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GENETICS

Hfr
3200 $\frac{F_{13}}{}$ × W3086 W

26

12/IV : 1959

REF:

Method

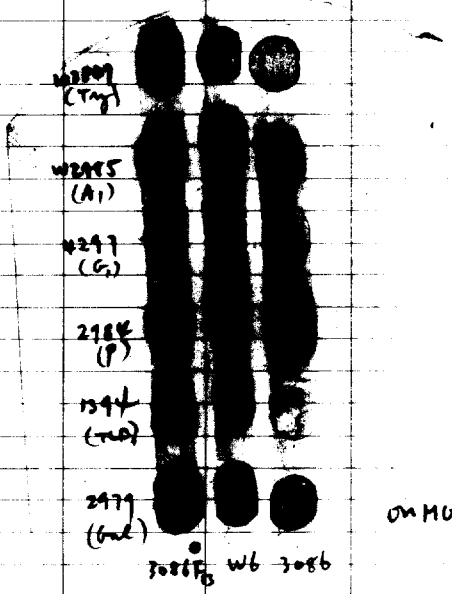
Overnight culture of W3200 5 ml/ pen + 0.1 ml W3086

Incubate overnight (20 hrs)

purify it on Blacsm-agar.

Replica plate it on MGal seeded W2979 on it.

Result & conclusion : F'_{13} is obtained from Hfr_{13} with the method described above. F' gives Hi for Gal; Medium for Try + A_1; Low for G_1 + TLB_1



on MGal

Comparisons on infectivity of F'^{+} and corresponding Hfr.

16/IV ; 1959. REF: cf. p.33. infectivity.

· cultural age : Overnight culture (Penassy)

· Strains: Hfr is isolated by purification of stab culture.
W4321 (Hfr_4 M^-) W3208 (Hfr_8 M^-)

· Experimental method:

Hfr or F'^{+} 1ml + 3086 0.2ml + Penassy 5ml.
↓
Incubate it overnight.
↓
Purify it on MMal Sm agar.
↓
Test sex-compatibility

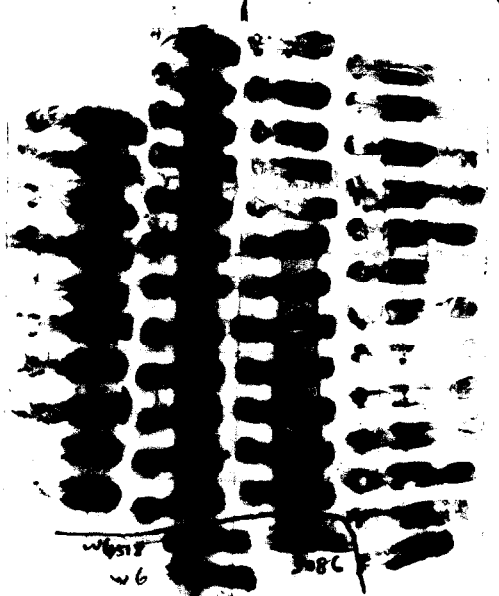
Result:

	Hfr/total.	% converted into F'	infectivity
W4321 (Hfr_4) → 3086	0/24	0	−
W4518 (W6 F_4) → 3086	22/22	100.	+
W3208 Hfr_8 → 3086	18/22 (not Hfr: F^+?)	82 (usual F^+?)	see back page. +
W4520 → 3086	20/25	80	+

This was not Hfr.

Conclusion : Original stab culture of W4321 contains 2 kinds of Hfr strains, infective and non-infective.
Infective Hfr may occurred by mutation of non-infective Hfr, F' is simply splited from chromosome.

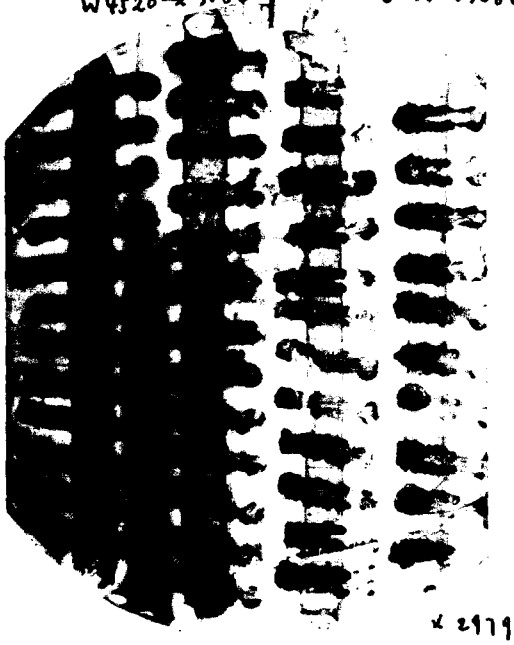
W 4518 → 3086 | W3241 → 3086



W4518
W6
3086

on Mlac.
x2985
A_1

W4520 → 3086 | W3208 → W3086



x 2979
on MGal.

I should use Gal marker, because no syntrophy.
try again.

$3086\,F_8 \longrightarrow \times\ W6$

$F_8 \longrightarrow \times\ F_1$

Is it co-exist or exclud with each others? P.29 P.48

16/IV ; 1959.

REF:

$F_4 \times F_1$

$F_8 \times F_1$

$3086\,F_8$: 1ml : F_1 (W6) 0.1ml : 5ml penassy.

↓

Incubate it for 24 hrs at 37°C.

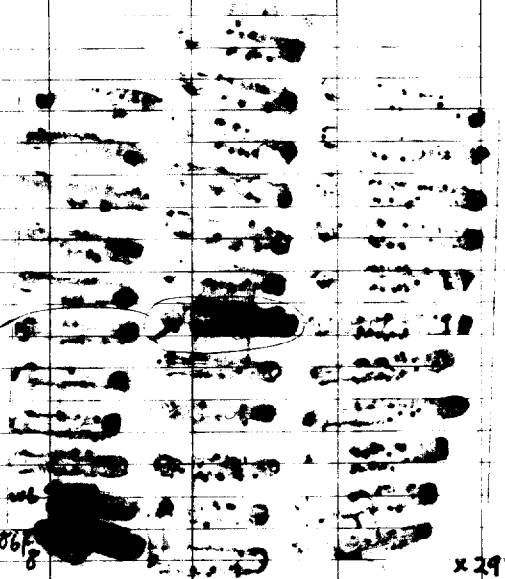
↓

Purify it on B Mal

↓

Test Mal^+ colonies on sex. in cross times 2979. on MGal.

$3086\,F_8 \longrightarrow \times\ W6$



F^+/total tested : $1/32 \times 100 = 3.12$

test infectivity of F_1 and F_8 to F^- stain (P29)

Does this clone still contain F_1?

$3086\,F_8$

$\times$ 2979 on MGal

Conclusion : F_8 is infective to $W6\,F^+$.

objection : $W6 \xrightarrow{\text{spontaneous loss of F}} W6\,F^- \rightarrow W6\,F_8$

$3086\,F_8$

W6(F_1 F_8) ⟶ 3086

23/1J ; 1959. REF:

Purpose of this experiment: Does W6F_1F_8 still retains F_1 or not?
Cf. P.28.

		W6F_1F_8	
Control 1.	or	W6F_1	⟶ W3086
control 2.	or	W6F_8	

1 ml + 0.1 ml + 5 ml penassy

↓

incubate overnight.

↓

Purify it on Blac Sm.

↓

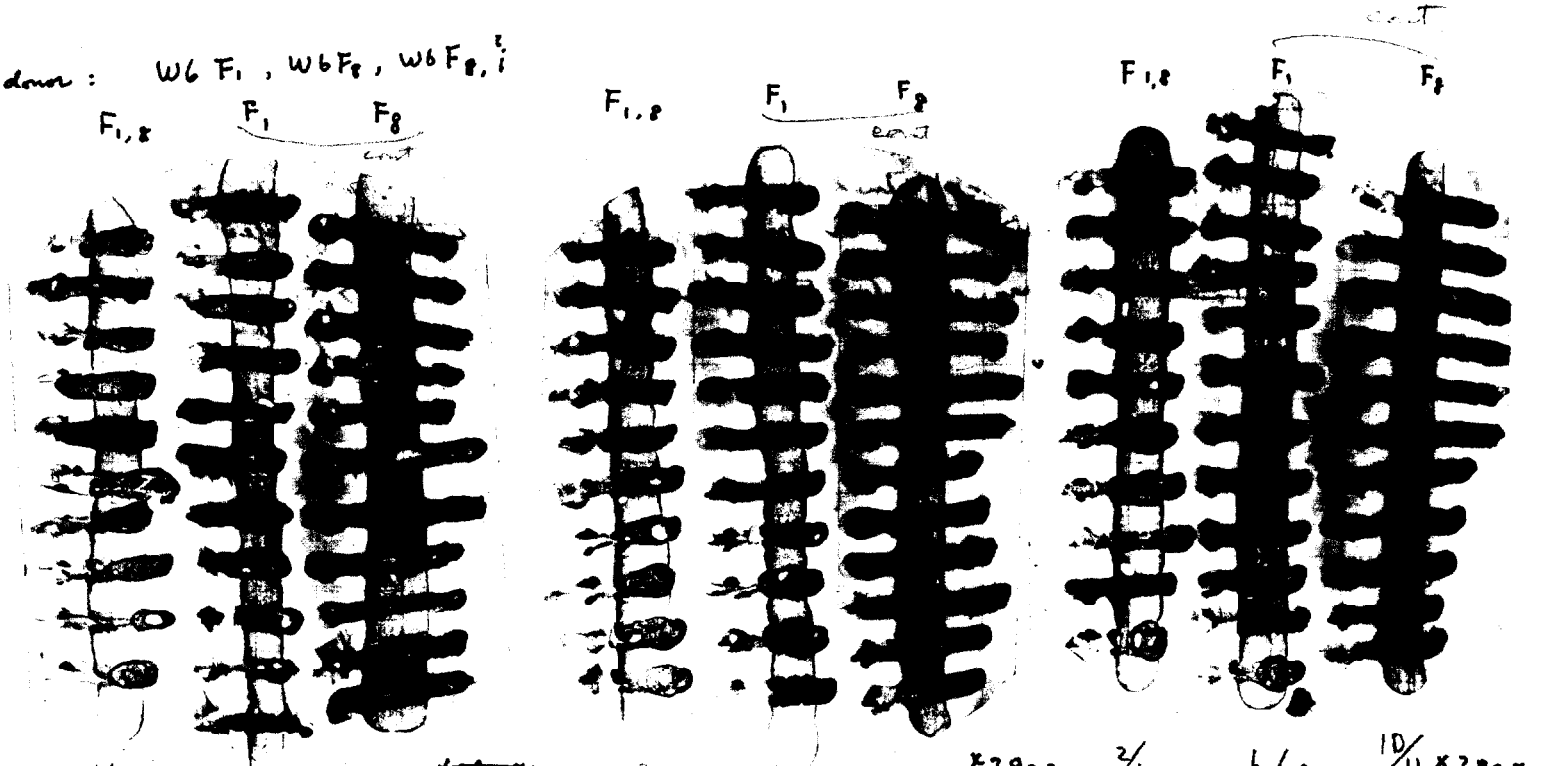
Cross-brush it in cross × 2979 on M Gal.

		F^8/total (%)	F_1/total (%)
Experiment:	W6F_1 F_8 ⟶ 3086	6/30 (20)	0/30 (0)
Control:	W6F_1 (W6) ⟶ 3086	0/34 (0)	17/34 (50)
	W6F_8 ⟶ 3086	30/34 (88)	0/34 (0)

Conclusion: W6$F_{1,8}$ (obtained by infection of F_8 to F_1^+ : W3086F_8 ⟶ W6) does not contain F_1, or not segregate in the time of infection.

~~F_1 and~~ F_8 may exclude F_1 after infection. $F_8 > F_1$ stronger than.

F donor : W6 F_1 , W6 F_8 , W6 $F_{8,i}$



1/10 5/11 10/11 ~~[illegible]~~ ×3828 on Mlec

3/10 6/11 10/12 ×3828 on Mlec

2/10 6/12 10/11 ×3828 on Mlec.

F recipient : 3086.

		%
$F_{1,8}$	6/30	20
F_1	17/34	50
F_8	20/34	88.

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Direct demonstration of true recombination.
Not syntrophy!

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Check syntrophy between Gal⁻ and M⁻
A and M.

20/IV ; 1959

REF:

1. Mix W6F8 1ml + W2979 1ml + 5ml pen. Ca. 10^8 cells/ml.

↓

incubate 1 hours at 37°C.

↓

dilute it into 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}.
and spread it on MGal and BGal.

↓

Incubate 40 hrs and see what happens.

See : back page.

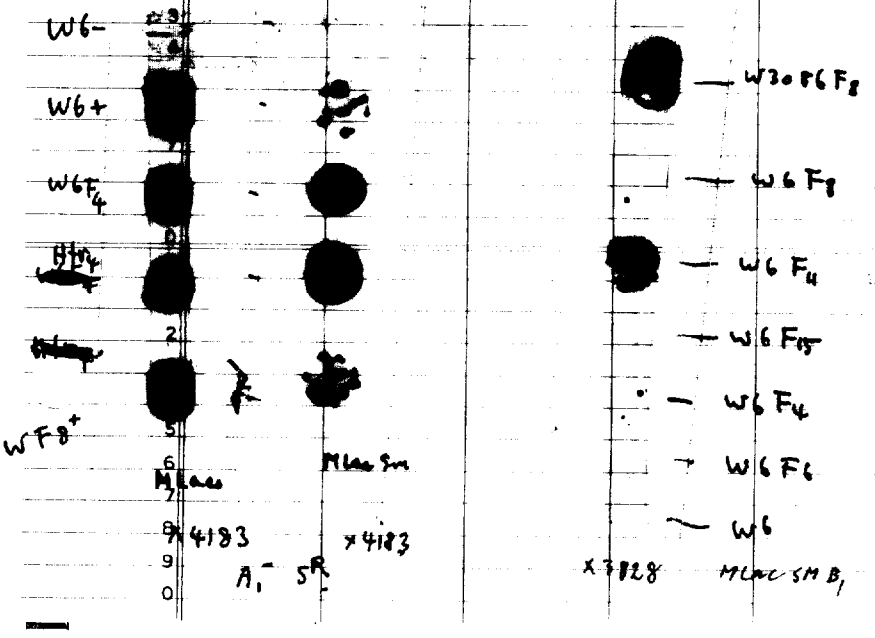
	Degree of dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	survival cont. 10^{-6}
# of colonies	too many	too many	too many	150	32	6	17

2. pick cells from blackspot and streak on BGal ~~[illegible] it Gal⁺ [illegible]~~, and test nutritional requirement on MGal (~~Replica plate from BGal to [illegible]~~)

		isolated on	on BGal	~~on MGal~~	Test nutritional requirement. aux/Prot.	% of X
W6F8 × 2979	on MGal	BGal	almost Gal⁺	tested Gal⁺	0/22	0.0
W6F4 × 2985 A⁻	on MLac	Bgal.	All gal⁺		4/21	16.0
W1394F8 × 2979	on MGal	BGal	2/3 gal⁺.	tested Gal⁺	2/23	8.0

young culture. 4 Spot it on MLac SMB1 seeded 3828
old culture

SM destruct male capacity of S^S F' male but not S^r F' male.



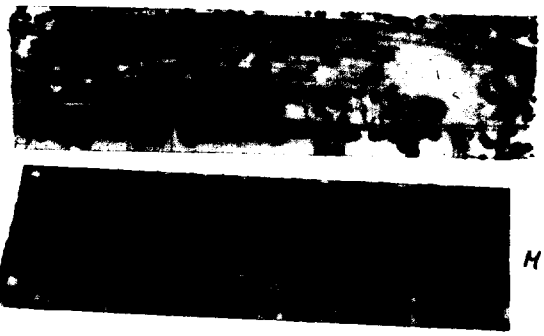
Conclusion : ① This phenomenon is not syntrophy but true recombination.

② ~~This~~ F8 × F⁻S^R recombination process is inhibitted by SM-treatment. (It may be interpreted by incorporation of S^S loci to F⁻S^R)

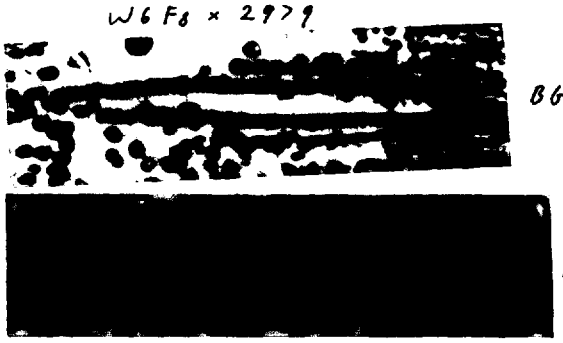
③ F4 × F⁻S^R is not inhibitted by Gm.

This phenomenon may be interpreted by incorporation of S^S to F⁻S^R.

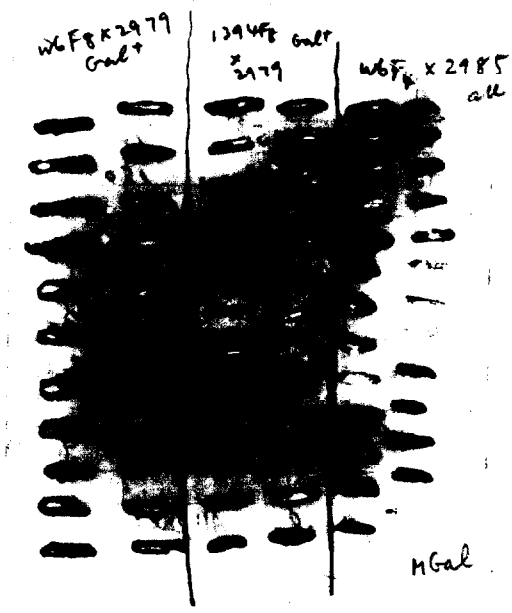
W6 F_4 x W2985



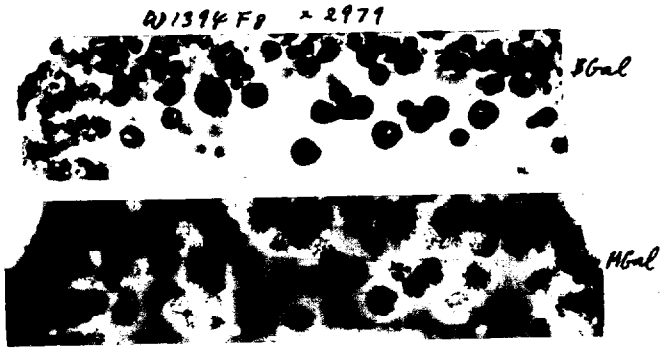
W6 F_8 x 2979



Test of the syntrophy.



W1394 F_8 x 2979



Conclusion:

almost of three Gal+ colonies are

X^+.

Therefore, this phenomena are not syntrophy but recombination.

Genetics.

Preliminary experiment for exclusion of F particles.

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W6 F_4 $\xrightarrow{F_8}$ × 3086 F_8

W6 F_8 $\xrightarrow{F_8}$ × 3086 F_4

16/IV. 1959 REF: cf. p 42 ~ p 50.

Ratio of mix.

Donor (W6 F') 1 ml + Recipient 0.1 ml + Penassay. 5 ml.

↓

Incubate overnight at 37°C

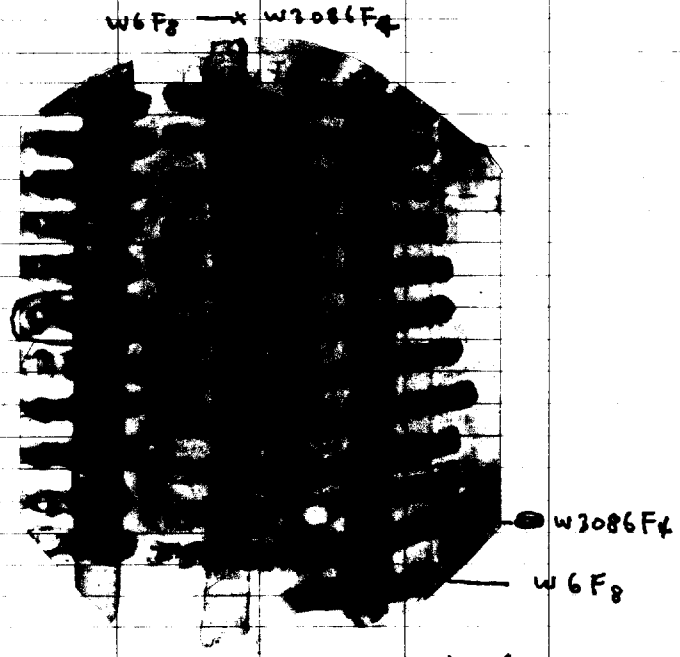
↓

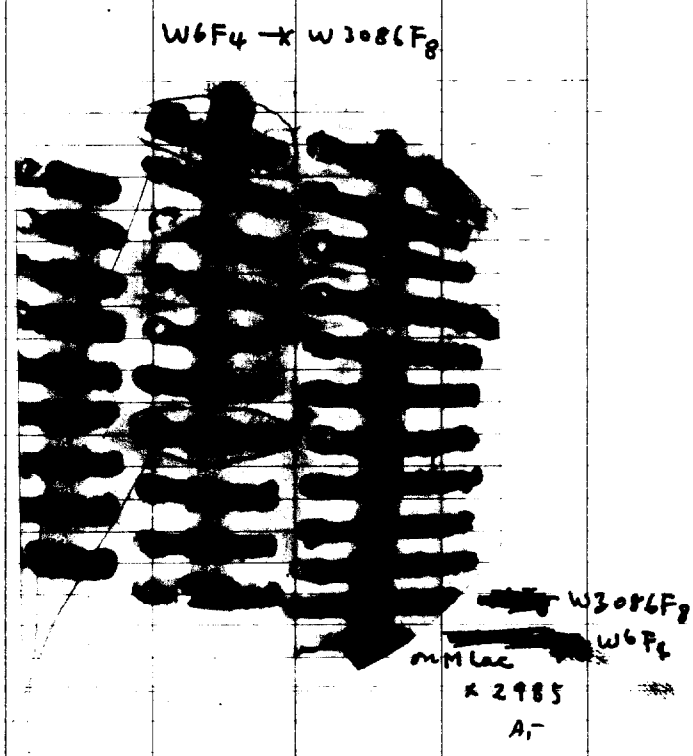
Purify it on B Hard SM.

↓

Test Mal⁻ colonies for sex-compatibility.

The method used in this experiment is not suitable to know the difference between two different F'+. Use spot method for this purpose. (See back page.)





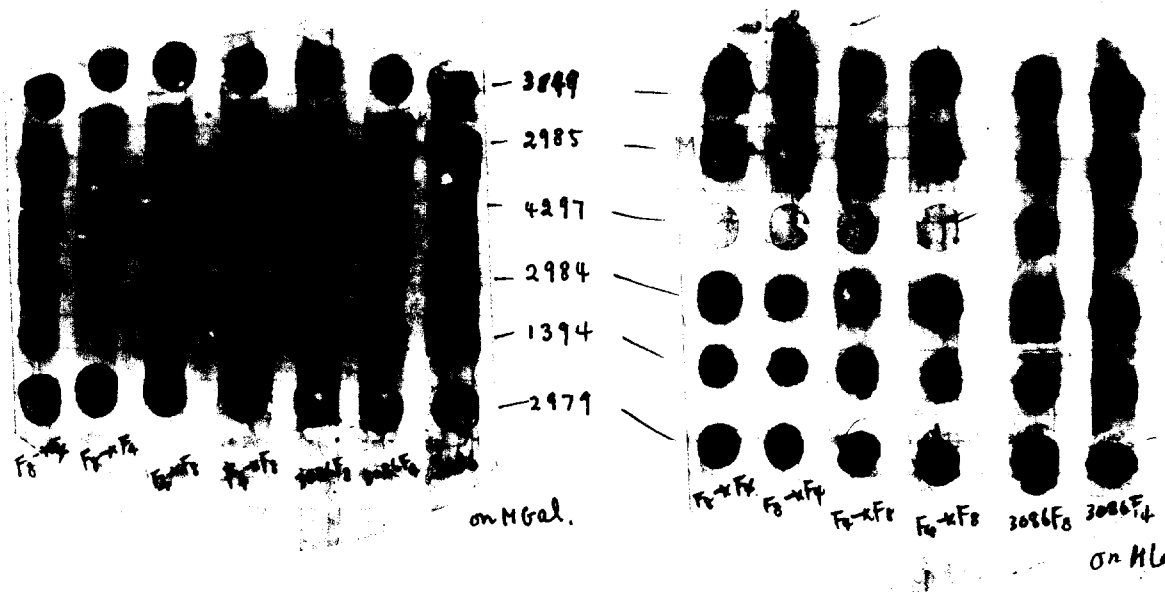
test each H1 markers. Retest H1 markers: See back page

conclusion : F_4 is stronger than F_8. F_4 seems expel F_8 after infection.

But, is it chromosomal level or cellular level? Does phenotypic F_8 (F_8) still contain F_8 or not? (See Back page.)

Next step : $F_{4,8}$ × F^- and see segregation of F_4 and F_8.

Fertility } patterns of F_4, F_8, and $F_4 \rightarrow\!\times F_8$, $F_8 \rightarrow\!\times F_4$ obtained by infection of F_4' to other F^+



$\underbrace{F_4 \quad F_4 \quad F_4 \quad F_4}_{F_4 \rightarrow\!\times F_8 \quad F_8^{\alpha} \rightarrow\!\times F_4}$ $\underbrace{F_8 \quad F_4}_{\text{control.}}$ F^-

Phenotype $\underbrace{F_4 \quad F_4 \quad F_4 \quad F_4}_{F_4 \rightarrow\!\times F_8 \quad F_8 \rightarrow\!\times F_4.}$ $\underbrace{F_8 \quad F_4}_{\text{control}}$ phenotype

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Isolation of F_2 from Hfr_2. 32

4068 ⟶ 3086.
Hfr_2 F^- M Mal S^R

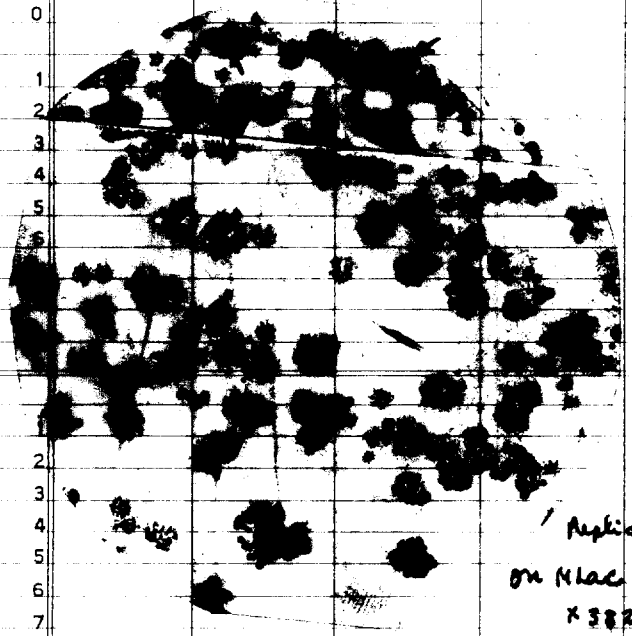
16/IV. 1959. REF:

4068 : From Gan. (15/IV) Exponentially growing. it is grown into pen. for overnight
3086 : old. broth

Ratio of mix.

4068 1ml : 3086 0.1ml : Penassy 5ml.
↓
Incubate then at 37°C. for overnight.
↓
Replica plated on MLac (x3828) or MLac B_1 SM. (1394).
↓
Pick High recombination colony and test Hfr quality (x1394) on MLac B_1 SM

Hfr_2
4068 — x 3086



Replica plated on MLac seeded x3828

Retest.

Master plate.

on MLac Sm.

Treatment of W1922F8 by AO.

22/IV. ; 1959 REF:

Time of treatment : Ca. 40 hrs. at 37°C. Conc. of AO 30γ/ml. 5 ml penassy.

Purpose : W1922 is Hfr1. Has W1922F8 Hfr1 or not. If Hfr1 is in W1922F8, it will gives Hfr1 after treatment. Tester : W2979 on MGal.

Result:

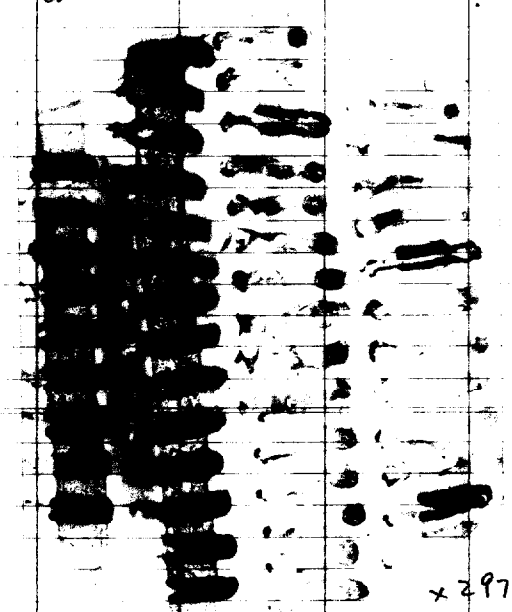
	AO treated W1922F8	Untreated W1922F8
♀/total	27/27	0/23
% of ♀	90	0

Conclusion :

W1922F8 does not give arised Hfr1 after AO-treatment. Only gives DF.

W1922F8 (Hfr1 F8)

Control AO-treated



x2979 on MGal

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FR. from F8 3086.

28/N — 1957

REF: cf. p35
P37 51 49

	1	2	3	4	5	6	7	8	9	10
		U.V.	10 sec.		Initial number of cell /plate : ca. 10^7–10^8 cells/pl					
					cultural age : overnight.		seeded on B Gal.			
							strain : W2979			
							med : on M Gal.			
	plate #	# of colonies/plate		# of Rec+ colonies.			% colony appeared.			
	1									
	2									
	3									
	4									
	5									
	6	19		18			1 ↙	pick and retest the leaks of		
	7	26		26			0	fertility.		
	8	36		36			0	and test immunity.		
	9	31		31			0			
	10	90		90			0			
	11	35		35			0			
	12	53		53			0			
	13	37		37			0			
	14	37		37			0			
	15	49		49			0			
	16	63		63			0			
	17	53		53			0			
	18	29		29			0			
	19	25		25			0			
	20	28		28			0			
	Σ.			610			1			

Interrupted mating experiment of W6F8.

18/IV ; 1959. REF: H is low mafer. bac.

F⁻ : W4352 F⁻ Try P M Gal₆ Lac₅₇ U_6^R Lp^+ Make S^R mutant.
Hfr : W4520 Fg H.
(W6F8)

Method.

Selective marker:	Medium	Purpose
Try	M Gal conc + Meth + Prol	Try incorporation.
Try & Prol	M Gal + Meth.	2 Which is earlier Try or Prol.
Try Lac.	M Lac + Meth + Prol	Which is earlier Try or Lac.
"	" + Meth + Prol + Trp	1 Lac incorporation
Try & Gal	M Gal + Meth + Prol	which is top. Try or Gal.
Gal	" " " + Trp	Gal.

Method:
1. Purity W4352 on Blac agar. (It was very small colonies).
2. Inoculate purified colony into peptone and incubate it at 37°C. for overnight.
3. Transfer it into peptone broth (10ml) and inoculate W4520 into 10ml peptone and shake it for 4hrs. (11:00 — 2:45)
4. Mix them (1:1) chilled take a sample, and dilute it into H_2O. ($\times 10^{-3}$, & 10^{-5} dil: ca 10^6 & 10^8 0time 0time viable/ml)
5. Spread over each selective medium at each time.

Selective Medium	Time / Selective marker	2:45 0 min. after mix. (# of cells ×100.)	2:50 5	2:55 10	3:00 15	3:05 20	3:10 25	actual time. min.
Gal-M-P	Try	0	0	0	0	0	0	
Gal-M-P	Try-Gal	0	0	0	0	0	1	
Lac-M-P-Try	Lac	0	0	0	0	0	0	
Gal-M-P	Try-Lac	0	0	0	0	0	0	
Gal-M-P	Gal.	3 ($\times 10^2$)	1	?	5	9	0	
BLac +	Survival Count							# of cells used in Exp one ($\times 10^2$).
F_8	# of 4520 a	0	0	0	0	0	0	
	# b	0	0	2	0	1	0	
F⁻	# of 4352 a	7	12	33	15	12	7	
	# b	12	24	38	18	27	11	

Method of dilution.

0.1/10 × 0.1/10 : 0.1 /plate : 5 min —— 25 min. (selective agar)

0.1/10 : 0.1 /plate : 0 min. only. (selective agar)

0.1/10 × 0.1/10ml × 0.1/10ml : 0.1ml : 0 min —— 25 min (EMB Lac)

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Crude Timing experiment for chromosome transfers from F'+ to F− marker observed : Lac.

24/10 ; 1959.

REF:

Cultural age : ca. 5 hr. on rotator. Penassay.

cell number : ca. 10^8 cells/ml.

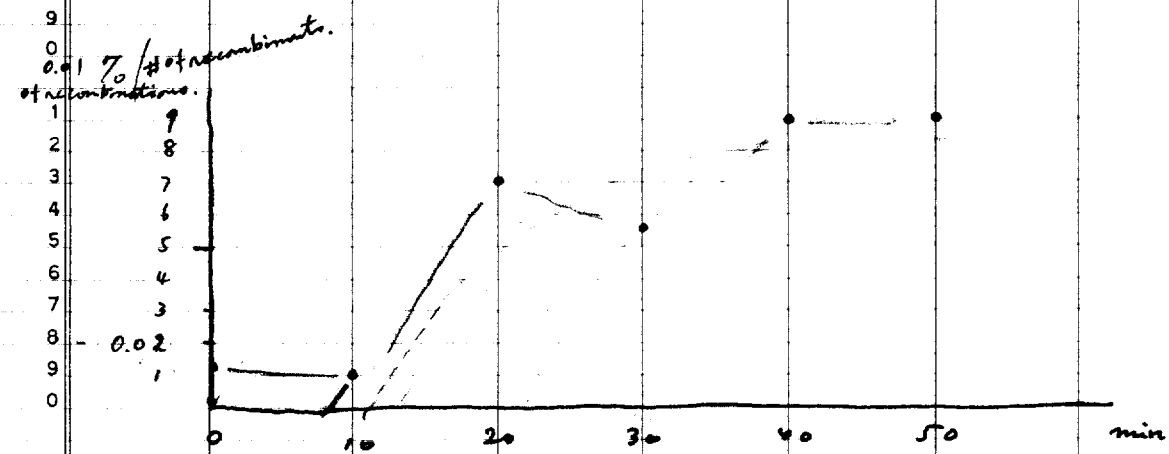
selective media : M Lac Sm.

Strain : W6 F8 ♀. W3828.

dilution

10^{-3}	10^8 : 0.1ml/10ml : 0.1ml/plate
10^{-4}	10^8 : 0.1ml/10ml : 1ml/10ml : 0.1 ml/plate

Time (min) / dilution	0	0	10	20	30	40	50		120
ca. 10^5/plate 10^{-3}	13	1	10	70	56	81	80		172.
ca. 10^4/plate 10^{-4}	0	0	0	1	4	5	7		30



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W6 F8 × W3828.

Is there 2 states in W6 F8 ?

23/10. 1959 REF:

W6 F8 × W3828. on MLac Sm, & MLac.
M⁻ F8 Lac⁻ S^R.

Hypothesis: Interpretation for low fertility of W6F8 on Sm-media.

: Almost of the F8 cells are in state I (cytoplasmic state), and not state (II).
Only state II can donate chromosome even in the presence of Sm.

: If it is true, all of W6F8 colonies show Hi on MLac, and only (clone) four W6F8 shows Hi fertility in the cross on MLac Sm. (W6F8 only shows the state II phenotype on (MLac) Sm-media) and almost of the W6F8 colonies are low fertile.

Method:

1) Make W6 F8 colonies on BLac agar. (10 plates).

2) Replica plate it on MLac and MLac Sm seeded W3828 on it,

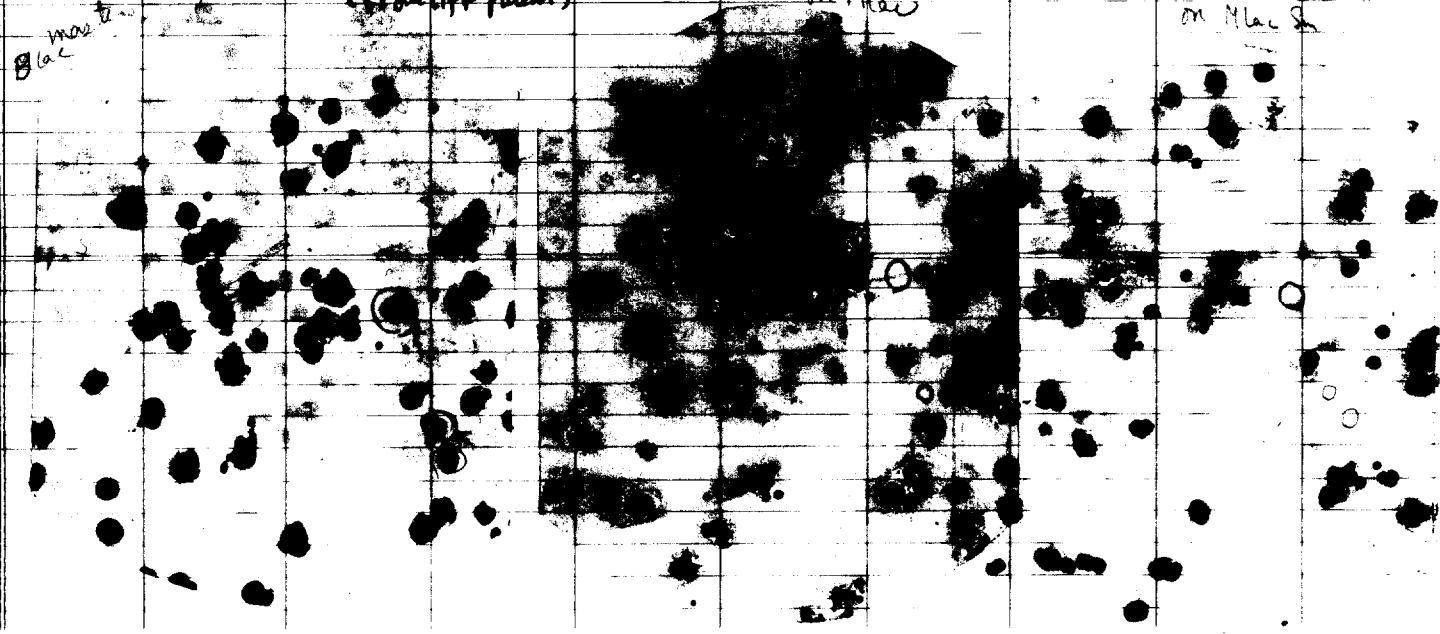
3) Incubate it for 30 hrs.

plate #	# of colonies	Hi # of Fertile colonies on MLac	on MLac Sm.
1	135	135	135
2	112	112	112
3	130	130	~~129~~ 129(1)
4	127	127	127
5	58	~~57~~ 58(1)	58(1)

Result.
apparently, fertility of F8 W6 (on MLac Sm) is much low than MLac. But there is almost no fluctuation among replicated colonies.
all State I ?

F⁻ or F^R =

Conclusion:
This phenomenon (inhibition of recombination formation by Sm.) may be interpreted by the incorporation of S^S segment to S^R F⁻.
(from Hfr parent)



one example.
Mlac
pickad

2nd trial of infection of $F_4 \rightarrow F_8$.

23/11, 1959. REF: Cf. P31. Cf. 50

- Method: (W6) (W3086) Control F_8 only.

Mix F_4 1ml : F_8 0.1ml : 5ml phassy.

↓

Incubate it overnight. (W6 F4 W3086 F_8 is purified on Blac. before using.)

↓

Purify it on B Mal Sm.

↓

Inoculate ~~[illegible]~~ Mal^- Sm^R colonies into 1ml phassy tubes. and let it grow for 5 hrs at 37°C

↓

Spot them on (~~[illegible]~~, W2985 (A_1), W2979 (Gal)) m. Gal.

and compair Hi-recombination pattern.

- Strain: W6 F_4, W6 F_8, W3086 F_4, W3086 F_8 all of them are purified. before using in this experiment.

- Result:

	HI for A_1 only (F_4)	HI for Gal_2 only (3086 F_8)	HI for both. (double)
Experiment W6 F_4 → W3086 F_8	1	38	5
	#28 (F_8 may be expelled by F_4)		1 2 15 31 34
Control W3086 F_8 only	0	40	0

Double F strains. (F_4 & F_8 in W3086)

- Conclusion:

1. F_4 is infectable to F_8^+ cell, and sometimes expel F_8 from the host cell and sometimes make double F strain.

2. These fertility may depends on the segregation of two types F'^+. (∵ If A_1 becomes higher than parent Gal will become lower or vice versa but not both.) See segregation of F' characters. by spot test and replica plating method.

[illegible] efficient method: application of Replica plating method.

W6 F_4 → 3086 F_8. Use Xyl to detect F_4

Seed on B Mal Sm.

Replica plate on MXyl seeded W2979.

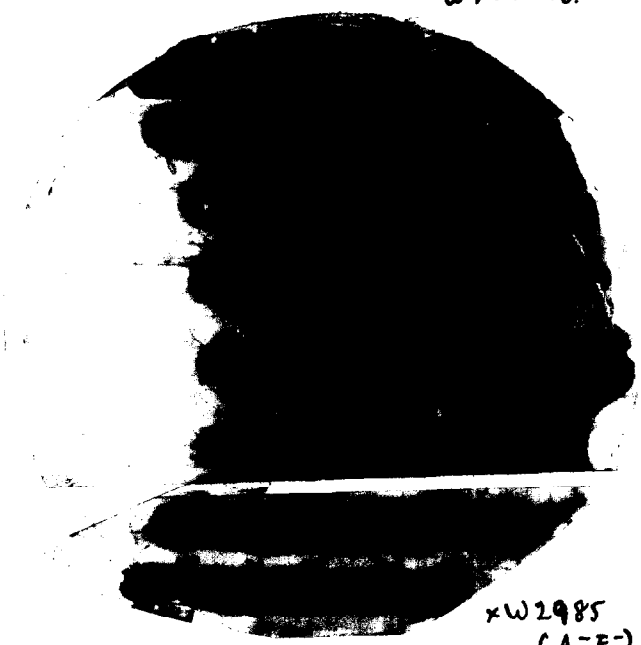
{ F_4 is hi for Xyl but F_8 is very low for Xyl, therefore it is easily differentiate between F_4 and F_8.

x2979 (Gal2) x2985 (A_1) on MGal

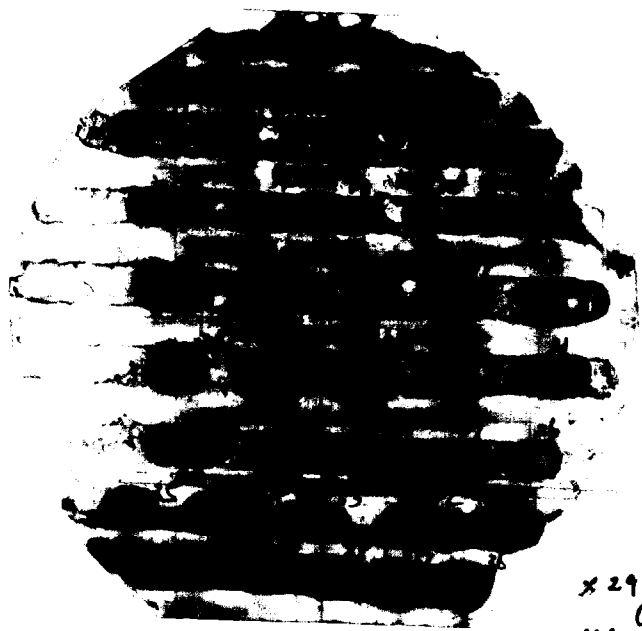
on MXyl W2979

Take HI colony, ~~[illegible]~~ on Xyl-transfer and see segregation of the marker by replica plating in same way.

control W3086 F_8.



x W2985 ($A_1^-F^-$) on MGal

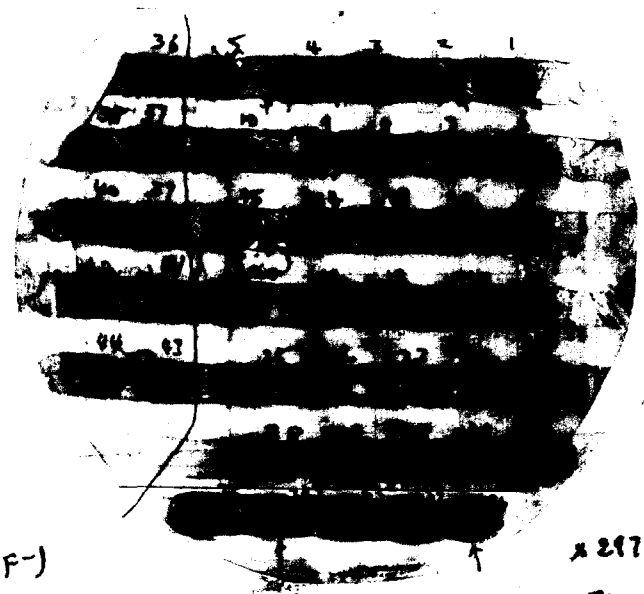


x 2979 (Gal^-X^+) on MGal.

infection. Look for double F.

W6 F_4 — x W3086 F_8

x W2985 ($A_1^-F^-$) on MGal



x 2979 ($Gal_2^-X^+$) on MGal

Test No. 28, [illegible] No. 34. and No 31. No 1.
on. Segregation, or [illegible] H1 for both.

2nd trial of infection of F_8 to F_4^+

$W6\ F_8$ —× $W3086\ F_4^+$.

23/IV 1959

REF: (cf. p 31.) $F_4 \rightarrow F_8$

Method: is completely same as $W6\ F_4$ —× $W3086\ F_8$.

Purpose: Is $W3086\ F_4^+$ accessible to the infection of F_8?

Result:

			H_1 for A_1 only (F_4 type)	H_1 for Bal_2 only (F_8 type)	H_1 for both (double F)
	$W6\ F_8$ —× $3086\ F_4$		35/35 (100)	0/35	0/35
Control	$3086\ F_4$ only.		35/35 (100)	0/35	0/35

All the colonies of 5 tested

Conclusion: [$3086\ F_4$] does not express F_8 character after mixed culture with $W6\ F_8$.

This may be explained as ① lack of infection from F_8 to F_4.

or ② it is infectable to F_4 but recessive for phenotypic expression.

or ③ it is infectable to F_4 but is expelled after infection by exclusion between F_4 and F_8. F_8 is weak to the competition than that of F_4.

Test for 2 states of fertility in F_8^+ ♀$_3$

W4525 (W3644 F_8^+)

1/N : 1959.

REF: TLB, Mal, SR ♀$_3$.
Cf p55, p70, p23

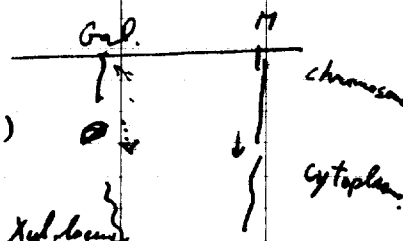
W4525.

① Purify F_8^+ ♀$_3$: (W3644 F_8). on B Lac.

② Inoculate it into penassy and let it grow. for overnight.

③ Make colonies (single colony) on B Lac. and replica plate it on M Gal plates seeded ~~W3244 F M Gal~~ W2979 ~~and~~ or W6 F−.

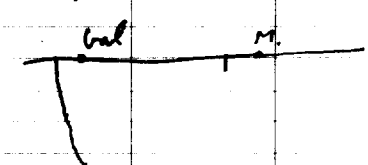
Purpose :
Does F_8^+ ♀$_3$ shows two states on Gal or M loci or not?
(only M or both or only Gal ?)



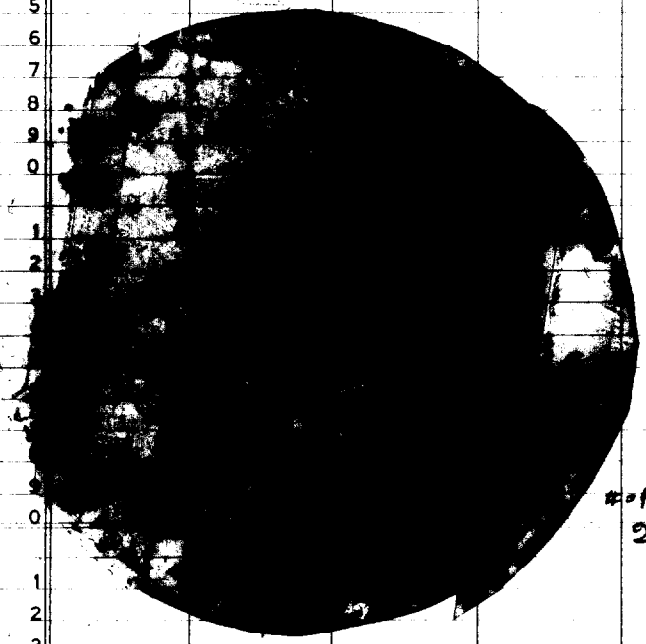
Result :
Only one state of fertility was observed. (see below)
in both cross. (× M−, × Gal_2−)

Conclusion : F_8 does not fixed on Rfd_3 locus. Only attach at the Xyl locus
(∵ Lo. for Meth (Rfd make hi). & Hi for Gal. and also only one state was observed.)

This means F_8 does not split off so easily as F_1.



W4525



# of colonies 220

× 2979 on M Gal

# of colonies 220

× W6F− on M Lac

Minimal cell number for infection of F' to F⁻.

5/6 ; 1959 REF:

1. Overnight culture (penassy culture) of W6F8 and W3086 are inoculated into penassy medium. (1 ml culture / 5 ml penassy).

2. Incubate it for 2 hrs. at 37°C. (To make new culture.)

4. Mix them in the ratio 1 : 10^6 cells / ml

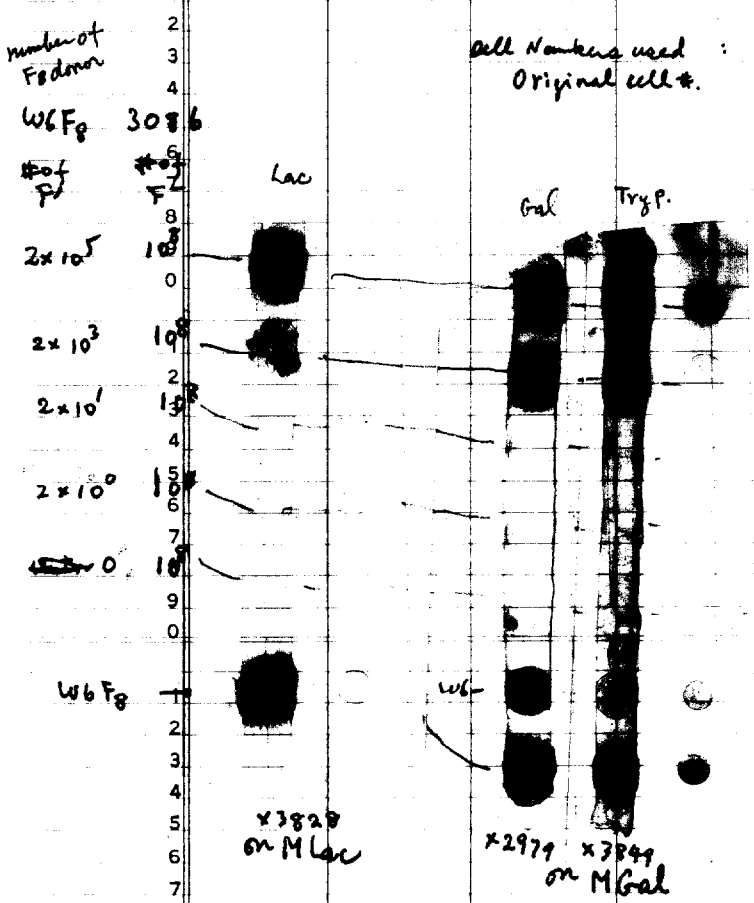
No of tube	1	2	3	4	Control
W6F8 :	$\times 10^{-2}$	$\times 10^{-2}$	$\times 10^{-2}$	$\times 10^{-1}$	
	2×10^6	2×10^4	2×10^2	2×10^1	0
	0.1 ml	0.1 ml	0.1 ml	0.1 ml	
	2×10^5	2×10^3		2×10^0	0
W3086 Ca. 10^8	1 ml	1 ml	1 ml	1 ml	1 ml.

5. Add Sm into the mixture at the final concentration of 1000 unit /ml. to kill F8 donor. 2 hrs after mixing, and incubate it overnight. 4:00 pm. 5/1/59.

2g/5ml H_2O $\times 10^{-2}$: 0.2 ml / 1 ml pen. final : 0.8 mg / ml.

6. Survival count of W6F8. & W3086.

	$\times 10^{-2} \times 10^{-2} \times 10^{-1} \times 10^{-1}$ 0.1 ml/plate	$\times 10^{-2} \times 10^{-2} \times 10^{-1} \times 10^{-1}$ 0.1 ml.
	230, 189	206, 185
Cell Numbers used : Original cell #.	2×10^8 cells/ml	2×10^8 cells/ml.



Summary of experimental conditions.

young culture : 2 hrs. culture in pen. at 37°C.

Inocular size :

Minimal cell number for infection of F' (within 2 hrs.)

$2 \times 10^3 \sim 2 \times 10^1$

2000 ~ 20

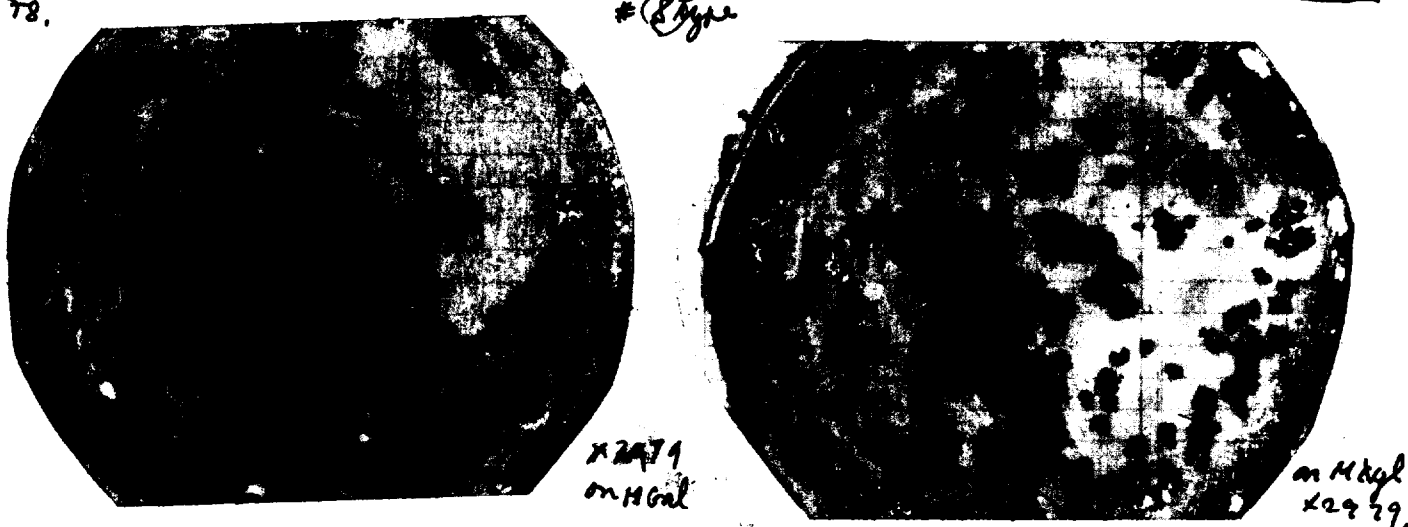
Ca. 100?

F4 → F8.

4 type.

F4 → F8.

8 type



Segregation pattern of F8 and F4. from double F. (F4,8.).
(judged by Replica plating method)

cf. P42

compair these two segregant. above 4 type; bellow 8 type.
Xyl plate is ok: F4 type is more futile on MXyl, than F8 type.
But MGal plate is not suitable for distination between F8 and F4.

Use #2 and #31. to detect recombinants between F'.