Chromosome Transfer in *Proteus mirabilis* Mediated by a Hybrid Plasmid

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SUMMARY

A previously-described fused plasmid, *P-lacR1drd19*, was found to mediate chromosomal transfer between cells of *Proteus mirabilis* strain PM5006; PM5006-*(P-lacR1drd19)* was usually the donor and various auxotrophs of PM5006 resistant to nalidixic acid and/or streptomycin were recipients. The donor was usually counterselected with nalidixic acid and/or high concentrations of streptomycin. Recombination experiments with single markers indicated a 40-fold variation in recombination frequencies for different markers. Mapping double-auxotrophic markers by their gradient of transmission confirmed this variation and placed each of two independent isolates of eight markers in a linkage group *his-ser-ura-pyrB-trp-cys-ade-ily*. Some donor markers did not register. Despite low recombination frequencies, interrupted mating experiments showed a polarity of early marker transfer. The segregation of unselected markers confirmed the order of some markers and showed that genetic material passed from the presumptive donor to the recipient. Recipients with two auxotrophic markers which could not be co-transduced by phage 5006M were converted to prototrophy by conjugation. The plasmid transferred to recipients at high frequency and all recombinants carried it. Recombinants could act as donors in further matings. Recombinants were fully susceptible to phage 5006M, unlike transductants of PM5006 by this phage. Direct involvement of the plasmid was indicated by drastically diminished recombination frequencies in crosses with recipients carrying *P-lac* as resident. *P-lac* had previously been shown to reduce the frequency of transfer of the hybrid plasmid to cells harbouring it. The histidine region was the first to register in recipients and recombined at the highest frequency of 5×10^{-6} /donor cell. Some temporary association of plasmid and perhaps only the histidine region of the chromosome is favoured as the mechanism of chromosomal transfer. This could explain why not all donor markers could be mapped. Transduction and transformation were excluded as the cause of results.

INTRODUCTION

Dienes (1946, 1947) likened the large bodies formed in the demarcation line between opposing swarms of *Proteus* to zygotes, but 'crossing or segregation' of properties was not observed. Stempen & Hutchinson (1951) observed fusion of *Proteus vulgaris* cells: a bud developed at the site of fusion and this grew into a large body which divided or ruptured. It could not be determined whether a hybrid phenotype resulted. Kvittingen (1953) maintained that swarms of *Proteus mirabilis* and *P. vulgaris* are sexual forms and obtained fermentation hybrids when different swarms were mixed on agar. This work has not been confirmed. Hutchinson & Medill (1954) and Hutchinson & Stempen (1954), with the use of various spontaneously arising markers, could not demonstrate recombination between strains of *P. mirabilis*, *P. vulgaris* or *P. morganii*. *Proteus* spp. can conjugate - they may receive and transfer a variety of plasmids both intra- and extragenetically (see Coetzee,

1972) – but it is generally accepted that chromosomal transfer has not been demonstrated in *Proteus*.

Certain R factors may mediate transfer of the *Escherichia coli* chromosome (Sugino & Hirota, 1962; Watanabe & Ogata, 1966; Cooke & Meynell, 1969; Meynell & Cooke, 1969; Moody & Runge, 1972; Moody & Hayes, 1972; Hirota, Fujii & Nishimura, 1966; Nishimura, Nishimura & Caro, 1973) and the chromosome of *Pseudomonas aeruginosa* has also been transferred by R factors (Stanisich & Holloway, 1971).

The plasmid P-lacR1drd19 investigated here, is a hybrid (Coetzee, 1974) produced by transduction of R1drd19 (Meynell & Datta, 1967) with vector phage PL25 (Coetzee, Smit & Prozesky, 1966) to Providence strain P29 carrying P-lac (Falkow, Wohlhieter, Citarella & Baron, 1964; Wohlhieter, Falkow, Citarella & Baron, 1964) as resident. The hybrid transmits to various *P. mirabilis*, Providence and *E. coli* strains at a frequency of about 10^{-1} /donor cell. Both P-lac (Falkow *et al.* 1964) and R1drd19 (Pearce & Meynell, 1968; Meynell & Ewins, 1973) can transfer the *E. coli* chromosome.

METHODS

Bacteria, plasmids and phages. These are listed in Table 1.

Media. Nutrient broth was Oxoid No. 2 code CM 67; nutrient agar was the same solidified with 1.2% (w/v) Difco agar. MacConkey agar was Difco. Minimal medium was that of Grabow & Smit (1967). This medium was supplemented with amino acids (20 µg/ml) to suit auxotrophic strains when necessary. Incubation temperature was 37 °C and media were brought to that temperature before inoculation.

Antibacterial drugs. The minimal medium contained streptomycin (7000 µg/ml) or nalidixic acid (50 µg/ml) or both drugs to counterselect donors in some mating experiments. Ampicillin and kanamycin (50 µg/ml) were added to MacConkey agar when necessary. Rifampin was used at 1000 µg/ml.

Phage absorption and transduction. Adsorption of phages to donor and recipient strains was determined by the method of Coetzee (1961). Transduction was performed by the methods of Coetzee, Datta, Hedges & Appelbaum (1973) except that the multiplicity of phage input was reduced to about 1.

Transfer of plasmids from Escherichia coli J62-1 to strains of PM5006. This was accomplished by the method of Coetzee *et al.* (1973) with the use of tetracycline to counterselect the *E. coli* strain.

Transfer of P-lacR1drd19 from strains of PM5006 to J62rif-r. This was done as above with contraselection of the donor with rifampin.

Bacterial conjugation. An overnight broth culture of donor was diluted 1:10 in broth and incubated for 2 h. The mating mixture contained 1 ml donor culture, 4.5 ml of an overnight broth culture of recipient and 4.5 ml broth. Controls lacked the donor or recipient and in some experiments neither culture carried the plasmid. In preliminary experiments 4 µg deoxyribonuclease I (Sigma)/ml were added at the time of mixing. The mixture was incubated for 2 h. Samples of the mating mixture and controls were then filtered through Millipore membranes (Sartorius, Göttingen, Germany), washed with a large excess of sterile warm saline, and the membranes then placed on appropriate minimal media to detect prototrophy and on selective MacConkey agar for determination of extrachromosomal plasmid transfer. Minimal media were incubated for 48 h and MacConkey media for 24 h before results were read. Other samples were also treated with 0.1 vol. chloroform and after evaporation of the chloroform the supernatants were titrated on PM5006 for phage detection.

Table 1. *Bacteria, plasmids and phage*

Bacteria	Properties	Reference(s)
PM5006	Recipient for generalized transducing phage 34.PM13. Restricts and modifies phage 34.PM13 T resistant. Cryptically lysogenic for generalized transducing phage 5006M. Susceptible to many <i>Proteus</i> bacteriophage tail-like bacteriocins but not bacteriocinogenic	Coetzee, De Klerk & Coetzee (1968); Coetzee & Smit (1970); Niemand (1971); Krizsanovich (1973); Coetzee (1974; unpublished)
PM5006(ϕ 5006M)	PM5006 lysogenized with phage 5006M	Coetzee (unpublished)
PM5006 <i>nal-r</i>	Nalidixic acid resistant mutant of PM5006	Coetzee (1974)
PM5006 <i>nal-r str-r</i>	Streptomycin resistant mutant of PM5006 <i>nal-r</i>	Coetzee (unpublished)
Auxotrophs of PM5006		
UPI	<i>trp-I</i>	Auxotrophs: <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine induced. Drug resistance: spontaneous selected mutants (Coetzee, unpublished)
UP2	<i>trp-I nal-r</i> mutant of UPI	
UP3	<i>his-I nal-r</i> mutant of PM5006 <i>nal-r</i>	
UP4	<i>pyrB1 nal-r</i> mutant of PM5006 <i>nal-r</i>	
UP5	<i>arg-I nal-r</i> mutant of PM5006 <i>nal-r</i>	
UP6	<i>his-I ser-2 nal-r</i> mutant of UP3	
UP7	<i>tyr-I str-r</i> mutant of PM5006 <i>str-r</i>	
UP8	<i>leu-I str-r</i> mutant of PM5006 <i>str-r</i>	
UP9	<i>pyrB1 trp-2 nal-r</i> mutant of UP4	
UPI0	<i>met-I nal-r str-r</i> mutant of PM5006 <i>nal-r str-r</i>	
UPI1	<i>ilv-I</i> mutant of PM5006 <i>nal-r</i>	
UPI2	<i>ade-I nal-r</i> mutant of PM5006 <i>nal-r</i>	
UPI3	<i>cys-I nal-r</i> mutant of PM5006 <i>nal-r</i>	
UPI4	<i>ura-I str-r</i> mutant of PM5006 <i>str-r</i>	
UPI5	<i>ser-I nal-r str-r</i> mutant of PM5006 <i>nal-r str-r</i>	
UPI6	<i>his-I nal-r str-r</i> mutant of UP3	
UPI7	<i>his-I pyrB2 nal-r str-r</i> mutant of UPI6	
UPI8	<i>his-I ser-2 nal-r str-r</i> mutant of UPI6	
UPI9	<i>his-I ser-2 ura-3 nal-r str-r</i> mutant of UPI8	
UP20	<i>his-I ura-2 nal-r str-r</i> mutant of UPI6	
UP21	<i>his-I trp-3 nal-r str-r</i> mutant of UPI6	
UP22	<i>his-I cys-2 nal-r str-r</i> mutant of UPI6	
UP23	<i>his-I ade-2 nal-r str-r</i> mutant of UPI6	
UP24	<i>his-I ilv-2 nal-r str-r</i> mutant of UPI6	
<i>Escherichia coli</i> K12		
J62-1	Nalidixic acid resistant mutant of J62, an F ⁻ <i>lac pro his trp</i> strain	Clowes & Hayes (1968); Coetzee, Datta, Hedges & Appelbaum (1973)
J62 <i>rif-r</i>	Spontaneous mutant of J62 resistant to 1000 μ g rifampin/ml	Coetzee (unpublished)
Plasmids and hosts		
PM5006(P- <i>lac</i>)	<i>lac</i> ⁺	Coetzee (1974)
PM5006(R1 <i>drd</i> 19)	ACKSSu	Coetzee (1974)
PM5006(P- <i>lac</i> R1 <i>drd</i> 19)*	ACKSSu <i>lac</i> ⁺ . Derived from P29 <i>str-r</i> (P- <i>lac</i> R1 <i>drd</i> 19)* by conjugal transmission of the fused plasmid to J62-1 and then to PM5006 with contraselection for J62-1 with T. Fully susceptible to phage 5006M	Coetzee (1974; unpublished)
J62-1(P- <i>lac</i>)	<i>lac</i> ⁺	Coetzee (unpublished)
J62-1(P- <i>lac</i> R1 <i>drd</i> 19)*	ACKSSu <i>lac</i> ⁺	Coetzee (unpublished)
Phage		
5006M	Serologically and morphologically identical to phage 34. Generalized transducing for PM13, PM5006. Converts hosts to non-adsorption of phages 34, 5006M	Coetzee & Sacks (1960); Krizsanovich (1973); Coetzee (unpublished)

A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphonamide; T, tetracycline.

* Superior line indicates transduced R factor.

Table 2. *Recombination experiments with three different donors*

Experiment no.	Recipient	Selected donor marker(s)	No. prototrophs/10 ⁸ donor cells*		
			PM5006- (P-lacR1drd19)	PM5006- (P-lac)	PM5006- (R1drd19)
1	UP3	<i>his-1</i>	511	0	0
2	UP15	<i>ser-1</i>	301	0	0
3	UP14	<i>ura-1</i>	219	0	0
4	UP4	<i>pyrB1</i>	111	0	0
5	UP2	<i>trp-1</i>	53	0	0
6	UP13	<i>cys-1</i>	30	0	0
7	UP12	<i>ade-1</i>	19	0	0
8	UP11	<i>ilv-1</i>	5	0	0
9	UP17	<i>his-1 pyrB2</i>	96	0	0
10	UP9	<i>pyrB1 trp-2</i>	70	0	0
11	UP6	<i>his-1 ser-2</i>	480	0	0
12	UP8	<i>leu-1</i>	0	0	0
13	UP5	<i>arg-1</i>	0	0	0
14	UP10	<i>met-1</i>	0	0	0
15	UP7	<i>tyr-1</i>	0	0	0
16	UP16	<i>his-1</i>	519	0	0

* Average of four experiments.

Mating mixtures and controls were constituted as described in text. One ml of the mixtures was filtered and the membrane placed on minimal medium containing streptomycin and/or nalidixic acid.

Recombination frequency was expressed with reference to the count of donor bacteria in the mating mixture at the time of mixing. To determine whether intact viable cells were necessary for recombinant formation and to further exclude possibilities of transduction and also transformation (Sugino & Hirota, 1962) either of the parent cultures (in separate experiments) was sterilized by treatment with 0.1 vol. chloroform shortly before constituting the mating mixture. The chloroform was evaporated by passing sterile air through the suspension at 37 °C and viability was tested by plating samples on MacConkey agar.

Interrupted mating. To 18 ml of a recipient culture in a 300 ml Erlenmeyer flask, 4 ml of donor (prepared as above) were carefully added at time zero, and incubated in a water bath for mating pairs to form. At 5 min, 200 ml warm broth were gently added. At intervals, 10 ml samples were carefully removed and mating pairs rudely separated by violent agitation in an MSE homogenizer for 1 min. The container of the homogenizer was then rinsed with a total of 40 ml warm saline (0.85 %, w/v, NaCl) and samples of this saline suspension immediately filtered through Millipore membranes. The membranes with impinged cells were incubated on minimal medium. Other samples, appropriately diluted, were plated on MacConkey agar containing ampicillin and kanamycin to detect plasmid transfer.

Unselected marker analysis. The clones were purified on the same agar. A single colony was then emulsified in 0.5 ml saline and loopfuls spotted on selective and control media.

Segregation studies. Suitable dilutions of overnight broth cultures of purified *his*⁺ recombinant clones were plated on MacConkey agar. After overnight incubation pale colonies were plated on this agar containing ampicillin and kanamycin and also on minimal medium.

Table 3. *Gradient of transmission of markers in relation to his⁺ recombinants in the same cross*

Three ml of the mating mixture were filtered and the membranes placed on minimal media supplemented with histidine for the selected donor marker. An equal volume of the mating mixture was filtered and the membrane placed on minimal media appropriately supplemented for detection of histidine recombinants in the same cross. Minimal media contained nalidixic acid and streptomycin.

Donor	Recipient	Donor marker	Recombinants/ 10 ⁸ his ⁺ *
PM5006(P-lacR1drd19)	UP18	<i>ser-2</i>	790
	UP20	<i>ura-2</i>	522
	UP17	<i>pyrB2</i>	311
	UP21	<i>trp-3</i>	190
	UP22	<i>cys-2</i>	150
	UP23	<i>ade-2</i>	56
	UP24	<i>ilv-2</i>	30

* Average of three experiments.

RESULTS

Recombination experiments with donor PM5006(P-lacR1drd19)

Initial observations are presented in Table 2. A variation of up to 40-fold in recombination frequency for inheritance of particular donor markers was encountered and could suggest polarity of marker transfer. Donor markers *leu-1*, *arg-1*, *met-1*, *tyr-1* did not register and work with these markers was abandoned. Recipient controls never had more than three colonies per plate and donor controls never had growth. Chloroform-treated supernatants of donor or recipient cultures never formed plaques on PM5006 and all of hundreds of recombinants tested plated phage 5006M with an efficiency similar to that of PM5006. The addition of deoxyribonuclease to mating mixtures had no effect on results. Chloroform-treated donor and recipient suspensions were sterile, and when mixed with corresponding viable partners only those with viable recipients sometimes had a few colonies which corresponded in number to those of the recipient controls mentioned above. Crosses between different auxotrophs not carrying the plasmid never yielded more colonies than appeared on control plates. The requirement for viability of both parental cultures was in agreement with the findings of Sugino & Hirota (1962) for R factor-mediated chromosome transfer in *E. coli* and differed from the F-mediated *E. coli* (Hayes, 1952) and *Vibrio cholerae* systems (Bhaskaran & Iyer, 1961) where viability of donor bacteria was not essential. However, the bactericidal agents employed differed. Table 2 also indicates that recombinants were not obtained with PM5006(P-lac) or PM5006(R1drd19) and experiments with these donors were discontinued. With the availability of double auxotrophic strains each derived from mutant UP16 bearing the *his* marker which appeared to recombine at the highest frequency (5×10^{-6} /donor cell), attempts were made to refine some of the results in Table 2. With these strains as recipients the number of recombinants for the other marker could be compared with the *his* recombinants in the same cross by appropriate selection. Results of this gradient-of-transmission mapping (Loutit, 1969) are presented in Table 3. Unlike ColI-mediated chromosomal transfer in *Salmonella typhimurium* (Ozeki & Howarth, 1961) and *E. coli* (Moody & Runge, 1972) the relative number of recombinants for different markers varied from 790 to 30. Comparison with results presented in Table 2 showed that the relative frequency of recombination placed these independently-isolated mutants *ser-2*, *ura-2*, *pyrB2*, *trp-2*, *cys-2*, *ade-2*, *ilv-2* in the same order. The frequency of transfer of the plasmid was 1×10^{-1} /donor cell and exceeded all

Table 4. Cross between UP9 and UP19: segregation of unselected markers

Three ml volumes of mating mixtures were filtered and membranes placed on appropriately supplemented minimal media.

Pattern of segregation						Supplement added to minimal medium	Recombinants	
<i>his</i>	<i>ser</i>	<i>ura</i>	<i>str</i>	<i>pyrB</i>	<i>trp</i>		No.	Percentage
o	o	S	o	o	o	Serine, histidine	20	12
o	I	S	o	o	o		8	5
I	I	S	o	o	o		131	80
I	o	S	o	o	o		3	2
o	I	S	o	I	o		2	1
I	I	S	o	o	I	Serine, histidine, tryptophan	o	o
I	I	S	o	o	o		55	100
I	S	I	o	o	o	Uracil, histidine	30	33
I	S	o	o	o	o		60	67
I	S	I	o	I	o	Uracil, arginine, histidine	6	5
I	S	I	o	o	o		51	39
I	S	o	o	o	o		74	56
S	I	I	o	o	o	Serine, uracil	29	25
S	I	o	o	o	o		68	60
S	o	o	o	o	o		17	15

UP9 = PM5006*pyrB1 trp-2 nal-r(P-lacR1drd19)*; UP19 = PM5006 *his-1 ser-2 ura-3 nal-r str-r*. I, Allele from donor; o, allele from recipient; S, donor allele selected.

other markers. Many minimal-medium plates which bore recombinant clones from different crosses were replicated to ampicillin-kanamycin-MacConkey agar: all recombinants were *lac*⁺ and drug resistant, and all clones tested transmitted the plasmid conjugally at the usual high frequency to *E. coli* strain J62rif-r. Similar situations exist in *E. coli* F⁺ × F⁻ matings (Hayes, 1953) and in the *V. cholerae* conjugation system (Bhaskaran, 1960), where all recombinants possessed the fertility factor concerned. Also, with ColV2 (Macfarren & Clowes, 1967- and R1drd19 (Pearce & Meynell, 1968)-mediated transfer of the *E. coli* chromosome, practically all recombinants received the corresponding plasmid. In a *Pseudomonas aeruginosa* system the number of recombinants which had received the sex factor FP varied from 0 to 71 % in various crosses (Holloway & Fargie, 1960).

All of five independently isolated *his* mutants of PM5006*nal-r str-r* were sired to prototrophy by PM5006(P-lacR1drd19) at the same frequency as indicated in Table 2 (results not shown) and the possibility was entertained that P-lacR1drd19 carried genes effecting the synthesis of histidine in the same way that *Ps. aeruginosa* sex factor FP39 possesses the property for leucine biosynthesis (Pemberton & Holloway, 1973). It was considered because of the higher frequency of transmission of these genes (Table 2, experiments 1 and 16). This was refuted however by the fact that many more recipients became *lac*⁺ and drug resistant than histidine independent. Also screening of about 5000 colonies each from six independently isolated *his*⁺ recombinants yielded 0, 3, 5, 7, 8 and 8 segregants, respectively, which had lost all markers of the plasmid but remained *his*⁺.

Recombinants from experiments 1 and 4 (Table 2) could sire strain UP17 in recombination experiments (not shown) with selection for prototrophy on streptomycin minimal medium. This result could influence recombination frequencies derived from prolonged mating experiments.

The plasmid was readily transferred from J62-1(P-lacR1drd19) to auxotrophs of PM5006

Table 5. Linkage of distal markers to *ura-I* expressed as no. prototrophic recombinants of selected marker which have incorporated *ura-I*

Three ml volumes of mating mixtures were filtered and the membranes placed on minimal media containing nalidixic acid and supplemented with uracil.

Donor	Recipient	Selected recipient marker	Unselected donor marker	Double recombinants* (%)
UPI4(P-lacR1drd19)	UP4	<i>pyrB1</i>	<i>ura-I</i>	41
	UP2	<i>trp-I</i>	<i>ura-I</i>	29
	UPI3	<i>cys-I</i>	<i>ura-I</i>	24
	UPI2	<i>ade-I</i>	<i>ura-I</i>	18
	UPI1	<i>ilv-I</i>	<i>ura-I</i>	12

* About 90 recombinants, each from two experiments, were examined for each cross.

Table 6. Cross between UP9 and UPI1: segregation of unselected markers

Three ml volumes of the mating mixture were filtered and the membranes placed on minimal medium or this medium appropriately supplemented.

Pattern of segregation					Supplement added to minimal medium	Recombinants	
<i>nal</i>	<i>pyr</i>	<i>trp</i>	<i>ilv</i>	<i>str</i>		No.	Percentage
o	o	o	S	o	—	44	91
I	o	o	S	o	—	4	9
o	o	I	S	o	Tryptophan	46	77
o	o	o	S	o	Tryptophan	11	18
I	o	o	S	o	Tryptophan	3	5
o	I	o	S	o	Arginine, uracil	13	14
o	o	o	S	o	Arginine, uracil	81	86

UP9 = PM5006*pyrB1 trp-2 nal-r(P-lacR1drd19)*; UPI1 = PM5006*ilv-I str-r*. I, Allele from donor; o, allele from recipient; S, donor allele selected.

without affecting chromosomal markers of the strains. Segregations of unselected markers in a mating between strains UP9 and UPI9 are presented in Table 4. Most of the alleles were those of the non-plasmid bearing parent (also see Table 6) indicating that transfer occurred to the latter organism. No recombinants of the donor allele of *str* were encountered. It appeared that when *ser* or *ura* was selected the transferred chromosome hardly ever extended beyond this region and *str*, *trp* loci of the donor never registered. Pearce & Meynell (1968) encountered similar phenomena with R1drd19-mediated chromosomal transfer in *E. coli*. The relative numbers of recombinants for *his*, *ser* and *ura* may mean that these markers were transferred in that order. Support for a fixed gradient of transfer in which *his* regularly preceded *ura* was provided by the finding that amongst recombinants selected for the distal marker *ura*, 82 % were also *his*⁺, whereas only 25 % of recombinants selected for *his*⁺ received *ura*⁺.

Table 5 presents results of attempts to determine linkage between distal markers and the proximal *ura-I* locus. Taking the number of recombinants between the two markers listed as a measure of linkage, it was seen that markers *pyrB1 trp-I cys-I ade-I ilv-I* are linked to the *ura-I* marker in the order predicted by results listed in Tables 2 and 3. The simplest conclusion to be drawn from the data is that at least eight of the loci are on one linkage group and the probable order is *his-ser-ura-pyrB-trp-cys-ade-ilv*. Attempts were made to locate *str* and *nal* loci by mating strains bearing late markers (Table 6). A few *nal* recombinants were

Fig. 1. Time of entry of donor markers. The crosses were PM5006(P-*lacR1drd19*) with UP19 and UP17. Mating mixtures were constituted for interrupted matings as described in the text. Samples were removed at intervals, blended and filtered. Membranes from the first cross were placed on minimal medium supplemented for selection of one of the markers. Membranes from the second cross were placed on unsupplemented minimal medium. All minimal media contained streptomycin and nalidixic acid. For plasmid transfer, membranes were placed on MacConkey agar for 60 min before transfer to agar containing ampicillin and kanamycin. ○, Plasmid; ●, *his-1*; □, *ser-2*; ■, *ura-3*; △, *his-1 pyrB2*.

found with selection for *ilv-1*, but the absolute numbers of recombinants were low and only suggestive evidence existed for linkage between *ilv-1* and the *nal* locus. These experiments also meant that results of recombination experiments reported in Tables 2 and 3 were possibly not distorted by close linkage of selected markers to *nal* or *str* loci used for contra-selection of donors.

Interrupted mating

These experiments were done in attempts to confirm the possibility of polarized transfer of markers suggested by the great variation in recombination frequencies presented in Tables 2 and 3. In Fig. 1 the times of entry of a few markers with relatively high recombination frequencies are shown. Nevertheless, owing to the low recombination frequency of even these markers, the mating mixture was diluted only 1:9 with broth. The probability therefore existed that new mating pairs were formed throughout the experiments. Concentrated parental mixtures were filtered in order to give significant prototroph counts. This raised the possibility that recombination occurred on the membrane during subsequent incubation on minimal agar. Also, Loutit, Pearce & Marinus (1968) found that, in a *Ps. aeruginosa* mating system, unless the mating mixture was diluted by at least a factor of 10^{-2} a delay occurred in the appearance of recombinants which was greater for distal markers. Nevertheless, the order of entry corresponded to that predicted by the results of the gradient-of-transmission mapping listed in Table 3 and the segregation studies of Table 4. Because of objections mentioned above, the times of entry must be regarded as approximate. It was evident that the plasmid P-*lacR1drd19* entered recipient cells early and at high frequency. Early and rapid transfer of R1drd19 occurred in *E. coli* crosses mediated by the latter plasmid (Pearce & Meynell, 1968) and in $FP^+ \times FP^-$ *Ps. aeruginosa* conjugation systems the

Table 7. *Crosses between PM5006(P-lacR1drd19) and recipients with P-lac as resident*

Experimental conditions were as for Table 2.

Donor	Recipient	Selected donor marker	No. prototrophs/10 ⁸ donor cells*
PM5006(P-lacR1drd19)	UP3(P-lac)	<i>his-1</i>	31
	UP4(P-lac)	<i>pyrB1</i>	5
	UP14(P-lac)	<i>ura-1</i>	11
	UP15(P-lac)	<i>ser-1</i>	22

* Average of two experiments.

factor was also transmitted at maximal frequency soon after conjugation commenced (Stanisich & Holloway, 1969). The conclusion drawn from these experiments was that a non-random temporal entry of the loci on a linear linkage group existed and that the probable order was *his*, *ser*, *ura*. This order was reproducible. The fact that the first marker, *his*, only registered in recipients after 8 min could mean that undetected markers were preceding it. Another explanation (Pittard, Loutit & Adelberg, 1963; Pittard & Adelberg, 1963) is that the delay was caused by interaction between plasmid and chromosome during transfer. Confidence that the polarity of transfer was due to a linear array of markers passing from donor to acceptor was increased by the observation that with the use of strain UP17 with markers *his-1 pyrB2* the appearance of prototrophs on unsupplemented minimal medium was delayed to possibly coincide with the time of entry of the more distal marker *pyrB2* (see Pemberton & Holloway, 1972).

Crosses between PM5006(P-lacR1drd19) and recipients with P-lac as resident

P-lac was readily transferred conjugally to strains UP3, UP4, UP14 and UP15 by mating with J62-1(P-lac). Results of conjugation experiments done with these exconjugants are presented in Table 7. Comparison with the corresponding experiments of Table 2 showed a marked decrease in recombinant frequency. The recombinants obtained were ampicillin and kanamycin resistant, lactose positive, and had inherited the plasmid as demonstrated by conjugal transfer of all markers to J62rif-r (not shown). Coetzee (1974) demonstrated that *P-lacR1drd19* registered at very much lower frequencies in cells harbouring *P-lac*.

Transduction

Results of transduction experiments are presented in Table 8. The chloroform-sterilized lysate of PM5006*nal-r str-r* by phage 5006M had a titre of 5×10^9 plaque-forming units/ml on PM5006. The phage transduced PM5006 to nalidixic acid resistance or streptomycin resistance but the markers were not transduced simultaneously. Also, single auxotrophic markers of strains UP6 and UP9 were readily transduced to prototrophy while the double auxotrophs were not similarly converted by the lysate. In contrast, results presented in Table 2 (experiments 10 and 11) show that these double auxotrophic strains were converted to prototrophy by conjugal recombination. Although the multiplicity of infection was always less than 1.0 and the adsorption period was only 15 min, none of hundreds of transductant clones plated phage 5006M. It was also evident that the lysate did not transduce lysogenized strains. This was a result of lysogenic conversion causing non-adsorption of the phage (Coetzee, unpublished) and was in agreement with findings of Krizsanovich (1973) and

Table 8. *Transduction of markers by phage 5006M*

Donor lysate	Recipient	Selected marker(s)	Transduction frequency/adsorbed plaque-forming unit
PM5006 <i>nal-r str-r</i>	PM5006	<i>nal-r</i>	5×10^{-7}
		<i>str-r</i>	1×10^{-7}
		<i>nal-r str-r</i>	$< 1 \times 10^{-9}$
	PM5006(ϕ 5006M)	<i>nal-r</i>	$< 1 \times 10^{-9}$
		<i>str-r</i>	$< 1 \times 10^{-9}$
	UP6	<i>his-1</i>	5×10^{-8}
		<i>ser-2</i>	8×10^{-8}
		<i>his-1 ser-2</i>	$< 1 \times 10^{-9}$
	UP9	<i>pyrBr</i>	1×10^{-7}
		<i>trp-2</i>	4×10^{-7}
		<i>pyrBr trp-2</i>	$< 1 \times 10^{-9}$

Phage input multiplicity was 1. Adsorption was for 15 min when samples were filtered. For drug-resistant markers the membranes were incubated on MacConkey agar for 3 h before transfer to selective media. For prototrophy, membranes were transferred directly to minimal medium or to this medium appropriately supplemented.

those for the closely related phages 34 and PM13(ϕ 34) (Coetzee, 1961). It was concluded that stepwise transduction of strains bearing double markers would not occur in recombination experiments should transducing phage have been present.

DISCUSSION

Proof that the conjugal chromosome transfer system described here was not masquerading under the guise of transduction or transformation has been obtained. The former possibility was excluded by the fact that free phage was never detected in parent cultures or in mating mixtures and that all recombinants were fully phage-susceptible. This has also been substantiated by the finding that lysogenized strains did not absorb phage and therefore excluded serial transduction of markers which could not be co-transduced. Transformation of strains of *P. mirabilis*, including PM5006, under stringent conditions has been described (Van Rensburg, 1970, 1971). Only DNA extracted in the cold with a chloroform–amyl alcohol mixture at pH 8.5 was effective and recipient cells had to be in a particular phase of growth. Transformation was excluded here by the demonstration that chloroform-killed donor (or recipient) cultures in mating mixtures did not produce recombinants and that the presence of deoxyribonuclease in mating mixtures had no effect on the outcome of crosses (Sugino & Hirota, 1962). The presence in single clones of more than one unselected marker from both parents (Table 4) could not be explained on the grounds of spontaneous mutation and was decisive evidence for genetic recombination. Furthermore, large segments of chromosome were involved and this excluded transduction and almost certainly also transformation.

Accepting conjugal chromosomal transfer as the vehicle for the formation of recombinants a number of problems require explanation. The hybrid *P-lacR1drd19* carries markers of both parent plasmids but cells harbouring it are not susceptible to phage MS2 which adsorbs to pili produced by *R1drd19* (Coetzee, 1974). *P-lac*-derived pili have not been demonstrated and phage, specific for strains carrying *P-lac*, has not been reported. The frequency of conjugal transmission of the fused plasmid equals that of the derepressed R factor (Coetzee,

1974). Why the hybrid plasmid and not one or both of the parents mediate chromosomal transfer is not known. Chromosomal mobilization also does not occur in a *Proteus* 'double' carrying *P-lac* and *R1drd19* as separate entities (unpublished). It could mean that particular regions of one or both plasmids now differ as a result of rearrangement in the fused plasmid. Further study of the plasmids concerned and, in particular, segregants of the hybrid (Coetzee, 1974) may help to clarify the position.

There was definite polarity of entry of some markers and this raised the question of the relationship between *P-lacR1drd19* and the donor chromosome. The frequency of transfer of the *his* marker, the first of the chromosomal markers investigated to enter, was reminiscent of the transfer of the *trp* region of *E. coli* by *R1drd19* (Pearce & Meynell, 1968) where it was claimed that the R factor had a special affinity for that portion of the chromosome. The latter authors as well as Cooke & Meynell (1969) suggested that this factor alternated frequently between integration near the *trp* region and the autonomous state. Moody & Hayes (1972) also favoured some mode of R1-determined interaction with the bacterial chromosome (see also Evenchik, Stacey & Hayes, 1969) and maintained that the efficient transfer of the *trp* region could hardly be explained on the basis of spontaneous fragmentation and passive transfer with the sex factor only providing effective contact, as has been suggested for factor ColI (Clowes & Moody, 1966; see also Edwards & Meynell, 1969; Clowes, 1972). Participation of *P-lacR1drd19* in the process of chromosome transfer was possibly indicated by results given in Table 6. The decrease in recombinant frequency could be due to entry exclusion and/or incompatibility of the plasmid by the resident *P-lac* (Coetzee, 1974) but the reason was not investigated.

Further work on the transfer of the *E. coli* chromosome by *P-lac* (Falkow *et al.* 1964) has not been published. Although many copies of *P-lac* (Wohlhieter *et al.* 1964) and *R1drd19* (Haapala & Falkow, 1971) may be present in cells of *P. mirabilis*, the state of the hybrid plasmid in *P. mirabilis* is not known. Some unstable association between the hybrid plasmid and the chromosome, similar to that proposed for *R1drd19* but with interaction in the histidine region, may be operative in the present system although recombination frequencies were much lower than *R1drd19*-mediated crosses in *E. coli* (Pearce & Meynell, 1968). This is disturbing, as transfer frequencies of the plasmids involved are similar (Coetzee, 1974), but may mean that affinity of the plasmid for the particular region of the chromosome was less and consequently only a fraction of the donor population was capable of chromosomal transfer (Adelberg & Burns, 1960). This also explained the high frequency of independent plasmid transfer, but it is not known what relationship exists between the autonomous and possible integrated states of *P-lacR1drd19*. Should it be analogous to the situation in *E. coli* (Adelberg & Burns, 1960) there would have to be frequent change between states to prevent loss of the autonomous plasmid. In the *Ps. aeruginosa* mating system, orientated chromosomal transfer has been demonstrated despite the absence of donors which correspond to stable Hfr strains of *E. coli* (Holloway & Fargie, 1960; Stanisich & Holloway, 1969; Pemberton & Holloway, 1973; Loutit, Marinus & Pearce, 1968). These authors also favoured unstable associations between sex factors and the *Ps. aeruginosa* chromosome.

Strain PM5006 was the initial choice for the investigation as, apart from having auxotrophic mutants of this strain resistant to nalidixic acid and streptomycin available, it differs from strain PM13 in that liberation of the cryptic phage occurs very rarely – in the course of this investigation phage 5006M was never detected. Host-controlled restriction and modification systems are operative in various strains of *P. mirabilis* including PM13 and PM5006 (Coetzee & Smit, 1969, 1970). For this reason the better-characterized arginine (Prozesky, 1968; Prozesky, Grabow, Van der Merwe & Coetzee, 1973), methionine (Grabow, 1972*a*) and cysteine

(Grabow, 1972*b*) genes of PM13 have not yet been employed in the present system (see also Stanisich & Holloway, 1969).

The fact that the *str* locus of donors did not register in recipients, even with selection for the most distal marker available, could mean that the donor chromosome proceeded from only one or a few origins and, as a result, not all regions of the chromosome were equally accessible to mapping (see Pemberton & Holloway, 1973). Certainly, results of mating experiments were not what would be expected if the plasmid had many potential sites of attachment to the *Proteus* chromosome as appears to be the case with F and the *E. coli* chromosome (Jacob & Wollman, 1956; Broda, 1967). The object of this investigation was to establish the existence of conjugal chromosomal transfer in *P. mirabilis*. A study of the complexities and niceties of the process (De Haan & Gross, 1962; Loutit, Pearce & Marinus, 1968; Pemberton & Holloway, 1972) was not attempted. It could be that, using different mating conditions, recombination frequencies of markers would be increased with possible extension of the linkage group.

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