Experiments on motile lines

1131 – 1151	Feb 15, 1954 – April 14, 1954
1212 – 1262	Jan 11 – June 2 1955
1272 (Leifson)	Sep 8 1955.

Salmonella Fla$^+$ ⟶ ✗ 1131

2/15/54

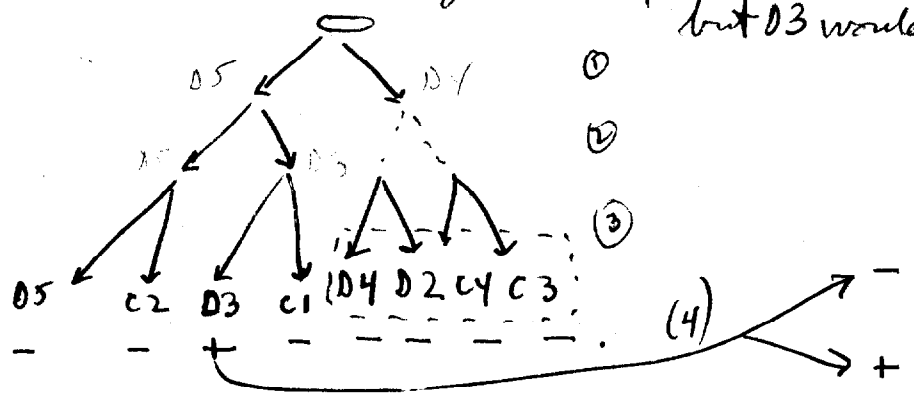
Mix FA12 (SW623) + SW666 + ca/vol. broth About 10^{30} Am — 33° etc. Search for motile cells in enc. droplets ~~Rare~~ Fla$^+$ seen (rare in FA22 ✗). Pick and set to allow clones as convenient.

Clone CD. Motile, but became immotile on transfer

ca 5:15 First division: separate At 7:50 both daughters had divided, shortly thereafter, before separation, second division in one line. C.410, after separation other one also divided, less regularly.

Slowest fission was D3 — C1 Add fluid

A16. Examined for motility. Thus most descendants are immotile, but D3 would have formed a stream.



Other isolates

- C5. Initially M+, then M−. Initial growth poor ⟶ NM. Lp^3
- D5 Removed motile for some time unless stuck ⟶ NM. (~~Fl~~ Lp$^+$)

E: 1 deep had many motile: E^{2}3, 4, 5 ⟶ all M E1 = mess ("NM").

(late). 1 clone? [E1, E4 Lp$^+$, others Lp^3.]

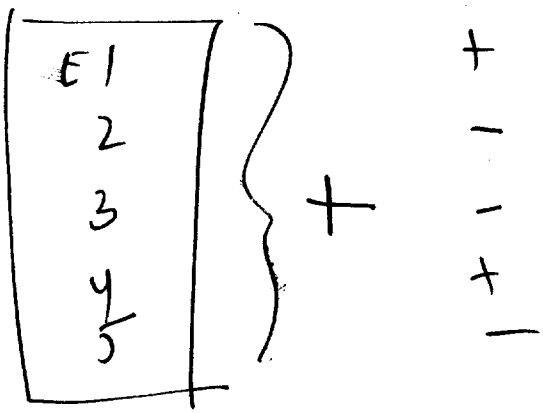
A: Controls: Lp$^+_2$, Lp$^+_3$, 4 ⟶ NM (~~Fl~~) (4 n.g.) 5 = deposit ⟶ NM. (Lp$^+$)

Blank control OK.

Pick for homogeneity test and serotyping.

Fla (anmotagan) 666

A2	−	+
A3	−	−
A5	−	+
B5	−	+
C1	−	−
C2	−	−
C3	−	−
C4	−	−
C5	−	−
D2	−	−
(D3)		−
D4	−	−
D5	−	−



all good T
just result would be weaker

A. Test $H_1^?$ of Fla_1^- conjugants of clone 1131CD. Grow motility tubes ~~+~~ D5, etc. and FA12. [Should have used 9!]. Look for $Fla^- H_1^i$ crossovers

~~D. + FA9 (es should be used!)~~ of course FA 9 → all gave us Fla^+

B. Test lysogenicity character on SW 666.

	Fla	H_1	Lp_{22}
C1	−		−
C2	−		−
C3	−		−
C4	−		−
D2	−		−
D3	+	i	−
D4	−		−
D5	−		−

C. Test single colonies from D3 for motility (incr. droplets under oil)

12 Fla^- : 8 Fla^+ Save 1, 2, 3, 4

(1, 2, 5, 6, 8, 10, 12, 13, 16, 17, 20) (3, 4, 7, 11, 14, 15, 18, 19)

∴ Conclude that final segregation occurred at (or since) the 4th fission. Phage apparently not penetrant (as noted frequently in this system).

B5, C5 were evidently stock formers.

1 ✓ = H^- ✓

3 + $i^+ + b^+$

4 + $i^+ + b^+$

✓ Alec finds same result + will check further. All are 1, 2...

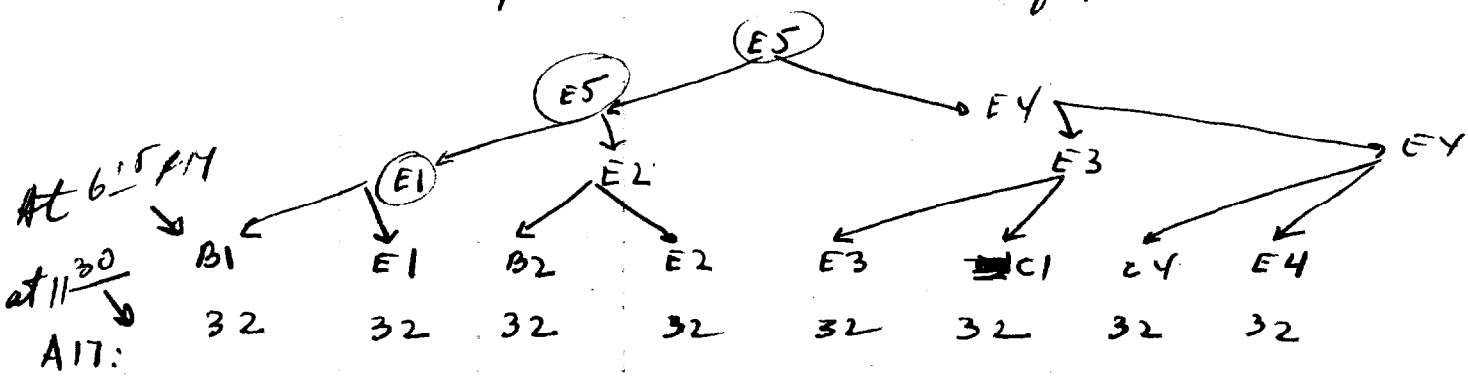
2/19 D FA 10 → 1, 2, 3, 4 probably b.

A

2/16.

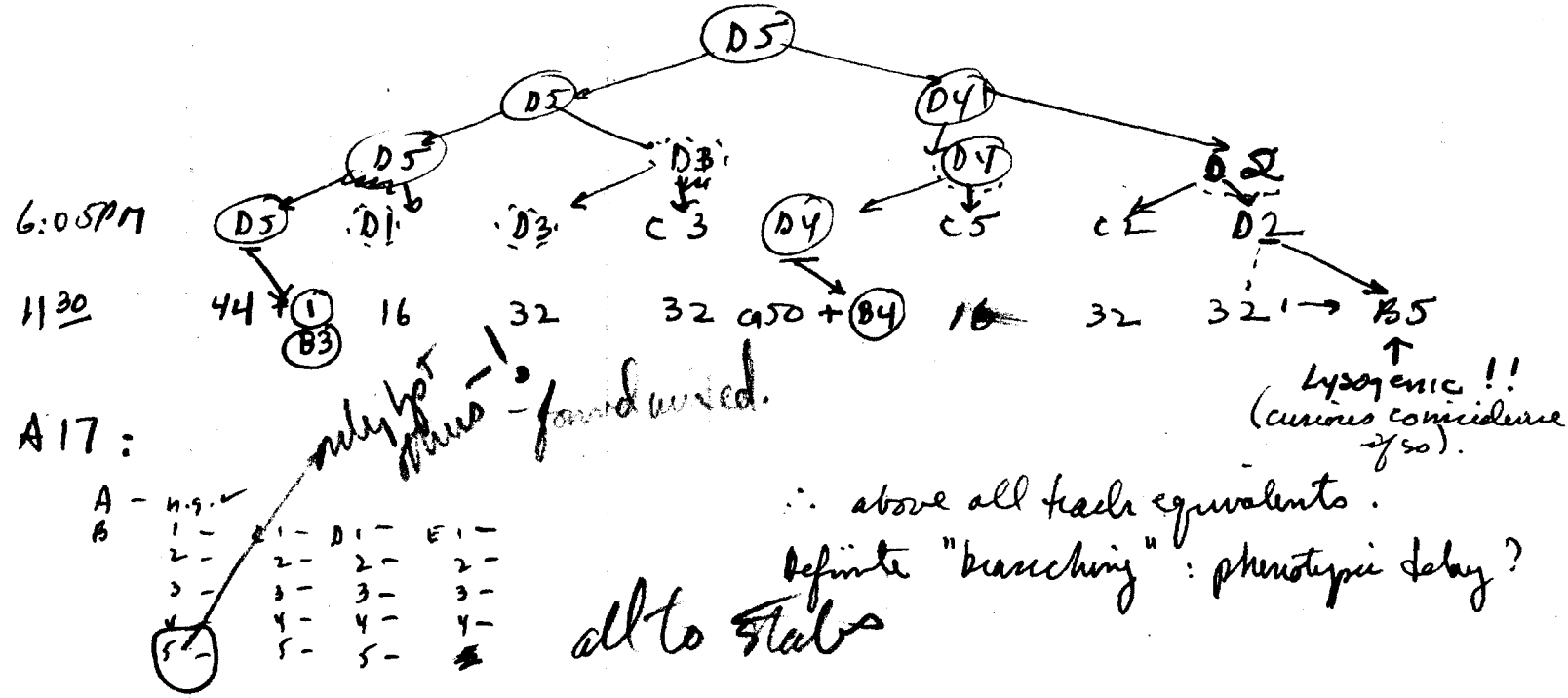
See 1131. 12^{25} to 3:50 PM before spotting drops. ○ = phen. motile

2 cells initially motile and at 1st transfer.



∴ at room temperature about 1 fission per hour. Had rested desiccators to warm perversely.

+ 8 generations of trails in each branch.



∴ above all trails equivalents.

Definite "branching": phenotypic delay?

added fluid ca 11^{45} PM. Picks all drops (no visible Fla$^+$) A17 to nutrient agar (i media).

[In future might be better to follow such trails more closely by chilling overnight ~~I.~~ order to find even late residual + by plating on soft agar!].

Fla+ →x

Febr. 17, '54

FA12 →x SW666. At room temperature, but 11:30 AM – 3:30(+) PM. probably allows too long. Rather large drops provide quite easy selection of Fla+ from large populations, Fla− falling to interface. See dictated record for details of manipulation technique.

Separate originals (E5), (D5), (E3), (C5), (B5), (E2), (E1), (B2) ← H_1^b

No pedigree on —.

○ motile when last seen or until fission + separation — all.

E5 < C1 ○ / [3] < E4 + H_1^i / [2] < D3 + H_1^i / E5 + H_1

E5: (E5) (D3) (E4) (C1) — x, i, i, 0 [they split off early, ?]

(D5) → D5 < (D4) — / (D5) —

~~(C5) C3~~

(C5) → C4 ± [?] / (C5) → C3 — / C5 —

(B5) → B4± — / (B5) —

A18: E3 —, C5 —, B5 —, E2 —

E1 + (? ted.), B2 —

[Plan somewhat disorganized. Probably best to collect a number of motiles early and not separate clones, or else isolate a few for full clonal analysis.]

B 2, 3, 4, 5

C 2, 3, 4

D 3, 4

E 2, 3

Lp22 ... all are + not necessarily pure

Streak before it Fla+ for purity

Test and cols.

E1 10: all −, 12: 1+, 11−. {Bluish says 6 for mess.

E4 7 all + i

E5 6 all − (b) i on pipette tube test

D3 5 all + i A.S. is ?

E5 pedigree ~~suggests~~ mixed clones! But finds 1133E1 $Fla^+ = Lp^+$, $Fla^- = Lp^-$

Feb. 18, 1954. 2/21/54.

FA12 – x SW66. Ihou 37°; Refrigerate.

(a). 2/18. Day 1. FIRST ISOLATIONS 4:45 – 5:25. In this interval also, A5, B5, had divided. All cells actively motile. But note that the two early divisions both gave lethal!

These isolations included:

A5
A4
A3
B5
C5
C3 } from 1 drop.
C4 }
D4
E5.

(b) At 10PM, ~10^4.5 cells had given clones of 2–25 individuals, 2/18. usually with 1 motile. This was separated as indicated. Leave at R.T

2/19 (c) 9:30 – 12N. Resample drops, leave to ~~4~~ second group as indicated. R.T

2/19 (d) Brief Exam. 5PM. Refrigerate.

(e) Resample A20. Where 1 motile cell already obvious, resample to another drop in same site. Incubate at 37° from ca 12N – 4PM, then R.T. (already too long!) Refrigerate ca 6PM – 10PM. (or at 9PM?)

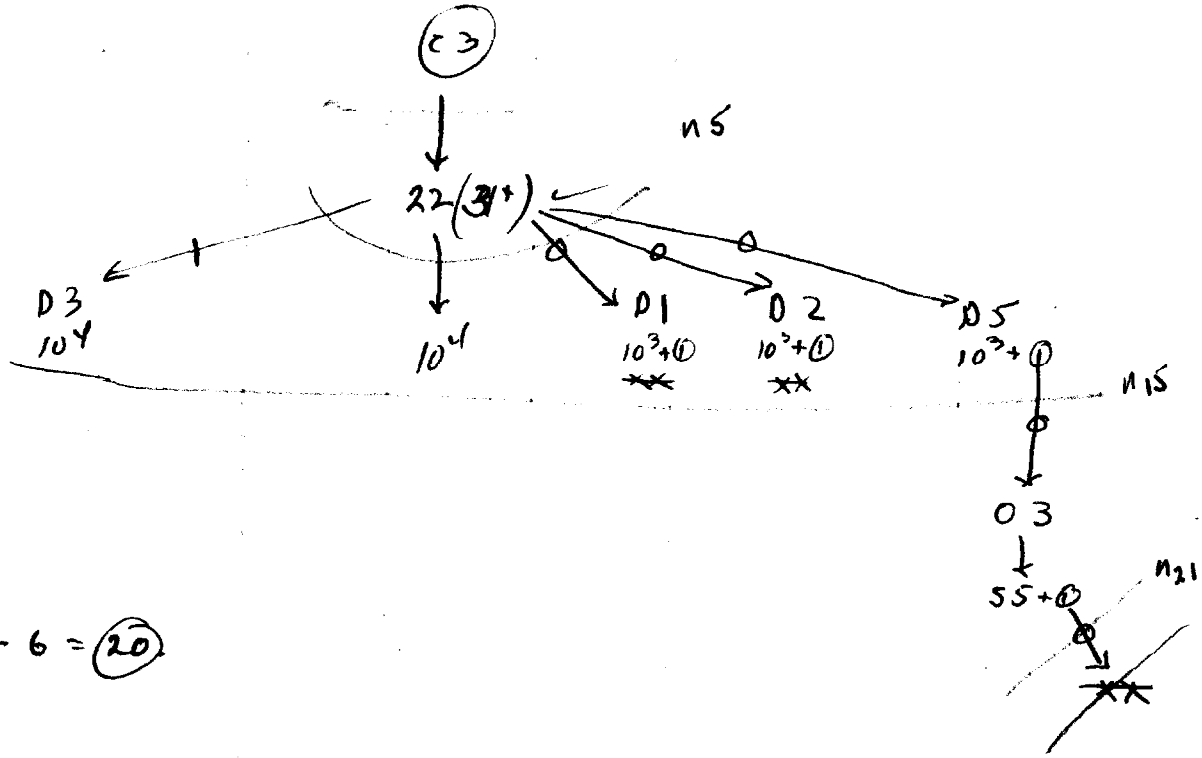
But this gave very large clones, perhaps also impaired motility. Some dried out. It is therefore uncertain whether this is natural termination or whether 37° played some part.

(f) Resample P20. Refrigerate for reexamination of those clones that are not too large, and for subsequent plating.

Note: when (1) dies due to [illegible] of lab, [illegible]

C3

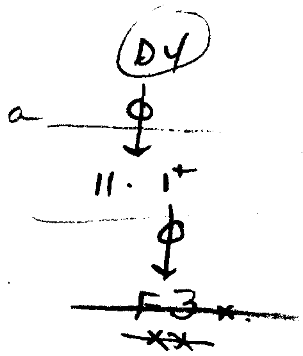
check notion time of isol. of D1-5.



∴ limited early replication only.

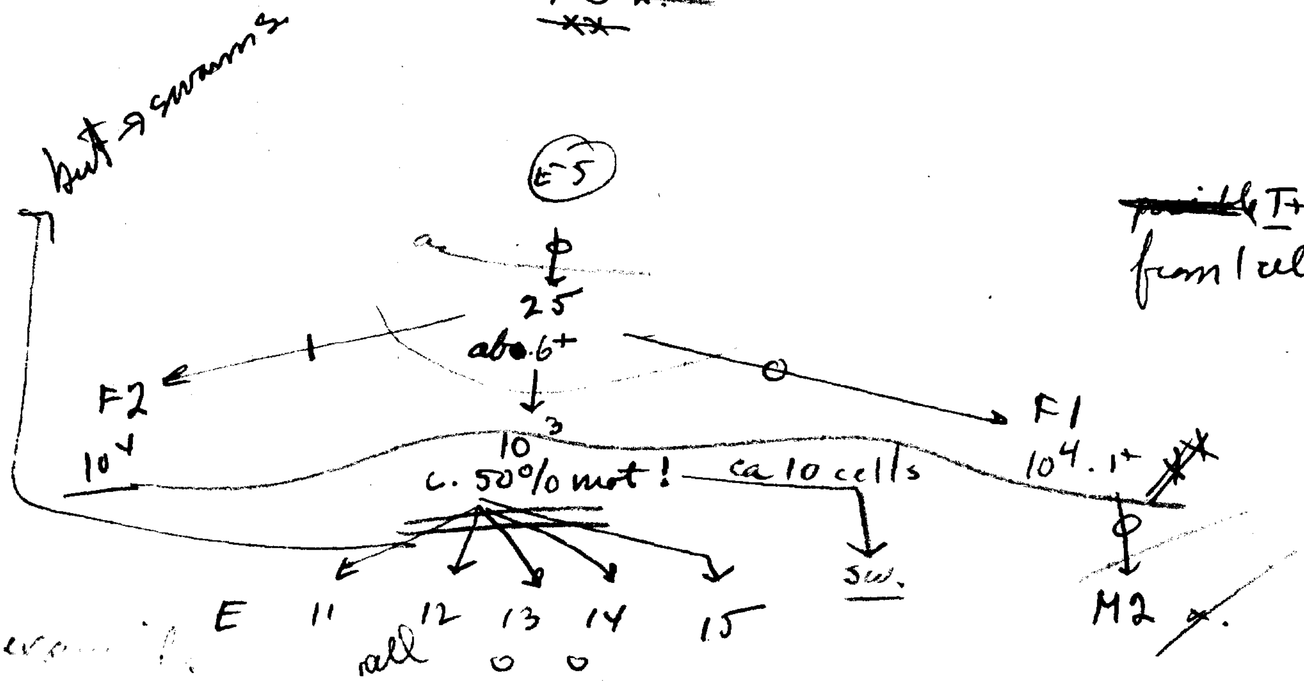
O3: $N = 4 + 10 + 6 = 20$

D4.



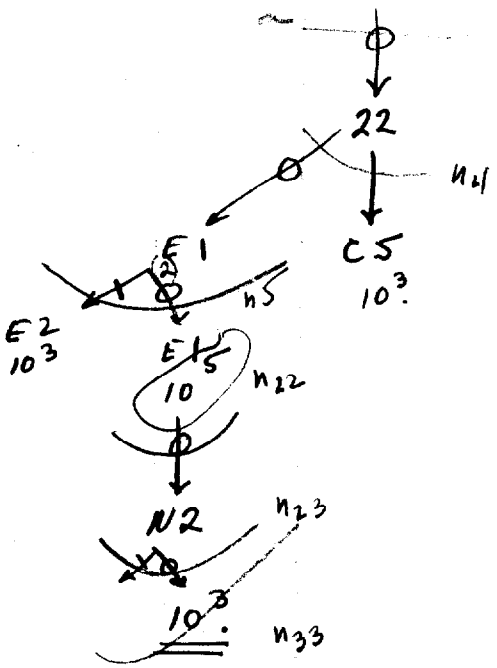
E5.

~~[illegible]~~ T+S. from 1 cell!



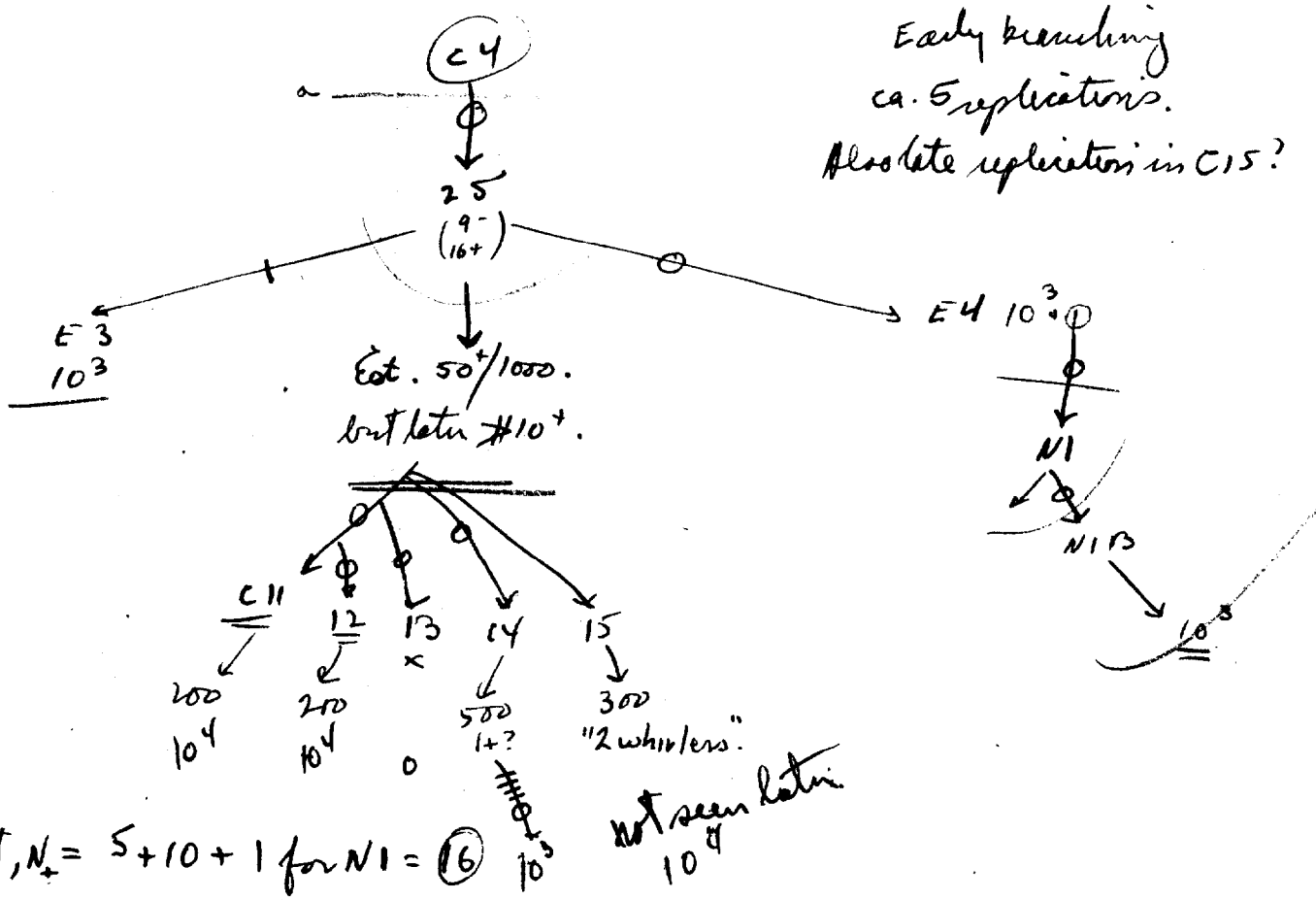
not yet exam…

C5



No branching.
Linear tracks =
4 + 1 + 17 + 1 = (23).

C4



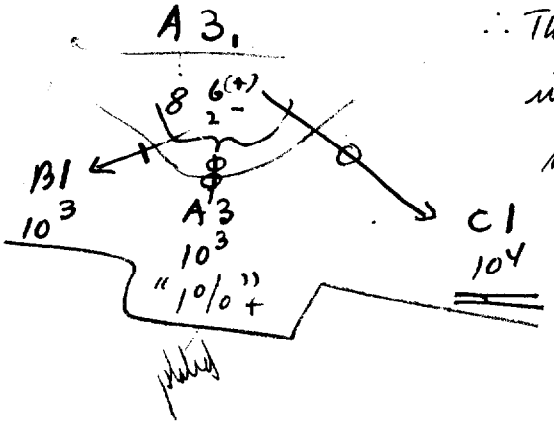
Early branching
ca. 5 replications.
Absolute replications in C15?

If correct, N_+ = 5 + 10 + 1 for N1 = (16)

C15: 5 + 10 + 9 = (24)

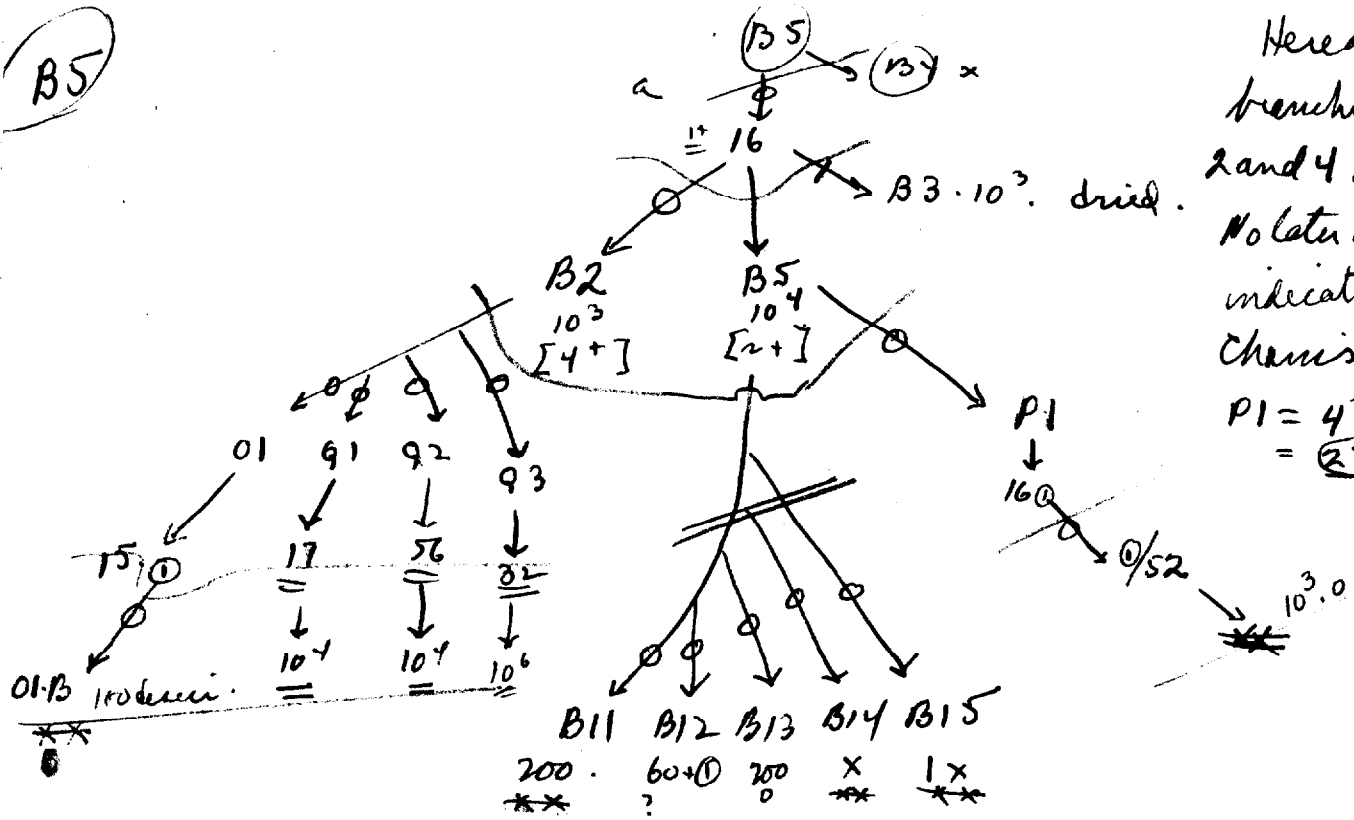
A3

A3 should have been included under 1135 but was plotted instead.



∴ This also probably an initial branching of semiclone, probably not quite completed at N = 8.

B5



Here again, covert branching between 2 and 4 divisions. No later branching indicated.

Chemis: with!

P1 = 4 + 13 + 4 + 6
= 27

Leaves fooled by such clones as B5 thinking that numerous + botolaneid stabilizations of a late clone.

Total N for All... →

is $4 \times 10^4 \times 70 \times 100 \times 4 \times 2 \times 6144$

$= 32 \times 70 \times 6144 \times 10^6$

$= 10^3 \times 10^1 \times 10^6 = 10^{13} = 2^{40}$.

Total transfers actually: 16.

Semimotile sibs: 8 actually followed;
estimate ca 20 total.

Latest replication: $\star n = 12$.

Should follow earlier divisions more closely.

Resume as of 2/21/54 AM.

1134

+ → 1 non motile
-o- → 1 motile cell
× n.g.

Platings on motility mixed.

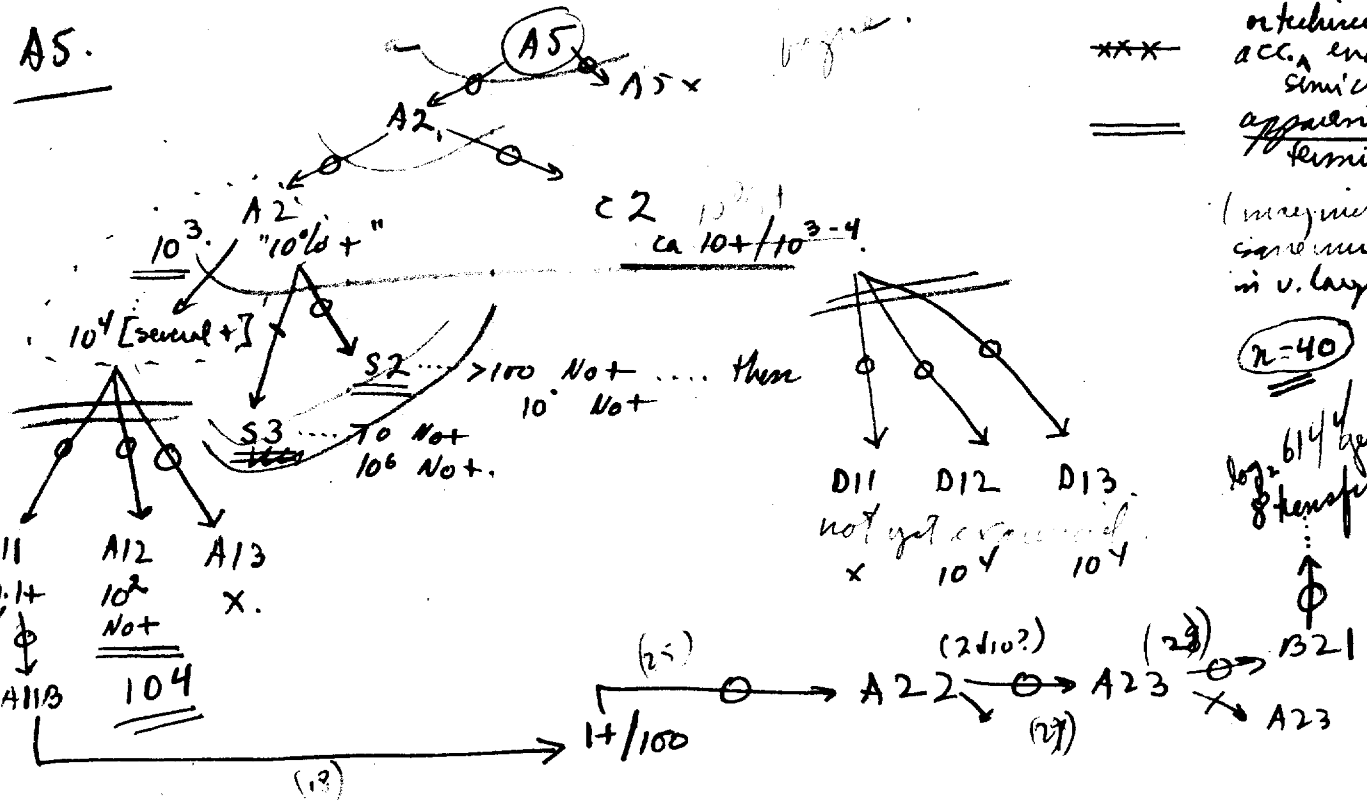
xxx — on technical acc. end of semiclone.

═ — apparent sp. termination (may include some misses in v. large pop.)

(n = 40)

$\log_2 614$ gen. 8 transfers

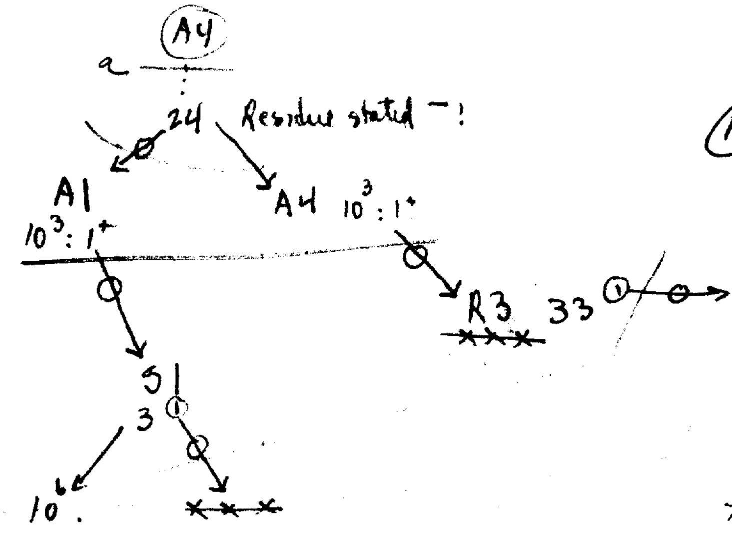
(1). A5.



∴ Semiclone branched at least at A2, – C2 and in these clones also gave ca. 10 each / 10^{3-4} descendants. Further branching not indicated and clonal propagation for ca. 5 generations would acct. for results.

Max. chain here is A11B : Generations = 1 + 1 + 13 + 6 = (21)

A4.



Max. chain (*) = S1

(16) = 4 + 10 + 2 generations

(19) = 4 + 10 + 5 R3 gens.

1 branching at (A4) and this originally covert!

1134n
plating

Reversed p23.

		Lp
S	1	
	2	+
	3	.
R	3	
Q	3	+
	2	+
	1	+
P	1	+
O	3	
	1	
N	2	+
	1	+
M	2	
A	11 → A22	+
	12	+
	13 x	.
B	11	+
	12	+
	13	+
	14 x	
	15 x	
C	11	+
	12	+
	13 x	
	14	+
	15	+
D	11 x	
	12	+
	13	+
E	11 0	
	12 0	
	13 0	
	14 0	
	15 0	

leave to Dot (DCC) for Lp tests.

2/20/54.

Recorded as 1135

at 6P20, inoculate motility tubes with residues of A-F: in toto.

E5 plated directly, and washings in 1ml, inoc 0.1ml, 0.01ml, and on agar p

	Growth	Tracks	Swarm	Lp22 (DCG) 2/24
A1	✓			+
A2	✓			+
B1	✓			+
2	✓			±
3	✓			−
4	✓			−
5	—			+
C1	✓			+
2	✓			+
3	✓			+
4	✓			+
5	✓			+
D1	✓			+
2	✓			+
3	✓			+
4	✓			+
5	✓			+
E1	—			+
2	✓			−
3	✓			+
4	✓			+
5	✓		✓ .1 ✓ .01 ✓	.1ml: − & phage .01ml colonies 10
F1	✓			−
2	✓			−

No tracks seen (60 hours)
Uncertainty of eff. of transfer
or whether motile cells
remained, as well as
efficiency of detection.
Try single motile cells
to agar!

Note numerous contamin colonies in E5! (Use anti-22 serum?).

February 20, 1954. see 1134

Data of 1134 reviewed P19, and evidence of multiple semi clones noted. ~~Reiso~~ Reisolate motile cells from indicated multiple semi clones about 3:15P20. ~~At 9P20~~, Incubate at 37°! At 9P20, examine.

Although taken at ≡ stage 1134 c, these will be treated separately under heading 1135.

Some examined at 9P20 ē discouraging results. Refrigerate [illegible] for later study. (No certain + semi clones. See note 1134 e.)

D, E examined 11A23. D11 0 D2, 3 10^4 no + seen.
E. all 0

A23
A 11B 1+/100 ~~HO~~→ A22 → (12) —0→ A23 ²—0→ B21 (28) → B21
A23 (from A23) ; 4:30P23. ; 5:15 ; B23 ; B24 ; 9PM ; B25

Δn: (7) (2) (1) (3)

11A24. 0 (5° overnight) to 9:30AM.

A.M. ✓ A31 ← 12:15 (1)

Hmod visit!

A32 —0→ A33 —0→ A34 —0→ A35 (4)
3:35 6PM 12 8:10PM Refr.

(2) (2)

warmed by lamp!

9:05 AM–12N R.T.
[illegible] fiber content, and 1 smaller only. (nonmotile). In course of day at RT gave 36 progeny after slow start, but none motile.

Plate semi clones, Fla+ × SW666

1136

DATE: 2/23/56

REF:

	1	2	3	4	5	6	7	8	9	10
A	FA12 × SW666 5 plated.			1 swarm 4 surface growth only (one or 2 loci). 7 Do not save						
10	Presumably few cells give tracks from surface plating. Try pour plate.									
B.	2.									
20										
30										
40										
50										

Feb. 26, 1954.

FA1(SW666) × SW967 11-12[30] 37° Refr. Collect Fly 3:30-4:20

4:25PM : A1-5, B1-5, C1, C5. (A1-2, B2-C5 prob. sibs).

By 5:45 PM most above had divided once, some gave 2 motile, some 1. Refr. 5:45 PM – 9:20 A 27. Follow clones at room temperature throughout the day., separating further. (Refrigerate overnight). Most clones no longer had descernable motility. Followed in separated subclones during 2/28. Overnight P28-A21, set out at room temperature, but the larger clones likewise were pure —. (Compare 1134-35)

This suggests that SW967 engenders many v. short generations or else conditions here used are unfavorable.

DATE: March 1, 1954. REF:

0: 60A (SW967) → SW666 12¹⁵-1⁰⁵.
Studied to 2:45 no Fla+. / >10⁷ Waiting

FA12 → SW666 3¹⁵-4¹⁵ 37°. No Fla+ seen at this time / >>10⁶
5:30 + 6 PM, several early isolates.
X Transfer to single drops at 6 PM refrigerate overnight
(A1-2-3-4-5 B1-2-3). A1-2 are prob. sibs; B1-2 ?Fla+
after transfer.

A2. Remic. under lamp 9:40 - 10:55. Resamm.
overheated! T.O.

3/2/54. 5-6 PM. From earlier mixture, refrigerated (ca 2 hours intermittent incubation previous). Isolate 12 cells. (some divided before being put away).

12:30 PM.

A1 a. × ① — xd.
A2 × ① — xd.
3 .. ① — xd.
4 ①① .. — xd.
5 ① ... — xd. (1 group only)

T.O.

B 1 lysed
2 ..
3 → 5. to D1 → 3. 3+, 7- lysed
4 large clone. By 5+ PM had formed 15 late pairs (total A11-F15) all pairs T.
5 .... ca. 16. each almost d.

C 1 ①
2 1. — 1. d.
3 1. — ca 8. each, some lysed
4 1.
5 1. — ca 20. some lysed.

B3 → D1
a = 2:10 PM 3/3. (Refr. overnight, at R.T. to 2:10)

.= unmot.

b. 5 PM 3/3 transfer the B4 isolates to new coverglass (ΣA11-F15) and incubate these overnight at R.T. Original mi Frig. to 10A-6; inc 30° 10A-6 —

p3.

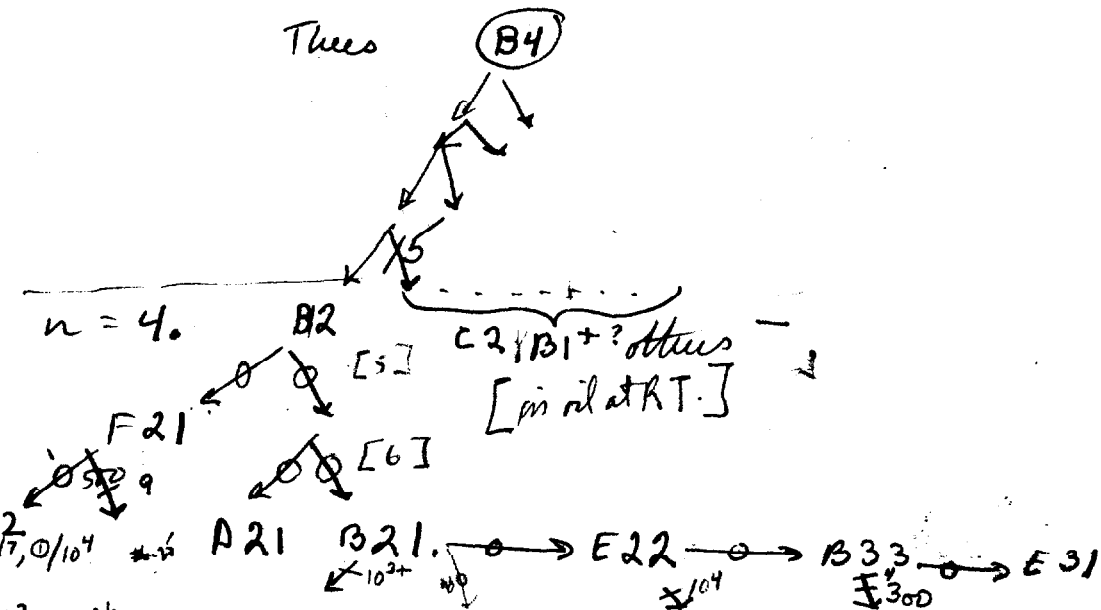
By 6PM, those subclones had undergone 1-3 further divisions and were separated within the agglaces.

(c) A4. Examine clones of A11-F15

But by this time, most were already immotile.

(e). Most have dried out. Of the 15 sites, only following were recorded as motile at this time:

C2, B2, B1. C2 probably inviable. C2 and B1 dried out, but B2 clones OK.



d. 2PM. B21. ca 10^3 total, 1/2 Fla+ = A21-25 B22-25 C21-22 E21, E22

D21 ca 400 ½ Fla+ = D22-24 E24 ? E21?

F21 ca 500 1 Fla+ = F22.

15 + 3

e. 4:20PM. Separate some subclones.
(Visit by Francis Bello from Fortune).

f. ~~9:03AM~~ 2:30-5:30 PM 3/5. Ca 10^4 clones in each.
From 10 clones identify + separate Fla+.

g. 8:30PM — Research remaining clones for +. Record clearly transfused for next cycle

over.

4-10A6 Kevannic clones
only B33, C32 still semiclonal.

These ranid to i = P6, j = A7.

Total n = 44, n = 42 resp.

involving 11, 7 actual transfers

Replication occurred beginning n = 3, 4? to
n = 10 (no greater than 19 poss.)

Detailed examination of early subclones seems now necessary. Also, repeat (n=36) transfer of cells to motility agar to verify multiple trail origins. (Assume that most semiclones never get into ~~agar~~ soft agar)

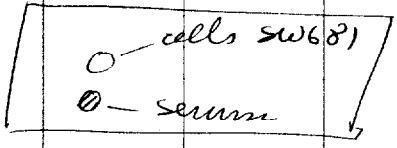
Serum eff; phenol ~~tactism~~ taxis

DATE: March 6, 1954. REF:

A). Test efficacy of immobilization of SW680, 681 by homologous antisera. Nearly complete agglutination in slide droplets under oil. Individual immobilized bacteria are also seen. Some seem stuck at one end. Other single bacteria still swimming at first. Suggest agglut. in tubes, then transfer under oil.

[10 Also try more dilute susp. for immobilization to determine whether single cells can be diagnosed by dilute serum! J.

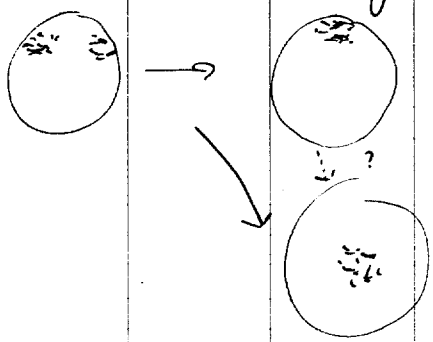
B) While setting up expt., separate droplets were set up as



& a polarization in the cell drop was noted.

20 As the serum droplet was entirely isolated, an oil-soluble component was presented, namely the phenol preservative. This was promptly confirmed by the arrangement resulting: The polarity was transient, and cells randomized again after 1-2 hours.

3/7/54. Experiment ± 1% phenol repeated ± clearcut results. No serious effect on non-motile bacteria (SW967). Also noted moving bands of cell concentration becoming either apical or (later?) central. Clustering very marked. These might suggest either an optimum conc. zone or faster diffusion through oil vs. water. SW681 diluted 1:50 in nonmotile SW967 showed same tactic effect but only for the motile cells (ca 5+ fold concentration distally.



Possible mechanisms? ∴ phototaxis (Clayton)

3/8/54. In agglutination tubes, definite ring formed over phenol (resorcin + lactose) 10% ring in 15-20 minutes. ← clearing Results are more clearcut under oil

March 8, 1954

Plate single motile cells in motility agar to learn possible multiple branching. Mixtures at R.T. 12⁰⁰ – 3⁰⁰ PM

FA92-X5W866 } not seen!
FA92-X8W967 }

Refr to 4:30 PM, Incubate 4:30 – 6 PM.

Most efficient isolation: let Fla⁻ settle, scan only top focal plane. Catches perhaps ½ all motile cells, but with a fraction of effort required for complete search.

By 6 PM, a dozen Fla⁺ picked out, some separated.

Refrigerate to 8:30 PM, then centrifuge. Separate 13 cells total.

at 8:40 PM:

2P9.

A	1	①	
	2	①	swam
	3	①	
	4	①	
	5	①	
B	1	②	3.
	2	①	
	3	①	
	4	1.	swam!
	5	①	
C	1	①	
	2	1	
	3	①	

No tracks.

Expt. rather sloppy

Ca 9–9:20 PM, Fleshout legs, plate to motility plates. Add fresh agar to embed. Then impress dizply in additional layers.

Incubate 37°. (0.5% agar formula)

7/13/55 Analysis → ϕ diff

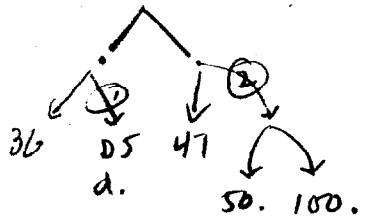
A1 ϕ

A2 ×

A3 ϕ

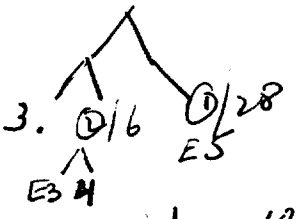
A4 see prologue

A5.

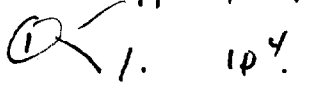


B1 — V.sh D (8.14.)

B2



B3



B4 v.i

B5 n.g., stayed motile

C1 diff ϕ

C2 —

FA12 → x SW666
single cells added.

March 9, 1954.

Further survey of pattern of establishment of semiclones
Terminology: coverglasses now ruled in advance, (A-H)(1-5)
and marked with serial numbers [101...]
12 × 666 kept overnight in frig to 9:45 AM.
Examined 10:20-10:55 : no Fla+ seen. [Sample refrigerated and examined again later also negative at 4:10 PM].
Pups. at room temperature 1:45 — 3 PM Isolations (ca. 3-4 hours).
Numerous Fla+ best isolated at uppermost focal plane.

[101]

at ca 3:40 PM. collected:

		a	b 4:50 PM	c 11 A10	d 4P10		e 10A11
A	1	(+)	(1) (1)	✓	1. (1)	D. (1)/32 → D3; C. (1) D4	A: (1)/10⁴; BCD: 10⁴.
	2	x ... Reservoir.					
	3	(1)	(+)	0	0	A. 15. → (D2),(D1); B. (2)/9.	
	4	(1)	(1) (+)	1.A (1).B	1.C T.D		
	5	(1)	(1)	→	(1)/36. → (D5); (1)/47 → (E),(E2)		d, 10⁴.
B	1	(1)	(1)	+ . 1. 1.	8. 14.	E5 ↑ (1)/28	10⁴.
	2	(1)	(1)	→ + . 3,	(2)/6, → E3 E4		10³. 10³. 10⁴.
	3	(1)	1, 1,	1. → 1.	32.		10⁴. 10⁴.
	4	(~)	T 1. 1.	2. 1. 1.	(1)/8 1.		(14)/10³⁺ → H2
	5	(1)	(1)	1.	1.		(1)
C	1	(1)	(1)	(1)	0		0
	2	(1)	(1) (1)	T_A T_C (1)_B (1)_D	16+ 8; 22 1. none motile		100. 10⁴.
	3				A2 → .		10⁴. 10⁴.

100 ... E3-4-5 probably abandoned inadvertently
Record lost

b. Refrigerate 5 PM to 9:30 A10.
d-e R.T. overnight
e 10A11.

FA92 — x 666 37° 9-12 N, Refr to 4 PM. No Fla+ seen.

FROM	10-12N11	N12	
D1 } A2B	②/10^{3} → G1G2	10^{4+}	
D2 } ②/11.	⑪/10^{3} ③ → H3	10^{4+}	
D3 ①/32 A2D	10^{4}.	10^{4+}	
D4 A2C ①	①	①	This all long note last in g.
D5 A5 ①/36	d.	0	
E1 } A5	50.	10^{2}.	
E2 } ①/47	100.	10^{4}.	
~~E3~~			
F1 B4A.	10^{3}.	10^{3}.	
G1 D1 } ①/10^{3}		10^{4}.	
G2 D1 }		+ 18/10^{3+} → 5P12 → 105 (d, 1+) (1-5)	
H1 }			by 3P13, each gave $20-10^{3}$.
H2 } B4A ①/10^{3}		25 (d).	
E3 } ②/6. B2		→ 10d.	
E4 }			no record on E4-5
E5 ①/28 B2			
H3 D2 ⑧/10^{3}		10^{5}. Not (mol trap)	

Confusion re G1-G2 H1-H2

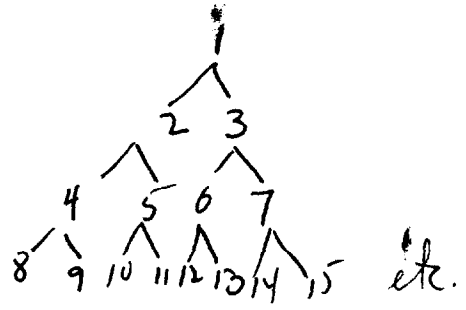
1 group is from B4, ⑥/10^{3}

1 group is from D1-D2, ①/10^{3}

Note: no H1 is listed.

∴ H2 is probably from B4. Whence F1? Maybe C3 - But unimportant.

Many of these pedigrees are there shortened! (Cf. 1142A-B!)

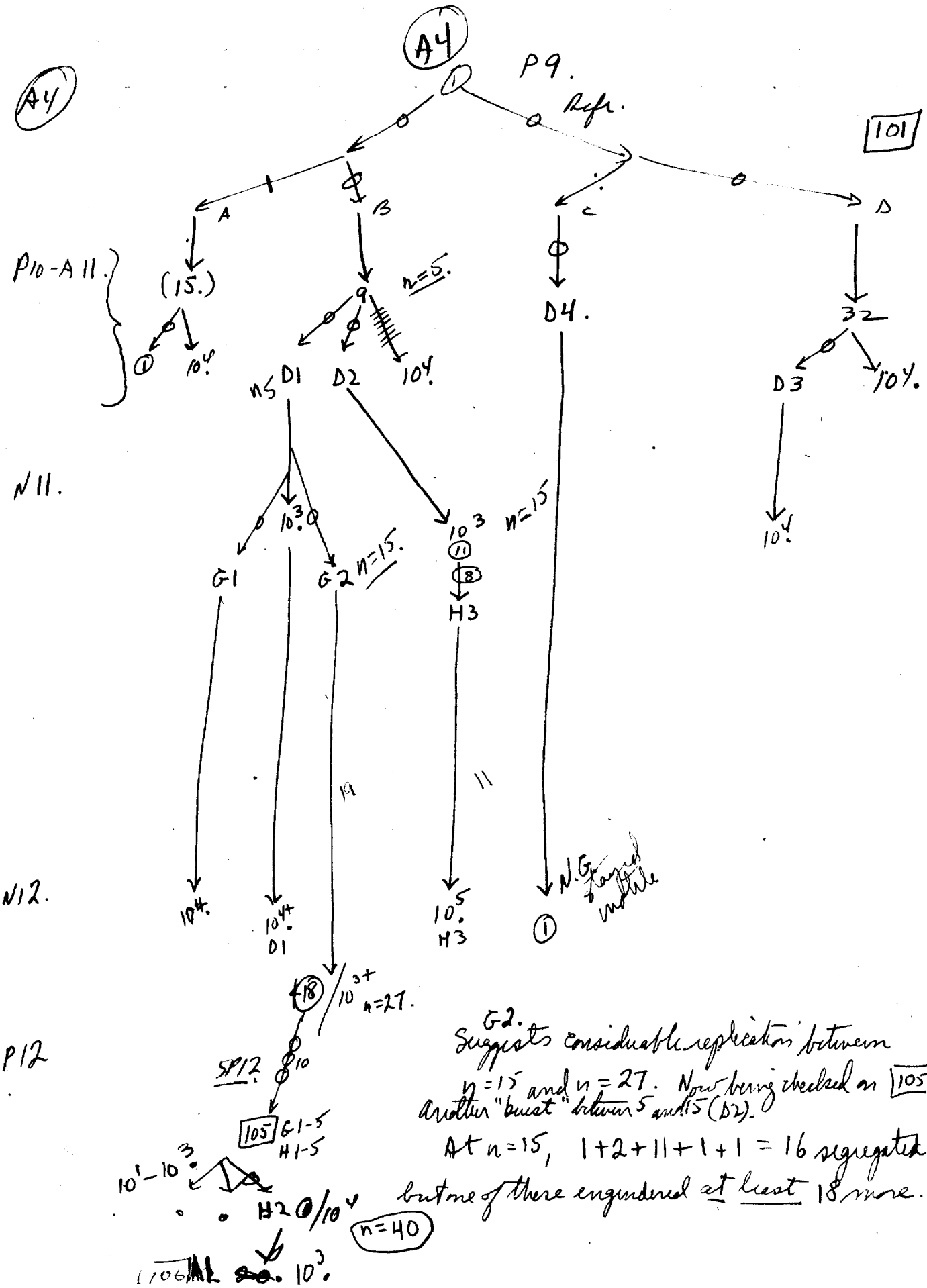


1141 A 4. line 6 2 showed replication 19 27. Then only semiclonal for up to n = 40.

Total semiclonal yield:

Cells	4	5	6	7
#	1	20+11	1	1
		31		
Min fate:	K6.	40,	15	7.
Time of division		17-27; 9-15.		

1141
pedigree

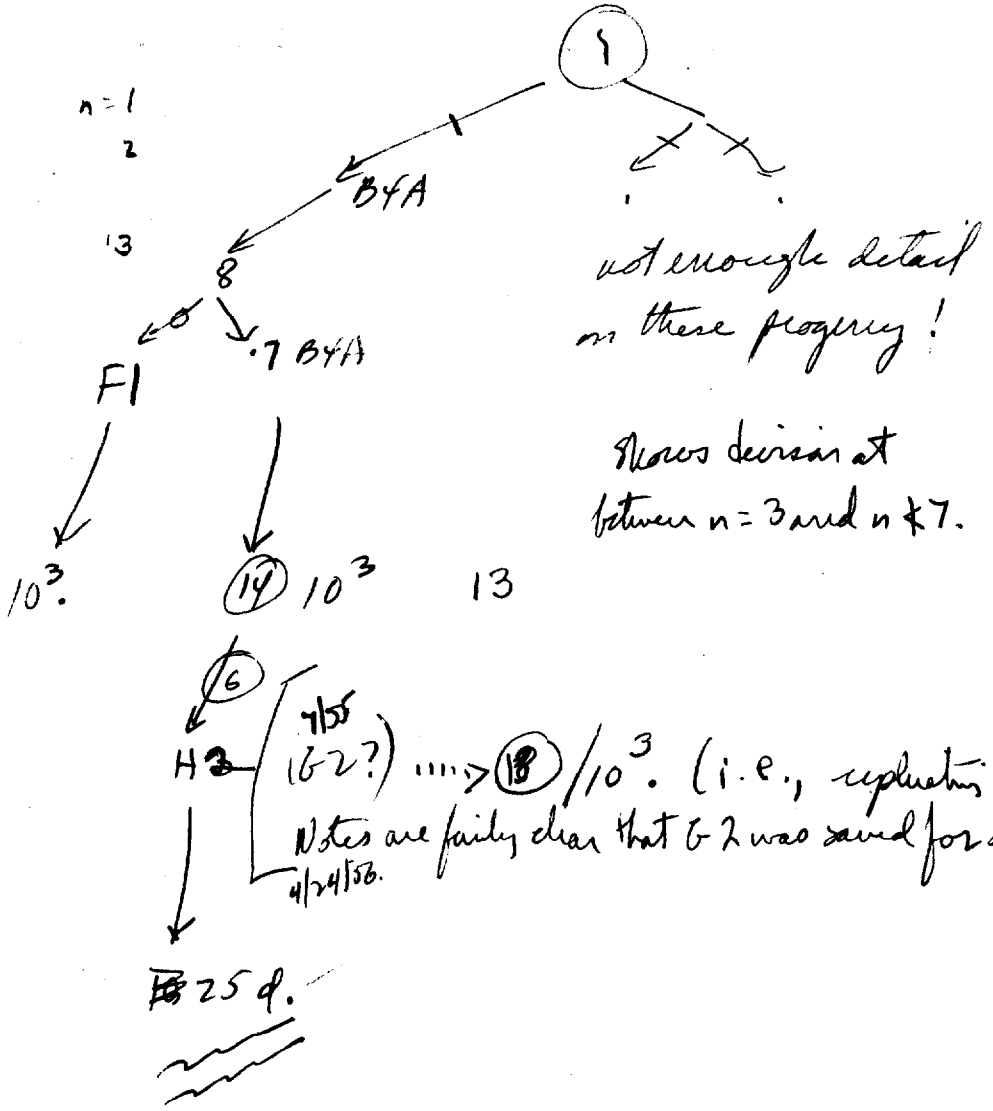


G2.
Suggests considerable replication between n=15 and n=27. Now being checked on 105 →.
another "burst" between 5 and 15 (D2).

At n=15, 1+2+11+1+1 = 16 segregated semidarks but one of these engendered at least 18 more.

B4

(B4)



COMMENT: I do not yet have a really adequate pedigree on senescence origins. Partly, it has been necessary to sketch out the background. Previous observations had suggested that division was restricted to $n < 10$.

Hyp: ① Irregular replication

② Segregation of particle

A: polytenic loci, aborted cytogenetics

B: gene product, autocatalytic but particles.

Best basis of distinction would be total extent of replication and irregular later mitoses or swarms to support A. Both might be possible!

From this plating, motile + non-motile recovered.
F4 (mislabelled 1142E3).

PA 60 → Fla^- gave +
9 → did not.
22 → Fla^- → H_1^b.
∴ Fla_1^- H_1^b ; sub is Fla^+ H_1^b.

No + found from > 40 isolates from "E3"

60 → × SW666.
and plating

104
105
106

March 11, 1954.

PA60B-×SW666 12·30 – 3:15 PM. Turn Refr. Ca 4:40 – 6:40 isolate + plate.
Use entrapment.
Plate EFGH on .4% motility agar. See over.
6:40 PM. 2P12

(a)

Screening yield of swarms (1)

Ca 6P11.

		a		
A	1	1.		
	2	1.		
	3	(1)		
	4 }	(1)		
	5 }	(1)		
B	1			
	2	(1)		
	3	(1)		
	4	(1)		
C	2	(1)	(2)/500	
	3	(0)	(50?)/10³!	→ 0 → 105 C1-5 5P12
	4	(1)	(18)/10³	
	5	(1)	(1)/10⁴	
D	1	(1)	10⁴: ~~…~~ ≮100 motile!	→ 0 → 105 (D,E)(1-5). 5P12
	2	(1)	(3)/10³	
	3	(1)	0/10³	platings also → no motile swarms.
	4	(1)	0/10³	
	5	(1)	(1)/10³	

A–B: Essentially N.G!!

E–H plated. ←

Plate (hastily, no flush) (on surface 0.4% agar) Res. on 104
A12 A13 P12

		A12	A13	swarms → DCG from plating E/H
E	3	0		
	4	1.	:	0
	5	1.	:	0
F	1	1.	.	Fla⁻
	2	0	≠	⊕/⁻
	3	1.	:	0
	4	swarm – to DCG to isol +/–.		0
	AH4	0		0
G	1	0		0
	2	0		
	3	smeared..	.	network.
	4	1.	3T?	0
	5	1.	2T?	0
H	1 [(4)]	1.	trails?	0
	3	0		Fla⁻ ⊕/–

But no appreciable trails were seen later. Thus v. few trails are manifested by the point count.

Good correlation "% picked" trails not very striking.

Fluid trap method (had been tried before on possibly negative samples) now found to be most effective.

Use FA60–× as including another factor possibly linked to H_1. Swarm isols wanted for crossover test.

5P12 — 3'15 P13 — 16° 4P13 — 4P15

I105 — not to 106 — 4P14

4C3 { C1 10^4 ; 2 10^4. ; 3 10^4. ; 4 10^4. ; 5 10^4 ① — C1 50. — no addl +.

C2 ①/100 ①/2 $50.\ 10^3$

4D1 { D 1 10^4 ② ; 2 10^4 ① — < C3 50. ; C4 ①/200 ①/2 $20.\ 10^3$; C5 0 — ∴ ended semiclones.

3 10^4 ; 4 10^4 ; 5 10^4 ① — B5 4.

E 1 10^{3+} ; 2 10^4 F1 — B1 28. ; 3 10^4 ① — B4 50. ; 4 10^4 E3 — B3 58. ; 5 10^4 F2 — B2 8.

3 ∴ 10

∴ each was 1141 H2 ① — A1 100. — 10^3

semidual not clone!

Pedigree results

By n = 10-15, many clones showed only a few +. Not followed further. However, C3 had ca. 50+. 5 sampled ~~etc~~ terminated by n = 23 except for 2 which ~~terminated~~ n ≥ 23 < 31 and 5 which was considered a semiclone ~~+++~~. +41.

a)

n): Thumps replication +6 +10. Semiclones ~~+~~ +41.

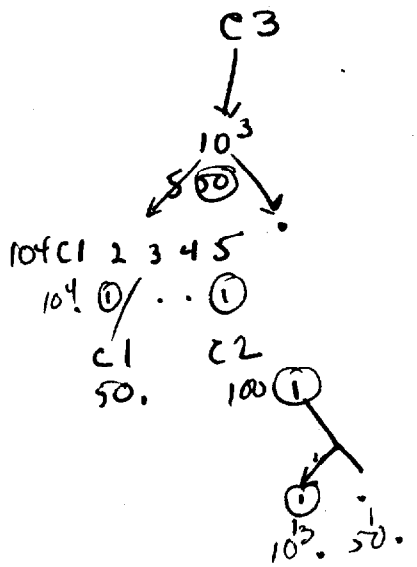
b) max semiclones

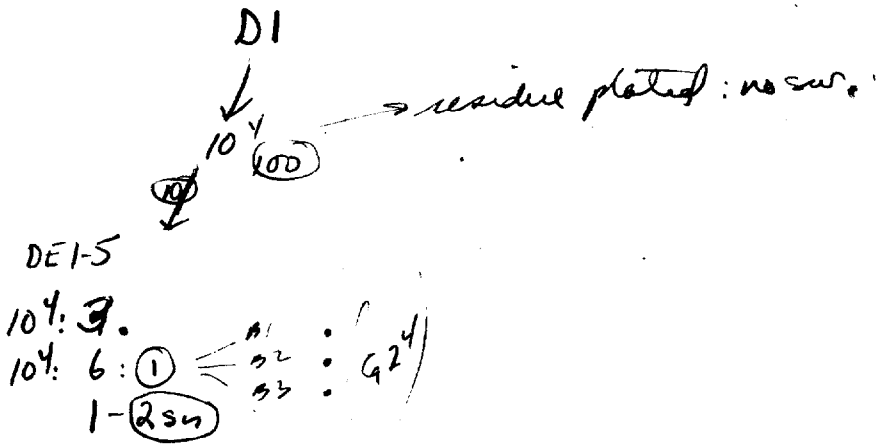
D1. at n = 13, ca 2⁷ motile. 10 tested ~~and~~ showed repl. at 14.

∴ replication to + 14. Semiclone then to +35. ~~the~~ others terminated earlier.

What was remarkable here was simply the very large crop of semiclones.

Need more detail on distribution of motile in earlier divisions. It is now quite clear that replication does not extend beyond n = 15, usually ends sooner.





110
107
112

DATE: 3/16/54. REF:

1 2 3 4 5 6 7 8 9 10

PA60B → SW666 (old susp) 11:25 – 1:45 at 37°. Refr. as 1143 stock

Isolate trapped motile cells to [107] A1–D5, E1 and plate.

[Should have labelled + followed residues but did not.]

D1 = 4 app. NM cells E1 = 8 motile cells pooled. For most platings; embed in layered .4% motility agar, ̄c chilling to immobilize. Layers seal perfectly.

E1: swarm D1 2 dup colonies.

not chilled 2/3 ̄c colonies. (contam?)

chilled: 14/16 had dup "colonies": no swarm, no distinct tracks at 10A17. Reincubate. Medium is quite fluid. Φ 1 plate ̄c contam. swarm

A19: no tracks. See over for results on swarms.

Also, using old, refr. susp. (1141-42) isolated A1–D3 on [110] but decide later not to use. However D4, 5, E1–5 (F1–5) isolated ca 2:30 PM from ~~new susp~~ 1143 stock above.

~~Re~~ Refrigerate at 5:55 PM. at 3:40 PM, all these were just ① except E2 (②) and F1 (②).

at 8P16, Check some of these, on [110] and transfer [110] E1–5 to [107]. 110 – F1 showed 0, ⊕; others not checked. Incubate [107] at 29°; Refr. [110] Residue 8PM – 9A17. Then RT till index.

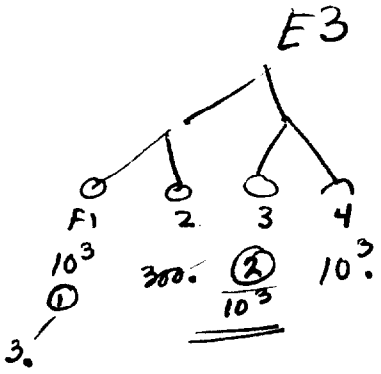
---

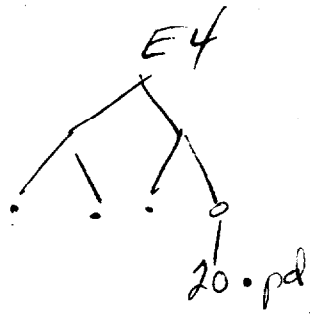
[107]: GH eso. n.g. (see protocol). 10:30A17

serb

E	2	42+/300	to [112] B1, B2	To test further replication.
	3	50.		
	4	47+/500	– 112: B3-4-5-C5.	Inc 30° 12:30P17
	5	~~part of 2.~~		
F	1	① /10³	→ A1.	
	2	250.		to ..
	3	① / 10³		
	4	10³.		

107





suff for E2

3/18 [107] 8P16 — 10^{30}A17 → [112] — [112] — 3P17 [112] — 6P17 [112] — 8^{15}P17 [112]

110 E2	E 2 +	4②/300 B1, B2	B1	①/19	①/8	①/8 →	D1	
	3 +		B2	①/4	①/8	①/6 n=2	D 2	v.sl.
	4 +	47 50/10^{2+} B3 4 5 C5.	B3	①/16	①/8	①/5 n=3	D 3	
	5 +	part d. 2	B4	①/8	①/8	①/5 n=2	D 4	
110 E3	F 1 +	①/10^3 A1 → immotile	B5	①/10	7.	40.		
	2 +	2-300.	C5	16.	50.	10^2.		
	3 +	②/10^3 . ..	A1	3.		50.		
	4 ±	10^3.						
110 E4	G1 −	p.d. 100						
	2 −	200.						
	3 −	60.						
	4 +	20 p.d.				to 16° overnight.		
110 E5	H1 +	50d						
	2 +	"						
	3 +	"						

110F1 ○

AT to 10^{30}A17

to 30°
at 12^{30}P17 − 3PM
Then R.T. various times

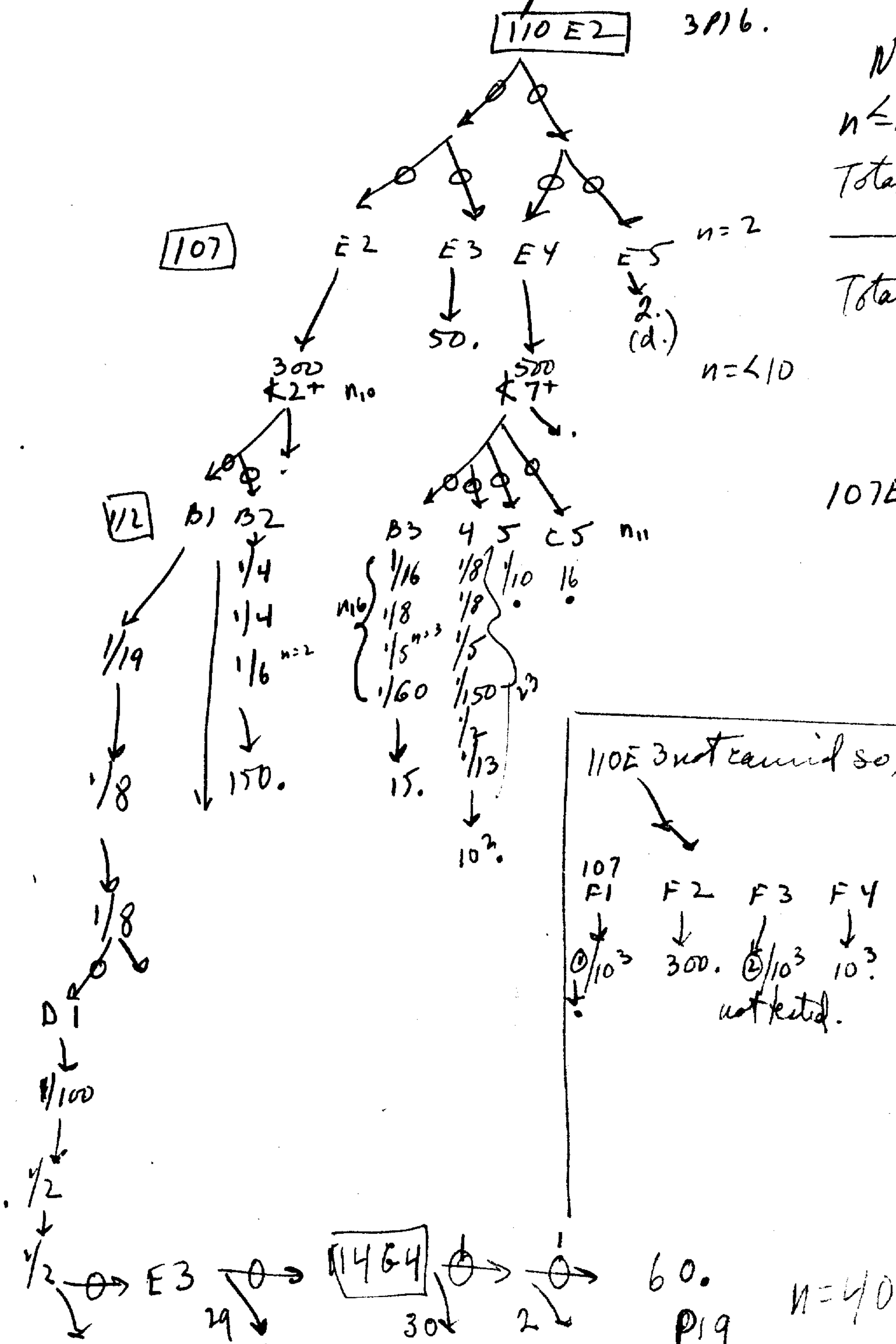
~~30 P18~~ [112] — 11A18 to 3P18 — AT to 6P18. Ref. to 9^{30}P18 — 16° — A19 — P19

D1	①/100	①/2 ①/2 → E 3.	①/29 → [114] G 4	①/30	①/2	60.
D2	150.					
D 3	①/10		ca 15.			
D4	150.	①/2 → E1. v.sl.	13. → [114] G 3		10^2.	10^4.

Summary.

a) Platings: cells grow out in depagor; no traces

b) 8 clones: fuo + at 10^{2-3}, 4 tested for later replication. 2 clones essentially n.g. (died) 2 clones followed:



No replication after n≤10, and mostly prior

Total reop. 2, 1, 7 (1).

Total fate: 107E2 = 2 + 8 + 4,3,3,7,1,1,5,5,1

40 = 10+30 in one and 10+6 in other

107E5. 10+16 = 26

10+20 = 30

10 + 3 = 13

10 + 0. = 10.

110E 3 not carried so much detail.

107
F1 F2 F3 F4
①/10³ 300. ②/10³ 10³.
not tested.

∴ here no ~~later~~ decisions seen after n = 3 but not properly tested.. F1 shattered!

109
111
H4

60B → × SW666

3/17/54. Refrigerated 1143 susp. 9 40 AM. – 10 20 AM.
Then 30° – 8^{30} P17 to 16°

all residues unless indicated

109

A2 ✓ 52. ① → 111·A1 dried out. 2^{45} P18 Res. 10^4. 114
3 ✓ n=6 ② ~~→~~ .. R 10^4; ①/300 ①/200 → D1, D2 see G1,2
5 ✓ 26 ① ~~→~~ . 10^4. no further +.
B1 ✓ 1? (+10)/60 (16)/100
2 ✓ 25 ⑩ 4 ~~→~~ ....
SWARM 3 ✓ (50) swarm 1? → + also. Pure + Pure gns (A.B.) save
4 50 ⑪ isolated in situ A–K see below. R 10^5.
5 57. ③ → ... (...) 3 not seen.

C 3 not exam.
5 " .

30° – 3^{40} clones already large. Refrig to 8 30 PM.

Note: manipulator broke down 9P17.
Ed Tatum visited A18.

3P18: ① from B4 to each of 11 droplets (A–K). No change
Ca. 250 each. Motile as indicated.

109 B4		114
A	0	D4
B	+15	F3 C3 - F3
C	0	E4 ✓
D	0	D3 ✓ ✓
E	0	
F	3	A1-2-3
G	2	A4-5
H	1	B5
I	1	C1
J	4	B1-4 B4?
K	1	C2

(recorded at first division but not necessarily this product).

	1^{6} P18 · A19	P19 (RT.)
A1	1.	1.
2	①/8	13.
3	1/25 1/2	10^3.
4	0	0
5	0	0
B1	~~①/4~~ 16.	200.
2	①/26	60.
3	82.	61.
4	31.	①/10^3 1/2 → H2
5	①/26	1/150 1/8 → H~~5~~
C1	①/9	40.
2	①/45 1/2	1/60 1/2 → H3
3	①/28	1/60 1/2 F4 114
4	52.	10^3.
5	①/20	44.
D1	71.	10^3
2	36.	10^3
3	73.	10^3

D4	21.	300
D5	17.	10^3.
E1	①/28	①/118 : 2? → F5
2	28.	32.
3	16.	10^2.
4	22.	10^2.
5	31.	400
F2	33.	200
3	①/34	70
G1	7150.	10^4
2	60.	10^3
3	10^{2+}	10^4
4	①/30	60

A20
H 1.
2.
3
F 4-5.

N19: Skeenegan visited

8 30 p 21

A 22

. . One line reached n = 59

others n = 33, n = 52 before

primogeniture ended.

Isolate residual non-motile sibs to check

their propensity to give long descendancies.

1144 F 2

G 3

1144 Pedigrees

Had planned to isolate clones at n = 3–5, but let things go too long. ∴ picked the motile from B4.

10 clones picked in consideration.

A2 1/52 – 4.

A3 2/2⁶ no further replication. Fate: n = 13±.

A5 1/26 → 10%. ! Limited semiclone (or others appeared and passed!) n < 13

B1 <10/60 not pursued. Grew late. Early division apparent
ca 16/100

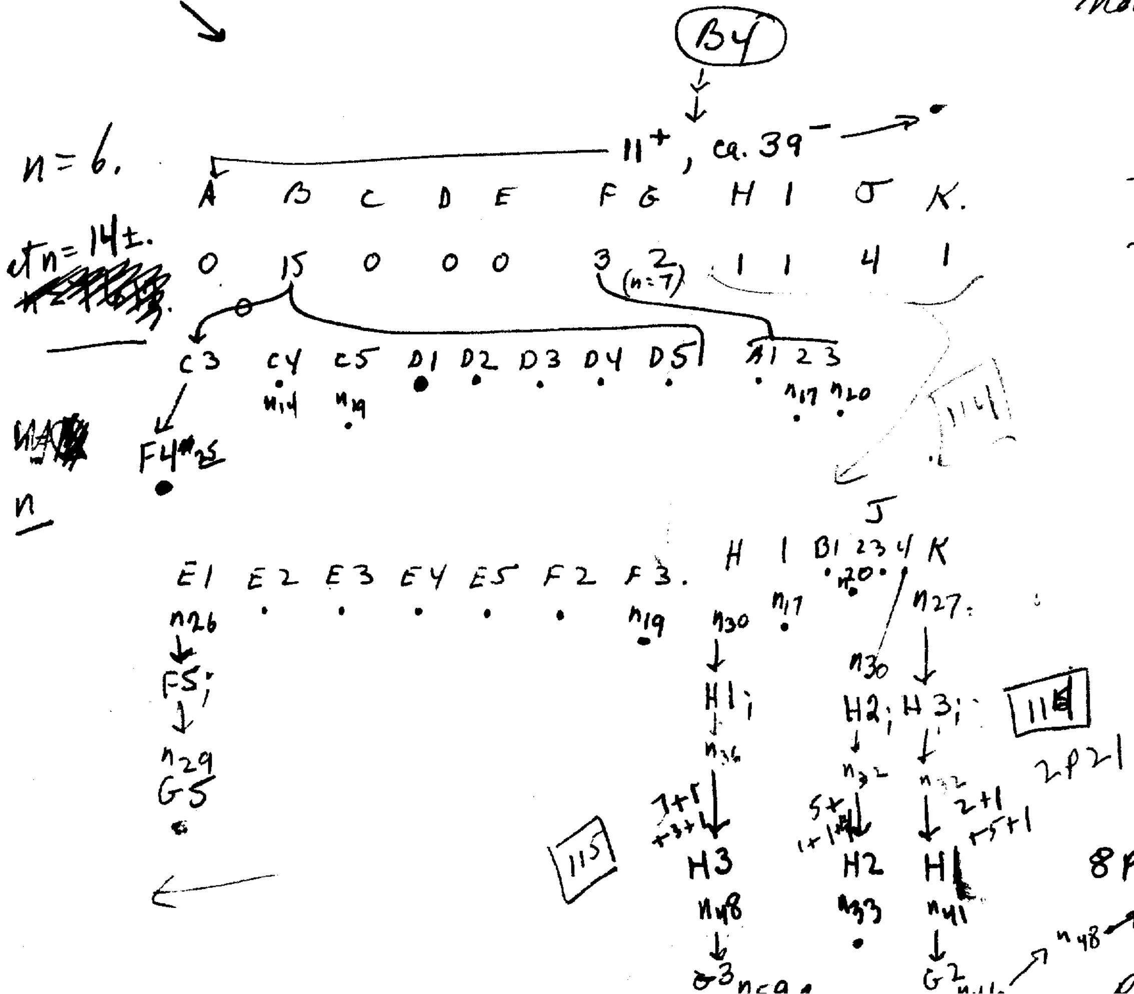
B2. ca 10/25 4 isolated / 10^3, A 0/10^3 B < 8 C < 6 D < 1⁺.

∴ substantial replication n > 5 < 8 but not pursued.

B3. Pure swarm. Check H, status.

B4. N.i. B5. ③/57 lost.

Thus all clones not notable except B4.



These some semiclones terminate, others divide to n < 6+4 = 10, but *14 not expounded fully. No later division seems to n as high as 59!

3/19/54.

5-6 PM. Isolates from pepsin refrigerated from 3/18. Plant at R.T. to see whether gross semi clone pattern applies here. A few previous isolates were discouraging, but agar this combination gives the largest tracks + should be the most suitable for transplant tests.

116 7 colds isolated 5P19, kept at RT overnight.

A 20. 6/7 were ca 10^3. C1 had 2+ to 115 H4, H5.

N21: H4: lysed H5... 1. lag.

Tumor Pla+ — mix growth

113

1145

3/17/54.

A; 2:05 PM Mix 60B, SRD466 (old) in c.g. R.T.
(Isolate cells singly & small groups) at 220–240.
Motile cells not seen in suspension at 2:40, (300), 3:35
Then R.T. By 5 40, ++ motile.
No substantial increase of isolated cells, but probably did in mass drops after all. Will have to be done by plate count.

swarms. SW666x —
1144 H3x —.

1147

DATE: March 30, 1954. REF: [118].

For past few days have had poor luck ē SW666x — to find motile cells. possibly suspensions too old, used to small drops?

3/28. Ca 3 hours then refrigerated to SW666x — FA60B

(1) 3/29. 2Fla+ → [118] E1, E2. P30: E1: 0 E2 ca (5)/20. leave as is.

(2). 3/30. Suspensions as above. 1144H3 x—60B. (= trail end sib of SW666x — 60B)
Isolations from 3:55 PM — ca 6 PM. Ca 1 division during this interval for some cells. Single motile cells to drops, a few sibs separated etc. 26° overnight. [118]

3/31

	log10 cells	mot.	to fate [119]					
A1	—			E1		0		
2				2		4+	K1	D2
3	2	1?	.	3	×	3+	K1	D4
4	2	0?		4		3	.	
5	2	2		5		3	1?	
B1	(2)	(1) → B2		F1		4	1?	
2	2	.		2	2	4, 4	K1	K2 E4 E3
3	2	.	1 → B1	3		1, 3	1+	
4	2 c	A, ca 50, 20+?		4	2	3, 2	1, 0	E1
5	0 A B	B, C 0		5		0		
C1	4	K 1 →	C1	G1		3	ca 50%+	
2	(2) 2			2		2, 3	1	F3
3	2	1+	C3	3		2, 3	4B	F1 (all 8)
4	(2.)		1+, 1-	4		3	2	F5 (?)
5	(2 swishes)			5		2	1?	
D 1	3. 3	4 4	(1) D1	H1		1, 3	.	
2	0			2	2	4-, 4-	>100+	K2 (5± each)
3	(0)			3	2	3, 3	all+. to H1, 2	
4	4		C. 4	4		4	1	G5
5	4	. 1 .	.	5	—			

mitotic G3 [119] ←

Did not search carefully for individ motile cells. . = 0 or 1 or 2...

This expt. designed for info on swarms.

Resumm. 4/5/54. 6PM

3/30/54.
35 motile cells to [118]. 8 clones had few or no +
3 failed
19 had a few
? 1 48/200 (118G3) →8 119F4 → 121F3
42/104... (5).

4 definite resumes

1. pure S variable uppr. 118 B4 → toto 119G3 (20)/50 → 121G3. (pure motile. ⊂ contaminant came in later)
2. pure S G1 → 119G1 → 121G1-G4. any non-motile? prob not.
3. T+S! A2 119H3, [H5, F1, F2.] — form non-motile branch
4. Pure S. H3 → 121 H1, H3. (2 initial sites. Both pure +.

Other lines were not followed up. [120] lost P3 interrupted analysis of possible segregating lines.

picked.

1. ~~not yet analyzed.~~ pure +. 119G3 Erratic expression of t. Same.
pure S 2: ~~121G3, G4.~~ 121G3, G4 same motile line.
3: 119 : H5, F1, F2 non-motile branch
S.T. + ^{119}H3 } motile branch ~~D1 single cell +.~~
– ^{121}D3
4. 2 branches: 121H1, 121H3 both motile
pure S

DATE: 3/31/54. REF:

1	2	3	4	5	6	7	8	9	10

See 1147.

SW666, overnight culture. X-60B R.T., 10^{30} – 1. (2½ hours).

~~Tube~~ Set up 4:10 – 4:45 [1120] No + seen. R.T.

5:20. No + . heat c̄ lamp (R.T. ~~too~~ too low now?

(ca 21-22° today)

5:45 Rare +

herring > numerous ca 6+ PM.

A 1
2
3 5:52
4
5

B 1
2
3
4 6:03
5 6:10.

P1. Three of 10 cells, 2 failed, 2 not +, 4 few +, 2: B1, B4: 10-20+/300 → d. few +/10^3. ✗30+/10^3.

no further multiplication on further transfer.

[120] lost P2. ∴ no swarms. 2 clones moderate exp. semi-dense

∴ 10 cells 8 viable no swarms.

Contiguity of 124F5 and 124H2-3.

F5. (122E4)

Initially, ① 6.

10^4. $>40/10^4$

$\therefore n = 13$

$Ca \; 20+ \; 20-$ → (40) → 123C2

10^4 K7+ → (7) → C5 $/10^4$.

$\therefore \Delta n = \log \frac{10^4}{40} = 8$

$n = 21$

122D5.

① ① ① ⑤

$n = 11$

10^3. K35. Ca 40 [(20)] → C1

$n = 19$

10^4 K8+ → 10[(7)] → 123 D5

$n = 29$

10^4 K4+ → 0 → 124F5 ②$/10^4$

[illegible] lost

① ① 0

Became contam. c coli accident recalled (stopped e.g. to fast, drops came off).

$n = 42$

125E1 4. → 10^4 overmolete

125D1. 40 10^4. 124H3

P6: 124H2

(might be contam coli) To be checked

probably contam. check OCFL

see next page

4/5/54 PM

April 2, 1954
60B-x666 10:00-12:20 at 30°. Refr.
~~? suspensions (same as 1148?)~~ Extra 20-30 mins at R.T.?
[122] ? Start to separate some at 2-4 cell stage, but too far along!

3:40-4:25 isolations [122]: A1-H4. F1, G5, H5 X.
37 single motile cells + 11 pooled at Z1.

all grew. SWARMS: 122 B5, F5, Z1. ∴ 3/48 swarms.

A few large semiclones also transferred:

[122] →	[123]	✓ = uninteresting
C2	A1-4	✓
D1	A5	✓
D3	B1-5	✓
D5	ABC(D) <35 → C1 (8)/10⁴ → D5 <4 ←	
E4	>40. C2 (7)/10⁴ → C5 ? {(1) → lost; (1) → [124] F5}	
F4	> 40+/10⁴ · C3. → (3)/10⁴ → C4 <40 [<42] → lost; (1) → 124-H1,2,3.	

ca 10⁴/clone. plated · · · no TTS! N4. P4 (ca 10³ each). P4 10⁴ each.

B5 ∴ pure + D1. Almost pure +. 0/22 non motile.

F5 ∴ + pure ↑ [rough! -] F1. Try to separate stationary cells.

3/24 stationary cells gave non-motile clones (E1, 2, 3). E3 →(1) E4⁺. Rough? [123]

~~GB...~~ F3 pure s.c+

see 1149E.

Also set up reconstruction mixture in E5, F5 (- from C1). Tried to give Fla > Fla⁺.

In this exp., I had intended to separate subclones, but these were too numerous and was finally abandoned. B5 and F5 are unseparated clones.

122FY

① ③

① / 10Y

K40/10Y → 123C3 (3Y) → C4

123C3 & 40/10Y

n = 13.

must have been considerable late replication

no further replication

n = ~~22~~ 21

42 lost

40 / 10Y

7..

124H2, 3. (A-F) → 10.3.

F 10^3 ①

125H1

n = 31

P5, some of these ①/1000 (n = ~~30~~ 31)

P6. n = 33 125 H2. → 10Y.

Crossovers in "back progeny."

1149B.
C

4/5/54. 35 non-motile clones from 1149 picked to broth P4. (Ignore rare semidomal cells). Test cntrl, ✗ 60C P5. (DCG. Cf. early expts. on macro-tracks.

C: also test 1147-3 branches: [119]-H5, #F1, F2 [121]-D3.
cntrl 60K. 60C ✗ F1, H5, D3 all +. 60B ✗ F2 +.

Note FA60C (22/967) transmutated each culture but also the current strains of SW666 and SW967. ∴ not valid test.

As a repetition, 60B ✗ 967 : 0 (most expts.)
60C ✗ 967 ++, Not tried to identify this by
60C. 0 type of strains.
967 +.

Tests continued by DCG — see her summary.

Not tried 90C ✗ 122 series. Each gave only b; each responded
JAN 24 1955
See note above on ambiguity of "60C", supposed to be 22/967

4/5/54. E3 E1-2-3 are clones from a few stationary cells isolated in transduction clones 122 ~~B5~~ and F5. They appear rather rough E4 is a motile clone from a cell in E3 at ca 200 cell stage. (Reversion?)

P5: test E1-3 for motility reversion and × FA60C. (cf 1149B.

However! E1-3 prove to be galactose positive! E4 is Gal⁻.

Unless a serious error has been made, at least E3 was ~~segregating~~ Fla⁺Gal⁻/Fla⁻Gal⁺. Could latter be SW967? Whence? (crossing?) Test on EMB/lactose.

There is a remote possibility that there are W2049 = Lac⁺ S^R. Droplets of 2049 + W2438 come on an adjacent coverglass that broke in course of expt. and conceivably might have contained some dego! Morphology of E1 is rough rods, ∴ diffuse from 2438

Great care must also be exercised in removing coverglasses for exchange.

✓ Lac⁺ S^R!

N6. FA60C = 22/967. But OCG finds control × 967 gave T+S. Possibly contaminated ∴ FA22? She is rechecking.

60C × E1-3 gave no swarms

P6 Try SW967 × SW666. Mixed culture plated gave numerous swarms! New transducing phage? (cf FA26!) — Asks Ahuka to check. — Yes. supernates × 967, not 666 to motility. Source of the phage? all ~~E~~ 6 were Gal+ pm.

Transduction of lac_3 - W435. [1150]

W1409

April 11, 1954.

Two coincidences of lp^s & lp-linked mutations have been recorded: W-518 and W-1650.

A. A third coincidental mutation, W435 lac_3^- has never been tested for transduction or linkage to lp.

B. Recurrent lp^s should be checked for other mutations, viz. auxotrophy. Cf. EML.

A: (1) Reisolate W435 from lyophil. First tube proved to be substantially Glu$^-$ but ca 1% Glu$^+$. Test against λ (Hfr Gal_2^-) and λ2 ex EMB Glu$^+$. Proved sensitive to both, no indication of transduction.

(2) Do. W1409 stock now repurified (lac_1^- lac_3^-) lp_2^s. λ2^s; No transduction.

A (1) should be repeated on repurified cultures; lp-Gal-lac_3 linkage should be tested. [Cf. W1741-1744]. (W618 = Gal_2^-).

Conclude mutation W435, W1409 not subject to transduction. EML will check lac_3 for linkage to lp-Gal, & DCG also will look for other mutations coupled c lp^s.

B) DCG isolated 3 suppressors (lac^+ Glu$^-$) from W1409. Tested by Boris - none of these are constitutive-lactase.

All EML program on isolated lp^s auxotrophs

~~PA 50 — x 666~~

April 12, 1954.

Setting up 5/12/54. No motile seen at 2 hours. First seen at 2½ hours!
Begin isolations 2½ hours – 3:30. Some divided at least once by this interval! 40 isolated to clones. As far as possible, separate at 2-cell stage. Most clone pairs showed very few, occ. none of motiles. Record ~~numerous types~~ clones with "numerous" motile at 10^3-10^4 cells.

A1 ① ① 134 →

A2 ① → Swarm studied stationary phase → 126 F4 (43) → F3 & F5. Save! pure swarm

See

E3 ① ① → (4) (8)
E4 " (7) (4)
E5 " (1) (9)
F3 " (30) (6)
H4 ① (50)
G1
F1
B2

(13)
G1 (20)
G4 (21)
H3 (24)
H4 (26)
G3 (27)
F2 (12)

No – formed from stationary cells of A2. (126 F3 – F5).
Save as pure swarm.

Note (re Stolon's question) Most numerous genus clones separate quite unequally. E3, E4 most exceptionally. But re should get more explicit data on congeners of swarms.
Need better method to isolate early predecessor lines.

→ paralytics

Spont. tracks?

April 14, 1954.

22 × 578. Pool A2 - F4. [127]

3h. 30° 9³⁰ - 12³⁰. Then isolate motile cells. Most inviable.

9 inviable + 5 — 14 dead

6 - nonmotile + 4 → 12 nonmotile at 10^3.

1 5+/20 - later 10^{35}.

3 (2-4).

1 ca 30 rough + (1?) → 10^3.

3 c semi clones. B1, C1, E2

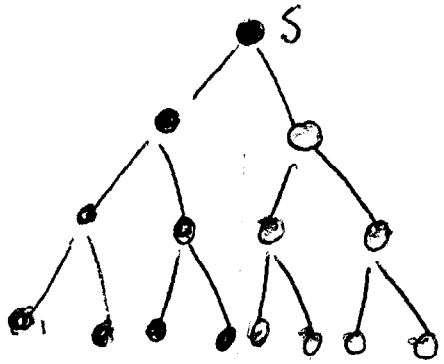
1 many + (how?) F1.

On transfer of single cells, motility very sluggish & petered out so that direct study of phenotypic delay was not successful.

Roughness?

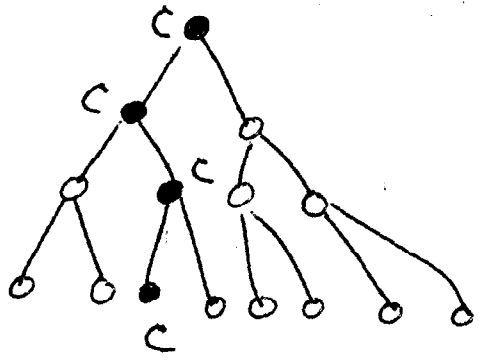
Platings:

FA 22 × 578	~~tr~~ trails and swarms.		578	0, 0, 1 sw no T.
60 × 578	1 S 0 T; 1 S 1 T.		580	0, 0
60 × 536	0		534	1 ? trail at 51 colonies of BHO sutes
534	0		536	1 sw, 2 T? relates sprinkling of subsurf. colonies.



Single clonal

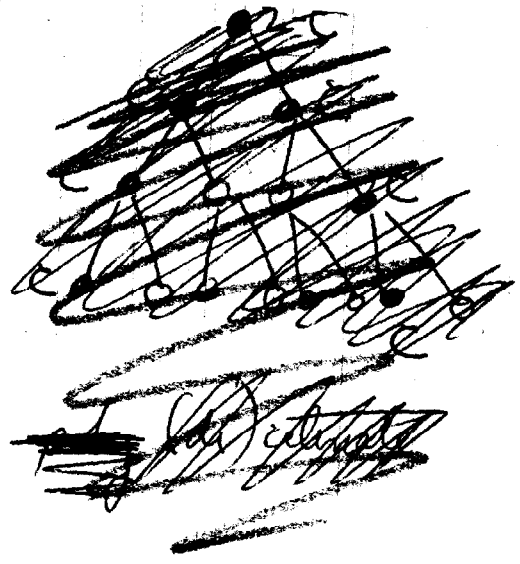
segregational.

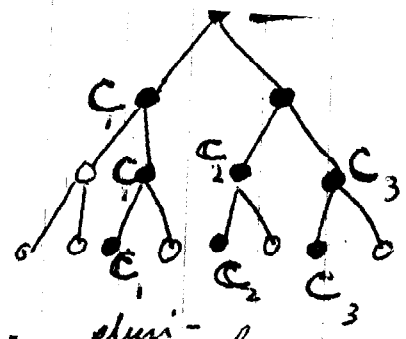


etc.

unicatenate

(single, unbranched chain)





etc.

pluri-catenate

(n = 3 in this case).

pluricatenate or ~~[illegible]~~

strain C.

~~1151~~
" 1151

Note away to ORNL week of 4/19/54.

4/4/54.

123. Isolate motile cells to G2. → 124 G1, G2. (ps. grow+)

pick for selection on mot. agar. Showed no motion.

W-2438 = C received from Murray for cytological comparison. strain C W2049 as rec'd from Weigle is ovally different (SR, rough). Had set up W-2438 for possibility of selecting a more bacillary form, but after several hours noticed occasional motile bacteria. Picked these as stated. Ones appear erratically motile + stalky. Possibility of selecting more motile variants? In mot. agar, C is stationary.

N6 123G2 in broth (37°): no motion at all. Incr. from this to necessary, incubate 11:30AM 37°, 30°, 19°. for temperature effect.

8:30PM: at 37°: homogeneous suspension; very few motile ±
30° rough mod. +
20° smooth fair. ++

Best chance for selection is at lower temperatures in agar & getting. Use 20° culture as inoculum.
= 115113.

N11: all cultures spanned appreciably, 20° < others. Retain for 30° and 37° spanners to the same, 3P11. P12: 30° showed faster spreading but still sluggish. P13 Both about ½ through tubes, 30 > 37.

4/30 4 transfers in getrin-motility, 1 tubes 37°.

DATE: 4/25/54. REF:

D. Leave plating of W2441 & Dot Boating.
She has separated EMB lac 24h.
1. Lac⁻ — (v. slow + ?)
2. Stable lac⁺ pure + ?
3. " " prob +, −
4. Reversion " strong +
5. " " +, −.

Restreak these + prepare cultures for constitutive test. Hand over to Bon
0, 1, 2, 4.

DATE: April 12, 54. REF:

W2441 = "Lac, Lac^c" as received from Morrod.

P12 Streak out on EMB Lac, Gal. All Gal+. Lac: colony types noted: separate #1 = Lac− (v. faint pink at 24 hrs) #2, 3 not pure. Re-streak

P15: both these lines showed sectored (slow Lac+), Lac− colonies. No pure + seen!

Crude NPGase test: intact cells — Enzyme

(Pneumococcus)	W1301 −	++	±
	#1	−	±
both r, − {	#2,	−	±
	3	±	± scè.

1151A. Restreak Lac− from #1 of W1427? Malt.

B. Lac−, Lac± from #2.

C Lac− " "

D Original W2441.

DCG purified Lac− from plate D = W2455− and Lac+ rerecessi from this (strong +) = 2456.

July 8, 1955. Resume

SRP tests on named cultures.

① Fredericq series = 776-96-108 (xW1177) 2 kept as wg 9, 10

W 1377, 1395-97 x W1177

11/17/50 B/6 W1362 W1376 W1113

1/1/51 Evening

? were Shapiro's, other strains (W1028 str.) ever tested ?

for first 1500, mostly only 1177 as parent.

→ × SW666

chains

DATE: Jan 11, 1955. REF: 214/217

Preliminary trials FA22 × SW666 (TA42) No notebook sum. Recall that this shows low transductant titer (phage compatability?)

FA37; and SW686(induced lysate) × SW666

Practice runs to help recruit what to look for.
(18) isolated. all grew; few gave any ① at n ≅ 10
see protocols.
1 gave 19 chains (vs 1 each in other subclones), did not continue to increase

1 swarm: F3 Probably gone but save for later review
other splits: 7/4 6/3 7/2 7/1 Suggest non-randomness or mis-identification of "E" cells.

Note >3 hours permutation. Most economical procedure: many isolates, 2 or 4 subclones (no more!) each. Merely count chains in subclones at n = 10-15.

Card

Effect of volume on stocks, etc. 1214

DATE: REF:

9-10... (use 1ml cells diluted ~~1:10~~ 1/10 + .1ml phage 1/10.) add .1ml phage + 1ml cells. Add 10ml broth. Spot ca 5×10^{-4} ml per sample ± addl ~~fluid~~ broth about 3×10^{-3}.
(9-10+)

cells are 24h. Sw666

No Tor's!

Recalibrate.

11-14 = .1ml FA37/1ml Sw666 (old; dil. 1:1 c broth) and 3 decade dilutions in Sw666! Use flat slope, ~~page~~ loop D.

1/24 Motagar (4.4% agar)

11	0^5	15^2	2^3	/8.	NM fall through agar
12	0^6	$1T^1$	2^3		swarm would be ill defined.
13	0^8				
14	0^8				

	1 or 2 s/2
	0
	0
	0

JAN 24 1955 Plates Reincubated 4h 5 plates each of 9, 10, x ca. 10 spots each (100) only 2 swarmers (late) no trails

Note, however, numerous poorly defined clusters under surface growth. Incidence should be compared c Sw666 s phage and prolonged incubation!

A24 15 hour tubes of .4% agar c ~~dilution 11~~ (.1ml FA37 + 1ml Sw666 (old)). Spot loop D.

FA 37 → SW666
quantitative : ratios of Trails Swarms

12-14

DATE: JAN 19 1955 REF:

Old susp. SW666 (Refr.).

A. 1 1ml SW666 + .1 ml FA37 . Test loopfuls (Dene's Coop) on motility agar. Preliminary test to define range for counting T+S and distributions of T.

2 .01 "

3 .001 "

4 .0001 "

5P19

JAN 20 1955 Plates somewhat smeared.
1: Each spot swarmed.
2: 7 swarms / 7 spots
3 1 swarm / 7 spots
4 No swarms: contam? } useful range?
wait for trails.

~~Each SW666 pedo tube #4 and compare with 10^{-3} dilutions of tube #1~~ (=5)
(diffuse ie number of cells.

Use $1/2 \times 10^{-3}$ as standard level.

∴ ~~5 = 5×10^{-4} ml FA37 + 1ml (fresh) SW666~~ (in 0.1ml)

6 = [(.1ml SW666 + .1ml FA) $\times 10^{-2}$] .1ml + 1ml broth.

5 = [(.1ml broth + .1ml FA37) $\times 10^{-2}$] .1ml + 1ml SW666

allow 6 to stand 20 minutes for adsorption.

1/21

7 ≐ 5.

8 ≐ 6

In re chemis

JAN 19 1955

1. Time affirms that isolated motiles do not form ~~chains~~ trails

Possibilities

① Only polycatenate cells form trails. Why no more branching than is seen?

② Any cell may form a trail if it is active enough and if it gets into cezar.

Tests: ① What is ratio of trails to swarms?

What is ratio of 1- catenates to swarms?

poly- "

② Get a trail from a chain of intermediate n to be sure it is unicatenate.

May need to develop tactic procedures.

③ Distribution of trails should not be random.

(Trouble: testing interfered by swarms. Use tubes? plates? spot plates?
(not dry enough)

Try 37-xSW666. Y dilutes i cells, broth, phenol.

Linkage. (Salmonella)

JAN 19 1955

JAN 1 1955

1. Discussion with Dino: search for Fla⁻ - linked markers, using Demerec' lysates, and prospective search for new markers.

2. From Bruce's letter, H, linked Fla⁻ are:

SL13 = SW1048 para A.*

SL28 = SW1092 = Heidelberg

SW543-666 PB

* SW544 = Schwarzengrund

SW553 - dublin

* SW966 (Have as b)

* rather poor; others are monophasic except SW1092.

3. Test spring (1130 ff.) to load for linkage & complementary reassortants as bases for pedigree analyses and thereby get into chemistry.

Recollection of some trials with SW1092 but cannot promptly find the notes on it. Should repeat to evaluate suitability for linkage study (& Dino). → of Alda Bertani.

DATE: 1/21/55 REF:

5: 2 6cm plates: 5 spots, 5?, (A, B)
2 10cm plates: 17 spots, 15 spots. (C, D)

A. 0 swarms, smeared no obvious T.

B " "

D 3 swarms. ~~0, 0, 0~~ O^{12}; $(^{13}IS\ IT)$; ^{14}IS; ^{15}IT

Note: 013 showed numerous flares; motile cells obviously entered at various points.

maybe too fluid!

C O^{15} IS; IT

6: A 6cm plate O^{4} (IS, T) (~~2~~ 2T, S) (IS) (IT) 8

B O^{3} IS^{6} IT IS^{1} 10

C 10cm O^{4} IS^{3} IS IT^{1} IT^{5}

one of these has multiple flares, maybe two swarms?

Totals		5	6
	O :	27	11
	S :	2	10
	T :	2	6
	ST :	1	3
	Σ	32	30.

T, S appear to occur ~~c~~ independently; note much higher incidence of both classes in 6 of 5. 6 is at very low multiplicity. Note also the overall low incidence of tracks! Any calibration data?

DATE: 11/22/55 REF:

A22

7: A (pre "diss" plat) 0^{10}

B	0^{9}		47-0	37-0
C	0^{10}		2-T	2-T
D	2^{9}	(1T)		
E	8^{9}	1T		39

8A c^{9}

B	0^{10}	1S	multiple Haves	41-0
C	0^{11}	1S		5-S
D	0^{9}	2S 1T		1-T
E	0^{11}	1S		47

aga.: note 8>7; reduced incidence of yesterday's dependence of Trails but low number res is disturbing. Cf. earlier date.

Note: present expt entails large fluid volume + potential strains might proliferate without ever entering aga. effect of inoculum volume

These experiments used loop B (dio's) whose volume, full, is about .00351. loop D, full, is about .0023#; flat = .00073; retention .00022, delivery from flat ∴ ≐ .00051 Call this 5×10^{-4} ml, and use about 10× conc of phage over previous expts.

Trails data: mine.

1215

DATE: 9/20/55 REF:

1	2	3	4	5	6	7	8	9	10

(1) Plate za 15 SW666 pyplate motility agar 1 pour. c/s
9/21/55 underlayer of NSA. In each case, discrete colonies with no doublets or spread observed.

Add FA37 0.1 ml to 1 plate: colonies still discrete 9/21
A22.

10

A25 No swarms. colonies becoming more radiate; initially rough to
spheroids (in all plates).

20

30

40

50

11.0/55

Comments: Work to date seems to have emphasized the prolongation of chains rather than distribution of their sets! No trouble should be taken now with settled chains but search instead for continued lines of increase.

For us, note we can detect division but not identity / multiplication. Present data do not define where units are between gene and final effect.

TRAILS: Grant.

JAN 2 4 1955

901 FA24 — x 666 23T: 6S

975 FA22 — x SW666 12T/15.

∴ Hasum SW666 x— FA12 38T: 3S

x— FA22 42T: 3S

x— FA24

Review data on trails

JAN 2 4 1955

Impression that T >> S in many cases. Is SW666 exceptional?

—X967 gives great excess of trails.

See 1033 designation.

Note "C" T not very numerous cf S. . "Rb" or "C" seems numerous swarms, no trails.

—XSW1048 120T: 10S.

See 999 T/S ratio of UV fixated FA12 UV$_0$:

29T : 0 swarms!

9T : 3 swarms!

"dimer Egar".

Has SW666 changed? Or is FA12 ≠ FA37?

cf SW543?

c UV, remarked that 13T: 14S! difference?

Use SW666 trail!

991: FA21 —X SW666 41T: 10S 5/7 Lp+

FA22 —X SW534 30T: 2S

JAN 2 5 1955

Salmonella Comment.

Spent the last couple of days reviewing notes on Salmonella.

Many problems are left in mid air, e.g.

(1) Monophasics
(2) H_1 duplications
(3) Fla^- mapping
(4) Specificity of transductions (Lysogenizations)
(5) Phase variations; exhaustion;
(6) Other phages as ×

(7) Pullorum
(8) Kunzendorf c
(11) Lysogeny - protection (coli groups) better.
(12) Heterogenotes (sw 686?)

(9) Misc H_1 → × monophasic (z28; lw;)
(10) Backcross data
(13) paralytics.
(14) especially chains.

Some of these are partly tackled

There is little in notes of immediate relevance to problem of trails except some inconsistencies in T:S ratios. See other comments for this and for comparison & pedigree data.

Trails: incidence

JAN 25 1955

, request comment that —x967 gives many, long trails.

But has SW666 changed?

note: 999 FA12—x gave 38T: 3S

FA12uv —x 13T: 14S.

BADS claim — SW541 [2×10^{-3} T/cell!]

.05ml FA22 (ca 10^{10}) gave ca 500 papillae! (973)

$\therefore \frac{500 \times 20}{10^{10}} = \frac{10^4}{10^{10}} = 10^{-6}$ papillae per phage.

BADS claim: 1ml FA = 5×10^6 T ! and ½ as many swarms!
$5 \times 10^{10(?)}$

SW541 is F.K. Copenhagen FK223
SW665 is stated to be Xyl- deri.

JAN 2 6 1955

Note - BAOS remarked that

534 — x553 gave T >> S (1T/17,102 gametes!)

LT2 — x541 many T, S. (claims 2×10^{-3} T/cell!
4×10^{-4} S/cell)

Try reciprocally!

compare pearl yields. Any other matings for 541? See notes. Ought to use 553 to demonstrate tactics from isolated tactiles!

(Consult Andy or Clive)

JAN 2 6 1955

MS comments.

Sw 54/1 x — TM2

3a. T > S. "several fold". counted 80-90 cols. in 15h. 37°.
∴ delay.

4b Trouble shooting (squirt oil at them.) Too much interpretation.

4g "expts in wh few T/plate majority were single" — data?

4/g he states adequate hyp. "prob. small".

5a earlier expts 9 & 6; later 24 isolates: 1 n.g. 1 sw

15 < 10 7 > 10 (generally about 1/5 are "E" cells").

5c 8 cells isolated: splitsome 1:0 2:0 2:1 2:1 2:1 6:3.
30:3 (E) 44:3 (E)

States random separation from non-E's how tell?

∴ E's catenate also.

6c how can n ≷ 10

7c ! ! critical

7d he has isolated E cells at 9th - 22d generation.

→ × SW967 pul.

1216 a

DATE: Jan 27, 1955 REF:

1	2	3	4	5	6	7	8	9	10

p26 FA22 ~~[illegible]~~ .1ml + 1ml SW967. decimal dilutions in SW967.

loop D. ~~#1 [illegible] sec tails / spot~~ #2: ~~[illegible]~~ / spots: $0^4\ 1^2$, strokes $0^1\ 1^1\ 3^1$
~~and 1 stream~~ /

#3 1T / 10 spots and strokes #4: 0

A #1: spots $0^3\ 1^1\ 3^2$ sec
strokes $0^2\ 1^1\ 5^1$

~~FA~~ 50 A

B ①: 11 spots $S+3T^2\ 1^2$ ca $5^1\ 2^3$
② 8: $1^6\ 2^2$
③ 8 spots $0^7\ 1^1\ 2^1$

---

p27 various media. Use FA 50A, tube ~~1~~ 2, flat loop D.

C

D = tube 3. #28, many trails appear to have ~~up~~ from ~~to~~ 30 to as much as 54 colonies per trail. (5^{30} - 9^{30} = 16 hours) which appears to be in excess of generation time.

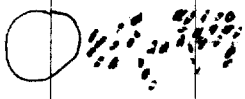
1216
6

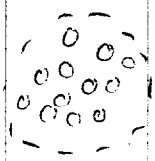
DATE: REF:

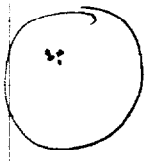
	1	2	3	4	5	6	7	8	9	10

A29.C

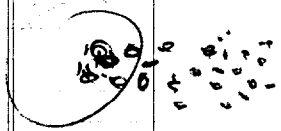
1 Motility agar standard
2 " .30% agar 2 brown
3 " .35 blue
4 " .40 red
5 " .5 gum
6 " .6 yellow
7 " .40 no gelatin.

C7:  clusters (& side chains?)

5 spots like this; 2  ; 2 have no lateral clusters but numerous colonies under main spot 

C6 or "clusters" under spots, short and small 

C5 clusters, larger than 6 

C4  not so large colonies nor as extended as C7. interspersed of small and large colonies suggests minor branching. In general has appearance of concurrent clusters rather than single trail. A few possible sister branches but had to extrapolate owing to numbers of swarms.

C3 swarms have more linear, or multiform appearance. appear more restricted than 4.

1216
C

DATE: REF:

C2 - numerous trails, somewhat fuzzy. # Clusters rather than linear origins. #

C1 Trails shorter, not readily diagnosed whether linear.

Conclusions 0.4% agar alone gives most extensive trails but somewhat fuzzy. Gelatin definitely retards motion. But 0.3% agar + gelatin though it has smaller microcolonies also shows up pretty well also. Considerable question on unilinearity of trails but will have to be settled by more precise methods. It is very difficult to say whether one trail or two.

D. distribution of T's:

plate	0	1	2	3	n	sw.
1	8+2	5			1 polyc.	
2	5	4	3			
3	1	5				
4	8	5	1	1	4:1	
5	8	4	2			
6	5	6	1			1
7	5	4	0	1		
8	5	5	1			
9	8	4	1			
10	6	6	1			
	63	48	10	2	1	

ignore smear.

Total: 1 swarm.
68 T
126 spots.

some definitely non linear:

(over)

some zero's have minute colony (ies) just under surface.

Exp. 63 48 10 2 1

$p = \frac{68}{126}$

12162

DATE: REF:

	1	2	3	4	5	6	7	8	9	10

E same Rel as D, 2 spots on agar c/s gelatin.

s = 1, 2

c = 3, 4

1. 4T+/30 v. thin agar

2. 6T+/18. somewhat fuzzed but clustered, not channeled.

3. 3T' 1T²/18 more "linear" (more overgrowth + tighter gradient)

4 9T'/24. ; 1 "cloud" - remainder

8a - percentage of chains.

JAN 27 1955

10a. "only E cells initiate trails"

Poisson formula applicable only when drops are of equal size + number of bacteria! Calculated 15 isolates and 3 trails =

11/5-1 E cells.

10c I have seen groups but would interpret them differently

10d Swirl.

Descussion - growing point.

Bruce found 3/15 motiles → trails

What are the glycolamate initials?

—x SW-967.
trails to agar

FEB 5 1955

1217

/216/

50B—x SW967 (fresh). 12^{30}-4^{40}.

FEB 5

motile cells fairly numerous. Let form single clones; H6 divided= a,b.

N6: examine for chains. Isolate as many motile cells as possible, and transfer these directly to ordinary motility agar, as individuals, as well as mass transfer from residual clone.(A) (B)

A B

Isolate		# of Fla+/-	Trails from clone G	T	pltd. Growth, trails from singlechains.
.4= 10^4					
A-6		0/.4	✓	O	
B-6		1/4			B4 1: 1g OT
C-6		0/.3+	✓	O	
D-6		0/5.	✓	O	
E-6		0/4.	✓	~~clg~~ profuse	
F-6		22/.4	✓	profuse	F1234E34 22: (10+12) 13g OT
H-6	a	6/4	•		H124 7 ~~6~~: not OT how many grow? small some 4L
	b	1/.4	•		H~~124~~5 1: O
A-5		1/.4	✓	~~•~~	A4 1: ng
B-5		4/.4	✓	O	B3 4: 3/4g OT
C-5		4/.4	✓	long profuse trail	c4 4: 1/4g !? T slow
D-5		13/.4	✓	profuse trail or cluster	D1234 13: 8/13g. not T ! ? slow
E-5		n.g.			
F-5		0/.4			
G-5		0/.5			
G-6		7/.4	✓	~~g~~ scattered cells	G34 7: (5+2) 4/7g. no T.

• not planted (picked ~~and~~ reserved by mistake)

Breeding of trails in column A vs B probably reflects chemotactic stimulus from neighboring Fla⁻. Compare inoculation with and without added SW 666 (for Fla⁻ mutant)

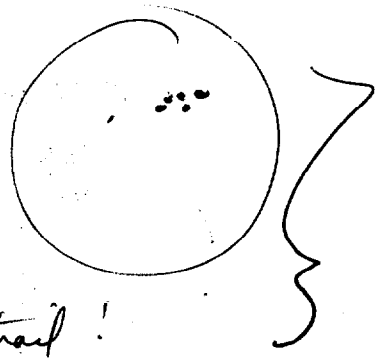
Recovery of cells ≐ 30/53 > 50%!

(om)

Prions cysts had been indecisive. Thought it better now to transplant directly to determine ① how many trails per initial and ② role of toxin.

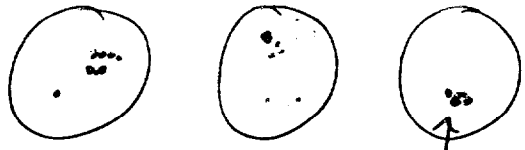
C1: 1 gun

5 colonies blow again!



note C5 also had long trail!

D1234 8 gun most 0, also



vent downwards definitely

1218

FEB 8 1955

226

Plate subdonal cells ε sw666.
5 = group of 5 Fla+ 1-3 ε sw666; 4-6 s
4-6: 2 prolific T #3 1 T.

add droplets to spots moistened again ε sw666

No def. advantage of plating ε sw666!

	spots	T.	
G1	6	0	swarm
G3 } G4 }	4	0	1 has 8 scattered subsample colonies

(crossing?!)

A2	2	
6	2	
B1	2	
3	2	
4	2	
6	2	
C1	2	
4	2	
D4	2	
5	1	
E1	2	
3	2	
A1(x)	1	

all 0

35+28+38+24+32

sp.		T:
F3	4	0
F4	4	0
5	4	0
G2	4	0
3	4	0
4	4	0
5	4	0

swarm mplete (entam?)

A3	4	0
4	4	1
5	4	1
B2	4	0
5	4	1
C2	4	
3	4	0
5	2	
6	3	
F1	2	
F6	1	
G6	2	0

(swarmed)

H1		
2		
3	ca 4 ends	0
4		
5		
6		

	spots	T
(swarm)	1	1
D1		
2	4	0
3		
E2		
4		
5		
6		
F2		

"157 s.c.i." #

This process a poor method indeed, only 1 clear track.

Misc: Serratia from D [illegible] A 1218
Cla^R for Lichstein B.

FEB 7 1955 + pre

A. W2745 = Edwards 55: 184 typed as "184 – Serratia marcescens 1/27/55.

B. Put expt i Cla 1mg/ml n.g. ditto Clam (chloroacetamide).
Both sterilized in autoclave; former buffered i NaHCO3.

2/8/55: 100% sols Cla, Clam; 20% Clh (Et<OH a). – ml/20ml NA plate.

Cla: .2ml heavy growth
.4ml spaces, no pop.
1 ml NG

Clam .2 small background Cl H .5ml: n.g.
numerous papillae + satellites
.4 few colonies in heavy growth.
1ml: n.g.

1 n.g.

W2754

Remistate.
Isolate 1218B 1 from Clam .4
Recodated and found to be:

	Glu		Form		M+l		Stl	
	A	G	A	G	A	G	A	G
W2754	+	–		+	±	±	±	±
W1485	+	+		+	+	+	±	±

See letter to Lichstein FEB 12 1955

See also notes on papers by Grey, EC Penfold + Harden.

(over)

Ad lib (see notes 180, 188) formate glucose EMB.

.4% – 1% formed n.v.g. ~~S. typhi OK~~

S. wichita = –

S. typhi – only weak =) not sharp enough

~~test~~

difference. Suggest OCG work it out.

From platings on Cla, ClH test a few cols: all glu: AG+.

Chloroacetate soln – is probably decomposed on autoclaving while ClH was not evenly mixed – gave poor purples in wells. Might try lower concentrations or purer material when F-G screening method is worked out.

APR 22 1955 Send Evans

APR 26 1955

W 2745

1218C1 = aerofoam

1218C2 = Johnson's steam BOK

3/28/-5

DATE: REF:

wet weight : 310 mg

suspend 310 mg in 10 ml dist H_2O = 31 mg/cc

dilute 1:10 = 3 mg/cc ; plate 300 γ #1 plate 0.1 cc onto NSA

dilute 1:10 = 300 γ/ml ; plate 30 γ #2 plate 0.1 cc onto NSA

dilute 1:10 = 3000 γ/ml ; plate 12 γ #3 plate 0.04 cc onto NSA

dilute 1:10 = 30 γ/ml ; plate 3 γ #4 plate 0.1 cc onto NSA

dilute 1:10 = 30 γ/ml ; plate 12 γ #5 plate 0.04 cc onto NSA

dilute 1:10 = 3 γ/ml ; plate 0.3 γ #6 plate 0.1 cc onto NSA

dilute 1:10 = 3 γ/ml ; plate 0.12 γ #7 plate 0.04 cc onto NSA

dilute 1:10 = 0.3 γ/ml ; plate 0.03 γ #8 plate 0.1 cc onto NSA

10^6/ml original → 1 on # 8 $10^7 \leftarrow 10^6 \leftarrow 10^5 \leftarrow 10^4 \leftarrow 10^3 \leftarrow 10^2 \leftarrow 10 \leftarrow 1$

10^8/ml original → 100 on # 8

incubate 1 series at 37°C, second series at 30°C

No.	after 2 days 30°C	37°C	average	#/mg
1	too crowded, i.e. nearly confluent			
2	– Not counted			calc
3				calc
4	~880	~784	~732	2.4×10^5
5	298	267	282	2.3×10^5
6	80	71	75	2.5×10^5
7	29	28	28	2.3×10^5
8	8	8	8	2.7×10^5

2.4×10^5 bacteria / 1 mg wet weight

no definite coloration yet (2 days)

replicate onto EMB lac for proportion of "coli"

Total	fem	non	% fem	
29	11	18	11/29	40%
68	19	49	19/68	28%
8	2	6	2/8	25%
28	4	24	4/28	14%
133	36			27%
225	61	164	27%	
455	97	358	22%	
813	194			24%

lac + (E. coli) 24% of population

no definite coloration yet (4 days)

1219

50B —x SW-967
transfer motile initials to mot. agar

FEB 8 1955

227

The primary purpose of this experiment is to evaluate to addition of extra SW-967 cells to the explants, and to estimate the fraction of trail-forming clones per initial.

50B—x SW-967 9AM-1130 AM. Concentrate mixtures and trap. (This procedure works very (SW-546) well. Its main limitation is that 30-60 minutes are needed to entrap the motile cells.)

A1-F3 were collected to about 12:30, deposited no later than 12:50.

After lunch, collect to about 2 PM, and deposit F4-H6 (2/square) by 2:10. At this time, earlier isolates were mostly 2-celled.

Ca. 3 PM, transfer isolates, at random, to motility agar, either alone or with supplement of cells of SW-666 or SW-967.

FEB 9 1955

	No. of transfers.	Grew	Trails
Series A-F3:	—	7	6	0
	SW666	7	—	0
	SW967	6	—	1
F-6-H	—	10	7	1
	SW666	10	—	1
	SW967	10	—	2
Totals:	—		13	1
	SW666		17	1
	SW967		16	3

of 1218: 1/157 transpl. why? Is SW666 inhib?

5/50 transpl. (46)

Result is indecisive owing to small numbers. SW967 might be worth making a bal- mutant in.
Probably use at 2-4 cell stage when exploited.

This test had been suggested by the result in 1217A where 10 clones had given 4 trails, whereas isolated cells had given four or more. This should be repeated by direct comparison: let cells form large clones. Examine for presence of motiles but do not isolate. Explant in deirided sampler. Compare clones with initial transplants.

single cells: trails 1221

FEB 9 1955 230

50 → SC 967 9→22 — 11 45 — 12 35

Collect individual Fla+. Explant series ACEG

Let remainder form large clones over my lit.

Seis	A		A'		C		C'		E		E'		G		G'	
0 germ	0	0	−	−	−	0	−	+	−	0	+	−	0	−	−	+
	0	−	−		0	0	−	−	0	0	−	−	0T	−	+	−
− n.g.	0	−	+		T0	0	+	−	0	−	+	−	0	−	+	−
	−	−	+		−	−	⊕	⊕	0	0	+	−	0	0	−	−
T trail	T0	−	+	+	−	0	+	−	0	−	+	*	0	−	+	−
A1-5	−	0	+	−	−	0	−	⊕	0	0	+	+	0T	−	+	−
	0	0	−	−												
	−	−	+	−												
34 clones viable:	−	0														
	0	0	−	+												
4 trails	0	0														

In A' .... 24 viable. Total isolated = 58 = 34 + 24 (accidental carryovers!)

Note general reciprocity between A, A'

Unfortunately a, b not precisely distinguished here; probably inverted

In second part of experiment, clones were examined and transferred in multiple drops to not again

+ = clone
⊕ may Fla+

In this series, 48 cells isolated, each was viable (sic!).
Motiles detected (probably some more): ① ① ~~③ ⑥ ⑩~~ ② ② ② ③ ① ① ⑳. Clones were about 10/each.

If at least 20 cells are coded for "E" type", then E = 1/48.
Detectable motiles after 13 generations = 9/48.
② although none of these gave trails, the apparent incidence would be about 4/34 ≐ 6/48. ⇒ number of clones ≥ ca 10 motiles!
No swarms seen so far. (?) — This expt was partly spoiled by motile (contaminant?) in second part.

Results: (over)

FEB 15 1955 — Despite much labor, the expt. was unsuccessful. Why no trails from the second group? Intent was to look for >1 T/cell clone. This seems patent from appearance of the trails in part I which appear to cluster or to flare out unlike earlier impressions (of other systems?)

FEB 16 1955

Note 2/12, 2/15 failed to get any Fl+ from "SW967" (= ? single colony isolate). 10→x, 9→x, 50→x }

Repeat, cf. "stock SW967" ĉ this isolate.

FEB 16 1955 φ + cells 9:35 AM —

A [231] → Picks as single cells (probably many at 2-cell stage) to Motility Isolation Agar. (MIA) ca 2×48=96 picked to two plates.

B [ ] → clones (small droplets).

FEB 17 1955 A: (2 plates). Unfortunately MIA > 8 days old & probably too dry. Colonies totalled 44 on first plate (sic!) [How so high?] only 1 trail = B3a (fairly strong) and 17 on second plate, 1 trail H6 (several mots.)

(Results not very telling pres. owing to the agar.)

Totals (note discrepancy — medium difference? — or does the pres. that some of these were non-motile — sup. ...) bear. On second plate, viables were: E6ab, F1a, 2ab, 3a, 5ab 6ab G2b, 3a, 5ab, 6b. H6ab/.

C. Pl also plated loops + ~~...~~ upip samples of FA 9, 50 → x SW967. Nothing trails seen (pres. agar!); unsurprising. In toto: from 50-x 1T, 1 swarm ~~...~~ (sic; ĉ cluster!) per 13 loop spots and nothing else. Save swarm as 1222C1.

D. Note "sci" suspension proved 'lysed-looking' and not further sens. to PLT22. SW967 and SW1139 are lp³. Store "sci" (see top of page) as 1222 D1. Spend no more time on it now: it may merely be contaminated.

~~Streaking of Spectinomycin~~ (1224)

Plate → x SW967

Spreading

FEB 17 1955

50 → x SW967 Usual routine.

Collect ca 50 Flat + in ~~100~~ ca .05 ml ~~H2O~~ broth, plate out on (old) ~~HE~~ MGA and MA no G (spread .01 ml samples)

	Colonies	Cotails
MGA	1	0
	5	3
	6	2
	12	5
MA	7	0
	3	0
	4	0
	14	0

This was remarkably successful if each colony is of single cell origin! Does spreading influence the agar? (Can be directly tested). Should be repeated on a larger scale with fresh agar.

Save 1 tail-forming colony as 1224-A

SD -XSW 967.

1225

Effect of spreading etc

FEB 18 1955

.5 ml + .5 ml $10^{15}-12^{25}$ 37°.

Then R.T., Centrifuge, decant and add 0.1 ml broth. Hold in Refrig. for subseq. use. (10^{5} PM).

3^{30} PM Isolate flat $-2/15$

500 (sic) isolates. transfer to 0.1 ml broth. Estimate final density at 2000/ml.

A). Effect of spreading: (Use loop D) etc.

see next page

FEB 19 1955 B) 96 micropip drops left under oil → 14 clones. (+2?)

plate these on pres spread agar. Microscopic scoring

2 clones were noted as ~~having~~ pluricatenate (Eq 10?)

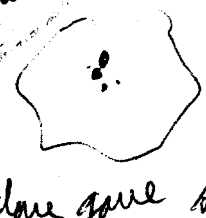
A 20, 23: 14 were streaked out (5-10 drops) c microspipette on 1 plate. ~~altogether~~, only 1 definite trail; some dubious isol. colonies.

From est. of drops up to end on c-7, 2/8 drops have cells.

14/96 in drops makes new.

2 clones (eq 10^{6+}) were spread out on ~~M~~ MG-A plates

1 clone gave about 6 isol small dry colonies and one cluster of 5-6



1 clone gave some indefinite isolated 1 colonies, and some definite but unimpressive: 1's:8 2's:5 3's:1 (If these were collected together they would probably be more impressive.

1225

FEB 19 1955

Sat The collected sample was used in various ways, partly divided by smearing of the plates. From yesterday's result it was wondered whether spreading the agar altered its surface to encourage tail formation.

1. Old plate 5 loops (D) then spread: 13 colonies, no T. ∴ est 2.6/loop

2. Fresh (poured Thursday) .01 ml, spread:

a. 6 tails 42 colonies (smeared).
2 " 48 " fairly discrete.

b. .01 ml not spread. (allowed to unmoor)
4 tails ? colonies (smeared)
35?
.02 ml little
5 tails badly smeared.

3. Spots (from micropipette: est ca 1 cell / 4 spots ?)
100, not prespread – colonies? (smeared)
only 4 tails (per est. 25 cells).

pre-spread: 48 spots → 13 colonies
6 tails !

" 43 spots → only 3 colonies
0 tails

sep. colonies per loop as noted:

∴ nonrandom distr. of cells in pipsamus certain

8 loops. prespread 5, 1, 2, 1, 3, 2, 3, 1, 0, 2 = 20 cols.
1 T

4 of 1. [illegible] tail at 16 h.

8 " not prespread. 3, 0, 2, 0, 3, 0, 2, 3, 1, 2 = 16 cols
3 T.

How account for so many descrepancies:

extreme variation: gave T/C

1. Old plate, spread 0/13.
(clear by loop).

2. Fresh plates, spread

2x .01 ml . 8/90

Clumped

.03 ml 9/? (assume 135)

3 Fresh plates, pre-spread & incr.

with A) loop 1/20C

B) pipette 0/3
6/13

4. Not prespread

A) loop 4/25?

B) p. 3/16

No clear effect of spreading.

Estimates per loop agree:
13/5 20/8 16/8.

How about mul fraction?
= 3/8 for 16/8 mean.

∴ ca 45 cells per .01 ml
(7 estimated 2500/ml)
and makes this loop now
ca. $\frac{49}{21}/45 \times .01$ ml

$\doteq .0005$ ml [< former estimates]

Note extreme variability (+ sampling?)

up. content est at .25/drop

∴ $= \frac{.25}{45} \times .01$ ml $\doteq .0005$

$= 5 \times 10^{-5}$ ml.

1226

FEB 21 1955
(Mon.)

Collected 895 motile cells from same conc. suspension as 1225 (refr. over weekend). Transfer to 0.2 ml broth for plating expts. (Transfer directly from pipette, in two runs, this time). Various platings.

1. Spread on MGA. (yellow = Fri poured / wh = Thurs.) 0.01 samples.

	Colonies	T
Y	37	7
W	46	7
W	55	5
W	50	6
Y	–	4
Y	–	9 + 3 wh.
	138 (3)	38 (6)

∴ mutants are not diffuint. Average T/C = 38/276 = .138 (≅ 1/7)

% 1225 = 8/90 ? 7/41.
Take 1/8 as rough average

FEB 22 1955

2. Pour in MGA. 0.01 ml

a. thin layer, then cover	23	5
b. thick layer.	38	5
	~~23~~ 61	10

all dup.

5 colonies had reached surface
2 ± trails

TRAILS ARE V. INTERESTING!

3. Spread .01 ml ± ca 10^8 SW967. → 9, 17 trails, many all very weak. Not unlikely the motile trails are provoked chemotactically.

4. Spots (loop).

4. Spots (loops) c/s prespreading plate surface.

		pres. C	pres. T	s pres. C	s pres. T	
a	w	3m (20)	1+1S		5	1 swarm on pres.
b.	w	10/12	1	13/13	2	— 1 def. branched? Reincubate
C	(+ ΦOH .05% drop) 20 spots laid, 2 def. blank.	18	4	(20)	13	no eff of ΦOH?
d.		4/4	1	3/4	0	
	large pip. drops	3/6	0	5/7	2	

1/c smeared from ΦOH side to other! may have carried cols. along.

altogether this plate shows 17/40; cf. 10/65 above. $\chi^2_1 = 7.4$ average cells ca 1+/loop $p < .01$

Try in shaker tubes

Reincubate after R.T. 2 hours for qual. exam. fails.

In (2) 1 fail = 125 colonies at 17 hours!

FEB 27 1955 Some errors noted in the dup platings too, though not long incubated.

FEB 23 1955
FEB 27 1955

Problem: ≯ 1 trait per clone? Would need to test clones of 10 – 100 cells.

A. T/C$_{limit}$ = ca 1/8.

I Approaches.

1. Most rigorous: Isolate single cells, let form clones and transfer individually. Too laborious!

2 Isolate single individuals. A. Transfer as singles to both tubes. Let grow to size n. Plate out

B. Let singles form clones before transfer. Then plate out. (Have uncertain what fraction of clones have developed although more clones are represented).

For this general approach 2A seems best. Can be contested with immediate platings of numerous inocula for concordance ratios.

II FEB 23 1955

Isolate motile cells but not singly. Plate out mixtals for T/C values. Dilute to samples of how many cells and let form clones. Plate these out at clone size 4.

how many? if $<<1$ then most samples will be wasted

if $\doteq 1$ then expect only 1/8 to have an initial, though no independent election events.

if >1 then too high expectation of coincidences

III. Methods of plating?

1. Spread – restriction in volume; may get away i respiratory
2. Pour plates } Try these now.
3. Shake tubes }

plating SW967x— clones 1227

see 1237 summary

FEB 22 1955
FEB 23 1955

P22 Mix SW967 .5 ml x— .5 ml FA 50 1^{45}. Then Refrig.
1224-A "

A23 Concentrate ca 10x for trapping.

try water? broth? buffer?

11 AM drops fused.

PM – n'very many Fla+ were found. Maybe too dilute some suspension or otherwise. Both tried. Altogether, ca 40 ⊕ were isolated singly.
Group A isolated ca. 12 N, → 2, 4 cells at 3 PM
B 2-3 PM → prev. 1 cell.
at 3-4 PM Isolate to 1 ml broth each
7:30-8 PM Plate in pour plates + shake tubes

A. tubes only 4 18mm (ca 20 ml) / 4 13mm (ca 10 ml) } ~~all had clones~~ no trails. Minor trails seen in each but only near air (over)

		plated	clones	blank	Comment
B.	tubes				
(B4)	~~broth~~	8	5	3	all show "minor trails"
(B1-2-3)	plates 10 cm	9	7	2	all but 1?(B2) show numerous "minor trails"
					no major trails (12 clones)

photographs at about 48 hours.

shake tubes probably OK for
major trails. For 16 hours, no growth
gradient. Later, colonies grow large
near air and minor trails mostly serum
culture.

8 tubes 7 large (20ml) – 2 blank
4 10ml – 1 blank

Thread

Clones ca 500 – 10^3 each!

So only 1T/20 clones! but note minor trails also.

FEB 23 1955

3¹⁰–5⁰⁰ PM SW967 (old) .5 ml + .5 ml FD50. Refr. ca 730 PM Enc. (+ thume) in centrifuge. Resuspn. when not available.

8⁵⁵ Resp. set up. By 10 PM all set. few ⊕. Dsol c. 15+2+2 and pour in shelsetube.

(27C)

Yields → 14/clusters – all flowery tails! + ? swans.

1228

FEB 24 1955

Stock of 1227 C. — 4·9. New stock

Incl. 34 tubes (10ml) inadvertently left in cold water P24-A25. See 9A25 -

Clone procedure:

(1) Store i motiles ca 2 hours mix fresh cells & phage, conc. in centrifuge ca 10x. (Takes 2½ hours). Then ~~free~~ set up on c.g. for manipulator and set up hap drops. Freeze. Takes ca. 1 hour more to find many motiles. Thus cysts, usually begins at ca 3½ hours! May keep "stock" after concentrating & store in refr. as indicated

(2) Collect up to 100 motiles. (A) Plant single hap drops (usually row in line on unmarked coverglass. Then promptly pick up from oil chamber with quartz pipette to .5 ml vols. of Penassay. Incubate (3 hours at 37° C.)

(B) Deposit ca 100 cells dumbly in .5 ml broth from pipette (removed from chamber) - (mount pipette syringe on stand & move the receptacle tube).

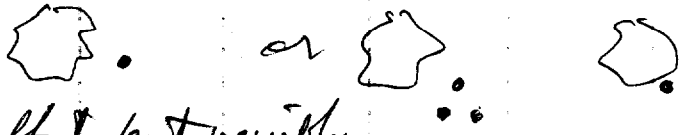
Add 10 ml MBA & pour plate at indicated time.

At last few weeks have been using hypodermic needles, syringes & coupling hardware etc. for convenience.

1228
2

FEB 26 1955

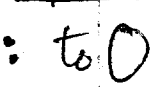
Reexamine plates.

N26 Practically every plate has occasional colonies with 1-3 satellites (minor tails)  about 1/20 colonies show effect, but variably.

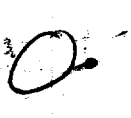
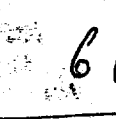
Three plates now show more definite tail possibilities

small plates

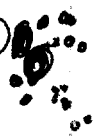
How many small plates are plated? ~~Ten~~ As stands now, 1 blank, 9 ± clones. Yesterday I scanned through and did not notice any tails but might have overlooked. Clone size is 50-100. (49, 62, 132, 74, 28, 82)

Plate 1. Total count is 143. Includes singles; minor ~~clone~~ tails:  {13 to  {4 also:  

and  (tight cluster regarded as major tail).

2.   61 singles and 

Total Count: 66

3.  and 7 minors.

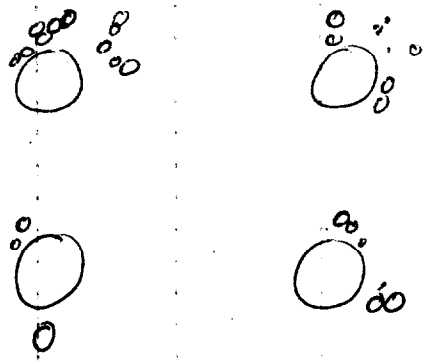
Total count: 50

12 28
3

FEB 26 1955

Large plates — 3 blanks; 6 clones.

plate 4: Several multiple minors (28)



Bottom plates similar.

1 has few if any minors.

marked as
5T/81 colonies. 1 is beautifully linear – should be photod. *

-28 D. dust plate of pool.

now count 11T/[illegible] cols.

left overnight & photographed N27. Occasional minors but

FEB 27 1955

not fully developed.

also plates 1–3 ready for phot. (left on bench overnight).

FEB 27 1955 Examine the tubes of this expt. 34 tubes; 28 clones None showed major tails, most had minor. (very few indeed – did low temp. interval have anything to do with it. Save a few tubes, i CH2O, as 28E.

FEB 25 1955

Prep Enc. 8[50] – 11[10] – ... 12[15] fixed drops RT. 37°.
fresh materials.

	Total.	No clone [used uninoc tubes?]	Clones i T.
tubes	5	[illegible]	1 (27.7)
plates 10 cm	6	5 "	1
plates 6 cm.	39	8	24

+ Plate out 5[15] – see next page. Later 4/more clones developed in tubes (call 29E)

Singles isolated 1[15] – 2[PM] + ~~pla~~ planted right away. (to ca 2:20 PM) over

3[30] PM – Collect 114 cells – 3[45] for B2. 4[25] ditto 100 cells for B1. Plate immediately.

FEB 26 1955

10 AM

B1 (100 cells gathered) 88 (114 gathered) isol colonies (incl. 5 pairs) + 12 major trails.

B2 68 isolated, 7 prs.; 1 8 ; 12 major trails.

totals.	151	1's
	12	2's
	1	3's
	24	TRAILS
	188	Total.

T/C = 24/188 = 12.8% (1/7.8)

Remvbate 10:15 AM.

/ clone size

D: 3 groups of (100) planted on c.g. for further growth

1 #0 p 76 — transfer to 30° inc. as clones are only ca 2-5000.

FEB 27 1955 29E - 5 clones 1 c major trail = E 1

all show v. prominent minor trails Why delayed, unless

unnoted.

(2's = ●●)

FEB 26 1955

C. SW967 plated as control on recurrence of minor tails. 5P25.
A 26: (2 plates) < 1 too dense (< 2's)
1 ca 400 cols. No MT, too dense to 2's

plating.

A 8: 31 clones. 7 (six) had major tails at 10⁴⁵ AM. (A3-9)
Only 1 tail per each of these clones.

Remaining 24 clones: 15 had all singles. 6 had 2's (3_1, 2_2, $)_3$ resp.) = A11-16

A-10. 1 had a ~~blotchy~~ pattern merging interface — definite cluster

call T.

A17 1 had a debonis surface pattern probably splatter

A18 1 had what looks already like a minor tail 

Remi 11 Bay.

Resume. 8 tails / 31 clones / 39 plates. All tails unique.

1229

FEB 27 1955

~~Makes case left on~~

1229C1 : This control also shows numerous "minor trails" — assume that SW967 produces spontaneous trails? Many are unmistakably distinct clones. These are therefore unrelated to transduction. Need to do more test platings with other Fla⁻ strains. (I had been suspicious of the very high incidence of clones with minor trails). Results on major trails are presumably still valid. Further comment on minor trails in the Transd. clones may therefore be superfluous.

1229A: (small plates) Reincubated & examined 12ˢᵗ P27.
A3-9 had major trails. Look at H 1-10, 17, 18.
No comment unless something new.

A3

4 def. terminal branching appearance (spontaneous minors?)

5 column of trails.

6 tight-ish cluster, tapering

7 edge of plate, loose cluster

8 loose cluster

9 edge of plate, branching vertical column

10 loose cluster & large vertical column.

Photograph
4, 5, 7, 8

see D

D MAR 6 1955 Plated, PH, each of 6

single colony isolations of SW967

from C. albicans (including stock SW967 earlier)

now show minor trails, though not

prominently (variations in fluidity of agar?))

Spontaneous trails from SW967 1230

DATE: MAR 3 1955 REF:

3/1 A. Repeat 1229C: SW967 plate/dilene: same result: numerous spont. minor trails.

3/1 B. Plate out

	Colonies	A3 Minor Trs. (40hrs)
SW967	~	+
666	~	—
1091	~	—
1092	~	—
11410	~	—
541	~	—
546	~	Swarmed (both plates).

under similar conditions (pour 50-100 per MBA, small plate).

P2 C. Concentrate SW967. look for motiles. Ca 1-2 formed per conc drop start clones from these. Reached ca 8-32 cells/each by 8 PM. Resistate motile where seen. mostly unicellulate; 1 di-cell. See protocols.

D. Reexamine same traps (left. a.f. overnight) N3: no + seen at this time.

see 1229D.

E C; did not test very long. Picks 3 of the ultimate clones = E1-3 (C5-D5-D4) and also pool others as E0 for comparison of incidence of spontaneous mt after selection.

~~X~~ SE 967

1231

DATE: MAR 3 1955 REF:

1 2 3 4 5 6 7 8 9 10

1228 Stock. Isolate ca 30 mins P2 — Phys. pattern.

A. ~~30~~ 47 clones (ca 25) plated in tubes + plates

22 were motile. Of 11 plates + 14 tubes viable,

P4 4 + 4 = 8 major trails seen, all singly.

But minor trails interfere + should/ start new-system (see 1232)

B. Restart same but abandon. Plate about 45 motile initials.

A5 (agar may have been fairly soft: profuse clusters were not at

Rec. ca 430 to- 10 AM (say 18 hours) Photograph/ hd

to show extent of motiles. * Not single clone

→ × SW666 → × 541.
→ × 1040

MAR 5 1955

A. TM2 × SW541
B. → SW1140 (paralyzed)
C. FA37 × SW666.

2 hours inc. 1 hour ph. Inc 10X. (to ca 2PM.)
1:1

Isolate ⊕ from C (2³⁰ - 3³⁰). Pick to Vinl penassay by 4/03
Incubate 3/30 to c. 6³⁰ - 6⁴⁵ PM.

A showed four ⊕ in traps and B, none
Plate conc. susp. of these on m6 A.

MAR 6 1955
Plates: A shows moderate T and S (ca 5 or 10 T:1 S)
B " none.

C + (2 plates, "101" ⊕ cells plated in each.

3/6: 1. Too clouded by swarms for ~~accurate~~ precise count. Not poss. to estimate swarms. Definite trails: 14
"Singles" and similar 67.
81.
These include about 13 clusters of a few colonies (● to ●●).

2. 1 (?) swarm occupies ca 1/5 of plate area.

Trails
"Singles" (Includes 2's 1, 3's 3, 4 6, 5 1) 13, 65

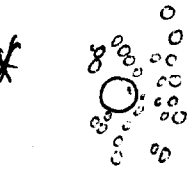
Overall T/C = 27/159 = 17% ≐ 1/6

78.

(over)

A third group of 100 ⊕ was diluted in 1ml and samples plated in shake tubes.

1) .2 ml	swarm throughout. Descem 7 single + 2 (+1?) trails*
2) .2 ml	No sw. 11 "single" 3 trails.
3) .2 ml	Swarm top half. 3 trails 14 singles
4) .1 ml	Sw. ~~tail~~ most of top. 6 s. 1 T. 1 large cluster
5) .1 ml	No sw. 6 s. 1 T. 1 med. cluster
6) 2 ml (residual).	1 sw. (bottom 1/2) 15 singles 2 T (semi linear)

1) 

not many linear

2). 1 more or less linear "branching"?

of est. 100 cells thought ~~pic~~ picked, there

20 inviable
5 swarms
13 tails
62± singles (incl. small clusters)

Totals (Est.)

sw.	TR.	Singles	
2	3	7	
0	3	11	
1	3	14	
1	1	6	+1?
0	1	6	1?
1	2	15	
5	13	59	2? / 79.

Some tubes w/ dark formation (11R6)

for sample of 300 plated!

cf. (13 : ~~72~~) 78. better.

1232

DATE: MAR 6 1955 REF:

Read individual clones (all in small plates).
16 plates negative ; 25 ± clones.

		count (2's = 2)
1.	1 trail plus several small clusters	~~65~~
2	1 trail only, terminal branch?	25
3	0 trail several 2's	73
4	several 2's, 15'. (would have been trail if aggreg.?)	8
5	1 trail (non-linear) 24's 13' ..	109
6	1 trail (non-linear) 13' several 2's ..	44

and remaining 19 have only 1's & occasional 2's.

∴ (4 trails)/25. Expectation = 4. } all singly but often accompanied by clusters
No swarms " ≅ 1. } ? oligoclonates ?

Although not very productive this expt. design is worth continuing.
Leave plates at RT for counting tmw.

Remember altus.

Counts on these ones (OCG). 81, 23, 21, 66, 87, 142, 44, 51, 69, 7, 143, 149, 140, 101, 32, 106, 10, 30, 17.

(Note variability - z/two / midnight selection) No addl trails seen.
Yes #7 however (count 66) one colony appeared like a solar system
hollow ball with studs of smaller pigmented colonies.

1232 X

opt sat:  Try to isolate to verify as Salmonella. prob not.

Having left at RT 24 hours, photograph some of above (2, 5, 6)
(2 shows trail; 5, 6 accessory clusters)

13

MAR 16 1955

Further tests on ~~X~~ SC 1140.

~~X~~ FA 22
766
37
84
85
—

no military oversight!

Would need FA 1140 to complete test, hold off now.

send
send

→ SW666 plate clones etc. 1233

DATE: MAR 7 1955 REF:

1 2 3 4 5 6 7 8 9 10

Same strain as 1232 (ref.) ① now very prolific (assume negligible continued growth in incubator resp., at 4°C.)

A. Harvest 400 ① to 2ml ca. 3 PM. Ref. to 502 PM. Plate .1ml samples. MA, MGA.

B. Single ~~all~~ transferred to ca 1:00 PM. Inc 37° to 4:30. Plate out. ~~to 5 PM.~~ of MA, MGA.

MAR 8 1955 B.

Group 1. MA (no gelatin) 2 plates negative 8 positive.

		Total Counts
1.	1. cluster c. 7 colonies all colonies tend to diffuse out.	20
	2 1 ? cluster at wall of plate	128
	3. & all singles	103
	4. " . 1 close cluster 4-5 cols.	125
	5. 3 2's	53
	6. 8 2's, 2 3's 17	125
	7. all singles sw 18 also.	170
10 negative plates.	8 (min MA MGA) all singles.	(141)
	9. 4 2's	118
	10 3 2's	335
	11 1 2' 7 total	47
	12 2 swarms. Salm.? No	
	13 2 2's	168.
	14 1 swarm, small trail 188 colonies total Salm? NO swarm	188
	15 Covered by swarm also c. 100 colonies, no trails seen. some lytic areas. Salmonella?	

~~other tubes as 2's, 3's, ...~~

A ~~totals~~ 100 mos,

4500/24 trails + clusters | 98 + 20%?

DATE: REF: 1233. 2

16 2 2's 200
17 2 2's 118
18 (MA): all singles
+ 16 addnl. plates c 2's or trails: Counts 208, 28, 95, 66, 75, 98, 207, 64, 198, 111, 214, 134, 32, 91, 89, 249.

34 plates c clones. 13 s.

also ~~10~~ tubes ~~5[illegible]~~ 5MGA.
(1 swarm + ca 100 colonies) — (n₁).
1 cluster of 10, 4, 2, ca 100 colonies.
3: singles.

— Excl. why so few trails? MA not v. satisfactory
(clones too large?) Compare A!

A (ca 20/plate scale.) = .1ml.)
1 MGA: (.05ml) 6 trails, 2 (minor?) trails; 22 singles / 30 nosw.
2 (.1ml) at least 2 swarms (obscure most of plate)
! also 1 trail or swarm origins (flare?)
3 3 swarms 1 surface trail 28 singles 2 3's 1.2!
MA ~~4~~ 15 single or 2-3'. 7 trail or cluster
4 (.2ml) 1 swarm 7 clusters 12 trails 31 singles)

5 1 swarm 5 trails 14 singles.
manifestation doubtless better than in MGA but nonmotile colonies also too fuzzy.

DATE: MAR 8 1955 REF:

new pygers. mixed 10^{40} – 12^{05} then centr. to 12^{35}. (stalk up.)

A left on slide RT to 2^{05} pm. Traps fused.
harvest + transfer 50 ① by 3^{30}.
(ca 3 hr). Plate 5^{00} – 6^{10} 48 transfers plated

B. New traps. Harvest 200/2ml. Plate .2 and .1 ml samples + plate on MGA, MG4A.

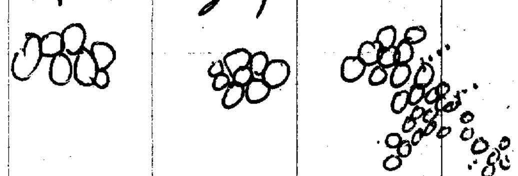
A. rather low recovery: 33 are negative (Remnant!)
Of 15 positive clones: 9 had only singles.
clone sizes v. small: 14, 11, 4, 7, 4, 24, 1, 18, 6

MAR 9 1955

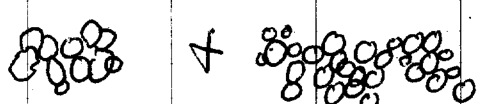
Try short deep. effect on motility

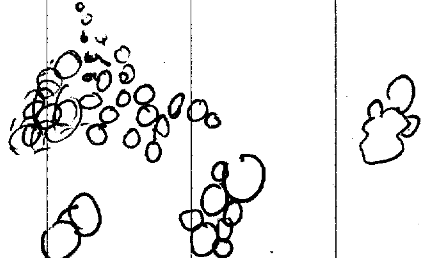
Remaining 6:

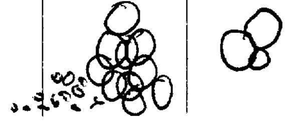
1. Cluster trail at edge, ca 40 colonies grading in size, adj. to cluster of about 5 large, + 5 singles + cluster of 7



Total: 8

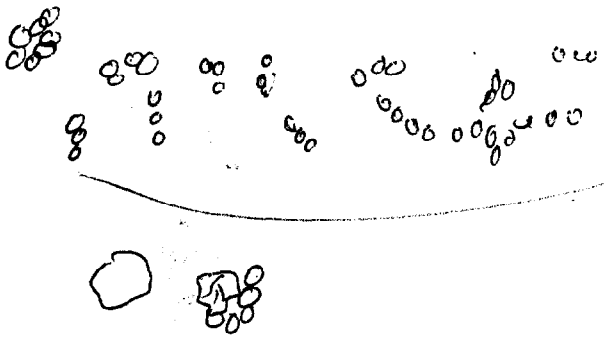
2.  + 8 singles + 8 8 8 (14)

3.  + 4 singles (9) (8).

4.  6 singles

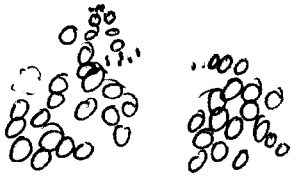
(over)

5 more diffuse trail reaching ground.



↑ + 6 singles +

---

6.  and 6 clusters of 3 – c. 8 colonies each.

photographs! 6, 2, 3.

MAR 10 1955

On incubation, 2 addl. positive clones appeared.
Count: 16 singles
4 "
Remaining plates show no change except surface overgrowths. No more trails or marked trail extensions.

3/9/55.

G4 = gel 4% rather than 8%

		ml	T	clusters	singles cols		sel.
1	} MGA	2		1	16	17	0
2	overlay MGA	2	2		13	15	1 ?
3	contaminated	2	2		12	14	
4	} MG4A	2	3	1	18	21/18	
5		2	1		7	8	
6		1	0		4	4	1?
7		1		1	6	7	
8		1	3		9	12	Beaut.-T.
9				3	6	9	
10							
11		1	0	0	8	8	
12	} MGA.	1	18	1	2	13	
13		1	2	5	5	12	
14		1	7	1	1	9	1+
						149	

Trails not greatly different MG4, MG8A.

13 sec! (18) trails 2 colonies Save for photog.
How such an odd one? Abnormal distr. of cells, or cooler agar?

14 ditto. Could they have been exch. betw. MGA, MG4A?
May have to repeat expt. of. when chilled

Conclusions: (1) Effect of gelatin concentration is indecisive
(2) 6 clones/15/48 had trails. But destinction between major and minor trails may not be so clearcut as most of these clones did have several aggregations. Note: clones small.
(3). Why low survival yield, but apparently selection

3) —X666 1236

9
MAR 1955

New preps.

A. Harvest 200 ⊕/sml + plate MGA. Inc. from c. 11AM to 5PM, then to RT. room, 4°C.

B. 57 single cells planted. c 11AM to be followed microscopically. Examine at 3PM — variable size; mostly quiescent. Cells have grown mostly by enlargement. At 6PM, clone sizes again v. variable (some only 1 or 2, others ~1000). 3 clones picked as most numerous motiles. These were plated c. 6:30 PM. Unfortunately the MGA was floccy + plates could not be accurately interpreted. 1 may have had + well-marked trail. Expt. needs to be repeated.

Limited incubation allows swarms to be limited and counted.

A.

Harvest:	T	SW	Clusters:	1-Cells.	Total
100	2	1	8, 4, 3, 2⁶	65	~~77~~
10				4	4
20		2	2'	15	18
10		2		1	3
20	2		3'	15	~~18~~
20	1	2	2 3 3'	33	40
~~*~~ 10			2, 4', 5'	9	12
190	5	7	18	142	~~184~~ 172

but again rather poor lumpy and hard to score. Why so few trails here?

Conclusions: plating of selected clones may be promising method but needs to be repeated.

MAR 11 1955

MC8A (pipe ~~...~~ del. dc) B

~~+ Tween. 0.1%~~ ~~Swarms~~

Plated from moculum cole. at 100/ml. Inc. ca 11-12 hours then R.T.

− Tween.

Est input	SW	T	C(2,3..)	1's	Total
40	2	3	2^5 3^1	35	46
20	2*	2	2^5 3^2	10	~~..~~ 21
25	3 **	0	2^2 3^1	14	20
85	7	5	2^{12} 3^4	59	8 ~~6~~ 7 ~~..~~

* sw had 1' in center

+ Tween

Est input	SW	T	C(2,3..)	1's	Total
10	~~2~~ 3	0	2^4	2	9
10	0	1	2^1	3	5
20	1*	3	2^1	17	22
40	1*	2	$2^2 3^1$	26	32
20	3*†	0	2^3	12	18
100	8	6	11 1	60	86

† ~~Trail~~ sw had 2' in center

‡ sw, at center had O8: and 8 nearby center.

Tween had no certain effect. how about survival?
but could be system. error of sampling?

86	~~..~~ 100
87	85

Swarms were all about 1-2cm diam, somewhat variable even on 1 plate.

Note very low incidence of trails. Too early selection of ⊕?

Should recover Fla subs from A swarms: plate out residues of the drops for full test.

PA37 → SW666
selected clones
Effect of tween 80.

Misc. observations

1237

MAR 10 1955
MAR 10 1955

[232]

Prepn. stock 3/9/55. (Quite fresh < 7 hours before incub.)

A. Freeze taps 11[0]. Isolate to [232]. 50 by 11[47] AM.

(A–D) 2/row (a, b); 1–6. = 48 isolates. Incubate at 12[40].

B. Isolate 100 (+) to 12[40]. To 2 ml pen assay. Plate samples (≐ 40, 20, 10 cells) in MGA c/s Tween 80 .01%. Incubate frozen 1[05] PM — 11 PM.

Examine ca 4[30] PM to select clones for plating. Isolate: (all have c. 10^{2+} cells and:)

1 A3a	sw	Inc to 11 PM then R.T.	sw. + 1's A hot. 10[30] AM.	
2 B6a	sw		sw. + 1's	
11 B3a	(20)		Shows 3 clusters (c. 2, 3, 4)	
4 B2b	(10)		sw. f 1's	
5 C1a	(15)	Inc overnight	1's	
6 C2a	(15)		1's	
7 C4b	(10)		1's, 2	
8 C3b	(20)		1's	
9 D2a	(10)		T	
10 D3b	(10)		T	
3 A5b				

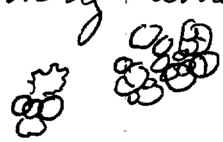
as well as 10 more quiescent clones as controls.

(107/366; 11/200; 7/19; SW/COL)

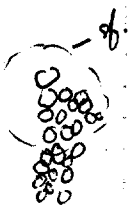
MAR 11 1955

A. Controls all had singles only. A3a, B2b, B6a all had swarms and colonies. A3a shows flares best: Isolate is selection for later plating. The other two do so less strikingly. Arrangement should isolate the Fla⁻ sibs.

Among remaining plates, 5, 6, 8 show singles only. 7 has 1 2'

9: 

TRAILS IN 9, 10

10 

Selected groups 2T/7
Unselected 0/10
hardly a dramatic result though warranting more extensive study.

Reexamine [232]

1) A3a - act. swarm ī residual Fla probable
4) B2b ✓
2) B6a act. sw.

3) A5b also has numerous s motiles (c. 20% or more) ✓ → swarms.

other clones are not remarkable
(most have no evident ⊕ on casual examination
some a few etc.)

Try, B5a,	has ca.	$10^4/10^4$	
B6b		$10^4/10^3$	No trails. MAR 12 1955
~~B~~			
C7a		$/10^5$	

MAR 12 1955 single colonies from residual drops of clones A3a ~~B~~2b B6a and A5b are being tested for motility and saved ī selection. ~~also~~ also stab originals as 1237-A.

4/1: 1237 - 1A +, 1B +, 2 + } all b not i

MAR 12 1955

Isolates F4+ and − from 1-4.

Streaked on NSA, test on glc colonies on MGA ca 4-5 hours.

1 {1+A: 16−
  1+B: 17−} Compose original plating counts

3 3+: 29−

4 0+: 25−

2 7+: 27−

Later a second +

can be used for flare production
in unselected mostly lines
diff may depend on selective residue.

~~Picked 24.~~ pool +,− to stab.

Isolate 4+ by selection. (original mixed clones all also preserved for possible later need).

MAR 13 1955

2/11/47 1+A is still mixed.

MAR 1 2 1955

Bearings:

Since Jan 1, I have been primarily occupied with Salmonella trails. An important question has been the uniqueness of the"E particle". This would be hard to establish by quantitative data on the clones directly, and I have been principally concerned with looking at platings of small clones in motility ggar.
The results with SW-967 are not fully reliable owing to spontaneous "minor trails". This work has been done only since Febr. 23. Before that, from Jan. 11, I was mostly fiddling around. I must have been preoccupied with otherkinds of experiments too, or writing or what not, since relatively few experiments are recorded. There are also some experiments on crosses of heterogenotes, but DCG did most of the routine on these. From Jan-Febr., there were a number of misc, experiments on conditions of plating etc., which amounted to very little. There were some indications of major and minor trails. Also developed technique of trapping from conc. cell susp.

Summary of clone platings. (trails per clones/ per platings) and comments

			mass pla.
1227:	1/20/25	T: unique	
1228	3/9/10	T: majors unique, noticed addl minors	13/84/ 100?
1229	8/31/39	All T unique	24/198/200
		1229C: apont minors	
Total —x 967	12/60/74	All major trails unique. some dist. non-linear however. Confusion with spontaneous minors.	

—x SW966 Almost as prolific source of motiles

1232	4/16/25	Single majors, but other clusters. Clone size c. 2^6	27/159/202 + 1sw.
			53w/13/79/100
1233	1/34/47	Single small trails; a few other clusters. c. 26	
1234	6/15/48	Definite concurrence of smaller trails or larger clusters; hard to define. Av.Cl.S. c. 23-4.	30/149/200 3 sw. (not random)
1236	57 clones, follow micr/osc. plate only 3 NVG.		5/172 /200 18 cl., 7 sw.
1237 (eff. Tween)		This prepn. seemsng. although very fresh. Should compare directly with 1234 prepn.	11/ 173 /185 15 sw.

48 clones. followed micros. 4 gave swarms (segr. non mot.)
10 quiescent clones gave only singles; 6 with fairly numerous motiles gave 2 T's, + 1 with clusters.

MAR 1 2 1955

The principal point is perhaps best met by experiments like 1234, plating fairly early. A correlation of trails with pluricatenates like 1237 might be worthwhile, but more laboraious.

In view of sluggish motility of early log phase cells, this should perhaps be done with earlier clones in aged medium.

In some prelim. expts. yesterday, I noticed that TM2 transferred to aged medium supernate was more actively motile, particularly showing a more jerky motion with shorter free path. Examination of TM2 in motility agar suggests that many cells are directly immobilized, others move in apparent interstices, but still more slowly than normal. There must be a considerable accidental factor, and cell with numerous mobile progeny naturally has best chance to propagate a trail. Since genetically competent TM2 are immobilized, there can hardly be immediate correlation of genotype (or pluricatenxtation) and ability to move. Should watch trails in situ if possible. Why not?

Plans: continue with experiments like 1234. Compare this prepn. with 1237 in yield of trails. Continue with medium effects. Set up trails in situ, selecting cells which remain motile in gel. to see if these form the most chains.

→ x1140 n.s. why?

Do not forget many other carryovers:

EM
Gal, Nelson
heterogenote crosses
Hfr x F- !!!

G&C
DA Gal Salmonella.

DATE: REF:

a) 3/14/55 Collected ca (100) from 1234, 1236 preps for comparison +
plated in MGA. (App. left out at RT so result is
misleading. Later by incubation showed 1234: 6 swarms/87/(100)
1236: 35/68/97.
T not detected.

3/15/55

b) New prep. 1238 (22 hours)
Freeze 1^40 Dool. (late) 3^20 – 3^50 A) 200/2ml ~~B) 50 ①'s but lost by smear.~~

Running out of gelatin!

Plate out A samples. (Noted remarkable incidence of trails, might be related to quality of medium. agar was granular, loose from reheatings!)

	Est. no.	C	T	Sw.	Cols.	Σ
SIC (B1)	100	2-3	82	5	4	95
	20	1	4	1	6	12
	20	11	4	1	3	18
	10	2	4		3	9
	10	3	3	1	4	11
α.	10					
	15	1	3	1	5	10
	185	21	100	9	25	155

See photographs. Note variability.

Many swarms were centered ē C or T

Suggests variation of 1238C

Trails in (B1) particularly florescent.

1238
C-D
E

DATE: March 16, 1955 (Wed.) REF:

New suspn. (ca 9-11) Fuse drops 12^30^

to 3^30^ coll. (500) in 2.5 ml Plate, .25 ml samples

a). in MGA variously diluted. (Plating yesterday had shown remarkable incidence of large trails) Incubate to 4PM. Then R.T., refrig. (Plates 0 c .25 ml). Figures indicate amount of NSB in this (-.25 ml). ~~½-1ml for samples.)~~

	NSB	Clusters	Cols.	T.	Sw.	Σ	
Est input 50 cells	0	2	53	2 (v. sh.)	2	59	
	1	2	55	1 "	0	58	
	2		59	2	3	64	Sw less compact
	5	5	46	6	0	57	
	7.5	18	39	11	3	71	sw diffuse
	10	11	19	19	3	52	" " ; TRAILS florescent!
Input (50)	0	1	93	1	8	107	
(100)	0		66	2	3		

Basic agar must have been unusually stiff. Swarms in NSB₀ v. compact also. Thus incidence and quality of trails increases with decreasing agar concentration!

---

also D) Fuse 12^30^ collect to 2^30^ D. P. drops to 3PM Incubate to ?5^30^PM. (Some refrig. to 12 15 PM Thurs for plating) E)

Isolated 12 15 Thurs.

C: 13 blank 4 contam) novis cols. 1 swarm + 18 clones } all except C1 singles only

C1 has 1 major (v. prof.) trail, 6 moderate 4 or 5 clusters + 20 clusters

D 8 blank, 5 clones. No [illegible] owing to contaminant also.

1238C1 – nepheto.

T_1 c. 400 ohms

$T_{...}$ << 50

DATE: Mar 17, 1955. Thurs REF:

1	2	3	4	5	6	7	8	9	10

Met Kornberg visit later in PM. ca (1000)/ml cool. but no used since 1240 prepn. was fresher next day.

1238 prepn. Freeze trap 12N. Spotted out 1:40 Clones picked and incubated c. 2³⁰ – 6PM (3½ hours). Plate in MGA ± diluent USB. (c. 10ml : 15 MGA). Also noted that these dessimulated cells showed internal structure (nuclear?)

MGA standard still .4% Agar 8% Gelatin

A). MGA straight. 1 contam, 12 blanks, 16 clones added. 2 swarmed < No def. colonies; 2 large plaques / " 0 " (vague sev. initials; 1 plaque

all singles. no trails

B) MGA 60%. 1 swarm – 7 plaques 4 single cols. 8 blanks, 8 clones add.

1. all singles
2 " "
3
4 " "
5 " "
→ (6) at least 11 trails, 1 major. * see photog. > 150 cols! / T_2 c. 30
7 all singles

Total 4[illegible].

3/22/55

Counts on motility plates:

1239A:

11	103
61	140
144	67
119	41
17	181
37	115
54	92
196	51

1239B:

97
225
60
134
100
102

1238D:

22	39
36	25
40	6
15	44
52	6
6	61
13	13
9	18
44	39

Plates marked "C"

9
9
2
31

DATE: March 19, 1957 Fri REF:

1 2 3 4 5 6 7 8 9 10

Pool (1000)/ml. and plate .1ml, .2ml samples. Inc c. 2–11 PM then RT

1. MGA (100). 4 swarms, 56 singles (remnants of yesterday's MGA equally diff Ref.
12 2's
8 3's
5 short tails c 6 clusters

2 MGA (200) (somewhat looser – cf. swarms) 6 swarms 23 Trails ext.
33 singles 4 2's 7 3's 6 clusters

? 3. MGA (200) [firm!) 4 compact swarms 2 or 3 short tails, rest singles
dilute 40%

4 MGA Wilson trails nebulous, swarms limited 47 tails 3 sw (1cT)
26 singles 6 clusters
5 3's

5 MA (4.5%) 3 sw: 1T, 1 center ;

Pc 6 mils

incubate 3:15 6 6 PC

Out of gelatin! Use MA (.45% agar) and Wilson gelatin (n.g) (a) (b)

two groups: (a). 9 plates (2 large) are blank ; 19 plates (6 large) have clones

a) (5) ① covered by a swarm ; 3 zones of lysis. In addition ca. 18
all tails of some extent, some considerable. May be hard to photograph.

19 clones

photo ② 7 + c^10, c^9, c^4 + pyramidal cluster, ± 50.

3. 7 + c^2, c^6, c^4;

4. 12 + c^6, c^6, c^4; c^9, c^12, c^7, (a T ca 100), c^9, c^6

⑤ 32 + (T^+), + c^7, c^8, c^6, c^3, c^11, c^2, c^4, c^6, c^7

also (14) 51 (1c?), 22 (1c), 9 + 2c [⚘ ⚘], 63, 94, 18 + ⚘ ;
15 ⚘ ; 23 ; 16 + ⚘ ; 33 ; 13 (incl 2'), 60 ; 11 ; 14 ;

(cont)

b) mill, wash gelatin again. (a sloppy - gelatin must bind again!). 9 blanks 10 clones.

	1.	5 short tails	+ 9 cols
*	2.	13 prol. tails	+ 4 cols.
	3.	4 short "	7 cols.
	(4)	12 prol.	7 cols
	5	2 mod. "	23 cols
	6	14 tails	11 cols
	7	1 good t.	+ 8 cols.
	8	12 " t.	18 cols
	9	1 linear t	15 cols
	(10.)		
	~~11~~.	7 tails	. 0 cols!

these tails off. linear

Conclusions 1938-40.

MAR 21 1955

among motile initials plated

1. Incidence of trails/varies with the fluidity of agar. Addition of 40% diluent gives very high incidence. In any event, agar that is hardening tends to be quite inhomogeneous, if maintained at critical temperature.

2. Single clones can give at least one trail + large clusters in harder agar, and numerous trails in softer. This is clairly an unreliable criterion for singularity of catenation of higher order.

Further plans:

1. A few more tests of fluidity and related variables for photographic documentation.

2. Shift studies to direct pedigrees; need some further data on irritants; inh. of cell size growth and chemotactics.

3. EM transfers.

4. Write it up!

Butterfield Doubtless spent this week cleaning up around lab.

APR 3 1955

What happened last week? N.G.

① Acetyl gelatin

② No guiding what to do next on chains!

③ Change medium < D(o) - flattens out too far (wets glass - how counteract?)
necessary 10% - poor growth.

Acet-serum in H_2O seemed limited. Metal poisoning? - Try pure water.

problem: don't want to follow mid. pedigree more than 3-4 generations but minimum size drops down too many cells ~~at~~ ultimately. Should have 1. inal clones of about 300 cells. Try partly exhausted medium.

④ Serum effect

i / TML of course diff. owing to H_2 1, 2

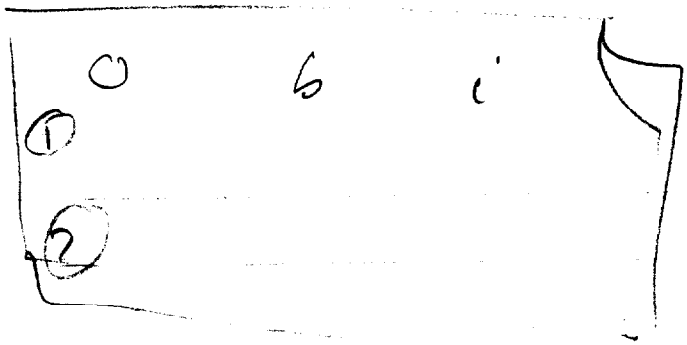
b < 1237-2(H_1 b). at first almost completely inhibited, but some probably inhibits by anti i at 1:100. with overnight growth, see active motility and agglutinated clumps - May still be worth trying at 1:100. (serum titer ca 10⁴).

APR 1 1955

Semm effects

① 1237-2 = b ② TM2 = i

1. Try against sums 1/100 in both.



0 cells almost completely ruled by b sems, partly by i

i cells partly ruled by i or b (1,2 comps?)

Plumb
Thames
Co. Eff

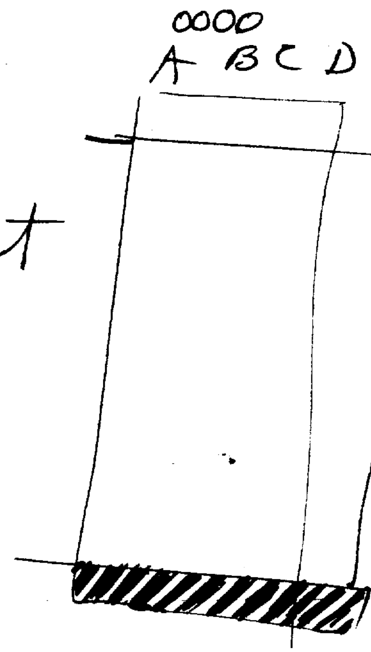
37-X866

1241 (E)
MAR 31 1955

New preps. 1/2 : 1/2 10:11 — Inc. 11:45 — Centr. 12:10 Decant. Refr.

Collect in 10% broth; D/0). (E, F)

Ecsetrops 2:45 (31) by 2:55 spot at A↓, B.
E) 22 deposited to 3:05



F (35) to 3:15 Spot at D, C, C'
to 3:25
Stet R.T.

5:30 ; 7:30 - 8:30 E: only 1-4 cells saw. mostly (f)

F 1-4 (often (2)) far more active!
spreads [illegible]

Viscosity?

9:30 A1 Poisoning to [illegible] very little growth.

→ Sc866

1241

Misc.

3/28 NG

3/29. 3) 10% broth dilution. A) Penassay 40 ①'s, same split, 1-4. No staining many chained, not closely examined (too many cells, >10⁴/.) cut 3 swarm-clones A < ⊕ 1 ⊕ 2 C / ⊕.

save for later check of identity + homogeneity

B < ⊕ 1 ⊕ 2

3/30 cf. 10%, 100% Penassay: middle tubes

F 12/58

DATE: APR 5 1955 REF:

New prepns. a): from aerated SW-666, b) from unaerated culture.
Both + 1:1 FA-37 10:20- 11:35-12:10 Refr. (Incub. in rot.)

Note: Rotator now standard for aeration.

A): Prepn. a). Fuse traps 12:30, collect ca. 100 motiles, but use to spot (2-2:15)
DCG pick to 3:30. Incub. in .5 ml Penassay to c. 6:15 PM. Plate in MGA +
40% NSA as diluent.

B): Same collection, plant in spent broth (SW666 Aer.) to cf. total clone size. St. RT.

C): Prepn. b) Fuse 4 PM. Collect 5:30-6:00 (some needle tr.) This prepn at least as good as a, probably better.

1000 (+) / 1 ml. Plate 0.1 ml samples in large Plates; .05 in small. Compare MGA, + 40% NSA dil., + 60% NSA dil. Incub. 37° c. 6:45 - 11PM/ Then RT to P6; then refr. for analysis. (dil. MGA very soupy!)

APR 6 1955
Hold A,C for study.

(Spent broth = overnight SW666A, 60° 20 mins., the sediment and decant. Numerous fine granules still present).

B

11:15 -12:15 AM Examine B). Note that clones are limited to 100-1000, while Penassay gives at least 10x as many. 18 clones (in spent broth) examined: (sequence not retained).

3 - 0's 2: about 100 Fla⁻. 5 had 1+/c. 100, of which 4 isolated to broth for plating clones. 3 (4?) had 2+ (+ Cl, 2,3), each isol.

C14 had 29+/1000 C13 23/1000 C6 12/-00. (These picked collected and plated without further growth.

C5 had 10+/-00. Plant individually, pick to broth for clones. (C5-1-2 maybe 2/0 instead of 1/1).

(Inc. 12:30 - 3:00 PM. Plate in MGA-40).

APR 6 1955

DATE: REF:

Note: OCG picked in sequence, but this was randomized for plating. Seems cold

#'s are empty:

(18) 1, 2, 5, 6, 8, 11, 12, 14, 23, 24, 25, 28, 31, 35, 41, 44, 45, 46.

note: 6n+1, 6n+3 ($n = 0-7$) were MGA all others MGA + 40% WSB.

(1, 7, 13, 19, 25, 31, 37, 43
3, 9, 15, 21, 27, 33, 39, 45)

swarms (or cart): 9 (plague c. 50 1's); 13 (c. 4 1's opp. ca 4 ⊕'s)

26: + c. 10 colonies, mil. 5-6 trails

29 + c. 20 trails, few 1's.

(7) 33: patchy lysis, swarm + 3 3's, 11'.

36: Prob cart.; No colonies — cart.

39 sw + c. 100 singles + short chains.

MGA:

3 ca 60 no T 2 2's.

7 6 1's

15 c. 60 1's

19 c. 45 1's,

21 6 1's 8 c's (3-6) 1 T35 1 T10 (closely).

27 Imagi trail + 9 c's (3-7) + 6 1-2's.

37 1 T ;8 1's 3 2-3's.

43 111's 2 2's

MGA 60% 40% WSB

4. ca 20-30 profuse trails ~~sw~~ 50 1's. somewhat detached

10 >100 all 1's

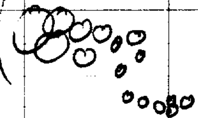
(11) save for plot. { 24, 10, 24, 23, 14, 6, 15, 10, 19, 24, 30, 47, 13, 11, 100, 150, 6, [illegible], 23, [illegible], 14, 15, 16 and others. (colonies per trail.) 37-1's 5 c (<10) 21 T's.

18 9 T's; 38 1's

18 seems somewhat tighter agg, 12 T; 3 clusters (3-7); 38 1's

1242A

DATE: REF:

20 1T () ; 60± 1's

22 (partly slopped) Profuse trails about 1:3 1's

(30) Profuse trails , 16T, 5C (3-5), 551's {T's include c.80,80}

32 " " 7T, 1C, 761's.

34 45 1's

38 17T's 3C's 45 1's

40 > 500/plate ; some prob. trails but too crowded to count.

42 14T's 33 1's (rough counts).

see in photos 11, 18, 30.

Test "swarmers" in B toc in order
9, 13, 26, 29, 33, (31) X, 39 others ✓

APR 9 1955

{ Note BADS letter – #11 illustrates clone c profuse trails. But this was incubated 15 hours. of "mcp trails" }

(Usually no progression over c. 8 hours but must be controlled! Need check on trail progression at R.T.)

37 → X 666

1242
+B

APR 5 1955

① New prepn A flask aerated SW666 × — FA37 } ~~[illegible]~~ Rotate 10^{20} to 11^{35}.
1:1
B non aerated " × —

(Rotator now in op'n and generally used for aeration rather than bubbling)

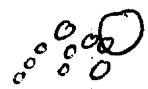
10^{20} - 11^{35} - 12^{10} refr.

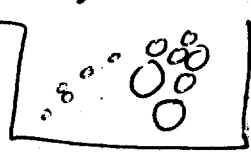
②. Freeze drops A - 12^{30}
see 1242

APR 7 1955 9^{30}AM Score series C.

Note: many plates have small swarms, doubtless contaminants. save for characterization + cf. 1243 A.

C1 2 plates A 10^2 1's; no clear T. 1 fuzzy stg of 8 mau glass. B. c. 60 1's

C2 A 60 1's 1?T (fuzzy: ) } terminal chains?
B ditto



C3' A. c 40 v.s. 1's & B the same — many cont? scr.

C3 a " "
b " "
c " " Heavy contams. } N.G.

(Q24)

C – ~30 1's no T

C – 0

C – ~40 1's. no T.

Terminal ⊕ have poor chance of starting a longish trail.
Medium OK.

~25 – 1 } 20 small 1's + cut swarm
2 } ditto
3 " "
4. " "
5 40 1's " "
6 " " " "
7 " "
8 0 " "
9 30 1's " "
10 30 1's " "

(small angles prob. also cut.

5/10 plants → clones. all of these had presumably petered out & gave no trails at this point.
cf. 6, 13, 14

1242B

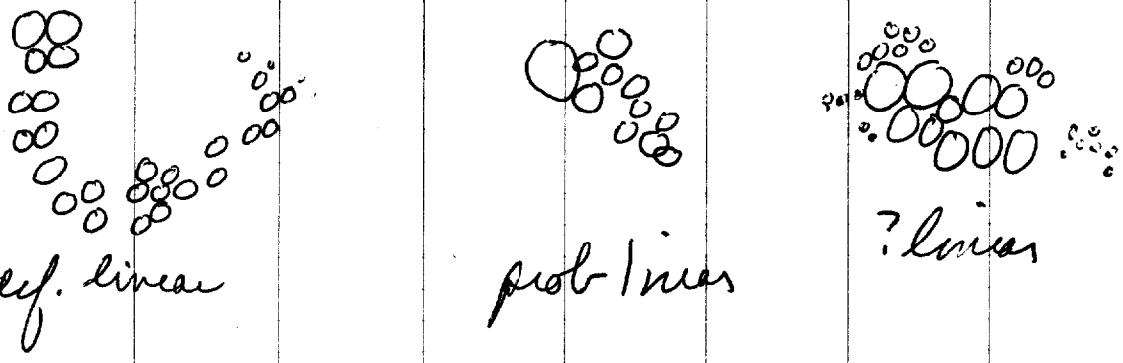
DATE: APR 7 1955 REF:

1 2 3 4 5 6 7 8 9 10

B6: 7 1's 6 clusters 3 trails

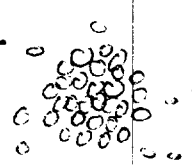
Σ 16

Input:



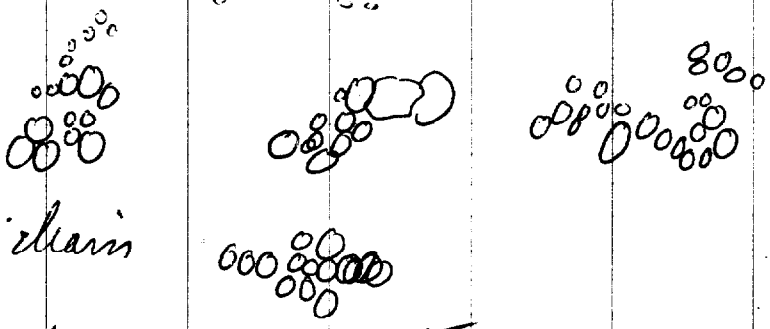
def. linear prob linear ? linear

B13 16 1's 1 globular cluster



3 linear clusters (4 B);

and



Σ 25

prob uni'dians

to total 16 1's, 8 "trails", 1 cluster

B14 See photog. Most trails linear in esput.

21 1's 12 T's 5 C.

But not C13 nebula!

DATE: APR 6 1955 REF:

3:40 – 6 PM Trap 1000 ⊕ / 1 ml. Plate 0.1 ml sampler
25 ml total volume.

				T	C	1's	SW	Σ
– 1	MGA			88	12	9	5	114
2	MGA		'0.03 ml'					
– (3)	10 ml	Penassay		129	0	8	12	149
4	"	H_2O						
– 5	"	Boiling						
– 18	"	NSB						
7	5 "	NSB	} mis-inoculated?					
8	7.5 "	"						
9	12.5 "	"						
10	15 "	"						

3 gave best development of tails. Use routinely from now on. Layers evidently too shallow for extreme gas.

Note: this batch of MGA already showed deposit of gelled agar and was probably inordinately soft to start with.

4-5-6 agreed in showing almost 100% tails! all worth photos.
St. room temp to bring out swarms more sharply.

This expt. n.v.g. for comparison of agar density owing to looseness of original MGA.

1244A

DATE: APR 8 1955 REF:

	1	2 i's	3 ℓ's	4 T's	5 sed	6 Σ	7
1				3^+		68	
				11		64	
				1		54	
			2^2	0		20	
2				1		43	
			1^{2-3}	0		78	
				0		62	
				2 linear		43	
3				0		59	
			3	0		67	
4				2 ~~…~~ v.s		12	
				4 s.		20	
5						0	
						0	
6	0					0	
				8 short		46	
7				0		49	
				0		47	
8				1 sh.		82	
				2 sh.		65	
9						0	
						0	
10				11 short		65	
						0	
11			✓	0		62	
			✓	0		0	
12				0		0	
			1^3	0		44	
13				0		0	
				0		6	
14				1		20	
				1 extens.		17	
15						0	
						0	
16				6 m.s. - linear		53	
				5 " ; 1 glob.		41	
17						0	
			1^2			24	

Inc. 5:30 - 11 PM, then RT. Scored 9 A 8. This dil. MGA may be stiff

Owing to probable stiffness of agar, expt is inconclusive.

1-2 prob. worth photographing.

Photography 1244'

APR 8

1243-3 DATE: (Lt. value 13-25) REF:

for calib f6.3 - f9 at 1/20 +X film found best (as indicated by light meter!) for large plates, on light box. Leica set-up no lens for large. c [struck through] in front. Develop D19 - 6 mins.

Use 1242 ~~#3~~ #1-6
#3 = sample.

f8 1/20 +X.

1243 f8. 1-6. 1 = #1 c 1½ secs.
2 #1 1/20 "
3
4
5
6 etc.

Same stops

EMB large plate (enlarger etc. for tiny?) 1/20 f4.5

1242C -0
-0
-40
-40
-60
-60

1238 B1 marked.

small plates ~~same~~ with lens #3, no ext tube.
1238C1
1244A1 (4), A2 (4), (marked).
1242 C13

4, 9. wavy solutions!

1244'

DATE: 4-9. APR 9 1955 REF:

Photography 35mm Leica astrogram per yesterday

Large plates ~~at~~ no fruit here.

(3)(4)(5)(6)(7)(8)(9)

1243 1-6 1243 : 3, 4, 5, 6, 1, 2 f8 1/20 sec.

7 λ-6A6

7

HF 7-6

ag came out beautifully

D19 clear 8+ minutes

EMB f3.5 2744-x last + 2769

EMB overexposed

1426A + 6.

~~1243-6 again~~

1238B1

1242C -0

-0

-40

-40

-60

-60

Small plates + 3 hrs.

1241-1 (4)

-2 (4)

1242A 11, 30, 18, 2, 1

1242 C6, C13, C14

1231B

Resume 1242 - 44.

APR 9 1955

1242

A) clones from single initials.

18 empty

7 had swarms (diffused) + trails [one certain.)

8 clones as MGA standard: some T's, usually poorly developed.

13 " " MGA 60%.

	*20T; 50 1's	100 1's
	9T 38 1	75 1's
*photographed.	* 12T 3C 38 1	>500 1's, T's.
	1T 60 1	
	>T	
	* 16T 5C 55 1's	
	#(32) 7T 1C 58 1's	
	17T 3C 45 1	
	14T 33 1.	
	*#11 21T 5C 37 1's (count complexity of trails).	

B) Plant in spent broth 17? 18 planted. Looks for F/a+ /c 10^3 F/a-

Terminals

3 0's. 3 0+ 5 1+ 3 2+ and

groups of 29, 23, 12 (C6) (C13) (C14) from 3 others. Plant these as groups or clone in MGA60. From singles, No clear trails from clones. Groups:

~~C13~~ C6, 13, 14 See photos. { 3T 6C 71 / 1T++ 8T 161 / not counted

Nothing at all!

ctd. APR 9 1955

1242C Platings in MGA, 60%, 40%. in small and large plates. ~~Swarms~~ large plates: rather messy but photographed. MGA-0 showed compact sw, no T. 40, 60 about equivalent development of trails

Small plates ~~not yet studied~~ equally messy, suggest that MGA40 is sufficiently dilute to bring out most trails; more profuse at MGA60.

1243. Is simply group initials, various media. See pictures Pen assay of stages as diluent. (1-6).

1244. (A) Like 1243 but excess nonmotiles interfered. MGA standard rather stiff; MGA 40-50 optimal

(B) Sib clones. Agar probably too stiff but photogr. sequence 1-4, 24.

New notes on Bruce - { abstract together? or MOB } c. 4/10/55 -

① my cells don't get stuck
② they stay motile - usually both n_1's are ⊕
③ don't like "replicas" of mine.

"We have never obs. E cells in > 1 sublin ~~[limited observations]~~". How many E clones have been similar

B claims one case of E at n_{22} only 1?

Need my own data on [E+E] or [E+S] in 1 clone

style; numerical calculations; first conclusions first.

Where are pedigrees?

No time now to clean up pedigrees.

37-X 666 / serum and clones.

1245

DATE: APR 12 1955 REF:

A. Misc. tests on serum, diluents.
Water in Sutter tap, this lab distilled, or enzyme institute double distilled appears to be suitable diluent (contra "spent broth"), to limit growth. Broth 2. 10 -20 cells retained motility, did not flatten unduly and growth was limited. (Unaware contamination with broth of isolation.)

B. 9, 27 ⊕ resp. were completely inhibited by 1: anti-b, 2 anti-i serum. Overnight in B1 (b) large clone of small cells, no motility except a single wriggling cell (planted out) A12 A.G. In B2, noted clone at center as above but at periphery, net and cords of long cells and filaments (some in serum?) This was also noted later in i-serum.

C 12 & 13 pupm. Free degas 12^20 Spot ① to 2^25
DCG picked 13 2^50 - 3^30 Inc. to 5^35 and plate in MGA 60%. Inc. 5^35 PM - 8^30 AM (15 hours).

35 (34 clones + 1 pure swarms) - held to 5/31/55 for photo and counting.
9 blanks. (probably picked late)

serum inhibition of trails

1246.
1246

digests fused 12 (4445 pages)

DATE: APR 13 1955 REF:

A. B. Serum effect microscopic. Britals isolated from sel 1245 (c. 2 30)
FA 11 × SW666; (A) FA 37 × SW666 (B) and put in 1:100 i, serums in broth. b serums inactivated both very gradually; i after 30–90 seconds (usually).

Then tried plating. (by c. 3 45). Harvest c. 300/ml A; 500

(but middle broke + numbers in B are doubtful.

A — .2 ml samples (est. count is 50 cells/plate). in MGA 60 + (1) 0 Plates somewhat messy but profuse T + S.
(6) b 1:100 3 inh. swarms, not all singles.
(5) b 1:1000 5 swarms (sl. inh.), 75 singles; No trails.
(4) i 1:100 Spread contam. But no trails
(3) i 1:1000 6 large 3 small swarms (b, i ?)} No t... why two kinds here ?

B .1 ml samples (do.) (1–6).

1 Swarms too messy; about 50% T.
2 (MGA) [5 swarms, 56 1's; 7 short trails]; [45; 77 1's;
3 No T or S.
4 No T or S
5 3 inh. S. No T
6 2 sw (1 spread contam?) No T.

1:1000 is adequate to inhibit trails!
i serum inhibits b trails!
abandon i × b system for this study

DATE: APR 13 1955 REF:

37 X 666

D. SW967 plate in MGA, MGA60 for ~~ne~~ mirror tails in <24 hours — 5PM. but too crowded.

ca 50 plates

E ~~do~~ inc. continuously ditto check SW666 MGA60 only — at 2 days, no mirror ta[ils] same diffusion: colonies are (•)

F 1237A+ for flares — Incubate 5PM — why. MGA only note singles, not pure?

ca 10 plate

G control SW673 "

H 1 MGA / 2 MGA 60 → excessive proportion of swarms. (? age of preparation ?). Do not use.

APR 14 1955 Platings of c. 50 initials each in MGA60 + .01 ml cea[...] all show swarms.

	tails
a	+
b	−
c	+
d	+
i	−
k	+
n	+
l2	+
B-O	±

a, c, or d would be quite good for further test.

(c. 5^30 PM)

D Replate P14 A15: excellent development of mirror tails 60% MGA; intact only in MGA but will probably show later.

12^40 DIA for photog. — Refrigerate 10 AM. 5/15. 60% MGA Reincubate sister plates

Needs to be done in my own expts.

APR 15 1955
13

	Today	
Serum effects	✓	
Swarm sibs		*
Flares	✓	
Terminals	start	*
E M.		
SW 967 / HA 10	✓ ✓	
Paired subclones for multiplicity of "L" (Use ± T ± ?)		*
Isolations in water?		
Viability pH 4		
Viscosity pH.		
Trails in 40+ ✓ (viability of subclones multi; strip smears)		

APR 15 1955

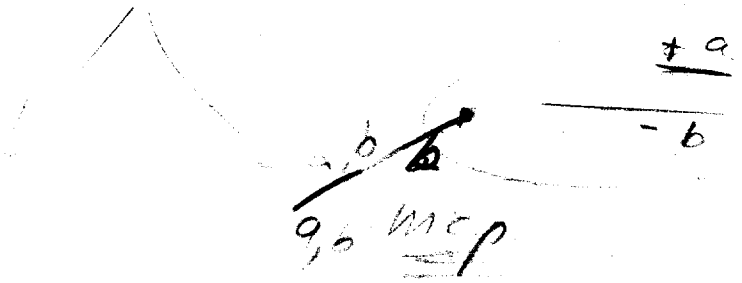
Notes: Phase var - TH2 ph2 monogh?

Mention to Bruce 6/i — of. burgmdof.

Fla^{+} $H.^{a}$

↓

Fla^{-} $H.^{b}$



Serum inhibitors a → x

1248

DATE: APR 14 1955 REF:

1 APR 15 1955	3	4	5	6	7	8	9

14: n.g.

4/15: Frese degps, FA76a (S. miami 9) → x SW666 11AM.
FA37 → x " "

Preliminaries
SW 1250, 1252

not tabulated but results indicate that a does not inhibit b trails, part with a/b trails. b inhibits all trails } at 1:10
eswella
1:16

10

20

30

40

50

Rescues # 4, 4

3 cells isolated

1131 ? ~~8 cells picked~~. ~~not thaw expt~~.

~~1) progeny to n_3~~

Flat: H_1^i ($8^+ : 12^-$)

1) progeny to n_3. 1/8 gave motile on transfer, found to be mixed +/- ~~H_1^i~~ H_1^b. ∴ segregation at n_{43} !

2) n_{13} : all −

3) n_{13} : all −

1132 2 cells. followed quitty to about n_3:

1) showed Ichem to n_3 ; n_{13}^-

2) 2 sibs both motile, catmated to n_3 ~~n_8~~ n_3, n_8 both. n_{13}^-.

11~3

1134 (C3): → 3/22., each then catmated

4/5,

1131) 1) 1 prolapse to n_3. 1/8 ~~full~~ gave swarm, mixed $F1a^+H_1^i$ $F1a^-H_1^b$. Segr & hy.

2) 2 cells → n_{13}^-.

n_0 = (Initial chem

1132) 1) 1 chain ~~$n_1 > n_3 < n_{13}$~~ $n_0 - n_3 < n_{13}$

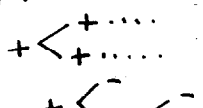
2) 2 chains $(n_0 > n_1 - n_8 < n_{21})(n_0 > n_1 - n_8 < n_{21})$

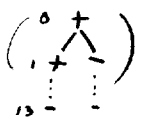
1133) 1) Swarm : pure (1/4 inviable; 3/4 → H_1^i) but late coal.

1) $n_0 > n_1 - n_1 > n_{13}$. (2 interm.)

1) $n_0 - n_1 > n_1$.

1) $n_0 - n_1 > n_{13}$.





initial only ↑

1134) E3) $n_4 > n_5 \begin{matrix} \nearrow n_{15} \\ \searrow n_{15} \end{matrix} n_{19} > n_{19}$. (3 interm.)

D4) $n_1 > n_4$. 1 interm — n_4 term.

E5) (sw) (T+S) from 1 cell. At n_5 —— n_{17}. chain (F1) F2 = nm. E11 = swarm in nurse

C6)$_1$ ~~n_0~~ $n_0 - n_{23} > n_{33}$.

C4)$_5$ $n_4 > n_5$ ····· $n_5 > n_{10}$ [S isolates $n_{10} > n_{18}$]. 16,50 interm ed.

$n_{16} > n_{26}$

A3)$_1$ $n_1 - n_3 - n_8$? not tested.

B5)$_{10}$ $n_{13} > n_{15}$ $\nearrow n_{15} > n_{20}$ (M)

(4) $\searrow n_{19} > n_{26}$

$n_{15} > n_{18}$ (6) $\nearrow n_{28}$. $\searrow n_{18}$.

Foci's 4/15/57

1134 A5, 7 {
- $n_2^{2} > n_{12} - n_{12} > n_{28}$ (3rd)
- $n_2 > n_{12}$ (+1?)
  - $n_{12} < n_{19}$
  - $n_{12} > n_{19}$
  - n_{40}.

(100θ ?)

(14 reisolations, 6 days [refr. ovens])

A4 {
- ~~$n_1' > n_{14} - n_{16}$.~~
- ~~$n_1' > n_{14} - n_{19}$.~~

$A4_2$ $n_1' > n_5 <$ n_{17}. / n_{20}.

1138 Mycelia dried. 1 clone saved (B4) also had trouble drying

$B4^{.F72}$ [illegible]

$n_4 > n_{14} - n_{42}$.

[illegible] n_5^2 [illegible]

$n_6 > n_{14} <$
- $n_{17} > n_{27}$
- $n_{18} > n_{23}$
- ~~$n_{?} > n_{?}$~~ 31 > n_{39}
- n_{27}.

$n_6^{3+} > n_{16} -$
19
22 > 27
17 > 30
32.
31.
32.
19 > 32
19 > 29
33 > 38
16
19 > 32
19 > 24
44.

∴ branching indeed not sooner than n_9 not later than n_{16}.

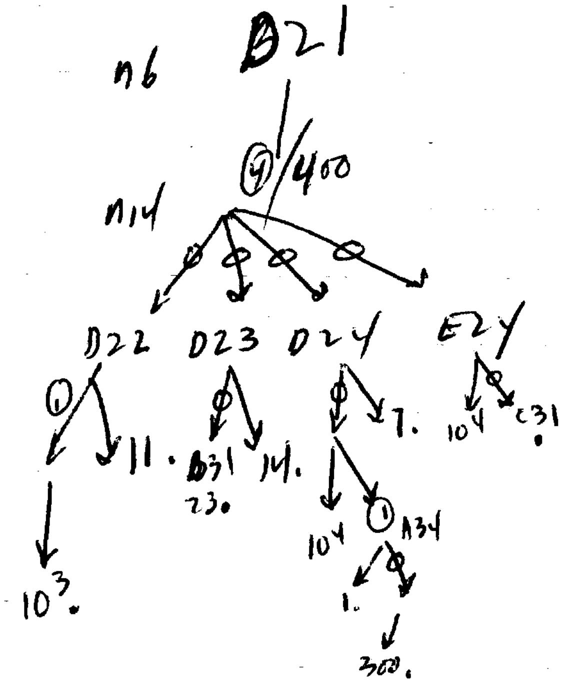
make to illustrate

14 + 13

$n_{27} + n_{15}$ n_{45}

1138 addl chain [struck out] of

n



B21

A21	3 C23 lost	
	4 C33. < 5	
22	2 C24. [struck out]	
23	1.	> 13
24	3 C25 13 A32.	> 1
25	2 D25 [struck out] 13 A33.	> 2.
B22	3, 13 A31.	
23	3.	> 13
24	3.	> 10
25	4 E23 13 B34.	< 5
C21	.	> 4
22	3.	> 13
E21	13 B32.	> 5
22	13 B33 2, 8 E31 1,	<

∴ total chainlengths

n16

A21	19.	
A22	22. [struck out]	> 27
A23	17 > 13.	> 30
A24	32	
A25	31	

B22	32	
23	19	> 32
24	19	> 29
25	33	> 38
C21	16.	
22	19.	> 32
E21	19	> 24
22	44.	

H_1^a —x

DATE: APR 20 1955 REF:

1	2	3	4	5	6	7	8	9

FA93 (SW940 4,5,12 a:–) —x SW666 . 11AM – 12¹⁵ — 12⁴⁰ [illegible]
FA37 " "

A) 93 —x 2³⁵ fuse drops. 5PM Harvest = 5x10³/10 ml.

B) Same dilute 500/ml. = 500/1 ml .2 ml samples.
B5 = 1 ml / 6.

C) 37—x (SW1257) 400/.4 ml
1. 0.1 ml MB-A60
2 ± "
3 .001 a
4 .001 b

Plate c 3³⁵ PM

5/31 The results here are not tabulated but come i below.

APR 22 1955 Repeated.

		T				T.	
B 1	–	++		C 1,2,3	–	++ >90%	
3	a	+ (reduced in % + extent)		3	a	++	large trail no in
4	b	–		4	b.	–*	– did not b any chains
5	b	– (Recheck carefully – seen					

* some small late trails?

JS 12/58

Conclusions: effect of anti-a serum is specific, as it works on H_1^a—x but not on H_1^b—x. The effect is, however, not complete and may be difficult to measure. Hold plates to photograph. It is possible that early chains are b and later are a or vice versa.

S... 1, 2, 3, B1, B3 for deleted photos 5/31.

5/31

from 5 plates of B5 and 2B4 looks for any trails or suspicions.

on 1B4 plate only, 3 susp. trails? or clusters.

B4/a 

b 

c 

These may have had early ~~B's~~ a phenotype with little enough b to swim in presence of anti b.
Plates to DCG to attempt reisolation of these trails.

6/4. DCG found (on replating isolates)

a: gave fuzz 1's and many clusters (ca 100 cols.) (after 3 hour incubation 37 then overnight at 23°. MG photographed.

b: pure 1's

c: mostly swarms – attempt to isolate any 1's (doubtless contain)

post partition of slants

123

DATE: APR 20 1955 APR 21 1955 REF:

Re 1257 pupa.

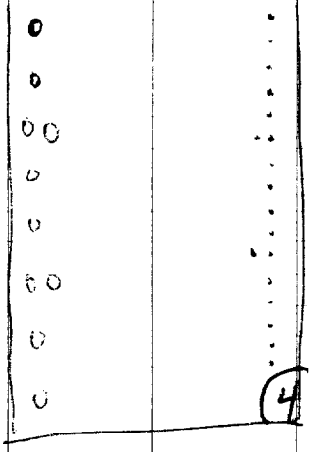
305 fuse dips

APR 21 1955

1252 pupa.

fuse dips, 4/5 2:10 - 2:35 spot 56 (1)

D C B A



A 1 -15

D 1 -11.

Examine 5:30 Most active without being handled

A 2, 3, 4, 5, 7 B1 B7 B9 C 1 2 3 4 8 14

D 2, 5. Check for circulation & swarm, trail.

5:10 P22 - Pickerman slides + plate samples

est 10⁴/ml/10ml = 10³/ml plate .02 ml .1 ml samples in M

activity is posting

DATE: APR 22 1955 REF:

	1	2	3	(F)	(-)	6	7	8	9
A	1 (F) uf			—	0				
	2 (1) (1) uf			3	4				
	3 (1) (1) F		v. active	10	4				
	4 (1)			10	4				
	5 (11) F			4	4				
	(1)			1 0 .	4	Research for (F) by trap:			0
	11.			11	4				
	10 uf 1.	vs		0 .	4		"		0
	F + . 1. 1.			— 6	0 4				
	11.	daily		14	4				
	15 11.			1 0 .	4				
B	1 (11) + .	v.a.		3	4 pd				
	uf 1.			4 —	0				
	11.			18	4				
	20 + .			1 0 .	4				
	11.			3+	4				
	(1) 1.			12 (1)	4				
	11.			3+	4				
	(F) + .			2	4				
	1. daily			1	4				
	uf F			2 8	4				
	1 (1) not			1	4				
	1 (1) slow			3 —	4				
	15 uf			c. 30	4				
	1 (1)			swarm	20% - 3+				
C	2 (1)			—	0				
	(11)			4 —	4				
	1.			—	0				
	uf F F			1	0				
	(11)			0 .	(200) 3				
	1.			20	4				
	40 + . daily			1	4				
C	11 + 1.			50% swarm	4				
	12 (1)			2	3+				
	13 uf			—	0		4/23.		
	15 (1)			3	3+				
	18 1.		"D1" →	40+	(500)		Proved to be swarm now		(10%)
D	1 (1)		2	1	3+				
	3 1.		3	swarm	<1	Inv. uf			
	3 1.		4	3	4				
	5 1.		5	0 .	3 typ.				
	1. (1)		6	8	4				
	1. F		7	swarm? 100	3		✓	10% met	
	1.		8	est. > 50%			✓		
	+ .		9	8	4				(over)
	+		10	10					

Save swarms

D1
D7
D8
C2
C11

① ferm samples directly to bioassay
(plated precise, over)
{ in stabs for whole clone
in MGA tubes for pooled motile
pool Pla⁻ cols. from MGA over for non

Replate
D1 - see infus
D8 - too crowded to count swarms
ca. 50%
to recover component strains for later colony tests.

37 [redacted] —x SW666
undivided clones

April 21, 1955

56 (1) isolates, grown to 2^{13} and summarily examined for motiles. Counts are underestimates. No tech losses

NG ~~12~~ 13 Swarms 4 No motiles 6 less than 9 22 10 or more 11

Maximum extimate: 40+. 5 clones were harvested for replating of the intermediate chains.

No.	Est motiles	harv. + nm.	mot left behind	Plate
B3	18	20	4	120 1's ~~many~~; 2 vs T. 800.
B12	28	22 4	10)	11 + 1 vs T 1 cluster
C9	20	28 6	5	1 tail, def. multiple but compact 28 1's.
C1	30	37		4 v.s. tails 000. 31 1's
D1	40	45 10	10)	34 swarms + 13 nm. No tails.

*Replate Residue
8 sw; 92 cols. (own)

Estimates on clones with many chains are therefore moderately low. Some of the nm may have had a motile but this was looked for. However, these drops were not search with a trap owing to shortness of time.

In addition, 4 drops had apparent swarms, but it was difficult to estimate incidence of non-motile elements. Therefore these were blind-picked and plated immediately. (picked to 10 ml, est. 60-70% recovery; plate .02 and .2 ml samples)(This will help evaluate estimate of clone size as 2^{13}.)

Swarm	% mot.	Found	clone size $\log_2$	Plate
C2	20	10%	11	.02 4 sw; 43 1's .2 33 sw — not counted
C11	50	20%	13	.02 4 sw 29 1's .2 5 sw ~ 250 1's
D6	100?	—	10	[No swarms or plated but not counted.]
D8	50+	10%	4	.02 3 1's .2 3 sw; [redacted]

Repeats

The data may be grouped as follows:

mots.	clones	12+ snl
inv	A1 9 11 B2 11 15 C3 5 6 14 D 10 11 ; D8 D5	12+ snl
0	A10 15 B5 C8 D4 A7	6
1	A6 14 B4 7 10 12 C7 10 D2	9
2	B9 C12	2
3	A2 B8 14 C14 D4	5
4	A5 B1 C4	3
m6	A12	1
8	D6 D9	2
"10"	A3 A4 D10	3
11-12	A6 D6	2
14 18 20	B12 B3 C9	3

C15-D1 conf. in tally but not pltg.

Initial activity:

D/swarm: Semi 45/55? Flat was removed from DI before plating, the count residue of 8 swarms: 92 colonies (in $\frac{.92\ ml}{10\ ml}$) is not fair estimate but the ratio must still have $\approx 10\%$. Late segregators?

April 21, 1955. Zur faire trails.

(1). Serum inhibitions. of anti-a, b // b → x b
a → x b
i → x b.

(a) Sm a does not inhibit b → x b, probably specific.
Is b effect specific? Would need a $F1a_1^- H_1^{\pi}$, e.g.
(Input c S. Heidelberg initials?).

(b) Should also be tried on intermediates as early trails might all tend to be H_1^b and agglutinated.

(2). Late branching? Pedigrees + platings of $\begin{cases} n_{1,0} \\ n_{1,3} \end{cases}$ isolates

(3). E-branching < platings of initial sibs
any large trails in sibs to swarms?

(4). Use all segregates H_1^b (a) trail ends
(b) swarm sibs — esp. of $H_1^b H_2^+$.
(c) look for b-resistant trails.

Today: (A) Replate a/. and of a/i → x b.
(B) start (2).

Tonight Dennis notes — summary for (1).

Does b (minnesota) serum also inhibit $H_1^a Fla^+ \rightarrow \times H_1^b$ trails.

any $Fla_1^- H_1^{unb}$?

DATE: APR 22 1955 REF: 1250

"b" New Preps FA10 →× sw666 10^{10} – 12^{15} – 12^{40} Ref.
Freeze trypsin 2:40. Harvest 4:45 c. 800/ml.
ml. c. 5^{30} – 10^{00}

A. FA10 →× sw666 (.1 ml) β a →×. (.2 ml) c. 100 cells/plate

9A23: trails

				A b→×b	B. a→× b.
1.	—		—	++	++. 10 swarms, none
2.	b minnesota (Edwards)	1/100	b	—	— no trails
3.	"	1/1000	b	—	— no trails
4.	a	1/100	a	++	± } much the same.
~~4~~5.	a	1/1000	a	++	± } appearance more ≈ MGA dilution.

B prep. may be late, segregated.

∴ b, minnesota also inhibits completely. Reaction may be specific for →× H_1^b but this cannot be verified unless a $Fla^- H_1$ can be isolated. (intent of 1250B5 plating?).

photog!

C Search for /b trailets. 1252A2 (1) (2) 1252B2 (3) each show → near edge (prob. serum not diff.)

2 ~~/~~ O° } no marked swarms.
1 ~~/~~. O°°°
3 O°.

1250B5 4 plates

See 1256.

5/31 ~~from 1250B4, B5 search carefully.~~

photos of 2?A ...

APR 22 1955
APR 23 1955

Tetrazolium- formazan chains preliminary

P22. Prepare stained cultures. Add 1 ml overnight culture to 7 ml broth + TZ .005%
Incubate c. 3-4 hours. Also (A) add TZ (1/200 .5%) to 1 ml culture directly.

Best method of preparation appears to be growth for short interval with TZ. Probably only older nongrowing cells willstain.

Refr. to 1 P 23. Test isol. to agar, small liq. drops. Maán traouble with agar is confusion from dirt even under oil. Probably better in fluid with a nonmotile culture.

1PM isol. 1) mot. W-2344 to A1

4 PM 3 more to A2

4PM 6 stained W-2802 to small drops near situs C. These were termihally marked. (Z = f[illegible] gra[illegible]
Hunt for rare medial marked= 7,8

2 W-1177,(dividing) 9 is terminal, 10 is medial.

of 10 clones found, 3/10 still located "Z". Fate limited - see protocol.

APR 25 1955 APR 26 1955 — Try out agar block methods to immobilize. Mostly a.i. owing to dirty agar — hard to refind individual cells. Try [illegible] method instead. (cf. test 1-2 [illegible]
Disadvantage of oil chamber is solubility of released Z in oil

But prel. observations above bear out earlier concept

(1)  as regular mode of fission

(2) most Z ~~to~~ unipolar

(3) Z dies out.

DATE: APR 2 5 1955 REF:

Standard system now: SW-940 (FA 93; H_1^a) —x SW 666.

37°

A. Overnight SW666. 1ml + 1 ml .01% TZ broth. Stained 9:00-10:30. Wash and add FA 93 to pellet. Inc 11 AM - 1 PM. (Cf. B); sediment pellet for harvesting motile :

A= cells prestained; 2hours+ phage/

B. 1:1 + FA93 9:30-11AM. Add = vol. TZ broth. Incubate till stained (1PM). Sedime to harvest pellet. (3i/2 hrs. + phage).

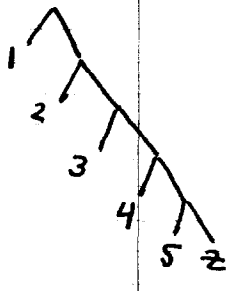
B= cells poststained.

Found: many motile initials in each, but almost no motile A were stained (overstai About 2% of motile B were labelled; c. 50% of parent population.

Summary: 28 isolates from B, 2 from A. 5 clones inviable. initially., only one Z (granule) chain died later. Z chains were followed for 4 to 6 fissions. 1 clone gave a swarm (c. 50% motile) = 31B/ E = preponderance of motiles (>10)

8 clones showed E. This appea

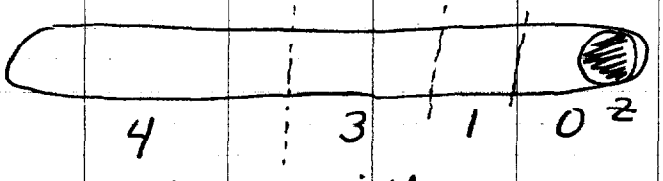
If clones are summarized :



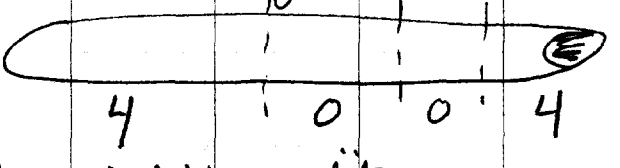
as follows :

(+) n	Found	Random expectation
1	4	4
2	3	2
3+	1	{1
Z	0	{1

or, if cell is



The result agrees with random expectation, but possibility of a negative correlation of Z (after n_3 or n_4) should be tested. Original notion if E were polar was expectation of :



if Z had random chance of marking the E or ~E pole initially. However, Z may either inhibit motility if it marks the E pole, or correlation may be one of selection (in terms of age correlation). Further study is made of Z-marked chains, in 2 senses. (over)

APR 25 1955

1254

does this necessitate? 6/56

DATE: 8PM.

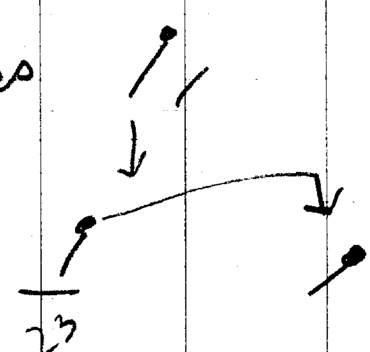
REF:

	1	app.	3	total	O. 5	6	7	8	9
1.	5	O shrunken.			4				
	4				4/1				
	4				4				
	3		no trans.		3				
	2 inf								
	8				4	→ 1/2.			
	4				4				
	–2				5				
	4, 5 (2³)				5	bites 4/16 mot.		c. 0/8	d 1/4
	4				5				
	4				4				
15	4		(all 4 mot)		4				
	8		only mot in a. bites 3/8		4				
18	2 inf.		2 mot in b.						
19	New	cool female temp 9PM							
21	7				4		(1⁺, 1⁻)		
22	5 2²		b. has 1/32		4	1/2 → . 6.			
23	5 2²				5				
24	8		3/8						
25	16 inf.				4				
26	4				4				
27	8 inf								
28	8 inf.								
29									
30	4				6				
31	11 2³				3				
32	4				6 (1 var. already)				

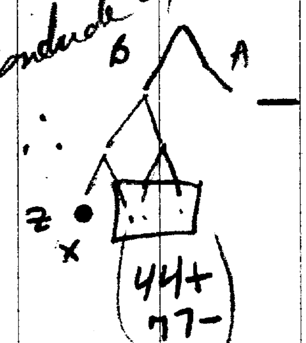
Reexamine at 8PM and isolate residue of Z from rid. # of nos Z. to rightmost drop.

St. RT.

E.G. 31 was



conclude 6/56

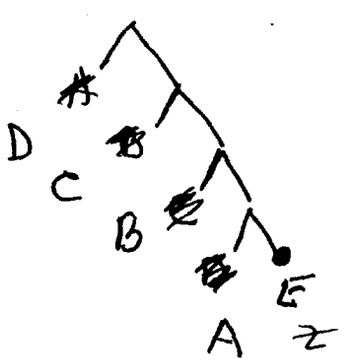


Reconstruction:

in general, rightmost is Z chain cell.

the others are successively later sibs of it.

e.g.



DATE: APR 26 1955 10:30 AM. REF: 2

(+) sign	#	A		B		C		D		E	Σ	F	+	n
4 nuclear 3?	1	0	4	4	4	(25)	4	0	—		29	22 → 3:00 PM.	2	4
	2	0	3	1	4	1	4	0	4		2			4
	3	0	—	~~3~~	~~4~~						—			0
	4	2	4	(15)	4	1	4	0	4		18	too close to pts.	1	4
	5	0	4	1	4	4	4				5			3
	6	1		3	4	(12)					16		2	2
	7		—								—			0
	8	0		3'		0		0		0	3			6
	9	1	4	1	3'	0	3	0	4.		2			4
	10	0		(23) 1	3	0					23	23 → tube for plating	1	2
	11	1		(18)	4	0		2		1.	22	18 →	1	5
	12	0		1		0		1		0	3	2		6
	13	0		3	4 3	0	3	0	3	0	3	3		5
	14	1		(30)	2	1	4	2	4	.	37	28 →	1	4
12:30 1:15	15	(44)	4	3	4	2	4	1	4		50	43 →	3-4	2
	16	0									—			0
	17	2	4	3	4	0	4	1	4		6			5
	18	(18)	4	2	4	6	4				26	17 → 6 →		2
	19	1./100, (3).												7
	~~20~~													
	21	1		0		1		0			2			4
	22	0		4		0		0			0			4
	23	1		0	3'	(22)	4	0		0	23	~~[illegible]~~ 21 →	2	6
	24	2		2		0	2+	0			4			5
	25	3		1							4			1
	26	1		0	3	0	3	0	4		1	~~41~~		4
	27	(42)		1		2		2			47	41 → 2 w/		0 Z
	28	1		5		0					6	2 w/		
	29	/									—			0
	30	0		2		0	3	0		0	2	0		5
	31	0	4	sw	3	0	.				sw no trails	Picked sw later - pure ?	1	4
	32													

× ↑ × ↑ × ↑ × ↑ total chains in whole clone.

× View ... gr...

(+) is chain in which pseudo... motiles appeared.

n = number of fissions for which Σ was followed. (not necess... isolated!)

Save + Recover Fla⁻ sibs in 31B swarm. Plating of .01 ml. sample from the clone gave (1 ml) 44 swarms : 77 colonies, no trails.

DATE: APR 28 1955 REF:

Preliminary expts 4/28 showed that 2% Methocel 4000 immobilized cells so they would stay together after fission and form subclones. Use to test distribution of Z in a chain, and to isolate after n_3 or n_4. Make up Methocel in Penassay. The methocel completely immobilizes the bacteria. ?What concentration allows Fla^+ to swim?

93 → x Sc 666 8:30 – 10 AM, add = vol TZ .01% to 12:05. Spin down and refr.

Abandoned.

B) Apr 29. 2 PM stained W-1177 (c. 2 hrs. mixture overnight + TZ broth).
Plate out in methocel broth on c.g., over oil. Also isolate a few definite anomalies.

Conc. Most cells stain unipolar. Rare (5% bipolar, subpolar). Most chains show terminal granule. Occ. cells lyse in random position.
Some exceptions with interstitial granules— probably from subpolar cells.
Need: observations at first division of subpolar and bipolar cells.
should also spot a fair number of unipolar controls.

Z chains 1255

APR 28 1955

DATE: 2-3PM 5PM REF:

(+)	1	2	3	4	5	6	7	8	9
A1 A	to A2A								
B									
C		(III) 1 to KU → A4A							
D		II → R							
B1 A		i							
B			0						
C									
D									
C1 A		II							
B									
C									
D									
A2 A	moves.								
B									
C									
D									
A3 A									
B2 A									
B									
C									
D									
B3 A									
B									
C									
D									
C2 A									
B									

Row B, col 2: II — to L. → ~~A4A~~ B5A

Exp. n.g. too much slippage perhaps fluid added Method drop or motility present. Let go — at key. c. 5P27

DATE: REF:

		(−) C.2 38M spotted	2	3	4	5	6	7	8	9
E1	A	(+)								
	B									
	C									
	D									
E2	A									
	B									
	C									
	D									
E3	A									
	B									
	C									
	D									

30

40

50

Fla⁻ $H_1^{non\,b}$ segregants. 1256

DATE: APR 2 8 1955 REF:

1 2 3 4 5 6 7 8 9 10

1 1252A2 } a-x b
2 " }
3 1252B2 } trails? w/ b serum.

Test by two agglutinins. Isolate $Fla^- H_1^x$

B) x— FA10 (H_1^b) + b serum ⟶ ? ⟶ $Fla^+ H_1^b$

A) x— FA93 H_1^a + a serum. If x = a, no swarms. If x = b, H_1^x = b.

Results:

1 - Not pure motile. Cover to DCC
2 - Pure motile. 3 { B no swarm
3 - " " A swarm ⟶

20

30

40

50

W1177 Z chains 1257

DATE: MAY 3 1955 REF:

A. Staining in situ W1177.
a. in mixture c .005% TZ under oil — No stg. overnight Some inhibition ?

b. In methocel 4000 2% (in Penassay) + c .1% TZ, isolated colonies only (resistant ?) — stained only in center.

? are these conditions too aerobic ? Otherwise potentiate toxicity of TZ

Should reisolate colonies; compare growth c .5 TZ under oil.

B. Chains from isolated cells. (Are c. 4-6 any Theresafter)

A4

1. In scattered region many chains showed intestitial lethals. (effect of cold ?)

2. Few Z granules now seen.

3. Isolates marred by spread of growth under them (film of moisture from drops !) A - B - C

D 2 is tangle
D 4 c 20 cells, Z term.
5 n.g.
6 n.g.
7, n.g.
10

E 1. tangle, Z term.
2 - 5 n.g.
6 no Z
7 no Z
8 no Z
9 n.g.

Z chains etc. 1258

Motility in Methocel solns.

DATE: MAY 4 1955 REF:

A. attempts at clearing cells in pectin, according in relation to 28μ MMM beads, in Methocel 4000 cps 2% is necessary.

General conclusions as stated ~~attached~~ - see 1258 protocol.

Some Z+ chains c. 100 cells terminal Z cell still intact. ~~Intercalated~~ detached noted probably more frequent after refrigeration, but this is not settled by direct observation.

B. 5/6/55. Set up to repeat 1254. Began OK but slow to divide at RT (though warm) & later lost c.g.

C. " Methocel 400 cps seems to show up motile cells (1257 A1+). Put trials as selector for E cells. But must used 4000 in trial.

D. See 5/8/55. E - a slump: did not completely inhibit motile cells upstairs.

1) Possible use of Scotchbrite MMM beads as reference markers - time is a slow drift; May be better to use 3% Methocel 4000 rather than 2%

2) Wasted expt. in completing E, Z chains

3) Pulmi expt. in screening E, S cells by reservoir medium strikingly successful. See ff.

4) But most time last week or 10 days was wasted exc. to improve general impression of technique.

5/6/00 1258

Lab plans: what to doS? Things are a mess.

1. Currently enmeshed in hhe fate of Z granules. Can these really givr any important information? By following a granule during the growth of a single cell, one might get a clue as to whether groth is interstitial or bipolar (jn a few cases). To distinguih, one might have to show increasing separation between two granules, before fission in a single cell and thismay be difficult.
It is already clear that 1) terminal granules usually remain terminal, and that this is the most common type, already suggesting a polarity in the cell. Occasionally, bi-antipolar cells are seen (more commonly that bi-synpolar), suggesting that the two poles share something distinct from the fissile center. However, the basic interest in the Z granule for the current problem is the possible correlation with E, and this, if anything is what should be pursued for now. Later it may be conveneint to try to bepeat experiments with a polar-flagellated organism.
Another sideline is to use the shaihs in stiff medium to study other problems, chiefly lethalitym both spontaneous and UV. Also look for data on growth of branched cells. (Twort)
2. More pertinent: 1),look for divided E further. 2) diagnose E,S sells by viscous media. 3) transfer intermediate chains for electron microscopy 4) clean up serotypes of co-segregants-- collect more? 5) For 4 and others need to complete review of data and write up.

3.TODAY: Clean up what is accumulated to look at and photograph.

Start new preps. of 93--x w/wo TZ. Use for divided clones and for Z correlation.

(Sat 5/7/55- Sun 5/8/55----)

Use TZ stained prepn. 5/6. 12n7 Checked first with 1237A1+ for swarm motility. In this series, used 2% methocel 400, diluted c. 1/10 with penassay.

a) use methocel for trap; b) isolate initials in broth trap, then trANSFER to mcl.

The latter was found ineffective (probably still too stiff); By 4 PM, had isolated 13 cells still sluggishly motile in mcl trap, and 7 addl. which were at a distance from reservoir but not now motile. swarm cells were sluggishly motile in this methocel conc., about 50-70% were directly inhibited. This oln. probably wets glass more effectively, at any rate it tends to spread, and a few of the motiles below may be contaminants from 1237A1+/

The motile residuals above were planted in individual drops of broth for class. as Sw. or E cells.

N8: found, in first group: 6 swarms, 3 E, 2 ng, 2 E.
second 4 E 1 ng 2 E

Total 6 S 7E 3ng 4 E

which demonstrates strong selection against E cells

Detailed counts:

	growth	motiles
1.	4+	9
2	3 + sw	swarm, 50%?
3	4+	2
4	ng	
5	4+	12 (from Z cell, but Z nf)
6	4+ trap 3+	0,1
7	sn, 1 mot cell	
8	20 sw	16, sev. shakes, prob. sw
9	sw sw	
10	sw sw	
11	like 8 sw	
12	500	12
13	200 sw sw	
21	4+	4
22	ng	
23	4+	18
24	4+	24
25	4+	2
26	4+	15
27	4+	16

(1o4)

The occasion was also used to plant about 25 single motiles (removed before test below--perhaps should have been left in it) for ~~opn~~ coordinance on immediate and later motility of dividing chain cell. About 12 usable cases no discrepancies, some to one or two later divisions. As none gave two motiles, pres, none of these were E . Of remainder, most gave two app. nm at this division-- it may be possible to reexamine these drops tomorrow. What is significance of this crisis in termination? Is is growth in fresh medium? (May still need a good exhausted medium to keep cell size small.)

P8 These were then used in tests for residual motility in mcl. Unf., 1,5 were washed in 5½% mcl 15 (calc. visc 200) which proved also to inh. swarms. Further tests were then made with mcl 400, 1.8% and 1% (1:1 penassay), the latter being adopted as it permits almost full motility of motile swarms(from above). (This may be too fluid for accurate discrimination against E, as will be seen). From E: 12,23,24 26, 27, ~~altogether~~ cells reisolated which remained motile were planted for further classification → none proved definite E cells. See further below.

Until this is worked out against misfutter predgries!

—x SW 666 initials in methocel and Z chains

b a serum.

DATE: May 9, 1955 REF:

Prestained pwepn. used. (st Z 9:45-12N; phage to 1:20, centr. and refr. 1:40) (also another preph. unstained = A.

Note immoderate spreading out of methocel droplets. isolate initials in 1% methocel 400 (1:1 2%: penassay).

A. isol. from unstained, plant out in droplets individually.

B. isol. Z-stained initials. to c. 3PM, some fresh isol. C to 4:30 me t in single drops on initial cg. first, transfer latter as families to isolation cg . Ditto for A— plant out descendants.

D. 5PM B above, in broth traps: pick c. 4000 initials (somewhat late now for tests) in serums.

(Klein visited 5/10-11.)

D: Almost all initials are inhibited in a or b serum, though cells may continue to spin for a few minutes. 7 cells did persist in b, planted out. 3 proved viable swarms. isolate as 1259 D1-3. See DCG for results of platings(after picking to broth)in MGA. 2 persisters in a, but neither viable.

E. same as B-D but not sublined

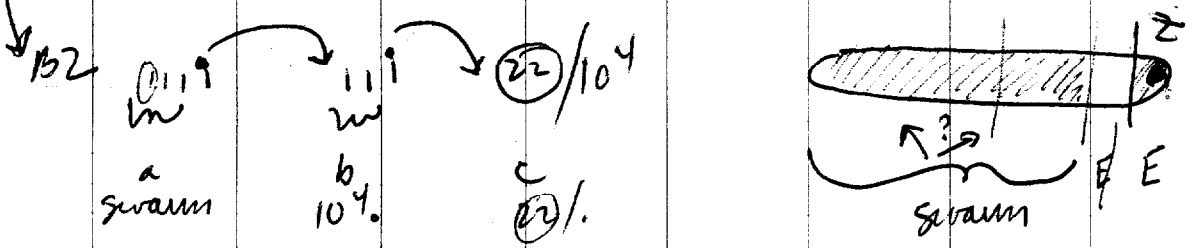
A: Held to 5/11 for examin, and may have partly diminished therefore.

13 clones 2? E clones. 1% Methocel probably too thin..

B: Most isolates grew out; had been separated once or twice at n_2-n_4. However, of
C: 38 isolations, 3 ng; 5 swarms; only 4E, none interesting except:
E-clones were reexamined for content. In B2, sib to swarms had 22 motiles, transferred to/238/ E2,3; 4; 5(sw). The motiles in E2 tested, all gave rise to inviable or E clones & therefore certainly not sw. cells/

not sublined

6% originally looked as if only c. 100+/10^4 but these later proved to be swarms. The clone was not recovered (owing to drying out) to verify originally low assay.



Otherwise, detailed numbers of intermediates were not recorded.
F+ 5/10 interm motiles tested in a serum: at least (28) from 5 clones were immbo. but 2/4 from B15 were not! However, two tested swarms were inhibited; specificity of serum should be rechecked.

Also saved 1259B1 (= b8). Swarm- test purity by plating
B2a,B (= c5 z cell removed at n_5 = nonmot,b) (not not certain record)

5/17

DCG found D1-3 all motile but with confusing clusters. B1: no definite swarms B2b "all clusters"; a pure non-motile. Will have to be rechecked on return (over)

E: 34 isolates planted w/o lineage afterward.

4E (9,11,15,16)
15E (1, 4,3,1,1,6,7,3,3,5,5,2,1,3,1,4,....)
5 Sw
5 ng.

Only conclusion: medium not adequately selective. Try 1½% methocel 400 (v.i.: 1260)

1259 summary to 5/16

5/12 Plated in MGA

5/13 Picked possible singles. Plates were incubated too short a time at 37°; D1 & D2 had singles, swarms, & clusters; D3, B1, & B2b singles & clusters only. Counts:

	Clusters & swarms	Singles	Singles picked
D1	51	3	2
D2	91	1	1
D3	59	5	4
B1	32	21	8
B2b	90	17	8

} these spotted on MGA

5/14 All "singles" picked 5/13 & spotted on MGA were motile (Spots had appearance of "clusters" rather than swarms).

Plated again: All original broths, + some of singles picked 5/13 (D1, 2; D2, 1; D3, 2; B1, 2; B2b, 2.) Incubated 3 hrs at 37°, overnight at 22°, then refrigerated until examined 5/16.

5/16 Results of 5/14 plating:

Original broths:

D1 Swarms, centered swarms, & col. ē "satellites"

D2 ~ D1, higher proportion of swarms.

D3 ~ D1.

B1 Trails, clusters, apparent singles; no swarms

B2a pure non-motile

B2b All clusters

Presumed Fla -:

D1(1) All clusters } no singles

D1(2) Clusters, swarms } no singles

D2 Clusters, swarms, no singles

D3(1) Clusters, swarms } no singles

D3(2) " " } no singles

B1(1) } Clusters, trails or satellites; possibly some singles;

B1(2) } no swarms.

Ni - 62 MAY 1 0 1955 *Best resume page*

- 1259B. ABCDE

In abc 3 swarms, 5E / 34 isolates

disappointing. No E comul. ?. But write out detail anyhow

B1 i (||||) → (37+) → (13)/104.

B2 i (1) ||| → i ‖ → (5)/104 later (22)

B10 i i → But only 1 drops seen.

B15 Rec. confused.

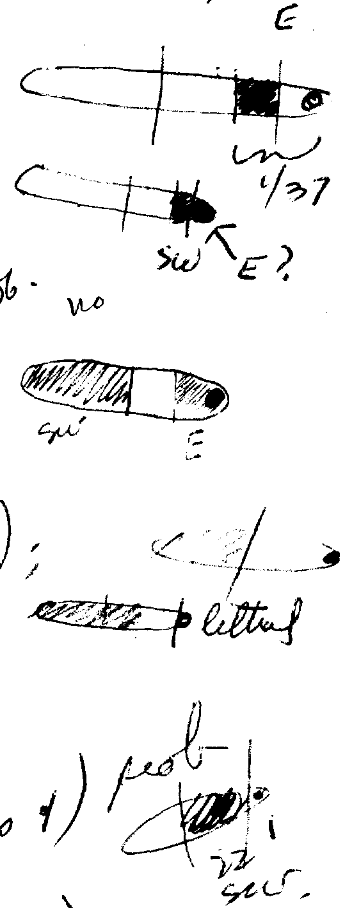
C1 ‖ i → why 3 drops? (prob Lng.);

C2 ||| i → i/9

C4 1 9 → But only 1 drop, + (100)/104!

C5 1 swf ·/22 → whence 3 drops? (3rd has 1)

C8 i i ||||.
sw sw



(over)

C4 (+) many. to [238] H2 } Hold for full analyses if needed.

Residue to F2, F3

C and B were evidently confused yesterday!!

MAY 1 0 1955

Do not save these swarms any to possible confusions.

But study closely B2 and C4.

B2 is swarm / 10%. / (5)/10%

(1)111/i → 11 10%

i (5)/10%

n_2 n_3 prob.

C4/is (1) → $\frac{>\times(100)}{10\%}$.

---

238 duid type pulsing

Partitions

May 13. New prepn., unstained. (probably usual, about 90-120mins.)

Fuse drops 2:30 Collect to 3:30. Cf 1259D motile.

No initial was nearly as active as 59D. Pick those that have moved the furthest, not necessarily v. active now. Estimated yield, 10% of broth yield.

Note : to compensate for spreading of methocel solution, use cg. that has been greased (human), then flamed. This works well, especially with larger drops, but smaller drops are too convex for best visualization. Intention was partly to look for early chains (E) in the methocel, but time did not allow and most isolates were made to broth directly/(A, B resp.) Lineages were separated at n_2-$_3$.

A: 1,2,3,6 ok. Partitions at n_1:

14+ :1 6:5 ng snakes. Later transferred entire clones to get fullest estimate of motiles.

Al came out +(14):6 Sepn at n_1 =

Bl-14,21-36. 4 ng. Mostly non E. Records show at first scanning:

2:4;1 14+ 2:1 3 7:20 0;4;1 1;3;d 3 2+ 2:1 1:0 5

sw;sw;sw;sw (1260B33 later DCG verified purity of each). 6:5 7:+

Underscores were rechecked (on ungreased slide!) and following definitobe values for splits on these:

1:20 8:20 2:2 4:12 3:2 7: 26 Therefore no equal splits.

General totals:

E 5
ng 4
sw 1
E

33

Little if any selection for E in 1½% methocel.400. Need 2% which probably totally stops many motile cells.

No new experiments after 5/14
Trip to NY 5/18 – 5/25. Resume lab work 5/3

→ × SW666
Methocel

MAY 31 1955
JUN 1 1955

Pupae 93 × SW666 (1:1) 10^{05}–11^{45} (– 12^{30}) SIC in antifyte. Rf. t c. 4 PM.
c 4^{30}–5^{45} isol residual motiles. Est discrimination factor ca 1%.

A) Note: to prevent spread of methocel, e.g. ~~slides~~ are lightly greased with fingers (on one side); flamed; oil added. However, motile selection seemed most effective where there was appreciable wetting and spreading of the drop on the coverglass.
Isolates transferred to fresh penassay drops c 6 PM, inc 30°

Counts of (+) / 10^{3}–10^{4}. : 4, 25, 20, 13, 46, 11, 6, 2, 50 ; 3, 20, 18, 3, 20, 7, 11, 13, 10, swarm!

JUN 2 1955

Σ : (6 E : 3 ∉ : 1 swarm ↓ 2 Invisible)

∴ with 2% methocel 400 there is effective discrimination. at this conc, Fla+ (1237A1+) was greatly slowed down (10 × ?) but most cells did continue to move.

[Note — to this point considerable interruption in continuity of work was occasioned by ① trip to NY for ascites meeting ② breakdown of manipulator — hole in diaphragm, temporarily repaired].

∴ continue pedigree studies on preselected initials.

swarm: indirect plating of clone, in 1 ml, .01 ml gave 44 swarms / 265 singles
again note low ratio. Schedule completion!
(see photo — plate had been held at RT overnight, inc 2½ hours 37°, then RT 4 hours.

Mcl 400 2% solution.

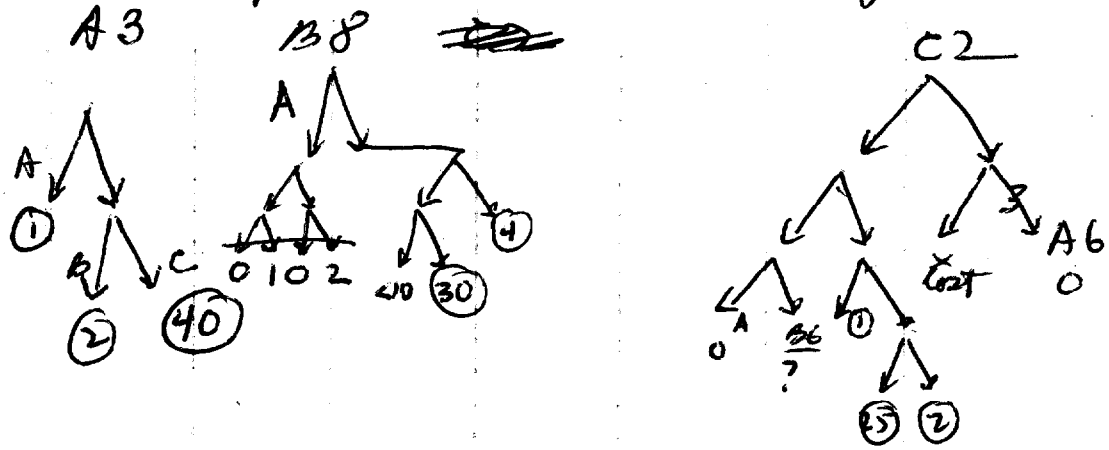
Pedigrees.

1262

JUN 2 1955

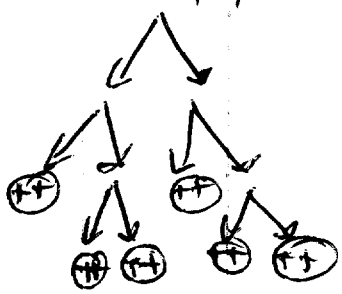
A). grow in penassay B. grow in Mcl. 1261 pups.
Plan to pure dups c. 12:10 PM.

No cells continuously motile like F1a+ clone were seen. Picked the most active. If continued, probably need to regulate the degree of wetting. Did pedigrees to 43-45 on 34 mutants transferred to broth.
Then pedigrees
P3, scan for E, ++, swarm. Found only 3 E clones.



∴ splits are 1:42 3:34 and 28?:0 (known 1:27)
2:25

One swarm clone C4, almost pure. DCC checked purity of each clone by plating.



Same! as 1262 C1-5
for H, check.

---

some plates were only 3E, 1S : 22 ++ and 10 ϕ (= lethal)
[11-0; 9-1; 2-2, 3, 4, 6, 7].,
43, 37+, 28
the experiment was quite unsuccessful.

Again review Salmonella data
to get paper out of the way.

July 13
1955

A) → Should first get general picture of experiments + what they were!

Write out 1138 B4

? → X SW666 4p+

Note diminished motility of large cells. Occ. early isol 1. → ①/10³...
(remarks on growth cycle) e.g. 1141 B4

7/55

1141 A4 v.p.

A5

B1

B3

B4 vp

A1, A3, B5, C1 n.q. (stayed motile)

C2

A4 (v.p.) Σ=33.

*① first suture!

note partition: 19:11
or 1:(19:11):1:1

But cannot use as G2 may be derived as from B4!

B4 If the latter, then

This datum is unreliable.

	Σ	a	b	c	d
3	1	1	5	—	
	2	6	6	11	
1	0	1	1	1	
	0	1	1	1	

	Σ	1	2	2	av 3.
A_2	1	2	2	19*	—
B_2 - D 15	18	6	16	16	29
- G2 15	1	20	27	27-29	37
				40	45
C_2	1 n.q.	2	2	2	2
B D 5	11	9	15	15	29
D_2	1	2	27		20
G2		16	23	33	47.
			23	24-26	34

subscript = point of this branch in the pedigree.

7/13/55

		Σ				
1142. ⑨	C 3	>50 5 tested	6	10	31	36
	D1	>100 10 tested	7	24	41	45
	C 4	10 2,1,3,1,0,0	3	10	—	— (no record)
1143	E 3	3	2	2	12	14
			3	12	—	—
	E 2	11	S_1 2	2	2	7
			S_1 2	2	3	3d
			S_2 3	10	16	23
			3	10	42	48
			S_7 5	11	27	31
			5	11	31	38
			5	11	14	—
			5	11	—	15
1144						

Leifson cultures.

1272
SEP 9 1955

all 6 cultures grow as well on both at 30° as at 37 except 205.

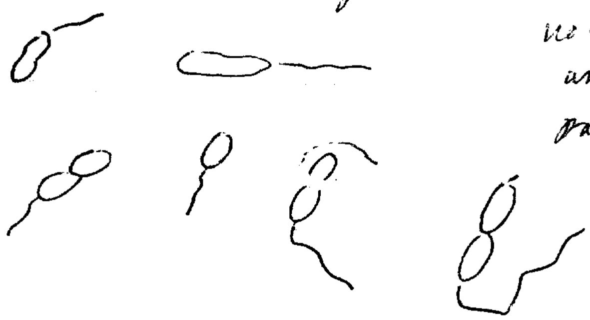
For preliminary comparisons, inoculate H1, H302, H32, H37 1:5 in broth + incubate 9AM —

1272

SEP 8 1955

Leifson's slides See his letter figure pairs priority

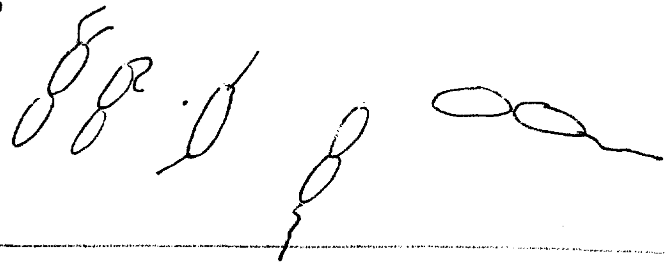
H1 A. 1200 x approx. P. aeruginosa typ.



no clear antipolar pairs. sprouting?

polar monotr. unip. ✓

H 300



ditto
cells larger. than above.

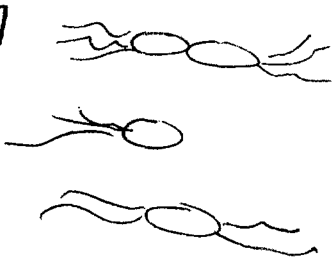
H32

both

vibrio?
multitrich, antipolar

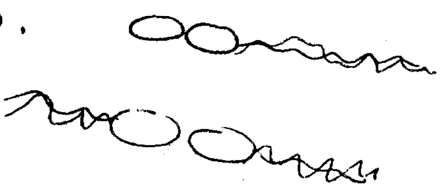
"alcaligenes"
(Lophobacter)

H37



large cells. ✓

H205.

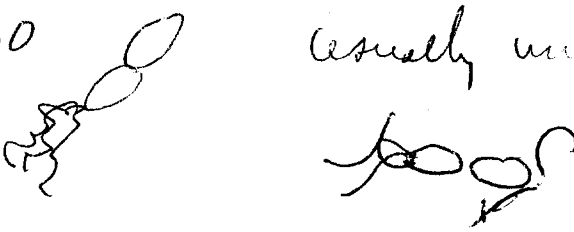


more usual

rare

prob. intermediate
polar multitrich
some bipolar!

H430



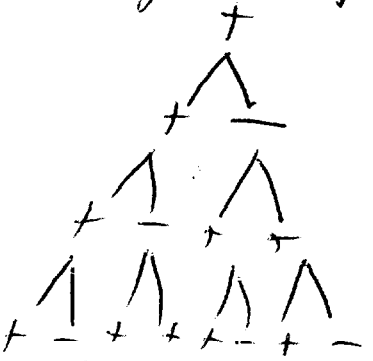
usually unipolar

(over)

All [242] protocols.

Conclusions

a) Pedigree possibly



b) No great regularity; some + < − / +

Some + < + / +

Could be studied further

It is just culture