Transmission of F+.

Jan 21, 1952

Clear Supernatant (2 centrif.) of 58-161 = A ~~Add ...~~ Assay: ca: 3×10^6

A. 1. A + W1177 (ca 10^7/ml) + sm 12° – 2:30 Inc. } v. little growth
~~2. A + W1177~~ Refrigerate
3. 58-161 cells ca 10^7/ml + W1177 10^7 + sm in Penassay Inc. } considerable growth.
4. " " 10^8/ml + W1177 + sm " Inc.

Streak out EMB lac after 2½ hours incubation.

1 : Ca 1% +
~~3~~ : No +
4 : Ca .1% +

Pool 20, lac – colonies as inoculum for crossing test.
For 'revis, pick as many colonies as possible.
Check all inocula by streak on EMB lac

A1	–	✓
A1'	++	✓
	Plate A1' alone: –	✓✓
A3	++	✓
A3'	+++	✓
A4	+++	✓
A4'	+++	✓

Cells are evidently very efficient in transfer of F+.

B 1/24/52. W1177 ca 10^8 + 58-161 in Penassay 4PM – (washed cells)

		A	B	C
1.	58-161 ca 10^7	} Incubate	2 colonies	++	++
2	58-161 ca 10^8				
3	58 161 supernatant (1:10) (ca 10^8 cells)		–	–	–
4	58-161 ca 10^8	Refrigerate in Penassay.	–	–	–

A. 2 cols } W1177 Reisolated and tested.
B 10
C ∞ cols

1/26. all plates bare except 2B, 2C.
∴ 10^8 cells transfer ca 10% F+ in 1 hour. But at best 2A.
F+ may not be all or none at first growth!

Cross each × W1607 (unwashed)

Assembled: 2B: +++ 2A, 1C, 3C, 4C: –
2 colonies: – probably contaminants

Sources of F+ ?

Jan. 21, 1952.

F+ may be produced by other bacteria, fertile and infertile. Test by growing [illegible] overnight in broth with W1177, followed by streaking out on EMB to separate W1177 component. Cross extracted W1177 with W1607 for F+ test.

		W1177 found ✓		F status of W1177 (x W1607)
1 wg 1 K12		✓		+++
2	2	2-3 cols.		—
(3)	3	✓	lac+. Reisol. ✓	+
4	4	ca ½		—
5	5	✓		—
6	6	✓		[illegible]
7	7			
8	8	later —		
9	9	✓	(Mal) ✓ ✓	—
10	10	✓		—
11	31	✓		—
12	B	✓		—
13	ML	—	Mal many tiny cols! ✓	—
14	W1811	—	~~lac+ Reisol~~ — also SR.	(Aff) [illegible] impure Recheck (secure)
KO 1	16	90%		—
2	17	—	✓ ✓	+ ? (Recheck)
(W-1551-5) 155 (3)	18	✓		1 col. Recheck
4	19	½		—
5	20	✓	Mal ✓	+++
15	W1305			
21	S. gallinarum	✓		—
22	pullorum	✓		—
23	LT2	½		—
24	LT22	<10		—
25	LT7	<10		—
26	coli 1	—	few cols. ~~[illegible]~~ ✓	~~[illegible]~~ —
27	coli 2	⅓		—
28	forma	½		—
29	coli 5	✓		—
30	coli 4	✓		—
31	coli 3	—	v. few Pickson ✓	—

M14 broth [illegible] in 10 ml Penassay 10:45 AM 1/22.
Add sm 10 μg/ml 4:30 PM. Streak out (A) 9 PM. Replicate lac or Mal— next day.

Transmissible F+ seems to be confined to K-12. Interfertile strains are evidently not restricted by this mechanism. Excerpt ?

#14 again scored Lac+ on check although care was taken to pick only Lac- colonies!

Repick from same plate. Streak out Lac+ check test.

Sources of F+ ?

902a

			suspensions of ex-W1177	× W1607	
32	wg 6			++ ✓	Restreak suspension for A1 test.
33	wg 7	lac⁻ - "peculiar" later -	++	T	
34	wg 8			—	
35	11			+++ ✓	
36	12		+	+++ ✓	
37	13		—	T why? ✓	check - 37 is lac⁻ prototroph; wg 13 is lac⁺ prot. of possible recst.
38	14			+++ ✓	
39	15			—	
40	16			+++ ✓	
41	17		+	3? ✓?	Prototroph not found on request
42	18			—	
43	19			—	
44	20	all Lac+	—		
45	21		++	T	
46	22		++	~~T~~ — sec!	46 is lac⁺ auxotroph but wg 22 is similar
47	23			—	
48	24			1? ✗	
49	25			1? ✗	
50	26		—	T	
51	27		—	T	
52	28	lac⁺ also -	—	T	
53	29			— ✗	
54	30			1? ✗	

Reticulas: all +++ (×1607) above EMS lac⁻ spots

3B
14B*
18B EMBlac: all + Pick + streak from EMBlac scr.
18A2: Repick from same plate ⟹ *
14A2 (Repick W1177 colonies from same plate as 14A1) → + ⟶ ∴ W1811 = F+
14A1 (see above): Mixture of lac⁺, lac⁻: evidently not clearly picked.

* When streaked out on EMBscr, the mixed culture of W1811 + W1177 showed plaquing in the thick streak. Origin of phage?

W G-16 also found to be lysogenic on same basis.

Confirmation of F+ transfer from thus team

9036

Jan. 28, 1952.

A) The following wg x are fairly clearly F+ :

WG: 1, 6, 11, 12, 14, 16, 21

B) The following are uncertain F+

~~WG~~ 3, ~~17~~ W1553

C) The following were inadequately tested

wg ~~7~~ + ~~13~~, ~~21~~, ~~(22)~~, ~~26~~, ~~27~~, ~~28~~, ~~20~~

Note: wg 22 is asporogenic. A Mal-Lac- prototroph was obtained from wg 13 + W1177's selection

---

B: Repeat or verify with W1177

C: Repeat transfer of F+ to W1607 for comparison of F+ agent number/flows wg.

Repeat 36, 41, 44

B

36	smeared.	+ ?	+
37		✱ — def.	—
41		±	+
44		±	—
46	all −.		—
50			—
51			—
52			—

C. F(wg x) to W1607: 6, 11, 12, 14, 16 Label W1607 (F wg 6 ...)
Note: ~~D12~~, ~~D14~~ OK; D6, 11, ~~12~~ not F+ on first test See 908

D Transfer F+ to W1177: wg 6, 11, 12, 14, 16 ~~3~~ 21 ~~17~~ 17 ~~D11 not F+~~

E Test Kauffmann O Types (W1551−) for F+ to W1177. Label per O-Type
example: E3 = W1553
Rebuilt 3, 6-12. all F−

F+ transfer from W3x

February 19, 1952.

(12 exps) all recent attempts to repeat F+ transfer from W3x have failed. Technique consisted of brushing on sm loop pure culture of W3x. Are there F+ perhaps reverted to sm?

Repeat 902-C-D by two methods: (a by picking single colonies from mixed culture to E/B lac (b by interrupted streaking on EMB sm.

lac+: D12, D14

a: C16 D16 C11 D11 — mass cultures prove ~~OK~~ F+ proceed to isolation from single col.

Test from washed cultures

C12, C14 turbid or impure. Reisolate

C-D 3, 6, — no signs of F+.

→ C3: 1 lysed? colony noted (lac-). No peculiarity in controls

b. C-D 3, 6 — no F+

C11 F+ (weak)
C12 F+
C14 F+ (weak)
C16 OK. F+.

D11 F+ strong
D16 no F+

Isolate F+ from a, b as follows

C11 a, D11 a
C12 b
C14 b
C16 a, D16 a

Try 3 and 6 again

C, D11, 16 single colonies F- by pencil repl. plate test.

Reisolate 2 single colonies via broth tubes.

	A	B		pool	single col.
D16	−	−	C3	−	−
D11	+, ±	+	✓ C6	−	−
C14	±	+	✓ D6	−	−
C12	±	±	✓ D3	± 2 col	−
C11	++	+	✓ C16	−	− −
			C21	+	−
			D21	++	+ −
			C17	±	−

F+ transfers from mg×. 902cc

March 16, 1952.

A) The following are F+ transduced, but single colonies not yet recovered as F+ :

		S. col.
D 3	•	none in pool.
C 21	•	2 cols in #1, [redacted]?
D 21	no F+ even in mass pool	
C 17	" • "	" " #4 pool?
~~D 35~~	— ✓	single colonies: 8/8 F-?

tests passing: old cultures some F+ unstable?

B) Not yet transduced even in mass culture:

C 3	✓ ×	#1, 2, 4, 6/8	
C 16	✓ ×		
D 16	✓ ×	#/8	1 pool ×
C 6	×		
D 6	×		
C 17	×		

C) Not yet attempted:

C 35 ✓ - 1 colony in F+ bit of pool!
× ×

D) accomplished Rec 923

C - D 11
C - D 12
C - D 14
D 17
D 35
C 21 ?

Can wg x acquire F+.

1/26/52

1. Y10
2. WG-3
3. WG4
4. WG31
5. WG24 (W1713 L+)

W.9. 6	900-F1	
7	"	G1
8	"	G2

AO 900

Grow prepositus in 1 ml Penassay overnight. Mix with W1817 12N26. (A) — A27. Streak out on EMB Lac, (B) reisolate Lac+. Grow these with W1177. Reisolate W1177 and test xx W1607.
streaks 1/29

Notes: #2 + overgrow — in first step
#8 W1817 carried along with Lac+ in second step.
Save 903 A 6–8 for fertility test

Results
x W 1607

B.		
1	++	
3	+++	Lac−
4	++	Lac−
5	turbid	
6	+++	
7	+++	

If reliable, this would indicate that wg 3-4-31 could become F+ if not so already.

A. 1.(xW1607) — ! Recheck. (cf. B1) No recheck, xW1607, F+

B			
1	+++		
3	+++	WG4	] can become F+ on contact if not already.
4	+++	WG31	
5	—		∴ WG24 is F−, remains so

Repeat: Compare original WG4, WG31 as sources of F+ vs. F+ deriv.
=C =D

to W1177

✓ WG4,31 originals do not donate F+ to W1177 (v x 1607)

WG4, WG31 after exposure to W1817 become F+ donors.

W1177		x W1607
(WG4)	C	—
	D	+++
(WG31)	C	—
	D	+++

Interactions of wg x F+. 9035

February 10, 1952

Cultures from 902. C are W1607 F+ D are W1177 F+. (All 902)

1	C6	x	D6	− −
2	C6	x	1177	− −
3	C11	x	D11	− −
4	C11	x	1177	− −
5	C12	x	D12	++ ++
6	C12	x	1177	− −
7	C14	x	D14	++ ++
8	C14	x	1177	− −
9	C16	x	D16	− −
10	C16	x	1177	− −
11	W1607	x	D6	− −
12	"	x	D11	− −
13	"	x	D12	+ ++
14	"	x	D14	+ ++
15	"	x	D16	− 1 col ?

~~16~~	~~58-161~~	x	W1177	
17	A	x	"	3
18	B	x	"	+++
19	C	x	"	2
20	D	x	"	++

A = aerated overnight B = remoistened 10 min. ea. 3 hours
C " " " 6½
D " " " noaer., 6½ hours

~~All~~ cultures harvested at 11 AM; 1:15 PM; 4:45 PM and refrigerated to 5 PM for plating

Reisolate W1607, W1177 (F+, wg x)

W1808	x	1177	+++	
		1817	+~~+~~	
1809		1177	−	} of 1809 F+.
		1817	+	

W1611	161	++	
	1607	1 col	
	1177	−	
	1817	~~+++~~ −	

wg 4

F states ?

1205	1607	}	−
	1875	}	−
	1611		++

W1611 x	1607	} tumbled	±
	1875	}	++
	1177		±
	1876		+

W1145	1607	} tumbled	−
	1875	}	+
	~~+++~~ 1611		−

behaves like a partial F −

Segregation & role of F+ in outcrosses

January 26, 1952

					1/29	
1/27 AM	A	W1446 wg4	x W1607		T	} n.g.
	B	"	W1816		++	T
	C	W1451 wg3	W1607		12	wg3 probably F+
	D	"	W1816		18	
	E	1446	x 58-161		28	
	~~F~~	~~"~~	~~x W1830~~		~~6~~	
PM	G	1451	x 58-161		40	
	~~H~~	~~"~~	x W1830		1	

900E1

~~Status of W1830~~ n.g.
~~is still doubtful~~.

~~Applies to EMS~~ anyway as fresh D (0).

In view of 903-3, "segregation" would not be meaningful (Unless F+ can not be transmitted extracellularly in minimal.)

W1868 (wg12) x	1808 · 31	+++
	1607 · 1-	+++
	1875 · 1+	+++
	1451 · 3	+
W1808 (wg31)	1451 · 3	++
	1818 · 31	+++

W1811 Stability: Summary & Expts.

Lysogenicity

1/28/52

In course of 902 - 14A, plaques were noted in W1177 that had been grown with W1811. The lysogenicity of W1811 / 1177 was confirmed. The plaques are readily seen on sm agar (suppressing W1811 bacterial growth.) Check other post-cultures from Maas! all of his strains proved to be lysogenic on W1177. Their history is given as K12 → * KIT (ts) → KI put aboard. Presumably phage entered (mutated?) at *. ↓ ↓ Since KIT series is futile, the phage is not related to W1811 stability.

1/28 Grow W1811 c and s 58-161, W1177.

1	58-161 + (W1177 · 1811)	+	W1811 stability is not absolutely transferred in mixed cultures
2	(58-161 · 1811) + W1177	++	
3	( ) + ( )	++	
4	58-111 + W1177 + W1811.	+	
5	See 897 for control, 902-14B.		
6	(58-161 + 1811) + W1817	+++	

2/1/52 Further work on this phage assumed by E.T.L. W1811 has been verified as F+ (902). The phage acts equally on W1177, W1817.

1811 ×	1451	wg 3	~~cont?~~
	1611	√	−
	1607	1 −	−
	1868	12	
	1808	31	! ? : Repeat → 4 colonies
	1205	wg 29	5 colonies
	1145	wg 2	

W1678 - aeration effects and F character

1/29/52.

1/26/52: 1678A × 1607 ++++ F− !
1816 + F+ !

Repeat 1/29-30/52.

			1/31	2/1
1/30	1 W1678 ×	W1607	++++	✓
	2 "	W1816	+++	✓
	3 "	W1177	+++	+++
	4 "	W1817	+	9 cds.
	5 W1678 aer	1607	+++	✓
	6 "	1816	±	+
	7 "	1177	++	++
	8 "	1817	−	−

1678	1830	−
1817	1830 900 E'	−
1177	1830	−

∴ Of aeration has any effect on W1678, it is to decrease its fertility with F+. Do W1678 a "super" F+.

pll 907

Growth conditions and the F- phenotype

58-161

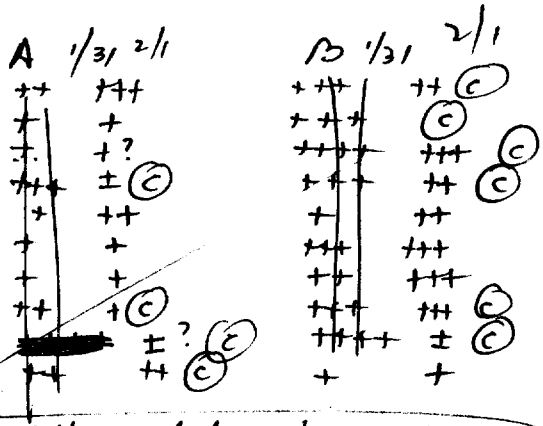
January 30, 1952

	Temp.	Vessel	Medium		
1	37	10 ml tube		aer	
2	"	Plate	- liquid		
3	"	tube		aer	
4	"	Plate	- agar		
5	30°	tube		aer	
6	"	"		aer	
7	"	plate		liq.	
8	"	"		agar	
9	44°	10 ml tube			v. poor growth
10	"	plate		liq.	

25-30° (5, 6)

A

× 1177 A
× 1817 B



As in previous expts., adjust approx. for cell density.

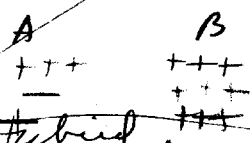
Note very high yields of 44° cells!

Many of these plates show contamination: (c)

Repeat comparison of 30°, 37° (30° thermostated).

58-161

1	37°	–
2		aer
3	30°	–
4		aer



plates ~~hybrid~~ : ~~contaminated~~

B

These results all n.g. owing to contamination

C

Growth conditions and F-phenocopy of various strains

2/1/52

	58-161 Temp.	Aeration		A: x W1177 x [illegible]	B: x W1817 [illegible]
1	37	+		— (2 cols.)	+++
2		−		+ ++	++
3	30	+		± (3+4 cols.)	++
4		−		++	++
5	1678-37°	+	x 1607	+++	+++
6		+	1816	−	7
7		+	1177	+	+
8		+	1817	−	−
9	1678-37°	−	1607	+++	++++
10		−	1816	+	+±
11		−	1177	++	+++
12		−	1817	+	4
13	1678	+	58-161	+	5
14		+	58-161 A	+++	++
15		−	58-161	−	40
16		−	58-161 A	[illegible]	++++
17	1816 ·		1177 ·		+++ (contam.)
18	1816A ·		1177 ·		−16
19	1816 ·		1817 ·		+19
20	1816A ·		1817 ·		+70
21	1817 [illegible] ·		1607 ·		+++++
22	~~1817A~~ 1177		1607 ·		−−
~~23~~	~~1817A~~		~~1816 ·~~		
~~24~~					

∴ 30° does not ameliorate aeration

1678 and especially 1678A are relatively infertile with F+, highly fertile with F−

Does 1816 respond to aeration?

2/2/52

23	161	~~W1177~~	−	++
24	161	W1817	++	+++
25	161A 37°	1177	+	++
26	161A 37°	1817	++	++
27	161A 26°	1177	+	+
28	161A 26°	1817	++	+++
29	161 Ag Plate 37	1177		5
30	" " 37	1817	·	+

(aeration was interrupted) [refers to rows 24–25]

} ? [rows 29–30]

W1817 crosses [illegible] show [illegible] W1177

2/1

31	161 44°	1177	++
32	"	1817	++
33	"	1177 44°	++

∴ 44° does not produce F− nor ameliorable fertility.

Conditions of aeration for F- phenotype 907b

February 4, 1952.

		A × W1177A	B × W1817	C = W1177	
1	58-161 37°	++	++	++	} inconclusive 1
2	" aer	++	+++	+	
3	" 26°	—	+++++		
(growth same as 3) 4	" aer.	—	++++		
5	W1816 × W1177A		+++		} inconcl.
6	W1816 A × W1177A		+		
7	W1816A × W1817		+++		
8	W1817 × W1607 A		+++		} inconcl.
9	W1817 A × W1607A		++		
10	W1817A × W1816		—		

× 1177. all cells washed stand in refrigerator 4 8PM

11	161 37° heavily grown	++	
12	161A "	6	
13	" "	±	} dilute media
14	" 1/10 "	—	
15	" 26°	1 colony	26°!
16	161A. Reinoc 10AM from 11 —	145 PM. +	
17	" " " 12	" +	
18	~~18=15 continued~~ to	—	
19	~~16 Reinoc~~ 2PM - 5PM	++	Moderate growth = unaerated saturation
20	17 " " "	+	

1. low temperature; dilute media do not mitigate aeration effect. Phase of culture cycle? (cf. 19-20 vs. 11; 16, 17)

Suggested experiment: Aerate cells to saturation. Assay. Reinoculate and assay at partial, complete saturation.

See 907c

A = aerated overnight B = reinoculated, aer ~~8~~ hours

C " " "

D = reinoc noaer.

21	58-161 × W1177	
22	" A	
23	" B	
24	" C	
25	" D	

cells harvested at 10:30 AM; , refr. to plating at PM.

2/10/52

A are aerated overnight (2/9 - 2/10 5PM - 12M).

1	58-161 × W1177	+	+
2	" A × "	−	−
3	909-1 × W1177	+	+
4	909-1 × W 909-4	++	++
5	909-4 × W 1607	+++	+++
6	W1607 × W1177	−	−
7	909-1A × W1177	−	−
8	909-4A × W1607	−	10 col.
9	909-1A × 909-4A	−	−
	909-1 pur × W1177	+++	
	909-4 pur × W1607	+++	

2/13.

11	1875 × 1177	++	++
12	1875 × 1876	+	++
13	1876 × 1607	++++	larger, more numerous than 12 & 11
14	1607 × 1177	−	−
15	1875A × 1177	±	+
16	1876A × 1607	±	+
17	1875A × 1876A	±	8
18	58-161A × 1177	−	1
19	58-161 × 1177	++	++
20	58-161B × 1177	−	6

} little effect of aeration.

B = 58-161A + Pennassay 4:30 − 6 . See 908-15,16

∴ no recovery in this interval.

2/15

21	58-161	× 1177	+	1
22	58-161A	× 1177	±	
23	58-161B	× 1177	+	

B = 58-161 aliquot suspended in supernatant of 58-161A for 2 hours, & recentrifuged.

∴ 58-161A supernatant had no effect on 58-161

Transduction F+.

2/1/52

ca. 10^9 each cell type in ~~Penassay~~ 3 ml. $3\frac{30}{}$ – 4^{30} PM.

			XW1607
1	58-161 + W1177	bre., Penassay	+++
2	58-161 A + "	" "	+++
3	58-161 + W1177	Defr. "	—
4	W1678 + "	bre "	+++
5	W1678 A + "	bre "	+++
6	58-161 + W1177	bre D(o)	—
7	" "	bre D(o) + MTLB,	—

For assays pool 2 (A) and 10 diffent (B) W1177 isolates.
XW1607. 2/6 A and B agund in each case.

∴ F is transfund in Penassay but not in synthetic medium under comparable conditions. This agrees with behavior of three-way crosses. Tryptophan in synthetic for longer periods. Brei extraction also seems to be necessary. Aerated cells, presumably phenotypically F–, still transfer F+.

8 58-161 + W1177 in D(TLB, BM) ~~#~~ aerated. ζ overnight.
9. " " " " "

Reisolate W1177 by streaking out and via sm.

The labels on 8, 9 were unfortunately lost. What was probably 8 failed to show transfer; in 9, 10/14 isolates were F+
Repeat experiment 2/11/52 (mor 10:20AM) – 5:10PM)

8 1F+/15 tot
9 5F+/12 tot.

also streak after overnight. 8-9A. 1st Replica tks n.g.

Transmission of F+

Feb. 12, 1952

11 —
12 sus 1000/ml. —
13 heat 56° 30m. —
14 boil 5m 1 colony ?

It has been previously established that W1811 washed cells do not evoke fertility in W1607 x W1177 on D(O) agar.

add ca 2×10^9 cells W1811 1 ml to 1 ml Penassay + ca 4×10^9 W1607.
Treat tubes as indicated. Incubate together from 12:30 — 2PM.
(Pretreat W1811 for heat experiments etc...). Rewash. Plate with W1177
This transfer technique; assay n.g. Phenotypic delay in F+ ??

15 58-161 + W1607 in Penassay
16 58-161A + W1607 " " } of 907 20.

test single colonies. Replica tests n.g.

Phenotypic ~~lag~~ + F+ transduction.

February 16, 1952. W1305 ⇒ W1177. Ca 10^9/ml in Penassay. 37° 2PM — 3:30

		x1607, immed.	
21	W1177 in Penassay	—	Single W1177 exctn.
22	(W1177 + W1305) in Penassay	+++	(Plates contam. but ca 10/15 F+)
23	W1177 original	—	
24	W1177 (Pen.) + W1305.	—	

1305 controls all —.

Phenotypic delay in F+ is not borne out by this experiment.

Bacteriocins [illegible]

90 S b

2/19/72.

W1305 from 2 TSA plates to 10 ml. 1 ml to 10 for assay. Later add W1177 = .
ca 10^9/ml each.

x ~~W1177~~ W1607

2 and 3 sterile
1 ca 2 1305 : 1 1177

1 No treatment. Incubate together 3PM – 5^{30}
2 Boil 5 mins
3 Heat 56° 30 mins.

1. showed many protoplasts; 2 and 3 – ?

but plates were contaminated!

Repeat.

4 Control
5 Heat 56° 30 m.

1305 + W1607.

x W1177.
+++ contam ?

W1305 : .1 ml sterile

W1305 stock ? or
plates contam.?

F+ from different sources.

January February 1, 1952.

Mix culture: Grow overnight. Streak out on EMB lac; EMB lac sm Rectivals from 1 to EMB lac → pure cultures. Pick from 1-5 cultures for pool for initial tests. Not s.c. pure!

✓ by crossing to W1177 or W1607 resp. all now F+

1	1607	K12
2	"	58161
3	"	1678
4	1177	K12
5	"	58161
6	"	1678

	×			
1	2	1177	++	+++
2	2	5	++	+
3	2	6	++	+
4	2	1678	+++	++
5	3	1177	±	++
6	3	5	±	+
7	3	6	+	+
8	3	1678	++	+
9	5	58-161	+	+
10	5	1607	++	++++
11	5	1678	—	4 cols
12	6	58-161	+	++
13	6	1607	++	++++
14	6	1678	—	2 cols
15	1678	1607	+++	++++
16	1678	1177	++	++
17	1678	58-161	++	+
18	1	4	+	+
19	1	1678	++	++
20	4	1678	+	6 cols

Summary.

	F^-	F^{161}	F^{1678}	F^{K12}	(BM test)
W1678 ×	++++	++	++ ÷ ++	÷ ++	÷
1607 ($\frac{F}{1678}$)		++	+	+	
1607 ($\frac{F}{58\text{-}161}$)		+++	+	+	

Note 1 × 4. Compare

1 × W1177
4 × W1607
1 × 4
1177 × 1607

∴ W1678 is relatively infertile with F+ chromosome derived from 58-161 or W-1678 or K-12.

F- reinfected with F+ from various sources behave in the same way. There is no evidence that W1678 carries a different F, but the infertility of F+ × F+ is emphasized especially in × W1177 F+.

2/5/52.

Grow 8 @ 16h 30° 3 TSH plates. Harvest, wash saline, dry overnight

2/5/52 Extract H₂O ca 5 ml. → A) Sup. B) Sediment

Extract B with 1½% saline overnight refrig.

Add 1 ml supernatant + 1 ml 10^9/ml W1177 to 5 ml Serum.

Inc 5:05 PM - 8 PM Streak out on EMB lac

2/6/52 No lact colonies seen. Pick individual and pooled colonies

(1) Replica xx test from EMB Streak

(2) Test 4 single colonies, and ca 40 pooled colonies.

x W1607.

all F— Crude extract: ~~neg~~ no transmission

Aeration effects

Febr. 19, 1952

I. Harvest 58-161 from 2 TS Agplates. Ca 3/4 of this into 10 mC fresh Penassay (~~broth~~ & 10^{10} cells/ml) aerate 12:15 – (3-4 PM ?) = B.

1. ~~58-161~~ x W1177
2. 58-161 A
3. " B plates contam: + x1876 +

Repeat: aerate in Penassay suspension of 58-161 A = B.

4 58-161 B
5 58-161 A
6 " + W1305 in Penassay. (Incl 58-161 ca 10^9/ml. 12^{25} PM –)
7 " —— " " (")
8 58-161

x W1177 — contam
contam.
++
+++

Compare transfer of F+

~~Transfer to~~

2/19/52.

W1177

1. 58-161 + ~~W1177~~ in Penassay — Ca. 10^8/inoculum.
2. " " aerate — 12 sc — 2P
3. 58-161A + ~~W1177~~ "

Stuehout and test results

1	2+/8
2	0/12
3	2+/12

∴ 58-161A donates F+. Aeration may inhibit transfer

1½ hr. min Penassay

1	58-161A	x ~~W1607~~ W1177	–	
2	" + W1305		2	
3	" + W1305A		1	
4	" + W1811		6	
~~5~~	" + W1811A		4	
6	" + W1607		5	=
7	58-161 + W1607		18	= 58-161 x comp?
8	58-161A - incubated		2	
9	W1607 + W1305		–	
10	" + " A		–	
11	" W1811		–	
12	" " A		–	
13	1305	no X	–	
14	1811		–	

From 4 and 5, strain F+ may have stimulated 58-161A but exchange even to W1607 was limited. Note: W1607 was aerated!

Todo: Compare 1607A ; 1607 as receptors of F+ from W1305. But note also low yields on 7.

W112 x W1435

Structure of diploids

2/19/52.

W112 x W1435
Lac 1b- Lac 1a- V_6^R Het.

EMS lac.
D(o) → EMS lac } ca 15 plates
ca 100-200/plate.

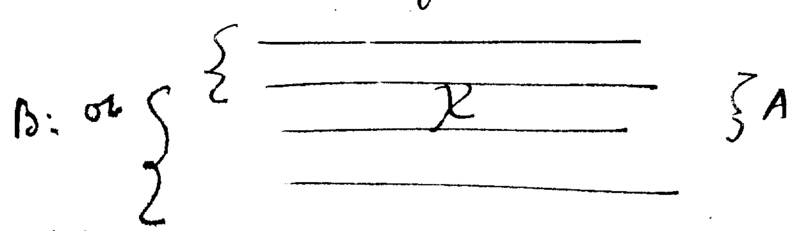
3 Lac+ found. 4+ in second set 4 in 3d.

			V_6		
	1	Lac ++	R	–	
	2	Lac ± slow	S	–	
	(3	Lac ++	R	–).	
H304	4	Lac v –	S		RS
	5	Lac ++	S		
	6	++	S		
	7	++	S		
	8	++	S		
	9	++	R		
H305	10	Lac v	S		
	11	++.	R		
	W112		R		
	W1435		S		

~~exd. #2. [illegible]~~
4R #7S
Lac- 1b Lac- 1b

Test segregants (mass streaking) of H-304-5.
H304 6 Lac+ all S 5 Lac- all R
305 7 Lac+ all S 5 Lac- all S sic !

H304 is therefore presumably A; H305 may be B.
(may have arisen by mutation in a $\frac{+\,-}{-\,+}$!)



If so, the Lac- of H304 should be stable, i.e. A-B-V_6^R
of H305 should be mutable A-B+V_6^S.

Pool the mutations of these – (pooled) and compare with parents.

Re-streak single clones, test +/+, 1- from each. I6.

H 304 7 lac- 1S 6R 8 lac+ all S

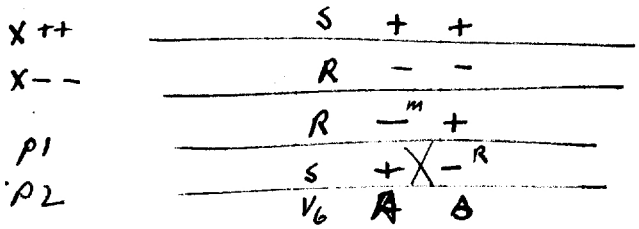
H 305 9 lac- all S 9 lac+ all S

This confirms previous page.

Check pooled lac- for unstability. cf. parents

	48 h. EMB	lac	
H 304	M	V_6^R	
H 305	S	V_6^S	
W1431	M	V_6^R	
W112	S	V_6^S	
lac+		V_6^S	

∴ These two lac- components appear to be parental, not complementary crossovers.



H 304 = x++ / p1

H 305 = x++ / p2

Repeat cross 3/13/52 8 plates ×400 EMBS lac 1? lac+ ✓

9 13-12 slow? lac+ 1-

Saved as H308

3/17/52. 4 × 600 2 lac+ 9 13: 13-14

13: probably lac v, sm. 12

14 lac+

3/27/52. 8 × 400 cols. 4 lac+ ✓ + v? → weak + few-sm? + + 15, 16, 17, 18.

5 × 300 0

February 22, 1952

Clearest comparison would be $W1368^{+R} \times W677^{-s}$ × $W677^{+s}F+$. Latter is being prepared.

2/22. Crosses on D(0) ± sm.

		D(0)	D(sm)	
1.	$58\text{-}161^{+s} \times W1177^{-R}$	++	+ =	] agrees with Hayes
2.	$W1368^{+R} \times W677^{-s}$	+++	— =	
3.	$W1802^{-s} \times W1876^{+R}$	+++	—	
4.	$58\text{-}161^{+s} \times W1876^{+R}$	1 ?	—	
(5)	$W1875^{+R} \times W677^{-s}$	+++	—	

2/25

			1-2 (0 / SM)	3-4 (0 / SM)	
11	1368^{+R}	677^{-s}	1, 2	3, 4 +++	| —
12	1368^{+R}	$677^{+s}F+$	1 + ±; 2 + ±	3 + ±; 4 ++ —	1
13	1607^{-R}	$677^{+s}F+$	1 +++ ✓; 2 +++ ✓	3 +++ ✓; 4 —	|(1-4): ± (14 cols.)

2/26

14	1802^{-s}	1876^{+R}
15	58-161	1876

F+ ? pooled. 1-4

∴ Hayes' observations are again confirmed. Also 677F+ becomes competent on sm agar, and the sm effect is related to compatibility.

677F1 - save F+ F+ F; use F1 hereto = W1896

			0	SM
16	1895	1177	+++	+++
17	"	1896	++	+
18	1811	W1177	—	—
19	1678	1177	+++	++ ±
20	1678 ~~1895~~	1876 1811	1 col. 2 ?	—

note mar sterility F+ × F+

Repeat a). (old 1368 susp.)

	0	SM
11	++	—
12	+	—
13	~~+++~~	—
14	+++	—
15	++	(—) ?

b) Result not confirmed!

0	SM	
~~8~~ +	1 col?	medium?
8	"	
~~8+~~	(3)	
++	—	
+	—	!

Try $1678 S^R$

			D(o)	D(sm)
3/10/52.	W1368 x W677		++	0, 0
		x W1896	+	2, 1

conclusion upheld (F+ and resistance to smuffle, but not shielding by).

3/12/52. Repeated:

		D(o)	D(sm)
W1368 x	677	+++	0
	1896	+	3

Again. Also note greater compatibility of F+ x F-.

~~The survival factors may be due in part to the low fraction of S^R protoplasts in the 1896 x. Better to use a comparison of~~

Review other test crosses for role of F+. F+ x F- combinations maybe more futile

				EMS/Mal	D(o)	D(sm)	
A	W1876 x	58-161	ca 15%	+++	—	—	! of W1177 x 58-161
B		W1802	"	+++	—	—	

Repl. to EMS 1a 2, Mal

as before, 1802 and 58-161 are similar x W1177 F+

Repeat x 1177 also! see 915.

				D(o)	D(sm)	
A	~~58-161~~	x	W1177	+++	+++	∴ Hfr behaves as F+
B	"	x	1876	+++	+++	in this context.
C	1802	x	1876	+++	—	

3/19	D	58-161	x W1177	+	3	Yields generally
	E	"	x W1876	++	=	=
	C	W1802	x W1876	+++	=	low. Bears repeating.

		D(o)	~~R~~ D(sm)	D(sm)	
3/22	D	++	5^S /49	~~4~~ 20	Note futility of C
	E	+++	56R /ca 200	—	on sm as expected.

Proportion of breakthrough / segregants S^R is evidently greater for D than E. F+ x F- S^R differs from F+ x F+ S^R.

This consistent with the concept of "relative potency" of BM and TL whereby BMF+ x TL is actually BMF-!

Equivalent of parents in F+ x F−

915

A. W1802 (BM F−) × W1876 (F+)

B ~~58~~ 58-161 (F+) × W1177 (F−) ± Sm (A+ ; B−)

C. 58-161 (F+) × W1876 (F+)

also see Genetics, 1947.

915a was conducted by Mrs. D. C. Gooding. It appears to show that W1177 F+ behaves like filial W1177! Repeat and of other F+ strains.

3-3-52
[illegible]

A

lac	mal	SM
+	+	S
+	−	S
+	+	S
+	−	S
+	−	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
−	−	R
−	−	R
+	−	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	−	R
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	−	R
+	+	S
+	−	R
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	−	S
+	+	S

B

lac	mal	SM
−	−	R
−	−	R
−	−	R
−	−	R
−	−	R
−	+	R
−	−	R
+	−	R
−	−	R
−	−	R
−	−	R
+	−	R
−	−	R
−	−	R

B cont.

lac	mal	SM
−	−	R
−	−	R
−	+	S
+	−	R
−	+	S
+	−	R
−	−	R
−	−	R
−	−	R
−	+	S
−	−	R
−	−	R
−	−	R
+	+	S

C

lac	mal	SM
+	+	S
+	−	R
+	+	S
+	+	S
+	−	S
+	+	S
+	−	R
+	+	S
+	+	S
+	+	S
+	+	S
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+	−	S
+	+	S
+	+	S

					D10) Rep		
			mEMS Mal			Vac	Mal
note near equal +/-	A	58-161 ×	W677	−	> +	24+ : 20− ;	20− : 5+
	B	"	W1177	−	> +	~~28~~ 18+ ! !	40− 6+
	C	"	W1817	+	> −	50% −	8− 62+
	D	"	W1876	+	> −	5% −	<100% −
	E	"	W1896	+	≥ −	61 + 6−	ca 10% −
~~⇄~~	≡	1368	1896			10+ 4−	
		"1868	677			23+ 39−	

. . . aberrant ♯ behavior of filial TLB, − stocks is entirely explained by their F+ character !! Instability of segregation ratios is fully explained.

Is phenotypic modification of TL F+ parent possible (by acetate ?)

See 915a for quantitative data here quantitatively confirmed.

				Lac	Mal	D/o	Lac Mal D/o), EMS Lac or Mal.	
Glyfed { A.	W1678	×	W1607	—??+ —??+		++	—> +	no effect of F++ on lac segregation obscured by linkage?
B	"		W1875	3- 2-		++	—>?+ !	
Prol/lac linkage? C	"		W1177	9-	3+ 12-	++	—> + ca 20% M+	
D	"		W1876	2- 1+	1+ 21		all lac-? 6M+ 12-	also mostly S^R
Expt Meth. E	W1687		1607		++			
F	"		1875		++			
G	"		1177		1-			
H	"		1876		++			

J. 58-161 × W1177F+ : 902 D 12 Mal: 13+ : 13- ! Partial effect?
K 14 Lac: ca 90% +.
L 17 Mal: ca 35- : 8+ minimal effect! numerous sectored cols.

M 1802 D12 ca 90% Lac+
N D14 " "
O D17 1 Mal- 2M+ low fertility!

Restreak D17 to verify purity.
Repeat D12.

A-B. Lac segregation similar in W1678 × W1607 — 1875 (F — F+)
C-D " " " " " ; Mal+ may? be increased in × F+ ?

C-D should be repeated.

	Lac	Mal	MtL
C	ca 10% +	4+ 1-	32+ 42-
D	2+ 10-		2-

		Mal	
1802	1177	ca 5% -	
1895	1177	ca 10% +	many sectored
"	1876	"	" "

Thus confirms:
a) 1802 ≅ 58-161 × 1177
b) 1895 × 1177, 1896 ≅ 58-161 × 1177

Repeat J-O ē single cols.

		#	%Lac	Mal
58-161	D17 -1		— ≥ +	
	-2		+ > —	
1802	D17 - 1	—	~~[illegible]~~	
	-2		+ > —	
161	D12		ca 30% Lac-	ca = +, -
1802	D12			ca 30% Mal-

∴ D17 result above probably due to admixture

D12 should also be repurified and studied

915
SUM

Conclusion: in following crosses the bacand Mel markers follow the parent indicated first:

	×		F (key fertility)	
	W1177, W677	58-161	−	+2
	58-161	W-1876, W1896 (also W477, etc.)	+2	+3
	W1802	W1876	−	+3
	W1607	W1678	-	+5
	W1875	W1678	+2	+5
	W1177	W1688	−	+5
	W1876	W1678	+3	+5
	W1687	W1875	+1	+2
	W1687	W1876	+1	+3
	W1177	W1687	−	+1 nearly infert.
	W1607	W1895	−	+10
?	W1177	"	-	"
	W1875	"	+2	"
	W1876	"	+3	"
1892C	W67	W1649	±	+..
	W1177	W67	−	±

~~Sa921~~
921, 915d. 916a.

Hfr. 916

2/27/52

"Hfr" received again from Cavalli ca. 2/24/52, after retests on W1033 showed no Hfr activity. Store as W1895.

a) Platings of W1895 at 10^8 and 10^6 per plate, in comparison with 58-161 showed 100-1000 x as many prototrophs

Prelim. b) Effect was same x W1177, x W1876. ∴ does not depend on F- Opposition;

c) In one experiment, aerated Hfr was still F+ (same yield as 1177, 1876) but not highly fertile as Hfr. Control showed unaerated Hfr still +++ fertile.

d) 1895 dil. x W1611, 1678 → few prototrophs! Repeat

e) 1895 of A. showed Hfr x W1177 ± aeration
also Hfr x 1876 does not accord with c).

3/10/52 f). 1895 - 1895A. x1678. Showed Hfr from dilute plating, but A +++ — ++
x 1177 A ++ — ++++

This may reflect a partial F- phenotypy effect of aeration.

g. Grow W1895 in broth with W1607, W1177, W1876. Streak out, recover, and test by replica plating

A. 1895/1607. 1895 12 cols. Hfr x W1177
1607 2 " Hfr
F- or Hfr? = 916G-2

C. 1895/1177/1876. → 11 cols } Hfr x W1177.
→ 11 " }
∴ No back-diminution

B. 1177/1876/1895. 1177: 8 cols. + Hfr? x 58-161
1876: 11 cols. No cols x 58-161
Both: No cols x W1607.

Is this W1177 F- Hfr? Recheck after isolation. = 916G-1

(These mixtures should be repeated)

W1607/1895 = 916G-2

3/12/52 M. Recetion: suburb.

	conc.	1177	1876	dil.	1177	1876
1895		++++	++++		+ all H+ - 2/3 Lac-	+ all H+ - ; 2/3 Lac-
1895A		+	+++		1	4

student out on NSA: By replica 20/20 all Hfr!

∴ Again, there seems to be ① an incomplete effect of aeration on the F+ character and ② a depression of Hfr in compatible crosses.

Note absence of F+ effect of W1876 x 1895 cf. 58-161

3/12 ∴ Repeat, using "moderate dilution"; ca 3×10^8 cells 1895 or 1895A plated to

	1177		1876
1895	1 +++	2	+++
1895A	3 ++	4	+++

Repl. to EMS lac. No marked effect.

lac-:lac+ Estem.

1	3:1
2	2:1
3	2-3:1
4	3:1

∴ again, W1895 x F+ shows no modification of segr. ratio!

916 "gA" 1-2. 1607/1895 Both gave seemingly very high yields with W1876, — with W1177. Same question arose with W1177/1895. See above. ? Hfr. gA1 = 916 & 2.

W1875 tester was day-old. Today's broke!

916 & 1	x 1607 —	
	x 58-161 ++	
	x 1875 +++	
dil.	x 58-161 29	
1177"	x 58-161 10.	

Closer comparison required!

as half-plates:

916 & 1	x W1875	— !	
W 1177		++	
916 & 2	x W1876	+++	
W 1607		+++	

Is close comparison they were very similar 3/21/52

Is 1895 x W1876 less fertile than 1607 x 1876?

M Repeat transfer of F+ from W1895: Grow with 1607, W1177 in broth as usual. Treat as EMS on agar. Pool 40+ colonies:

C	W1607 / 1895 /	x 1177	—
D	W1177 / 1895 /	x 1607	—

∴ again Hfr does not transfer F+!

① Is F+ found in Hfr? ② Is it absent? Test pooled prototrophs from 1895 x [redacted] Y10

Summary

3/11/52. h. aeration of W1895 → ~~[illegible]~~ a partial effect on F+, Hfr.
In streak plates, 1895A on nutrient agar gave 20/20 all Hfr.
1895 gave normal Lac, Mal- ratios × 1177, or 1876

3/12. i Similar result.

j. W1607/1895
W1177/1895 Neither were modified, either re F+ or Hfr.

k. Similar result. → l. do. But 916 G1 on half plate test was −. (presumably error of k.)

m Retrial of j, via sm agar, for F+ from W1895.
Both W1607, W1177 (pools of colonies) remained F−.

3/16 n Retrial of m. 1607
1177 → Remain F−, with +++, +++ × F+ testers.

o 1876/1895. ✓OK. 1876 remains F+.

p. Retrial m:

		1607	1875
A	~~[illegible]~~ 1177/1895	+++	++
B	1177 / 1033	++	+
C	1177 / 58-161.	++	+

[Results (1607)! − Is this correct? A] + proved to be impure (Lac+) presumably residual 1895! Repeat]

q. Is 1895 more fertile than 1607 × 1876? Kinetic study may be required.
Hfr does seem more highly fertile Test 2.
about =. Test 2

R. Repeat p

		× 1607	× 1895
A	~~58~~-161 (1177)	+++	++
B	1033	+++	++
C	1895	−	+++

∴, as before, Hfr does not transduce F+!, but W1033 does.

Methyl-naphthaquinone effects.

3/8/52.

Y. Colwell 1946 J. Bact. 52: 417.

(D/O) + MNQ (from 1% solution in acetone). Strain K12.

A 24h. B 48h.

1	0		++
2	.0002 %		+
3	.0005		+
4	.001		+
5	.002		−

A. Streak out on nutrient agar. No "petites" observed.

B. Re-streak. No petites " . #5 was sterile.

Critical concentration may be between 4 and 5.

		A) 24h	B) 48h	A	B
C.	1. 0	+++	+++	A No petites	
	2. .001 %	+	++	" "	no petites.
	3. .0015	−	−	Few saw. pet?	Streak
	4. .002	−	−	"	"

3/19/52 Colwell's observations are not confirmed with K-12! Write him for details

3/27/52 Repeat with Colwell's #II strain = 776-1763 = W1939

		24h	colonies	48h.	colonies
1	0	++	++	+++	−
2	.0005	+	++	++	++
3	.001	−	++	−	mostly +, some dw? Restreak.
4	.002	−	some dw?	−	1+ 1 dw? (practically streak)

"dw" grew out to full size.

Repeat 4/21/52

		24	48h	colonies:
3/31/52 1	0	++	+++	
2	.001	−	++	
3	.0015	−	−	one small but gave +++ on re-streak.
4	.002	−	−	

Colwell specifies previous transfer on minimal. Try this with W1939 A (single col. isol.) No luck.

MNG ... continued

917A

September 16, 1952.

9/13. Colwell sent 4 transfers again: #1 = original coli transferred on minimal medium. = W1939A
#2, 3, 4 = "SCV" selected with guinine.

9/14-15. Characterization of SCV verified. #1 grew promptly to give large colonies on NSA. #2-4 gave hardly visible colonies in 24 hours. By 48 hours, ca .2 mm colonies.

9/15. Inoculate 4 agar plates to D(o); Penassay:

9/16: All cultures grew very well, #1 perhaps slightly denser
By D(o) #1 grew more poorly than others! for further work, use 1 and 2 only.

9/16. Inoculate 917-1 and -2 from Penassay to:

	1	2	2 days
D(o) liq.	++	+	
Penassay	+++	++	+++
NSB	++	+	
NSB + glucose	+++ acid agglut	++	
D(o) Agar	++ 2 days	±	
NS Agar	+++	±	
EMB Glu	+++	+	
EMB Lac	+++	+	

Also, mix 1 and 2 ca 1:5 and streak out — no satellite effect obvious.

Growth on D(o) comparable to NB, NA. ∴ not likely single tryptophane requirement. In her paper, Colwell refers to Mac Leod's "synthetic medium" but does not specify whether HC was added.
In experiments with W1571 (HLB yeast) MNG .002% was lytic in standing tubes. 2 unique was lytic only in aeration. Usefulness in place of penicillin still to be verified.

Walesman strains
= W1906

March 10, 1952.

A. W1177 + W1906 in Penassay; streak out on EMB Maltose. Walesman phage noted again
Pick single colonies for F + test. x W1607. 5 singles — | 6 singles
Reisolate single colonies = 902 D35 pool ++ ++. | 3 + / 3 — / pool ++ (+3)

B. Crossing tests: auxotrophs.
W1907 - 1908 - 1909 control platings — — .
1907 x 1909 —
1907 x 1607 —
1875 —
1909 x 1177 ~~++~~ contaminant coli? Replica to EMS lac: poor growth. prob. contam. Repeat
1876 —

C. W1846 — —
" x 1177 —
A " x 1876 4?
B " x 1607 1 } → all Lac— Mal+ prototrophs. Fertility suggested.
" x 1875 —

D. Walesman. SRP x
1177
1876
1607
1875 } ca 20 Mal+ no — each! This would have been classified as doubtful fertile to be retested.

E - F (W1852; W1909)

		E	F	
1	1607	—	1?	ng
2	~~1875~~ 58.161	—	~~||||~~	
3	1177	(1) —	—, —	
4	1876	—, 1		
5	1808	—	4 —	ng
6	1678	—		

E 3 grew others did not

∴ part TS strains may be detectably fertile, others not (yet)

1177 + 1909 : grown sep and together : —, —

1875 x . . ?

WG-35 x

918a

March 28, 1952

1.	W1852 ×	W1177	37°	=	
2	"	"	+ pant.	=	
3	"	"	+ pant. 30°		ca 20 cols. eventually
4	" ×	W1895			
5	W1846 ×	"	+ phage-ridden		
6	W1907 ×	"	+ " "		heavy background lysis
7	W1908 ×	"	ca 1000		

∴ Hfr does allow crossing of wg35 × wg 1., but W1908, not W1907! (φ^R tests should be used)
EML is working on the extraction of Wollman phage and transfer to wg-1.

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ca. 4/10/52. EML noted Wallas. to be sucr + - ± on EMB sucr.
Comparison of W1906; W1811; W1852; K12 shows the first 3 to be alike, strengthening conclusion of origin from wg 35. Cf. Shaw for cross-reaction (H?) of wg-1 × wg-35.

4/15/52. Strains received from Maas: K1t-p A) K-1t h2 B).
Grow together in Penassay 3h. Plate ca. .3 ml per plate

W1177 · A	No prototrophs	
W1177 · B	"	
A · B	Minute colonies; background growth (cytotoxicity?)	
A ·	1-5 " "	
B.	" "	

?? Was K1t-p or -h2 ever properly crossed with # wg 1? as claimed by Maas? WG-35 behaves in my hands throughout as a nearly sterile wg×, but there is some likelihood of crossing with other wg's.

March 10, 1952.

W1903 (= W1678 S^R) x		EMB Lac	Pick
A W1325	40-50/plate	ca 2-:1+	ca 30
B W1178.	5-10/plate.	ca 5-:1+	ca 10

Pick Lac+ and streak on EMB Lac to look for Lac v.

A. 1 Lac v → Lac v Mal-

B. 5 Lac v → Lac v 4 Mal- 1 Mal+. No Mal v!

Presumably Mal- are hemizygous and Mal segmental elimination also occurs in these "outcrosses".

C. W478 x W1876 (for formal statement). EMS Lac, Mal. + >>-.
EMB poor - differ. uncertain 40 tests - 8? rechecks.

Recheck from EMS Lac.

4 more? / 40 tests.

✓ V, P photograph for W1927 Wahl

		Lac	Mal
from EMS	1	+ ✓	+ ✓
	2	+	−
	3	+	−
	4	+	−
	5	+	−
	6	V	+
	7	+	+
	8	V	+
From EMB	9	+	−
	10	+	−
	11	? ✓	+
	12	+	−

None of these are useful for reversion analysis.
cf. Mal status of W478 x W1177

Should try W1178 x Y10F+

March 14, 1952.

58+161 + W1177. Contacted and separated. ERL micromanipul.

3/12/52 Crowing cells contacted on Nutr. agar. Pools assayed x W1607

			count		F
1.	On needle	5 min	59		−
2.	colonies mixed	2 hours.	65−	303+	−
3	control.		50		−
4			50		
6	⟩ → ≪	30"	42		−
5	control		88		−
9	⟩	10"	57		−
11	Near	30 min	79		−

3/26/52.

1	−
2	−
3	−
4	++
5	−
6	−
7	−
8	++
9	−
10	−
1876	++

3/21/52

#	no cells positioned	no. after 3 hrs.	no. on plate (5-6 hrs) Lac −	Lac +	hrs. in contact
1	3	8, 20	13	30	½ - 2 *
2	2	32	16	crowded	1½ - 2
3	2 (1 dead?)	17	14	0	4
4	3	16, 16, 16	27	crowded	0 - ¾ *
5	2	16, 16	27	65	½ - 1 **
6	2	38	14	crowded	1½ - 2
7	3	27, 13	7	"	0 - 2
8	2	60 (4 hrs.)	22	53	1½ - 2
9	2	14, 12	17	crowded	¼ - 1
10	4	15, 13	35	50	1 - 2

Dividing cells from 58-161 and W1177, placed near each other, grown at room temp. 2-4 hrs before the microcolonies coalesced. Hrs. in contact is the time from the first observance of coalescence until the mixed col. was picked up and plated on E+B. agar.

* When >1 cell was present originally, it was not definite which coalescence brought + and − together.

** #5 2 microcolonies were mixed with [illegible] after 4 hrs. when each was about 30 cells.

Inheritance of Hfr.

3/17/52

A W1895 x W477
B " x W677. Very crowded. Pool and restreak.
C " xW1896

A. 12 picked. 2 probable Lac v. Restreak. (2 Lac-). → 1 Lac v = 921A

Crossover?
1 Lac- M-
2 Lac+ TLB₁
3 Lac- M-
4 Lac+ TLB₁

B. Pool tested for transduction to W 1607. All F- by transduction test! See over

C.	Lac	Mal	streak on EMB sm	Pretty high transduce F
1	+	+	Lac	+
2	+	+	Lac	+
3	+	−	Lac	+
4	+	+		
5	+	+		
6	+	−		
7	+	+		
8	+	−	Lac	+
9	+	−		
10	−	−	Mal	+
11	−	−		
12	−	+	Mal	+

Test for transmission of F+ to W1177. Test exposed W1177 / W1607

921 B should be tested for fertility x W1177, W1817

A)
1 A1 x A2 — 3
2 A1 x 1177 — 2
3 A1 x 1876 — 16 →R 14 S^s

Therefore $F_{A2} > 1875$, $F_{A1} < 1876$
4 A2 x 1607 — ++ →R (1/0) lac sm all − R
5 A2 x 1875 — + " 1+R.

∴ A1 behaves like a weak F+

A2 ~~hats~~ like a moderate F+. A1xA2 not acctd for (unless F ÷)

Replica A1-1876, A2 x { EMS lac sm

In these experiments, Hfr behaves like an F −, with F+ Hfr not Hfr.

921B #1 = lac+
2-8 lac-

Prior to Permissory for
SRP x 1876
1177

Prot. (W1895 x W677) F states by SRP test

921B

~~3/30/52~~ 4/3/52

#1 Lac+ 2-8 are Lac-. Run 1 ml Penassay to prepare SRP x 1177, 1876

		1177		1876
1	-	0	+	46
2	-	0	+	22
3	-	0	+	47
4	-	0	+	101
5	-	7	+	42
6	-	0	+	38
7	-	4	+	109
8	-	0	+	77

Second test

1177	1817	control	
0	370	0	
0	108	4	* prec. SR mutants.

Can these be re-F+'d ?

	1177	1817	control
11-1	236	27	54
11-2	128	7	21
1	ca 150	5	0
2	21	2	0

Hfr x F- → F- prototrophs which can be transduced F+.

✓ on EMB Mal.

F+(wg x) Crosses 923

W1177 x		1876	1678
c11	−	++	+++
c12	−	+++	+++
c14	−	+++	+++
1875	+	+	++
1678	+	!	
1607		++	+++

d 11		d12		d14	
c11	3	c12	+	c14	+
1607	2	1607	+		+
1875	++	1875	+++		+++
1678	++	1678	++		++

c11-12-14 have evidently became again F−

d " " " have retained F+ character, but are much weaker F+ than W1876. D12 (see above ≠ 915 d) may have became mixed F+/F−

March 26, 1952.

R = rutin Q = quercitrin.

Cells from 24 hr Pennazzay. 58-161 and W1895 1 ml / 10 Ben sup
1:15 PM — 9 PM.
× W1177

1.*	58-161	Sup. —	+
2.	"	Illuminated (Hanovia/glass) 60 secs.	++ (I)? Increased over ?
3.	"	Rutin 1/2 × 10^{-4} = .05 mg/ml	++
4.	"	Quercitrin "	++
5.	1895	—	++++
6	"	Rutin 1/4	+++
7	"	Rutin 1/2	++++

B = plated with .25 mg rutin per plate : 1 +, 5 ++++

*tube broke/ Use residual cells from inoculum tube.
in centrif.

Rutin had no effect!

March 27, 1952

					2 HL/10	11AM – 2 30 PM	
1	58-161				+ (19)	1B (Rutin) +I 65	1C + (15)
2	"	Light 60s.			+		
3	"	Rectin	1/4		+		
4	"	Quer	1/4		+		
5	W1895	—			+++	5B +++	(5C: illum Hanovia-glass)
6	"	Rutin	1/4		++++		

Recheck i control for Rutin effect on 58-161 x W1177!; light on 1895x.

58-161, W1177 grown in dark (red glass).

b.

1.
2. Fluorese lamp 10 min.
3. Hanovia (through glass) "
4 58-161 x W1177 grown without dark precautions

1:	56, 57
2:	64, 61
3:	54, 85
4:	98, 74

no significant effect of light.

F+ Hormone from W 1895

March 26, 1952..

A Cross W1895 with W1607. Plate on D(o), D(sm), EMS lac. x Y10

B. Control septum, grown separately

C. " components. 1 1895 x ~~W~~ Y10

2 ~~W~~ 1607 x Y10

D streak out 1607/1895. ca ½ % lac+ ! SR

A. D(o) +++ $\xrightarrow{R}$ EMS lac sm 4 SRP. later ca 20 addtl. SRP, some lac− !

D(sm) No SRP. 3 days. ca 100 !

EMS lac ① ca 1000 lac+ No lac−

B. D(o) +++ $\xrightarrow{R}$ ② 500 + ① ? 925A1 streak out + check

D(sm) ①

EMS lac all + 6 SRP, lac+, −

C1 D(o) +++ $\xrightarrow{R}$ EMS lac sm 0.

C2

In view of possibility of intercalary crossing of W1607 x W1895, these data provide no support for a hormonal control of F+ grade in a situation where F+ transduction does not occur. See 928

Delayed crossing probably occurs on EMS sm plates.