A NEW KIND OF FERTILITY VARIANT IN *STREPTOMYCES COELICOLOR*

DAVID A. HOPWOOD, RICHARD J. HAROLD, ALAN VIVIAN AND HELEN M. FERGUSON

*Institute of Genetics, Glasgow University, Glasgow, Scotland and John Innes Institute, Norwich, NOR 70F, England**

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A class of fertility variants, called "ultra-fertile" (UF), has been identified in the actinomycete *Streptomyces coelicolor* A3(2) (HOPWOOD, HAROLD, VIVIAN and FERGUSON 1968); these variants differ strikingly in their sexual capabilities from the strains that had previously been encountered in *S. coelicolor* genetics (HOPWOOD 1967). It seems likely that UF variants will contribute to an understanding of the normal process of genetic transfer in this organism, about which little is yet known, just as the well-known fertility variants (Hfr and F^-) of the original F^+ strain of *Escherichia coli* K-12 led to the elucidation of the sexual process in that bacterium. The present paper describes the isolation of UF strains and some of the characteristics of genetic recombination in crosses involving them.

MATERIALS AND METHODS

General: Media and standard techniques of *S. coelicolor* genetics were those described by HAROLD and HOPWOOD (1969). Treatment with N-methyl-N′-nitro-N-nitrosoguanidine (NTG) was by the schedule of DELIĆ, HOPWOOD and FRIEND (1969). Bacterial strains were mutational or recombinational derivatives of *S. coelicolor* A3(2) (HOPWOOD 1959); the characteristics of the strains referred to in this paper are listed in Table 1, and the linkage relations of the markers are shown in Figure 1.

Isolation of UF variants: Most UF variants were recognized by an indirect selection procedure, which is a modification of that described by SERMONTI and CASCIANO (1963), as follows. A spore suspension of the strain in which variants were sought, sometimes after treatment with UV or NTG, was plated on the surface of dishes of non-selective medium (minimal medium supplemented with required growth factors) at a dilution yielding 100–150 colonies per plate. After incubation until the colonies were sporulating (3–4 days), the plates were replicated by velvet to plates of complete medium on which a dense suspension of spores of a tester strain of normal fertility (NF), bearing complementary genetic markers, had been spread and allowed to dry. These 'lawns' of spores were prepared on rather thick plates (25 ml of medium per 9 cm dish). After incubation for 3–4 days these 'plate-crosses' were replicated to dishes containing a medium selecting recombinants. These plates were compared with the original plates after 2 days' incubation, when patches of recombinant colonies occurred in positions corresponding to the colonies on the original plates (Figure 2). A small proportion of colonies gave rise to denser patches of recombinants, and such colonies were purified by streaking and then retested for fertility by inoculating them in defined areas on a 'master plate' of non-selective medium, together with control patches of the original strain, and repeating the indirect selection procedure (Figure 3). UF variants were clearly distinguished from the original strain by this test.

* Present address

TABLE 1

Characteristics of strains

Strain number	Phenotype in respect of the markers*													Fertility
	tps-30	*cysD18*	*leuB5*	*strA1*	*metB4*	*adeA3*	*pheA1*	*uraA1*	*nicA3*	*cysA1*	*proA1*	*hisA1*	*argA1/C4*	
358	+	+	+	R	—	—	+	+	+	+	+	—	+	NF
749	+	—	+	S	+	+	+	—	+	+	—	+	A1	NF
791	+	—	—	R	+	+	+	+	+	+	—	+	A1	NF
923	+	—	+	S	+	+	+	—	+	+	+	—	+	NF
928	—	+	+	S	+	+	+	—	—	—	+	—	+	NF
933	+	+	+	S	+	—	+	—	+	—	+	—	C4	NF
949	—	+	+	S	+	+	+	—	—	—	—	+	A1	NF
1105	+	—	—	R	+	+	+	—	—	+	+	—	A1	NF
12	+	+	+	S	+	+	—	+	+	+	+	+	+	NF
1098	+	+	+	S	+	+	—	+	+	+	+	+	+	UF
1117	+	+	+	S	+	+	—	+	+	+	+	+	+	UF
1120	+	+	+	S	+	+	—	+	+	+	+	+	+	UF
1121	+	+	+	S	+	+	—	+	+	+	+	+	+	UF
1122	+	+	+	S	+	+	—	+	+	+	+	+	+	UF
1125	+	+	+	S	+	+	—	+	+	+	+	+	+	UF

* See HOPWOOD (1967) for marker characteristics, and Figure 1 for linkage relationships.

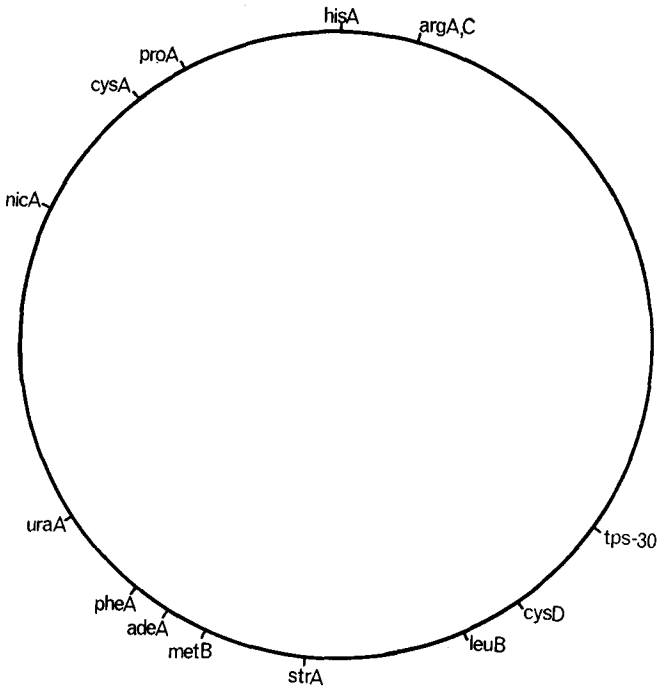

FIGURE 1.—Linkage map of *S. coelicolor* A3(2) showing the approximate relative positions of markers referred to in this paper. Locus symbols have conventional meanings (HOPWOOD 1967).

A few UF variants were isolated by replicating the original plates of colonies directly to plates of the selective medium bearing 'lawns' of spores of the tester strain; that is omitting the intermediate 'plate-cross' on complete medium. In this test, NF colonies usually failed to produce any selected recombinants, whereas UF colonies gave rise to clusters of recombinant colonies as a result of mating on the selective plates.

Non-selective analysis of recombination: Crosses were usually made on slants of complete medium in 15 × 2.5 cm tubes, as follows. About 0.2 ml of water was added to the slant and loopfuls of spore suspensions of the two strains to be crossed, one UF and one NF, taken from young, vigorously sporulating cultures, were added to the water and mixed thoroughly. The liquid was then spread over the entire surface of the slant and the tube incubated in an inclined position with the agar surface horizontal. After incubation (for 4 days unless specified), spore suspensions were prepared in the usual way (HOPWOOD 1967), except that centrifugation was omitted, and plated at suitable dilutions on nonselective medium (minimal medium supplemented with all required growth factors of the parental strains). Samples of the resulting colonies were characterized in respect of parental markers in the usual way, and some were tested for fertility by the method described above and illustrated in Figure 3. In the experiments in which one parent was inoculated 12 or 24 hrs before the other, the crosses were made on plates instead of on slants.

RESULTS

Strategy for the isolation of UF strains: It has been deduced (HOPWOOD 1967) that each zygote of *S. coelicolor* contains a complete, probably circular, chromo-

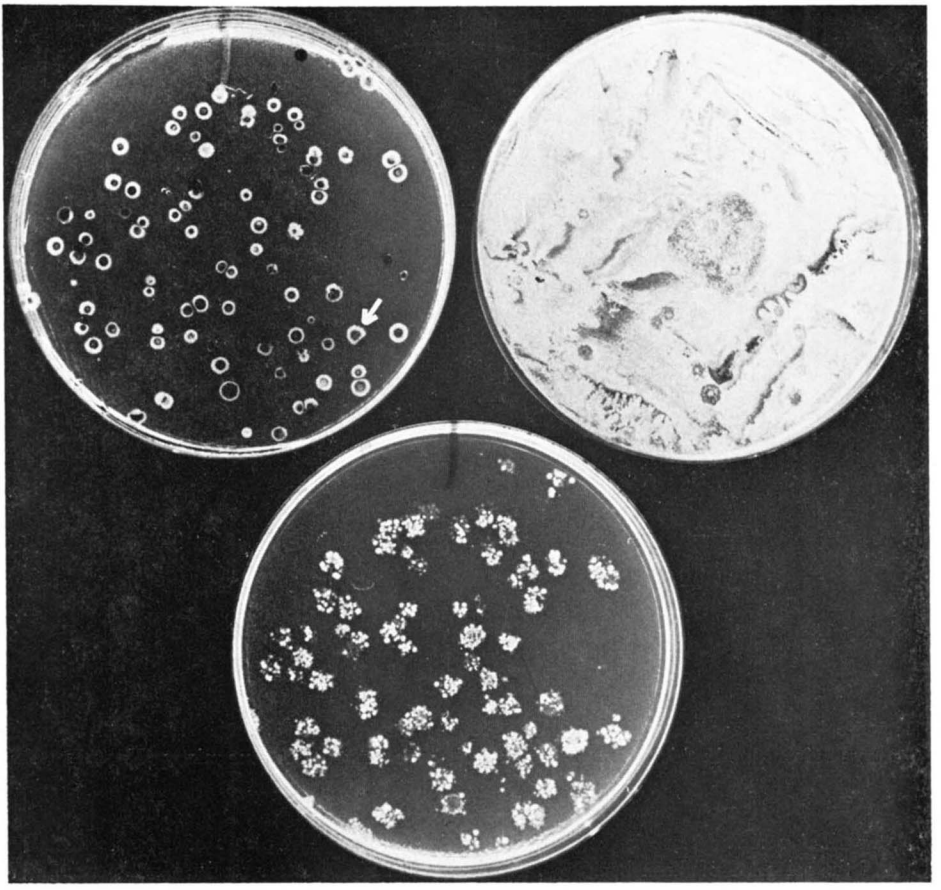

FIGURE 2.—Isolation of UF variants from strain 12. Survivors of UV irradiation growing on non-selective medium (top left-hand plate) were replicated to non-selective medium spread with a 'lawn' of spores of the tester strain 923; after incubation, the resulting 'plate-cross' (top right-hand plate) was replicated to selective medium (lower plate). Each colony on the original plate gave rise to a patch of recombinants on the selective medium (the recombinant colonies are white, on a grey background due to leaky growth of strain 12). A UF variant (arrowed) was identified because it produced a denser patch of recombinants.

some from one parent and a random chromosome fragment, on the average about one-sixth of a genome in length, from the other. In a typical cross involving two strains of normal fertility (NF), each strain contributes the complete chromosome to about half the zygotes, and the chromosome fragment to the remainder; such crosses have been called "nonpolarised" (HOPWOOD 1967). Certain crosses, in contrast, are "polarised" to varying extents in that the complete genomes of all, or most, of the zygotes come from the same parent; the causes of such polarisation, which is distinct from the phenomenon of ultrafertility described in this paper, remain to be recognized.

It should theoretically be possible to obtain progeny preferentially from one of

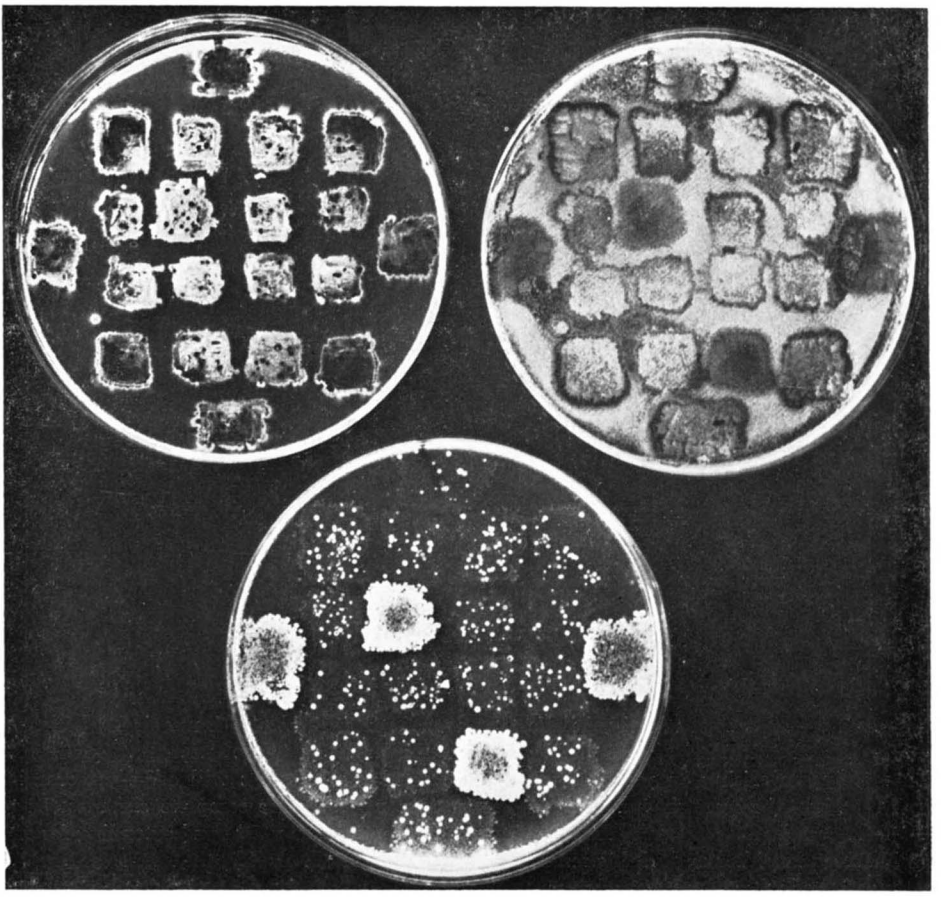

FIGURE 3.—Fertility-testing of presumptive UF variants isolated from strain 12 by the procedure illustrated in Figure 2. Sixteen possible variants were inoculated on a 'master-plate' of non-selective medium (top left), together with standard patches of strain 12 NF (north and south) and strain 1098 UF (east and west). A plate-cross was prepared by replicating the master-plate to a lawn of strain 923 (top right), and the plate-cross was replicated to selective medium (lower plate). Two of the 16 possible variants turn out to be UF.

the two classes of zygotes, even if the cross is nonpolarised, by selecting a combination of markers that arises by a simple pattern of crossing over, and therefore abundantly, from one class of zygotes and by rare crossovers from the other; this principle was applied in isolating the UF variants. The UF strains so far investigated were isolated from strain 12 (*pheA1*), with strain 923 (*hisA1 cysD18 uraA1*) as the complementary tester strain. The selective medium on which recombinants were recognized was unsupplemented minimal medium, so that the wild-type alleles of all four auxotrophic markers were selected. As we see in Figure 4, the three selected markers of strain 12, *his*$^+$, *cys*$^+$ and *ura*$^+$, are widely separated on the linkage map. Therefore, if the mating was to be highly fertile, this strain had to have an enhanced capacity for contributing the *complete* chro-

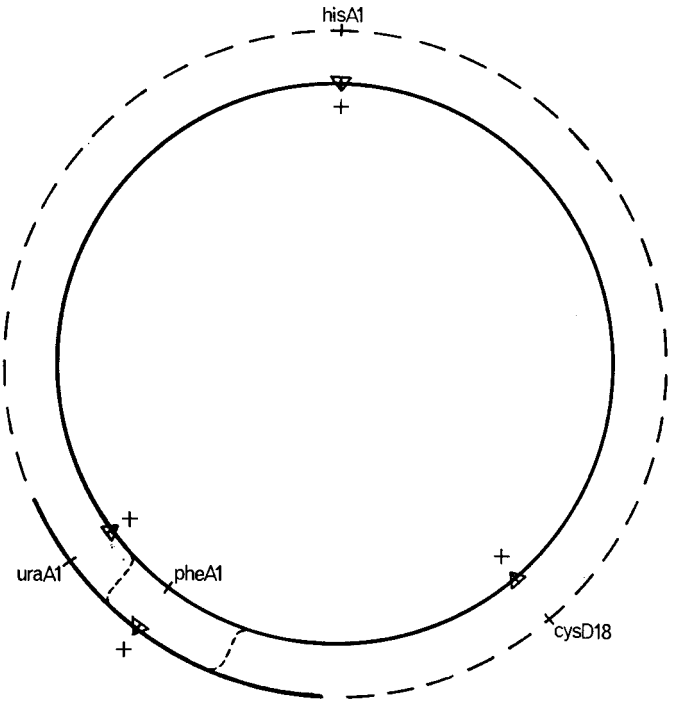

FIGURE 4.—Pattern of selection in a fertility test of strain 12 *pheA1* (inner circle) with strain 923 *hisA1 cysD18 uraA1* (outer circle) as tester. Triangles indicate the selected wild-type alleles of recombinants arising on minimal medium. Selected recombinants will come preferentially from the class of zygote shown, to which strain 12 contributes the complete chromosome and strain 923 the chromosome fragment, bearing at least the marker *phe*+, and extending for varying distances on either side of this marker (dotted region of the circle); the half-crossovers generating selected recombinants are drawn.

mosome to the zygotes, while strain 923 would contribute chromosome *fragments* obligately carrying the single selected marker *phe*+; zygotes of opposite polarity would very rarely give rise to selected recombinants since chromosome *fragments* long enough to carry all three selected alleles from strain 12 would be very rare.

This strategy was successful in isolating variant strains of enhanced fertility. Moreover the zygotes produced in crosses of such UF strains with NF strains appear to have, to an extreme degree, the polarisation demanded by the test used to isolate them: the NF strain contributes the chromosome fragment to *all* zygotes. There are, however, further unexpected features of these zygotes, as we shall see later.

Frequency of UF variants: Table 2 summarises semi-quantitative data on the frequency of occurrence of UF variants in strain 12. There is some evidence that both ultraviolet irradiation (about one in 300 surviving colonies was UF) and nitrosoguanidine (one in 700) increased the frequency of UF variants over the spontaneous frequency of about one in 3700. However, the small numbers of UF variants obtained in each experiment, as well as the somewhat subjective nature

TABLE 2

Isolation of UF variants from strain 12 tested against strain 923

Treatment*	Number of colonies examined		Percent UF
	Total	UF	
[UV	1550	3	0.2
[NTG	1500	2	0.1
[UV	1700	5	0.3
[NTG	350	1	0.3
[—	3600	1	0.03
[UV	5250	18	0.3
[—	3800	1	0.03
[NTG	2550	3	0.1

* Brackets denote samples of the same spore suspension.

of the isolation procedure, make conclusions as to the relative efficacy of different treatments inadvisable.

Extreme fertility of UF × NF crosses: Some of the unusual features of crosses in which one parent was UF, as compared with crosses of two NF strains, will be illustrated by the cross of strain 1105 (*argA1 hisA1 nicA3 uraA1 strA1 leuB5 cysD18* NF) with strain 1098 (*pheA1* UF). The parental arrangements of markers are shown in Figure 5. The cross was made and analysed nonselectively as described under MATERIALS AND METHODS and a sample of 643 progeny was characterised in respect of all markers.

The most striking characteristic of such crosses is their extreme fertility. In the example under discussion, only 81 of the 643 progeny had the parental arrangements of markers, 19 resembling the 1105 parent and 62 the 1098 parent: the remaining 87% of the progeny showed themselves to be recombinants, and therefore sexually produced. It was of course to be expected that some, at least, of the 81 progeny with parental arrangements of markers were also sexually produced. The following observations suggest that all, or almost all, of the 19 *arg his nic ura str leu cys* progeny were asexually produced, whereas the larger *phe* class contained sexual as well as asexual progeny.

Evidence on the origin of the *arg his nic ura str leu cys* class is provided by a consideration of allele ratios and the distribution of alleles among the various classes of progeny. It is at once apparent that the percentage allele ratios at different loci among the full sample of 643 progeny (the numbers in brackets in Figure 5) are very different from one another and from 50%. The *cysD18* allele originating from the 1105 (outer) parent has the lowest frequency (3%); proceeding in a clockwise direction, the frequencies of alleles from this parent increase to values well over 50% for *pheA1*, *uraA1* and *nicA3*, and then fall again to 4% for *argA1*. It is evident that the 'parental' class *arg his nic ura str leu cys* is peculiar in accounting for a disproportionately large share of the rare alleles: 100% of *cysD18*, 76% of *argA1*, etc. It is concluded that this class there-

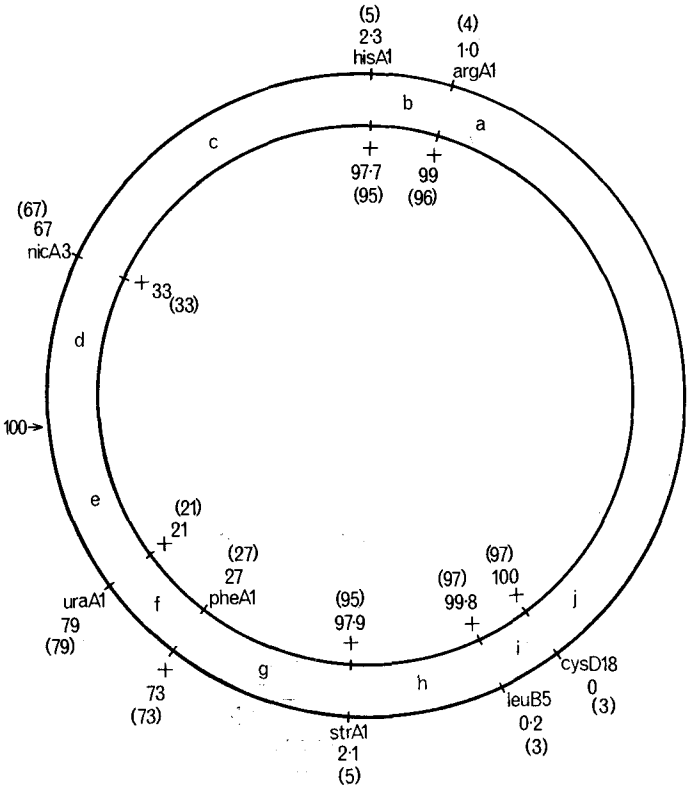

FIGURE 5.—Representation of a cross of 1105 *argA1 hisA1 nicA3 uraA1 strA1 leuB5 cysD18* NF (outer circle) with 1098 *pheA1* UF (inner circle). Numbers in brackets are the percentage frequencies of alleles in the whole sample of 643 progeny (Table 4); unbracketed numbers are the percentage allele frequencies in the 619 sexual progeny. The arrow indicates the 'required region' of the NF genome, deduced to be present in all sexual progeny (see text). Letters indicate map intervals.

fore has a different origin from the others and is tentatively identified as consisting of asexually produced progeny. (It is worth pointing out that none of the important conclusions of this paper are changed if this deduction should be incorrect.)

The *phe* class turns out to be unique in a different way, in being heterogeneous in respect of fertility. Samples of progeny from this and other crosses involving strain 1098 *pheA1* UF were tested for fertility by the plate test (Figure 3) with NF tester strains and found all to have normal fertility, *except for some members of the* phe *class*: in the cross under discussion, 57 of the *phe* segregants were NF and the remaining 5 were UF. These 5 segregants are the only members of the *phe* class that are parental in respect both of their standard markers and their fertility, and they have therefore been taken as being asexually produced, while the 57 NF *phe* segregants which show recombination of standard markers with fertility, are classed as sexually produced.

These conclusions are supported by the results of experiments in which the

TABLE 3

Constancy of relative frequencies of various classes of sexual progeny when frequencies of asexual classes are varied by inoculating one parent before the other, or by harvesting the cross prematurely; cross of strain 749 NF × 1098 UF

Time (hrs)			Asexual progeny			Sexual progeny*					
Inoculation						Recombinants					
NF parent	UF parent	Harvest	*pro arg cys ura*	*phe*(UF)	Total (percent)	*phe*(NF)	*ura*	*phe ura*	Others	Total	Total progeny
0	0	96	13	15	28 (2.8)	315	390	150	107	962	990
24	0	96	0	175	175 (88)	8	8	4	3	23	198
0	12	96	47	1	48 (28)	41	55	15	14	125	173
0	0	48	31	30	61 (66)	12	11	5	3	31	92
0	0	48	5	66	71 (79)	3	11	2	3	19	90
						379	475	176	130	1160	

* χ^2_{12} for the frequencies inside the box = 6.22; P~0.90.

frequencies of the two presumed asexual classes were caused to vary independently of one another and of the recombinant classes, within wide limits. Table 3 lists the frequencies of six classes of progeny in crosses of strain 749 *argA1 proA1 uraA1 cysD18* NF with strain 1098 *pheA1* UF; these six classes are the two presumed asexual (that is parental) phenotypes, the NF *phe* class, the two commonest recombinant classes (*ura* and *phe ura*), and a class containing all the rarer recombinant phenotypes. When both parents were inoculated at zero time and the cross was harvested after the normal period (96 hrs) of incubation (top line of Table 3), both presumed asexual classes were rare (less than 1.5% each). By inoculating one parent at zero time and later spreading a spore suspension of the second parent over the growing culture of the first, then harvesting the cross at the usual time, asexual progeny occurred at high frequency: with suitable time lags, one or the other parent could be caused to predominate (second and third lines of Table 3). Another way of increasing the asexual contribution to the progeny was to inoculate both parents at zero time and to harvest after only 48 hrs of incubation, when the culture consisted almost entirely of substrate mycelium (last two lines of Table 3); in one experiment the two asexual classes happened to be equally frequent, while one greatly predominated in the other. Under all these conditions, the frequency of the NF *phe* class was proportional to those of the three recombinant classes (see the test of homogeneity in Table 3), supporting the conclusion that this class represents "recombinants" that, because of the particular pattern of crossing over in the zygotes, come to have a parental combination of markers.

Allowing for the existence of this class, the fertility of the cross we have been considering is even more extreme: only 24 of the 643 progeny (19 *arg his nic ura str leu cys* NF and 5 *phe* UF) were asexually produced, the remaining 96% being the products of sexual reproduction. This result is characteristic of crosses of UF with NF strains; the total progeny parental in respect both of standard markers and fertility ranged in frequency, in a series of crosses, from zero to about 14%, with an average of 3.7% (see later: Table 6). The upper limit is arbitrary; as we have seen, much higher values were obtained if one strain was allowed to grow for a period before the second was inoculated, or if the cross was harvested prematurely. It seems likely that, at least as a limiting case, 100% of the progeny of UF by NF crosses are sexually produced; in practice a few asexual progeny are usually encountered, and these are perhaps explained by factors such as local imperfections of mixing of the two inocula, or by a chance lag in germination on the part of one strain.

Nature of zygote genomes in UF × NF crosses: The remaining characteristics of recombination in UF × NF crosses concern the distribution of recombinant classes within the sexually produced progeny. The percentage allele ratios within this group of progeny for the cross of 1105 NF with 1098 UF are the unbracketed numbers in Figure 5. We see that, in the upper half of the map, the frequencies of alleles contributed by the NF parent approach zero in a clockwise direction, and rise well above 50% (perhaps tending towards 100%) in an anticlockwise direction; the opposite result is found in the lower half of the map. This result

appears to indicate that all sexual progeny produced in a UF by NF cross derive a part of their genome near the 9 o'clock position from the NF parent and a part diametrically opposite from the UF parent. In order to account for this finding it is proposed that all effective zygotes (that is zygotes that give rise to viable progeny) in such crosses receive a chromosome fragment from the NF parent, and that all such fragments must include a point or region (the 'required region') between *uraA* and *nicA*, which is incorporated into 100% of the progeny. In other words the corresponding region of the UF genome is invariably *excluded* from the progeny. Conceivably it is excluded also from the zygotes so that they contain two incomplete genomes: a usually short fragment from the NF parent, and an almost complete genome from the UF parent. Alternatively, the UF parent would contribute a complete genome to the zygotes and a post-zygotic event would lead to inheritance of the required region of the NF genome by all progeny.

The fall in the frequencies of alleles inherited from the NF parent, with increasing distance from the required region, would reflect the operation of two factors: (a) the increased likelihood of crossing over with increased distance, leading to incorporation of UF markers instead of NF markers into recombinants (this factor alone would allow the frequencies of NF markers to fall to 50% but not below); and (b) the decreased chance of the fragment being long enough to include NF markers further from the required region (this factor would cause the frequencies of NF markers to approach a low value, determined by the average fragment length).

On this hypothesis, genomes capable of recovery in viable segregants from these zygotes would arise only when *odd-numbered* crossovers occurred on *both* sides of the required region; that is in the upper and lower halves of the map. In Table 4, the sexual progeny are listed according to the map intervals (Figure 5) in which crossing over would occur to produce them; the region between *uraA* and *nicA* is divided into two intervals, *d* and *e*, on either side of the required region. We see that 603 out of the 619 sexual progeny are explicable by the simplest patterns of crossing over: one exchange in the upper and one in the lower half of the map. The remaining 16 (only 2.6% of the sexual progeny) require three crossovers in one half and one in the other; none requires a more complex set of exchanges. Thus the hypothesis is adequate to account for the pattern of recombination observed.

Previous studies summarized by HOPWOOD (1967) have suggested that the chromosome fragments in the zygotes arising in crosses of two NF strains have ends defined by approximately random breakage points in the circular linkage map, rather than by breakage at a small number of fixed positions. The data from the cross under discussion indicate that, in a UF × NF cross, the ends of the chromosome fragments contributed by the NF parent to the zygotes are likewise not fixed, although their average mid-point is defined by the required region. In Table 5, the recombination percentage in each nonterminal interval, that is each interval bounded by two segregating loci, in this cross is divided by the standard map length of the interval, to give the frequency of recombination per unit length. This frequency becomes very low in the intervals farthest from the required

TABLE 4

Segregant classes from the cross depicted in Figure 5

Lower region		Single crossover classes: Upper region, genotype* and crossovers†				
Genotype*	Crossovers†	.... d	*nic* c	*nic, his* b	*nic, his, arg* a	Totals
phe	e	57	57	1	1	116
ura, phe	f	11	42	0	0	53
ura	g	131	281	8	3	423
ura, str	h	3	8	0	0	11
Totals		202	388	9	4	603 (93.8%)

Multiple crossover classes

Genotype*	Crossovers†	Number observed
nic	c; e, f, g	1
nic, str	c; e, f, h	1
....	d; e, f, g	2
ura, leu	d; g, h, i	1
arg, nic, ura	a, b, c; g	1
arg, nic, ura, str	a, b, c; h	1
his, ura	b, c, d; g	1
Total		16 (2.5%)

Parental classes

arg his, nic, ura, str, leu, cys	19
phe (ultrafertile)	5
Total	24 (3.7%)

* Wild-type alleles omitted.
† In the intervals in Figure 5.

region in both clockwise and anti-clockwise directions (intervals *b* and *h*). The simplest explanation of this finding is that the ends of the fragment are not fixed; thus distant intervals are duplicated in a much smaller proportion of zygotes than intervals closer to the required region, and hence the likelihood of crossing over in these distant intervals is proportionately reduced. (The tendency for recombination per unit length to be higher in intermediately placed intervals than in

TABLE 5

Recombination in each map interval in the cross depicted in Figure 5

Interval	Standard map length (percent recombination)	Percent recombination in this cross	Percent recombination per unit length
b	11	1.94	0.18
c	31	64.8	2.09
d	19	33.3	1.75
e	17	20.7	1.22
f	11	10.5	0.96
g	20	72.2	3.61
h	18	2.26	0.13

intervals adjacent to the required region, which is apparent in Table 5 (values for intervals *c* and *g* higher than those for *d*, *e* and *f*) is reproducible and so far lacks a unique explanation.)

Comparison of different NF and UF strains: Several different NF strains, arbitrarily chosen from the HOPWOOD stock culture collection, have been crossed with the same UF strain, 1098. The data for eight NF strains, bearing between them a series of 13 markers, are summarized in the first eight lines of Table 6. The NF markers are listed along the top of the table in the map order obtained by opening the linkage map in the 3 o'clock position and proceeding in a clockwise direction. The percentage frequency of each marker among the total sexual progeny (that is excluding the asexual classes as defined previously) is remarkably constant from cross to cross, indicating that all eight NF strains behaved in an equivalent fashion; all must have provided a similar population of chromosome fragments to the effective zygotes. (These same strains, of course, contribute a quite different population of fragments to the effective zygotes when crossed with other NF strains.)

Also included in Table 6 are the results of crossing five other UF strains, 1117, 1120, 1121, 1122 and 1125 derived from strain 12, with the same NF strain, 923. The marker frequencies in these crosses resemble those in the crosses involving strain 1098 UF, suggesting that all UF strains so far isolated from strain 12 have the same characteristics.

The average percentage NF marker frequencies derived from Table 6 are inserted approximately to scale in Figure 6.

Fertility in mixed cultures of different age: Table 7 summarises experiments investigating the effect of harvesting mixed cultures of UF and NF strains after only two days' incubation, instead of the usual four, when they consisted almost entirely of substrate mycelium. The experiment was done three times, with two different strain combinations. In each experiment, the cross harvested at the mycelial stage contained a much higher frequency of asexual progeny (66% or more) than the cross allowed to grow to the stage of sporulation, by which time only 4% or less of the total plating units developed into colonies manifesting asexually-produced phenotypes.

Phenotype of UF strains in pure culture: The peculiarities of UF strains described above are manifested in mixed cultures with NF strains. So far no phenotypically observable characteristics distinguish the UF strains in pure culture; they have not been found to differ in such things as growth rate, capacity to sporulate or in any other aspect of colonial morphology from the NF strain which gave rise to them.

DISCUSSION

Non-selective analysis of recombination: In all the experimental genetic systems that have been exploited in bacteria, whether based on genetic transfer by cellular conjugation, by transduction, or by transformation, the efficiency of recombination has been comparatively low. This has meant that recombinants have had to be *selected* from populations consisting predominantly of asexually

TABLE 6

Features of the progeny of crosses involving UF strains

Parent strains		Percent sexual segregants carrying each marker from the NF parent												Number of sexual progeny examined	Percent asexual progeny		
NF	UF	*tps-30*	*cysD*	*leuB*	*strA*	*metB*	*adeA*	*pheA+*	*uraA*	*nicA*	*cysA*	*proA*	*hisA*	*argA/C*		NF	UF	Total
358	1098	..	..	..	1.8	13	39	50	..	..	..	..	3.0	..	168	1.7	0	1.7
749	1098	..	0	..	..	..	..	49	64	..	..	8.5	..	0.6	962	1.3	1.5	2.8
791	1098	..	0	0	2.5	..	..	53	..	..	..	8.7	..	0.6	161	2.7	10.8	13.5
923	1098	..	0	..	..	..	..	64	78	..	..	..	0.6	..	176	1.7	3.4	5.1
928	1098	0	..	..	..	..	..	84	88	73	13	..	0.9	..	212	0	0.5	0.5
933	1098	..	..	..	..	..	31	49	66	..	15	..	0.4	0	255	0	0.4	0.4
949	1098	0	..	..	..	..	..	66	80	67	13	5.2	..	1.9	212	3.9	3.1	7.0
1105	1098	..	0	0.2	2.1	..	..	73	79	67	..	..	2.3	1.0	619	3.0	0.7	3.7
923	1117	..	0	..	..	..	..	53	72	..	..	..	0	..	179	0	2.7	2.7
923	1120	..	0	..	..	..	..	73	84	..	..	..	1.2	..	82	0	7.9	7.9
923	1121	..	1.2	..	..	..	..	59	69	..	..	..	1.2	..	85	0	2.3	2.3
923	1122	..	0	..	..	..	..	57	71	..	..	..	0	..	86	0	1.1	1.1
923	1125	..	0	..	..	..	..	74	79	..	..	..	2.1	..	95	0	0	0
Average		(0)	0.04 (0.14)*	0.13 (0.36)*	2.1	13	34	60	73	68	14	7.9	1.4	0.77	3292	1.6	2.1	3.7

* These estimates were obtained by *selecting* 187 *str his*+ progeny in a cross 1105 × 1098, classifying them in respect of *leuB* and *cysD*, and relating the frequencies of these markers to 2.1% for *strA*.

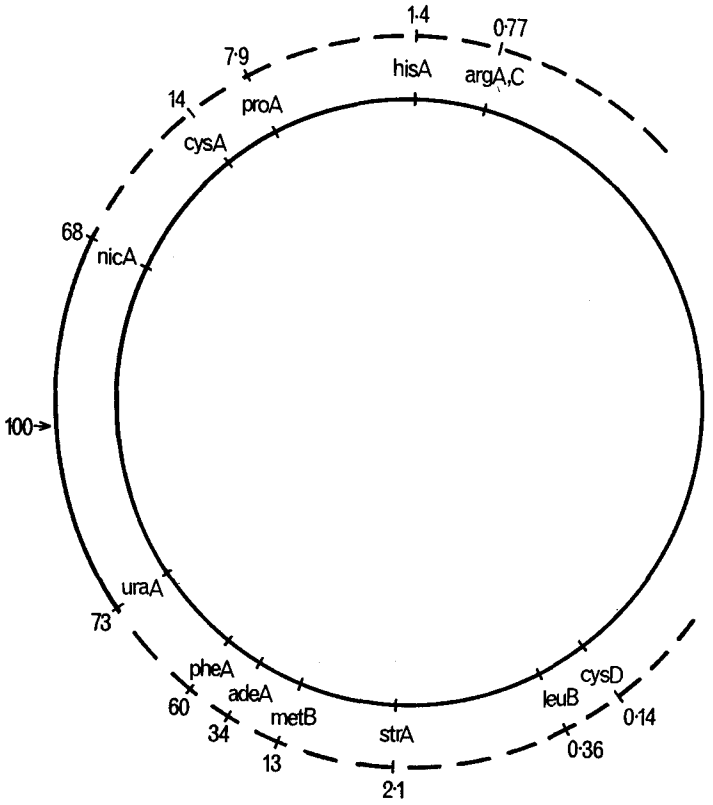

FIGURE 6.—Diagrammatic representation of the effective zygotes in UF × NF crosses according to the proposed model; each contains a complete circular chromosome from the UF parent (inner circle) and a chromosome fragment from the NF parent (outer arc), always including the 'required region' (arrow) between *uraA* and *nicA* and extending for varying distances on either side of this region (dotted lines). Numbers are the percentage frequencies of NF markers among the sexual progeny (from the pooled data in Table 6). (Note that an alternative interpretation, in which the zygotes lack a region of the UF genome corresponding to the required region of the NF genome is not excluded: see text.)

TABLE 7

Effect of age of mixed culture on proportions of asexual and sexual progeny

Experiment number	Age of mixed culture (days)	Percent asexual progeny			Percent sexual progeny	Total progeny examined
		NF parental	UF parental	Total		
I*	2	34	32	66	34	92
	4	2	0	2	98	177
II*	2	5.5	73	79	21	90
	4	0	1	1	99	193
III†	2	1	84	85	15	93
	4	2	2	4	96	92

* NF parent: 749; UF parent: 1098.
† NF parent: 1105; UF parent: 1098.

produced organisms by culturing them on media on which one parent (in the case of transduction or transformation) or both (in the case of conjugation) could not grow. On such selective media, only certain classes of recombinants could be recovered. An exception to this state of affairs was provided by the technically exacting pedigree analyses of LEDERBERG (1957) and ANDERSON (1958), in which single zygotes of *Escherichia coli* K12 were recognised microscopically and the products of their first few divisions in a non-selective medium were isolated by micromanipulator, cultured separately, and characterised in respect of parental markers.

The genetic system involving ultrafertile (UF) strains of *S. coelicolor* described in the present paper is operationally unique in bacterial genetics because, in a mixed culture of a UF strain with a strain of normal fertility (NF), asexual reproduction is virtually abolished. Consequently the products of the cross arising on *nonselective* medium are, almost without exception, sexually-produced; among these progeny the frequencies of all classes of segregants can be determined, and the yield of information is correspondingly increased.

The nature of the UF character: The UF phenotype consists of a syndrome of characteristics manifested in mixed cultures of UF with NF strains. The components of this phenotype are: polarisation of the (effective) zygotes so that they invariably derive a chromosome fragment from the NF parent and a complete, or almost complete, chromosome from the UF parent; the obligate inheritance of a certain "required region" of the NF genome by all haploid progeny descended from these zygotes (zygotes that lack this region either do not arise or leave no haploid descendants); and the almost total suppression of asexual reproduction by both parental strains.

It is premature to speculate at length on the mechanisms determining these characteristics in view of our ignorance of the normal mode of chromosomal transfer and zygote formation in *S. coelicolor*. We can, however, predict that polarised zygote formation in UF by NF crosses will be experimentally suited to investigating these phenomena more effectively than hitherto.

If the difference between UF and NF strains is chromosomally determined, the responsible site on the chromosome would most simply be located between *uraA* and *nicA* in the region of the UF genome that is excluded from the progeny; hence all progeny, as observed, are NF. There is nothing to indicate the nature of the change at this site on the UF genome, whether a mutation, the integration of an episome, or some other event. It may be significant that this site lies in the middle of one of the "silent regions" of the *S. coelicolor* map (HOPWOOD 1966), that is regions devoid of known genes. We must, however, remember the absence of direct evidence for a chromosomal location of the factor that determines UF behaviour. The finding that (almost) all progeny of mixed cultures of UF and NF strains are NF is equally compatible with the notion that NF strains harbour cytoplasmic factors, missing or inactive in UF strains, which come to be present in all sexually produced progeny because of a mixing of the cytoplasms of the two parents. The evidence tends to exclude an infectious conversion of UF to NF strains in mixed culture, independent of the recombination of chromosomal markers, because the frequency of the NF class bearing the chromosomal markers

of the UF parent (which would be the converted class) is proportional to the frequencies of the various classes manifesting recombination of chromosomal markers under a wide range of conditions (Table 3). This does not, however, exclude the possibility that a single event, like conjugation, leads to two independent processes, conversion of UF to NF cytoplasm and recombination of chromosomal markers, each with efficiencies approaching 100%.

The experiment summarised in Table 7 helps to define the problem of suppression of asexual reproduction in these crosses. In the substrate mycelial mat arising from mixed growth of NF and UF inocula, genomes of both parental types evidently reproduce themselves effectively and can be recovered by harvesting mycelial fragments. On the other hand, the spores arising in the aerial branches produced on this mixed substrate mycelium almost all arise by sexual interaction between the two parents. Conceivably recombination is obligatory at the initiation of the aerial phase of the mycelium, so that only aerial branches containing recombinant genomes are able to proceed to sporulation. Such a possible association between recombination and a morphogenetic event promises to be one of the interesting implications of the novel fertility variants described in this paper.

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SUMMARY

The isolation of "ultrafertile" (UF) variants of *S. coelicolor* A3(2) is described. These strains are so fertile that the spores produced on a mixed culture of a UF strain with a strain of normal fertility (NF) are almost exclusively sexually produced. Such crosses are "polarised," in that the UF strain contributes a complete (or almost complete) genome to all zygotes, and the NF strain a chromosome fragment. All haploid progeny arising from these zygotes derive a region of their genome, between *uraA* and *nicA*, from the NF parent.

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