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The time course of recombinant production in *Streptomyces coelicolor*

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SUMMARY

The process leading to gene recombination can be interrupted in the filamentous bacteria *Streptomyces coelicolor* by growing mixed cultures on cellophane disks lying on complete medium. The mycelium is harvested, broken, diluted and the broken hyphae plated at different time intervals. By this means some markers can be excluded from heteroclones or from recombinant progeny in early samples. The recombinant pattern clearly changes with time, with an increase of markers contributed to the recombinant progeny. In crosses between male (NF) and female (UF) strains, the maleness is the first donor trait to appear in the cells of the recipient parent. The fertility factor does not produce a transfer origin on the donor chromosomes; the donor contribution may extend on either side or on both sides of the factor which appears to be compulsory for zygote formation. The longer the time of contact between parental cells, the longer the segment of the donor chromosome contributing to the recombinant progeny. When spores are formed they contain almost exclusively recombinant nuclei derived from segregation processes.

1. INTRODUCTION

It is well known that chromosome transfer is gradual and unidirectional in crosses of *Escherichia coli* K 12 involving Hfr and F^- strains. This has been exhaustively proved in mating experiments during which the conjugating cell pairs have been dissociated at various time intervals by means of violent mechanical stirring (Wollman, Jacob & Hayes, 1956). Similar evidence has recently been provided for crosses $F^+ \times F^-$ (Curtiss III & Renshaw, 1969). Progressive transfer of chromosome has been shown by interrupted mating experiments in *Pseudomonas aeruginosa* (see Holloway, 1969). A comparable situation is likely to occur in any bacterial mating but it turns out to be hard to prove in a filamentous bacterium like *Streptomyces*, whose fertilization takes place in a network of intertwined hyphae growing on the surface of a solid medium (Sermonti, Mancinelli & Spada-Sermonti, 1966). The occurrence of heterokaryosis in the genus makes it also possible that chromosome transfer occurs between nuclei present in the same hypha which could not be dissociated by breaking the mycelium mechanically. The two processes – heterokaryosis and fertilization – appear, however, to be independent of each other (Sermonti, 1969), and preliminary evidence has been

reported on the interruption of chromosome transfer during conjugation by abrupt change of growth medium (Sermonti *et al.* 1966).

A new method of interrupted mating has been devised, based on fine grinding of mycelium from mixed cultures. The changing pattern of recombinant progeny after different periods of mixed culture provides evidence that, whatever the cause of mating interruption, the effective formation of the merozygote is gradual in *Streptomyces*. The analysis of the mating process has taken advantage of the discovery of various types of fertility variants in *Streptomyces coelicolor* (Sermonti & Casciano, 1963; Hopwood *et al.* 1969; Spada-Sermonti & Sermonti, 1970; Vivian, 1970; Vivian & Hopwood, 1970). Mating pairs involving a high frequency donor (NF) and a high-frequency recipient (UF) have been particularly considered in this work.

2. MATERIALS AND METHODS

(i) *Strains and media*

The mutant strains used derive all from the wild-type *Streptomyces coelicolor* A 3(2) (*Streptomyces violaceoruber*, according to Kutzner & Waksman, 1959) the genetics of which have been thoroughly investigated (for a recent review, see Hopwood, 1967). The mutant loci are the same as located and described by Hopwood (1967), to whom we are indebted for having provided us with most of the strains adopted. The meaning of the mutant symbols is reported in Table 1. The location of the relevant loci is shown in Fig. 1. The media used are those already described in previous papers (Hopwood & Sermonti, 1962), i.e. a richly supplemented complete medium (CM) and a Czapek-Dox minimal medium (MM).

Table 1. *List of strains employed in the present work*

Code no.	Fertility type	Genotype*
053	IF	*hisG11*
80	NF	*argA1 hisA1 leuA1 uraA1 strA1*
39	NF	*hisA1*
219	UF	*metA2 pheA1 strA1*
2	IF	Wild-type
316	UF	*pheA1 strA1 hisD3*

* *his*, Requirement for histidine; *arg*, requirement for arginine; *leu*, requirement for leucine; *ura*, requirement for uracil; *str*, resistance to streptomycin; *met*, requirement for methionine; *phe*, requirement for phenylalanine.

(ii) *Fertility types*

The strains used belong to various fertility types. To avoid ambiguities we have accepted the symbols employed by Hopwood *et al.* (1969) and Vivian & Hopwood (1970). The three classes of fertility variants are as follows: NF, normal fertility, high frequency donors; IF, initial fertility, low frequency recipients or donors; UF, ultrafertile, high frequency recipients. A detailed description of the fertility behaviour has been given by Vivian & Hopwood (1970). As to the present work,

the recombination rate of some of the crosses is of interest. Crosses NF × UF yield from 50 to 100% recombinants, crosses IF × UF yield from 0·1 to 1 per cent recombinants; UF × UF crosses appear to be virtually infertile.

(iii) *Interrupted mating*

The strains to be crossed were streaked together on to celophane disks, about 3 cm in diameter, lying on complete agar medium, and incubated at 30 °C. When the mating had to be interrupted, the growing mycelium was harvested by repeatedly streaking a loop over the cellophane surface and shaking it in 2 ml sterile water.

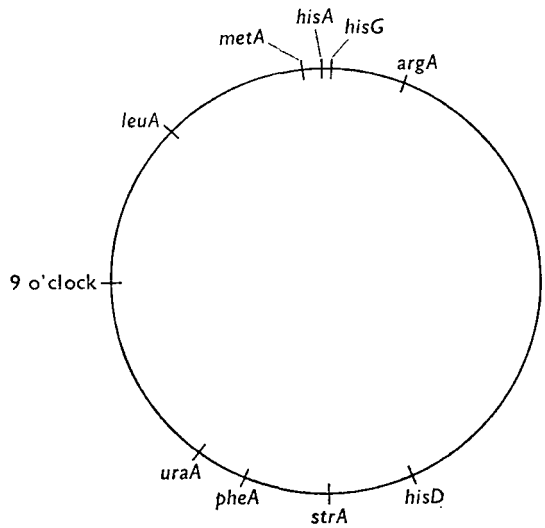

Fig. 1. The linkage map of *Streptomyces coelicolor* A 3(2), according to Hopwood (1967). Only the loci considered in this work are reported. The 9 o'clock position is the presumptive site of fertility factor (Hopwood *et al.* 1969).

The mycelium suspension was centrifuged and resuspended in 5 ml sterile water, and then finely ground in a Potter homogenizer. After 30 strokes the number of viable plating units reached a plateau, and this procedure was used throughout the work. The size of the fragments was rather uniform and measured under the eyepiece an average length of $3{\cdot}7 \pm 0{\cdot}76\ \mu$m (fiducial limits, $P = 0{\cdot}05$). Rare tiny filaments appeared longer, but never exceeded 20 μm. The hyphal suspension, properly diluted, was immediately plated on to media selective either for heteroclones (Hopwood *et al.* 1963) or for recombinants (Hopwood & Sermonti, 1962). The plating efficiency of the hyphal suspension obtained by this procedure was almost 100%.

(iv) *Characterization of recombinants and heteroclones*

The phenotype of the clones derived from various stages of mixed cultures have been classified by replica plating on diagnostic media, i.e. on a series of media containing all possible requirements but one, and on complete media containing

streptomycin. The heteroclones have first been identified by replica plating on the same medium on which they had been selected (Hopwood, Sermonti & Spada-Sermonti, 1963), then assayed on master plates of complete medium and classified by replica plating for the presence of some wild-type genes (Sermonti, Bandiera & Spada-Sermonti, 1966; Hopwood, 1966). The presence of such genes was in some cases directly inferred from the medium on which the heteroclones had been selected. A heteroclone growing in the absence of uracil, for instance, must be carrier of the wild-type allele of the *ura* gene.

3. RESULTS

(i) *Heteroclones isolated after different preincubation times*

By heteroclone we define a self-reproducing merodiploid formed in mixed cultures of *Streptomyces* (Sermonti *et al.* 1960). Selection of heteroclones require the use of two parent strains bearing two closely linked nutritional markers in

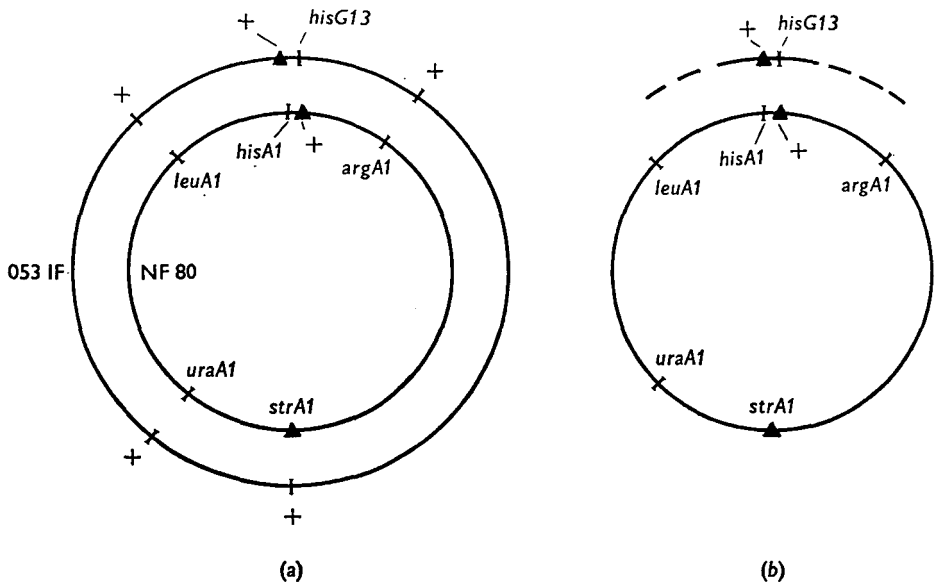

Fig. 2. Cross 053 IF *hisG13* × 80 NF *argA1 hisA1 leuA1 uraA1 strA1*. (*a*) Representation of the cross. (*b*) The simplest merozygote giving heteroclones on a medium supplemented with all the requirements but histidine.

repulsion and a plating medium selecting for the presence of the wild-type alleles of the two loci. In many experiments we observed that heteroclones obtained by plating the ground mixed mycelium from earlier samples were, as a whole, *more incomplete* than those obtained by later platings, i.e. the former bore a lower number of markers in the heterozygous condition. A model experiment will be described, representative of a consistently recorded situation. The parent strains were marked as shown in Fig. 2 (*a*). The *argA1* marker was disregarded throughout. The two *his* loci are very closely linked (actually adjacent, according to Piperno,

Carere & Sermonti, 1966). The selective media were always lacking histidine and containing streptomycin, so that the *hisG-hisA* region was selected to be heterozygous, while the *strA* region was hemizygous. The simplest merozygote structure fulfilling these conditions is shown in Fig. 2(*b*). It involves the shortest possible disomic region and two broken ends in the genome of only one parent, the outer one. Any other structure would require some breakage in both parental genomes. Since *hisG-hisA* and *strA* are on opposite sides of the circular map of *S. coelicolor* (Fig. 1), the loci considered mark only one half (the conventional left arc) of the total map.

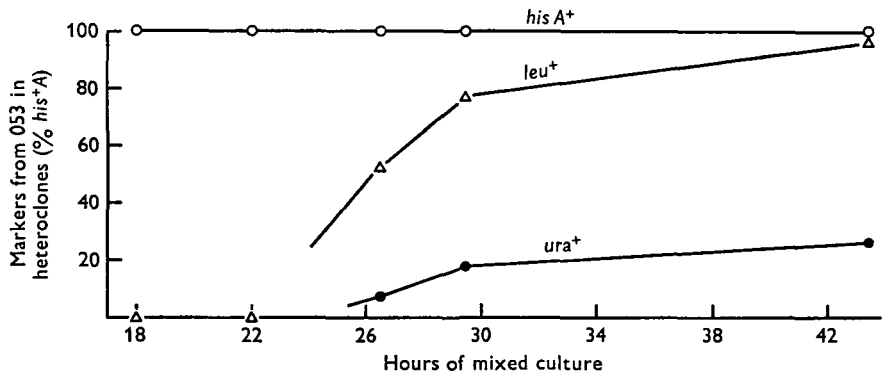

Fig. 3. Changing pattern in populations of heteroclones, obtained from mixed cultures at different time intervals. Cross: 053 *hisG13* + 80 *argA1 hisA1 leuA1 uraA1 strA1*. All heteroclones were selected in the absence of histidine and in the presence of streptomycin. The relative frequencies of *leu*+ and *ura*+ bearing heteroclones increase with time.

As shown in Fig. 3, the heteroclones selected from the early samples (18 and 22 h) may all be traced back to the simple merozygote structure sketched in Fig. 2(*b*). They all require leucine and uracil for growth. No heteroclones may be selected before 26 h in the absence of either supplement. In later samples leucine-independent heteroclones start to appear at a rate increasing with the increase of preincubation time, until they reach almost the whole of the heteroclones selected. Uracil-independent heteroclones appear at the same time as the leucine-independent ones, but at a significantly lower rate. All the *ura*+ heteroclones are also *leu*+, while most of the *leu*+ are *ura*−: thus three main phenotypes of heteroclones appear:

(1) *his*+ *leu*− *ura*− (2) *his*+ *leu*+ *ura*− (3) *his*+ *leu*+ *ura*+

Heteroclones (1) are formed earlier, types (2) and (3) are formed later, the latter at a lower rate.

These results are compatible with a contribution of the 053 (outer) genome to the zygote, increasing with time. The segment contributed elongates from the compulsory marker (*hisA1*+) towards the counterselected marker (*strA1*+). Strain 80 was bound to act as 'recipient' by the selective conditions, favouring heteroclones tracing back to merozygotes with a complete contribution of its genome,

and preventing the growth of heteroclones bearing the whole genome of the latter parent (053), through selection against the *strA1*$^+$ allele.

(ii) *Interrupted mating in NF × UF mixed cultures*

The very high rate of recombinant formation in NF × UF crosses allows the recovery of recombinant hyphae even after plating the ground mycelium on to complete medium. The fertility pattern of the colonies isolated from hyphal fragments at different time intervals can thus be studied in unselected populations.

The general picture of the interrupted NF × UF matings is as follows (Fig. 4). In the first half-day novel phenotypes appear roughly after 12 h and are represented by colonies bearing the recipient markers and the fertility ability of the donor parent. Their number increases steeply until a plateau is reached after about 1 day

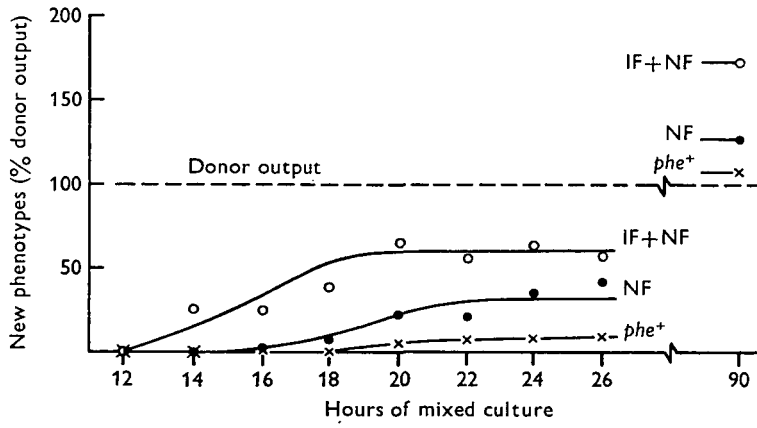

Fig. 4. Increasing rates of fertilized strains (IF + NF) and of *phe*$^+$ recombinants among colonies obtained from hyphae (or spores) of a mixed culture NF *hisA1* × UF *metA2 pheA1 strA1*. The new phenotypes, scored on complete medium, are given as percent of the donor (NF *hisA1*) output. A very high level of recombinants and/or fertilized strains was recorded among spores (90 h).

from the start of the mixed culture. After 2–4 h from the first appearance of the fertilized recipients recombinants start appearing, bearing from the donor parent only the marker closer to the 9 o'clock region of the map (where the fertility factor is assumed to be located, according to Hopwood *et al.* 1969). Later on new phenotypes appear with various combinations of donor and recipient markers. Nearly all recombinants are fertile (see also Hopwood *et al.* 1969) (Table 2).

A model experiment is reported in Table 2 and represented in Fig. 4. In the figure the rates of fertilized and recombinant hyphae are given as percent of the donor NF *his* hyphae recovered from the mixed culture at the same time. Fertilized recipients appear first after 14 h and reach a plateau of 60 % donor output after 20 h. It is remarkable that the first fertilized clones show only a moderate fertility, much lower than that of the donor parent. NF recipients appear later and represent an increasing proportion of the fertilized strains with the proceeding of the

Table 2. *Complete analysis of hyphal fragments from a mixed culture NF × UF at successive time intervals*

Cross: 39 NF + + + *hisA1* / 219 UF *strA1 pheA1 metA2* +

Time of sampling (h)	Parental types: Donor *NF his* (no.)	Parental types: Recipient UF *met phe str* (no.)	Fertilized recipients *met phe str*: IF no.	Fertilized recipients *met phe str*: NF no.	Recombinants (donor contribution*): *phe*+ IF no.	*phe*+ NF no.	*met*+ IF no.	*met*+ NF no.	*phe*+ *met*+ IF no.	*phe*+ *met*+ NF no.	Non-parental types: Total no.	Non-parental types: % donor output
12	94	69	0	0	0	0	0	0	0	0	0	0
14	67	80	16	0	0	0	0	0	0	0	16	23·8
16	101	52	25	2	0	0	0	0	0	0	27	26·7
18	100	48	29	8	1	0	0	0	0	0	38	38·8
20	82	44	31	19	2	1	0	0	0	0	53	64·7
22	101	51	29	15	3	4	2	1	0	0	54	53·5
24	101	47	26	25	3	6	0	0	2	0	62	61·3
26	90	35	11	29	2	4	0†	3	0	2	52	57·7
90‡	61	15	13	10	9	21	1†	13	5	31	114	186·8

* The *his* and *str* markers are not recorded.

† Plus one UF colony.

‡ The culture is fully sporulated.

mixed culture. The first recombinants are detected in the sample taken after 18 h (as shown in Table 3, they are indeed already present in previous samples, though their number may not be detected on unselective media). They are all of the *phe*$^+$ *met*$^-$ *his*$^+$ *str*$^-$ phenotype, i.e. of the recipient phenotype, in which only the *phe*$^+$ donor marker has been included, as well as the donor ability. The *phe*$^+$ recombinants, to which other donor markers are added later (Table 2), reach a level of about 10% donor output after 24 h.

If the mixed culture is allowed to develop to complete sporulation and colonies obtained from spore samples are tested for fertility and recombinant rates, a surprising picture emerges. The rate of fertilized clones largely exceeds the donor output (190%) and most of them are of the NF type. Also the recombinant (*phe*$^+$) rate exceeds the donor output (105%). This phenomenon may be accounted for by assuming that the sporulation phase involves the preferential (or even exclusive) inclusion into the spores of the products of merodiploid nuclei or of recipient nuclei bearing the fertility factor, the latter being possibly referable to merodiploids with the donor contribution restricted to the fertility factor and to the adjacent unmarked donor chromosome arc (see Hopwood *et al.* 1969; Vivian & Hopwood, 1970).

The changing pattern of recombinant progeny is better studied among recombinants arising on selective media after plating the ground hyphae at successive time intervals. Incidentally it may be noticed that the plating efficiency of recombinants on selective media appears constantly lower than that observed on complete media (Table 3). Three plating media were used, all supplemented with streptomycin to select for the *strA1* recipient markers. One medium was selective for the *phe*$^+$ donor marker, another for the *met*$^+$ donor marker and a third one for both markers. Recombinants appear first (14 h) on the first medium, then in the second (16 h) and later on in the third one (18 h). The three classes of recombinants, *phe*$^+$ *met*$^-$, *phe*$^-$ *met*$^+$ and *phe*$^+$ *met*$^+$, were easily scored by replica plating the colonies grown on the less-restrictive selective media, on minimal medium, and subtracting the prototrophic phenotypes (*phe*$^+$ *met*$^+$) from the number of phenotypes grown on media selecting for either *phe*$^+$ or *met*$^+$.

As has already been noticed, the first donor marker to appear among recombinants is *phe*$^+$, i.e. the one closer to the location of the fertility factor, in the counterclockwise sense. Later on the *met*$^+$ donor marker appears, farther from the location of the fertility factor, in the clockwise sense. The first *met*$^+$ recombinants *do not bear the phe*$^+$ *donor marker*. They reach a plateau of about 80% of the *phe*$^+$ *met*$^-$ recombinants. Recombinants bearing both *phe*$^+$ and *met*$^+$ appear only later and reach a final level of 25% the *phe*$^+$ *met*$^-$ recombinants (Fig. 5). This order of the donor marker's appearance clearly shows that there is not a regular progression of the donor markers' contribution, starting from a fixed origin, as is the case in *E. coli* K 12 Hfr × F$^-$ crosses (Wollman *et al.* 1956).

The longer the preincubation time, the longer the donor segment contributed, though its ends are not fixed and its arc always includes the fertility factor site. It may be assumed that at the very beginning of mating, the shortest possible

contribution from the donor parent to the merozygote only includes the fertility-factor site (see Fig. 4).

Fig. 5 shows that, after 24 h, the recombinants' relative frequencies stay constant with time and do not change even during the sporulation phase. This observation supports the assumption that merozygotes are definitely established after 24 h and the great increase of the recombinants' rate recorded in Fig. 4 must be accounted for by a preferential contribution of segregation products to the spore nuclei. The pattern of segregation does not appear to differ significantly in growing basal mycelium and in sporulating hyphae.

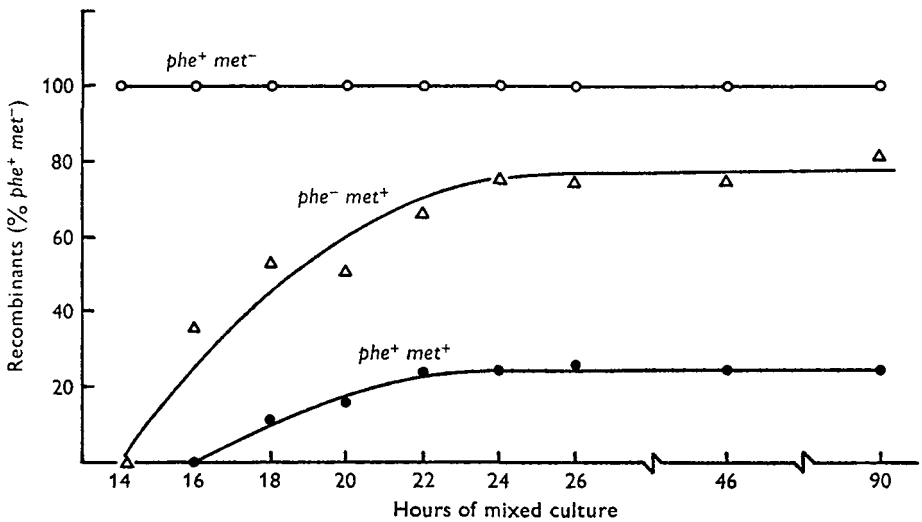

Fig. 5. Relative rates of three classes of streptomycin resistant recombinants from cross of Fig. 4, given as percentage of the most frequent class (*phe*$^+$*met*$^-$). Recombinants were scored on selective media. Their relative frequencies are changing with time, until about 24 h from the starting of the mixed culture.

(iii) *Segregation of fertility among recombinants*

All the recombinant spores in a cross NF × UF have been reported by Hopwood *et al.* (1969) to be of the NF type. As we have seen, a fraction of them is better referred to as the IF type. We have thoroughly checked the progeny of a cross 39 NF *hisA1* × *219* UF *metA2 pheA1 strA1* looking for possible occasional segregants of the UF type. They have been detected among the rare *phe*$^-$ *met*$^+$ recombinants, the two markers being in the following position around the NF site:

+	NF	+
metA2	UF	*pheA1*

Most of the recombinants (130/197) are indeed of the UF type while the remaining ones are of the NF (or IF) type. The NF/UF alternative segregates thus among the

recombinants as a pair of allelic loci. This confirms the chromosomal location of the fertility factor in NF strains, in a site between *pheA1* and *metA2* (Hopwood *et al.* 1969). However, the NF/UF ratio among recombinants shows variations from experiment to experiment, possibly due to the ratio between the parental strains in the inoculum. The presence of the fertility factor appears not to be compulsory for growth and replication of the recombinant nuclei, but it may be indispensable for zygote synthesis and/or recombinant formation.

Table 3. *Recombinant types* per cent hyphal fragments of a mixed culture NF × UF, scored at different time intervals on selective and on complete media (cross as in Table 2)*

	Donor contribution					
	phe+		*met*+		*phe*+ *met*+	
Time of sampling (h)	Media selective (*HIS, MET STR*)	Media complete	Media selective (*HIS, PHE, STR*)	Media complete	Media selective (*HIS, STR*)	Media complete
12	0·00	0·0	+0·00	0·0	0·00	0·0
14	0·005	0·0	0·00	0·0	0·00	0·0
16	0·03	0·0	0·01	0·0	0·00	0·0
18	0·19	0·53	0·11	0·0	0·02	0·0
20	0·50	1·65	0·25	0·0	0·08	0·0
22	0·64	3·39	0·42	1·45	0·15	0·0
24	0·78	4·28	0·60	0·0	0·19	0·0
26	0·80	3·38	0·60	2·2	0·20	0·0
46	1·08	—	0·78	—	0·27	—
90†	3·5	17·0	2·8	4·5	0·83	1·5

* Only streptomycin-resistant recombinants grow on the selective media and have been scored on complete medium. 10^5, 2×10^5 and 3×10^5 fragments have been plated on selective media at 12, 14 and 16 h respectively, and larger numbers thereafter. The unselected samples (complete medium) numbered about 200 colonies (see Table 2).

† The culture was fully sporulated.

4. DISCUSSION

The data reported provide a significant demonstration that the pattern of recombinants recoverable from a mixed culture of two strains of *Streptomyces coelicolor* A 3(2) is variable according to the age of the culture. This hypothesis had already been supported by some preliminary data by Sermonti *et al.* (1966).

As a rule, one or more markers appearing in later samples are fully missing in earlier samples. We may thus argue that such markers have been in some way prevented from contributing to the early recombinant progeny by the operation performed in order to interrupt the mating process.

Different markers are affected to a different extent by this exclusion phenomenon, some being absent in the first samples containing recombinants, others being present as soon as recombinants are recoverable. The progressive involve-

ment of markers in the sexual process is thus evident and it is also evident that the sampling procedures interfere with such progression. However, it is not possible to state that the samples drawn at a given time actually derive from merozygotes bearing the markers expressed in the recombinant colonies. The completion of the zygotes may be hindered but not cut down, or markers already contributed to the merozygote may be prevented from participating further in the recombination process. An operational definition of a merozygote is indeed hard to provide. We eventually deal with recombinants but these are the result of a three-step process (Sermonti *et al.* 1966):

$$\text{merozygote} \xrightarrow{2} \text{heteroclone} \xrightarrow{3} \text{recombinants.}$$

The last two steps involve a crossing-over mechanism (Hopwood, 1967). The progressive appearance of markers in the recombinants along with the ageing of the mixed culture may be accounted for by a progressive completion of the merozygote (step 1), though a gradual spreading of the segment liable to crossing-over may also be assumed, e.g. due to an extension of the effective pairing region in the merozygote (step, 2) or in the heteroclone (step 3). When recombinants or heteroclones are obtained on selective media the delayed expression of the phenotype may also play a role. If the rate of recombinants obtained from the same hyphal sample on selective and on complete media are compared, it turns out that their number is roughly three to five times higher in unselective media, even when mature spores are plated (see Table 3). Such reduced expression of the markers on selective media does not, however, alter the general picture of the progressive contribution of donor markers to the recombinant progeny.

Whatever the process involved, the mechanical or biochemical mechanism determining the markers' exclusion is still far from being understood. Sermonti *et al.* (1966) obtained a mimicry effect similar to mating interruption just by transferring intact cultures from complete to minimal medium on cellophane disks. In the present work the hyphae were finely broken to small pieces to make the interruption more convincing and drastic but it would be wholly arbitrary to assume the occurrence of the breakage of some bridge or pilus connecting a mating pair of hyphae. Anyway, the mating appears to be effectively interrupted by the series of operations performed.

The study of heteroclones selected from mixed cultures of various ages provides unquestionable evidence that the pattern changes with time. The disomic region appears to be confined to the very segment selected for heterozygosity in the heteroclones present in the first two samples. Further markers from the parent selected to act as donor (053) appear only in the third sample (8 h after the first one). Thereafter the heteroclones turn out to be more complete, the longer the preincubation time. The long delay before the appearance of a second marker suggests that two different recombination processes are involved, one concerning the simple transfer of the *his* region (possibly the *his* operon) and another involving the transfer of the large chromosome segments. This idea is supported by the observation made by Sermonti & Carere (1969) that the polarized recombination within the *his* operon does not affect, as a rule, the remaining part of the genome.

A better evidence of a gradual chromosome transfer during fertilization comes from interrupted mating in mixed cultures of NF (donor) and UF (recipient) strains. Various recombinant types make their first appearance after various pre-incubation times. A precise time of entrance cannot, however, be determined for each marker, due to the long time elapsing between the seeding of spores and effective contact among growing hyphae. As a rule the closer a donor marker to the 9 o'clock region of the map, the sooner it appears among recombinants, the higher the final level observed. The donor markers, however, do not make their first appearance in a time sequence corresponding to their clockwise (or counterclockwise) order in the map, starting at the 9 o'clock position. After a marker at 7 o'clock position (*phe*$^+$) has appeared among recombinants, a marker appears at the 12 o'clock position (*met*$^+$) and still later recombinants appear, bearing both markers (*phe*$^+$ *met*$^+$). The simplest assumption explaining these results is that the 9 o'clock region is compulsory in the donor contribution (Hopwood *et al.* 1969; Vivian & Hopwood, 1970) but its function is not that of producing a transfer origin. The chromosome segment donated may extend on either side or on both sides of the obligate tract. Moreover it seems that a longer donor segment requires a longer time to be contributed to the recombinant progeny.

As a matter of fact, the donor ability is the first donor character to be transferred to recipient cells. This transfer is, however, unique, in so far as the donor ability is achieved gradually by the fertilized recipients. The initial level of fertility (IF) turns out to be a self-reproducible condition since clones are isolated with initial fertility, which breed true. In other words, the first result of the contact between an NF and a UF cell is the formation of an IF cell. The point has been discussed by Spada-Sermonti & Sermonti (1970) and it deserves further investigation. A point clearly established by the data obtained in this work is that the 9 o'clock region is very likely compulsory for the merozygote formation, but is not required for recombinant reproduction, since very few recombinants are found with a UF fertility type. The 9 o'clock region is the presumptive location of a fertility factor, whose features might be profoundly different from those of the F factor in *E. coli* K 12 (Wollman *et al.* 1956).

Its nature seems rather comparable with that of the FP factor in *Pseudomonas aeruginosa*, which turns out to enter first into the recipient cells in interrupted mating experiments (Loutit, Marcinus & Pearce, 1968) and appears to have perhaps only two or three possible locations on the chromosome (Holloway, 1969). There seems to be only one location in *S. coelicolor* (Vivian & Hopwood, 1970).

On the basis of the results reported several phases may be distinguished in the process eventually leading to the formation of recombinant spores in an NF × UF cross. First, the parental spores germinate and produce hyphae which come into effective contact (about half a day in our experimental conditions). Then the merozygotes are gradually formed (another half day). They are self-reproduced in the basal mycelium, very likely in the form of heteroclones, undergoing segregation (one or two days). Finally the sporulation process starts, during which the products of segregation of the heteroclones are preferentially included into the spores, which

eventually contain almost 100% of segregation products (Hopwood *et al.* 1969). In NF × UF mixed cultures the spores formed may well be considered as sexual spores, and the whole surface of the culture as the surface of a fruiting body or hymenium.

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