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Genetic Analysis and Genome Structure in *Streptomyces coelicolor*

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INTRODUCTION

In a previous monograph (38), we gave as one reason for interest in the genetics of *Streptomyces coelicolor* a desire to know the features that characterize the genomes of prokaryotes. It is an unfortunate fact that many interesting groups of prokaryotes remain almost or completely unknown from a genetic standpoint: spirochetes, myxobacteria, blue-green algae, to name only three. Therefore, in the absence of information on the genetics of the majority of prokaryote groups, the problem can be provisionally expressed in the following question: which peculiarities of the structure and behavior of the *Escherichia coli* chromosome, the best known prokaryote genome, are shared by that of *S. coelicolor*? Since these two microbes are near the opposite extremes of morphological complexity to be found among prokaryotes, they are likely to be phylogenetically separated to a more than average degree. Therefore, common features of their genetics stand a good chance of being shared by the majority at least of prokaryotes.

Since the last review, some progress has been made in answering the question posed above. There is now quite good evidence that *S. coelicolor* resembles *E. coli* in all of the following: the zygotes are merozygotes, that is, disomic for only part of the genome (see later in this article); the genome consists of a single chromosome (31), which is circular or circularly permuted (33); and functionally related loci are frequently

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clustered on the genome (29, 30). The notion that these features are indeed prokaryote trademarks is supported by positive evidence from some other bacterial species, and lack of contrary evidence from others: no bacterium has so far been shown to manifest complete diploidy in its zygotes; *Salmonella typhimurium* (60, 66) and probably *Bacillus subtilis* (17, 53, 74) have single linkage groups, the former circular and the latter (still) linear, and the chromosome of *Mycoplasma hominis* consists of a single ring of deoxyribonucleic acid (DNA; 4). The only discrepancy in the chain of similarities was the apparent lack of clustering in *Pseudomonas aeruginosa* (26), but more recent studies have indicated that certain genes may be clustered also in this organism (54); it would appear that clustering, although common in bacteria, is highly inconstant from group to group and has probably been free to vary quite extensively during prokaryote evolution (*see* section on *Meaning of Gene Arrangement on the Linkage Map*).

Apart from helping to elucidate these comparative questions, the past 5 years has seen a considerable refinement of genetic analysis in *S. coelicolor*. Previously troublesome irregularities in segregation are now explicable to a considerable degree, as we shall see in this paper, so that genetic analysis is no longer tedious. Moreover, the linkage map is abundantly marked. Thus, the basic requirements of an experimental genetic organism are satisfied. This article will be concerned for the most part with describing the techniques currently employed for genetic analysis in *S. coelicolor* so that the reader may judge the advantages and limitations of this organism for studying any particular problem. Some speculations on the kinds of questions to which *S. coelicolor* might be able to contribute answers are made in the final section.

NATURAL HISTORY OF A CROSS

A brief outline of a cross at this point will make it easier for the reader unfamiliar with the peculiarities of *Streptomyces* genetics to understand what follows. Practical details are deferred until later sections of this article. A cross usually consists of the following steps.

(i) Two parent strains are chosen, differing in a minimum of two characters, and usually more. (Outcrosses of single mutants to the wild type cannot so far be analyzed since sexually produced progeny can be recognized as such only when they differ from *both* parents, and therefore from the large excess of asexually produced progeny which are always present.)

(ii) Inocula of the two strains, consisting of spores or hyphal fragments, or both, are mixed on a slant of nonselective agar medium ("complete medium").

(iii) The mixed culture is incubated (at 28 to 30 C) for a minimum of 2 to 3 days, during which time the complete cycle of development takes place, comprising the following stages: spore germination; growth of the substrate mycelium; development of the aerial mycelium; sporulation. [Outlines of the structure and life-cycle, with references to original papers, were presented by Hopwood and Sermonti (35) and Sermonti and Hopwood (64). A schematic drawing of a colony, summarizing its main organizational features, is given in Fig. 1.] During one or more of these stages, probably the second, zygotes arise; these zygotes are merozygotes, heterozygous for some, but normally not all, of the characters in which the parent strains differ. No absolute barriers to recombination have been encountered among the hundreds of strains—all descended by mutational and recombinational steps from the same single-spore wild-type culture (27)—employed in Glasgow; that is, all crosses have given rise to recombinants, although the yield of recombinants, expressed as the ratio of nonparental to parental spores arising from a cross, varies widely between different pairs of strains. Some of these same strains and their immediate descendants appeared, after subculture for a few generations in Rome, to form a group of intersterile cultures, fertile with a second group of universally fertile strains (63). The manifestation of sterility by the first group of strains was, however, somewhat irregular, since they were reported to regain a moderate fertility on further subculture. Thus, for all practical purposes, almost any strain can currently be crossed with any other.

(iv) As soon as enough spores are produced on the mixed culture, they are harvested as a suspension in water.

(v) The suspension is spread on the surface of plates of one or more selective media, that is, media on which neither parent can grow by virtue of a lack of a growth factor required by one strain or because of the presence of an inhibitory substance to which one strain is sensitive.

(vi) On incubation of the plates, a small but variable proportion (between 10^{-2} and 10^{-7}) of the spores in the suspension are able to develop into colonies on the selective medium, because they combine characters originally present in different parents.

These recombinant colonies are of two kinds: haploids and heteroclones. The relative proportions of the two types can be varied according to

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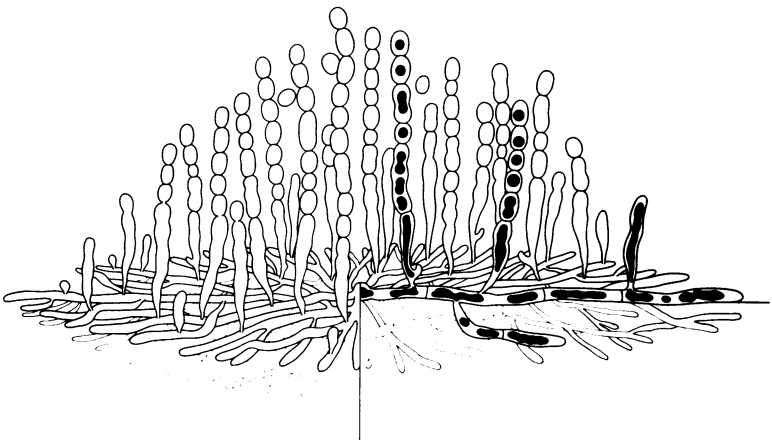

FIG. 1. *Diagram of a Streptomyces colony growing on agar medium, showing the spore that initiated the colony (center), hyphae of the substrate mycelium (horizontal and below the medium surface), aerial hyphae (vertical above the medium surface), and spores. The right half of the colony is sectioned to show nuclear regions and septa. Reproduced from Hopwood and Sermonti (38) by permission of Academic Press, Inc.*

the pattern of selection imposed by the plating medium. Haploid recombinant colonies develop from spores containing haploid genomes and appear genetically homogeneous: subculture from a single colony gives rise to cultures all of the same genotype. Heteroclones, on the other hand, develop from plating units (possibly spores) containing partially disomic genomes (which are postulated to be terminally redundant heterozygotes; see later in this paper), and are genetically heterogeneous: subculture by suitable methods from a heteroclone gives rise, not only to further ("higher order") heteroclones, but also to a wide spectrum of haploids having the markers for which the heteroclone was heterozygous in new combinations. When the spores of the heteroclone are subcultured, only a small minority (ca. 2%) of the resulting cultures are heteroclones because segregation within the heteroclone, producing haploids, is almost complete by the time sporulation has occurred; when the mycelium is subcultured, the proportion of haploids is much lower.

(vii) In a genetic analysis, haploids or heteroclones are studied, depending on the scope of the experiment. Haploids are analyzed by classification according to their nonselected markers. From the resulting segregation pattern, the linkage relations of the markers, etc., can be deduced. Heteroclones are analyzed individually by plating their spores on a nonselective medium and then classifying a sample of the resulting haploid segregants for all the markers in the cross. This classification reveals which markers were heterozygous in a particular heteroclone, in addition to providing other information, as we shall see in a subsequent section.

MATERIALS OF A CROSS

Media

Formulas for the two basic media are slightly modified from those given by Hopwood and Sermonti (38).

Minimal medium (MM) has the following composition (quantities per liter): asparagine, 0.5 g; K_2HPO_4, 0.5 g; KOH, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; agar, 15 g; and glucose (autoclaved separately as 50% solution), 10 g. Selective media are supplemented as follows (per liter): amino acids except histidine, 50 mg; histidine, 70 mg; adenine and uracil, 10 mg; vitamins, 1 mg; dihydrostreptomycin sulfate, 50 mg; acriflavine, 15 mg.

Complete medium (CM) contains (per liter): K_2HPO_4, 5 g; NaCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; peptone (Difco), 2 g; yeast extract (Yeastrel), 1 g; Casamino Acids (Difco), 1.5 g; yeast nucleic acid hydrolysate (boil 2 g of nucleic acid in 15 ml of 1 N NaOH for 10 min; boil 2 g of nucleic acid in 15 ml of 1 N HCl for 10 min; mix the two solutions; adjust to *p*H 6.0; filter hot; make up to 40 ml with water), 5 ml; vitamin solution (riboflavine, 0.1%; nicotinamide, 0.1%; *p*-aminobenzoic acid, 0.01%; pyridoxine HCl, 0.05%; thiamine HCl, 0.05%; biotin, 0.02%), 1 ml; agar, 15 g; glucose, 25 g; histidine, proline, threonine, tryptophan, and tyrosine (if dealing with the relevant auxotrophs), 50 mg each. CM is adjusted to *p*H 7.2 with 1 N HCl and can

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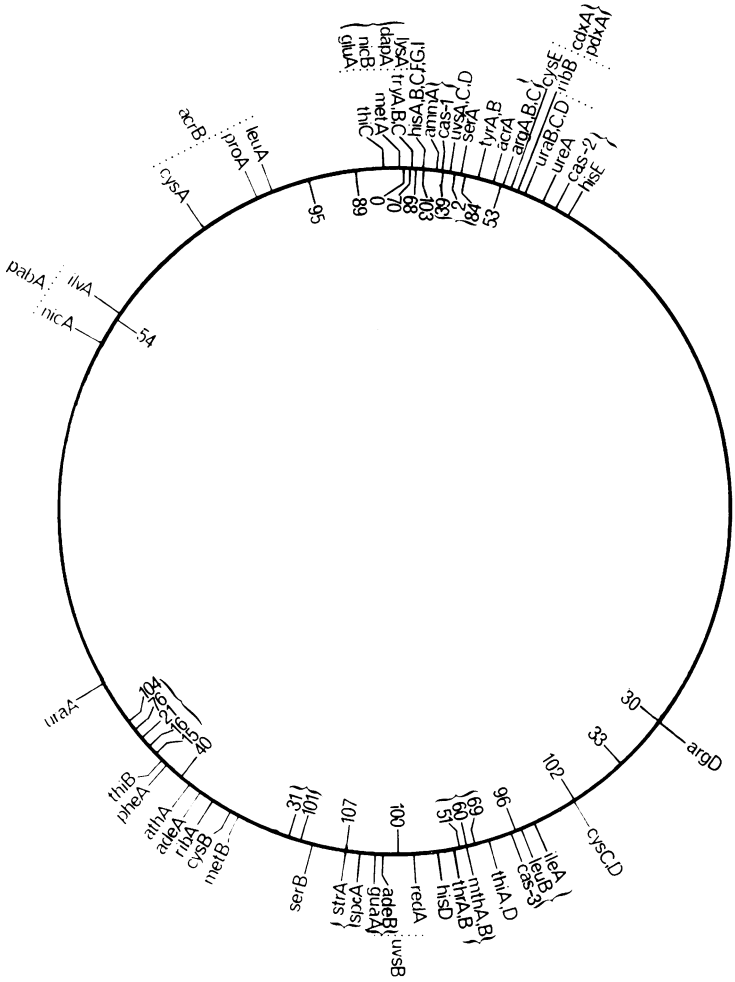

FIG. 2. *Linkage map, from Hopwood (32), with later additions. The map is drawn to scale, the circumference corresponding to approximately 260 cM [1 cM (centimorgan) is a unit of map distance corresponding to one per cent recombination when corrected for the effects of multiple crossovers (24)]. Symbols of loci on the outside of the circle are explained in Table 1. Numbers on the inside of the circle indicate the loci of indispensable temperature-sensitive mutants. The orders within groups of bracketed loci are unknown. Certain loci have not been ordered relative to groups of loci covered by dotted lines.*

be autoclaved at 115 C for 10 min without excessive decomposition of the glucose.

Mutagenic Treatments

Ultraviolet light (UV) remains a very convenient routine mutagen (*for details, see* 38).

Few chemical mutagens have been tested for convenience and efficacy in *S. coelicolor*. Spores treated with *N* - methyl - *N'* - nitro - *N* - nitrosoguanidine (NTG) at 1 mg/ml in tris(hydroxymethyl)aminomethane (Tris)-maleic acid buffer, essentially according to the schedule of Adelberg, Mandel, and Chen (1), have yielded mutants, but not in the spectacular proportions reported for some organisms. Experiments currently in progress (V. Delić and D. A. Hopwood, *unpublished data*) indicate that good mutant yields can be obtained by exposure to NTG at high *p*H (8 or higher instead of 6). J. H. Coats (*personal communication*) obtained higher mutant yields in another species of *Streptomyces* by allowing the spores to germinate during incubation for 5 hr before treatment with the mutagen. Ethyl methane sulfonate (EMS) has been used successfully as a mutagen by P. Engel (*personal communication*), who followed a procedure similar to that described by Freese (21).

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TABLE 1. *List of marker loci with their mutant alleles*[a]

Locus	Alleles[b]	Characteristics[c]
acrA	*acr-9*, (*50*)	Resistant to acriflavine
acrB	*acr-3*	Resistant to acriflavine
adeA	*ade-3*, *7*, *22*, (*14*), (*15*), *h3*	Requirement for purines
adeB	*ade-h1*	Requirement for adenine
ammA	*amm-1*, *2*, *3*, *4*, *5*	Unable to utilize nitrate
argA	*arg-1*	Requirement for arginine (or citrulline) or ornithine
argB	*arg-t74*, (*2*)	Requirement for arginine
argC	*arg-4*	Requirement for arginine (or citrulline) or ornithine
argD	*arg-v8*	Requirement for arginine (or citrulline) or ornithine
athA	*ath-2*, *8*, *10*, *11*, *12*, *h1*, *h2*, *h3*, *h4*, *h5*	Requirement for purines plus thiamine
cas-1, *2*, *3*	*cas-1*, *2*, *3*	Requirement for "casein hydrolysate"
cdxA	*cdx-vk62*	Requirement for carbon dioxide
cysA	*cys-15*, (*17*), (*19*), (*20*)	Requirement for cysteine
cysB	*cys-4*, *6*, *22*, *23*, *24*	Requirement for cysteine or S_2O_3
cysC	*cys-3*, (*14*)	Requirement for cysteine or S_2O_3 or S_2O_4
cysD	*cys-5*, *7*, *9*, *11*, *13*, *18*, (*16*), (*21*)	Requirement for cysteine or S_2O_3 or S_2O_4 or S_2O_5
cysE	*cys-24*, *v1*	Requirement for cysteine or S_2O_3
dapA	*dap-1*	Requirement for diaminopimelic acid
gluA	*glu-v1*	Requirement for glutamic acid
guaA	*gua-1*, *h1*	Requirement for guanine
hisA	*his-1*, *120*, *132*	Requirement for histidine (*histidinol dehydrogenase*)
hisB	*his-2*, *11*, *12*	Requirement for histidine or histidinol (*imidazole glycerol phosphate dehydrase*)
hisC	*his-9*, *10*, *14*, *119*, *127*, *128*	Requirement for histidine or histidinol (? *cyclase*)
hisD	*his-3*, *4*, *15*, *121* (*h2*)	Requirement for histidine or histidinol (*histidinol phosphate phosphatase*)
hisE	*his-6* (*5*)	Requirement for histidine or histidinol or purines (*PR-AMP 1,6-cyclohydrolase*)
hisF	*his-116*, *123*	Requirement for histidine or histidinol (? *isomerase*)
hisG	*his-8*, *13*, *117*	Requirement for histidine or histidinol (*imidazole acetol phosphate transaminase*)
hisI	*his-129*	Requirement for histidine or histidinol (? *amidotransferase*)
ileA	*ile-h1*	Requirement for isoleucine
ilvA	*ilv-1*	Requirement for isoleucine plus valine
leuA	*leu-1* (*3*), (*4*)	Requirements for leucine or α-ketoisocaproic acid
leuB	*leu-5*, *t92*	Requirement for leucine or α-ketoisocaproic acid
lysA	*lys-1*	Requirement for lysine
metA	*met-2*	Requirement for methionine
metB	*met-3*, *4*, *5*, *6*	Requirement for methionine or homocysteine
mthA	*mth-1*	Requirement for methionine plus threonine
mthB	*mth-2*	Requirement for methionine plus threonine; or homoserine
nicA	*nic-1*, (*2*), (*3*), (*6*), (*h1*)	Requirement for nicotinamide
nicB	*nic-v1* (*h2*)	Requirement for nicotinamide
pabA	*pab-1*	Requirement for *p*-aminobenzoic acid
pdxA	*pdx-v1*	Requirement for pyridoxine
pheA	*phe-1*	Requirement for phenylalanine
proA	*pro-1*, *3*, (*2*), *h1*, *h2*	Requirement for proline
redA	*red-1*	Red pigment instead of blue/red indicator pigment
ribA	*rib-h1*	Requirement for riboflavine
ribB	*rib-1*	Requirement for riboflavine
serA	*ser-1*	Requirement for serine or glycine
serB	*ser-2*	Requirement for serine (or glycine)
spcA	*spc-1*	Resistant to spectinomycin
strA	*str-1*	Resistant to streptomycin
thiA	*thi-1*	Requirement for thiamine; does not crossfeed *thiB*

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TABLE 1—*Continued*

Locus	Alleles[b]	Characteristics
thiB	*thi-3*, (*7*), (*9*), (*h2*)	Requirement for thiamine or thiazole
thiC	*thi-t106*, (*2*), (*4*), (*5*), (*6*), (*h1*)	Requirement for thiamine; crossfeed *thiB*
thiD	*thi-8*	Requirement for thiamine; crossfeeds *thiB*
thrA	*thr-1*	Requirement for threonine
thrB	*thr-2*	Requirement for threonine or serine
tryA	*try-1*	Requirement for tryptophan; accumulation of indole
tryB	*try-2*	Requirement for tryptophan or indole or anthranilic acid
tryC	*try-5*	Requirement for tryptophan or indole
tyrA	*tyr-t98*	Requirement for tyrosine or phenylalanine
tyrB	*tyr-1*	Requirement for tyrosine
uraA	*ura-1*	Requirement for uracil
uraB	*ura-2*	Requirement for uracil
uraC	*ura-3*	Requirement for uracil
uraD	*aur-1*, (*arg-3*, *7*)	Requirement for uracil plus arginine
ureA	*ure-1*, (*3*), (*20*)	Urease-negative
uvsA	*uvs-2*, *4*, *9*, *15*, *19*, *22*, *23*	UV-sensitive
uvsB	*uvs-6*, (*12*), (13), (*21*)	UV-sensitive
uvsC	*uvs-7*, *8*, *10*, *14*, *16*, *17*	UV-sensitive
uvsD	*uvs-1*, *3*, *5*, *11*, *18*	UV-sensitive

[a] Sources of information on the location of loci mapped by others: *acrA*, *acrB* (I. Spada-Sermonti); *adeB*, *ileA*, *ribA* (R. J. Harold); *argD*, *cdxA*, *gluA*, *nicB*, *pdxA* (72*a*); *hisF*, *hisG*, *hisI* (55); *uvsA*, *uvsB*, *uvsC*, *uvsD* (25).

[b] Parentheses indicate uncertainty; *h*, R. J. Harold; *t*, temperature-sensitive; *v*, A. Vivian; *his* alleles over 100, (55, 59).

[c] Tentative association of histidine biosynthetic enzymes with *his* genes is based on growth and accumulation studies summarized by Russi et al. (59), and enzymological studies (especially for *hisE*) of P. Engel (*personal communication*).

Isolation and Description of Mutants

A linkage map is given in Fig. 2, and a list of the characteristics of the markers on it is given in Table 1.

The great majority of current markers are provided by auxotrophic mutants. Other mutants are resistant to various agents (*acr*, *spc*, *str*), sensitive to radiation (*uvs*), or deficient in a dispensable enzyme revealed by a plate reaction (*ure*). The map also shows (by numbers on the inside of the circle) the loci of a collection of indispensable temperature-sensitive (*tps*) mutants (32), a few of which (not more than one per cross) serve as useful markers in regions of the map poorly provided with markers of other kinds (especially *tps-30*).

Auxotrophs. A much wider spectrum of auxotrophs has been isolated and mapped since the previous review (38). Many define "clusters" of loci concerned with different steps in the same pathway of biosynthesis (29), including at least the following groups: *argA*, *B*, *C*; *cysC*, *D*; *guaA*; *adeB*; *hisA*, *B*, *C*, *F*, *G*, *I*; *mthA*, *B*; *thiA*, *D*; *tryA*, *B*, *C*; *uraB*, *C*, *D*. These clusters greatly facilitate the isolation of heteroclones disomic over particular regions of the genome (see section on *Heteroclone Analysis*).

Resistant mutants. To streptomycin resistance have now been added resistance to spectinomycin and (by Sermonti and Spada-Sermonti) two loci for acriflavine resistance.

Enzyme losses scored by plate tests. Details of a plate test for urease were given by Hopwood (30), who mapped a locus for production of this enzyme.

Temperature-sensitive mutants. These mutants are readily isolated by comparing replica plates, incubated at 30 and 38 C, from the same plate of colonies, and picking those colonies that fail to grow, or grow slowly, at the higher temperature, although they are normal at the lower temperature (32). As in other organisms (18), they are expected, and found, to map at many different loci.

Ultraviolet-sensitive mutants. These mutants have been isolated by comparing two replica plates, one of which had received, before incubation, a dose of UV sufficient to kill about 50% of wild-type spores. The mutants so far mapped identify four loci: *uvs A*, *B*, *C*, *D*, of which *uvs A*. *C*, *and D* constitute a closely linked cluster (25),

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PROCEDURES OF GENETIC ANALYSIS

Two procedures are central to a serious genetic investigation of any organism: mapping, and testing for dominance and complementation. In this section, analyses of the two kinds of recombinant colonies, haploids and heteroclones, are described, particularly from the point of view of these procedures.

Haploids were the first class of recombinant colony to be discovered, so that preliminary mapping had perforce to make use of them (27). Later, when heteroclones were identified, these were preferred for mapping all except very closely linked markers because segregation data from heteroclones could be demonstrated to be free from differential viability and other sources of error (38). Heteroclones have now been overtaken again by haploid recombinants, since more complete knowledge of the linkage map and identification of zygote incompleteness as a cause of peculiarities of segregation have enabled the ordering of loci by haploid recombinant analysis to be carried out reliably, even over long map intervals (*see below*). Heteroclones, apart from being more laborious to analyze, are ill-suited for this purpose, owing to the comparative rarity with which they are disomic over more than a small proportion of the genome. On the other hand, heteroclones provide a means of carrying out tests of complementation and dominance; they were also useful for estimating the lengths of key intervals of the map in standard recombination units, since haploids, unless analyzed by a rather laborious procedure (27), yield only *relative* map lengths.

Methods

In this section are outlined some of the simple procedures that have proved useful so far in *S. coelicolor* genetics. The schedule that follows is partly based on the description of techniques given by Hopwood and Sermonti (38). It consists of a series of alternative procedures having certain steps in common; alternative steps in the schedule have the same letter, and are distinguished by superscripts. The procedures described are: *mapping by selecting haploids* (steps a, b, c, d, e); *detection of heteroclones—complementation tests* (a, b, c′, d′); *complete analysis of heteroclones* (a, b, c′, d′, e′, f′, g′, h′, i′); and *"phenotypic" analysis of heteroclones* (a, b, c′, d′, e′, f″, g″, h″). Times are, of course, subject to some variation.

(a) Day 1. Streak the two parent strains together on a large slant of CM.

(b) Day 4. Make a spore suspension, by scraping the surface of the culture with a loop in 10 ml of water, shake vigorously, filter through cotton wool, centrifuge at 1,000 × *g* (or more), resuspend pellet in a small volume of water, shaking vigorously. Spread 0.1 ml of the suspension, undiluted and at 1:10 and 1:100 dilutions, on plates of MM with suitable supplements.

(c) Day 8. Make master plates on the same medium as the selective plates, e.g., 50 colonies per plate.

(d) Day 10. Classify any visible markers by inspection. Classify auxotrophic markers and resistances by replica plating on a series of diagnostic plates of suitably supplemented MM.

(e) Day 12. Record genotypes.

(c′) Day 7. As soon as colonies have developed aerial mycelium but before any background parental growth has developed, make replicas from the selective plates on plates of the same medium to identify heteroclones. Incubate replicas, and store originals in the refrigerator.

(d′) Day 9. Compare replicas and originals. Identify as heteroclones those (small) colonies on the original plates which fail, completely or partially, to give growth on the replica, even though they have aerial mycelium (powdery to the naked eye); i.e., exclude any colonies that fail to replicate merely because they lack aerial mycelium.

(e′) Having identified heteroclones, choose those that completely failed to give growth on the replica plate; i.e., reject those that were partially "overgrown" by a recombinant clone that originated early in the development of the colony. Suspend spores from each heteroclone in a small volume of water, by means of a drop of water on a loop.

(f′) Spread 0.1 ml of the suspension, undiluted and at 1:10 dilution, on plates of CM (or fully supplemented MM).

(g′) Day 12. Make master plates on nonselective medium (CM or fully supplemented MM).

(h′) Day 14. Classify auxotrophic markers and resistances by replica plating on a series of diagnostic plates of suitably supplemented MM.

(i′) Day 16. Record genotypes.

(f″) Make "heteroclone master plates" by spreading a loopful of the undiluted suspension from each heteroclone in a sector of a plate of nonselective medium, e.g., eight sectors per plate.

(g″) Day 12. Replicate master plates to diagnostic media, each completely lacking a growth factor (in the case of leaky mutants) or containing a suboptimal concentration of it (in the case of nonleaky mutants; 33).

(h″) Day 14.. Classify each heteroclone for

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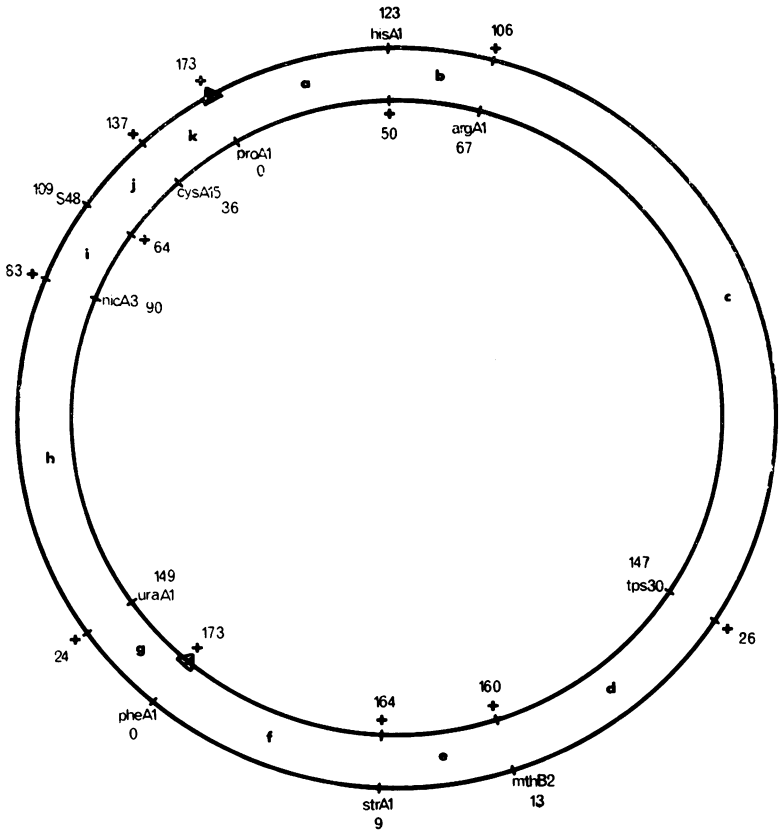

FIG. 3. *Location of a morphological marker, S48. Markers of the two parents are indicated on the two circles. Recombinants were selected on minimal medium supplemented with histidine, arginine, homoserine (for mth), uracil, nicotinamide, and cystine (that is, the alleles pro⁺ and phe⁺, indicated by triangles, were selected) and classified for the nonselected markers at the remaining nine loci (see Table 2). Marker symbols: tps, temperature-sensitive; others in Table 1. Letters a–k indicate map intervals. Numbers indicate the frequency of each allele in the sample of recombinants analyzed.*

heterozygosity or nonheterozygosity of each marker. If the former, a mixture of normal and slow-growing colonies is seen on the replica plate testing that marker (hence, the importance of the markers being leaky); if the latter, the colonies are either all normal (wild type) or all slow-growing (mutant).

Mapping by Selecting Haploids

Ordering of loci. Let us suppose we are to map a mutant gene about whose location nothing is previously known. Two strains are chosen, differing by a number of well-separated markers. One of the strains may be the wild type, provided the other strain carries a resistance marker (*str*, *spc*, *acr*), which is used as one of the selected alleles. The mutant gene to be mapped is obtained in one of these strains, and crossed with the other strain. Spores from the cross are plated on a medium selecting one allele from each parent, at distant loci. In the example illustrated in Fig. 3, *pro*⁺ and *phe*⁺ were the selected alleles; markers at all the other loci, including the mutant gene to be mapped, were nonselected. A sample of recombinants from the selective plates was characterized according to the nonselected markers. The results (Table 2) are tabulated systematically according to the crossovers required to produce the various genotypes. Note that, because the map is circular, a single (or odd multiple) crossover is required in *each* of the two arcs separating the selected alleles. The position of *S48*, the morphological marker to be located, has been chosen to minimize the total crossovers required.

The position of the new marker can be deduced systematically from the data as follows. Allele frequencies at each locus are first calculated by adding the numbers of recombinants bearing each

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TABLE 2. *Location of a morphological mutant, S48*[a]

Single crossover classes

Left hand arc		Right-hand arc, genotype[b] and crossovers[c]						Totals
Genotype[b]	Crossovers[c]	*arg, tps* a	*his, arg, tps* b	*his, tps* c	*his* d	*his, mth* e	*his, mth, str* f	
S48	g	7	2	11	3	1	0	24
ura, S48	h	11	6	30	7	0	3	57
ura, nic, S48	i	6	1	14	1	2	1	25
ura, nic	j	8	2	13	3	0	2	28
ura, nic, cys	k	14	7	11	0	1	0	33
Totals		46	18	79	14	4	6	167

Multiple crossover classes

Genotype[b]	Crossovers[c]	No. observed
ura, nic, cys	a, b, d; k	2
mth, str, ura, nic	a, b, f; j	1
arg, mth, str, ura, S48	a, c, f; h	1
his, arg, mth, str, ura, S48	b, c, f; h	1
his, arg, tps, ura, nic, S48, cys	b; i, j, k	1

[a] The cross is illustrated in Fig. 3. Recombinants containing *pro*$^+$ and *phe*$^+$ were selected and classified for nonselected markers at the remaining nine loci, the observed numbers in each class being given in the table.

[b] Wild-type alleles omitted.

[c] See intervals in Fig. 3.

allele. The allele frequencies of each parent must (in the absence of viability disturbances) form a continuous gradient in each arc, falling from the total number of recombinants in the sample (173) for the selected allele, to zero for the counter-selected allele. The frequency of the marker to be located fits into this gradient at one possible position in each arc; in the example under consideration, *S48*, with a frequency of 109, could lie either between *arg*$^+$ (106) and *his* (123), or between *nic*$^+$ (83) and *cys*$^+$ (137). The two possible locations are now tested in turn against the frequencies of the different recombinant classes: if *S48* lay between *his* and *arg*$^+$, all *S48* recombinants arising by single crossovers would be *his* (25 are not), and all *S48*$^+$ would be *arg* (33 are not). On the other hand, if *S48* were between *nic*$^+$ and *cys*$^+$, as indicated, all *S48* recombinants arising by single crossovers would be *cys*$^+$ (there is only one exception), and all *S48*$^+$ would be *nic* (no exceptions). The location of *S48* in the position indicated minimizes, by a large factor, the number of segregants needing to be explained by multiple crossovers, and is therefore chosen (only 6 of the total of 173 are multiples: lower part of Table 2).

As we see, the rarity of multiple crossovers in each arc in this type of analysis results in very unambiguous ordering of loci, even though some of the intervals are very long. (Interval *c* is nearly 80 map units.) This is partly due to the automatic exclusion of doubles by their not giving rise to haploid genomes, so that triples (and higher odd multiples), with low frequencies, are the only multiples to be taken into account. There is, however, another factor which results in "shrinkage" of the map: incompleteness of the zygotes.

Effects of zygote incompleteness on genetic analysis. In a later section, a model of zygote genomes is proposed in which the contribution from one parent is a complete chromosome and that from the other is a chromosome fragment, averaging perhaps about one-sixth of the genome in length. As we see later, such zygote incompleteness results in relatively more crossing-over in intervals near to selected markers than in more distant intervals; crossing-over in more distant intervals is depressed because the zygotes are frequently not disomic for these intervals. In the present example, the real length of interval *c* (80 units) is about 3.6 times that of interval *a* (22 units); yet there is only 1.7 times as much crossing-over in *c* as in *a* in this experiment. Thus, very long intervals, provided that the selected loci are chosen not to be adjacent to them, are reduced to

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manageable proportions, and a single cross comfortably monitors the whole linkage group.

In the example just described, the cross is not strongly "polarized": crossing-over per unit map length is relatively frequent in intervals flanking *both* selected markers. In many cases, however, a different result is observed in this kind of selection: crossing-over is very frequent in intervals on either side of one of the selected markers, but infrequent in intervals flanking the other (*see below*). This is interpreted to mean that, in such crosses, the great majority of the zygotes receive their complete chromosome from the same parent. Thus, of the two possible classes of zygote that could contribute progeny capable of growth on the selective medium, that is, with one or the other parental chromosome complete, only one is present with an appreciable frequency. This matter will be discussed in more detail after the nature of the zygote genomes has been considered more fully. What factors determine the "polarity" of crosses are still largely undetermined.

A consequence of the progressive shrinkage of map intervals with distance from the selected markers consequent upon zygote incompleteness is, of course, that the *lengths* of intervals are not readily estimated in this kind of selection, since recombination frequencies are not proportional to map distances: in general, we have to be content with precise information on order, with only a very approximate indication of the distance of a new marker from its neighbors. However, once preliminary locations for new markers have been obtained by selection between distant markers, further selections can be made in which recombinants between two loci more closely flanking the new markers are recovered. In this way, the order within a group of loci mapped by preliminary crosses between the same pair of known loci can be established efficiently (Table 3). Moreover, the relative distances of the new markers from the flanking loci can be determined with reasonable accuracy, provided the selected markers are close enough for the zygotes nearly always to be complete over the intervening region (Table 3).

Most of the loci in Fig. 2 were mapped by selecting haploids after the lengths of key map intervals had been established from heteroclone segregation data (*see below*).

Heteroclone Analysis

A prerequisite for the selection of heteroclones is the availability of pairs of closely linked, complementing markers. The closer the two markers the better, and therefore ideal pairs are provided by markers in neighboring genes in the same "cluster," several of which are now available. The most extensively used pair of markers is *hisA1* and *hisC9*; other pairs include representatives of the groups *argA*, *B*, *C*; *cysC*, *D*; *mthA*, *B*; *tryA*, *B*, *C*; *uraB*, *C*, *D*, etc.

Hopwood and Sermonti (38) discussed two reasons for the requirement of closely linked markers in heteroclone selection: (i) to increase the proportion of heteroclones to haploid recombinants on the selective plates by restricting the number of haploids capable of growth to those having arisen by crossing-over in the short interval between the pair of markers in question; (ii) to minimize the chance of haploids capable of growth on the selective medium arising early in the life of the heteroclone, by crossing-over between the selected markers, and "overgrowing" the heteroclone colony as fast-growing "sectors." It now appears that only the second consideration is important, for the following reason. If spores from a cross are plated in parallel on two media, one selecting for markers at opposite sides of the map, and the other for very closely linked complementing markers, the majority of the colonies on the second medium are heteroclones, and the total colony count is lower than on the first medium, but usually *by a factor of only 2 or 3* (the younger the mixed culture, the smaller the factor; *see below*). This means that a large proportion, sometimes approaching, and possibly exceeding, 50% of the total plating units capable of forming colonies on selective media, have the potentiality of developing into heteroclones under suitable conditions. When plated on a medium which selects for closely linked markers, they do so, but on a medium selective for distant markers they do not, because, soon after initiation of the colony, crossing-over between the markers generates a recombinant genotype which emerges as a fast-growing "sector." Thus, the colony is scored as a haploid. Presumably, the colony often contains more than one genotype of recombinants, but one is so greatly in excess that heterogeneity is not detected unless it is deliberately sought.

Operationally, at least, this state of affairs resembles the common situation in *E. coli* in which zygotes from a mating mixture plated on a selective medium give rise to colonies; to a first approximation, these colonies resemble pure recombinant clones, whereas, in fact, at least when certain Hfr's sire the progeny, a high proportion of the colonies contain more than one class of segregant from the same zygote (*see below*).

Complementation tests. The presence or absence of heteroclones in appropriate crosses

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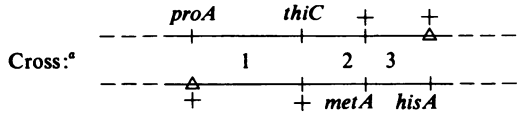
TABLE 3. *Mapping of two loci with respect to outside markers by selecting haploid recombinants between the outside markers*



Genotype with respect to nonselected markers	Observed no.	Crossover in interval
thi +	269	1
+ +	5	2
+ *met*	30	3
thi met	0	1, 2, 3

[a] Triangles indicate selected alleles. A sample of recombinants was classified with respect to the nonselected markers *thiC* and *metA*. Occurrence of the genotype + + but not *thi met* indicates the order of markers shown. Relative frequencies of the three single crossover classes give approximate relative lengths of the three intervals.

provides a test of complementation between recessive mutants. For example, suppose we have two independently isolated histidine-requiring mutant genes, *his-x* and *his-y*, which are to be tested for complementation. In order for a negative result in the test to be meaningful, two strains, each bearing one of the mutant genes, must differ in two further selectable markers, if possible at rather distant loci: for example, the strains might have the genotypes *his-x argA* and *his-y pheA*. Spores from the cross are plated on two media, one containing arginine and phenylalanine and therefore selecting for recombinants between *his-x* and *his-y*, and the other containing histidine and selecting for recombinants between *argA* and *pheA*; these loci lie at approximately 180° to one another on the map. The colonies on the first medium are classified into haploids and heteroclones; this may be possible on the basis of size alone, but it is usually desirable to confirm the classification by replica plating, to the same medium lacking histidine, when any heteroclones fail, completely or partially, to give growth on the replica plate, because nearly all spores carry either *his-x* or *his-y* (65). There are three possibilities: if colony counts on the two media are approximately equal, and no heteroclones are identified on the medium lacking histidine, we conclude that *his-x* and *his-y* are not closely linked; if the total colony count on the second medium greatly exceeds that on the first, and no heteroclones are found, *his-x* and *his-y* originated by mutations in the same cistron; if the total colony count on the second medium exceeds that on the first (though usually not by a large factor) and heteroclones are identified on the medium lacking histidine, *his-x* and *his-y* are closely linked and complement one another.

A special case of the use of heteroclones in complementation is a recent study of a group of 22 UV-sensitive (*uvs*) mutants isolated as described earlier (25). The mutant genes were mapped by the method of haploid selection: four were located close to *guaA* at the bottom of the map (*uvsB*), and the remaining 18 fell between *hisA* and *serA* at the top of the map. This group of mutants was tested for complementation as follows. Approximately half of the mutant genes were obtained in strains carrying *hisA1* and the other half in strains bearing *hisC9*. Members of the first group of strains were crossed with members of the second, and spores were plated in duplicate on selective plates lacking histidine. Before incubation, one member of each pair of plates received a dose of UV corresponding to a survival of about 50% for wild-type spores, but of less than 1% for *uvs* spores. After incubation, the numbers and types of colonies on duplicate plates were compared.

The rationale of the experiment was as follows. Since the cluster of *uvs* markers was close to the pair of *his* markers, almost all heteroclones selected to be heterozygous for the *his* markers would also be heterozygous for the pair of *uvs* markers in the cross; on the other hand, haploid *his*$^+$ recombinants would almost always carry one or other of the *uvs* markers. Therefore, after irradiation, the frequencies of haploid colonies should always be reduced to a small fraction of controls, but the number of surviving heteroclones would depend on the presence or absence of complementation between the *uvs* markers: with full complementation, heteroclone counts should be about 50% of controls, whereas with no complementation they should be reduced to a very low level.

This expectation was fully borne out by the results of the crosses, which fell unambiguously into the two classes predicted. The pattern of complementation is consistent with the existence of three cistrons, which have been designated *uvsA*, *C*, and *D*. In Table 4, the percentage of *total* colonies surviving UV is given for each cross: that is, the distinction between heteroclones and haploids, which is sometimes subjective on irradiated plates, is ignored. Since haploids represent about 5% of total recombinant colonies when selection is for *hisA1*$^+$ and *hisC9*$^+$ (*see below:* Table 13), total colony counts after UV should be reduced, in cases of full complementa-

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TABLE 4. *Complementation tests between uvs mutants: identification of three cistrons, A, C, and D*[a]

uvs mutant in *hisA1* parent	*uvs* mutant in *hisC9* parent							
	A15	A19	A22	A23	C14	C16	C17	D18
A2	<0.4	<0.2	0.3	0.3	**45**	**78**	**52**	**47**
A4	<0.5	<0.3	<2	<0.9	**28**	**37**	**28**	**36**
A9	<1	<0.1	<0.4	<0.1	**53**	**36**	**40**	**42**
C7	**23**	**32**	**54**	**55**	0.2	<0.3	<1	**60**
C8	**23**	**60**	**53**	**83**	<0.1	<3	0.3	**77**
C10	**52**	**63**	**38**	**33**	<0.3	<2	<0.3	**54**
D1	**33**	**40**	**44**	**51**	**49**	**52**	**59**	<3
D3	**30**	**48**	**46**	**65**	**27**	**57**	**59**	<1
D5	**47**	**47**	**45**	**57**	**35**	**54**	**46**	<1
D11	**32**	**66**	**41**	**40**	**57**	**51**	**51**	<1

[a] Pairwise crosses were made between *uvs* mutants in a parent strain carrying *hisA1* and *uvs* mutants in a strain carrying *hisC9*, and selection was made for *his*$^+$ recombinants. Each figure is the percentage *total* recombinant colonies (haploids plus heteroclones) surviving a mild dose of UV (see text). Where no colonies grew after UV, an upper limit of survival is given. Figures in bold type are interpreted as indicating complementation. Data of Harold and Hopwood (25).

tion, to (100 − 5) × ½ = 48% of controls and this is, on the average, what is found.

An alternative (rapid) method to the use of heteroclones in complementation tests between auxotrophic mutants has been described (59), based on a cellophane-transfer technique (55); this method depends on the production of tufts of aerial mycelium, presumably resulting from heterokaryotic growth, in pairwise combinations of complementing (but not of noncomplementing) auxotrophs growing on a limiting concentration of the growth factor. The test is performed by plating a suitably diluted spore suspension containing a mixture of the two mutants to be tested on a disc of cellophane laid on a plate of complete medium. After 24 hr of incubation, the disc is transferred to a plate of selective medium lacking the growth factor required by the mutants, and incubation is continued. If the mutants complement one another, tufts of aerial mycelium appear, after about 2 more days of incubation, along the lines of contact between colonies of different genotype, whereas in the absence of complementation no such tufts are seen.

Complete analysis of segregants from heteroclones. This technique furnishes considerable information on the genome of an individual heteroclone, since the genotype of each haploid segregant in a sample derived from a single heteroclone is determined. The technique was previously used extensively for routine mapping. It was valuable at that stage in the formal genetics of *S. coelicolor* because it provided the simplest means of estimating the lengths of map intervals in standard recombination units. This is because, unlike recombinants from a mixed population of zygotes, the segregants from an individual heteroclone arise from a (to a first approximation) uniform class of partially disomic genomes. Provided the map interval in question is not close to more than one end of the disomic region of the heteroclone, the recombination percentage calculated directly is a good estimate of interval length, since parental and recombinant classes are modified proportionately by the effects of heterozygote incompleteness, as discussed in previous papers (*see* 38). Some data of this type are given in Table 5.

With enough key intervals estimated by this procedure, mapping has now been largely taken over by selective analysis, as mentioned above. More recent studies of individual heteroclones have been concerned with the structure and behavior of these heterozygous genomes rather than with mapping. Further discussion of this topic is therefore deferred until later.

"Phenotypic" analysis of heteroclones. This technique (33, 62) furnishes a rapid method for determining which loci are heterozygous and which are nonheterozygous in each of a sample of heteroclones, without the necessity of analyzing the genotypes of individual segregants. This makes it possible to study a substantial population of heteroclones, thus facilitating an experiment demonstrating that the genome is circular or circularly permuted (33).

GENOMES OF ZYGOTES AND HETEROCLONES

In this section, some formal models are put forward to account for the results of genetic analysis and to act as a working hypothesis for

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TABLE 5. *Estimation of recombination percentages from heteroclone data*[a]

Pair of loci	Parental classes						Recombinant classes						Per cent recombinants
	Genotype		No.	Genotype		No.	Genotype		No.	Genotype		No.	
proA	+	*hisA*	146	*pro*	+	19	+	+	26	*pro*	*hisA*	2	14.5
hisA/C	+	+	158	*pro*	*hisC*	52	*pro*	+	21	+	*hisC*	8	12.1
	pro	*hisC*	158	+	*hisA*	81	+	*hisC*	52	*pro*	*hisA*	20	23.1
			58			38			22			7	23.2
			83			19			3			20	20.0
			55			73			12			8	13.5
			50			28			12			6	18.8
			60			72			15			18	20.0
Pooled[b]													18.1 (22cM[c])
cysA	*cys*	+	59	+	*pro*	56	*cys*	*pro*	9	+	+	1	8.0
proA			13			103			0			9	7.2
			83			54			9			2	7.5
			77			75			3			10	7.7
Pooled[b]													7.6 (8cM[c])

[a] Each line in the table represents data from a single heteroclone. In each case, the ratio between the frequencies of recombinant classes does not differ significantly from that between the frequencies of parental classes, indicating that not more than one end of the disomic region of the heteroclone is affecting the data.

[b] Data for *proA-hisA/C* are somewhat heterogeneous ($\chi^2_7 = 17.5$); those for *cysA-proA* are homogeneous ($\chi^2_3 = 0.064$).

[c] Calculated from recombination percentages by Haldane's (24) formula.

future work. It is not possible, of course, in the present state of knowledge, to arrive at definitive molecular models of genome structure, and of the events which relate one genome type to another.

Nature of the Normal Genome

By "normal" is meant the genome before recombinational events are initiated and after they are completed. We can make the following statements about its nature. (i) All known markers map on a single circular linkage group; therefore, it is reasonable to conclude that the genome is represented by a single chromosome (31), although we cannot exclude the unlikely possibility that other, as yet unmarked, chromosomes exist. (ii) The disomic region of each heteroclone can be formally represented as an uninterrupted arc of the circular linkage map defined by breaks in the circle. The evidence suggests that, to a first approximation, the ends of the arcs are randomly distributed (although there may conceivably be small-scale restrictions), indicating that the parental genomes from which the arcs originate do not have ends at a unique point on the circumference of the circle; otherwise, arcs spanning this point would not arise (33).

Especially armed with knowledge of other microbial genomes, it is possible to imagine more than one plausible model of genome structure compatible with these findings: for example, the genome might be a closed circle, as appears to be the case in the other bacteria *E. coli* (7) and *M. hominis* (4), or it might be an open structure, but with ends distributed in a circularly permuted fashion within a population of genomes, as in bacteriophages T4 (70) and P22 (72).

There is no direct evidence against either of these alternatives. It seems a simpler hypothesis to assume for the time being that the normal genome is a closed circle, but we must bear in mind the lack of direct evidence on this point. If the genome were not closed, it would be necessary to make further hypotheses to explain why closed circular genomes were not generated during recombination, or if generated did not remain closed (*see below*). It is, of course, conceivable that an equilibrium exists between open and closed configurations, so that the argument, closed versus open, loses much of its significance.

Nature of Zygote Genomes

Information on zygote genomes derives mainly from crosses in which recombinants are recovered on media selective for various pairs of parental markers, with other markers nonselected. The relationships between crossovers in different parts of the map are analyzed. For

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example, the products of the cross depicted in Fig. 4 were plated on six media, each selective for a pair of adjacent markers (all other markers being nonselected) as follows: *hisA*$^+$, *argA*$^+$; *cysD*$^+$, *mthB*$^+$; *mthB*$^+$, *strA*; *strA*, *pheA*$^+$, *pheA*$^+$, *uraA*$^+$; *proA*$^+$, *hisA*$^+$. Thus, the media recovered recombinants produced by crossing-over in intervals *a*, *c*, *d*, *e*, *f*, or *h*, respectively. A sample of colonies from each medium was classified according to the nonselected markers.

The data may be analyzed in various ways. In Table 6, the percentage of the total colonies showing nonselected crossovers in each interval was calculated, and each percentage was divided by the standard length of the interval in per cent recombination units to give an index, in arbitrary units, of the relative frequency of crossing-over per unit length in each interval. These figures show that the likelihood of crossing-over per unit length is always highest in the two intervals

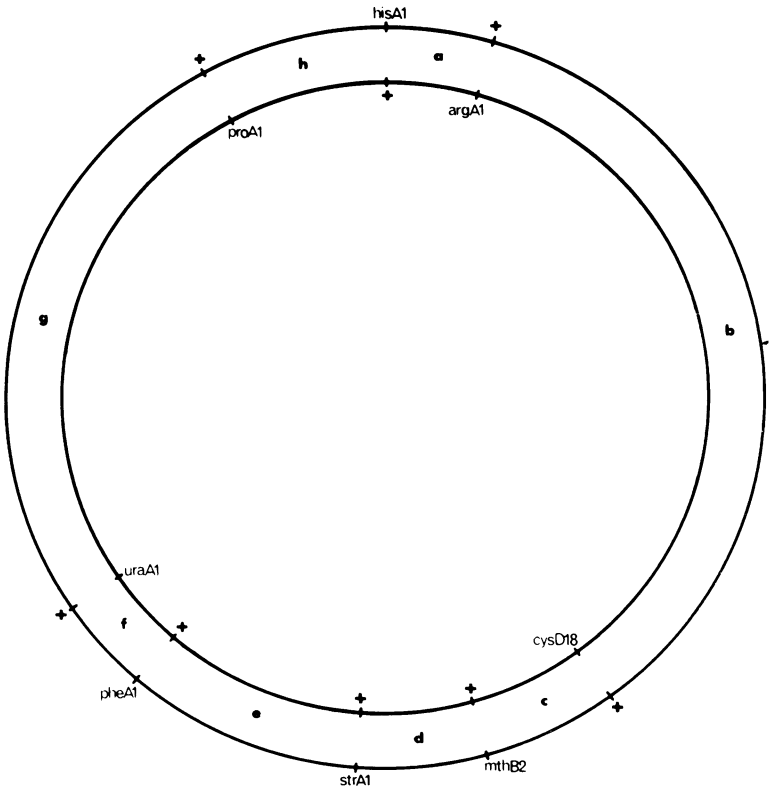

FIG. 4. *Diagram of a cross referred to in the text.*

TABLE 6. *Effect of selecting crossovers in an interval on the frequencies of nonselected crossovers in neighboring intervals*[a]

Interval in which crossovers were selected	Relative frequencies of nonselected crossovers per unit length in interval								No. of recombinants analyzed
	a	b	c	d	e	f	g	h	
a	—	1.28	0.43	0.41	0.41	0.39	0.44	1.30	706
c	0.17	1.71	—	0.96	0.22	0.30	0.39	0.08	258
d	0.49	0.54	1.16	—	1.57	0.40	0.50	0.35	276
e	0.16	0.30	0.71	1.52	—	2.42	0.86	0.23	220
f	0.31	0.25	0.37	0.51	1.80	—	1.19	0.23	269
h	1.64	0.72	0.24	0.36	0.41	0.59	1.18	—	657
Interval length (per cent recombination)	11	42	14	14	18	9	38	18	

[a] See diagram of the cross in Fig. 4.

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TABLE 7. *Lack of interference in individual heteroclones of Streptomyces coelicolor*[a]

Markers	No. of segregants observed				Expected no. of double crossovers
	No crossover	Single crossover	Single crossover	Double crossover	
cysA, hisA/C, argA	134	87	27	14	16
proA, hisA, argA	147	23	18	5	3
proA, argA, hisE	73	16	7	1	1
strA, mthB, cysC/D	134	10	31	5	3
pheA, strA, mthB	154	11	14	1	1

[a] From Tables 3 and 4 of Hopwood (30).

TABLE 8. *Lack of interference in zygotes of Escherichia coli K-12*

Markers	No. of segregants observed				Expected no. of double crossovers
	No crossover	Single crossover	Single crossover	Double crossover	
T, L, T$_1$[a]	164	16	18	2	2
L, T$_1$*, Lac*[a]	157	17	23	3	3
T$_1$*, Lac, Gal*[a]	124	19	50	7	7
Lac, Gal, Try[a]	94	42	49	15	18
thr, leu, proA[b]	906	89	462	55	49

[a] Calculated from data in Table 35 of Jacob and Wollman (42).
[b] Calculated from data in Table 3 of DeHaan and Verhoef (13).

flanking that in which crossing-over was obligatory, and is much lower in distant intervals. On the average, crossing-over per unit length is five times more frequent in intervals flanking the selected markers than in intervals diametrically opposite them on the map.

This result amounts to a localized high negative interference. The simplest explanation of negative interference, over regions as long as this (the shortest map interval is 9 units) is zygote incompleteness: the negative interference is due to heterogeneity in respect of disomy within a population of zygotes. All zygotes must be disomic over at least part of the interval in which crossovers are selected, and the probability of disomy, and therefore of nonselected crossing-over, will be high in adjacent intervals, but low in more distant ones. If this explanation is correct, negative interference should be reduced or absent whenever a heterozygote is disomic over all of the intervals in question. In individual heteroclones, we can determine which intervals are disomic, and, considering only these, no interference is found over regions of length comparable to those in the experiment just described. The numbers of double crossovers in three-point data are those expected if crossovers in the two intervals are independent (Table 7).

Support for this interpretation is provided by data from other prokaryote systems. In most published data from Hfr × F^- crosses, there is no significant interference between crossovers in adjacent intervals (all 9 units or more in length) in *E. coli* zygotes when these are made disomic over all intervals by selecting an Hfr marker distal to the whole region (Table 8); negative interference appears, however, in a mixed population of zygotes disomic over varying intervals, as in F^+ × F^- or F^+ × F^+ crosses (58). Negative interference is also present in one set of published data from Hfr × F^- crosses (47); why these data should differ from the others remains unexplained. As in most *E. coli* zygotes, negative interference is absent in individual heterozygotes of bacteriophage T4, but present among segregants from a mixed population of heterozygotes, disomic over varying portions of the genome (15). It is thus most likely that, as in these other systems, negative interference among segregants from a population of zygotes of *S. coelicolor* reflects incompleteness of the zygote genomes.

Partial disomy of the zygotes could theoretically involve: incompleteness of both genomes, but for different regions (Fig. 5a); or incompleteness of one genome only (Fig. 5b). Since the average contribution of markers to the progeny of a cross by both parents is often equal, although

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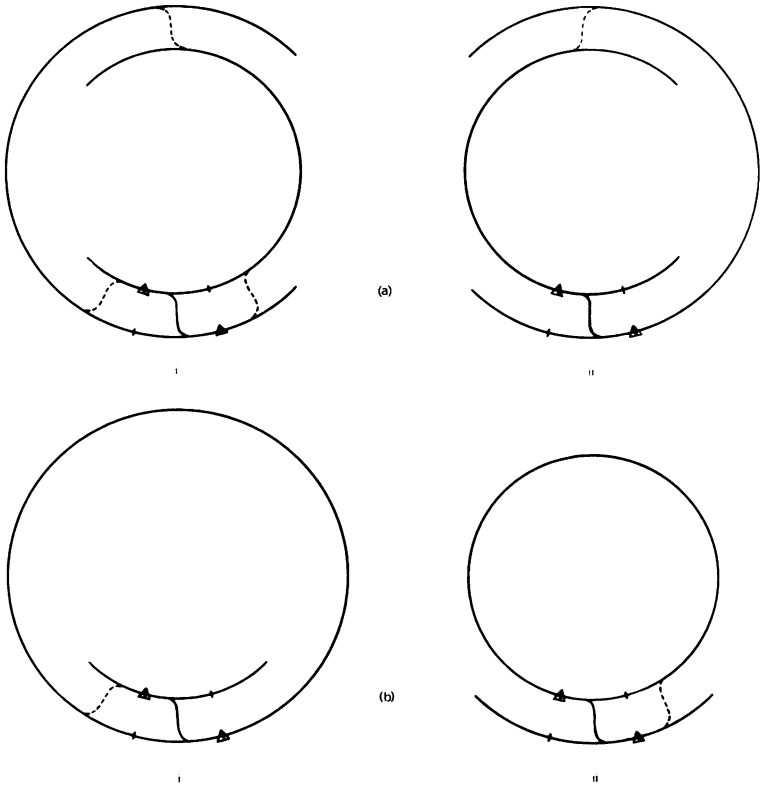

FIG. 5. *Two alternative models for the structure of merozygote genomes. In (a), both parental chromosomes are incomplete, but for different regions; in (b), one parental chromosome is complete and the other incomplete. The two variants (i and ii) of each model are reciprocal with respect to the patterns of incompleteness of the chromosomes coming from the two parents. Triangles indicate selected markers. The half-crossovers giving haploid recombinant genomes are indicated: solid lines = selected; broken lines = unselected.*

		Interval d	
		n.c.o.	c.o.
Interval e	n.c.o.	179	24
	c.o.	76	77

$\chi_1^2 = 63.6$

		Interval d	
		n.c.o.	c.o.
Interval e	n.c.o.	41	24
	c.o.	52	20

$\chi_1^2 = 1.31$

it is not by any means always so (*see below*), we have to postulate two classes of zygotes, reciprocal with respect to the patterns of incompleteness of the genomes coming from the two parents (classes i and ii). On both models, it is assumed that the incomplete genome(s) can vary in length, and may possibly be complete as a limiting case.

A choice between these models can be made by considering the outcome of crossing-over within the disomic region of the zygote. Note that one selected crossover occurs in this region in the experiment under discussion. On model (a), a disomic region is an overlap between partial genomes, and within it the total number of crossovers must be odd to produce a haploid recombinant genome. It follows that, depending on the polarity of the overlap relative to the markers used to define the selected crossover, single nonselected crossovers must occur on each side or neither side of the selected crossover (Fig. 5a; i and ii, respectively). On model (b), on the other hand, the total number of crossovers within the disomic region must be even, so that a single nonselected crossover is always required on one side or the other (but not both) of the selected crossover (Fig. 5b, i and ii). Thus, on model (a), there should be a positive correlation between nonselected crossovers in intervals flanking the selected markers, whereas on model (b), there should be a negative correlation. (In the real situation, the correlation will not of course be complete, because the markers used

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to define the flanking intervals will not coincide with the ends of the disomic region.)

Table 9 summarizes data from the experiment described above, this time analyzed to reveal the correlation of nonselected crossovers in intervals on either side of that in which crossovers were selected. For each selection, there is a highly significant negative correlation between crossing-over in the two intervals, or in other words an enormous excess of single crossovers on one side or the other of the selected markers, in agreement with model (b) for the structure of the zygotes, and in contradiction to model (a).

Let us consider what would happen if the complete genome in Fig. 5b were not circular, but circularly permuted. Sometimes the genome ends of the complete genome would lie in the region overlapped by the incomplete genome. In this case, double crossovers in the disomic region should often span the ends of the complete genome, and should give rise to a closed circular recombinant genome.

The cross just discussed is not strongly "polarized": that is, the numbers of zygotes of classes i and ii are not very different, since nonselected crossovers are frequent on *both* sides of the selected crossover (though not in the same zygote). Sometimes, however, nonselected crossovers are much more frequent on one side of the selected markers than on the other, and these cases are interpreted as crosses in which one class of zygote (Fig. 5b, i or ii) predominated over the other. How far the "polarized recombination" described by Piperno, Carere, and Sermonti (55) is due to this cause, rather than to polarization of the recombination process itself, remains to be seen.

Figure 6 summarizes some data illustrating the differences between "polarized" and "nonpolarized" crosses (involving the same markers), when selection is made for markers on opposite sides of the map: *his*$^+$ and *str*. The percentage frequency of each allele is indicated on the diagrams. It is obvious that in the polarized cross (Fig. 6b), crossing-over in the intervals *a* and *h*, flanking the selected marker *his*$^+$, was much reduced compared with the nonpolarized cross: the frequencies of the alleles *arg*$^+$ and *pro*$^+$, in *trans* to *his*$^+$, are very low. This is interpreted to mean that, in the nonpolarized cross, zygotes of the two possible classes shown in Fig. 7, i and ii, were present in roughly equal numbers, whereas in the polarized cross zygotes of class i were absent, so that crossing-over in intervals *a* and *h* was possible only in those (rare) zygotes of class ii in which the incomplete genome was long enough to extend into these intervals. If this interpretation is correct, the polarized cross should not show any correlation of crossovers on either side of the selected marker *str*, since all zygotes would be of the same class, whereas the nonpolarized cross should show strong positive correlation of crossovers ("negative interference"). This is precisely what is

TABLE 9. *Correlation of crossing-over (c.o.) and no crossing-over (n.c.o.) in intervals flanking selected markers*[a]

Selection in interval c

		Interval b	
		n.c.o.	c.o.
Interval d	n.c.o.	45	182
	c.o.	27	4

Selection in interval d

		Interval c	
		n.c.o.	c.o.
Interval e	n.c.o.	155	43
	c.o.	76	2

Selection in interval e

		Interval d	
		n.c.o.	c.o.
Interval f	n.c.o.	128	44
	c.o.	45	3

Selection in interval f

		Interval e	
		n.c.o.	c.o.
Interval g	n.c.o.	65	83
	c.o.	117	4

Selection in interval a

		Interval b	
		n.c.o.	c.o.
Interval h	n.c.o.	184	356
	c.o.	144	22

Selection in interval h

		Interval a	
		n.c.o.	c.o.
Interval g	n.c.o.	264	100
	c.o.	274	19

[a] See diagram of the cross in Fig. 4. Note that in each case there is a highly significant shortage of simultaneous crossing-over in both intervals (probability of homogeneity always <0.005).

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found, as shown in the lower part of Fig. 6, where the correlation of crossovers in intervals *d* and *e* is analyzed. Criteria such as these for the recognition of polarization should aid in the identification of the factors controlling it.

Nature of Heteroclone Genomes

In the first investigation of heteroclones (65), it was noted that allele frequencies at some loci, but not all, among the haploid segregants from

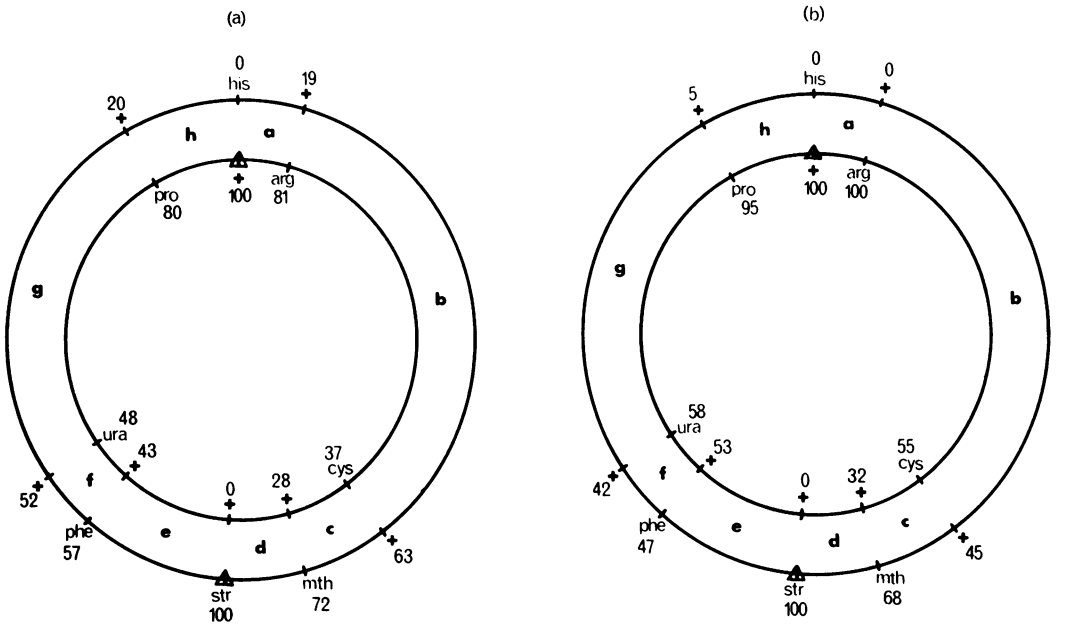

FIG. 6. *Allele frequencies and correlation o f nonselected crossovers in (a) "non-polarized" and (b) "polarized" crosses. Percentage allele frequencies are indicated by numbers on the diagrams. Triangles indicate the selected alleles.*

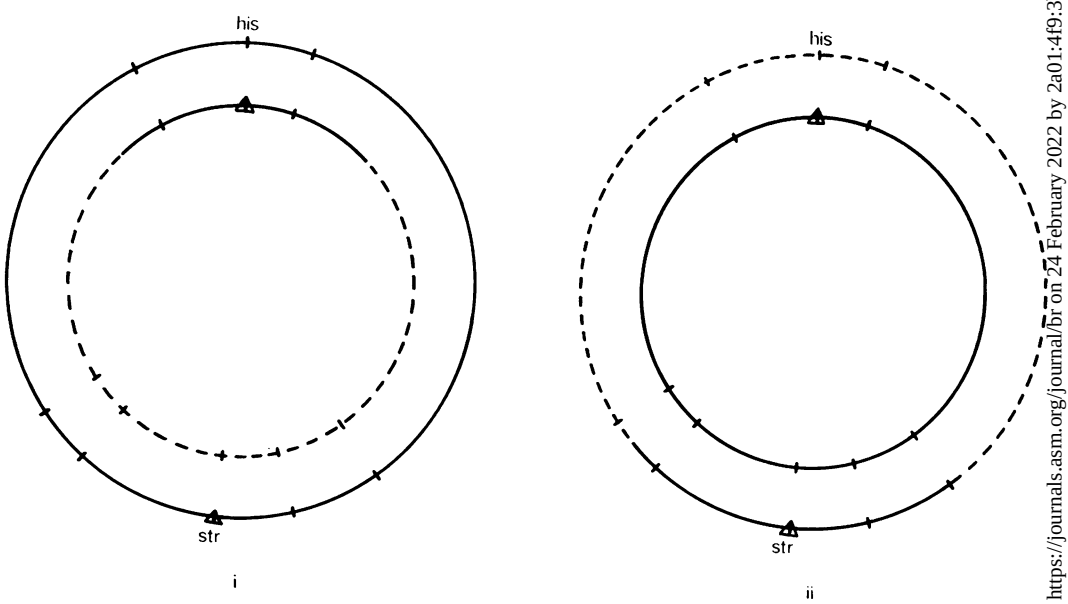

FIG. 7. *The two classes of zygotes contributing selected progeny in the crosses shown in Fig. 6. In i, the inner parental chromosome is incomplete (dashed) and in ii, the outer.*

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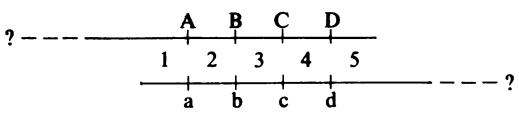
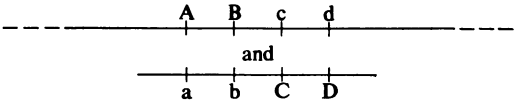
individual heteroclones were very unequal. For example, in a cross involving markers at the loci now designated *metA*, *hisA*, *argA*, and *strA*, although allele frequencies at the first three loci were approximately equal in all heteroclones, those at the *strA* locus were usually very different, often with 99% or more of the segregants carrying the allele for streptomycin sensitivity. The reduced frequency, or complete lack, of certain markers among heteroclone segregants was later found not to be a property of particular alleles: the same allele could be deficient in some heteroclones and in excess in others from the same cross. This led Hopwood, Mancinelli, Sermonti, and Spada-Sermonti (37) to propose that inequalities in allele ratios were due to incompleteness of the diploid nuclei of the heteroclones, which were thus heterogenotes (51). This interpretation was substantiated in numerous subsequent analyses of segregation in heteroclones (30, 38, 39, 64).

In these earlier studies, description of the nature of the heterogenote genomes was hampered by incomplete knowledge of the linkage map, which consisted at that time of two independently segregating linkage groups. Two typical features of segregation from the heterogenotes were, nevertheless, established. First, within a heterozygous region, there was a gradient of allele frequencies of each parent from one end to the other of a series of linked markers. Second, within a heterozygous region, one parent characteristically contributed the majority allele at some loci and the minority allele at others. These two features gave rise to the representation of a disomic region of the heterogenote as an *overlap* between two partial genomes, as follows (capital letters represent alleles contributed by one parent and small letters those contributed by the other):



(This model was referred to in earlier papers as "deletions in *trans*," since it can notionally be derived from a complete diploid containing two linear chromosomes by deleting a segment from one end of one chromosome and from the opposite end of the other.) Only odd numbers of crossovers within such an overlap would give rise to viable haploid segregants, and even then only one of the two products of reciprocal crossing-over would survive, the other being incomplete. For example, a single crossover in interval 3 would give products as follows:



of which only the upper one would be recovered in a viable haploid segregant. The frequencies of particular alleles in a sample of haploid segregants would reflect these restrictions on the recovery of crossover products. Thus, allele ratios in such a sample might be as follows:

95	65	35	5
5	35	65	95

Allele *a*, for example, would find its way only into those haploid segregants which arose by crossing-over in the short interval 1—hence, its presence in only 5% of segregants. Allele *b* would have a higher frequency, 35%, since segregants arising by a crossover in the longer region comprising intervals 1 plus 2 could carry *b*, and so on.

What was unclear in these earlier studies was the constitution of the heterogenote outside a region of heterozygosity, that is, in the dashed areas of the above diagram. Such questions as the number of overlaps in a single heteroclone and the mode of association of the two parental genomes remained unanswered. With the finding of a single circular linkage group by Hopwood (31), interpretation of the nature of the heteroclones became easier, and it is now possible to propose a plausible model of their genome structure.

Figure 8 summarizes an experiment, some of the conclusions of which have already been described (33). Markers of the two parents are indicated on the two circles. Heteroclones were selected to be heterozygous (and therefore disomic) at the closely linked loci *hisA* and *hisC* by plating the products of the cross on a medium lacking histidine, but containing all the other growth factors required by the parent strains. The extent of the disomic region in each of a sample of 447 heteroclones was then determined (Table 10) by scoring each marker for heterozygosity, or otherwise, by the method of "phenotypic" analysis (*see above*).

Two main conclusions can be drawn from the results, the first of which is evident in Table 10. (i) In all except 4 (classes w, x, y) of the 365 heteroclones in which *two or more* markers were not heterozygous (that is, excluding classes 02, 13, 24, 35, 46, 57, z), the heterozygous loci formed an uninterrupted series: in other words, the heterozygous region of each class of het-

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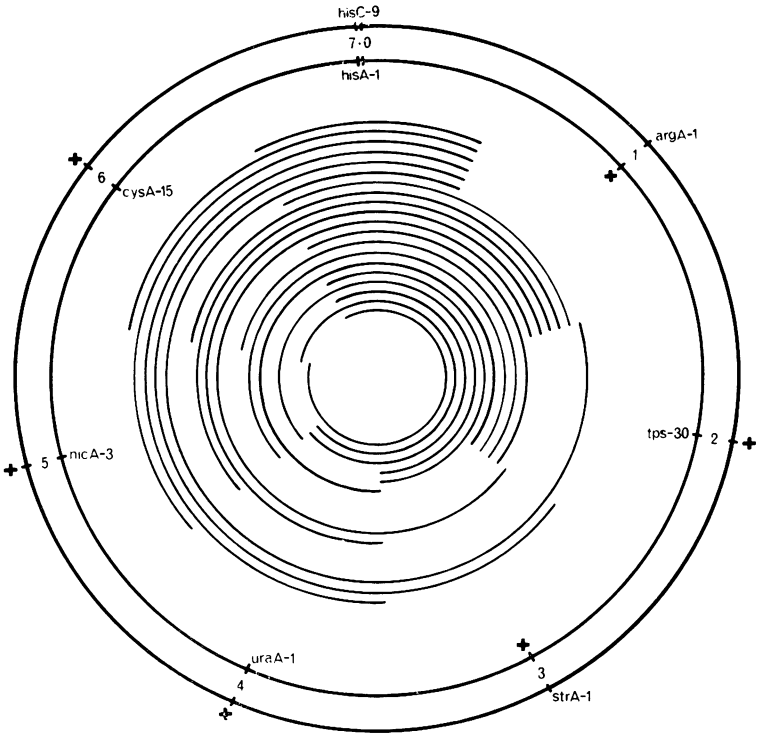

FIG. 8. *Representation of the disomic regions of a sample of heteroclones (33). The constitutions of the parents in the cross are indicated on the two circles; map intervals are arbitrarily drawn of equal length. Loci are numbered clockwise from 0 to 7. Each of the arcs in the central part of the figure represents the extent of the disomic region in one of the classes 07 to 57 of heteroclones in Table 10.*

eroclones (including, of course, those with fewer than two markers not heterozygous) could be represented by an arc corresponding to a particular segment of the circular linkage map, as in Fig. 8. Thus, each heteroclone seems to contain a single disomic region. (ii) Within the group of loci that were *not* heterozygous in a particular heteroclone, the allele present at each of the loci always came from the *same* parent, with only two exceptions. Thus, changes of coupling of markers outside the disomic region do not normally occur. What about changes of coupling within the disomic region or between it and the monosomic region? It appears that there is typically one such change in each heteroclone.

The evidence for this conclusion is obtained from the analysis of individual heteroclones by classifying a sample of their segregants according to all the parental markers (*see above*). Table 11 gives the allele ratios, derived by summing the numbers of segregants with each allele, in several heteroclones from this same cross (*pro*, unscored in Table 10 and Fig. 8, is here included). As in the large sample of heteroclones in Table 10, the heterozygous loci in each heteroclone are all adjacent to one another, and the alleles at the nonheterozygous loci all come from the same parent: in heteroclones (i), (ii), (iii), (v), and (vi) from the upper parent, and in heteroclone (iv) from the lower parent. Two further features are evident in these data. First, the parent contributing the alleles at the nonheterozygous loci frequently contributes the *minority* allele at some or all of the heterozygous loci. Second, in each heteroclone, there is a gradient of allele frequencies from each parent, and this gradient is reversed at one place in each heteroclone (marked by an arrow in Table 11). For example, in heteroclone (i), progressing from right to left, the frequencies of alleles coming from the upper parent gradually fall: 164, 101, 90, 86, 80, 67, 66, 35, until there is a rise again at the arrow to 164. The point of reversal is very often at one end of the group of heterozygous loci, as in heteroclones (i), (ii), (iv), (v), and (vi), but sometimes within it, as in heteroclone (iii).

These characteristics of the heteroclones can be summarized in a formal model of their origin and structure. Figure 9 illustrates this model for heteroclone (iii). The genome of the merozygote

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TABLE 10. *Patterns of heterozygosity in a sample of heteroclones from the cross represented in Fig. 8*

Class of heteroclones[a]	Loci at which heteroclones are heterozygous (+) or not heterozygous (·)							Observed no. of heteroclones
	ura (4)	*nic* (5)	*cys* (6)	*his* (7, 0)	*arg* (1)	*tps* (2)	*str* (3)	
07	·	·	·	+	·	·	·	48
06	·	·	+	+	·	·	·	12
05	·	+	+	+	·	·	·	12
04	+	+	+	+	·	·	·	8
03	+	+	+	+	·	·	+	1
02	+	+	+	+	·	+	+	3
17	·	·	·	+	+	·	·	132
16	·	·	+	+	+	·	·	49
15	·	+	+	+	+	·	·	60
14	+	+	+	+	+	·	·	12
13	+	+	+	+	+	·	+	3
27	·	·	·	+	+	+	·	14
26	·	·	+	+	+	+	·	5
25	·	+	+	+	+	+	·	6
24	+	+	+	+	+	+	·	6
37	·	·	·	+	+	+	+	1
36	·	·	+	+	+	+	+	0
35	·	+	+	+	+	+	+	2
47	+	·	·	+	+	+	+	1
46	+	·	+	+	+	+	+	1
57	+	+	·	+	+	+	+	3
w	·	+	·	+	+	·	·	2
x	+	·	·	+	+	+	·	1
y	·	+	·	+	+	+	·	1
z	+	+	+	+	+	+	+	64
Total								447

[a] Numbered according to the terminal heterozygous loci (except complex classes w–z). Data from Hopwood (33).

from which this heteroclone arose is shown in Fig. 9a; it consists of a complete chromosome from one parent and an incomplete one from the other, including the loci clockwise from *nicA* to *argA*, but not the remaining three marked loci. A single (reciprocal) crossover in interval *b* between *nicA* and *cysA* converts this merozygote genome into that of the heteroclone (Fig. 9b); this crossover is responsible for the reversal of the gradient of allele frequencies in this interval when these are tabulated according to the parent originally carrying each allele (Table 11). However, when the change in coupling resulting from the crossover is taken into account (Fig. 9b), the gradient of allele frequencies progresses uninterruptedly both within the heterozygous region and from it into the nonheterozygous region in both directions.

This is an example of the comparatively infrequent class of heteroclones in which the crossover converting the zygote genome into that of the heteroclone has to be postulated in an interval between two heterozygous loci. More often, the crossover occurs in a terminal segment of the disomic region, possibly indicating a preponderance of crossing-over associated with genome ends, as demonstrated recently for T4 by Doermann and Parma (16), confirming an earlier conclusion of Shalitin and Stahl (65*a*), based on rather less direct observations. A similar enhancement of crossing-over associated with genome ends has also been demonstrated for phage T1 (50*a*, 51*a*), and there is some slight evidence that this may be true also in *E. coli* [discussed by Glansdorff (21*a*)].

Note that, on this model, the extent of the disomic region in the merozygote and the heteroclone it produces is the same; only the coupling of the markers is changed. Therefore, since the average length of the disomic region of a heteroclone is of the order of one-sixth of the genome (33), the same would be true of the length of the chromosome fragment of the merozygote.

We can thus make a unified hypothesis about the events following merozygote formation as follows. A merozygote gives rise either to haploid recombinant genomes, or to genomes capable of initiating heteroclones, depending on whether crossovers within the disomic region of the zygote are, respectively, of even or odd number. Haploid recombinant genomes are circular, like the "normal" genomes, whereas genomes initiating heteroclones are terminally redundant heterozygotes. The stability of the latter during subsequent reproduction would depend on the balance between replication and crossing-over. In principle, at least, such heterozygotes could replicate without losing heterozygosity, since heterozygosity resides in a single structural unit (a semiconservatively replicating DNA molecule?). We know that the genome initiating a heteroclone must be able to give rise to numerous heterozygous genomes like itself, since thousands of daughter heteroclones, many of them, although not all, heterozygous for the same markers as the parent heteroclone, can be obtained from a single original heteroclone (38, 64). Also in principle, such terminally redundant heteroclones should be capable of giving rise to haploid segregants by further, odd-numbered crossovers within the disomic region during head-to-tail synapsis. The relative lengths of the various intervals would determine the allele ratios among these segregants at the different marked loci. For example, the ratio of *cysA* to *cysA*$^+$ (34 to 104) among segregants from the heteroclone in Fig. 9b would

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TABLE 11. *Allele ratios in six heteroclones*

Heteroclone no.	Allele ratios at loci[a]								
	tps	+	*ura*	*nic*	*cys*	+	*hisA*	+	*tps*
	+	*str*	+	+	+	*pro*	*hisC*	*arg*	+
i	164	35	66	67	80	86	90	101	164
	0	129	98	97	84	78	74	64	0
ii	148	148	148	148	92	85	81	148	148
	0	0	0	0	56	63	67	0	0
iii	138	138	138	137	34	43	60	81	138
	0	0	0	1	104	45	78	57	0
iv	0	0	0	0	0	0	28	47	0
	121	121	121	121	121	121	93	74	121
v	156	156	156	156	156	156	45	57	156
	0	0	0	0	0	0	111	99	0
vi	24	24	24	24	16	16	7	2	24
	0	0	0	0	8	8	17	22	0

[a] *tps* is repeated at each end to indicate circularity; arrows indicate points of reversal of allele frequency gradients (see text).

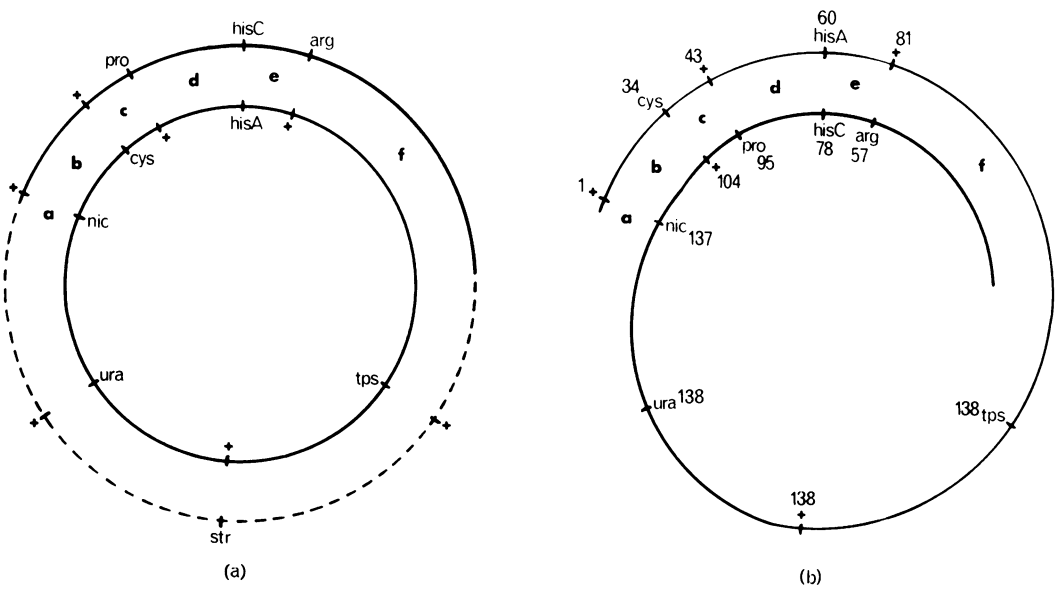

FIG. 9. *Origin and constitution of a heteroclone genome according to the proposed model.* (*a*) *The genome of the parent merozygote; the dashed region of the outer parental genome is missing.* (*b*) *The heteroclone genome, arisen by a single crossover in interval b. Numbers indicate the frequency of each allele in the sample of segregants analyzed* (*Table 12*).

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reflect the relative lengths of intervals (a + b) to (c + d + e + f).

We know little about the molecular problems involved in replicating a bacterial genome, whether closed or open (8). However, it may be that genome closure is a prerequisite for replication. If so, it would be necessary to postulate closure of the terminally redundant genome represented as an open structure in Fig. 9b by joining of the free ends, giving in effect a tandem duplication of the genes in the disomic region. Such closure could, in principle, occur *before* crossing over in the zygote, so that the tandem duplication would arise by crossing over between two circles, rather as in the Campbell (9) model of prophage integration. Note that a precisely similar gradient of allele frequencies among haploid segregants produced by odd numbers of crossovers within the duplicated region of the heterozygote would be expected, whether the genome were open or closed, so that segregation data do not help to discriminate between these alternatives. Since the limits of the duplication in different heteroclones would have to be, to a first approximation, randomly distributed over the genome, closure of ends could be mediated by specific base pairing of a nucleotide sequence only if this were repeated at many points on the genome.

Even numbers of crossovers in heteroclone genomes should not result in segregation of haploids, but should cause changes in coupling of heterozygous markers, and should also lead to a loss of heterozygosity at some loci, one of the alleles becoming homozygous, provided crossing-over occurred at a multistrand stage (50). Homozygosity does indeed occur among higher-order heteroclones (38, 64), as well as very occasionally among primary ones; homozygosity for an interstitial marker is presumably the explanation of heteroclones of types *w*, *x*, and *y* (Table 10), in which heterozygous loci are separated by one or more loci which are not heterozygous.

Thus, a growing heteroclone cannot be regarded as a clone of identical heterozygous genomes all with precisely the same pattern of segregation open to them, but rather as a pool of heterozygous genomes, the major class being that which initiated the heteroclone, but with a number of different subclones with varying rearrangements of markers (38). The final pattern of segregation observed among a sample of haploids from a heteroclone would therefore represent a somewhat heterogeneous pool of segregants from different classes of heterozygous genomes; it would reflect in a general way the structure of the major heterozygous component of the heteroclone, but would show varying discrepancies when analyzed in detail. Such discrepancies are often found when a long enough series of linked markers is available, although they were not obvious in previously published data which analyzed groups of markers covering only a small proportion of the whole linkage map. For example, in Table 12, in which the segregant frequencies from the heteroclone of Fig. 9b are listed, some multiple crossover classes are larger than one of the component singles.

TABLE 12. *Segregation in a heteroclone*

Genotypes of segregants[a]	Observed no.	Crossovers in intervals[b]
nic cys hisA tps ura	34	2
nic hisA tps ura	2	3
nic pro hisA tps ura	20	4
nic pro hisC tps ura	23	5
nic pro hisC arg tps ura	52	6
hisA tps ura	1	1, 2, 3
nic hisC tps ura	1	3, 4, 5
nic hisC arg tps ura	2	3, 4, 6
nic hisA arg tps ura	3	3, 5, 6
Total	138	

[a] Wild-type alleles omitted.
[b] See Fig. 9b. Data of A. Vivian.

On the hypothesis just described, and in the absence of special assumptions which might predict an excess of even-numbered crossovers in the zygotes, heteroclone genomes should arise within the zygotes at least as frequently as haploids. The relative frequencies of the two classes have not been measured experimentally. In a normal cross, recombinant genomes are not collected as they arise; instead, spores and fragments of aerial mycelium are harvested from the mixed culture hours, or possibly days, after the first zygotes originated in the substrate mycelium. Thus, recombinant genomes have ample opportunity for reproduction before they are collected, during which the ratio of haploids to disomics may change. On the present hypothesis, the proportion of disomics would be expected to decrease, since haploids would reproduce to give rise to further haploids, whereas disomics would produce a mixture of further disomics and haploid segregants.

Table 13 gives the results of an experiment to determine the ratio of haploids to heteroclones in crosses harvested after different periods of incubation. Two crosses were performed, with the same markers in different coupling arrangements. Replicate tubes of each cross were harvested after 2, 3, and 6 days of incubation and plated on

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TABLE 13. *Proportions of heteroclones among total recombinant colonies in two crosses*

Parents of cross	Age of the mixed culture (days)	Colony counts[a]			Proportion of heteroclones: ratio of columns (3) and (1)
		Selection for *pro*$^+$ *ura*$^-$ (1)	Selection for *his*$^+$		
			Large colonies (2)	Small colonies (3)	
hisA uraA	2	69	2.5	31.5	0.46
×	3	1,625	21	451	0.28
hisC proA	6	1,685	19	246	0.15
hisC uraA	2	441	8.5	160.5	0.36
×	3	254.5	3	39	0.15
hisA proA	6	740	4.5	81.5	0.11

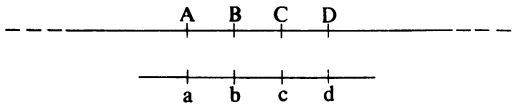
[a] Average counts for two plates; relative magnitudes of counts on different lines are arbitrary since they represent different dilutions of the spore suspensions.

two media, one selecting for distant markers (*proA*$^+$ and *uraA*$^+$), and the other for very close markers (*hisA*$^+$ and *hisC*$^+$). On the second medium, as expected, a majority of the colonies were heteroclones, identified on the basis of small size. The data show that the proportion of heteroclones (estimated from the small colony count on the second medium, column 3) to total recombinants (estimated from the total colony count on the first medium, column 1) may exceed 40%, and that the proportion of heteroclones falls steeply with incubation of the cross; clearly, the proportion of heteroclones at 2 days is a minimal estimate. These findings are too indirect to establish any particular model for the origin and structure of heteroclone genomes, but they certainly indicate that genomes capable of initiating heteroclones arise with a high frequency, which is probably related to the frequency of haploid recombinants, since a similar range of proportions is found in crosses between many different pairs of strains; it is unlikely that heteroclones arise by events unrelated to the "mainstream" of recombination.

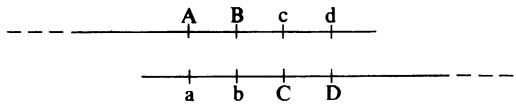
In earlier papers (30, 38, 64), some minority classes of heteroclone were described, besides the common class (described as "two deletions in *trans*") in which there appeared to be an overlap between partial genomes from the two parents. As was pointed out then, the class of "single deletions" could very well have comprised limiting cases of the common class, in which the group of marked loci was far enough from the "second deletion" for its effects not to have been felt on the segregation at marked loci. A remaining (rare) class, referred to as "two deletions in *cis*," probably comprised heteroclones in which, on the present model, the crossover giving rise to the heteroclone genome had occurred within a group of marked loci, resulting in a gradient of allele frequencies such as:

95	65	65	95
5	35	35	5

This was previously interpreted on the basis of a heterogenote structure of the following type:



On the present model a change in coupling within the overlap region would be postulated, leading to the following structure (the dashed ends being connected):



Thus, all classes of heteroclone would be referable to the single type of genome structure proposed in this article.

The data of Table 10 and Fig. 8 are compatible with the notion that there might be a certain region of the genome, close to *hisA/C*, that is obligatorily disomic in all heteroclones; because all heteroclones were *selected* to be disomic at *hisA/C*, the existence of such a region would have been obscured in this experiment. This possibility has been excluded by a limited analysis of heteroclones selected by means of a pair of markers on the other side of the map, *thrA/mthB*; these heteroclones were always, of course, disomic for *thrA/mthB*, but disomy usually did not extend to the *hisA/C* markers.

Comparative Considerations

It will have been noticed that some of the features of the formal model of heteroclone structure and behavior just described are reminiscent of current ideas on the structure and behavior of bacteriophage genomes, especially those of T2 and T4 (for a summary of these ideas, see 68). T2 and T4 have the advantage that some of the experiments required to convert a formal model into a molecular model have been carried out. In particular, the notion of terminal redundancy (70) has received striking support from the results of physical experiments (49). In T4, terminally redundant genomes are supposed to undergo recombination during synapsis between the redun-

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dant regions of different genomes, resulting in the production of "polygenomes" which later fragment to the unit-sized pieces characteristic of mature phage. Physical evidence for the existence of such giant DNA molecules within T4-infected bacteria has been presented (20), and criticized (43). For *S. coelicolor*, there are no comparable data, but cytological observations (35) are compatible with the idea that multiple genomes might exist, possibly as a normal part of the life cycle, particularly in the sequence of events immediately prior to sporulation. Thus, although for simplicity the terminally redundant heteroclone genomes postulated for *S. coelicolor* have been considered to synapse with their own tails, conceivably they sometimes or regularly do so with the tail of another genome instead.

The events postulated to occur within the zygotes of *S. coelicolor* may also find comparisons with those taking place in other bacterial zygotes. The zygotes of *E. coli* K-12 formally resemble those of *S. coelicolor* in containing a complete genome derived from one parent and a partial genome from the other. In both systems, the length of the incomplete genome varies in an approximately random fashion (with the restriction that one end occurs at a fixed position in matings between Hfr and F^- strains of *E. coli*), and appears to represent about the same fraction of a full genome. Since nothing is known about the molecular events occurring during recombinant formation in *S. coelicolor* zygotes, we have to ignore the information beginning to be obtained about these events in *E. coli* (52) and confine ourselves to possible analogies between the two systems at a more superficial level.

Recombinants in *E. coli*, as in *S. coelicolor*, must, in formal terms, result from even-numbered exchanges (consider two for simplicity) within that region of the genome that was disomic in the zygote. In *S. coelicolor*, it is postulated that the two crossovers may or may not occur contemporaneously. If they do, then a haploid recombinant genome originates within the zygote itself (presumably together with a recombinant fragment which is broken down or diluted out). If, on the other hand, only one crossover occurs within the zygote, a terminally redundant genome arises, which can give rise to a heteroclone. The second crossover is then delayed by a period of replication of the terminally redundant genome, and occurs at different positions in different descendants of this genome. Unless some presently unknown mechanism acts in *E. coli* zygotes to ensure that crossovers are always of even number, odd numbers of crossovers should occur at least as often as even numbers, and should give rise to genomes corresponding in topology to those capable of initiating heteroclones in *S. coelicolor*. In principle, such genomes might be broken down or diluted out on cell division; they might survive long enough for further odd-numbered crossovers to cause segregation from them of haploid recombinant genomes; or they might replicate and subsequently undergo segregation, as a result of odd-numbered crossovers, in the way postulated for *S. coelicolor*.

There is considerable evidence that a sizeable fraction of zygotes arising in matings between certain combinations of *E. coli* strains give rise to more than one recombinant clone, and frequently to several (3, 6, 45, 73). Thus, heterozygosity can undoubtedly be prolonged after zygote formation long enough to allow more than one opportunity for recombination. In this, *E. coli* resembles *S. coelicolor*, but it remains to be seen whether the mechanisms responsible for prolonged heterozygosity are the same in the two organisms. Even if they were, heteroclone *colonies* would not necessarily be expected in *E. coli*; their development would depend on the particular balance between replication of and segregation from the heterozygous genomes.

As noted above, strains of *E. coli* K-12 in common use differ considerably in the extent to which recombinant colonies developing from zygotes are heterogeneous with regard to recombinant genotypes; for example, HfrH strains seem to sire mixed progenies much more often than HfrC strains (73).

More prolonged heterozygosity has been described in a number of situations involving particular strains of *E. coli* K-12, apart, of course, from the rather well understood F′ strains in which duplication of a segment of chromosome results from its integration into the autonomously replicating F factor. Lederberg (44) was the first to describe a persistently heterozygous strain, and this and its derivatives were studied in some detail (45*a*). The main characteristic of these strains, which had a particular segment of the genome duplicated, was their comparative stability, indicating some mechanism resulting in coordinated replication of the duplicated genes. However, with an easily detectable frequency, these disomic strains segregated recessive markers, which sometimes became homozygous and sometimes segregated in hemizygous condition. In the latter case, the segregants were completely haploid; partial elimination of the duplicated segment was never observed. All of these features are shared by the heteroclones of *S. coelicolor*; the information required to build a specific model of the genome structure of these heterozygotes of *E. coli* ap-

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pears, however, to be lacking, so that a more detailed comparison is not possible.

Horiuchi, Horiuchi, and Novick (39*a*) later isolated some strains of *E. coli* which were disomic just for the *lac* region; the strains lacked the F factor and behaved very much as if the extra *lac* genes were integrated into the chromosome as a tandem duplication, since P1 phage was able to transduce both copies of the *lac* region simultaneously. Like the other heterozygous strains, they segregated both homo- and hemizygous derivatives. Perhaps these strains were qualitatively different from those studied by Lederberg in being simple duplications; alternatively, the two kinds of heterozygotes may have had the same kind of structural basis, but the much longer duplication in the Lederberg strains may have allowed a greater complexity of segregation behavior.

More recently, Curtiss (12) has also obtained rather stable partial diploids of *E. coli*, in matings involving strains with some kind of structural aberration (possibly a deletion) in the *pro-1-pro-2* region of the chromosome; this aberration appeared to be necessary if the strains were to be stable (less than 1% segregants per bacterium per generation). The duplicated segment, which did not carry the aberration, extended anticlockwise from the *pro* region for about 10% of the genome. Many features of these strains are extremely reminiscent of those of the *S. coelicolor* heteroclones; in particular, allele frequencies among haploid segregants for duplicated loci showed a gradient, from almost 100% for the "exogenote" marker at a locus at one end of the duplicated region to nearly 100% for the "endogenote" marker at the other terminal locus. However, one finding appeared to exclude the possibility that this gradient was a consequence of integration of the "exogenote" into the entire chromosome in the manner postulated for *S. coelicolor* heteroclones: the F factor appeared not to mediate any *linked* transfer of markers located on the "exogenote" and on the nonduplicated part of the genome. Therefore, it was proposed that the "exogenote" was an independent chromosomal segment, associated non-covalently with the complete chromosome, but in such a manner that it always replicated with it. Moreover, it could be lost only as a result of recombination, and such recombinational events had the special property of requiring an obligatory crossover at one end, but not the other, of the "exogenote." If this model were to be substantiated, it would of course offer a plausible interpretation also of the *S. coelicolor* heteroclones, in the absence of the means for demonstrating a covalent connection between the duplicated genes, such as has been postulated on the basis of segregation ratios alone.

Maas, Goldschmidt, and Low (46) have studied partially diploid strains of *E. coli* K-12 obtained by mating Rec$^-$ females (11) with a double male strain (10). The genomes of these heterozygotes may have the same structure as that postulated for *S. coelicolor* heteroclones, carrying the piece of chromosome originating from the donor integrated as a terminal redundancy or tandem duplication. However, segregation of recessive markers from the *E. coli* disomics, unlike that in *S. coelicolor* heteroclones, appears not to result from the simple occurrence of crossovers within the duplicated region; this may, of course, be related to the fact that the strains are phenotypically Rec$^-$.

PERSPECTIVES

The gaps in our knowledge of almost every aspect of *S. coelicolor* genetics will have been obvious to the reader of this article. Hopefully, the interest attracted by this organism which offers a prokaryote genetic system with certain unique features will have been no less obvious. This final section puts forward some guidelines to future work, pinpointing some gaps in the present picture and some of the more obvious possibilities for future exploitation of the system.

Chromosome Transfer and Fertility

We are still to a large extent ignorant of events preceding zygote formation. It can be deduced that the zygotes, as well as the heteroclones to which they give rise, are disomic for random segments of the genome, averaging about one-sixth of the total. It is a reasonable conjecture that, as in other bacteria, this incompleteness is a consequence of partial transfer rather than of subsequent loss from a complete diploid zygote. Since (almost) a whole genome can be transferred as a limiting case, it is fairly certain that some kind of "conjugation" mechanism is involved. It is of course possible that transfer is orientated, as in *E. coli*, and that it occurs through a similar organelle, whatever that turns out to be. Some recombination data in *S. coelicolor* have already been interpreted in terms of such a model of orientated partial transfer (62).

The question of sexual polarity in *S. coelicolor* and the related one of whether chromosomal transfer is episomally mediated likewise remain largely open. As mentioned earlier in this article, pairs of strains differ greatly in the frequency with which they produce recombinants. Numerous environmental and genetic factors could be

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expected to influence the "fertility" of a cross as commonly measured by the ratio of nonparental to parental spores produced, and progress in analyzing the situation will probably depend on devising a precise measure of the frequency of chromosomal transfer itself. Sermonti and Casciano (63) have gone farthest in drawing an analogy between some universally fertile (R^+) and mutually (almost) sterile (R^-) strains of *S. coelicolor* and the corresponding F^+ and F^- strains of *E. coli*, but the crucial factor of transmissibility of fertility by an episome was not demonstrated.

Heterozygotes

Much has been said in a previous section of this article to indicate, on the one hand, the extent to which the model of heteroclone genome structure proposed is still only a working hypothesis, and, on the other, some possible analogies with heterozygous genomes of other bacteria and phages, which promise to lead to mutually illuminating comparisons in the future. To mention a further specific example: *ex hypothesis*, the heteroclone genome of *S. coelicolor* arises by a single reciprocal crossover (or higher odd number), and contains, in an integrated form, the products of this exchange; that is, it is a kind of "half tetrad." This situation, if exploited, could contribute to the answering of some of the questions about crossing-over raised, and partially answered, by Meselson (50) in his elegant studies of another prokaryote system.

Heterokaryons

A complicating factor in genetic analysis of some members of the genus *Streptomyces* is the occurrence of heterokaryons. Operationally, these are phenotypically prototrophic colonies, arising on selective media from platings of spores from mixed cultures of auxotrophs, and segregating a mixture of the two parental phenotypes only. At one extreme, for example, in the strains of *S. griseus* and *S. cyaneus* studied by Bradley and Lederberg (5), heterokaryons appeared to constitute the only class of colony arising on selective media. At the other extreme, crosses performed under normal conditions between substrains of *S. coelicolor* A3(2) (27), the strain which is the subject of this article, produce almost exclusively recombinants (haploids and heteroclones); however, heterokaryons, as operationally defined, do occur (38, 65). Intermediate behavior, with heterokaryons and recombinants more or less evenly balanced, has been found in some other streptomycetes. The factors determining the proportions of heterokaryons and recombinants are largely unexplored.

Heterokaryosis would seem to be unique to *Streptomyces*, among genetically well-known prokaryotes (although F′ strains of *E. coli* are in a sense partial heterokaryons), and as such should be a promising field for investigation from several points of view (64). Because of the apparent lack of correlation of heterokaryon formation and recombination in different strains, it is a reasonable speculation that (complete) genome transfer leading to heterokaryosis proceeds by a different route from (incomplete) genome transfer leading to zygote formation. One process might, however, be dependent on the other in that complete transfer *between* mycelia of different parents leading to heterokaryosis might be a prerequisite for subsequent incomplete genome "transfer" *within* the mycelium to produce a merozygote. Such possibilities should now be open to investigation.

From a physiological point of view, a comparison between gene interaction in heterokaryons and (partial) diploids of a prokaryote might provide a useful contrast to such studies in eukaryote systems (56). However, quantitative complementation and dominance tests in *S. coelicolor* may be contingent on the development of more stable disomic strains than the presently known heteroclones; there is no reason to believe that such strains will not eventually become available.

Transduction and Transformation

Although there have been sporadic reports of the isolation of prototrophs from auxotrophic strains of various streptomycetes treated with phage- or DNA-containing preparations from prototrophic strains (64), none of them has led to a useful system of transduction or transformation, possibly because the experiments were performed in species of *Streptomyces* in which information on the genome and linkage map was lacking. Some preliminary attempts to demonstrate either process in *S. coelicolor* A3(2) have given negative results, but the matter merits a more intensified effort, if only because one or the other process should make it easier to achieve a high resolving power in genetic analysis than the conjugation system currently available.

Meaning of Gene Arrangement on the Linkage Map

The significance of any arrangement of genes on a chromosome might be: historical, i.e., the present arrangement of genes might be only one

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of very many equally compatible with optimal gene function and largely reflect "accidents" in the origin and subsequent transposition of genes around the genome as a result of chromosomal rearrangements; or functional, i.e., the present arrangement of genes might have been selected to optimize their mutual functioning and preserve little trace of historical events. The truth, of course, must lie somewhere between these two extremes.

There is no doubt that, on the scale of single biosynthetic sequences, gene arrangement in "clusters" in bacteria (14) has a functional significance (41). This makes it the more surprising that such clusters appear to be inconstant from group to group of bacteria. For example, the well-known histidine operon of *Salmonella* (2) contains all the structural genes for the pathway uniquely leading to histidine, whereas the evidence is reasonably strong that the corresponding genes are separated in *S. coelicolor*, although still partially clustered (see below).

Perhaps partial clustering has come about as a result of duplication of an original cluster of structural genes, complete with its switchgear (promoter, operator, etc.), followed by loss, through mutation or deletion, of certain structural genes from one of the pair of duplicated operons and some from the other. A "wholesale" version of such a process has been suggested by Hopwood (34) to account for a possible circular symmetry of the linkage map of *S. coelicolor*, with members of the same class of genes tending to lie at 180° to one another on the map; the whole genome was postulated to have duplicated in tandem as a result of crossing-over between homologous circles. A "retail" version of this cluster dispersion is, of course, conceivable, and poses fewer problems; that is, duplication of a single operon might occur as a result of a transposition of a more orthodox kind (48).

Meaningful (from a functional standpoint) gene arrangements at a grosser level than individual operons are hard to discern on any linkage map, with the striking exception of phage T4 (19), and even here the significance of the finding is open to more than one interpretation (69). In *S. coelicolor*, there *may* be a functional specialization of parts of the genome, in that two quadrants of the linkage map are almost devoid of known genes (32). However, attempts to identify a class of genes in these regions have so far failed, and it remains possible, in the absence of physical estimates of interval length, that the regions represent crossover "hot spots" rather than regions rich in an elusive class of gene.

Comparative Gene-Enzyme Relationships

Although the sequences of enzyme reactions leading to the biosynthesis of particular metabolites are largely uniform between different groups of microbes, both eu- and prokaryotes, the relationships between the genes and the enzymes concerned, and between the different enzymes of the same pathway (enzyme aggregation, etc.), are more variable [*see* Roberts (57) for references]. Even within a group such as the fungi, there is variation in gene-enzyme relationships in, for example, the tryptophan pathway (40), and differences must be widespread also among bacteria. The addition of an actinomycete to the select group of genetically amenable bacteria widens the scope for comparative studies of gene-enzyme relationships. So far, the only biosynthetic pathway whose gene-enzyme relationships have been investigated in *S. coelicolor* is that leading to histidine. The functions of several genes have been identified confidently, and others tentatively; these gene-enzyme relationships, based on the work of Russi et al. (59) and, for *his*, P. Engel (*personal communication*), are summarized and compared with those in *S. typhimurium* (Table 14). The map locations of the genes in *S. coelicolor* (27, 30, 38, 55, 59) are also compared with those of *S. typhimurium*. We find that, in contrast to the situation in *Salmonella*, where all nine genes are contiguous, at least two genes in *S. coelicolor* are far from each other and from a cluster of six genes; presumably two other genes are still to be discovered. There is no clear correspondence between the order of the six genes in the cluster and that of the corresponding genes in the *Salmonella* cluster; moreover, two enzymes specified by one gene (*hisB*) in *Salmonella* appear to be specified by two widely separated genes (*hisB* and *hisD*) in *S. coelicolor* (59). Whether the six clustered genes correspond to an operon is not yet known.

Although *S. coelicolor* resembles the ascomycetes *Aspergillus nidulans* (3*a*), *Neurospora crassa* (1*a*) and *Saccharomyces cerevisiae* (19*a*) in having some histidine genes scattered and others clustered, there is no evidence that the resemblance is more than superficial. In all three of these fungi, the same three genes, specifying enzymes 2, 3, and 10, form a cluster, and the polypeptide products of the three genes form an enzyme aggregate; no such aggregates involve any of the histidine enzymes of *Salmonella*. These three enzymes are not aggregated in *S. coelicolor*, since ammonium sulfate precipitation readily separates them (P. Engel, *personal communication*); moreover, if the gene-enzyme relationships

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TABLE 14. *Gene-enzyme relationships of histidine biosynthesis in Streptomyces coelicolor and Salmonella typhimurium compared*[a]

Step	Enzyme	*Salmonella* gene	*Streptomyces* gene
1	PR-ATP pyrophosphorylase	G	?
2	PR-ATP pyrophosphohydrolase	E	?
3	PR-AMP 1,6-cyclohydrolase	I	E
4	Isomerase	A	?F
5	Amidotransferase	H	?I
6	Cyclase	F	?C
7	IGP dehydrase	B	B
8	IAP transaminase	C	G
9	Histidinol phosphate phosphatase	B	D
10	Histidinol dehydrogenase	D	A

Linkage relations of genes

Salmonella:									
Gene:	E	I	F	A	H	B	C	D	G
Enzyme:	2	3	6	4	5	7,9	8	10	1
Streptomyces:									
Gene:	B	I	A	G	C	F		E	D
Enzyme:	7	?5	10	8	?6	?4		3	9

[a] For references, see text.

in Table 14 are correct, the corresponding three genes (only *hisA* and *hisE* have been identified) are not contiguous in this organism.

We can hope that analysis of the histidine system of *S. coelicolor* will be taken further in the future and that gene-enzyme relationships in other biochemical pathways will be explored.

Mutational Analysis of Morphogenesis

In the same way that nutritional mutants have contributed to the recognition of steps in the pathways of synthesis of metabolites, so mutants defective in normal morphogenesis must be able to contribute to the recognition of unitary processes in the "synthesis" of structures. Whereas the choice of material for study is embarrassingly wide amongst eukaryotes, the prerequisites of a well-developed experimental genetics, combined with a suitably complex morphogenesis, are currently found in a very limited number of prokaryote systems. Ideally, the subject of study would be a dispensable structure, thereby increasing the range of morphological mutants available. The endospore of *B. subtilis* is one such structure that has already been subjected to a limited mutational analysis (61, 67, 71); the sporogenous phase, that is the aerial mycelium and spores, of *S. coelicolor* (22, 23, 28, 35, 36) is another, and preliminary observations in my laboratory indicate that mutants with lesions at different stages in their development can be isolated by various visual and selective procedures. The whole structure is dispensable, in the sense that mutants lacking aerial mycelium altogether can be propagated indefinitely by substrate hyphal transfer, and even such extreme departures from normal morphology cause no serious difficulties in crossing and genetic analysis (for example *S48*, Table 2).

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