

## Gene conversion: remarks on the quantitative implications of hybrid DNA models\*

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### SUMMARY

Some quantitative implications of the gene conversion models of Holliday and Whitehouse & Hastings are discussed. These models predict two kinds of mispairing when a heterozygous mutant is in hybrid DNA. It is pointed out that the latter model, in contrast to statements in the literature, does not require identical repair rates in both kinds of mispairing.

Furthermore, some peculiarities are discussed which result if, among the corrected fractions of hybrid sites, the frequency of repair to wild-type is the same in both kinds of mispairing ( $r = s$ ). In the Holliday model a minimum results under this condition for the frequency of asci which show a normal 4:4 segregation, but which have originated by repair from meioses with hybrid DNA.

### 1. DIFFERENCES BETWEEN THE HOLLIDAY AND WHITEHOUSE & HASTINGS MODEL

The main difference between the gene conversion models of Holliday (1964) and Whitehouse & Hastings (1965; Whitehouse, 1965) is the mode in which the hybrid DNA is supposed to be formed (Figs. 1, 2). Furthermore, it is frequently said (e.g. Emerson, 1966, 1969) that the Whitehouse & Hastings model requires identical repair rates at both mispaired sites. This opinion seems to have originated from Whitehouse's (1965) original scheme in which he only assumed one frequency for each of the three possible events (repair to wild-type, repair to mutant, no repair) at the hybrid sites. In his diagram Whitehouse did not consider the actual base mispairings in the hybrid DNA but used the general symbols  $+ / +$ ,  $+ / g$ , and  $g / g$  for wild-type, hybrid site, and mutant, respectively. If one considers the actual kinds of mispairing as Emerson (1966, 1969) has done for the Holliday model, identical repair rates are no longer implied in the Whitehouse & Hastings model.

In a cross between the wild-type and a single site mutant, the wild-type will have the base pair A–B, and the mutant the base pair X–Y. If at the site in question hybrid DNA is formed according to the Holliday model, the mispairing A\*Y will result in one chromatid, and B\*X will result in the other (Emerson, 1966) regardless of the DNA strands in which the breaks occur (Fig. 1). In contrast to

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this, in the Whitehouse & Hastings model the mispairings are identical in both chromatids in an individual meiosis, but depending on the way the hybrid DNA is formed, meioses with either two A\*Y or two B\*X will occur (Fig. 2).\*

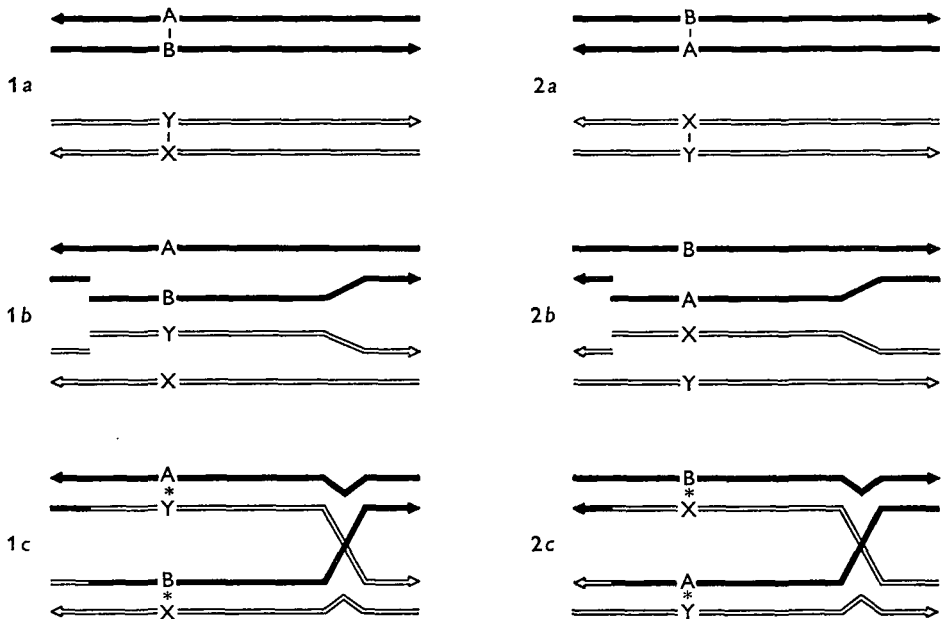


Fig. 1. Hybrid DNA formation according to the model of Holliday (1964). Only the two chromatids participating in recombination are shown; each chromatid consists of one DNA double helix. The base pairs at the heterozygous site are indicated by A-B (wild-type) and X-Y (mutant) (1a and 2a). The formation of hybrid DNA is initiated by breaks in DNA strands of the same polarity. Regardless of the strands in which these breaks occur (1b or 2b), one chromatid will have the mispairing A\*Y and the other the mispairing B\*X (1c, 2c). The half-chromatid chiasmata are resolved later on.

Emerson (1966) assumed that the mispairing A\*Y is repaired with the probability  $p$  and B\*X with the probability  $q$ ; if no repair occurs, postmeiotic segregation results. In the case of repair, Emerson assumed furthermore that the repair frequencies to wild type are also different for both hybrid sites ( $r$  and  $s$ , respectively). On the basis of these assumptions, he derived equations for the expected fractions of the different types of aberrant asci. Emerson's equations for the Holliday model are shown in Table 1. The segregations shown in the table refer to ascomycetes with eight-spored asci.

With respect to the Whitehouse & Hastings model, Emerson (1969, p. 311) has pointed out that meioses with either two A\*Y or two B\*X occur, but later (p. 357) he says that this model requires  $p = q$  and  $r = s$ , and he gives for the Whitehouse & Hastings model equations which result from the ones for the Holliday model by

\* In the present discussion it is always assumed that the mutation site is included in the hybrid region of both chromatids participating in recombination; this condition might not be true in all instances in the Whitehouse & Hastings model (Whitehouse, 1967).

introducing the above equalities. As I have already stated, the conditions  $p = q$  and  $r = s$  are not implied in the Whitehouse & Hastings model if the actual base mispairings are considered.

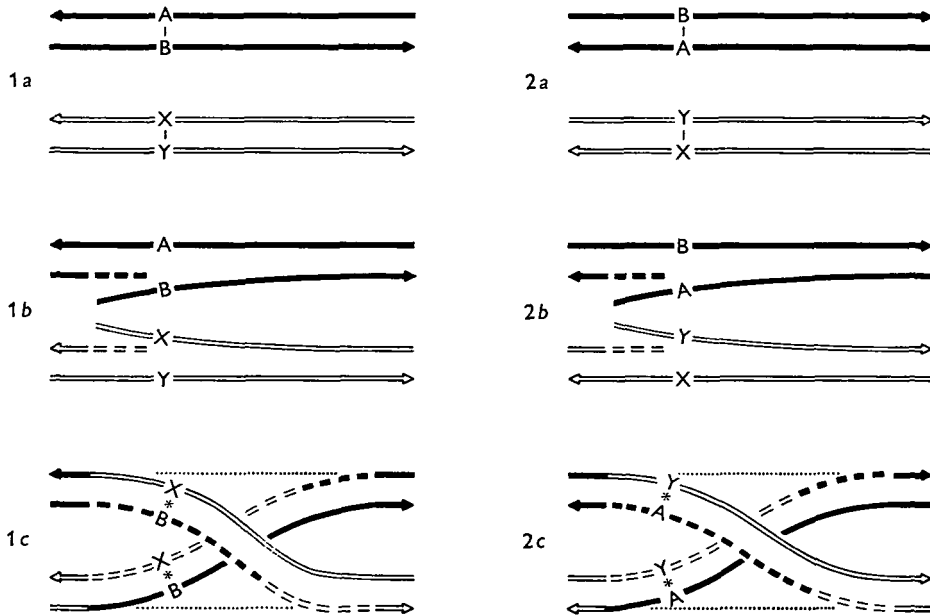


Fig. 2. Hybrid DNA formation according to the Whitehouse & Hastings model (Whitehouse, 1965). 1a and 2a as in Fig. 1. The formation of hybrid DNA is initiated by breaks in DNA strands of opposite polarity. Depending upon the strands in which these breaks occur (1b or 2b), meioses with either two  $X*B$  (1c) or two  $A*Y$  (2c) will result. ---, Newly synthesized strands; . . . . ., broken-down strands.

Under the assumption that the molecular events leading to genetic recombination proceed as postulated by Whitehouse (1965; see Fig. 2), aberrant asci would originate either from meioses with two  $A*Y$  or two  $B*X$ . If  $k$  is the fraction of the former meioses and  $(1 - k)$  the fraction of the latter meioses, the equations shown in the last column of Table 1 result for the expected fractions of the different types of aberrant asci. Each equation is a sum of two terms. The terms with the factor  $k$  can be derived from the corresponding equations for the Holliday model by substituting  $p$  for  $q$  and  $r$  for  $s$ , the terms with the factor  $(1 - k)$  by substituting  $q$  for  $p$  and  $s$  for  $r$ .

The derived equations for the Whitehouse & Hastings model are more complex than the ones for the Holliday model because of the parameter  $k$ . Only if  $k = 1$  or  $k = 0$ , equations would result which would be equivalent to those given by Emerson (1969) for the Whitehouse & Hastings model.

In the above considerations the main emphasis was put on the mode in which hybrid DNA is formed, and different equations resulted for both models. The question whether  $p$  might equal  $q$  and/or  $r$  might equal  $s$  is, in my opinion, of no

significance for discriminating between the two models unless one considers the omission of Whitehouse (1965) to contemplate on the real base mispairings in the hybrid DNA as an essential aspect of his model. Depending upon the type of mutation involved in the cross (e.g. transition or transversion) and the base pairs neighbouring the hybrid sites, the condition(s)  $p \sim q$  and/or  $r \sim s$  might result in the Holliday as well as Whitehouse & Hastings model.

Table 1. *Expected fractions of aberrant asci resulting from hybrid DNA in the models of Holliday (1964) and Whitehouse & Hastings (1965)*

Ascus types		Expected fractions*	
Fraction	Segregation†	Holliday model‡	Whitehouse & Hastings model
<i>a</i>	6+ : 2m	$prqs$	$kp^2r^2 + (1-k)q^2s^2$
<i>b</i>	2+ : 6m	$p(1-r)q(1-s)$	$kp^2(1-r)^2 + (1-k)q^2(1-s)^2$
<i>c</i>	5+ : 3m	$pr(1-q) + (1-p)qs$	$k2pr(1-p) + (1-k)2qs(1-q)$
<i>d</i>	3+ : 5m	$p(1-r)(1-q) + (1-p)q(1-s)$	$k2p(1-r)(1-p) + (1-k)2q(1-s)(1-q)$
<i>e</i>	4+ : 4m§	$(1-p)(1-q)$	$k(1-p)^2 + (1-k)(1-q)^2$
<i>f</i>	4+ : 4m	$prq(1-s) + p(1-r)qs$	$k2p^2r(1-r) + (1-k)2q^2s(1-s)$

\* In accordance with Emerson (1966), two different mispaired sites A\*Y and B\*X are assumed:  $p$  = fraction in which repair occurs at site A\*Y, among which  $r$  = fraction repaired to wild type,  $1-r$  = fraction repaired to mutant,  $1-p$  = fraction in which no repair occurs at site A\*Y;  $q$  = fraction in which repair occurs at site B\*X, among which  $s$  = fraction repaired to wild type,  $1-s$  = fraction repaired to mutant,  $1-q$  = fraction in which no repair occurs at site B\*X;  $k$  = fraction of meioses in the Whitehouse & Hastings model with two mispaired A\*Y sites,  $1-k$  = fraction with two B\*X sites.

† + = wild type, m = mutant.

‡ Algebraic formulations of Emerson (1966).

§ With postmeiotic segregation in two spore pairs.

|| Normal (one hybrid site corrected to wild type, the other to mutant).

Is it possible with the help of the equations shown in Table 1 to discriminate whether a set of experimental data fits one but not the other model? If one compares experimental data on gene conversion with the theoretical ascus patterns, a difficulty arises: the asci showing an apparent normal 4:4 segregation, but which have originated by repair from meioses with hybrid DNA (fraction *f* in Table 1), cannot be distinguished experimentally from 'true' normal asci.\* As a consequence, in the Holliday model the parameters  $p$ ,  $q$ ,  $r$ , and  $s$  cannot be determined directly from the observed ascus pattern. It is only possible to obtain partial solutions from the relative frequencies of the different aberrant asci: for each set of experimental data, a range of values for the four parameters can be computed which would all agree with the data but other ranges of values can be excluded (Emerson, 1966).

In the equations for the Whitehouse & Hastings model the parameter  $k$  is necessary in addition to  $p$ ,  $q$ ,  $r$ , and  $s$ . Due to the additional parameter  $k$ , it is with this model practically impossible to estimate five parameters (or a range of para-

\* Ascus of fraction *e* can be observed only if it is possible to detect postmeiotic segregation.

meters) which would be in agreement with a given set of experimental data. Thus, it does not seem possible to make any decision for or against the Holliday or the Whitehouse & Hastings model on the basis of different ascus patterns in single factor crosses.

## 2. THE SPECIAL CASE $r = s$

In spite of the insufficiencies mentioned, the equations of Table 1 are useful for testing whether or not experimental data fit some special conditions. As is shown below, a condition of special interest is  $r = s$ . For the discussion of this case, some comments are necessary on the symbols used for the different types of aberrant asci.

In Table 1 the *fractions* of the different ascus types which have originated from meioses with hybrid DNA at the site in question are symbolized by small letters  $a$  to  $f$ . The sum of these fractions equals unity. In contrast to these fractions, the *numbers* of aberrant tetrads found experimentally will be symbolized by the corresponding capital letters  $A$  to  $E$ . Because asci of the fraction  $f$  cannot be detected, an experimental number  $F$  is not available. It is therefore only possible to determine the relative frequencies of different observable ascus patterns. These relative frequencies can be obtained by using the found numbers of the different aberrant asci, because  $A/B = a/b$ ,  $C/D = c/d$ , etc. As shown below, a minimum value for fraction  $f$  can be calculated in certain cases; the symbol  $F$  will therefore be used for the theoretical number of these asci in a set of data.

Emerson (1969) has pointed out that for the conditions  $p = q$  and  $r = s$  the following relationship results:

$$\frac{a}{b} = \frac{c^2}{d^2} = \frac{A}{B} = \frac{C^2}{D^2}. \quad (1)$$

However,  $p = q$  is not a necessary condition for this relationship. Equation (1) results whenever  $r = s$  alone. This is true for the Holliday as well as for the Whitehouse & Hastings models as given in this paper (Table 1).

*Holliday model,  $r = s$ :*

$$\frac{a}{b} = \frac{pqr^2}{pq(1-r)^2} = \frac{r^2}{(1-r)^2}$$

$$\frac{c}{d} = \frac{r[p(1-q) + (1-p)q]}{(1-r)[p(1-q) + (1-p)q]} = \frac{r}{1-r}.$$

Thus it follows  $a/b = c^2/d^2$ .

*Whitehouse & Hastings model,  $r = s$ :*

$$\frac{a}{b} = \frac{r^2[kp^2 + (1-k)q^2]}{(1-r)^2[kp^2 + (1-k)q^2]} = \frac{r^2}{(1-r)^2}$$

$$\frac{c}{d} = \frac{r[k2p(1-p) + (1-k)2q(1-q)]}{(1-r)[k2p(1-p) + (1-k)2q(1-q)]} = \frac{r}{1-r}.$$

Thus it follows again  $a/b = c^2/d^2$ .

Therefore, with the help of equation (1) it is possible to check whether data fit the special condition  $r = s$  ( $p \neq q$  or  $p = q$ ), but the equation does not allow a discrimination between the two models under discussion.

In the Holliday model the condition  $r = s$  implies an interesting consequence with respect to fraction  $f$ : if  $r = s$ , a minimum results for the value of  $f$ . This can be shown in the following way:

From the fraction

$$\frac{a}{b} = \frac{A}{B} = \frac{prqs}{p(1-r)q(1-s)}$$

one obtains

$$r = \frac{A(1-s)}{A + (B-A)s} \quad (\text{Emerson, 1966}). \quad (2)$$

From  $f = prq(1-s) + p(1-r)qs$  (Table 1) and equation (2) one obtains

$$f = pq \frac{Bs^2 + As^2 - 2As + A}{Bs - As + A}. \quad (3)$$

For given values of  $p$  and  $q$ ,  $f$  is a function of  $s$ ; its minimum value can be obtained from equation (3) by differentiation:

$$\frac{df}{ds} = pq \frac{(Bs - As + A)(2Bs + 2As - 2A) - (Bs^2 + As^2 - 2As + A)(B - A)}{(Bs - As + A)^2} = 0.$$

It follows

$$(B - A)s^2 + 2As - A = 0. \quad (4)$$

Equation (4) can be transformed to

$$s = \frac{A(1-s)}{A + (B-A)s}. \quad (4a)$$

The right part of (4a) is identical with the right part of equation (2). Therefore, in the extrema defined by (4),  $r = s$ . For  $A \geq 1$  and  $B \geq 1$ , and in the biological meaningful range  $0 < r = s < 1$ , the quadratic equation (4) has one real solution which corresponds to the minimum of  $f$ . From  $A > B$  follows  $r = s > 0.5$ , from  $A = B$  follows  $r = s = 0.5$ , and from  $A < B$  follows  $r = s < 0.5$ . It is noteworthy that equation (4) is based only on the numbers of  $6 + : 2m$  and  $2 + : 6m$  asci; the parameters  $p$  and  $q$  are not included in the equation.

The above calculations are done on the basis of the Holliday model. Because of the greater complexity of the equations for the Whitehouse & Hastings model, I did not succeed in devising an analogous mathematical test for this model.

In studies on gene conversion, the occurrence of postmeiotic segregation seems to be mutant-specific; in a number of crosses this phenomenon was not observed (Paszewski, 1970). The absence of asci with postmeiotic segregation in a cross would mean  $p \sim 1$  and  $q \sim 1$ . In such cases the data cannot be compared with equation (1). However, on the basis of the correlation that a minimum results for  $f$  if  $r = a$  (Holliday model), the minimum number of meioses can be estimated in which the site in question must have been included in hybrid DNA. This will be demonstrated in the following example.

The *ade6* mutant M26 of *Schizosaccharomyces pombe* yields relatively high frequencies of gene conversion. In crosses of M26 with the wild-type, 52 asci out of 1018 (5.1%) were aberrant. From the aberrant asci, 46 showed a segregation 3+ : 1 M26(A), and 6 a segregation 1+ : 3 M26(B); no postmeiotic segregation was detected (Gutz, 1968, and unpublished results).<sup>\*</sup> Disregarding statistical variations and assuming  $r = s$ , one obtains from equation (4)  $r = s = 0.738$ . Under these conditions ( $p \sim 1$ ,  $q \sim 1$ ,  $r = s$ ),  $f = 2(r - r^2) = 0.387$ . Because

$$a + b = (1 - f) = 0.613$$

and

$$\frac{F}{A + B} = \frac{f}{a + b} = \frac{f}{(1 - f)},$$

it follows

$$F = \frac{(A + B)f}{(1 - f)} = 32.8.$$

Thus, on the basis of the Holliday model, in *at least* 33 meioses hybrid DNA must have been corrected in a way that asci of fraction  $f$  resulted, and in *at least* 85 meioses out of 1018 (8.5%) the mutation site of M26 must have been included in hybrid DNA.

### 3. CONCLUDING REMARKS

In the current literature on gene conversion the models of Holliday and Whitehouse & Hastings are favourably considered by many fungal geneticists. In particular, Emerson (1966, 1969) has done extensive theoretical work on the basis of these models. It was the purpose of the present paper to draw attention to some features of both models which seem not to have been recognized so far. It should, however, be emphasized that at the present state of our knowledge the assumptions made in the Holliday as well as the Whitehouse & Hastings model are still very speculative. This is also true for the ideas on gene conversion expressed by Taylor (1967), Stahl (1969), and Paszewski (1970). Definitive conclusions as to the mechanism of gene conversion seem to be possible only if biophysical methods can be devised which would allow direct studies of the recombining DNA molecules.

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<sup>\*</sup> Due to the red colour of *ade6* mutants, postmeiotic segregation is observable in this system by sectored (white/red) spore colonies.

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