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COGITATIONES

The Interpretation of Genetical Data

Six assumptions are commonly made in the calculation of linkage relations: (a) That the gene is stable in the heterozygous condition. (b) That crossing-over in the 4-strand stage is at random (chromatid interference does not occur and the frequency of 2-: 3-: 4-strand double exchanges is 1:2:1). (c) That a low frequency of recombination indicates a low frequency of crossing-over. (d) That positive chromosomal interference occurs (one cross-over inhibits crossing-over in an adjacent region of the same chromosome). (e) That the centromeres segregate at random. (f) That crossing-over on one chromosome is independent of crossing-over on any other chromosome. It is the purpose of this communication to point out that these assumptions usually are made implicitly rather than explicitly, and since their validity is in question it may be advisable (until the present confusion has been clarified) to state explicitly the assumptions which are made in any given calculation.

The assumption (a) that the gene is stable is contradicted by the work of LINDEGREN[1] and others[2] who have reported numerous instances in which genes in the heterozygous condition undergo 'conversion'. LINDEGREN and LINDEGREN[3] have recently shown that this may may be due to the irregular distribution of the nutrilites required to construct nucleic acids. The analysis by LINDEGREN and LINDEGREN[4] of chromatid and chromosomal interference in Neurospora revealed that chromatid interference (deviation from 1:2:1 ratio of 2-:3-:4-strand double exchanges) occurs, invalidating assumption (b). The most frequent type of double exchange was the 2-strand double, making the relation between the frequency of recombination and crossing-over obscure since a high frequency of 2-strand double exchange makes a low frequency of recombination not inconsistent with a high frequency of crossing-over, invalidating assumption (c). The high frequency of multiple exchanges found by LINDEGREN and LINDEGREN indicates that chromosomal interference is negative rather than positive across the centromere (simultaneous adjacent 2-strand double exchanges occur much more frequently than would be expected), contrary to assumption (d). Since yeast centromeres segregate preferentially[5], it is improper to assume, as in (e), that the centromeres assort at random. SCHULTZ and REDFIELD[6] showed that interchromosomal 'coincidence' may differ significantly from unity[7]. Recent findings[8] indicate that inferences involving assumptions of independence of crossing-over between different chromosomes need to be reevaluated (f).

Testing for Significance. In testing a statistical hypothesis (for example, in testing for significant deviation from a 1:2:1 ratio among 2-:3-: and 4-strand double exchanges), two possibilities of error are involved: (α) the acceptance of a false hypothesis and (β) the rejection of the correct hypothesis, just as the two errors of a murder trial may be (α) hanging an innocent man or (β) the release of a murderer. In testing for chromatid interference, the statistical hypothesis being tested is the proposition that double exchanges are proportioned according to a 1:2:1 ratio. The χ^2 test yields the probability P (or $1-p$) that the accepted hypothesis is false.

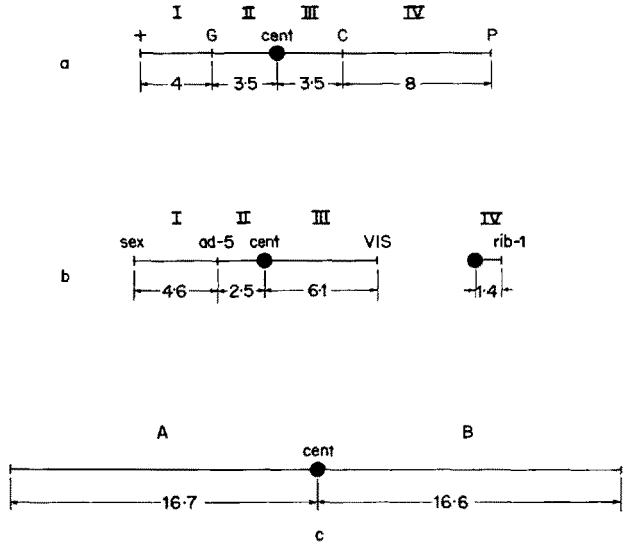

Fig. 1.—Maps of *Neurospora* chromosomes over regions analyzed for chromatid interference (*a*) by LINDEGREN and LINDEGREN[4], (*b*) by HOWE[13], and (*c*) by DAVID STADLER[14]. The maps are drawn to scale in centimorgans.

Data showing a significant deviation from a random proportion of double exchanges[9] means that it is quite likely that the hypothesis of random exchange is false, on condition that the hypothesis was accepted. Other distributions of double exchanges which do not deviate from a random proportion[10] show that the probability of accepting a false hypothesis of random double exchange is not so high with regard to the latter distributions. It has been customary to assume that these data constitute a refutation of LINDEGREN and LINDEGREN's conclusion that double exchanges do not occur randomly. However, to accomplish a logical refutation it is necessary to compute the probability (β) that LINDEGREN and LINDEGREN had rejected a true hypothesis (not that they had somehow accepted a false hypothesis). Computation of β involves the prior knowledge of the *actual* proportion of double exchanges in a population so that β represents the probability of rejection of the hypothesis on the basis of specific data (population sample). Since knowledge of this kind is not available before sample data have been collected, such a computation cannot be performed.

Chromatid Interference in Neurospora. LINDEGREN and LINDEGREN concluded that locally specific patterns of chromatid interference are characteristic for different pairs of regions (Fig. 1*a*) and that an excess of 2-strand

[1] C. C. LINDEGREN, Science *121*, 605 (1955).

[2] B. D. MUNDKUR, Ann. Mo. Bot. Gar. *36*, 259 (1949). – P. ST. LAWRENCE, P.N.A.S. *42*, 189 (1956). – M. B. MITCHELL, P.N.A.S. *41*, 215 (1955); *41*, 935 (1955).

[3] C. C. LINDEGREN and G. LINDEGREN, Nature *178*, 796 (1956).

[4] C. C. LINDEGREN and G. LINDEGREN, Genetics *27*, 1 (1942).

[5] C. C. LINDEGREN and E. E. SHULT, Exper. *12*, 177 (1956).

[6] J. SCHULTZ and H. REDFIELD, Cold Spr. Harb. Symp. quant. Biol. *16*, 175 (1951).

[7] E. E. SHULT and C. C. LINDEGREN, Nature *175*, 507 (1955).

[8] D. MAZIA, P.N.A.S. *40*, 521 (1954). – D. STEFFENSEN, Abs., Genetics Society of America, East Lansing, Mich. (1955). – R. P. LEVINE, P.N.A.S. *41*, 727 (1955).

[9] C. C. LINDEGREN and G. LINDEGREN, Genetics *27*, 1 (1942). – C. L. HUSKINS and H. B. NEWCOMBE, Genetics *26*, 101 (1941).

[10] G. W. BEADLE and S. H. EMERSON, Genetics *20*, 192 (1935). – D. R. STADLER, Science *122*, 878 (1955).

double exchanges occurs in regions symmetrically placed across the centromere. To quote from their paper: 'Either overlapping of the spindles or equational division of the

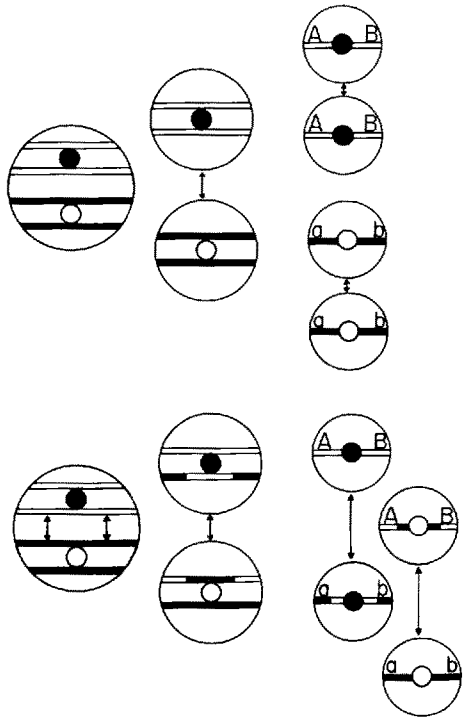

Fig. 2. — If misassortment occurs 'more frequently in double exchange bivalents than on others', as PERKINS assumed, most 2-strand double exchanges in regions II and III would be undetected, since the orientation of the spores would be the same in a 2-strand double which had misassorted as in a non-cross-over. Simultaneous second division segregation of the genes bounding regions II and III would occur only when either misassortment or 2-strand double exchange occurred alone, both classes being assumed by PERKINS to be relatively rare in comparison with the class in which both events occurred concomitantly. On PERKINS' assumption the observed number of simultaneous second division segregations of genes in regions II and III (which were interpreted by LINDEGREN and LINDEGREN as 2-strand double exchanges in regions II and III) are usually either misassortments or 2-strand double exchanges and most of the 2-strand doubles are concealed by misassortment. He was obviously unaware of this consequence of his hypothesis since it greatly increases the frequency of 2-strand doubles in regions II and III over the observed ratio of 20:3:1 of 2-:3-:4-strand double exchanges and it was *this* deviation from randomness that he was really trying to reduce.

centromere at the first meiotic division would produce an arrangement of spores in the ascus interpreted as a 2-strand double exchange in regions II and III. Therefore, this particular type of exchange has alternative interpretations. If the asci were reclassified and the arrangements interpreted as 2-strand double exchanges in regions II and III were *reinterpreted* as overlapping of the spindles... 4 of the 13 cases of overlapping or equational separation of the centromere at metaphase I occur among the 28 double exchange asci. This is an extremely improbable distribution as the χ^2 test shows, and we may conclude that either the before-mentioned arrangements are not due to overlapping or equational separation of the centromere at metaphase I, or we are forced to take the highly improbable view that overlapping or equational centromere separation usually occurs coincidentally with a double exchange in regions I and IV... There is no *a priori* reason why equational division of the centromere at metaphase I or spindle overlapping should occur coincidentally with crossing-over. In fact, it is a reasonable assumption that misassortment occurs independently of crossing-over.' PERKINS[11] chose one of the alternatives, stating that '*misassortment must be assumed to occur more frequently in double-exchange bivalents than in others*... in order to account for observed frequencies of apparent quadruples.' The consequences of this assumption are shown in Figure 2. A double exchange occurring in regions II and III coincidental with misassortment would be an apparent non-cross-over tetrad although crossing-over had actually occurred. PERKINS failed to consider these in his recalculation. He did not realize that although his assumption reduces the number of calculated quadruple exchanges it increases the number of 2-strand double exchanges in regions II and III above the ratio of 20:3:1 of 2-:3-:4-strand double exchanges. We have chosen to assume that *misassortment, if it occurs at all, is independent of crossing-over.*

The excess of 2-strand double exchanges found by LINDEGREN and LINDEGREN between regions I and IV are independent of interpretations involving misassortment of the centromere since two markers adjacent to the centromere define the events occurring in regions II and III irrespective of misassortment of the centromeres. The ratio of 2-:3-:4-strand double exchanges between regions I and IV was 15:7:6. In the discussion following Dr. PERKINS' paper [11], Dr. LINDEGREN pointed out that 'Since the p values (by χ^2) for regions I–IV and II–IV, based on PERKINS' recalculated data, are 0·025 and 0·0001, respectively, the evidence for chromatid interference is as positive on the hypothesis of misassortment of the centromere as on the hypothesis that the centromere segregates regularly at meiosis I.' PERKINS admitted, 'It is true that two or three deviations' (from a total of six!) 'that indicate chromatid interference still remain...' (after recomputation of the data on the hypothesis of misassortment of the centromeres), thus establishing the nonrandomness of the events occurring between regions I–IV and II–IV by his own calculations.

PERKINS' assumption of a 1 per cent frequency of misassortment is in conflict with the available cytological data. DODGE[12] in an extensive cytological investigation of *N. sitophila*, did not find any overlapping spindles and noted a partition of vacuoles in the cytoplasm of the ascus at the four-nucleate stage effectively separating the nuclei and preventing change of position. LINDEGREN (unpublished) had a similar experience with *N. crassa*. In *N. tetrasperma*, DODGE had described the overlapping of the spindles (which is easy to identify because the central bodies remain attached to the nuclei after telophase) to occur regularly in every ascus. The attached central body makes it possible to distinguish the orientation of the spindles even after the spindles themselves have disappeared.

LINDEGREN and LINDEGREN (Table I) showed that the distribution of 2-:3-:4-strand double exchanges for regions I–IV, II–IV and III–IV each differ significantly from a 1:2:1 ratio and from each other. If PERKIN's correction were valid and were applied (see above) the ratio of 20:3:1 in regions II–III would diverge even more from 1:2:1 ratio. These data prove that chromatid interference is locally specific for any given pair of regions and that it is not permissable to lump data from different pairs of regions. In spite of this, HOWE[13] has

[11] D. D. PERKINS, *Symposium of Genetic Recombination* (Oak Ridge, Tenn., April 1954), p. 119.

[12] B. O. DODGE, J. agr. Res. *35*, 289 (1927).

[13] H. B. HOWE, Jr., Abs., Records of the Genetics Society of America (1954), p. 45.

Table I.—LINDEGREN and LINDEGREN's Data.

	I and II	I and III	I and IV	II and III	II and IV	III and IV
2-strand	1	2	15	20	0	0
3-strand	2	4	7	3	0	8
4-strand	3	1	6	1	5	5
2- or 4-strand	6					2
2- or 3- or 4-strand			1			
2-:3-:4-	1:2:3	2:4:1	15:7:6	20:3:1	0:0:5	0:8:5

lumped data involving three pairs of regions on the same chromosome studied by LINDEGREN and LINDEGREN (Fig. 1 *b*) and presented the summation of these data (Table II) as evidence for random distribution of 2-: 3-: 4-strand double exchanges. He arbitrarily "corrected" the data on the assumption that crossing-over on a given chromosome is independent of crossing-over on any other chromosome. The data from his regions I–II and II–III are not significant, and since it is not permissable to lump data, only the 3:8:1 distribution for region I–III may be considered. There seems to be no point in publication of this information since the conclusion is unsupported by the data. This criticism disregards both the validity of his arguments for "correcting" his data and the obvious difficulties he encountered in dissection. He records 55 cases of translocated spores. Considering the fact that most transpositions are undetectable in non exchange tetrads these errors were accumulated in about 300 tetrads. This can only mean either (*a*) that his technique was not adequate or (*b*) that the spores were much smaller than the ascus, since gross aberrations of this kind were never encountered by LINDEGREN and LINDEGREN.

Table II.—HOWE's Data. Regions

	I–II	I–III	II–III
2-strand	0	3	2
3-strand	1	8	2
4-strand	1	1	2

Randomization of Nonrandom Data. DAVID STADLER[14] analyzed the effects of segregation of two spore markers located, respectively, 16·7 and 16·6 units on opposite sides of the same centromere (Fig. 1*c*). Even if his data involved the chromosome studied by LINDEGREN and LINDEGREN, STADLER's markers are so far from the centromere that the effects of crossing-over would be randomized over the regions described by him, concealing the nonrandom events which LINDEGREN and LINDEGREN demonstrated to occur across the centromere. STADLER concluded that his observations 'failed to confirm the observations of LINDEGREN and LINDEGREN'. The negative evidence presented by STADLER over a single large region is not pertinent to the significance of the positive nonrandom data presented by LINDEGREN and LINDEGREN over four, short, well-marked regions (Fig. 1*a*). STADLER's inability to recover all chromatids due to inviability further reduced the significance of his conclusions. There is no point in STADLER's statement that his data are 'in good agreement' with those of HOWE, since HOWE's data are too scanty to show significant deviation from either the nonrandom data of LINDEGREN or the randomized data of STADLER; HOWE's data are significant only with regard to the interdependence of crossing-over between heterologous chromosomes.

Somatic Crossing-Over. STERN's[15] demonstration of somatic crossing-over was based on two assumptions: (a) That the gene is stable in the heterozygote and (b) that, if gene-conversion occurs, it is unlikely that linked genes would undergo conversion concurrently. LINDEGREN[3] has shown recently, however, that linked genes do, in fact, tend to undergo conversion concurrently. It may be necessary to reevaluate inferences based on assumptions concerning somatic crossing-over.

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Biological Research Laboratory, Southern Illinois University, Carbondale, June 28, 1956.

Note added in proof. A recent paper by STADLER[16] expanding the information cited above claims to present further data "in complete agreement with those of HOWE", but points out that "HOWE's experiment involved short map regions"... and "because the numbers are small the result is compatible with quite a wide range of ratios of the three types".

STADLER's analysis of both chromosomal and chromatid interference in 3-point half-tetrad data assumes that both types of 3-strand doubles occur with equal frequency. If the one type of 3-strand double in his sample preponderates excessively over the reciprocal type, it is possible that his ratio of 43:54:33 is nearly a 1:1:1 ratio even when chromatid interference (in the form of an excess or deficiency of 3-strand double exchanges) is present. Critical evidence on this subject is not available from *Neurospora* data, although the indications from Table I[4] suggests that it may not be a permissible assumption. Unpublished data from yeast genetics show 14:2 and 8:1 ratios for the two types of 3-strand doubles indicating that they do not always occur with equal frequency. In half tetrad data, involving 2 loci and a centromere, STADLER's conclusions that chromosomal interference is absent and that chromatid interference is absent (so that his data do not require WHITEHOUSE's correction) rest on the more general assumption that 3-strand double exchange comprise one-half of the double exchanges. Since much of STADLER's analysis depends upon these assumptions, his inferences concerning the absence of chromatid interference are prejudicated.

STADLER points out that "double crossovers involving regions I and III" (in the material studied by him) "would not be affected by meiotic nuclear passing".... (since)... "such an event would be scored double crossing over involving regions I and II". In comments on LINDEGREN and LINDEGREN[4], STADLER states that "they reported

[14] D. R. STADLER, Science *122*, 878 (1955).

[15] C. STERN, Biol. Zbl. *51*, 547 (1931).

[16] D. R. STADLER, Genetics *41*, 623 (1956).

that among the double crossovers involving regions in opposite chromosomes arms, the majority involved only two strands. Recent work has not confirmed this report of an excess of 2-strand double crossovers." LINDEGREN and LINDEGREN pointed out that the ratio of 2-:3-:4-strand double exchanges occurring in regions I and IV analyzed by them were independent of events occurring in regions II and III, and it is clear that STADLER understands the significance of this statement by the quote above. Between regions I and IV, LINDEGREN and LINDEGREN reported a frequency of 15:7:6 of 2-:3-:4-strand double exchanges making STADLER's comments on the absence of evidence for chromatid interference described by LINDEGREN and LINDEGREN incomprehensible.

Throughout his discussion, STADLER has failed to discriminate between recombination and crossing-over. Since it is obvious that undetected multiple exchanges must be involved, WHITEHOUSE's correction must be applied before any conclusive interpretation can be drawn from his data.

Zusammenfassung

Für die Interpretation genetischer Ergebnisse wird den Autoren vorgeschlagen, Grundannahmen jeweils ausführlich zu formulieren.

STUDIORUM PROGRESSUS

The Use of Radioactive Bands in Tracing Hibernating Bats

By A. PUNT and P. J. VAN NIEUWENHOVEN[1]

Introduction.—In the southern part of the province of Limburg (the Netherlands) a number of caves are found, which are formed by excavating the limestone for building purposes. These caves provide an ideal shelter to hibernating bats. Hundreds of bats of 11 species can be found in these large labyrinths during the winter and it is clear that Dutch zoologists have studied physiological, ecological and ethological problems concerning bat hibernation (BELS[2]; SLUITER *et al.*[3]; DE WILDE and VAN NIEUWENHOVEN[4]).

When bat behaviour during the winter was studied some difficulties were met, which were not easy to overcome. The aim of this paper is to point out a method to deal with one of these problems and to report some results recently obtained by using this method.

As is generally assumed bats do not remain asleep for the whole winter season but get awake periodically. DE WILDE and VAN NIEUWENHOVEN tried to determine the mean duration of the sleep-period which was estimated to be a few weeks. They also studied the migration of hibernating bats, depending on this fact. They found that especially in the second half of the winter animals, being marked with aluminium bands disappeared from the cave and that new bats, without bands, appeared. It may be noticed, that this winter migration could have been forwarded by the act of banding itself, which may alarm the animals considerably. But the appearance of bats without bands, which probably never had been handled, was considered to be natural behaviour.

So it was very likely that local migration, from one cave to another took place during the winter, which was in accordance with observations of FOLK[5], ANCIAUX[6], VERSCHUREN[7], and KOWALSKI[8].

Much bats are hibernating in fissures of the rocky walls of the cave and they may enter into these crevices so far, that they cannot be detected visually. Now the disappearance of banded bats and the appearance of unbanded ones could also be explained by displacements of animals to and from this invisible bat-reservoir. So there are two possible explanations for the change in population of a cave: local-migration to and from other caves and movements within the cave to and from unaccessable fissures in the rock. In order to determine which of the two possibilities was the most likely, we provided bands with radioactive material, which made it possible to trace the animals even when they were quite invisible.

Methods.—Bands of the usual size (width 4 mm, weight 120 mg) were made of aluminium and stamped with a number. One of the ends was lengthened and folded back to form a small tube. In this tube a small rod of radioactive antimony (Sb^{124}) (approximately 2 mm long, diameter 1 mm, weight 10 mg) was inserted and fixed by pinching the aluminium edges of the tube.

Sb^{124} was used for some special reasons. In the first place the half life of this material is 60 days. So its radioactivity was lost after not to long a period. Our experiments had a duration of some months and we thought it better that the activity of the material vanished soon after that. As some banded animals escaped it was not possible to remove all bands at the end of the experiments. But now the escaped animals were not exposed to the radiation for a long time and the 'radio active' material was not scattered over the country. Moreover experiments in the next season could not be spoiled by the remained activity from earlier investigations. In the second place Sb^{124} produces γ-radiation, ranging from 0·12 to 1·7 MV, with a large quantity of the short wavelengths (up to 39% of the 1·7 MV rays). These hard rays penetrated sufficiently through the limestone, the animals were detectable even when hiding at a considerable distance in crooked fissures.

The rods of antimony were made by us and afterwards activated in the cyclotron at Kjeller, Norway, by the mediation of Philips-Roxane N. V., Holland, which firm also estimated the activity to be about 250 μc per rod of 10 mg. No bands were used till three weeks after the process of activation was finished, the material having lost in this time most of the β-radiation caused by equally formed Sb^{122} (half life: 2·8 days).

As far as we know, no damage was done to the animals. At the end of our experiments the bats were as normal as they could be. This was as we expected for by a very rough calculation the dose of radiation was estimated to be 16 r/week in the beginning of the experiments (clinical dose in man: 20 r/week). The radioactive bats were traced by means of a portable battery monitor, provided with a low voltage halogen quenched gamma-counter tube, Philips No. 18502. The monitor was built according to GODFREY[9], with some modifications of minor importance. The Geiger-Müller tube was housed in an aluminium cylinder (Philips Probe PW 4101) which was attached to the end of a bamboo rod, which

[1] From the Laboratory of comparative Physiology University of Amsterdam.

[2] L. BELS, Publicaties van het Natuurhist. Genootschap in Limburg *V* (1952); Thesis, Utrecht.

[3] J. W. SLUITER, P. F. VAN HEERDT, and J. TH. DE SMIDT (review of literature), *De Levende Natuur*, Linders, Arnhem (1956).

[4] J. DE WILDE and P. J. VAN NIEUWENHOVEN, Publicaties van het Natuurhist. Genootschap in Limburg *VII*, 51 (1954).

[5] G. E. FOLK, J. Mammal. *21*, 306 (1940).

[6] F. ANCIAUX DE FAVEAUX, J. Mammal. *16*, 148 (1952).

[7] J. VERSCHUREN, Bull. Mus. Hist. nat. Belg. *25*, 1 (1949).

[8] K. KOWALSKI and R. J. WOJTUSIAK, Cracova Bull. *3*, 33 (1952).

[9] G. K. GODFREY, Ecology *35*, 5 (1954).