Genetic Control of Fertility in *Streptomyces coelicolor* A3(2): the IF Fertility Type

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(Accepted for publication 28 September 1970)

SUMMARY

Wild-type *Streptomyces coelicolor* A3(2), and many mutant and recombinant derivatives of it, are of the IF (Initial Fertility) type. At an early step in the production of recombinant strains from some of the first derivatives of A3(2), a variant fertility type arose (NF: Normal Fertility), and subsequently IF and NF segregated within the pedigree of stock cultures. IF $\times$ IF crosses are about 100-fold less fertile than NF $\times$ NF or IF $\times$ NF crosses, but the clearest distinction between IF and NF is achieved by crossing with a strain of the previously described UF (Ultra-Fertile) type, when the difference in fertility approaches 1000-fold.

The IF strains give rise to UF strains with a high spontaneous frequency, and the frequency is increased by ultraviolet or X-irradiation but not appreciably by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. NF strains do not give rise to UF variants with a high frequency.

The difference between IF and NF is determined by a chromosomal locus near the 9 o'clock position on the linkage map. There is no evidence for the infectious conversion of one type of strain to the other in a mixed culture.

In crosses with an NF strain, both IF and UF strains contribute the whole chromosome to the effective merozygotes, and the NF strain contributes the fragment, which obligatorily includes the 9 o'clock region. However, whereas in UF $\times$ NF crosses there is obligate inheritance of the 9 o'clock region of the NF genome by all sexually produced progeny, this is not true of IF $\times$ NF crosses.

INTRODUCTION

All the strains of *Streptomyces coelicolor* used recently in genetic studies were derived from a single spore isolate; this wild-type is called A3(2) (Hopwood, 1959). Mixtures of the first mutant and recombinant derivatives of A3(2) were characterized by a rather low level of recombinant production, or 'fertility'. Later, with the introduction of further genetic markers into the stocks and the enlargement of the culture collection by the isolation of more and more recombinant descendants of the earlier strains, the range of fertility levels encountered in the crosses increased (Hopwood, 1958). However, no recognizable pattern of fertility variation was seen, and it fell to Sermonti & Casciano (1963) to group the strains into two classes, which they called R⁺ and R⁻ (R = recombination). R⁺ $\times$ R⁺ and R⁺ $\times$ R⁻ crosses yielded recombinants, whereas R⁻ $\times$ R⁻ mixtures were sterile, or nearly so, under their cultural conditions.

Although the R⁺ and R⁻ strains of Sermonti & Casciano (1963) derived directly, or after further recombinational steps, from the Hopwood stock culture collection, Hopwood (1967) found no sterile combinations amongst his strains. It was possible

that some of the strains had undergone a heritable change during their subculture for a few generations under the possibly different environmental conditions employed by Sermonti & Casciano.

In the hope of throwing further light on the nature of any variation in sexual capabilities that existed amongst the *Streptomyces coelicolor* strains, Hopwood, Harold, Vivian & Ferguson (1968, 1969) attempted to isolate strains of altered fertility after mutagenic treatments, rather than relying on spontaneous variations that might arise by chance in the stock culture collection. They were rewarded by the isolation of a class of 'Ultra-Fertile' (UF) variants displaying remarkable properties when crossed with each of eight strains chosen at random from the stock culture collection. Since all eight of these strains behaved identically, they, and the strain from which the UF variants had been isolated, which had likewise been chosen at random, were regarded as belonging to the same fertility type, called NF (Normal Fertility). The characteristics of NF $\times$ UF crosses were described by Hopwood *et al.* (1969). Almost all the viable spores produced by the mixed culture were recombinant. Moreover, all recombinants were of NF fertility, and all inherited a particular region of the NF parental genome, corresponding to 9 o'clock on the linkage map. The NF parent was deduced to be a 'donor' in the sense that it obligatorily contributed a chromosome fragment to each merozygote, while the UF was a 'recipient', contributing its entire genome to all merozygotes. (Throughout this paper we shall refer to the constitution of merozygotes in particular crosses. It is understood that we are discussing those merozygotes effective in yielding progeny, since only these are at present open to study. It is possible that in certain cases other classes of zygote arise, but fail to yield viable progeny.)

The results described in the present paper arose from experiments to test the fertility of UF $\times$ UF crosses, which might have been expected to be sterile if UF strains were obligate recipients. They showed that the UF strains were powerful indicators of the sexual capability of other strains, and that the stock culture collection contains strains of at least two fertility types, NF and IF. The IF (Initial Fertility) type, which is that of the wild-type A3(2), is the subject of this paper. The relationship of our NF and IF types with the R⁺ and R⁻ strains of Sermonti & Casciano (1963) is discussed.

METHODS

General. Complete (CM) and minimal (MM) media and standard cultural techniques were those described by Hopwood (1967).

Strains. The strains, which are all mutational and recombinational derivatives of *Streptomyces coelicolor* A3(2) (Hopwood, 1959), are listed in Table 1. The linkage relations of their genetic markers are shown in Fig. 1.

Mutagenesis. Ultraviolet (u.v.) irradiation was carried out as described by Harold & Hopwood (1970). Treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was by the schedule of Delić, Hopwood & Friend (1970).

Testing of fertility by 'plate crossing'. This is an indirect selection procedure, originally described by Sermonti & Casciano (1963). For the isolation of UF variants it was performed as described by Hopwood *et al.* (1969). To test their fertility, existing strains were inoculated in defined areas of a 'master plate' and tested in the same way as presumptive UF variants (Hopwood *et al.* 1969), but this time against UF as well as NF tester strains.

Genetic analysis. Haploid recombinant selection was done in the way described by Hopwood (1967). Non-selective analysis was performed by the method of Hopwood *et al.* (1969).

Fig. 1. Linkage map showing the locations of markers referred to in the paper. Marker abbreviations have conventional meanings (Hopwood, 1967); *tps* = temperature-sensitive (Hopwood, 1966a).

RESULTS

Heterogeneity of the culture collection in respect of fertility

The recognition of the IF type arose from experiments to test the fertility (or sterility) of mixtures involving only UF strains. All the UF strains obtained by Hopwood *et al.* (1969) derived from a single strain (12) and all were therefore isogenic in respect of standard markers, carrying only the mutation *pheA1*. To select recombinants from UF × UF crosses we had to introduce other markers into one of the UF strains by two steps of mutagenesis:

12 IF —————> 1098 UF —————> 1143 UF —————> A 200 UF
pheA1 u.v.   *pheA1* NTG   *pheA1 str-5* NTG   *pheA1 str-5 ade-v10*.

The final strain (A 200) could now be crossed with strain 1098, and recombinants inheriting *ade*⁺ and *str* selected. A similar cross was made between strain A 200 and the progenitor of 1098, strain 12.

The results (Table 2) show that the UF × UF cross had a low but easily detected fertility. Unexpectedly strain 12, which had been assumed to be NF, was almost as infertile with strain A 200 as was strain 1098, whereas if 12 had been NF the fertility should have been about 1000-fold higher. (These low levels of recombination, only some ten times higher than the frequency of *ade*⁺ reverse mutations in this experiment, have subsequently been shown unequivocally to involve recombination rather than

Table 1. *Characteristics of strains*

Strain	Relevant phenotype													Fertility
	<i>tps-30</i>	<i>cysD18</i>	<i>strA1</i>	<i>pheA1</i>	<i>uraA1</i>	<i>nicA1</i>	<i>ade-v10</i>	<i>cysA15</i>	<i>proA1</i>	<i>hisA1</i>	<i>hisB12</i>	<i>hisC9</i>	<i>argA1</i>	
A3(2)*	+	+	S	+	+	+	+	+	+	+	+	+	+	IF
11	+	+	S	-	+	+	+	+	+	+	+	+	+	IF
12	+	+	S	-	+	+	+	+	+	+	+	+	+	IF
28	+	+	S	-	+	+	+	+	+	-	+	+	+	NF
29	+	+	S	+	+	+	+	+	+	-	+	+	+	NF
32	+	+	S	+	-	+	+	+	+	+	+	+	+	NF
104	+	+	R	+	-	+	+	+	+	+	+	+	+	IF
424	+	+	S	+	+	+	+	+	+	+	-	+	+	IF
749	+	-	S	+	-	+	+	+	-	+	+	+	-	NF
923	+	-	S	+	-	+	+	+	-	+	+	+	+	NF
949	-	+	S	+	-	-	+	+	-	+	+	+	-	NF
1098	+	+	S	-	+	+	+	+	+	+	+	+	+	UF
1100	+	+	S	-	+	+	+	+	-	+	+	+	-	NF
1143	+	+	R†	-	+	+	+	+	-	+	+	+	+	UF
1258	+	-	R	+	-	+	+	+	-	+	+	-	-	NF
1190	+	+	R	+	-	+	+	+	+	-	+	+	+	UF
A200	+	+	R†	-	+	+	+	+	+	+	+	+	+	UF
A317	+	+	R	+	+	+	+	+	+	+	+	+	+	NF
A332	+	+	S	-	+	+	+	+	+	-	+	+	+	NF

* The wild-type. † *str-5*, assumed to be allelic with *strA1*.

mutation in crosses in which non-selected markers were available.) Therefore strain 12 was of a fertility type quite distinct from NF (as well as UF). This fertility type was designated IF (Initial Fertility). The main criterion for distinguishing it from NF strains is that in a plate-test (Pl. 1) with a UF tester it gives few or no recombinants, whereas an NF strain gives a confluent patch of recombinants.

Table 2. *Frequency of selected recombinants in crosses of strains 12 and 1098 with a UF tester, strain A 200*

		Number of colonies on media selective for				
		Parentals			Recombinants or mutants <i>pheA1</i> <i>str-5</i>	Recombinants or mutants* (%)
Strains		Fertility	<i>pheA1</i>	<i>ade-v10</i> <i>pheA1</i> <i>str-5</i>		
Controls {	12	IF	1.24×10^8	—	0	$< 8 \times 10^{-7}$
	1098	UF	2.74×10^8	—	0	$< 3 \times 10^{-7}$
	A 200	UF	—	3.6×10^8	37	1.0×10^{-5}
Crosses {	12 $\times$ A 200	IF $\times$ UF	3.7×10^8	2.1×10^7	79	3.2×10^{-4}
	1098 $\times$ A 200	UF $\times$ UF	5.6×10^8	1.2×10^7	22	1.3×10^{-4}

* Per total parental colonies.

Diagnosis of fertility of some key strains within the descendants of the wild-type A 3(2), using the plate-test (Pl. 1) incorporating both NF and UF tester strains, revealed that the culture collection contained both NF and IF strains. Strain A 3(2) and all its mutational derivatives so far tested, together with a few recombinants, are of the IF type (Fig. 2). Unfortunately some of the strains in early parts of the pedigree, which were isolated some 15 years ago, have since died or been discarded and their fertility cannot be determined; nevertheless the origin of the NF character from IF has been traced to strain 4 or one of its immediate ancestors.

Segregation of fertility in IF $\times$ NF crosses

Crosses were made between suitably marked NF and IF parents and the progeny, either selected or non-selected, were characterized in respect of fertility. An example of a cross of strains 949 NF and 12 IF (Fig. 3), analysed non-selectively (Hopwood *et al.* 1969) is given in Tables 3 and 4. The fertility of all the progeny was tested against a UF tester, strain A 200, selecting *ade*⁺ and *str*.

In this analysis (Table 3), 13 % of the progeny were recombinants, the remainder being parental *in respect both of standard markers and of fertility*. The proportions of the two parental genotypes were equal within less than a factor 2, indicating a well-balanced mixture of the parents. There is clearly no evidence for the infectious conversion within this intimate mixture of one parental fertility type into the other.

Amongst the 132 recombinants there was also a clear classification of the IF/NF phenotypic difference. The segregation indicated a chromosomal location for the IF/NF determinants in the 9 o'clock region of the genome between *nicA* and *uraA*. The simplest evidence for this conclusion derives from three-point analysis of the data (Table 4). This location is compatible with the allele ratios at the various loci (Fig. 3);

the explanation of the particular pattern of allele ratios observed will be considered in the final section of the results.

Fig. 2. Interrelationships and fertility type of some mutant and recombinant derivatives of the wild-type A3(2). Numbers are stock collection numbers; strains without numbers were dead and unavailable for fertility testing. Lower case letters refer to genetic markers, as follows: a = *argA1*; ad = *adeA3*; h = *hisA1*; h₆ = *his-6*; h₁₂ = *hisB12*; m = *metA2*; p = *pheA1*; s = *strA1*; u = *uraA1*.

Production of UF from IF strains

Table 5 summarizes quantitative data on the spontaneous and induced production of UF variants from two well-characterized IF strains. An example of the test is shown in Pl. 2; the relative fertility of UF and IF colonies under the conditions of this test was such that UF variants could be identified rather objectively. The spontaneous frequency of UF variants within IF strains varied, not unexpectedly, between different clones, ranging in these experiments from 0.03 to 0.4 %. Ultraviolet and X-rays induced UF variants with frequencies of up to at least 1 to 2 %. There is evidence for an increase in UF frequency with u.v. dose (decreasing survival). On the other hand NTG had only a marginal effect on UF frequencies, even at survival levels at which u.v. and X-rays

were very effective, and under conditions of very effective general mutagenesis (Delić *et al.* 1970).

UF strains have also been obtained, with comparable frequencies, from IF strains 11, 13 and 14, in addition to 12 and 104; the interrelations of these strains are in Fig. 2. Table 6 (top two lines) shows the negative results of attempts to isolate UF variants from two strains (28, 29; Fig. 2) classified by the plate test as NF. We conclude that, if UF variants arise from NF strains, their induced frequency is at least 100-fold lower than from an IF strain.

Fig. 3. Representation of a cross between strains 12 IF (inner circle) and 949 NF (outer circle). Letters indicate map intervals. Numbers are the frequencies of each allele in a sample of non-selected recombinants (Table 3).

Also in Table 6 are the frequencies of UF production from the *uraA1* recombinant progeny of the NF $\times$ IF cross (Table 3). Three of these had been classified as NF by the plate test and six as IF. All six IF strains produced UF variants with frequencies, after u.v. treatment, within the previously determined range, but no UFs were obtained from the three NF recombinants. These results thus confirm the original fertility typing of progeny by the plate test. They also indicate that the IF character had segregated from an NF $\times$ IF cross unchanged in phenotype in regard to UF production.

Frequency of recombination in crosses homologous and heterologous in respect of fertility type

We made two sets of strains, one set carrying the marker *pheA1* and the other the markers *hisA1 uraA1 strA1*, each set consisting of IF, NF and UF types. The UF strains derived by mutation from the corresponding IF strains. However, because we have not been able to derive NF by mutation from IF or UF strains, the corresponding NF strains were derived by appropriate marker selections from crosses involving only

strains known to be NF. These sets of strains have allowed the levels of recombination between different fertility types to be studied systematically.

The experiment consisted of plating the crosses on three media, selecting respectively recombinants and the two parental phenotypes. To minimize the effects of an inconsistent parental imbalance in the crosses the results (Table 7) are expressed in terms of recombinant frequencies per *minority* parental phenotype. We see that the IF $\times$ IF, IF $\times$ UF and UF $\times$ UF crosses had a low, and similar, recombinant frequency, rather greater than one in 10^4 of the minority parent. In contrast, NF $\times$ NF and NF $\times$ IF crosses had

Table 3. *Non-selective analysis of a cross between strains 12 IF and 949 NF*

Fertility was tested against strain A 200. The marker arrangements, map intervals and allele ratios amongst the recombinants are in Fig. 3.

Genotype	Fertility	Crossover in interval	Number
<i>tps</i> + <i>ura nic cys pro</i> +	NF	a, h	1
+ + + + +	IF	b, c	7
+ + <i>ura</i> + + + +	IF	b, d	6
+ + <i>ura</i> + + + +	NF	b, e	4
+ + <i>ura nic</i> + + +	NF	b, f	21
+ + <i>ura nic cys</i> + +	NF	b, g	18
+ + <i>ura nic cys pro</i> +	NF	b, h	45
+ + <i>ura nic cys pro arg</i>	NF	b, i	17
+ <i>phe ura nic</i> + + +	NF	c, f	1
+ <i>phe ura nic cys</i> + +	NF	c, g	1
+ <i>phe ura nic cys pro</i> +	NF	c, h	1
+ <i>phe ura nic cys pro arg</i>	NF	c, i	1
+ <i>phe</i> + <i>nic cys pro</i> +	NF	d, h	1
+ <i>phe</i> + <i>nic cys pro arg</i>	NF	d, i	1
+ <i>phe</i> + <i>nic</i> + + +	IF	e, f	1
+ <i>phe</i> + + + <i>pro</i> +	IF	g, h	1
+ <i>phe</i> + + + + <i>arg</i>	IF	h, i	2
<i>tps</i> + <i>ura nic</i> + <i>pro arg</i>	NF	a, b, h, i	1
+ + + <i>nic cys</i> + +	IF	b, c, e, g	1
+ + <i>ura</i> + <i>cys pro</i> +	NF	b, e, f, h	1
Total recombinants			132 (13%)
<i>tps</i> + <i>ura nic cys pro arg</i>	NF	Parental	359 (34%)
+ <i>phe</i> + + + + +	IF	Parental	564 (53%)
Total colonies			1055

Table 4. *Ordering of IF/NF between nicA and uraA*

Data from Table 3. The marker arrangements, map intervals and allele ratios amongst the recombinants are in Fig. 3. Crossovers in intervals outside d and e are ignored.

Marker combination	Crossover in intervals	Number
<i>uraA1</i> NF <i>nicA1</i>	— } No crossover	107
+ IF +		10
<i>uraA1</i> IF +	d } Single crossover	6
+ NF <i>nicA1</i>		2
<i>uraA1</i> NF +	e } Single crossover	5
+ IF <i>nicA1</i>		2
<i>uraA1</i> IF <i>nicA1</i>	d, e } Double crossover	0
+ NF +		0

Table 5. Isolation of UF variants from two IF strains after treatment with ultraviolet light (u.v.), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) or X-rays

The test strain was 923 for strain 12 and 1100 for strain 104.

Strain	Treatment	Survival (%)	Number of colonies examined		% UF	
			Total	UF	Total	Induced*
12†	Control	100	3600	1	0.03	—
	u.v.	3	5250	18	0.34	0.31
12	Control	100	5057	3	0.06	—
	u.v.	3	3682	12	0.33	0.27
104	Control	100	5255	13	0.25	—
	u.v.	0.1	2918	40	1.37	1.12
104	Control	100	5309	22	0.41	—
	u.v.	0.3	1219	18	1.48	1.07
12†	Control	100	3800	1	0.03	—
	NTG	8	2550	3	0.11	0.08
104	Control	100	1608	2	0.12	—
	NTG	5	1483	1	0.07	0.05
104	Control	100	1685	1	0.06	—
	NTG	6	1344	1	0.07	0.01
104	Control	100	988	1	0.10	—
	NTG	3	2850	3	0.10	0
104	Control	100	2401	0	< 0.04	—
	NTG	2	2436	3	0.12	> 0.08
104‡	Control	100	2893	8	0.28	—
	X-rays	4	2763	72	2.61	2.33

* Total minus control; a genuine induction, rather than a selection, is indicated by the finding that the ultraviolet sensitivity of strains 104 IF and 1190 UF were the same (R. Baumann, personal communication).

† Data from Hopwood *et al.* (1969).

‡ Data kindly supplied by Mr T. Schupp.

Table 6. Attempts to isolate UF variants from NF strains, and segregation of ability to yield UF variants

The strains were irradiated with u.v. to the survival levels indicated.
The test strain was 749 for strain 28 and 1100 for the remaining strains.

Strain	Fertility	Survival (%)	Number of colonies examined		% UF
			Total	UF	
28	NF	2	11731	0	< 0.009
29	NF	0.5	4255	0	< 0.02
A 321*	NF	0.4	1458	0	< 0.07
A 323*	NF	6.8	2101	0	< 0.05
A 329*	NF	0.8	900	0	< 0.1
A 322*	IF	0.4	751	10	1.3
A 324*	IF	0.1	1392	32	2.3
A 326*	IF	0.2	4009	44	1.1
A 327*	IF	0.2	3773	42	1.1
A 328*	IF	0.3	3941	50	1.3
A 330*	IF	0.4	6523	62	1.0

* These strains are *uraA1* recombinants from a cross of strain 949 NF × 12 IF (Table 3).

frequencies between 2 and 17 %, the NF $\times$ IF crosses being rather more fertile than the NF $\times$ NF. This greater fertility of NF $\times$ IF was confirmed in crosses analysed non-selectively (Table 8: see next section), in which NF $\times$ IF crosses gave an average total recombinant frequency per total progeny some sixfold higher than a corresponding NF $\times$ NF cross. The determination of fertility by *selective* plating, as in Table 7, while clearly differentiating between grossly different fertility levels, gives only semiquantitative results, since the fertility indicated depends on the particular selection applied, in relation to the pattern of merozygote polarization and asymmetry (see next section); this is doubtless the explanation for the different recombinant frequencies in the two crosses involving NF and IF strains in Table 7.

Table 7. *Frequency of selected recombinants in crosses involving strains of various fertility types*

Strains				Fertility	Recombinant frequency*
<i>pheA1</i>		<i>hisA1 uraA1 strA1</i>			
A 332	$\times$	A 317		NF $\times$ NF	1.6×10^{-2}
A 332	$\times$	104		NF $\times$ IF	4.0×10^{-2}
12	$\times$	A 317		IF $\times$ NF	1.7×10^{-1}
12	$\times$	104		IF $\times$ IF	1.5×10^{-4}
1098	$\times$	104		UF $\times$ IF	3.9×10^{-4}
1098	$\times$	1190		UF $\times$ UF	2.9×10^{-4}

* Ratio of selected recombinants (*his*⁺ *str* or *ura*⁺ *str*) to minority parental type (*phe* or *his ura str*)

Merozygote polarization and asymmetry in crosses heterologous in respect of fertility

We shall use the terms polarization and asymmetry in the following senses. Polarization (Hopwood, 1967) refers to the roles of the two parents in merozygote formation, a polarized cross being one in which the chromosome fragment comes preferentially (or exclusively) from one particular parent and the complete chromosome from the other. Asymmetry refers to non-randomness of the chromosome fragments in the zygotes, such that they preferentially include some particular region or regions of the genome. The zygotes in an NF $\times$ UF cross (Hopwood *et al.* 1969) are polarized, since the chromosome fragment comes exclusively from the NF parent, and asymmetric, since the fragment always includes the 9 o'clock region of the genome.

The allele frequencies at different loci in the non-selective analysis of the NF $\times$ IF cross (Fig. 3) were very unequal: in particular there was a shortage of NF markers at loci in the right-hand half of the map (*argA1* and *tps-30*) and an excess of NF markers in the left-hand half. This result suggested a polarization and asymmetry of the merozygotes, possibly resembling that in NF $\times$ UF crosses (Hopwood *et al.* 1969). In order to investigate this merozygote polarization and asymmetry more specifically, two sets of crosses were made, each involving strains isogenic for standard markers and having a common NF parent, the other parent being in turn of the three fertility types, NF, IF and UF. The first set of crosses was analysed non-selectively (Table 8) and the second selectively (Fig. 4).

In Table 8, data for several replicates of the NF $\times$ NF and NF $\times$ IF crosses indicate the relatively small effects of clonal fluctuation between crosses. The data are presented in summary form, with omission of the many individual recombinant classes from which the frequencies of the various markers of the common NF parent (strain 749) were

Table 8. *Non-selective analysis of NF × NF, IF × NF and UF × NF crosses*
pheAI strains of different fertility (IF, NF or UF) were crossed with NF strain 749 *argAI cysDI8 proAI uraAI*.

<i>pheAI</i> parent		Numbers of progeny					Recombinants (%)	Allele frequencies of strain 749 amongst recombinants				
Stock no.	Fertility	<i>pheAI</i>	Parental		Recombinants†	Total		<i>cysDI8</i>	<i>pheAI</i> ⁺	<i>uraAI</i>	<i>proAI</i>	<i>argAI</i>
A 332	NF	282	443	4	729	0.5	2	2	3	1	3	
		73	304	3	380	0.8	3	2	1	2	3	
		66	296	9	371	2.4	2	8	8	3	3	
		67	290	6	363	1.7	3	6	4	4	2	
		33	339	5	377	1.3	2	4	4	2	3	
I 1	IF	521	1672	27	2220	1.2	44%	81%	74%	44%	52%	
		275	34	60	369	16.2	2	55	38	6	2	
		212	149	35	396	8.7	1	32	30	18	4	
		63	18	6	87	6.9	0	5	5	3	1	
		164	59	20	243	8.2	2	15	10	5	2	
I 2	IF	352	14	12	378	3.2	2	11	10	6	3	
		226	178	9	413	2.2	2	8	8	4	2	
		1292	452	142	1886	7.5	5%	89%	71%	30%	10%	
		330	13	647	990	67	< 0.2%	73%	95%	13%	1%	
		1098*	UF									

* Data recalculated from Hopwood *et al.* (1969).

† Includes only recombinants in respect of standard markers; in the case of the UF × NF cross, the large class of progeny parental in respect of standard markers but recombinant in respect of fertility have been tabulated under 'parentals'.

calculated. Considering these frequencies first for the NF $\times$ NF cross, we see that they are not markedly different from one another or from 50 %. This agrees with previous conclusions (Hopwood, 1967, 1969) that in normal crosses, now known to have involved NF strains, the mixture of merozygotes is non-polarized and symmetrical. (The moderate excess of the marker *pheA1*⁺, and to a lesser extent of the nearby marker *uraA1*, in these data probably reflects a selective disadvantage of the allele *pheA1* compared with *pheA1*⁺, which is consistently observed.)

In marked contrast to these data for the NF $\times$ NF cross, those for the NF $\times$ IF cross (Table 8) show a very significant shortage of the NF markers *cysD18* and *argA1* reminiscent of, although less extreme than, the comparable figures for the NF $\times$ UF cross (Table 8). As discussed in detail by Hopwood *et al.* (1969), the NF $\times$ UF data are compatible with the hypothesis that the merozygotes are completely polarized, and also asymmetrical in that all NF fragments include the 9 o'clock region. Moreover, in such NF $\times$ UF crosses this region of the NF genome is obligatorily inherited by the progeny derived from the merozygotes. The low frequencies amongst the recombinants of such markers as *cysD18* and *argA1*, far from 9 o'clock, follow from their poor chances of being carried on the NF fragment. The data in Table 8 suggest that the merozygotes in an NF $\times$ IF cross resemble those in an NF $\times$ UF cross. However, the data of these crosses and those analysed selectively (Fig. 4) indicate that the resemblance is not complete.

In Fig. 4 the data are again reduced to the allele frequencies at each locus. Considering the NF $\times$ NF cross (Fig. 4*a*) as a basis of comparison for the heterologous crosses, we see the effects of merozygote symmetry expressed in the allele ratios at the four non-selected loci, which are considered in pairs, each pair flanking one of the selected loci. The frequencies of crossing-over in the intervals on either side of these selected loci, a and f for *hisC* and c and d for *strA*, are reflected by the frequencies of the alleles *argA1* and *proA1*, and *cysD18*⁺ and *uraA1*⁺ respectively. Interval a is known (Hopwood, 1966*a, b*) to be only some two-thirds of the length of interval f, and this difference is reflected in the frequencies of *argA1* and *proA1*; on the other hand the lengths of intervals c and d are not very different (Hopwood, 1966*a, b*), and the allele frequencies of *cysD18*⁺ and *uraA1*⁺ are in agreement. Thus there is no evidence for asymmetry in the zygotes, although there is a slight polarization of this particular cross in favour of strain 1258 (represented by the outer circle in Fig. 4*a*) contributing the complete genome; this is indicated by the frequencies of crossing-over per unit length in intervals a and f being somewhat higher than in c and d.

The data for the NF $\times$ IF cross (Fig. 4*b*) differ strikingly from those of the NF $\times$ NF cross in respect of the non-selected markers in the right-hand half of the map. There is a conspicuous shortage of crossing-over in interval a (a low frequency of *argA1*) and a large excess of crossing-over in interval c (a high frequency of *cysD18*⁺). From this result we deduce that the effective zygotes in this NF $\times$ IF cross have the constitution shown in Fig. 4*d*, with the 9 o'clock region of the NF fragment obligatorily represented and the duplicated region extending, in different zygotes, for varying distances on either side of this region. Owing to the pattern of selection in this cross, the NF fragment in all zygotes contributing selectable progeny had to include the selected marker *strA1*; crossing-over in interval c was very frequent since the strand carrying *strA1* would very often not have extended beyond the locus *cysD* into interval b. The low likelihood of crossing-over in interval a reflects two factors: many zygotes will have

Fig. 4. Selective analysis of (a) NF × NF, (b) IF × NF and (c) UF × NF crosses. Strains carrying the markers *pheA1* and of varying fertility (NF = A 332, IF = 12, UF = 1098) were crossed with a common NF strain (1258, *argA1 hisC9 proA1 uraA1 strA1 cysD18*). Recombinants inheriting the alleles *hisC9*⁺ and *strA1* (indicated by triangles) were selected and classified in respect of the non-selected markers. Numbers are the percentage frequencies of each non-selected allele among the selected recombinants (sample sizes 191–199); only two markers flanking each selected locus have been considered for simplicity. The asterisk denotes the 'required region' of the NF genome inherited by all recombinants in a UF × NF cross (Hopwood *et al.* 1969).

(d) Postulated constitution of merozygotes in an IF × NF cross. The IF strain contributes the complete genome (inner circle) and the NF strain contributes a fragment (outer arc) obligatorily including the 9 o'clock region and extending for varying distances on either side (broken lines). Triangles indicate the alleles selected in the cross in (b); thus in all zygotes leading to selected recombinants in this cross the NF fragment must have included the region between 9 o'clock and *str*.

contained fragments that did not extend into this interval; and from those that did, *argA1* could have been inherited by recombinant progeny only when two non-selected crossovers occurred, one in interval a and one in b.

Fig. 4 shows very clearly the difference between $\text{NF} \times \text{IF}$ (Fig. 4*b*) and $\text{NF} \times \text{UF}$ (Fig. 4*c*) crosses. First, the low frequency of the allele *uraA1*⁺ in the UF cross follows from the obligate inheritance of the 9 o'clock region (the 'required' region: Hopwood *et al.* 1969) of the NF fragment by all the progeny; thus any segregant inheriting *uraA1*⁺ must have arisen by two crossovers, one in interval d and one in that region of e between the *uraA* locus and the 'required' region. The much higher frequency of *uraA1*⁺ in the $\text{NF} \times \text{IF}$ cross tells us that the 'required' region is not obligatorily inherited by the progeny, although it is obligate in the zygotes, in such a cross. Second, the frequencies of the alleles *argA1* and *cysD18* are even lower in the $\text{NF} \times \text{UF}$ than in the $\text{NF} \times \text{IF}$ cross. This finding might indicate the average NF fragment length in $\text{NF} \times \text{UF}$ crosses to be shorter than in $\text{NF} \times \text{IF}$ crosses.

DISCUSSION

The results described in this paper provide an explanation for some hitherto puzzling observations made early in the study of the genetics of *Streptomyces coelicolor* A3(2). The earliest crosses (Hopwood, 1957, 1959) were characterized by the production of complementary recombinant genotypes with statistically equal frequencies. This finding indicated an equal contribution of genetic material by the two parents to the progeny, although not necessarily to each zygote (Hopwood, 1959). In later crosses, involving further recombinant descendants of the early strains, complementary genotypes in general had unequal frequencies (Hopwood, 1961; Hopwood & Sermoniti, 1962), and some crosses were also considerably more fertile than the early crosses (Hopwood, 1958).

It is now clear that this changed behaviour of the crosses reflected the origin of the variant fertility type, NF, from one of the IF descendants of the wild-type, and its inheritance by other strains in the culture collection (Fig. 2); some crosses were thus heterologous in respect of fertility ($\text{NF} \times \text{IF}$). Whereas $\text{IF} \times \text{IF}$ crosses, like the $\text{NF} \times \text{NF}$ crosses described in the present paper and previously (Hopwood, 1967), are characterized by random merozygote formation, each parent contributing markers to the zygotes with statistically equal frequencies, in $\text{NF} \times \text{IF}$ crosses the roles of the parents differ: the IF parent contributes the complete genome to all merozygotes effective in recombinant formation, and moreover the fragments contributed by the NF parent are non-random. The result is that markers of the IF parent tend to be inherited preferentially by the progeny, particularly at loci far from the 9 o'clock region of the genome, and of course complementary recombinant genotypes must therefore have unequal frequencies.

We have deliberately designated the fertility types in *Streptomyces coelicolor* A3(2) by terms (IF, NF, UF) that do not recall the plasmid-determined mating types of Gram-negative bacteria, such as F^+/F^- (Hayes, 1968), R^+/R^- (Meynell, Meynell & Datta, 1968) or $\text{Col}^+/\text{Col}^-$ (Nomura, 1967) in *Escherichia coli*, FP^+/FP^- in *Pseudomonas aeruginosa* (Holloway, 1969), or P^+/P^- in *Vibrio cholerae* (Bhaskharan, 1960) since, as we have seen in this and a previous paper (Hopwood *et al.* 1969), there appear to be no close analogies between *S. coelicolor* and these eubacteria in respect of the known fertility types. In particular we have so far implicated exclusively chromosomal,

rather than plasmid, determinants of fertility, with no evidence of infectious conversion of one fertility type to another, although an interaction between chromosomal alleles and one or more plasmids may of course remain to be recognized.

At least some of the strains designated R^- and R^+ by Sermonti & Casciano (1963) appear to correspond with our IF and NF types respectively. It is not possible to identify the derivation from our strains of all the cultures classified by Sermonti & Casciano (1963), but their strains numbered 8 R^- , 109 R^+ and 118 R^- must have been derived by subculture from the Hopwood stock cultures 424, 32 and 109 respectively. The derivations of these strains are in Fig. 3 and their fertility types are IF, NF and IF respectively. Moreover the patterns of marker inheritance observed in $R^+ \times R^-$ crosses (Sermonti & Casciano, 1963), which were difficult to interpret in the absence of the later acquired knowledge of the location of the markers on the circular linkage map (Hopwood, 1965, 1966*a, b*), seem compatible with those we observe in $NF \times IF$ crosses. The only important discrepancy appears to be the conclusion by Sermonti & Casciano (1963) of infectious conversion of an R^- strain to R^+ by mixed growth with an R^+ strain, on the basis of which an analogy was claimed between R^+ and R^- *Streptomyces coelicolor* and F^+ and F^- *Escherichia coli* strains (Spada-Sermonti & Sermonti, 1970). As we have shown in the present paper, there is no evidence for conversion of IF to NF in our mixed cultures (Table 3); instead there is a clear segregation of fertility attributable to a pair of chromosomal 'alleles'. The explanation of Sermonti & Casciano's result is conceivably that the R^+ strains emerging from their $R^+ \times R^-$ cross with the standard markers of the R^- parent were in fact recombinants which, because of the particular pattern of crossing-over, inherited a parental combination of standard markers. It was stated (Spada-Sermonti & Sermonti, 1970) that the frequency of recombinant production in the cross was only 1×10^{-4} per total progeny, but if the parental balance had been very unequal, with the R^- parent in the minority, the frequency of the 'converted' R^+ class could have been comparable with those of other, overtly recombinant, classes.

We feel that the R^+/R^- nomenclature for the NF/IF strains of *Streptomyces coelicolor* is inappropriate. Historically, NF arose from IF (Fig. 3), rather than the reverse, indicating no close analogy between NF and IF *S. coelicolor* and F^+ and F^- *Escherichia coli*. Furthermore, the designation R^- (recombination-less: Sermonti, 1969) for the IF strains could cause confusion with the very different *rec* $^-$ (recombinationless) mutations of *E. coli* (Clark & Margulies, 1965) and other microbes. In any case, $IF \times IF$ (and even $UF \times UF$) crosses are far from sterile, at least under our conditions: the early crosses must have involved exclusively such strains, and they allowed quite effective recombinant selection (Hopwood, 1959). The levels of fertility observed by Sermonti & Casciano (1963) in $R^+ \times R^-$ and $R^- \times R^-$ crosses were some 100-fold lower than those we observe in $NF \times IF$ and $IF \times IF$ crosses, perhaps because of a difference in media. Thus their $R^- \times R^-$ crosses yielded recombinants at barely detectable frequencies and were described as sterile. However, this has always been a relative term (Sermonti & Casciano, 1963; Sermonti, 1969).

In this paper we have merely defined in genetic terms some of the fertility variation in *Streptomyces coelicolor* A3(2). An understanding of the mechanisms involved in the change of one fertility type to another, in determining the polarization and asymmetry of crosses, the exclusion of the 9 o'clock region of the UF genome in $NF \times UF$ crosses (Hopwood *et al.* 1969), and the abolition of asexual reproduction in such crosses

(Hopwood *et al.* 1968) must await further work. It seems likely that the control of sexual reproduction in this morphologically complex bacterium, particularly in relation to morphological differentiation (Hopwood *et al.* 1969), may provide an insight into biological problems that do not arise in the much simpler eubacterial systems.

It is a pleasure to acknowledge the able assistance, during successive periods of this investigation, of Mrs Anne Rush and Mrs Helen Tovell. We should particularly like to thank Mrs Helen M. Wright (née Ferguson) for her skilled performance of the experiments in Table 8 and Fig. 4, and for the preparation of the specimens in Pl. 1. One of us (A. V.) gratefully acknowledges a post-doctoral stipend from Science Research Council grant number B/SR/4060.

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EXPLANATION OF PLATES

PLATE 1

Diagnosis of the fertility of NF, IF and UF strains by a plate test involving NF and UF tester strains. The top plate is a non-selective streaking of the strains to be tested. The middle two plates are non-selective 'plate crosses' prepared by replicating the top plate to 'lawns' of spores of the tester strains. The bottom two plates are selective for recombinants and were prepared by replicating the plate crosses to selective media.

PLATE 2

Isolation of UF variants from an IF strain. The top plate bears the survivors of u.v. irradiation of strain A 327 (see Table 6). The middle plate is a plate cross involving NF tester strain 1100. The bottom plate is selective for recombinants; five UF variants have given rise to dense patches of recombinants (arrows), whereas each IF colony produced only a few recombinants on a background of slight parental growth.