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Note

On Spo16 and the Coefficient of Coincidence

Franklin W. Stahl[1] and Henriette M. Foss

Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403-1229

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ABSTRACT

spo16 mutants in yeast were reported to have reduced map lengths, a high frequency of nondisjunction in the first meiotic division, and essentially unchanged coefficients of coincidence. Were all crossing over in yeast subject to interference, such data would suggest that the "designation" of recombination events to become crossovers is separable from the "implementation" of that crossing over. In the presence of coexisting interference and noninterference phases of crossing over, however, lack of change in the coefficient of coincidence may show only that *spo16* reduces crossing over in the two phases by a similar factor.

Be careful. Coefficient of coincidence is a slippery concept.
A. H. Sturtevant to a young geneticist

Shinohara *et al.* (2008) report on *spo16* strains of yeast with meiotic linkage map distances that are 0.32–0.73 of the wild-type values. These mutant strains had approximately wild-type values for indicators of interference, which, like the three-factor coefficient of coincidence (C_3), assess the effect of crossing over in one interval on crossing over in an adjacent interval.

Because Shinohara *et al.* (2008) detected no appreciable change in the indicators of interference accompanying the *spo16*-induced reduction in linkage distances, they concluded that *spo16* affects the "implementation" of crossing over and, in so doing, eliminates any hypothetical "assurance" of at least one crossover, without disturbing the "designation" of sites that, in wild type, would have received a crossover. They point out that their observation is compatible with a "stress" model for interference. This assertion caught our interest because of the implied possibility that the observation might be incompatible with a counting model for interference (*e.g.*, Foss *et al.* 1993).

In this note we first indicate a version of a counting model (aka chi-square, gamma, or Erlang model) that, too, is compatible with the observation of Shinohara *et al.* (2008), and we then discuss the possibility that their observation has little bearing on models for interference or on issues of designation and implementation.

The expectations for *spo16* under a counting model: The counting model proposed by Foss *et al.* (1993) hypothesized that double-strand breaks (DSBs) occur independently of each other and that there will (ordinarily) be a fixed number of noncrossovers between adjacent crossovers. In one development of the model, counting is achieved by "sweeping" adjacent DSBs, or precursor structures, into clusters of fixed size, with one particular position in the cluster designated to yield a crossover (Stahl 1993; Stahl *et al.* 2004). Below, we show that the lack of a *spo16*-induced phenotype with respect to indicators of interference is as expected of such a system, as long as the *spo16*-induced reduction in linkage distances represents random loss of designated crossovers.

For simplicity, our indicator of interference in this section is the factor by which the presence of a crossover in interval 1 reduces the map length of adjacent interval 2 from its value in the total population, *i.e.*, (crossover frequency in interval 2 among crossovers in interval 1)/(crossover frequency in interval 2 in the total population). (Since, in the *spo16* mutant, DSBs are not diminished and appear to be repaired, we presume that a fraction of the DSBs that, in wild type, would have been repaired as crossovers will, in the mutant, be repaired as noncrossovers or by intrachromosomal repair.) For a *spo16* mutant eliminating about half the crossovers, a DSB that had been destined by position in the cluster (or some other mechanism) to give an interhomolog crossover now has a probability of about one-half of actually doing so—crossover designation occurs but implementation fails about half the time. As long as the distribution of such failures among designated DSBs

[1]*Corresponding author:* Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229. E-mail: fstahl@uoregon.edu

is random, *i.e.*, independent of the presence or absence of a crossover in interval 1, the mutation will reduce the map length of interval 2 to about one-half that of wild type both among recombinants for interval 1 and in the total population, leaving the measured coefficient-of-coincidence-like indicator unchanged.

We conclude that the observation made by Shinohara *et al.* (2008) is compatible with such a version of the counting model, as it is likely to be with any model in which designation and implementation are separable events. However, as described below, the result may be irrelevant to all models for interference as well as to the conclusion that *spo16* cripples implementation without affecting designation.

Evidence of two crossover phases complicates the interpretation of the coefficient of coincidence: The concept of two wild-type phases (or pathways) for crossing over was based on the oft-reported lack of interference among the crossovers remaining in the *zmm* mutants *msh4* and *msh5* (reviewed in Stahl *et al.* 2004). Zalevsky *et al.* (1999) proposed that these crossovers represent a class of crossovers in wild-type yeast that serve to promote chromosome pairing. That proposal has received its most direct support from the observation of a class of crossovers in wild-type yeast, identifiable by their frequent evasion of mismatch repair, that lack positive interference and have a frequency that is independent of Msh4 (Getz *et al.* 2008).

We now show how the coexistence of interference (*disjunction*) and noninterference (*pairing*) phases of crossing over makes it plausible that the absence of detectable *spo16*-induced change in the coefficient of coincidence shows nothing about designation and implementation.

In this demonstration, our operational indicator of interference is C_3, the familiar coefficient of coincidence for adjacent intervals 1 and 2; *i.e.*, (observed frequency of double crossovers)/(frequency expected on the assumption of independence),

$$C_3 = R_{12}/R_1R_2. \tag{1}$$

Our algebra assumes linkage-map intervals that rarely involve more than one crossover, allowing us to approximate recombinant frequencies R_1 and R_2 with the probability of a single crossover. For each interval, R is the sum of the contributions from the two phases, R_D and R_P. To simplify the algebra, and with no serious loss of generality, we let the two intervals be of approximately equal length. Then, the frequency of double recombinants expected on the assumption of independence approaches $(R_D + R_P)^2$. Expanding this binomial gives $R_D^2 + 2R_DR_P + R_P^2$. These three terms describe three classes of double crossovers: one with both events derived from the disjunction phase, one with one event from each phase, and one with two events from the pairing phase, respectively.

Because the two-pathway interpretation restricts interference to the disjunction phase [ignoring the possibility of negative interference between interfering and noninterfering crossovers (Getz *et al.* 2008)], the observed frequency of double crossovers becomes $S_3R_D^2 + 2R_DR_P + R_P^2$, where S_3 is the coefficient of coincidence for double recombinants involving disjunction-phase crossovers in adjacent intervals, and the operational coefficient of coincidence is

$$\begin{aligned} C_3 &= (S_3R_D^2 + 2R_DR_P + R_P^2)/(R_D^2 + 2R_DR_P + R_P^2) \\ &= (S_3R_D/R_P + 2 + R_P/R_D)/(R_D/R_P + 2 + R_P/R_D). \end{aligned} \tag{2}$$

Since, for purely algebraic reasons, S_3 can be expected to approach unity with increasing R_D, experimental tests for interference appropriately involve intervals with small S_3 values. Equation 2 implies that, when $S3R_D/R_P$ is negligible compared to $2 + R_P/R_D$, the value of S_3 (the measure of interference between crossovers in the disjunction phase) is of little consequence for C_3, so that an observed change, or lack of change, in C_3 may be uninformative with respect to S_3 and represent, instead, a change, or lack of change, in R_P/R_D. C_3 *vs.* S_3 is plotted in Figure 1 for select values of R_P/R_D.

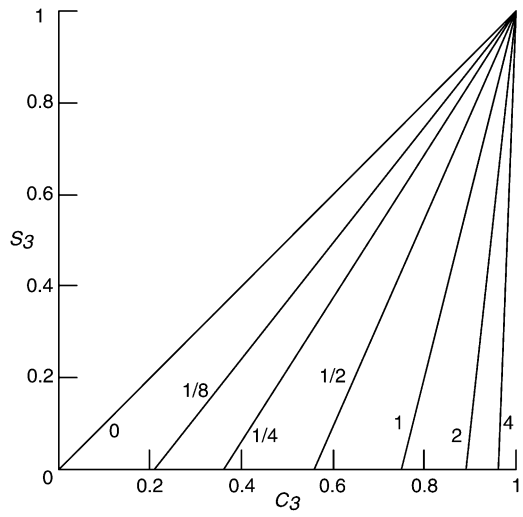

Figure 1.—S_3 *vs.* C_3 for select values of R_P/R_D, calculated from Equation 2, which assumes genetically short, adjacent intervals. R_P/R_D values are indicated by each curve.

Testing the data of Shinohara *et al.* (2008): Whether it is legitimate to test the data presented in Tables S5 and S6 of Shinohara *et al.* (2008) for compatibility with C_3 as presented in Equation 2 depends on the extent to which the experimental conditions match the hypothetical conditions stated above. A nearly inevitable difference between theory and experiment is that some of the interference data collected by Shinohara *et al.* (2008) involve intervals within which double exchanges, as assessed by nonparental ditype (NPD) tetrads, were detected, leading potentially to a data-based C_3

value that is larger than the hypothetical value. A second potential issue is that the "C_3's" calculated from the tetrad data by Shinohara *et al.* (2008) are not identical to the classical C_3, which was defined for random meiotic products. However, they are easily related to C_3, as shown below.

In Table S6, Shinohara *et al.* (2008) defined their indicator of interference as (observed frequency of tetrads that are not PD in either interval)/(frequency of tetrads not PD in interval 1)(frequency of tetrads not PD in interval 2), where PD means parental ditype tetrads. It is well known that this indicator, when applied to short distances under the presumption of no chromatid interference, is equivalent to C_3 as written (Equation 1, and hence Equation 2). In Table S5, the authors used (map length in the presence of crossing over in an adjacent interval)/(map length in the absence of crossing over in the adjacent interval) as their indicator of interference, where map length is calculated according to Perkins (1949). This indicator is not equivalent to C_3, but the tetrad data in that table may be used to calculate (map length in the presence of crossing over in an adjacent interval)/(map length in the total population). Since R and map length are equivalent for short intervals, this indicator, at short intervals, is equivalent to C_3 in Equations 1 and 2. The C_3 values in Table S6 are 0.51 and 0.8 for wild type and 0.55 and 0.65 for *spo16*, while those calculated from the data in Table S5 range from 0.54 to 0.85 for wild type and 0.31 to 0.65 for *spo16*. (The value of 0.55 for *spo16* represents 3 observed double crossovers divided by 5.5 expected. Other values are more precise.)

The remaining obstacle to testing the possibility that the data of Shinohara *et al.* (2008) may reveal only the lack of *spo16*-induced change in R_P/R_D is the lack of means for determining values for S_3, the "interesting" coefficient of coincidence. However, since Equation 2 was written for adjacent intervals within which double exchanges are rare, even in the face of some noninterfering crossovers, we may reasonably assume that S_3 is essentially zero, as it is for short intervals in Drosophila (see Foss *et al.* 1993). When $S_3 = 0$,

$$C_3 = (2 + R_P/R_D)/(R_D/R_P + 2 + R_P/R_D). \qquad (3)$$

Now we can ask whether the C_3-like values, based on chromosome III data from Tables S5 and S6, are compatible with the S_3-independent C_3 values predicted by Equation 3. Values of the ratio of interfering to noninterfering crossovers, needed to evaluate Equation 3, may be obtained from data on *zmm* mutants such as *msh4*, and presumably *zip1*, where the density of residual crossovers reflects the density of noninterfering crossovers (Getz *et al.* 2008). According to the *zip1* data of Shinohara *et al.* (2008) and the *msh4* data of Stahl *et al.* (2004), the relative densities of the two types of crossovers on chromosome III appear to be close to 1/1, perhaps varying among intervals over a range from ~1/3 to 3/1. The C_3 values at these relative densities (Figure 1 at $S_3 = 0$) indicate that, for both wild type and *spo16*, the C_3 values reported by Shinohara *et al.* (2008), above, are compatible with values that are essentially independent of S_3. Within the framework of our two-pathway analysis, this suggests that deletion of *SPO16* reduced R_D and R_P on chromosome III by about the same factor.

Discussion: In our first interpretation of the *spo16* phenotypes, based on the assumption of a single recombination phase, we agreed with Shinohara *et al.* (2008) that the mutants apparently failed to implement some of the designated crossovers and proposed that such failures must have occurred in a random manner. As a result, the mutation certainly decreased the uniformity of intercrossover distances. Thus, even in a one-phase model, the lack of a significant *spo16*-induced change in the coefficient of coincidence reported by Shinohara *et al.* (2008) does not necessarily imply that the strength of interference in the mutant remains intact unless "interference" is redefined as designation, as indicated by Shinohara *et al.* (2008).

In the second example, we learned that the coexistence of an interference and a noninterference phase of recombination might render the coefficient of coincidence (C_3) uninformative regarding strength of interference, especially when S_3 is small. Under such conditions, C_3 would reveal primarily the relative frequencies of interfering and noninterfering crossovers, as suggested by Kitani (1978), but nothing about designation or implementation. In fact, S_3 might be reduced by the *spo16* mutation, as would be expected for models (*e.g.*, Foss *et al.* 1993) in which interference is determined by genetic linkage distance rather than physical distance, but the reduction need not register as a detectable change in C_3.

The lack of a *spo16*-induced change in C_3 for intervals on chromosome III suggests that the mutation lowered crossing over in the two phases of recombination by a comparable factor. (Because R_P/R_D is close to unity in these data, the underlying mechanism of the *spo16* phenotype might be a reduction in number, rather than factor.) This phenotype differs from that of *zmm* mutants *msh4* and *msh5* (Getz *et al.* 2008) and, presumably, *zip1*, which are understood to lack disjunction-phase crossovers without suffering a loss of pairing-phase crossovers. A testable prediction of this view is that double mutants such as *spo16 msh4* or *spo16 zip1* would be reduced for crossing over to a somewhat greater degree than either single mutant. Shinohara *et al.* (2008) used isolated DNA to measure crossing over for the double mutant *spo16 zip1* at the *HIS-LEU* construct and found a phenotype like that of *zip1*, in possible contradiction to our two-pathway analysis. However, quantification of the density of DNA bands in gels may lack the precision needed for that test. Furthermore, the sensitivity of yeast meiotic recombination to experimental conditions (Cotton

et al. 2009) adds to the uncertainties of conclusions based on data derived from different strains sporulated under different regimes (SHINOHARA *et al.* 2008).

The Spo16 work has raised interesting questions that need more study (A. SHINOHARA, personal communication).

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