DATE: July 7, 1984 REF: Y61

	1	2	3	4	5	6	7	8	9	10

W25 83 x 2639 .5 : .5 : 10 12 15 PM – ca 2 30 PM

(So many pairs became stuck there is no advantage to using excess of non-motile parent!)

		cell	drop	lac
A 1	4	1	1	
2	2	28	28	
3	1	1	1	
A 4	x	B	1	1
B 1		00.	28	
2		1	0	
3		1	1	
B 4		0.	28	
5		1	1	–
C 1		00.	28	
2		1	1	
3		1.	1	–
C 4 5		00	28	
5 6		①①	1	1 –
D 1		①	1	
2 3	X	B	1	X
3		①	1	28 ..
D 4		① ① 4/ ㉘ ①	1	1
5			1	
6		①	1	–
E 1	5 00!	1.	1	
2		1.	1	–
E 4		0000.	28	
5			①	–
F 1		00	28	
2		1.	1	
3		1.	1	–
F 4		0.	28	
5		1	1	
6		1	1	–
G 1		00	28	
2		1	1	
3		1	1	–
G 4		00	28	
5		x		
6		1	1	–
H 1	very close	0000	28	
2		① lost?	0	
H 4		0.	28	
5 6		0 ① 8?	1 1	–

1 - Lac –
28 - Lac + except E5. lac ±

prob not curved

♂ × ♀

162

~~10~~ – 105 – 250 +

July 8, 1954

(Rickenberg demonstr.) ♂ × ♀ 1:10. Note v. high incidence of pairs.

		cell	drop	lac
A 1		0	28	–
2		(1)	1	+
3		0.	●	
4		(1)	1	+
5		0	0	
6		0	0	
B 1		(1)	0	
2	uniform	0	28	–
3		0		
B 4	✓	(1)	1	+
5		(1) 0.	28	–
6		0.	28	–
C 1		?	1	+
2		(1)	1	+
C 4	✓	(1)	1	+
5		0.	28	–
D 1	✓	(1)–(1)	1	+
2			28	–
D 4		(1)	(1) 1 (28) — D3 + –	
5		0.	–	
6		0.	–	
E 1		(1) (1)	0	
2		0.	0	
E 3			0	
4			1	+
5			–	
6			0	
F 1		(1)	●	
2		0.	28	–
3		0.	0	
D3R1		0. 0.	● ●	
G 1	✓	(1)	1	+
2		0.	0	
3		0.	28	–
G 4		(1)	1	+
5		0.	0	
H 1		(1)	1	+
2		0.	28	–
3		0.	0	
H 4		(1)	1	+
5		0.	28	–
6		0.	0	

but see scores after 1179

scores dubious not combined

B4L not spec. Looked for presumably absent! (large body?)

Many movable. Complete marked – No (2)!

July 10, 1954.

164

W2640 : W2639   1:50. 1^{05} - 3^{20} pairs ~~suff~~ infrequent
Either plated at ca 3^{30} while I isolated.

			dep	#	Interest	
A	1	0	28	39		
	2	1)	0			
	3	1	1	1	P	F+ .... ✓
A	4	00	28	40		
	5	1	1	2	P	
	6	1	1	3	P	
B	1	~~x~~ — 0	1	4	P ??	
	2	,	1	5	P	
B	4	all motile	1	6		
	3		0		1P	
	5		1	7		
	6		1	8		
sig C	1	0	28	41		
	2	(1)	1	9	P	
	4	00]	0			
m.	5		0			
	6		1	10	P'	
D	2	[illegible]	0			
	3		1	12	X	

15 del. random. 0 F+
7 del pairs 2 F+

D4-6 (13-15), E4-6 (18-20), F1-6 (21-26), G1-6 (27-32) are random isolates of motiles (at end)

			#		
H	1	all motile not pair.	33	} 1P F+ }	
	2		34		
	3		35		
H	4	[illegible] 0...]	1-28	36	x	(28+1).
	5	(1)	1	37	P.	F+ ✓
	6	(1)	1	38	P'	F+ ✓
E	1	0	28	42		
	2	1)	1	16	P	
	3		1	17	P	

∴ 1-18 and 37,38 should be checked as pair F progeny
39-42 are pair F+
6-8 and 33-35 may be illegitimate pairs

to EML (picked to [illegible] for assay P11)

DATE: July 12, 1954 REF: 156 165

× 2 - 2+ hour intervals as in pre-pair experiments. Crosses might have been confused?

Note fairly numerous colonies type 28 Lac+ and −

Lac type (28) O Recorded as 28 digs

156

C4 −	A1 −	B5 −	D6 +	G4 −
E1 −	2 −	6 −	E2 −	5 −
F2 −	3 −	C1 +	3 −	6 −
G2 −	4 −	2 −	4 +	H2 −
H1 +	5 ?+	3 −	5 −	3 −
→ 1178.	6 +	5 −	F1 −	4 −
	B1 −	D1 −	4 −	5 −
	2 −	2 −	5 −	6 −
	3 −	3 −	6 −	
	4 −	4 +	G3 −	

10/8 what is this expt? Where are prototrophs. presumably occurrence of (Z) among non-paired ♀ isolates.

165

A1 −	A3 −	D4 −	F5 −
2 −	5 −	5 −	G1 +
4 −	6 −	6 −	2 +
B3 −	B1 +	E1 −	4 ?
C1 −	2 −	3 −	6 0
C2 −	4 −	4 −	H1 −
C3 −	6 −	5 −	2 +
C6 −	C4 −	F1 −	6 −
D2 −	5 −	2 ?	
E6 −	D1 −	4 +	
H3 −			
→ 1178			

all + should be checked for motility

DATE: 7/18/54 REF:

162 | 165

	mal 1	lae 2	Eal 3	sill 4	Sy 5	6	7	8	9	10
1	–	–	+	–	R	–	–	+	–	R
2	–	–	+	–	R	–	–	+	–	R
3	–	–	+	–	R	–	–	+	–	R
4	+–	–	+	–	R	–	–	+	–	R
5	–	–	+	–	R	–	–	+	–	R
6	–	–	+	–	R	–	–	+	–	R
7	–	–	+	–	R	–	–	+	–	R
8	–	–	+	–	R	–	–	+	–	R
9	–	–	+	–	R	–	–	+	–	R
10	–	–	+	–	R	–	–	+	–	R
11	–	–	+	–	R	–	–	+	–	R
12	+	–	–	+	S	–	–	+	–	R
13	+	–	–	+	S	–	–	–	+	S
14	+	–	–	+	S	–	–	–	+	S
15	+	–	+–	+	R	–	–	–	+	S
16	+	–	–	+	S	+	sl	–	sl	S
17	+	–	–	+	S	–	–	–	+	S
18	+	–	–	+	S	–	–	–	+	S
19	+	–	–	+	S	–	–	–	+	S
20	–	–	+	–	R	–	–	–	+	S
21	±	–	–	+	S	–	–	–	+	S
22	–	–	+	–	R	–	–	–	+	S
23	+	–	–	+	S	–	–	–	+	S
24	+	–	–	+	S	–	–	–	+	S
25	–	–	+	–	R	–	–	–	+	S
26	+	–	–	+	S	–	–	–	+	S
27	+	–	–	+	S	–	–	–	+	S
28	+	–	–	+	S	–	–	–	+	S
29						–	–	–	+	S
30						–	–	–	+	S
31						–	–	–	+	S
32						–	–	–	+	S
33						+	sl	–	+	S
34						–	–	–	+	S
35						+	sl	–	+	S
36						+	sl	–	+	S
37						–	–	–	+	S
38						+	sl	–	+	S
39						–	–	–	+	S

(col. 2:) prob. miscord by virtue of Bal.

DATE: 7/18/54 REF:

156 mal	156 lac	156 gal	156 malt	156 SM	160 mal	160 lac	160 gal	160 malt	160 SM
1	2	3	4	5	6	7	8	9	10
-	-	+	-	R	+	-	-	+	S
-	-	+	-	R	+	-	-	+	S
-	-	+	-	R	+	-	-	+	S
-	-	+	-	R	+	-	-	+	S
+	+ ± -	+ ± -	slow?	R	+	-	-	+	S
+	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	sl?	-	+	S	+	-	-	+	S
+	sl?	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	sl?	-	+	S
-	-	-	+	S	+	-	-	+	S
+	sl?	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	-	sl	sl?	-	R
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
+	sl?	-	+	S	+	-	-	+	S
+	sl?	-	+	S	+	-	-	+	S
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	+ ± -	R
±	sl?	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S					
-	-	-	+	S					
-	-	-	+	S					

Sept. 20, 1954

At my request for "the aerogenes strain used in the Baskett-Hinshelwood2 expts. (PRS 139:58-73, 1951) received a culture labelled simply "Aerogenes aerobacter" 19.7.54" This is stated to have a lag of about 5-6 days in synthetic-arabinose medium.

Initially it was streaked on EMB-L-arabinose and found positive.

Aleck Bernstein received culture and stored it as W-2654. For first experiments, slant from single colony on L-arabinose was used. Subsequently, used slant directly from Hinshelwoods vial.

9/20. PM. Inoculate D(m) (citrate!) and D(0) for inocula. Latter grew well in 24h; former shows slight initial growth.

p21: From D(0) above, streak out EMB-Darabinose (Dar) and inoculate: (.1ml /10)

(to avoid citrate until D(A)= D(m) s/citrate mix is made up)

	A22	P22	A23	P24	P25
D(m)	±	✓	+	+	
D(0)	+++	✓	✓		
D(m,Dar)	±	± ··+ ?	+	+	
T(m)	÷		±	±	
T(glu)	++		—		
T(Dar)	÷		±	+	

÷ is faint turbidity scarcely more than inoculum. Should try smaller.

A1. EMB-Dar plate all negative. (faint pink by afternoon)

A2. P22. Streak out from D(Dar) above which shows some growth progress? all negative. No papillae seen.

B1 Restreak original (BLac) W2654 for single colony for initiation. Prepare current slant and D(0) inoculum tube from this.

P24. Streak out ① from T(dar) ② sole papilla on 1 colony of A2 ③ sole papilla on 1 colony of A1. to EMB Dar.

C: In future expts., minimal medium D(Ar) is based on salts s̄ citrate.

D. Inoc fresh D(0) culture from B1 to D±Ar for new selection. 1=.1ml 2=.01ml

N24 E. TAr. to D¹Ar, D²(0) P25: +, +++. Hence. TAr is +++ (E0)

N26 E-1 is +++

	P25	N26	A27	P28
D2: D(0)	+++		✓	
D(-)	−	✓ ∓		
D(Ar)	±		✓	✓
D1: D(0)	+++; +++		✓	
D(-)	± ±	(Bnor?) ∓		
D(Ar)	± ±	✓	✓	✓

P25 and
C1-2-3 no + but some diffuse in shading

More papillae now seen on A1-A2.
C56 ~~C6~~ C7

P25
F1 = EO on EMB Ar.

N26: C1 } mostly slow +
(48h.) 2 }
3 mostly −, 1 colony "+"

no stray +.

(24h.) F1 } mostly (heterogeneous) weak +
C5 }
C6
C7. distinctly two colony types − and +.

New platings N26: E1 is still slower on arabinose than on glucose. Wait on the D series for definitive series; meanwhile sub. E series to look for fast arabinose. Note: C3 is a + from first stage papilla of A1. Replate C3, C7 +/− and ✓ on D(0), D(Ar.)

	EMB	D(0)	D(Ar) P28
A27: C3A	−	+++	− −
C3B	± centers	+++	± +
C7A	+ and ++ }	+++	+ +++
C7B	− }	+++	− −

D0, D(Ar)
E1 A +++ +++

1 ml/10
Ar. P28 C7A'

DATE: REF:

N3. Since yesterday, 1 tube of D1 has begun to grow. (other D1 and D2 still negative).

C7A'' seems now to be as rapid ~~on~~ on glucose and arabinose. On EMB agar, still weak +.

<u>Plan</u> (1) Restreak D1A, C7A on EMB, DAr and mix. liq. D(Ar) for comparison.
P3–P4 (24 hours) — C7A'' has formed colonies, other two are similar, pinpoints., D1A' has scarcely begun.

P4: In 26 hours, C7A'' has grown optimally (lag = glucose)

Plate mix 9/30, D(Ar). shows C7A' forming good size colonies (two sizes). W2654 forms numerous pinpoints c̄ definite stimulation from Ara+.

Conclusions (1). EMB probably not a good indicator for this problem. However papillae on EMB suggest a non-homogeneous response (contra Hinshelwood). (2) possibility of trying indirect selection on DAr.

Take C7A'' single colony to NSA as <u>1181 A</u>.

P4: Note original 9/30 DAr plate shows no papillation yet for W2654 stock.

P6: On D(Ar), D1A' shows about <u>1%</u> denser colonies

P7 picks + and − to D(0) for check.

P6: D2A is now ++.

P7: D2A ++ | 1 strain as G1-3 | not adapted ?
D1A (++) | 2
D1B now +. | 3

P11:
1 all v.s.
2 mostly +, few v.s.
3 " "
} still heterogeneous

P#7			A12	A14
D1 +	H 3	+ by P11	++	+++
D1 − colony to D(o).	2	−	−	−
W2604	1	−	−	−

∴ these are distinct.

Mor D(o). all +++ in 24h.	D(A) ~~all~~ − still less

P14
1. + and − colonies. ○ o about =
2. ++ and a few − ◯ o. any +?
3. " "

∴ heterogeneity is still obvious.

None of these grows nearly as well as an D (blu). What was Dean's finding? Write H?

10/15/54 Conclusions

(1) Initial strain, Ara⁻ grows v. slowly on agar, gives v. small flat colonies which appear to decay slowly as original.

Mutants not observed on O(Ar) (except possibly after 3-4 cycles) but heavy inocula have not been tested

(2) In O(Ar) liquid, Ara⁻ grows initially to ca 10^{6}-10^{7}/ml. Then stationary turbidity for 4-5 days, then slow growth.

(3) Platings of these first cultures show mixture of Ara⁻ and Ara⁺ (denser, faster colonies) on O(Ar) agar. Only trial for completion of colonies with subsequent lag time.

(4) These cultures still have long lags on Ara, but successive transfers are gradually shorter. After 4-5 transfers, fully adapted cultures are found.

(5) No critical experiments on deadaptation except from 1st stage.

(6) Platings on O(Ar) and EMB Ar at various stages suggest several mutational steps.

A. Cultures adapting in Ara liquid are not homogeneous.
B. Not proven whether induced or selected.
C: Needed: (1) Platings of dense suspensions & hope of identifying

the first step mutants ⊘ lac$^+$/lac$^-$ markers for confirming heterogeneity of response.

DATE: Sept. 22, 1954. REF:

c̄ Luca.

P21 Inoc 10 ml Penassay c̄ W6, W1177, W2207, W1305, W2437
M-F+ TL-F- M-F+ MTL-F+ MTL-F-.

10:20 A22 Inoc .5 ml per. 10 ml Penassay c̄ and s̄ aeration.

	Aer.	
W1305	−	A
"	+	B
W2437	−	C
"	+	D.
W6	−	1
"	+	2
W2207	−	3
"	+	4

Plan: set up all combinations (A,B,C,D)(1,2,3,4) and wash, cross mixtures to W1177 (anaer.) to test interactions. Plate comparable aliquots on D(0) agar

3:45 mix 1.5 ml each culture + 7 ml Penassay. - 4:45 spin down

5:30 Resuspend in 1 ml water each. 20 ml culture (inoc above s̄ aeration) W1177 to ~~[illegible]~~ 5 ml.

Plate .1 ml W1177 each per plate.
c̄ 1 ml other

		1 W-6 −	2 W-6 + aer	3 W2207 −	4 W2207 +
A	F+ W1305	22	17, 17	30, 84	153,
B	" Aer.	3	7, 2	2, 5	18,
C	F- W2437	60	15, 6	0 0	0
D	" Aer.	29	10, 10	0, 0	0
E	Penassay.	131	22, 25	0, 0	1, 0

Controls

E2-			
E3-	0	A ×	0
E1-	0	C ×	0
C-	0		
A-	0		

This expt meaningless
Continuation by Luca.

Preliminaries.

1182 revertants

1) Recovery of aerated cells in Penassay.

2) Effect of 1 hour aeration on unaerated cells.

3) Effect of aerated ~~E~~ 1305$^-$ on ~~W~~ Waerated.

Comparisons

① E2: either aeration was ineffective in recovery in Penassay.

②. 4 series: a. W1305 converted. W1305 aerated still converted but qualified by ①

3 series: ~~W~~ W2207 aerated also converted, less effectively ~~=~~ by aerated W1305.

③ 2 series: see ①. However B2/E2 suggests that 1305F$^+$ aer. inhibits recovery.

④ 1 series of A1/B1/E1: W1305 aerated may transmit mutagenic to W6 unaerated.

W1305F^a ~~/~~ × W6F$^+$?

F$^+$ —× F^a

Isolate pairs W2344/M1 (♂) × W2401 ♀ 1183.

DATE: Sept. 27, 1954. REF: 173-174

Obj: Begin to look for pedigrees on the ♀ side. Do not try to obtain ♂ pedigrees but record viability and isolate pools.

9:50AM. Mix overnight cultures 1ml ♀ : ·1ml ♂ : 10 ml Penassay 37°.
9/28 (PM) Plate drops. See protocols for pick schedule and pedigree details.
Plate to EMB Lac. Score P29 and A30.

A30): all parental on Lac except A2 and E16. Pending further tests, the results may be summarized:

save A2, 4, 5, 6

A: 100/00 → 10 → → 10 and 0 0 0 / ♀ ♀ ♀ ; 000 ♀ ; (z) ♂ (Ara+)

≠ still segregating Lac! Might represent n=0, n=2 or n≠4.

B: 10 all died

C: 10 → ♂ / ♀ parental. ["1/4" ♀ died)

all other isolates concordant (♀ par.) on Lac, Lacsm, Mal, Mtl, Xyl, Ara, Gal ~~(Not tested on ...)~~. ~~(...)~~.

D: 10 → ♂ / ♀ died.

save E16 and pools representing E11, 13, 14 D6, E50

E: 10 → 18 → ♀ ; → 01 → ♀ [still paired; several generations]. ; ♀ lost (z)

∴ n ≠ 2, still segreg. [Note 2/4 sibs lost].

F. 10 < parental. Note: [3/4 ♀ died] [several gen from "4"].

try H♀ × H♂ for interfertility

G. 01 → ♂ / ♀ died.

∴ 2(z) from maximum of 5 possibilities. Considerable mortality in the latter.

? ≠ W2303?

H 01 → ♂ / → ... ♀ (only "1/8" survived). parental.

Summary on pairs.

(1184)

9/23/54

NOV 28 1955

total pairs [illegible]

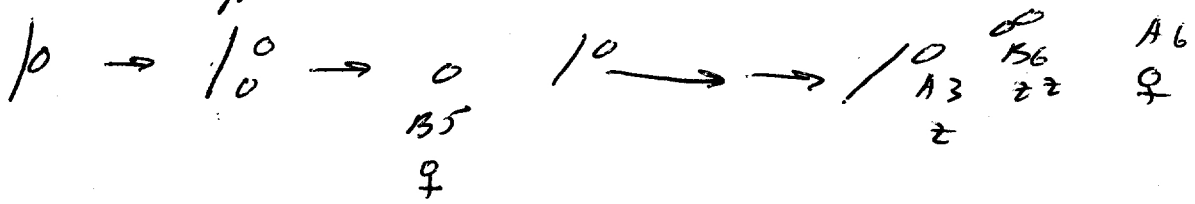
— presumably no loss on ♀

DATE page | REF:

page	2	Isol.	Intact	c̄ ♀	Zygotes	7	8	9 pairs in Brig.	♂♀ both died
Expt.									
1153D	129	4	1	4	2	✓	1	0	
55A	130	4	3	4	4	✓	3	3	
56	131	16	5	9	7	✓	11	5	2 dead pairs
[57 W1177X		7	2	3	0		5	0]	
58 sip	10		8	8	0		8	0	3
SS	5		2	4	2	✓	3	1	0
59		14	4	8	3	✓	12	2+1?	1
60		5	2	3	1	✓	5	1	
61	142	17	–	8	2	✓	14	1	2
~~74~~	~~160~~	~~16~~	~~10~~	~~12~~	~~1~~				
~~76~~	~~161~~	~~14~~	~~9~~	~~11~~	~~1~~				
77	162	15	5	10	0	E	~~do not label comp. pairs~~		
66	153	8	8	8	2	✓	8	1	( )
62	137	16	2	6	1	✓	8	1	5
63	150	12	6	7	3	✓	11	3	1
64	151	15	5	10	3	✓	14	3	0
65	152	18	8	9	4	✓	12	4	3
Σ.		166 ✓	62	98 101	34		see further for details		
	corrected	158 ~~166~~		~~101~~ 93	~~34~~ 32		107	25	
Reverse.									
68B	140	4	4	4	0				
73A	158	13	2	6	1				
B	159	16	9	13	0				
74	160	16	10	12	1				
76	161	14	9	11	1				
		63	34	46	3.				
		229	96	144	37				

19. 2/11/64 151	B3	+	—·< x z		Lac+
20. 28	C6	+	—·< ♀ z		Lac+
21. 29	D3	+	>·< ♀ z	✓	4 types: [Ar, Lac]$^{+}_{-}$ [Mal, MH, S]$^{+S}_{-R}$ Xyl?
22. 30 1165 152	A3	+	>·< ♀♀ z A, z B6, z B	✓	{ Lac+
31 A	B6.			V.i.	{ Lac+
32 B					
23. 33 34	D2	+	>·< 6, 2 < z A, z B	✓	{ A: Lac+ (Mal−) MH−? { B. 4 types [Lac$^{+}_{-}$][Mal, S]$^{-R}_{+S}$ MH−? all V, S Ar−
24. 35	E3	+	>·< ♀ z	✓	Lac+
25. 36	H3	+	4—·—4< ♀ z		Lac+
26. 1166 [153] 35 37	C1	+	≡·≡		Lac+
27. 38 39	C3, 4	+	10—·< 6 z C3, z C4, x	✓	{ Lac+ { Lac+
28. 19 1159 1116 C3		−	pair to C lump c5		Lac+
29. 9 1156	C1	+	>·< ♀ z	✓	Lac+
30. 10 131	C3	−	—< c4 ♀, z		Lac+
31. 11	D1	+	>·—		Lac+
32. 12	F3	−	—·—		Lac+
33. 13	G5	+	—·< z G5, ♀ H3 ?		Lac+

1165 A3-B6 (Hyp. reconstruction)

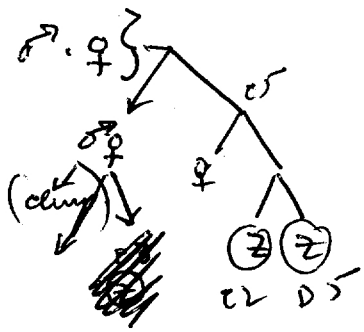


Enumerate zygote pairs. 9/24/54.

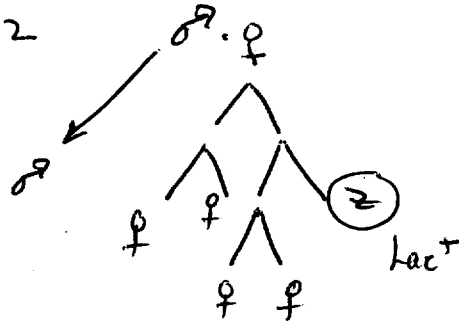
F − parent plus [orthotype re Gal, Mal, Xyl, Mtl, S unless noted]

#	F	♂ surv?	♂ . ♀ Pedigree notes (✓ if interesting)	Recomb notes
1 1 53D2	129	−	[illegible] — . —	Lac+ ....
2. 2 "D1	"	−	— . < ♀ z	Lac+
3. 3 57A	130 D3	+	8— . < (4) z (1) x	Lac+
4. 4 "	" F2	+	— . —	Lac+
5. 5 "	" F5	+	4— . (2) ♀ (3) z	Lac+
6. 6	H5	no rec.	. 2 ♀ 2 z	Lac+
7. 7 56	131 ✓B2	+	— . —	Lac+ Xyl+ Mtl− Mal− Gal+
8. 8	B5	+	2 . < x z	{ Lac+ Xyl+ Mal+ Mtl− S^R ; Lac+ Xyl− Mal− Mtl− S^R } { Lac− Mal+ ; Lac− Mal− S^R } ↖ +/− [Lac] [Mal, Xyl] +/− all Ara− S^R Mtl− Gal+
9. 14/58	136 B1	+	— . —	Lac+
33) 15)	H2	−	— . —	Lac+
10 16 59.	146 A3	+	— . < ♀ z	Lac+
11 17	{ C2 [illegible] D5	+	v.i. ✓ (most of descent)	Lac+ " "
48				
12 20 60 (1160-2)	143 A2 (1160-2A?)	+	v.i. [illegible], stiel segr. after 2 div. ✓	Lac+
13 21 61	M1 C4	+	— . < x ♀ ; < ♀ z ✓	Lac+
14 22 1161	142 G1	−	— . —	"Lac+/− Gal+ Mal, Xyl −/+, S^R (Lac−)!" (pure.) No Mal+ S^R (unless chick)
15 23 1162	137 H5	+	— . —	Lac+
16 24 1163	150 C2	+	> . —	Lac− Gal+ Ar− Mal. Ttfr... W2502 ([illegible]
17. 25	1 D6 (1164)	+	> . < z ✓	Lac+
18. 26	G5	+	— . < x z	Lac+

1159 - C2



1160 - 2



but > 34 zygotes!

Notes on summary:

(1) all zygotes must to be tested for segregation of V_1, Ar. (cf. notes on colony segregation).

(2) of 38 zygotes, S^s survived in 26. Probably as high as controls.

(3) Pairings after division analyzable in 8, 7 are completed. (Exc. 56 65)

(4) Pedigrees 2 or more generations in 7. all still segregating!

Following in #/total:

11592	calc.	2/8	(sibs)
1160-2	"	1/4	
61-64		1/4	(1x)
65-#3, 36.		3/8	(sibs)
6502		2/8	
H 3		1/4	
27 C3		≠2/8	

(5) Review distributions of Mal. (#16 uncertain). 3 cases of Mal+ all segregating! B5 should be examined for S^s also say 2 cases of S^s and both show 4 phenotypes!
(Recall test for recurrent recombination).

(6) of 34 zygotes. 28 are Lac$^+$...

Retests on zygotes

DATE: REF:

1	2	3	4	5	6	7	8	9	10

A see review for numbers (1-39). # 40, 41 = 1183 A2, E6 resp.

B rev specific isolates

C Replicate 1-41 on D(o), D(M), D(H). (4 types!)

29 grow on histidine 1164D3 none on D(o).

19 " " methionine 1159C3 Lac⁺ ...

OCG tests.

DATE: REF:

all zygotes presumably contained same Lac − V_1^R Ara−. In addition, found (for Lac+):

	Lac+: V_1::	Ara	Notes:
1	S	−	
	S	−	
	R	+	
	S	−	
	R	−	any −S?
	R	+	
	R	+	
	S	+	
	R	−	
10	R	−	−R?
	S	−	and −R!
	S	−	
	R	+	
	S	−	
	S	−	
	S	−	
Clump: [	S	−	
	S	−	
	R	+	−R?
20	S	+	
1	S	−	
2	R	+	
3	S	+	
4	Lac− S	−	(6302?)
5	S	−	
6	R	−	
7	R	−	
8	S	−	
9	R	+.	
30	S	−	
!	S	−]	
	R	+	
	S	−]	
	S	−	
	S	−	
	S	− +	any Ar+?
	R	+	
	R	+]	
	R	+	
40	R	+	−R!
	S	−	

Deenver examined 6/12/54

over: ignoring complications & assuming that recombinants are all Lac+, following table:

DATE: REF:

	Ara+	Ara−	
among lac+:			
V_1^R	(12)	5	(17)
V_1^S	3	20	23
	(15)	25	40

Ara and V_1 are clearly linked to each other. Are they linked to lac? The parentals in coupling with lac+ are excited. There is a definite excess of recombinants, possibly significant (no!) However, incidence of couplings may be exaggerated by admixture.

~~Data~~ Table above is uncorrected for a few sib zygotes. Note especially 1165 B6A, B (31, 32).
30

Pedigree Hfr × F⁻ 1185

DATE: Sept. 30, 1954 REF: 175–177

Second run. overnight cultures, 1♀ : .1♂ : 7ml broth 32°

10²⁰ – 11⁴⁰ to set up 8 pairs isolated initially, 12:20–12:40 PM.

B1 was initially suspicious and proved illegitimate. No viable ♀ from A1; (see over) paired exconjugant from B1 also inviable. Other pedigrees to 4–6 generations.

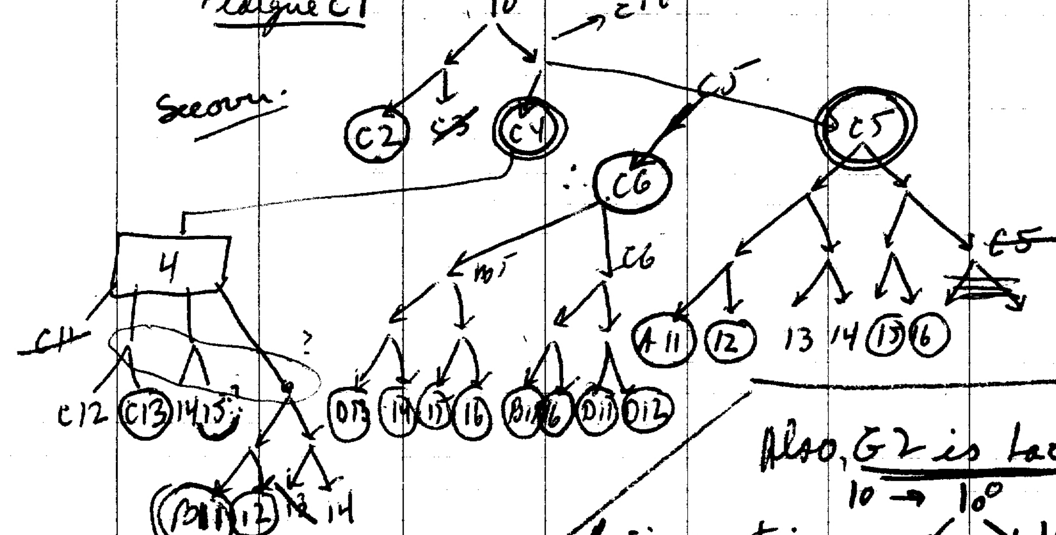
See protocols for picking schedule on rows I – II – III / VI – VII (Lac – or Lac⁺ parentals)

			Lac	Lac streak	Mal	Mtl	Xyl	Gal	Sm	Ara	V_1	
IV	A	11	+	+ (pure)	−	−	−	++	R +	+	} V_1^R	
		12	+	+ "					R +	+		
		13	−	− "					R −	−	} V_1^S	
		14	−	− "					R −	−		
		15	+	+					R +	+	} V_1^R	
		16	+	+					R +	+		
	B	11	+ −	−, +					R +	−+	$+V_1^R$ $-V_1^S$	
		12	+	+, −					R +	+ −	$+V_1^R$ − V_1^S	
		14	−	−					R −	−	V_1^S	
		15	+	+					R +	+		
		16	+	+					R +	+		
V	C	12	−	−	−	−	−	++	R −	−	V_1^S	
		13	+	+, −					−+	−+		$\pm V_1^R$ V_1^S
		14	−	−					−	−	V_1S	
		15	− +?	−, +				"	−+	−+		$+V_1^R$ $-V_1^S$
	D	11	+	+					+	+	} V_1^R	
		12	+	+					+	+		
		13	+	+					+	+		
pure?		14	+	+				"	+	[illegible]		
		15	+	+					+	[illegible]		
		16.	+	+					+	+		

Pedigree C1

/ = 10 m

See over.



○ has intact
○ has Lac – affinity

Also, G2 is Lac^+ Ara^- S^R V_1^S.

10 → 10⁰ → 10 → G1

G2 ↘ long pedigree

Pairing exception

Save G1, G2, pool = G3456 H11 – 16

except for this pedigree, other isolates are concordant in the respects indicated

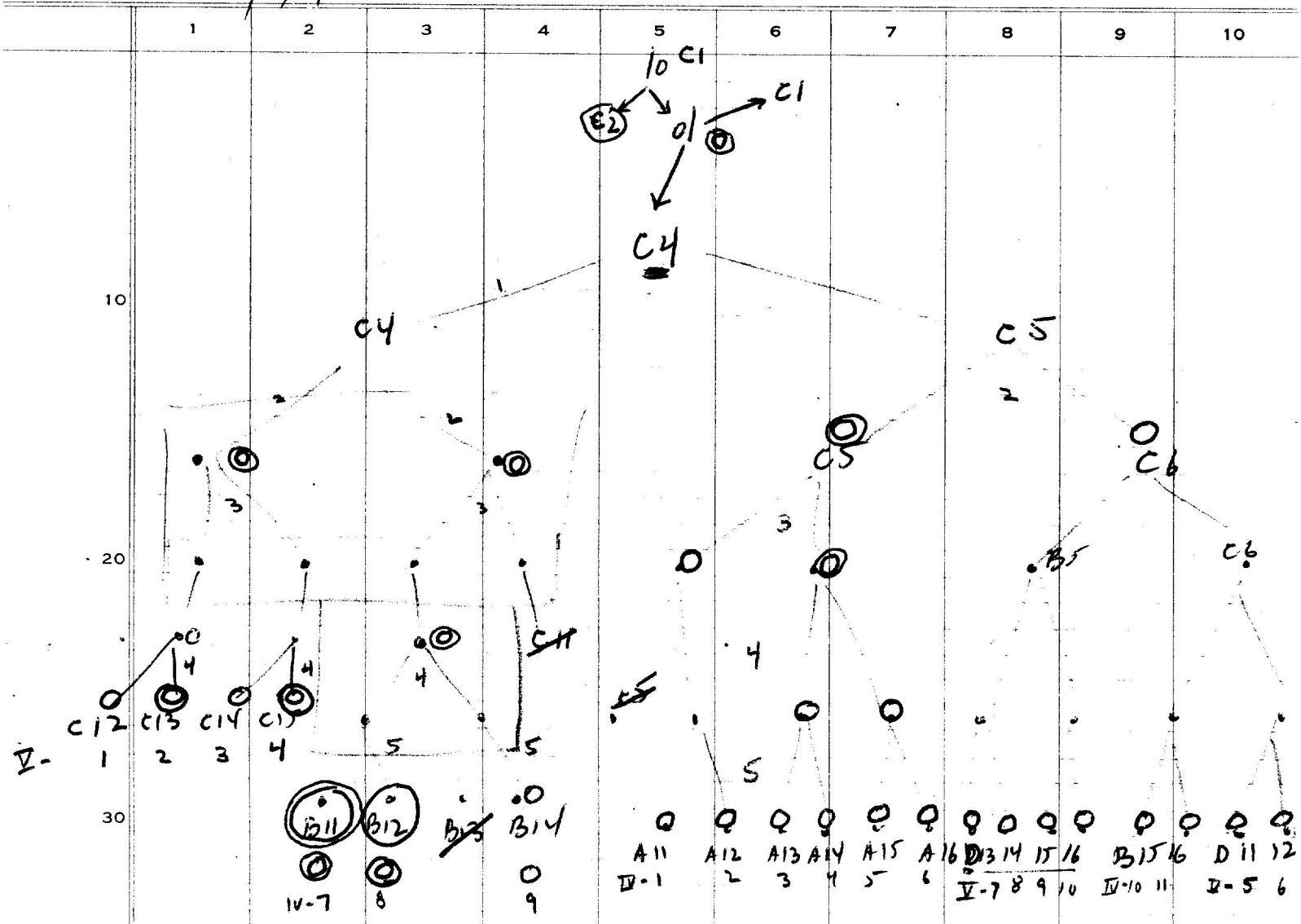
---

In all likelihood, a single recombinant is represented, though of the most common type:

$$\left(\frac{\text{Lac+ Ara+ V}_1^R}{\text{Lac- Ara- V}_1^S \cdots}\right)$$

(absence of other recombinants argues against double meiosis)

DATE: 10/2/54. REF:



∴ B11 shows two lines still segregating after the 5th generation, while the C6 clone seems to have segregated at the 2d. A13-14/A15-16 probably at the 4th. Pedigrees generally should probably be carried to 4 generations.

Note: both C4, C5 are 2n, tr. Time of fertilization?

1186

	♂	♀	(2)	
A1	×	✓	✓	Lac$^+$... ; Ara$^+$...
A4	aband			
B4	✓	×		
C1	✓	✓	✓	motile.
C4	× without	✓	✓	Lac$^+$Ara$^+$
E1	✓	✓		SIP
E4	✓	×		
F1	✓	✓ part		
F4	✓	✓	✓	Lac$^+$..., Mal$^+$...
D4	✓	×		
B1	✓	×		
G4	aband.	maybe silly.	♂ rec. no ♀	
D1	✓	✓	✓	Lac$^+$...
G1	✓	✓	✓	
H4	✓	✓	✓	Lac$^+$Ara$^+$ Lac$^-$Ara$^+$ no Lac$^+$Ara$^-$

DATE: Oct 5 1954 REF: 178 - 179 - 180

Cross ~~no~~ 10: Ratio W2401:W2344 1/4. ca 8^{45} A.M. Cross is therefore somewhat old when picked (10^{30} - 11^{15}) = 1:45 - 2:15 hours.
16 pairs were picked initially. Results:

B12: Lac+ Ara- ../♀
A2: Lac- Ara+ ./♀

A1 — o → o —o → ♂ died
↘ (2) ~~[illegible]~~ B12
(A2.) Ara+

A4. — ✕ abandoned as too complex (B1) ?

B4 —o s.p ♀ died

save C56 } C5
A56 }

C1 complex ~~[illegible]~~ ♀. motility What is C3 - originally listed as motile. ~~[illegible]~~ no!

C4 —o → 1oo
↙ ↘
o o'
C12 C11.
(2)

C11: male withered away.

Note C12 exception. C12: Lac+ Ara+ / ♀ only

E1 —o s.p. both survived → E1 ♂
↘ E2 ♀

E4 o— → o/o' ♀ died ♂ E4

F1 o/o— ...→ cluster o/o o' → F1 ♂
↘ o/o F3 ♀
↓
X

F4 o— → o/o
↙ ↘ → F4 ♂
o ↘
F5 F6 (2)

Note pairing correlation!

F6: { A ♀
B Lac+ Mal+ ~~[illegible]~~ S R
C Lac+ Mal- ~~[illegible]~~ S R
D o } Ara- Xyl-

no SS . (23 + 5) Mal+ tested no Lac+ Mal+ found

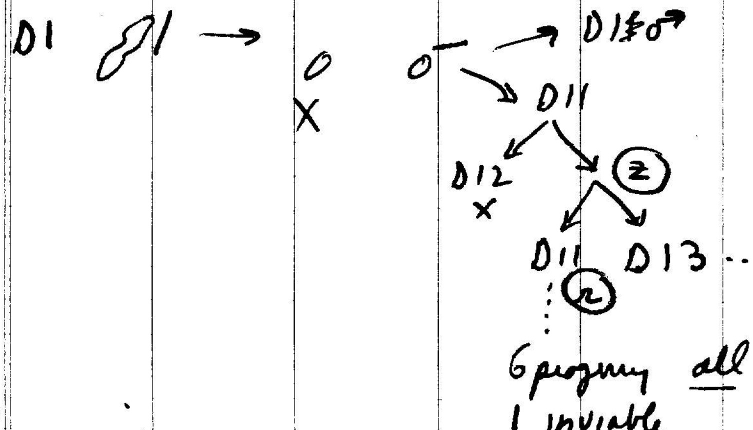
DATE: REF:

D4 ⚬ ..... → d3 ♂ all ♀ exc. eventually died, not before dividing

H1 ⚬ → H1 ♂ (fully spont sep.)
↘ all 6 ♀ died!

G4. conferred ⚬' → G4 ↘ G5 both ♂ ♀ died?

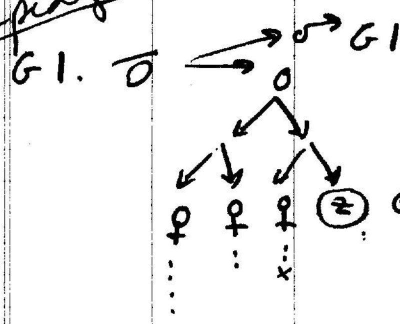
For fuller pedigrees see below.

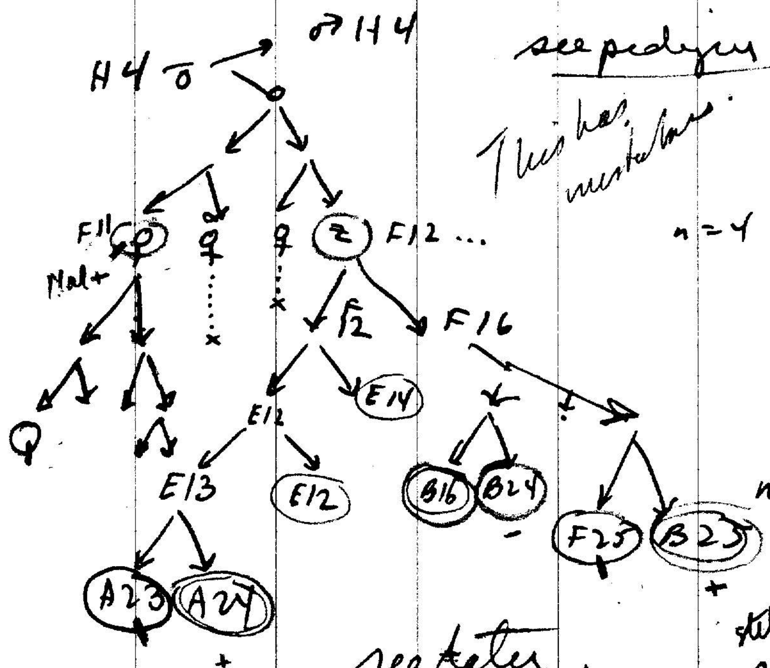


Presume conjugation
D11
D21-22-23-26 } Lac+ ...
D26

D13, 16, 24, 25
B13, 23
C16

see pedigree





see pedigree

This has mistakes.

n = 4

n = 8

still segr!

see later.

Zygote summary:
viable exconjugant in
A1, C1, C4, E1 (+,p), F1, F4, D1, G1, H4.

z in ◯ = 1/9

pairings seen in C4, F4, D1,
and probably conjugation in 2/3.

DATE: REF:

		#	Lac*	Ara	Mal	Xyl	Mtl	Gal	S/Lac	D(M) D(H) 9	Note 10
I	1	A 2	–	+–	–	–					
	2	8	–	–	–	–					
	3	6	–	–	–	–					
	4	3	–	–	–	–					
A.	5	B 2	–	–	–	–					
	6	C 5	–	–	–	–	all others –	all others +		all others types S or R resp.	
	7	6	–	–	–	–					
	8	E 2	–	–	–	–					
	9	F 3	–	–	–	–					
	10	5	–	–	–	–					
	11	6	–+ •	–	–+	–			–+	–	{ – – – + ; + – ; no ++
II	1	G 4	±	+	+ ••	+	+	–	S	+	
	2	B 12	+– •	–	–	–			+		
	3	13		–	–	–			–		
	4	C 11	~~–~~	–	–	–			–		
	5	12	+ •	+–	–	–			+		++/-- only
	6	D 11	+ ✓	–	–	–			+		
	7	13	–	–	–	–					
	8	16	–	–	–	–					
	9	E 14	–	–	–	–					
	10	15	–	–	–	–					
	11	16	–	–	–	–		all others +			
		C 16	–	save for later check	–						
	1	F 11	–	–	+ •	–	/		Mal – only		
III	2	"	–	–	–	–					
	3	"	–	–	–	–					
	4	"	–	–	–	–					
	5	"	–	–	–	–					
weak Lac+	6	16	–+ •	–	–	+ ✓			–+		
	7	G 11	–(+)	+ pink spots	–	–	/		✓		
	8	12	+–	+–	–	–			+		++/-- only
	9	14	–	–+	–	–					
	10	15	–	+	–	–					
	11	16	–	+–	–	–					
IV	1	A 21	–	–	–	–					
	2	"	–	–	–	–					
	3	"	–	–	–+ •	–			Mal – only		
	4	"	–+ •	–	+ ✓	–			S!		
	5	"	–	–	–	–					
	6	26	–	–	–	–	/				
	7	B 23	–	–	–	–					
	8	"	+ ✓	–	–	+ ✓					
	9	"	+	–	+–	+			(Mal Lac+ – only)		
wk. Lac+	10	26	–	–	–	–					

* if streaked, ✓ = pure; mixture as indicated

all R exc. as noted

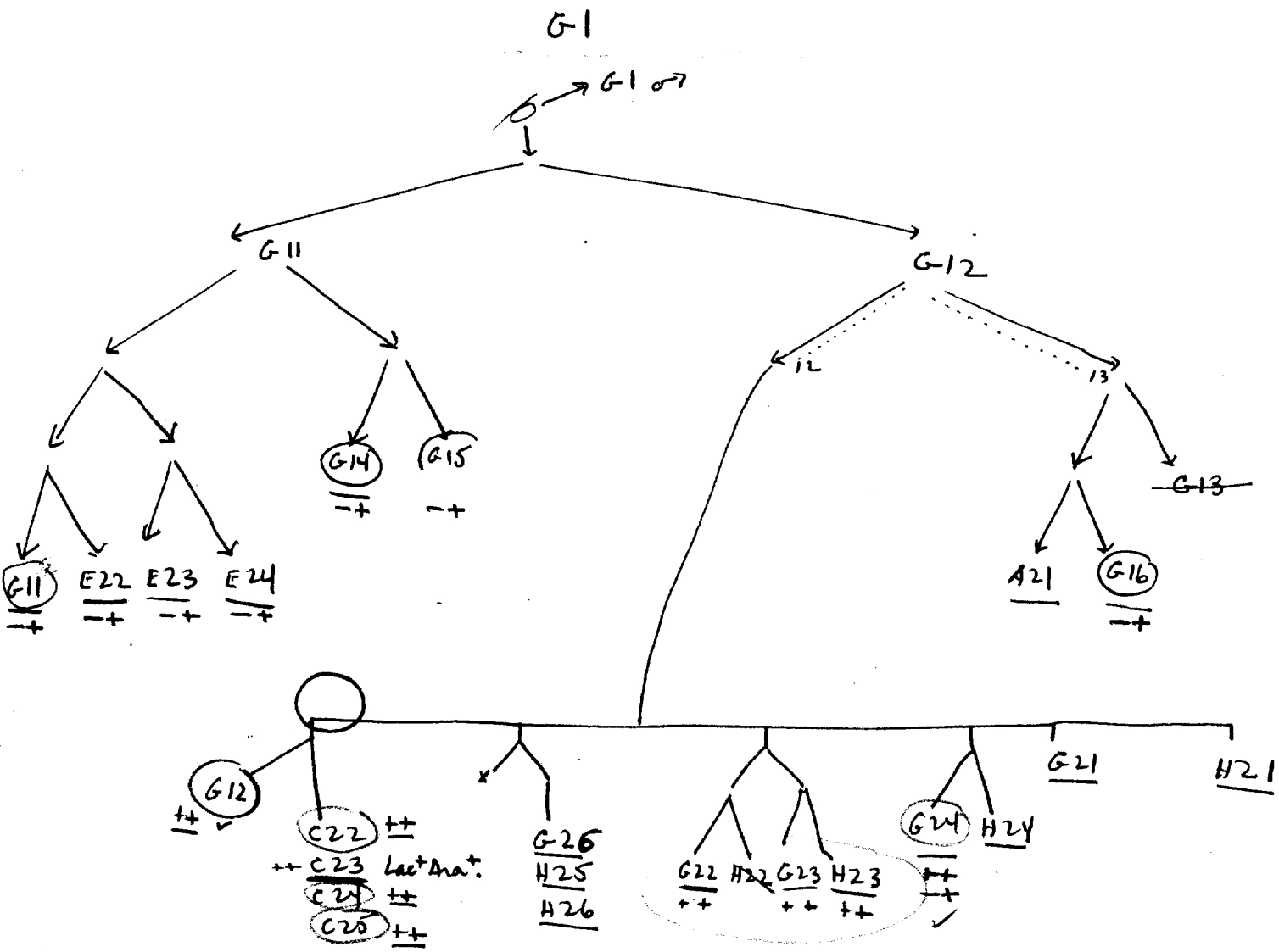
_singular lac colony.

c 2 2 (A) - pure Zal +
lac ±. save

c3: un? mottle. 2 colony sizes, pure Zal +. ∴ typ. ♀.

DATE: REF: D(H) D(H)

		#	Lac	Ara	Mal	Xyl	MH	Gal	S/Lac	9	10
V	1	C21	+-	-						4 S, irregular colony A [?]	
	2		+- ✓	+							
	3		+	+							
	4		+-	+							
	5		+-	+							
	6	26	-	-	all -	all -	all -	all +			
B.	7 } D	21	+ ✓	-							
	8 } P		+ ✓	-							
	19		+ ✓	-							
P2	10		-	-							
	11		-	-							
	12	26	+ ✓	-							
VI	1 E	21	-	-	-				do Lac except		
	2		-	+	-						
	3		-	+	-						
	4	24	-	+	-	all -					
E21P {	5 F	21	-	-	-						
	20 6		-	-	-						
F15P	7		-	-	-						
	8		-	-	-				↓		
	9	25	-	-	+ ✓	-	-	+	S orig.		
V	1 G	21	-	-	-						
	2	22	+ ✓	+	-			all +			
G22P	3	23	+ ✓?	+	-		all -				
	4	24	+-	+	-						
	5	26	-	-	-	all -					
G21P	30 6 H	21	-	-	-						
G22P	7	23	+ ✓	+	-						
	8		-	-	-						
G26P {	9		-	-	-						
	10	26	-	-	-				↓		
VI	1	B4	±	+	+	+	+	-	S		
	2	C1	±	+	+	+	+	-	S		
	3	C3	-	-	-	-	-	+	R	M-H- ~~[illegible]~~ not yes!	
	40 4	D1	±	+	+	+	+	-	S		
	5	D6	↓	↓	↓	↓	↓	-	S		
	6	E1							↓		
	7	E4									
	8	F1									
	9	F4									
VII	11	G1									
	12	G5									see also G4
	13	H1									
	14	H4									



— ♀

♂ Lac- Ara+/Ara-

○ Lac+ / Lac-

- pure as ind
all Mal-Xyl-Mtl-SR

Still to be characterized:

- ① G11 purity on Ara (Lac-Ara+). Try V1 also
- ② Look thoroughly for recombinants of Lac/Ara in the G12 progeny. ~~(Use V1)~~

✓ ③ G24 any complementary Lac+ Ara- ?
(No - of 45 Ara-, all Lac-; do. Lac+)
other three types definite = A B C
-- ++ -+

$\varphi = Lac^- Gal^+ Ara^- (Mal^- S^R)(Xyl^- MH^-)$

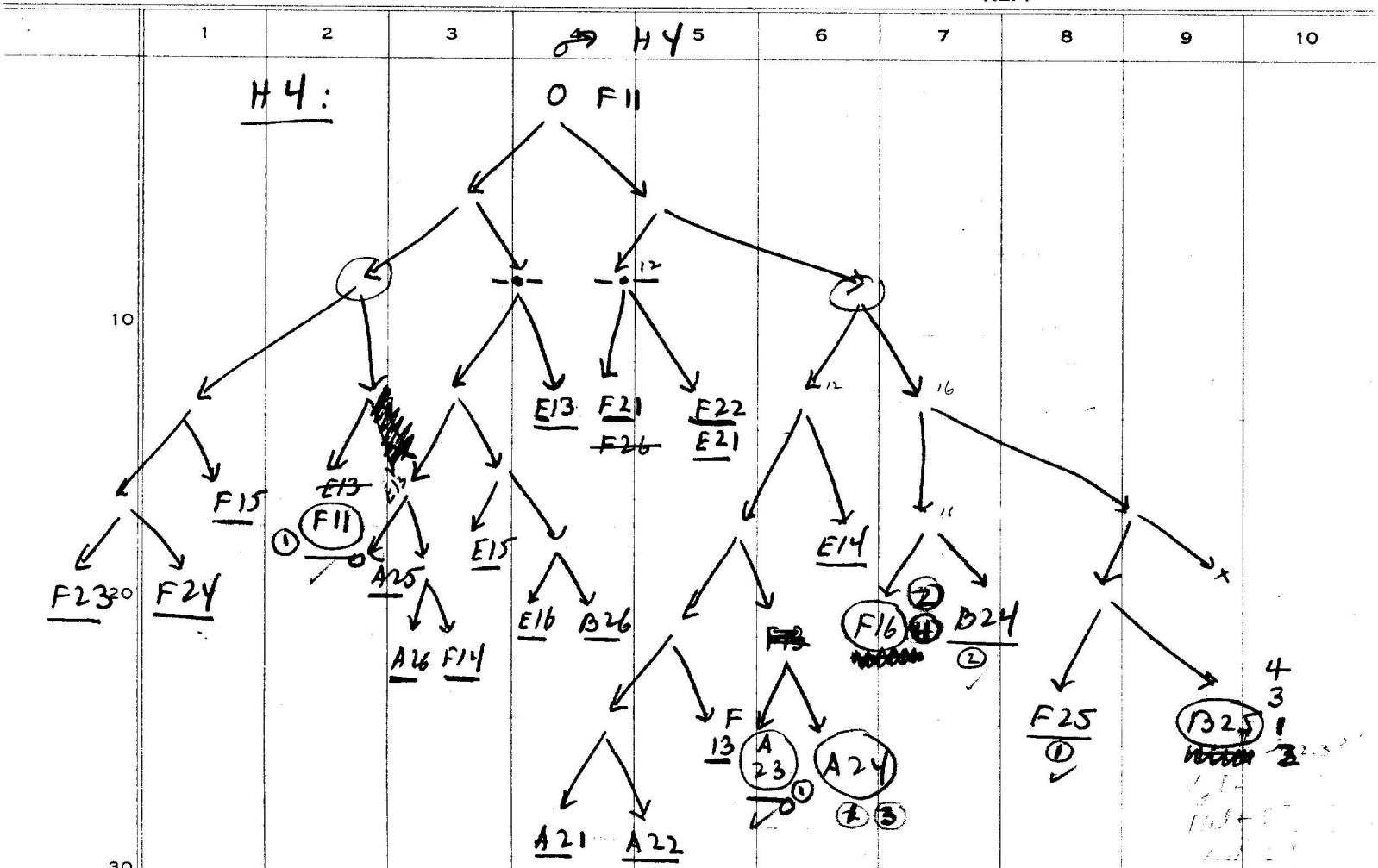
of ... + − + − s x +

Pure $Ara^- Gal^+ MH^-$

Redraw pedigrees

1186

DATE: REF:



— = Lac⁻ ... S^R
o verified in mixture
— pure
◯ segregating

① Mal⁺ S^S Lac⁻ Xyl⁻ ② Mal⁻ S^R Xyl⁺ Lac⁺ ③ Mal⁺ S^S Lac+ Xyl⁻ ④ Mal⁻ S^R Lac⁻ Xyl+

F25: Mal+ S^S Lac- pure

B24: Lac+ Xyl+ S^R Mal⁻

F16: Lac-,+ S^R; Xyl+ Mal⁻ (pure)

all are Mtl- Gal+ M⁻ H⁻ Ara⁻

F11 Mal+,- ; Lac- S^R/S^S.

A23 " "

A24 Mal+ S^S < Lac+ / Lac- > Xyl-

B25 any Xyl- S^R ~~[illegible]~~ NO
Lac+ S^R: all Xyl+

Record details

Caulescens

a) heterocaryon vs. heterozygote

b) double meiosis?

B25 etd. 10/14: 2 plates replicated to Lac ± Sm
Xyl
Mal

① all Lac$^+$ S^R are Xyl$^+$

② all S^R = Mal$^-$

③ all Mal$^+$ = Xyl$^-$
Mal$^-$ = Xyl$^+$

④ 1, 2, 3, 4 types seen.

also, since no Xyl$^-$ S^R, 7 is absent.

Further tests on content of clones

DATE: Oct. 11, 1974... REF:

Lac+ and −, to check Mal, S, Xyl.

C: F6.

		Xyl	S	Mal
F6	10 Lac+	all −	R	all −
	10 Lac −	all −	R	5+ 5−.

Xyl

∴ F6 includes Lac+ Mal− Xyl− S^R ✓

Lac− Mal− Xyl− S^R

Lac− Mal+ Xyl− S^R

Not excluded: Lac+ Mal+ (pres. Xyl− S^R) S^S.

		Xyl	S	Mal	
B25	12 Lac+	−	S	Mal +	should be same S^R! repeat (might have confused F25)
	16 Lac−	−	S	all +	
		medium?			
B12	4 +	−	R	−	
	4 −	−	R	−	
F16	2 +	−	R	−	
	2 −	? → −	R	−	
A24	1 −	−	S	+	
	1 +	−	S	+	
		medium n.g.			

do. in feet of replicates

to isolate Xyl− Lac + Mal + S^s, Lac − Mal + S^s

C22 {Lac 3+ 3− Ara 3+ 3−} C12 4++, 4−−

24 " " G12 " "

25 " "

G24 5 Lac− 2 Ara+ 3− C21 1−+?) 4+−

all S^R 1 Lac+ Ara+

∴ C22-24-25 have Lac+ Ara+ / Lac− Ara−

G24 has ++, −− and −+ ?

in first pick Lac+ were not pure

All Mal+ are S^R
Mal− S^S

B25

						Type
Mal−	: 5 Lac−	[illegible] Xyl+	S^R		B25A	(4)
Mal+	7 Lac+	} Xyl−	S^S		B	(3)
	3 Lac−		S^S		C	(1)
					D	(2)

any ♀ ?

no Xyl− S^R or streak

A23 4+ Mal } all Lac − Xyl− (1) A23B = C
4− " ♀ A23A = ♀

F11 ditto } Lac− Xyl−

A24 1 Lac + } Mal+ S^S Xyl− (3) A24A
1 Lac − (1) A24B

F-16: A (2) − 2 (Lac+) both Xyl+ ✓
B (8) − 4 (Lac−)

Types seem to include

	Lac⁻	Xyl⁻	Mal⁻ S^R
♀ =			
(1)	−	−	+
(2)	+	+	−
(3)	+	−	+
(4)	−	+	−

Note complementarity

two lac classes included here!
~~lac+ possibly weaker~~

In replicas of two plates
mil Lac, Lac sm
Mal, Xyl,
only (1–4) found, no ♀, no Mal/Xyl
no S^R...

Selected and unselected Hfr: fertility; Hfr × Hfr.

DATE: Oct. 8, 1954. REF: 181

overnight cultures:

A 11851 C1 ♂ (fertile)
B C2 ♀
C D1 ♂ (infertile)
D D2 ♀

E W2582.

M ♂ W2344M1
F ♀ W2401

Conclusion - no gross difference in fertility of a fruitful vs. unfruitful pair! Also no prolonged adolescence required for remating.

1:40PM. Mix in 7ml broth 0.1 ml each of ♂'s and ♀'s. (E+M at 1:1). 4:10 plate out on EMS lac ± sm.

Counts are lac+/total on Lac sm

AB 5/293 (1.7%)
10/493 (2.0)

A9. CD 16/648 (2.5) 39/1090 (3.7)

EM. ca 3/1000 Lac+Sr. Try for pair isolation.

A9. Mix same (1 day) cultures ~~10~~ 1E 10E:1♂ 945 AM

About 10 "pairs" isolated (181).

But all proved illegitimate. However, cross was very late (1:15), i.e., at least 3½ hours.

Hfr x Hfr

1188

DATE: October 1~~7~~, 1954.

REF: 182

W2582 x W2344M1. ("♂") 9:30 – 11 AM mix.

Most pairs illegitimate. 3 Legit, 1 obvious recombination (W2582 as ♀); F1 indeterminate because mixed.

		Lac	Gal	Mal	Xyl	Swarm.	Ara	S	Note
A1	♂	± ✓	−	+	+		+		S Illeg.
× B1	•	++ MIX	+	+	+	+	+		+ Not illeg.
C1	♂	± ✓	−	+	+		+		S Illeg.
C2	♂	± ✓	−	+	+		+		S T
[D1	♂	± ✓	−	+	+		+		S
D2	♀	± ✓	+	−	−		−	−	R Legit
E1		− ✓	+	−	−		−	−	R ×
E3 — E2		+ MIX	+	+	+	+	+	+	
E F1		+ MIX	+	+	+	+ (−)	+	+	Recomb?
F2	♀	− ✓	+	−	−		−	−	R
F3	♀	− ✓	+	−	−		−	−	R
G2A	♀	− ✓	+	−	−		−	−	R
G2(B)	♀	− ✓	+	−	−		−	−	R
G3	⊗	+ ✓	+ ✓	−	−	−	+ −	+	R (Lac+)
G4	♂	± ✓	− ✓	+	+	+	± ✓		S
H1	♀	− ✓	+	−	−		−	−	R some ♂+?
× H2		+?	+	+	+	+	+	−+	R +, − mixed how?

mixed ±, ++

parents are W2344 = Gal− Lac+ + ... "♂"
W2582 = Gal+ Lac− ... "♀"

Interest: G3 = Lac+ Gal+ Ara+ Mal− Xyl−
(118 (C3 = W2401))

may need a reisolation for motility of W2344

Restreak Lac and Gal # B1, E2, F1, G3, H2

B1 – "both motile"

E2 – "x" ~~(E3?)~~

F1/0 < 1 / 00 F2 } may be recomb in opposite sense?

G3 } 10 → 0
G4 } 10 → 1 → ♂ ? check purity in Lac

F1 may represent
~~lfac~~ W2344 x W2582 ♂

G3 is evidently W2582 x W2344 ♂

} Probably not. Dominations of Gal+Lac- and Gal-Lac+ merely require

	Lac	Gal	
B1	mostly ±	mostly -, few +	F1: Gal+Lac- pool all non motile
E2	± and - ? are all Lac- Gal+?	- and +	
F1	mostly or all ±	mostly -	since F1 is mostly Lac- Gal+, it is presumably ♀ + fuss?
H2	"	" "	
G3	pure +	pure + ; Agar on Aga	
G4	all ±	~~all +~~ not tested plus. -	

· terminate
· continue

♂ × ♀ for prolyne

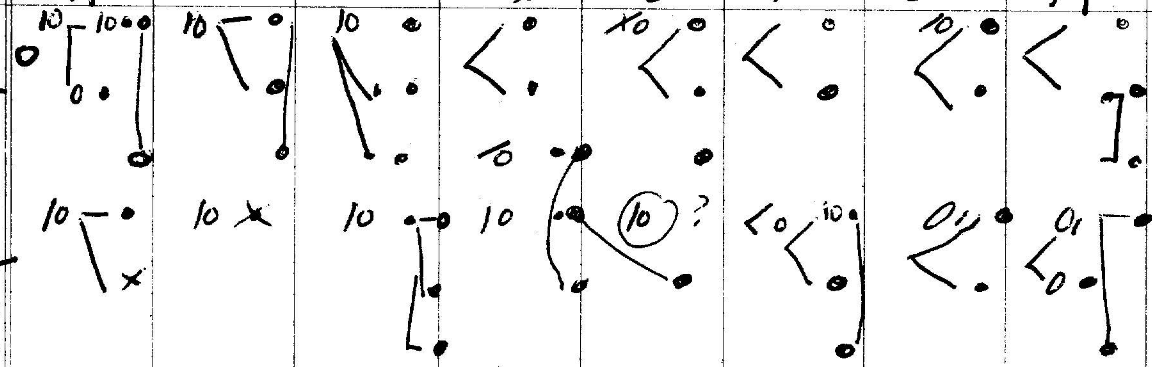
1189

OCT 14 1954

DATE:

REF: 183

183



grow:?

183

p18.

	A	B	C	D	E	F	G	H	9	10
11	O ,d	O,d	O,d	+ nm	+ m	O:	+	O		
12	O ,d	+ ,d	O	+ m	O	+ m	O	+		
13	O	+ nm	O	O	O	O	O	O		
14	not	O	+ m	O	O	O	O	+ sparse		
15	O		O	O	O	+ nm	O	+ nm		
16			O			O	O	O		

21

22

23

24

25

26

abandon.

why poor or no growth

① low temperature

② capillary tip too sharp? or too acutely bent

but same as 1190, 1191.

Abandoned owing to growth failing (temperature?)

1190 Succ.

	♂	Zyg.	types.
B4 ✓	✓	no	
C4 ✓	✓ part	—	
✓	✓	0	

DATE: Oct 19, 1954 REF: [185-186-187]

1 2 3 4 5 6 7 8 9 10

W-2344M1 x W2401 10:30- 12N 10;1 ♀ Collect numerous pairs and follow for 1-3 exconjugant generations. P19: refrigerate[185] after transferring three pedigrees to [186-187]
B4, C4, E4

1 2 3 4 5 ~~B~~ L

B4 → B4 ✓

B5 — B5 — A15 — A15: A15 ✓ 1, A24 ✓; ~~B15~~: B15 ✓, B13 ✓
B5 — B5 — A16 — A16: A16 ✓, A22 ✓; B16: B16 ✓, B14 ✓
B5 — A2 — A13 — A13: A13 ✓, A25 ✓; A14: A14 ✓, A23 ✓
B5 — A2 — < — A11: A11 ✓, A26 ✓; A12: A12 ✓, A21 ✓

B6 — ~~C11~~ — C11 — ~~C11~~: C11 ✓, D12 ✓ 18; D11: D11 ✗, D23 ✓ 19
B6 — ~~C11~~ — ~~C11~~ ~~D11~~ — C12: C12 ✓ 20, C26 ✓ 21; C13 ✓ v slow 22
B6 — < — C14 — ✓ V.S. ~~C14~~ 23; D14: D14 ✓ V.S. 24, D22 ✓ 25
B6 — < — ~~C14~~ C15 — C15: C15 ✓ 26, C25 ✓ 27; ~~C15~~ D15: D15 ✓ 28, D13 ✓ 29

These 3 pedigrees: all ♀ on Lac Gal Ara Mal MH sm.

[initials]

C14? B26? exchanged with C25, C26? mistranslation?

29 cells

DATE: REF:

1	2	3	4	5	6	7	8	9	10
	1	2	3	4	5				
						[redacted]M			
		E15 • X							
	c5								
c4̄ → c4 ✓ c5			E13	✓	E13 ✓ 1				
				E26	E22 ✓ 2 B26 ✓ 3 E26 ✓ 4				
			E14	✓	E14 X F21 ✓ 5				
				F15	F15 ✓ 6 F23 ✓ 7				
c5			E11	✓	E11 ✓ 8 E23 ✓ 9				
				E25	{too many} ✓ 10				
				✓	✓	✓ 11			
			E12	E24	E21 ✓ 12	13 14 E24 B23 B24 B25 15 16			
	c6			✓	X				
			c16	D16	D16 ✓ 17 D24 X				
			E16	✓	E16 X 18 F24 ✓ 19				
				F16 X					

X did not

1190.

DATE: REF:

1	2	3	4	5	6	7	8	9	10

K

E4 → E5, 6
E4 ↗ E4

E5
- G11
  - G11 — G11 ×; G24 ✓ 1
  - H11 — H11 ✓ 2; H25 ✓ 3
- G12
  - G12 ×
  - H12 ×
- G13
  - G13 ✓ — 4
  - H13 ✓ — 5
- G14
  - G14 ✓ — 6
  - H14 ✓ — 7

E6
- F11
  - ✓ — F11 ✓ 8; F25 ✓ 9
  - G16 — G16 ✓ 10; G25 ✓ 11
- F12 ⟷
  - ? NF — G15 ✓ 12
  - G15 ✓ — G15; G26 ✓ 13
- F13
  - ✓ — F13 ✓ 14; F26 ✓ 15
  - H16 — H16 ✓ 16; H26 ✓ 17
- F14
  - ✓ ×
  - H15 ×

DATE: Oct 22, 1954 REF:

By UV/EMB, got obtained 3 Lac⁻ mutants from W2654.
A is nearly full⁻ ; B + C are slow but entirely scoreable

P21 Inoc D(0) c̄ W2654, ~~[illegible]~~ W2663. Keep A as W2663

2:15 P22 Prepare mixture of W2654 + W2663 1:1
Inoc D(Ar) with .01 and .001 ml/10 of each + mixture. 37°.

		ml	Tubes	
A	W2654	.01	1-3	A1 became + 11/7 AM. A2 ± 11/8. → almost pure ~~[illegible]~~
B	"	.001	1-3	
C	W2663	.01	1-3	
D		.001	1-3	
E	Mix	.01	1-5	11/8: 1: ± 2: ± 3: 0 → almost pure Lac⁺ mutants
F		.001	1-5	

also tube #0 = D(0).

P23 (out on bench all day). Streak on EMB Lac - 0-tubes and DA tubes. Turbidity 0 except in D(0).

A-D (0) as parent pure E ca 1:1 F > 20:1 Lac⁻ : Lac⁺

F(?): (DA no arabinose) ca 1:1
E, F1 (Ara.) ca 1:1

P24 0 turbidity.

~~P24~~ P9 streaks of E1, E2
E1 now ++ E2 ± E3 0 E4 ±
~~[illegible]~~ now + B1 + B2 ± B3 ±
P2? A2, 3 ±

stratifont P9:

E 1 } almost pure +
2
3
4

F 1 } almost pure +
2
3
4

A 1 ++
C 1 − −

E 0 almost pure +
F 0 almost pure +

clear that "haz − " does not persist i [illegible] some
(cf S^R!)

DATE: 10/23/04 see 1190 REF: 188 - 189

	1 A	2 B	3 C	4 D	5 E	6 F	7 G	8 H
11	✓ (o)	✓ •	x •	•	(o)	•	x •	x (o)
12	x (o)	x x •	x (o)	x (o) x	(o)	•	x •	x •
13	✓ (o)	•	x (o)	(o) x	(o) x	x •	x •	(o)
14	✓ (o)	x •	x	x (o)	• x	•	•	(o)
15	✓ •	x •	x •	(o) x	• x	• x	•	(o)
16	✓ (o)	• (o)	x	x (o)	• x	• x	x •	(o)
21								x
22								x
23								(o)
24								x (o)
25								(o)
26		(o)		(o)				(o)

188 (rows 11–16); 189 (rows 21–26)

A24. — no growth
x n.g.

Pick 188: A 11, 13, 14, 15, 16; B 11, 13, 16; D 11, 14, 16; E 11, 124 [redacted]; F 11, 12, 14; G 14, 15; H 13, 14, 16, 16; B26, D26, H23, 25, 26

also visible: ♂: A4, ~~B4~~, (C1), ~~C4~~, ~~E4~~, F1 G1 H1 H4 second
♀: A6 B6 D2 D5 D6 F...

[185]

~~dope~~ ~~pilot~~ pulled

A26 { all ♀ except G15 are Lac −
all ♂ Lac ±.
G15 pure? Lac+

No good pedigrees after 3 days refr. storage

over.

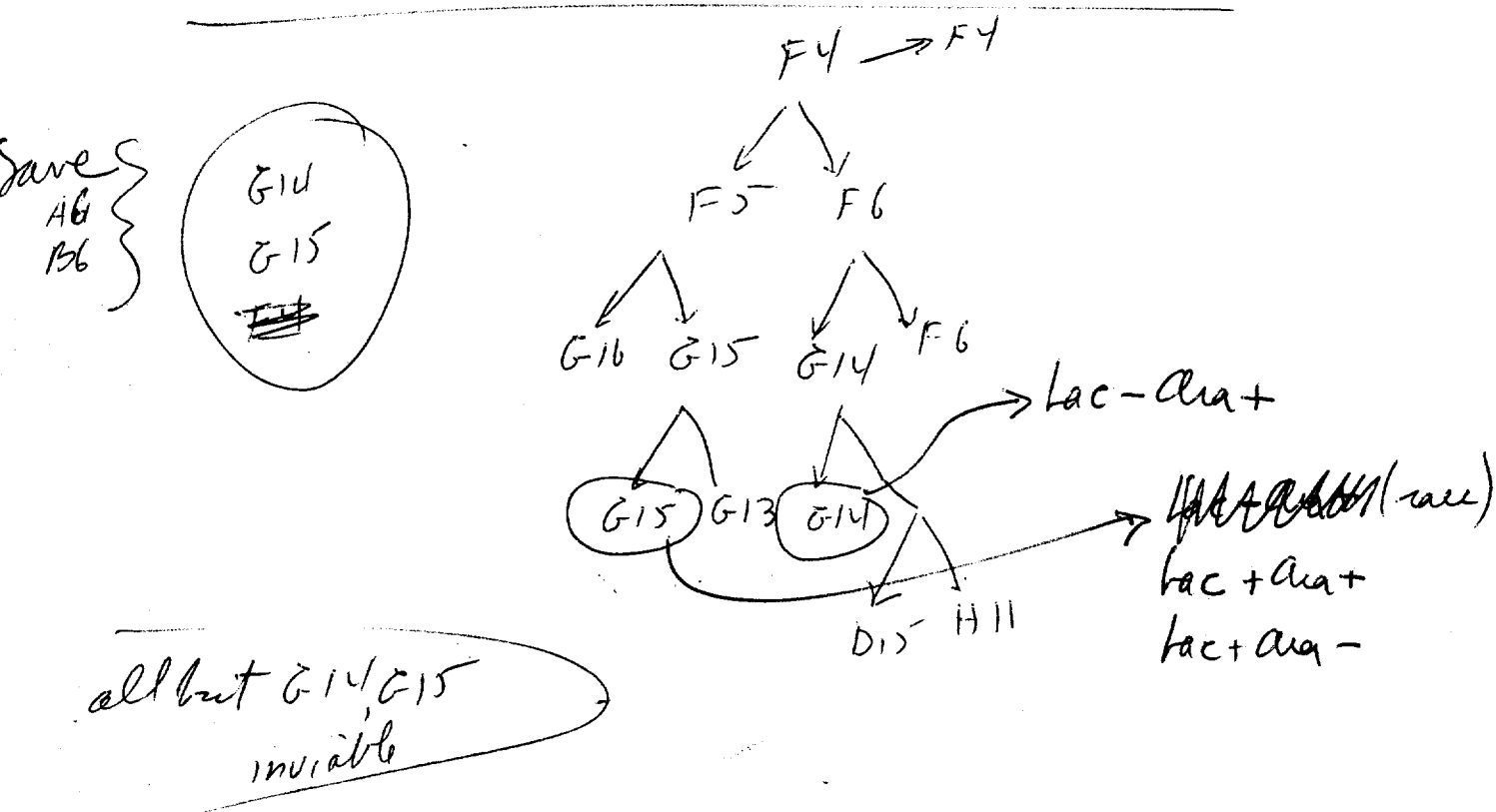
A27 all viable clones from 185 188 189.

are parental on Lac, Mal, Xyl, Mtl, Ara, Gal sm.

except # 19 = Lac+ S^R Ara+ } Gal+ MXM−

18 = Ara+ Lac−

185-1-2 should be rechecked on EMS Gal for purity.



all but G14/G15 inviable

G14: pure Ara+ Gal+ Lac−

G15 Ara+/− "

For future tests, use "MXM" (Mal+ Xyl+ Mtl) EMS in preliminary screening.

also

ca 7/14 – 16

Look at Caulobacter.

(1) slant from Hutner is contaminated with interesting motile rod ($\frac{1}{2}$) (2) grows better ~~than~~ in peressay than NSB, and OK at 37.°

26-2a FEB 25 1955 Photo

26-2b

27B4 (small plate, range #)

27 B1

27 B2

27 B3

---

29 B2

29 B1 FEB 26 1955

28D

should do 28D1.

DATE: REF:

G14: pure Lac− Gal+ Ara+
G15: pure Gal+. Reported rare Lac− among many Lac+.

of 12 Ara+ all Lac+
12 Ara− all Lac+.

at least 1 Lac− Ara+ identified. Reexamine for Lac− Ara−. V_1 should also be scored: both V_1^R on Lac.

On restreak, G15 was pure Lac+. (Previous Lac− colony probably came from G14.) Absence of Lac− Ara− is notable but might be in other parts of pedigree, unstudied. If sequence is Lac − V_1 − Ara, implies crossover of V_1 − Ara. Ara− should be checked also. Since Lac? did come from late [illegible] restreaks! — These are all Lac+ V_1^r

Ara− are Lac+ V_1^s. ∴ Types recovered are

Lac+ Ara+ V_1^r Lac+ Ara− V_1^s and Lac− Ara+ V_1^r as if 3/4 strands from $\frac{+\ +r}{-\ -s}$ hybrid with c.o. between Lac/Ara. Unfortunate that others are not recovered.

Note A5 and A6 also had mottled appearance on EMB Gal, but DCO failed to find any evidence of segregation.

Reciprocal, if one c.o. is recovered, should other be also?

Luca - J L. Indirect selection 1193

DATE: P25 10/5/54. REF:

7-36 assays at 125/ml. (wrong: per 0.1 ml)

8- Therefore samples of .5 ml will have 62 R.

dilute .2 + ~~6.1 ml per assay~~ + 12.2 ml Pen

Distribute .5 ml samples to 10 ml H_2O, sal

Found most cultures had mutants — note error in planning above! (average inoculum was 1 mutant per tube!

DCG assayed all tubes (1-10 = ~~H2O~~ sal) (11-20) = water.

8-1 = 169, 160 × 10^6 8-11 = 142, 144 × 10^6

(1.65×10^8) (1.43×10^8)

DCG notes 1/4 of colonies Gal-!

Sensitive

on SM, per .1 ml: presumably variability in lag.

8(1-11)		11-20	
	7		27
	32		31
	15		12
	34		9
	17		27
	3		1
	12		16
	9		0
	11		12
	23		17

Note adverse selection! Parent cultures had ca 125/.1 ml.

8(2) series: dilute 7-36 by 1:6250, then .5 ml into each of 20 tubes of saline. Found 1 tube (8(2)-3) to have cols. by loop assay. But 0.1 ml assay shows only 34, 20 colonies per 0.1 ml!

Remember: 8(1)-3 = 8-C 8(2)-3 = 8-D (over)

Do periodic selection coming in?

Reconstruct[illegible] by Luca

stage 14-5

11/27 Pick 109 singles from EMB-0
also 8 clusters of 5, to EMB lac I sm.

None of singles, 2 of 5's showed S^R.

Restreak these on EMB lac for final isol.

= total of 2/149. A, B.

(102716.) 10 colonies on EMB lac. Pick at random
3/10, 2/10 SR

Parallel platings at last stage (+ replicas) showed S^R = Gal⁻
(cf S^R suppresses $Su^+_{Gal_4}$). This isolate has weak if any response to $S14$.

Note C Gal + and ±? together rated as
Gal - — not on second haul, probably matter of fading

~~C1 = S^R Gal -~~
~~C2 = S^S Gal?~~
C3 = reportedly S^S.

Gal+ reversions of W2716 were noticed to be Gal $\underline{v}$!

---

passed through 20 passages (ca 10^{-3} ml/10 - 10^{-4} / mo.), i.e. for

1/10/55 about $\log_2 10^{80}$ = about 250 generations, then plated.

DCG examined ca 1000 colonies (5 plates); all were S^R on replica. One isolated as W2716-20 for quantitative comparison.

About 0.1% of colonies at this stage were weak Gal+. Proved still SR and, as above, unstable +. Save ①.

TZ and crosses

1195

DATE: OCT 27 1954

REF: Some preliminaries (1194) not recorded!

Grow ♂, ♀ overnight.

8:55 add 1ml broth culture to .1ml .05% TZ

10:05 – treatment 2: spin down + resuspend

" 3: use stained cells per se

ca 10^{25}

(1)	♀ + ♂3	1ml : .5	7ml Penassay	
(2)	♀ + ♂2	" "	"	
(3)	♀2 + ♂	1ml : .5	"	37°.
(4)	♀3 + ♂	" "	"	

Also note .2ml TZ went slightly faster.

addition of 1ml fresh broth (.2ml TZ) at 8^{05} delayed coloration about 1 hour

♂ reduced TZ > ♀.

Exp. n.g. – label (TZ) insufficient for low power determination.

Conclusions – so far, TZ label has not been satisfactory. In growth overnight in TZ, much of the label is extracellular. In briefer periods so far, there has generally been just too little label to be workable. Needed: some better system incorporating the label, especially in line 28. This should not be allowed to interfere with pedigree work and cytology

DATE: Nov. 2 1954 REF: 192

11/1 Prepare ♂♂, ♀ T2 for prel. study

General conclusion. ① T2 label can be introduced in ca 2 hours (in old broth .005% T2)

② T2 interferes considerably with motility but some pairs may still be obtainable.

P1 prepare labelled cells. Let stand in frig:

A2 Most ♂ T2 had settled. Supernatant may contain the most active labelled ♂♂. Take off about .4 ml and mix with ♀ unlabelled + ca 1 ml broth 10:30 AM.

(also prepare freshly labelled ♀♀ and fresh ♂♂

In general, only a few labelled ♀ proved satisfactory.

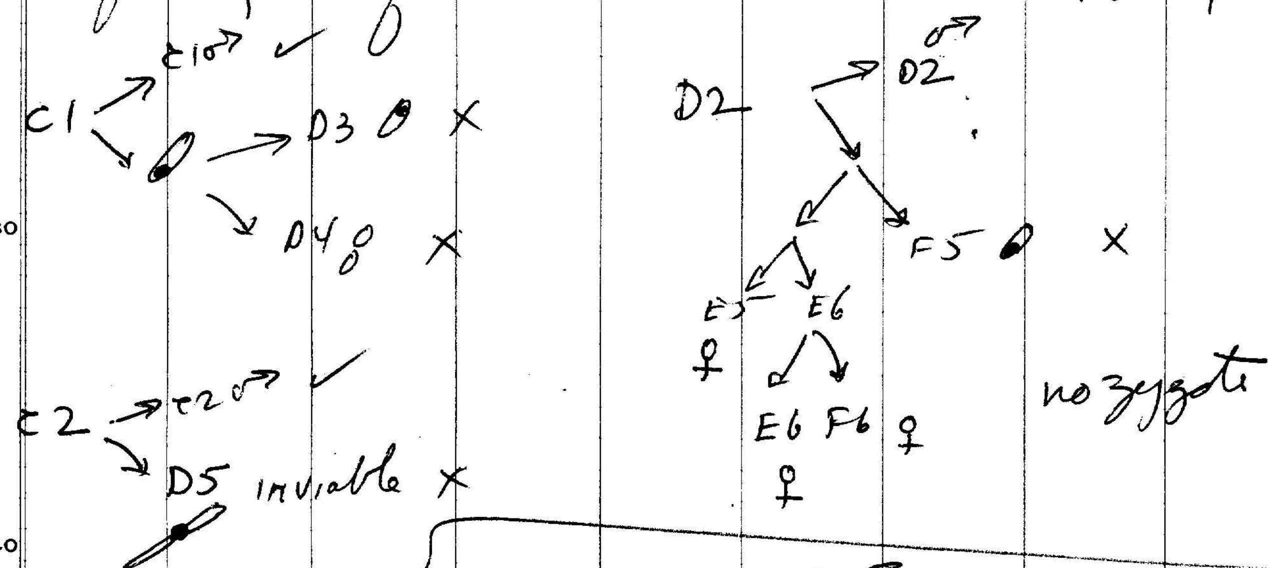
DATE: REF: 192 - 193

Comment: [192]

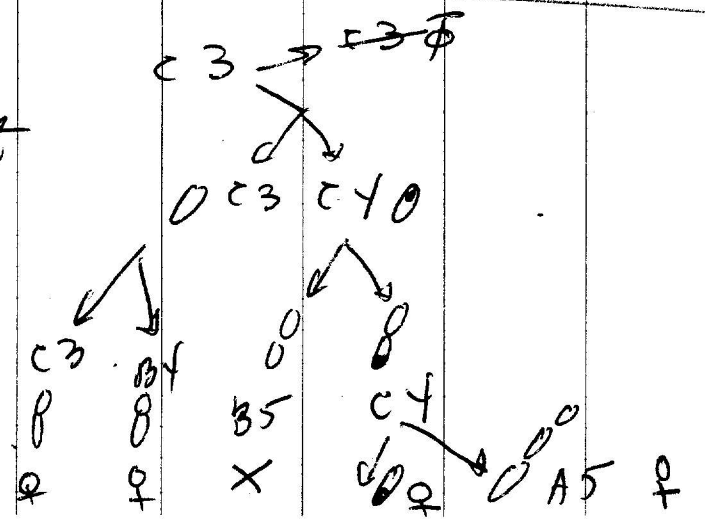
E16, 6, are ♀ & ♂
C2, C5 } are ♂♂
F2, F3 }

[193] C5 was given as non motile unless contaminated. Not examined in detail; may have been silly. pair ~~C5 was given as non motile unless contaminated. Not examined in detail; may have been silly. pair~~ (Examine now)
C5 & OK. But examine for motility of present culture.
F2 - F3 no record!

[192] was attempt to follow pedigrees c TZ only the following are of interest: Most 0 did not grow



0 survived at least 3 generations but no zygotes.



DATE: REF:

	1	2	Lac	Gal	Ara	MxM	Sm	8	9	10
1	C1		+	−	+	+				
	D2		+	−	+	+				
	E1a		+	−	+	+				
→	42 [illegible]1b		±	+	−	−	R Lac −			
→	E1b		−	+	−	−	R Lac −			
♂♂	193A1		+	(+) error?	+					
	2		↓	↓	+					
	3				+				∴ all ♂♂ OK	
	4				+					
10	5				+					
	A6				↓					
	C1									
	C2									
	C4									
	C6									
	E2									
	3									
	4									
	5									
20	6									
	G1									
	2									
	3		↓	↓	↓					
	193H2			+−			R −+			
25	192E2		+	[illegible] −	+		R −+		[illegible]	
31	192 A2		−	+	−	−	R −			
2	3		−	+	−	−	R −			
3	5		−	+	−	−	R −			
4	B4		−	+	−	−	R −			
0 0 / + + → 35	192C2		+	−	+	+	S			
36	3		−	+	−	−	R −			
37	4		−	+	−	−	R −			
→ 8	192C5		+	−	+	+	S			
9	E5		−	+	−	−	R −			
40	E6		−	+	−	−	R −			
N.R. 41	192 F2		+	−	+	+	S			
2	192 F3		+	−	+	+	S			
3	F4		−	+	−	−	R −			
4	F6		−	+	−	−				
45	193 B1		−	+	−	−	"			
6	2		−	+	−	−				
7	5		−	+	−	[illegible] −				
8	6		−	+	−	−	"			
9	C3		−	+	−	−	"			
50	C5		−	+	−	−				
51	D3		+ −	+	−	−	R +−	im		
2	4		−	↓	−	−	R −			
3	5		−		−	−				
4	6		−		−	−	↓			
55	F2		−		−	−				
6	3		−		−	−				
7	F4		−		+	[illegible] −	R −	m		
8	5		−		−	−	↓			
9	6		−		−	−				
60	H1		−		−	−				
61-62	H4-H5		+− / −	↓	−	−	R+− / R−	m		

DATE: REF:

1	2	3	4	5	6	7	8	9	10

Conclude: It has no particular value and tends to impair viability as well as motility.

[192] E2 needs review! Mated as mixture! Only part may have been picked up!

[193]. Here random pairs were picked & pedigree analysis.

H2 mixed as recorded! ~~What are H4 · H5?~~ ~~pick A6~~

Pairs candled are

	♂	♀	♀2		
	A2 ✓	B1 ✓	B2 ✓		1
A1♂?	A3 ✓	B3 ✗			
	A4 ✓	B4 ✗			
	A5 ✓	B5 ✓			2
	A6 ✓	B6 ✓	H6? ✓		3
	C1 ✓	D1 ✗			4
	C2 ✓	D2 ✗			
A1	(C3 ♀) ✓	D3 (R)	H5	may have E♂?	5 4
	C4 ✓	D4 ✓	H4		6
	~~C5~~	C5 ✓			7
	C6	D5 ✓	D6 ✓		8
	E2 ✓	F2 ✓			9
	E3 ✓	F3 ✓			10
	E4 ✓	F4 (R)			11
	E5 ✓ ~~F5~~	~~[illegible]~~ illegitimate			
	E6 ✓	F5 ✓	F6 ✓		12
	G1 ✓	H1 ✓			13
mixed	G2 ✓ ~~[illegible]~~ →	H2 mixed.	~~[illegible]~~		
	G3	~~[illegible]~~ (R)			14

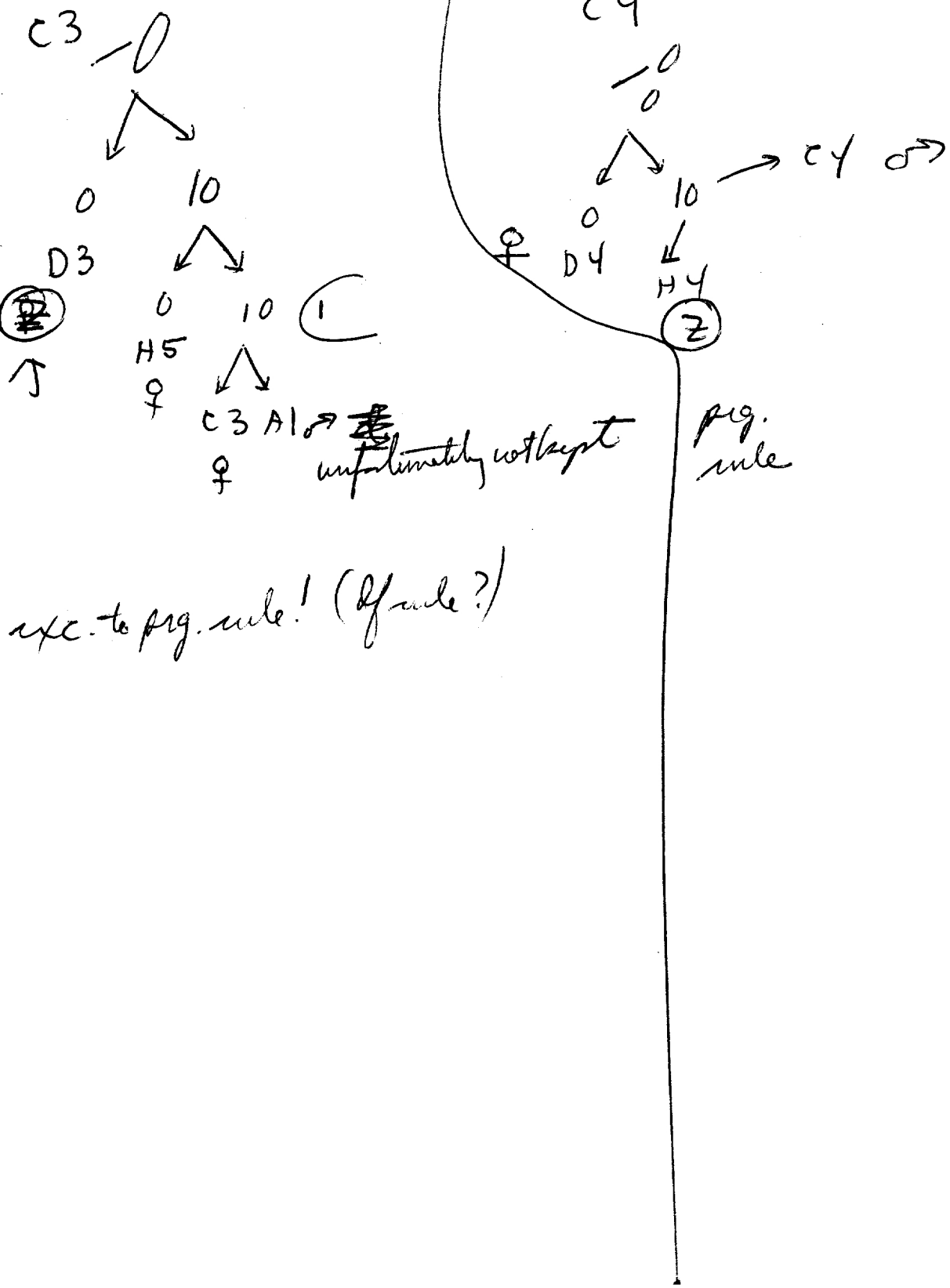
Total score then is:

3 (R) from 14 reasonable pairs! Why so low? Pedigree analyses have indicated a higher incidence! Maybe based on selection for clonal integrity in the pedigrees!

14 viable pairs
4 inviable ♂♀
no pedigree

Owing to temporary mislaying of one protocol sheet, not all pedigrees were clearly stated and some sibs thrown out. See over.

A1-C3-D3-H5
C4-D4-H4
~~C4~~ -F-~~G~~



DATE: 11/7/54. REF:

Productive pairs were

C3 - D3 — 8 large 1♂ OD3 → C3 (pres! Fate of male?)

E4 - F4 1♂, 3♀ 1 E4 OF4.

G3 - H4 1♂ : (11) G3 O H4

There is no ambiguity in the history of these, though it is unclear why H4 should have been chosen for G3, unless preempted. Note inversal of pairing relationships in C3. (Zoxo was ~~prepared~~ copied rather late)

Recombinants in ♂♂ x conjugants would be difficult to detect. Should routinely restreak ♂♂ on EMB Lac.

Save ♀♀ above and ♂♂ for recheck

C3 ♀ D3 Lac+/- MxM - Ara - SR. Score V, R/S; V,s V,s

E4 ♂ F4 Ara+/- Lac - MxM - S^R ♀ $V,^S$ ♂ $V,^R$

G3 ♂ H4 Lac+/- MxM - Ara - SR. ♀ $V,^S$ ♂ $V,^R$

Rec. not of very great interest.

192E2 - mixed! But no record of separation of clones.

DATE: Nov. 4, 1954. REF: [193]

J: 10 :7ml ♂: 9 ca 12^45 - 12^15 setup. Ca. 24 pairs isolated and allowed to separate. Minimum of pedigree analyses except in re intercurrent fission during conjugation. Pick mixtures with [192] for tests.

pairs isolated as.

17	♂ surv.	♀ surv.	Zyg.	~~types~~
10	✓	✓	no	
0	x	✓	no	
3	✓	✓	✓	
4	✓	x	—	

11/55

DATE: Nov 8, 1954 REF: Jacob letter, mo. & CRAS article

cf 1079

24 hour cultures as mornings 1ml ♀ : .2ml ♂ : 7ml Penassay

A W1603 (W1177 Lp^s)
B W1177
C W1895M1 ($\sigma_2^?$)
D W2344M1 (♂)
E W2401 (♀)
F W2578 (W1607 Lp_2^r).

Inc. 37° 8^30 – 10^10 PM.
Plate ca 10^3 / EMB lac sm.

Est SR+/SR−

also got W2588 = Lp^r Lp^r as SR

AC	54/1000
AD	37/1000
BC	22/300
BD	28/300
EC	10/300
ED	8/300
FC	
FD	too weak lethal +

Note no marked difference in efficiency of recombination of Lp^+, Lp^s Hfrs!

Try Hayes' Hfr.

A10 Repeat with W2[illegible] (= G) (old cultures)
1:1:7ml 11^15 AM – 12 PM.
Refrigerate to 3PM.

1603 A, 1177 B, 2401 E } { C 1895, D ♂, G 2344

	SR+/R−	Plaques/518
AC	17/300	ca 50
AD	14/300	
AG	3/300	ca 20
BC	15/200	++ (10^2–10^3)
BD	14/150	
BG	7/200	ca 100.
EC	10/300	
ED	6/300	
EG	0/300+ ←	

Results: inconclusive if W2344M1 and λ^s × W2401

Note $Lp^+ \times Lp^+$ gave more λ than $Lp^+ \times Lp^s$. In all combinations W1895 was more fertile than W

(over)

also plate AC and AG, BC, BG on λ^s indicator. (2401)

Repeat P15 use old cultures as inocula 1:1; 7 ml
2 1/2 hours.
streakout EMB lac sms. Score SR+

W2324 Motilized	x W1177	0	
"	x ~~W2344~~ W2401	0	
W2324	x W1177	++	(>1%)
"	x W2401	++	also note plaquing!
W2344	x W2401	++	

---

again P17 (A) old cultures 7:30-9PM
1:1:7 (B) fresh cultures (from above) 9PM-10PM

ca 1% in all B. In A, ~~♂~~ W2324 x ♀ was ca 1/10 % SR+ but x W1177 and ♂ x ♀ gave ca 1%. ∴ ? W2324 is more affected by aging than is W2344.

(C) A18. overnight cultures 1:1:7 9[50]-11[30]

♂ x ♀ ca 1%
♂ x 1177 >1%
W2324 x ♀ >1%
W2324 x 1177 +, <1% Some plaquing again.
What is this phage Ecoli acts on W2401?

Then why no zygotes from pairs?

Conclusion: W2324 may be slightly less fertile than W2344. No clear evidence here of zygotic induction. Should use Jacob's conditions, count inf.c.

DATE: 11/19/74. REF:

Motilize W2324 for crossing purposes — 1 passage.
~~too~~ Cross culture was almost sterile
S.C. ī gave no SR+ × W1177. Conclude probable F − (W2696)

P20	W2693	× ♀	SR+: 1 papilla only in thin's brain!	Recheck for fertility!
1 hour	W2324	♀	ca 0.2%.	
1:1:7	"	W1177	2%	
	2344M1	♀	2%	

W2324 (Hayes Hfr) x W2401
single cells

1199

DATE: Nov 11 1954 REF: 194

Cross ① 9:40 - 10:30 thersametime at RT
② 11:15 - 11:35.

A1. Unseparated pair → ♀♀ only.
~~A~~ - 6 -
~~B1~~ - 2 ✓
B3 - A3 - B4 ~~illegitimate~~
C1 - C2 ~~C4~~ C5
C3 - C4
D1 - D2
D3 - D4
~~E1~~ - E2
~~E3~~ - E4
F1 - F2
F3 - F4
G1 - G2
G3 - ~~G4~~
H1 - H2 - ~~H5~~
- H3 - G4 - H4 - H6

Complete pairs: 9
Pro (♀OK) :
—
no zygotes

No lysis seen! Numerous pairs despite indifferent motility of W2324. Cells of latter are shorter than W2344, & harder to distinguish from W2401.

all parentals are lac± ser, 14 x M. (Gal+).
~~exp~~ except B3 which is lac+/- (lac-SK) pres. mixed.

lac- : A1, A6, B2, B6, C1, C2, C4, D2, D4, E2, E4, F2, F4, G2, H4, H6.

lac+ : D3, F1, A3, B4, B5, C3, C5, D1, F3, G1, G3, G4, H1, H2, H3.

Non-motiles in F1? (mix. segr.)

presumed just mixed (not c chain)
B3 - A3 - B4

Save F1, C1-C2-C5, D1-D2 as examples.

single cross ~~[illegible]~~ × ♀
W1895M1 ♂

DATE: Nov 13, 1954 REF: 1917

Gal ≡

D1 - 10 → (1) , 1 0 0 0 Presume.
D1 2 3 6

→ D1 → (D6) ; D2 × 3

all these Gal+ Lac+/- V_1^s

E1 - 0 → (1) 0E2 0E6 ; 10 → E1. 0 → 0E3 0(A3)

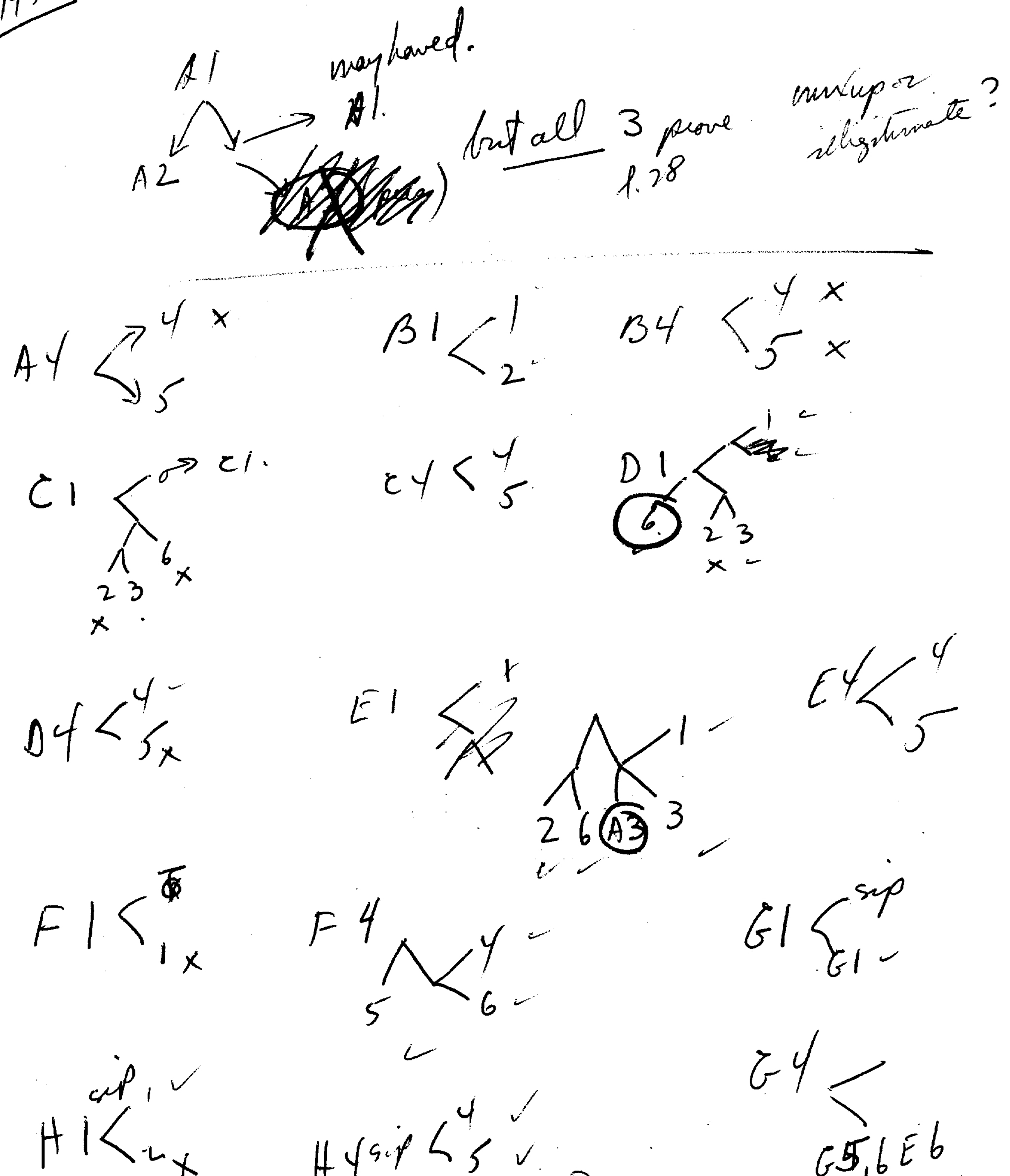
A3 E3 3 E6

parental ♂, ♀ resp except A3 Lac+/- and D6 S^R Lac+/- Lac+/- B4/n.g.
all parental on MXM ~~Ara~~

Presumed to have been W2344M1 × W2401. However, all ♂♂ were Gal+ and at least D1, E1 were $T1^s$. ∴ must have been W1895M1 instead, ~~[illegible]~~ which had been set up concurrently! Confirms as D(0).

Yield 1/9 1/2 c. Inc. } 2/11 zygotes

195



♀♀ complete: A5, B2, C5, (E 2,3,6 (A3)) E5, (F5, 6), G1 (sip), H4 (sip),
(G5,6,E6) : (9)

♀ partial: (C3), (D2 (3) 6) : (2)

∴ 2/11 zygotes. save E1-2-3-6-A3 and D1-3-6