1	f_2	$f_1 + f_2$	Additive	Proportional	
1	<i>pn-v</i>	<i>abo</i>	SR†	32.2	18.5	37.2	32.5	23.5***	21.4***	Complex
1	<i>pn-cv</i>	<i>mei-9</i>	SR	11.9	1.17	25.1	2.64	14.4***	2.46	Proportional
1	<i>cv-m</i>	<i>mei-9</i>	SR	26.5	2.62	34.5	5.03	10.6**	3.41***	Complex
2	<i>w^d-cl⁶</i>	<i>mei-1</i>	X-rays‡	20.4	7.69	16.2	3.49	3.49	6.11***	Additive
2	<i>ec-cl⁶</i>	<i>mei-1</i>	SR	18.6	3.65	24.2	5.79	9.25**	4.75	Proportional
2	<i>cl⁶-v</i>	<i>mei-1</i>	SR	14.1	2.78	16.5	5.14	5.18	3.25***	Additive
1	<i>pn-cv</i>	<i>mei-218</i>	SR	9.27	0.302	22.9	0.674	13.9***	0.746	Proportional
1	<i>cv-v</i>	<i>mei-218</i>	SR	21.2	0.655	32.3	0.990	11.8***	1.000	Proportional
3	<i>pn-cv</i>	<i>mei-S282</i>	SR	11.4	6.68	18.7	7.78	14.0***	11.0	Proportional
3	<i>cv-m</i>	<i>mei-S282</i>	SR	22.6	14.4	26.1	14.2	17.9*	16.6	Proportional

References: 1: CARPENTER and SANDLER (1974); 2: VALENTIN (1973a); 3: PARRY (1973).
 † SR: Interchromosomal ("Schultz-Redfield") effect of heterologous, heterozygous inversion (s).
 ‡ X-rays: days 10-12 after treatment of females with 1800 r.
 Asterisks denote conventional significance levels for comparisons of expected frequencies with observations in $(f_1 + f_2)$ series.

shown in Table 2, give perfect agreement with proportionality regarding $m-f$, but a significant difference at the $P=0.05$ level for $f-\gamma^+$. It should be noted that the empirical variances behind these significances take stochastic but not biological variation into account—which means that the significance tests are slightly too rigid). Obviously, the proportional interaction observed is exactly what would be predicted since one factor should affect exchange, the other one preconditions. CARPENTER and SANDLER (1974) have instead suggested that inversions have no real effect in the *mei-9* series. That *mei-9* plus inversions cause a higher recombination frequency than *mei-9* alone does is by them attributed to nonrandom elimination of noncrossover strands. In contrast, the present interpretation suffices to explain the results without resort to the hypothesis of differential gamete recovery.

Both *mei-1* and X-rays should affect preconditions distally. Therefore, an additive interaction between them is expected. This is also observed (Table 2).

The interaction between *mei-1* and inversions should also be additive. This is observed for the *ct-v* region. Interaction is altered as one moves toward either chromosome end, and is proportional at telomere and centromere. In part, this could be due to effects of genes within the particular inversion chromosome used (see VALENTIN 1973a).

On the other hand, *mei-218* is believed to be a precondition mutant, but the joint effect of *mei-218* and inversions agrees very well with a proportional model, indicating that *mei-218* and inversions affect different events.

This is by no means a contradiction to the suggestion that both *mei-218* and inversions affect preconditions. It appears most probable that preconditions may themselves be broken down into a sequence of consecutive events (see, e.g., MOENS 1973). One such event could, as pointed out by VALENTIN (1973b), be specific pairing in homologous regions, mediated by specific pairing proteins perhaps as suggested by COMINGS and RIGGS (1971).

Finally, the recombination frequencies obtained with *mei-S282* and inversions are not significantly different from expectations on a proportional model. However, for all regions (including those not shown in Table 2), the observed frequencies are somewhat lower than the expected ones, so that cautious interpretation is warranted.

Thus, analysis of additivity/proportionality resolves "preconditions" into (at least) two steps, where one is the target for inversions, X-rays and *mei-1*, and another is the target for *mei-218* and possibly *mei-S282*.

CARPENTER AND SANDLER'S NEW MODEL

SANDLER *et al.*'s (1968) model, the parameters of which were defined above, leads to a coincidence of

$$C = \frac{x^2 d}{x(a+d) x(b+d)} = \frac{d}{(a+d)(b+d)}.$$

Thus recombination frequency is a function of both precondition and exchange

probabilities, but coincidence is a function of precondition probabilities only. Therefore, mutants affecting exchange should not disturb coincidence.

CARPENTER and SANDLER (1974) point out that coincidence is usually increased, not decreased, when at all affected. They also observe that coincidence remains unchanged in *abo*, a mutant which probably affects preconditions. To explain these observations, they suggest a more complex formula for coincidence. This assumes that preconditions are established in a polar way: proximal regions would tend to establish preconditions "before", or "more than", distal regions. It is unfortunate that the validity of this cytogenetic assumption is not discussed by them, since there are strong indications that chromosomes, at least in some respects, do not follow such a polar precondition establishment pattern (RAMEL 1962).

Accepting, however, the primary assumption for the moment, the following probabilities are defined by CARPENTER and SANDLER (1974): $\gamma = P$ (preconditions established in *neither* of two segments), $x = P$ (preconditions in proximal segment only) and $(1-x-\gamma) = P$ (preconditions in both segments); and conditional probabilities $p = P$ (exchange proximally), $d = P$ (exchange distally) and $m = P$ (exchange both segments). It is noteworthy that the definition of m as something possibly different from p or d must imply that interference may be a property of exchange, not only preconditions!

Using these probabilities, recombination frequencies and coincidence can be calculated. They are

$$\begin{aligned} RF_{\text{dist}} &= (1-x-\gamma) (d+m) \\ RF_{\text{prox}} &= (1-\gamma)p + (1-x-\gamma)m \\ C &= \frac{(1-x-\gamma)m}{(1-x-\gamma) (d+m) [(1-\gamma)p + (1-x-\gamma)m]} \end{aligned}$$

CARPENTER and SANDLER (1974), who obtained a slightly different result, claim that C becomes independent of x if $\gamma = 0$. In other words, if the mutant investigated is "weak", so that establishment of preconditions always occurs at least proximally, coincidence would not be affected by the altered distal conditions. Their model would thus provide an explanation to precondition mutants not affecting coincidence. Furthermore, if both $x \neq 0$ and $\gamma \neq 0$, coincidence would always be increased.

However, as can be seen here, the correct C formula does depend on x even if $\gamma = 0$.

This mathematical model therefore does not explain one of the phenomena (precondition mutants without coincidence effects) for which it was suggested, and furthermore rests on cytogenetic assumptions which are not self-evident and need not be universally valid.

Constant coincidence with precondition mutants may instead be explained along the "old" model of SANDLER *et al.* (1968) if it is assumed that d (the probability of preconditions established in two regions) can change at a rate other than a and b (preconditions in regions A and B respectively), as stated in VALENTIN (1973a).

The "old" model, while idealized, makes no controversial cytogenetic assumption and has proven a useful tool for characterization of meiotic mutants. We thus believe that the "old" model of the SANDLER group is of more general validity than the new one.

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