5/27/56.

1212 → early partitions

1214 calibrate loop volume. + trails in motag. no trails, only clusters.
ratio of trails to swarms F+37 × SW666

		Exp 5	6		
Σ spots (diluted)	0	27	11		
	S	2	10		
	T	2	6		
	T+S	1	3		
		32	30.		
total T		3	~~10~~ 9 }	~~12~~	12
S		3	~~9~~ 13 }	~~16~~	16

In these conditions, S > T. (question of earlier data). See 1216

1215 - First platings of SW666 in dilute motag

1216 SW967 × ; plating & dil. agar. noted satellites.
16 long trails with 30-54 colonies per trail
vary motag. Not reliable. Looked for evidence on non random
distr. trails per spot

BBDS*

2/5/58 .1217 → ~~early partitions, date~~ Clones from undivided initials; plate
1218 Deconseq
1219

5/27/56.

1212 → early partitions

1214 calibrate loop volume. + trails in motag. no trails, only clusters.
ratio of trails to swarms F#37 × sw666

		Expt 5	6		
Σ spots (diluted)	0	27	11		
	S	2	10		
	~~T~~ T	2	6		
	T+S	1	3		
		32	30.		
total T		3	~~1~~9	~~Σ~~	12
S		3	~~4~~13	~~Σ~~	16

In these conditions, S > T. (question of earlier data). See 1216

1215 - First platings of sw666 in dilute motag

1216 sw967 × ; plating & dil. agar. noted satellites.
16 korea trails with 30-54 colonies per trail
vary motag. Not reliable. Looked for evidence of non random
distr. trails per spot

BDDS*

2/5/58 1217 → ~~early partitions, plate~~; Clones from undivided initials; plate
1218 Deconseq
1219

5/27/56.

~~1219~~ – 1221 – 1222

1219 SW967 x – 50B. ① effect of extra cells ② trails per initial.

added	initials	Crew	trails
–	17	13	1
SW666	17		1
SW967	16		3

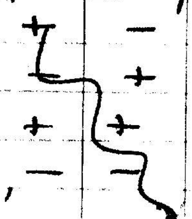
} 5 T/50 initials

No decisive difference.

~~58~~ isolates

1221. A) ~~[illegible]~~ initials planted out. A C E F
58 ~~Ab kept [illegible] drops.~~ 34 viable → 4 trails motag.
~~24 [illegible]~~

Ascarisn. expt res. drop



Also, 48 initials formed clones,

following had ⊕ at n_{10} 0^{39}....; 1^4; 2^3; 3^1; 20^1 ∴ 1E/48.

Sign. of initial ratio of E so low! None of these gave trails. c/ $\frac{1}{48}$, 4/34 above.
No swarms.

44 initials → 1 trail rather old Motag.
17 " → 1 trail

1224 50 – x Sw967. Plate ~~on~~ spread motag. Initials pooled.
M & A 5 T/12 colonies MA 0 T/14 cols. Hegh!
effect of spreading considered (spots before now).

1225 ditto. and control on residues.
a) 96 initials → 14 clones (low viability!) 2* duplicate mats
b) 14 " stroked out on [illegible] → 1 trail
c) 2 complete clones (A?) spread out → small clusters under lenses.

1225

d. pool inoculated various ways, media

967x	2x .01ml spread →	90c, 8T		
	" not "	35c 4T		
	.02 " "	—c 5T		∴ not effect of spreading
	Spots from pipet	25c 4T.	16 3T.	" "
	" pre-spread plate	16c 6T	20c 1T	

∴ No effect of spreading; rather variable ratios.

Totals: 202C 31T.

1226. Pool 895 initials & plate samples. Media diff in stages.
967x

altogether 38T, 138 colonies = 1/4.

In poor plates, 10/71 No difference.

also misc effects. possible ΦOH → 17/40.

1 trail: 125 ucolonies in 17 hours

later known: (But 967 has spont. "minors")

1227 40 initials to broth for plating in tubes + plates.
12/17 pure clones No major trails; all slow "minor".
+ 8 500–1000 some photographed
→ 1 trail
pooled: 19 → 14 flowery trails

1/20/75 = 1 trail/20 viable clones/25 initials

1228 – 9 – plated clones see 1237 summary. 1229. discovered that SW987 produced spontaneous minors. Other Fla⁻ did not.
– 30. – spontaneous motiles.

5/28/56.

1230: some clones from sp motile SW967. noted fluidity effects.

1231 all major trails occurred singly.

1232 dropped SW967 x—.

SW666 x— Plate pooled initials:

1233 plated clones.

1236 plated selected single clones
"Flares" also noted. Genotypic Fla+ are also immobilized in motag.

Cloneplatings (problem of quiescence at large cell size)

1227 } SW967 x—. Clones: in motag 100%.
1228 } 12 had major trails (uniquely)
1229 } 60 were viable (clone size ~ 150)
74 were plated. definite nonlinearity noted for first time. (tactic orientation is diffuse)

SW666 x—

1232 4/16/25 Major is single, but others clusters 2^6
1233 1/34/47 2^6
1234 6/15/48 Concurrence of trails! 2^{3-4}

"selected" clones (most active on mucic agar following).

1236 3/57 plated: floccy MGA. — not recordable.
1237

48 clones followed: 4 swarms + single — 3 sibs tested; all 6 Fla- note ratio. (all 4) and flare effect.

In selected group 2/7/7 (grown before picking) } expt to test "quiescent vs active" clones
control 0/10 incl 1 swarm
2/17/17

Would S/T concurrence be obvious?

5/28/56.

1227 – 1237

pooled - initials: platings in motag.

SW967x —	1228	~~#/10~~ 11/81/~~xx~~100
practically no swarms	1229	24/198/200
		35/279/300

SW666x —

		SW	TRAILS	COLS.	POOL plated
	1232	1	27	159	202
B)	1233	5	13	79	100
	1234	3	30	149	200
	1236	7	5 Bcl	172	190
	1237	15	11	173	185
		31	86	732	877

old prepn.

character of swarms - any comment given - often too large to be readily studied at origin.

1237 of 15 swarms, 8 were clear, 5 had singlet center, 1 had c^2, 1 had $\circ^{\circ}_{\circ}$; $\circ$ at near-center. no recorded attempt to isolate (see A)

5/28/56. 1238

FA37 x SW666

a) ~200 plated, but not incubated. Include 9 sw / 157 cols / 197 moc.
recisc fluidity effects.

155 { 100 T 21 Cl 9 sw 25 1's / 175 moc. "many swarmers were centered: Col T" Most trails in one plate.

Effect of agar on T: S... 250 samples:

NB/25ml motag. Input ~250

	Clust.	Cols	T.	Sw.	Σ
0	2	53	2	2	59
1	2	55	1	0	58
2	–	59	2	3	64
5	5	46	6	0	57
7.5	18	39	11	3	71
10	11	19	19	3	52
0	1	159	3	11	174.

Also plated some clones see 1238 C1 photo for major + minor trails.
C 13 heads 4 contam. } all but C1 single only. } 9, 9, 2, 31 = (4)
D 8 heads 5 clones } 6 to 52 (18)
see protocol notes for clone sizes ↑

22 viable clones

1239
5/28/06.

plate clones in 60% MGA.

mutation not limited for these

a) MGA
1 contains 12 blanks 16 all singles 2 swarms no def. 1-colonies; 2 large plaques.

b) MGA 60
8 blanks 1 swarm + 4 1-cols.
6 all singles
1 11 trails (100; 30 elements). See photo 1239-6

see notes protocol for clone sizes: ~~from 6~~ - from 11 to 225.

[1240] used Wilson gelatin n.g. but have photos.
plated clones. 9 blanks 19 viable

also many platings
3 sw - 1 c T
3 sw {1 T, 1 e}

a) 1 swarm + cols + trails. See for pictures + cluster sizes.

40 ① to 10⁴. "No E" - too many cells.

[1241] part pedigrees: saved 3 Fla+ clones.
49.

[1242]

(A:) Single cell clones plated in MGA+40%NSB

18 empty

7 swarms, all had additionally: 50 1's; 41's; 10 cols incl 5-6 trails; 20 trails, few 1's; 3 3's 2'; 100 singles + short clusters

1 was contaminant ∴ all 6 segregated!

clones on MGA:

8 clones most had 1's + 2's

#21: 61's 8 clusters 3-6 1 T^{35} 1 T^{10} ←

#37: 18-1 3-C 1T

#27: 6-1 9-C 1T

MGA+40 #11: 37-1 5-C 2 1 T (>10/t; 2 had 100, 150 sectors) phot ←

10#: >100-1

48: 50-1 20-30 profuse trails

16: 9-T 38-1

18: 12-T 3-C 38-1. phot

20: 1-T$_{16}$ 60-1

22: partly sprayed; 1/3 T 2/3 1

30: 16-T [80, 80, ...] 5-C 55-1 ← phot

32 7-T profuse; 1-C; 56-1

34 75-1

38 17-T 3-C 45-1

40 too crowded

42 14-T 33-1

incubated 15 hours.

6/7/56.

1242B
-C

-1244

B: plant cells in spent broth.
growth limited to 100-1000. 18 x examined

3 - 0
3 - 100 Fla⁻
5 - ①/100
3 - ②/100 each isol.

4 isol. { 30-1, 0, 40-1 }

C1-2-3 < 160-1; 1 fuzzy T8 / 120-1; 2-T / all -1's

C14	1	㉙/1000	plated ⊕ 45.15.
C13	1	㉓/1000	
C6	1	⑱/1000	
C5:	1	⑩/100	Plant individually

gave some linear but also globular trails.
see photogr. ⇐
no profuse seen. note doubling of trails!

C. Plate pooled initials   Readings? See photos 1244. messy plates.

[1243]

Plate pooled initials various media.

	T	C	I	SW	Σ
MGA	88	12	9	5	114
MGA + 40% penassay	129	0	8	12	149.

but MGA itself seemed soft.

Single clones
12 blanks; See protocols   1244

6/7/56.

Plate 5b clones.

• 15 pairs; 24's

Trail distribution: (clusters as subsc.) x = no clone

3 – 11 – 1 – 0_2

1 – 0_1 – 0_x – 2

0 – 0_3

2 – 4

1 – 2

11 – x

0 – 0

0 – 1

x – 0

1 – 1

6 – 5

x – 1

x – 8

need more data of this kind. omit x – x (5)

[some photos] Note that agar too stiff.

6/8/56.

37 — × 666 serum effects.

A) also noted that H_2O ok for trap medium as dilute nutrient.

B) 36 ⊕ picked were completely inhibited by anti b or anti i.
overnight, clones in i, b serum: 1 wiggling cell isol but n.g.

C) ~~35~~ 43 clones plated. 35 viable incl 1 swarm (pure) MGA 60.
readings: ?

see photos

6/8/56 [1246]

Serum inh of trails [b, c.]

SW666x – FA11; x – FA32 initials viol to 1:100 i, b serum.

b serum inhibited both very quickly; i after 30–90 secs. Therefore serum n.g. in this combination.

similar plating trials: no trails here either.

[1247]

Check serums for inhibiting of trails 37–x666

a	+
b	–
c	+
d	+
i	–
k	+
n	+
1,2	+
B-0	±

∴ used a for further tests on this point.

TRAILS ∴ i almost only serum which inhibits. i did not give inhibited swarms.

(FA76a) a → x SW666

[1248]

a { no inhibit b trails; part. " a/b } b { inh all trails } at 1:1000 as well as 1:100

E coli Fla⁻

note problem of no absolute Fla⁻; better to use serologically well defined Fla⁻.

6/8/56.

1250
1252
1256
1258E

a → × SW666; serum effect: platings of ⊕

Confirmed   b inhibit all   see photos
a partially inhibit all

conclude all are initially inhibited. Some terminals may develop b, none a phenotype. May be able to conclude that no fragments are Fla⁺ s b effect.

b (minnesota) serum to check specificity; plating   1252

10 → × 666   results as above.

see phot.

no further test of specificity availability.

~~No expts c a in microclones?~~   1256

from 1202.

Tested residual ? trails in b serum. This would be one way of picking up Fla⁺ ex // Fla₁⁻ $H_1{}^a$. These were probably areas of nondiffusion of b serum. proved $H_1{}^b$.

Many $\frac{+}{}$ $\frac{+}{-}$ $\frac{+}{}$ $\frac{-}{}$ seen.

1258E a serum as a → × 666 initials: inhibition slow and incomplete.
Some aggl. pairs continue to swim.

1259D 3 oz ⊕ each tested in a, b serum. a inhibited more slowly than b but initials all inhibited except c 2% which persisted at all.
4 all persisted in b, 3 viable were swarms.
2 a 0 viable.

of later intern isolates, e.g. B615 ④/a serum → 2 remained motile. most others immobilized. Conclude some terminals not affected by a. while all initials are.

Correlation of initial motility & progeny; segregation in swarms

56 ① isol for microcolonies Examined for ⊕; underestimates

NG 13 swarms 4/+1 0/2^{13} 6 ⑨/2^{13} 2 2 >10/2^{13} 11

high incidence of ε

5 clones harvested for replating of intermediates ⊕.

B3
B12
c9
c1
D1 } 1's and v.s. tails only

D1 swarms. Replate residue: 8 sec/92 colonies. ∴ only ≈ 10% of sec. not tails from init isol.

Note: this clone scored as ⑩/10^4; must have had (10^3)/10^4!

∴ Take segregation ratios as:

	$F/a^+/F/a^+ + F/a^-$	
D1	~~8%~~	8/100
c2		4/47
c11		4/33
D7	~~100%~~	10% not repl.
D8		3/28

Note swarms were flared but not entered.

Note distribution of clones by ⊕ was

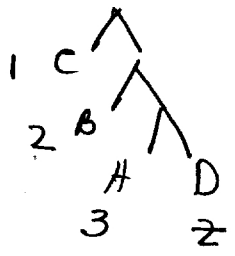
	inv	0 ≠ 1	2-5	6-9	10	>10	sw	Σ
Overall all	13	6 9	10	3	3	8	5	
scored as not active	2	1 2	6	0	2	1	1	

No obvious correlation between progeny + initial motility of method.

inv	0				sw	
broader clones. 13	6	22		11	5	57
2	1	8		3	1	15
—	0	1-9		10-40	sec	

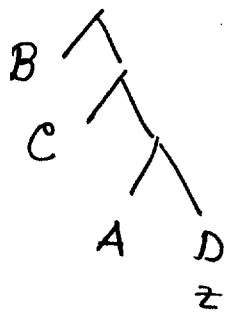
1254. a

According to my reconstruction of 1254, the indicated pedigree is



But, e.g., in #1, E chosen is listed as 2.

as if



Can this be right?
I think I must be correct now!

---

partition of E

for n_4 minimal

E	#	E+P.
1	~~1~~ +1	4
2	~~3~~ +1	2
3	~~3~~	1
4=Z	~~0~~ +1	1

# coer.	
3+1	= 4
2+1	3
1	1
0	0

+ 

= (3-4)

and one clone c/ Z disappeared.

contemporary summary is either wrong or my interpretation of the ~~result~~ argument!

→

See Summary

IGNORE

1253-4

Tetrahymena

Z and Fla+ chains.

6/8/56

pub. in W 1177, W 2802
superseded by later tests in methocel.

1254.

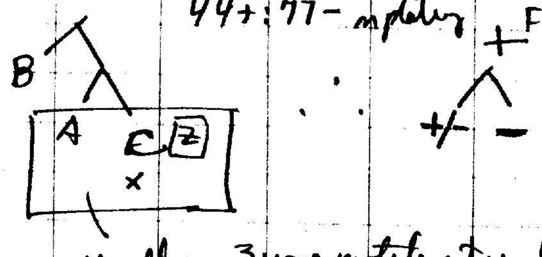
1 Released Tz dissolves in oil
2 Z taken up only by stationary cultures.

(a) 93 → x 666 A prestained cells B) poststained cells.

Almost no motile A were stained; only 20% of motile B were stained; not random sample; 50% of parent population was! Z chains followed 4-6 fissions

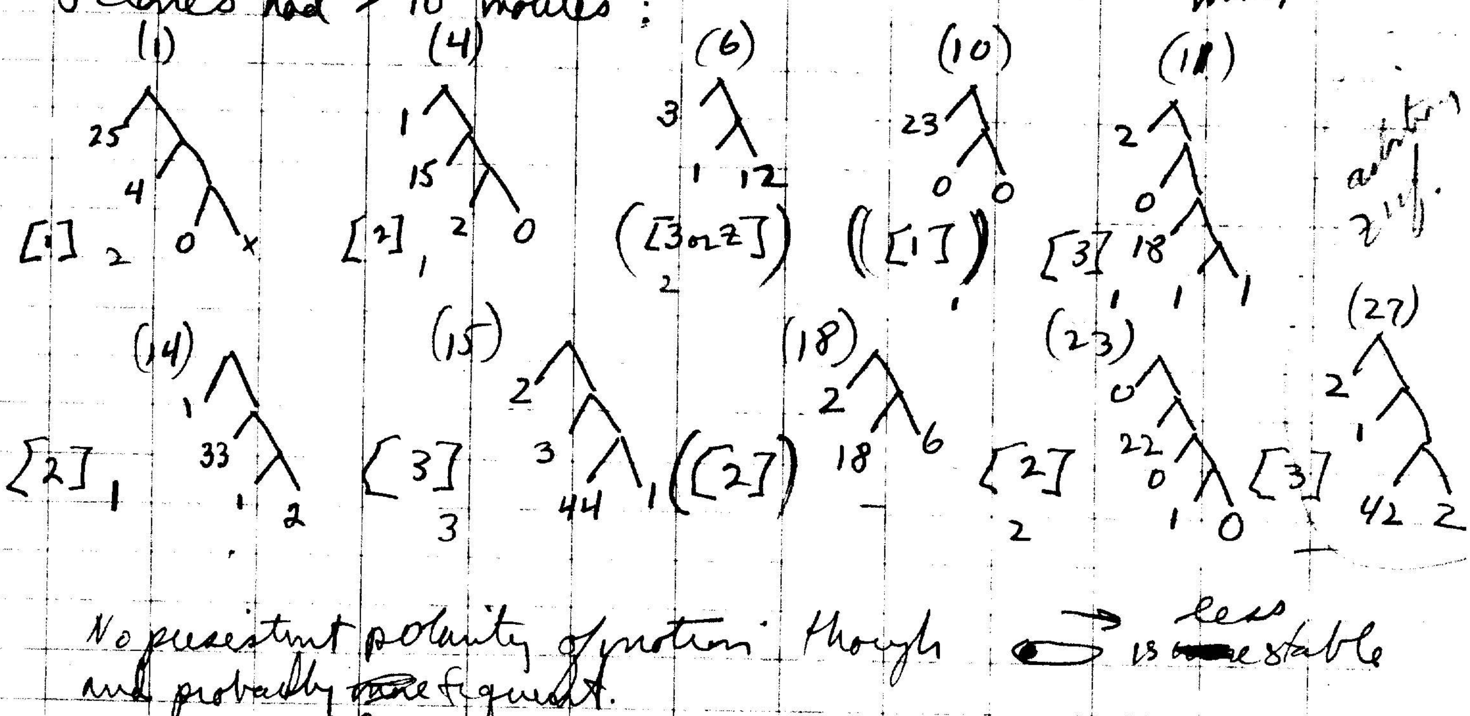
28 isolates B, 2 from A.

1 swarm = 31 B. What is pedigree? Need to reconstruct the tabulations.
44+ : 77 - in plating + Fla



Halls. 3 were motile at isol; none later.

8 clones had > 10 motiles: See correction next page

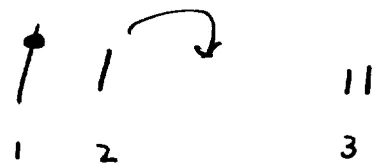


No persistent polarity of motion though ⟶ is less stable and probably less frequent.

6/9/06.

The problem is how to read protocols.

E.G.



Does |↷ (2) mean this cell is interpolated between 1 and 3 or put to right of 3. As practical matter, almost certainly the latter. Then sequence becomes:

A	B	[C	D]	E
4	1	[2	3]	2

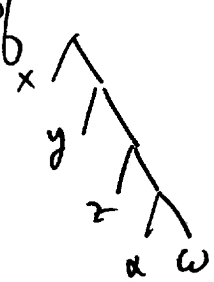
rather than

A	B	C	D	E
4	3	2	1	2

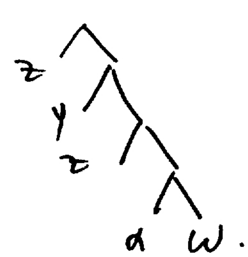
To translate pedigrees as given (own:), invert the central items.

A and E still have to be the the terminal sides.

i.e., instead of



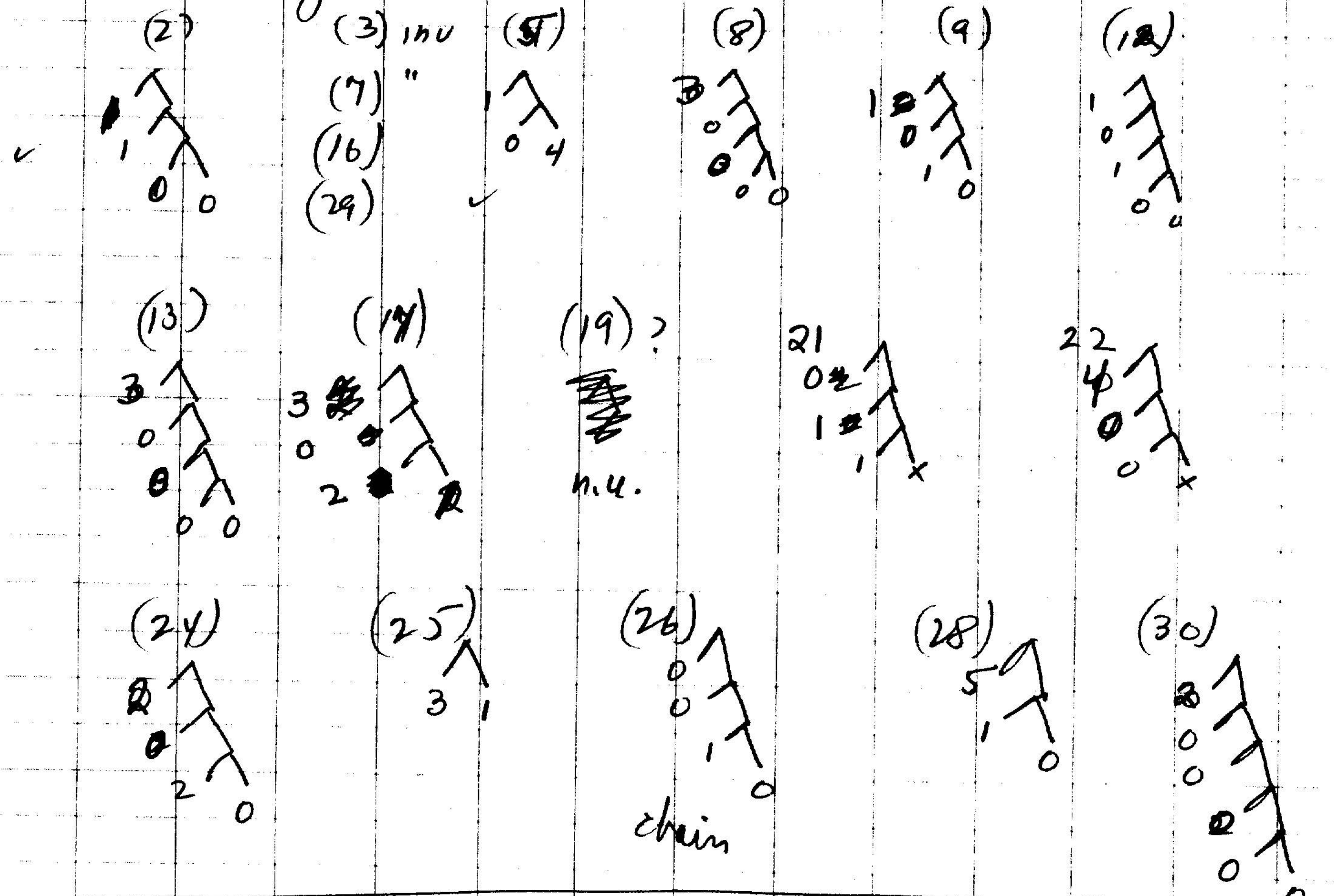
read



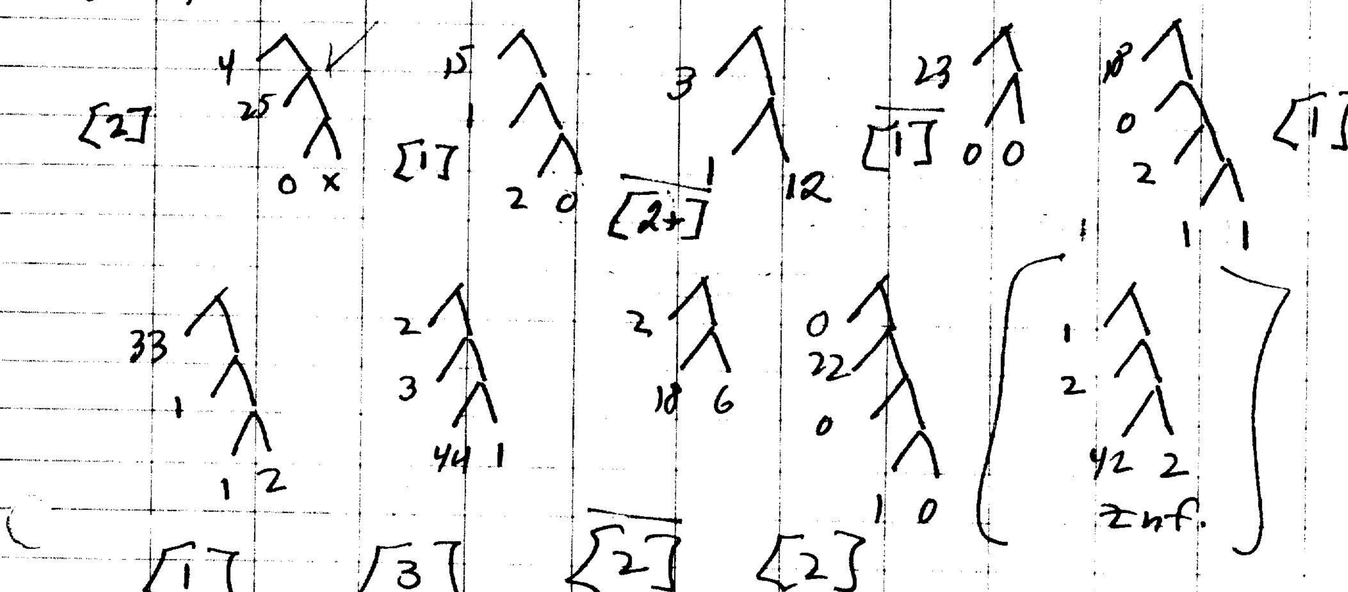
This now agrees w/ previous summary.

— SEE SUMMARY ~~IGNORE~~ of partition of chains counted.

Remaining classes are non-E.

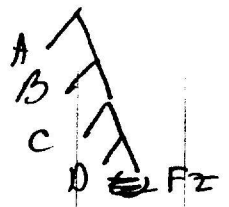


Counted E classes:



SUMMARY

1254
tabulate.



E clones.

	A	B	C	D	W	Σ	
✓	4	25	0		x	29	
✓	15	1	2		0	18	
= (	3	1	.....		12	16	) ≡
✓	23	0			0	23	)
✓	18	0	2	1	1	22	
✓	13	1	1		2	17	
	2	3	(44)		1	50	
(	2	18			6	26	)
	0	22	0	1	0	23	
Z inf. → (	1	2	42		2	47	)
Distributions #	4	3	1+1	[1]			
swarm	0	⊞	+		x		
nest	1	1	0		0		
	1	0			4		
	3	0	0	0	0		
	1	0	1		0		
	1	0	1	0	0		
	3	0	0	0	0		
	3	0	2		1		
	0	1	1		x		
	4	0	0		x		
	2	0	2		0		
	3				1		
	0	0	1		0		
	5	1			0		
	2	0	0	0/0	0		

Forward 4:3:1:0
Random Exp: 4:2:1:1
Poker Exp 4:0:0:4

Neg. correlation of Z and E not excluded. But

1	
5	see 1259

see 1259 B2 for more

Initials with Fz granule. Follow the Z chain to W.

10 E clones
1 swarm
14 E.
4 inviable

T2; Methocel.

1255
7
8

DATE: 6/7/56. REF:

	1	2	3	4	5	6	7	8	9	10

W1177 Used 2% methocel 4000 to form necklaces i usually terminal 2. See sketches. This particular series too slowly.

W1177 much same. 1257

10 ditto tried glow beads as positional markers but this n.g. 1258
Note interstitial lysis of necklaces after refrigeration.
some 2 ~~lysis~~ chains i terminal 2 still intact.

~~Suggest~~ +10 cps methocel to slow up Fla+
Noted that swarm cells are much more active than initials. Residual motiles are 6 sw 7E 4 # 4 ng. suggesting selection in favor of #. These were ↓ at 10^{3}-10^{4} dilutions of ⊕
9, 2, 12, 1, 12, 4, 18, 24, 2, 15, 16.
Also attempted to isolate E from interim clones in methocel, unsuccessful.
2% MeC 400 stops 80-90% of Fla+. No selections are E
1% ~~stops~~ none; move more slowly

~~A Sketch~~

A. No Z. 1% methocel 400 (probably too thin) (15% silated)

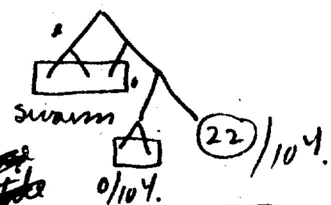
13 clones 2?E in cursory study. most active 10, 11, 12, 14.

B. Z. Partial separation of early fissions.

(-C) 38 initials 3ng. 4 E 5 sw.

some partition data E: (E) 15, 10, 11, 6 See for partition info.

SW: B2 interesting



C8 |||| |

0/104. sw

3 others uninformative on segregation.

D see 1250 serum effect.

E. Like B but no separation. 34 isolates

4E 9 11 15 16

25 ∅ 0^3 1^5 2^1 3^4 4^2 5^2 6^1 7^1

sw 5 not plated.

inv 5

conclude no selectivity for E cells in 1% methocel.

(1259 B-C)

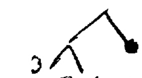
ΦE   Φsw /34 isolates.

b 1   ≡ III { ♀ → 37/ i 10^4.   (15)/10^4. 10^3.

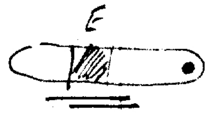
b 2   swarm seen

b 10   no sepn. ♀ → (10)/10^4.

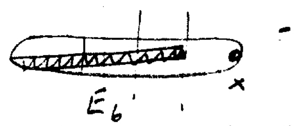
c 1



10^4. 3 10. (11)/10^4.



c 2   III (6)   ♀/9 → x 10^4.



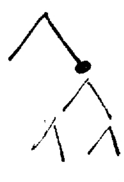
c 4   ♀ not sep. → sw? > (10)/10^4. ~~before~~ picking not plate

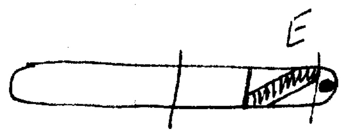
c 5   x sw x. ∴ ♀ → swarm ~~not plated~~ prime.

c 8   IIII.I / ♀ < .sw .sw

Fla⁻ 0/10^4.   swarm.

preserve   b1 10^4.





(36) ♀

↓

(15)/10^4 10^3.

MeC 400 1½%

1260

6/10/13

4 partitions.

DATE:

REF:

1	2	3	4	5	6	7	8	9	10

Treated slide — 10% selected by MeC — No initial as active as 54D Fla+

3rd ~~24~~ 20 lobes — splits — Some partitions at 4,

A. (4) : 2 inv 14:1 6:5

15 11

B. (1)/x (1) (1)/4/1/x (14) (1) (1) (3) 0/0/0

4 inv (1)/(1) 2/1 0/(1)/0 0/4/1 1/3 3 0 0

1 sw. 0 0 2/1 1/0 (5) sw 0/0

20 ≠

1 E

++++

and ~~6/5~~ 1/20 8/20 2/2 4/12 3/2 7/26.

4 E

2 ≠

∴ Totals.

E ~~5~~ 7

≠ ~~23~~ 23

sw 1

x 6

~~37~~ 37.

no marked selection for E

distributions

0	6
1	4
2	2
3	4
4	2
5	3
~~4 5~~	1
~~11~~	1
~~14~~	1
inv	~~4~~ 6

~~15~~ 16 ~~2~~ ~~1~~ 12

leads: 7, 11, 14, 15, 16, 21, 28, 33

Methoul 1261

DATE: REF:

1	2	3	4	5	6	7	8	9	10

2% MeC 400. discrimination factor about 1% 8/1258.

A. (Takes too long to establish differential) + writing problem

Clones ' partition :

⊕/10^{3}-10^{4}:

25, 20, 3, 16, 1, 6, 2, 50, 3, 20, 18, 3, 20, 7, 11, 13, 10, sev

and 2 inviable.

10E 7F

44+ / 285 -

Remarks: better to wait until linkage organization is better understood or for similar strains ε crossing systems.

Fla+ also greatly slowed but must not matter.

McCl 400 2% 1262

pedigree attempts.

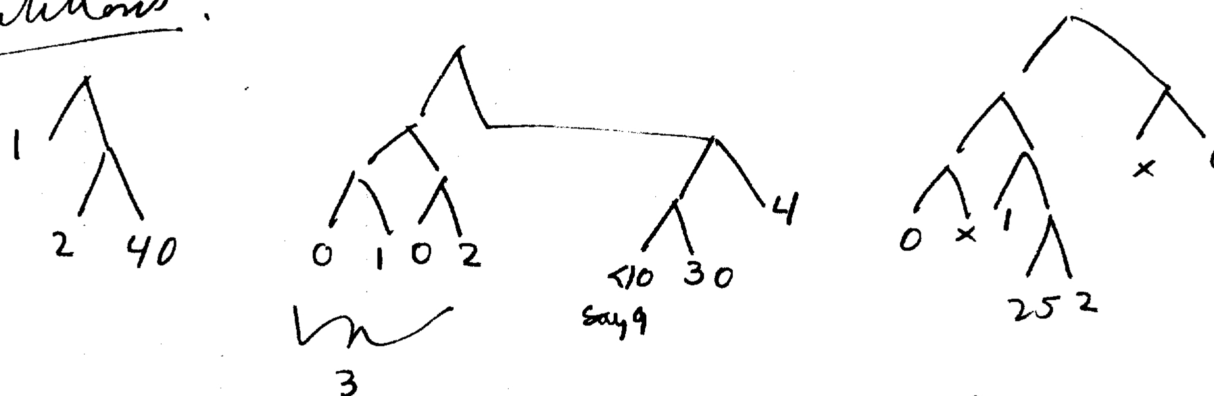
No cont. motile cells (like Fla+) seen.

34 initials; only 3 E's and 1 swarm (pure at n_2 n_3)

distr.

	n.g	10	
22E	0	11	
	1	5	
	2	2	
	3	1	
	4	1	28
	6	1	37
	7	1	43.

Partitions.



42:1
40:2

40: 3
39: 4
30: 9

28:0
28:0
27:1
25:2

no selection by McC.

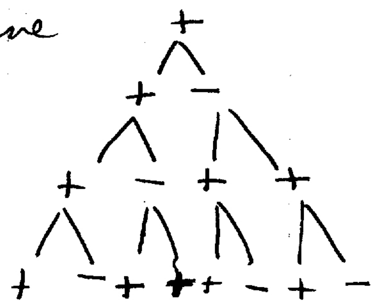
Leifson cultures. 1272

6/10/56.

H1. Ps. aeruginosa typically —OO probably charact.

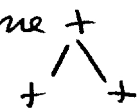
H32 vibrio =O and =OL

did have



see [242] before writing.

but same

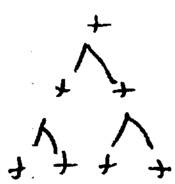


also.

— later → +.

Salmonella typically is

from many observations



Summary of splits

Exclude vague splits

DATE:

REF:

Column 1

1134 A1 1 10

A5 10? 50

1138 B4 { 1 17
4 13 }

1141 A4 { 2 31
1 30
11 19
1 18 }

[B4 1 14 but sep 1 cell from 7]

1143 E2 2 7

1144 B4 [illegible] 27

A1 0 16

1147 F2 1 12

1149 B5 { 4 12
2 10 }

D3 1 12

D5 { 2 35
0 30 }

E4 1 40

F4 2 40

F2 2 20

1150 F2 6 30
E3 4 8
E4 7 13
A1 1 43
E3 6 30
E5 1 20
F1 4 27

– possible ambiguity but I think OK.

Incl (15) from intermediate 12 l.

Columns 4–5

1212 C1 6 7
B3 6 3
D1 4 7
F1 1 7
C5 { 2 24
3 21
1 20 }

1244 { 1 14
3 11 }

6 5

1254 { 4 25
0 25 }

3 15

3 13
1 12

23 0

4 18

4 13

2 48
3 45
1 44

2 24
6 18

0 23
1 22
~~[illegible]~~
1 46
2 44
2 42

1259 0 22

Columns 7–8

1259: 0 36
0 36

0 11
0 11

1260 1 14
5 6

1 20

8 20

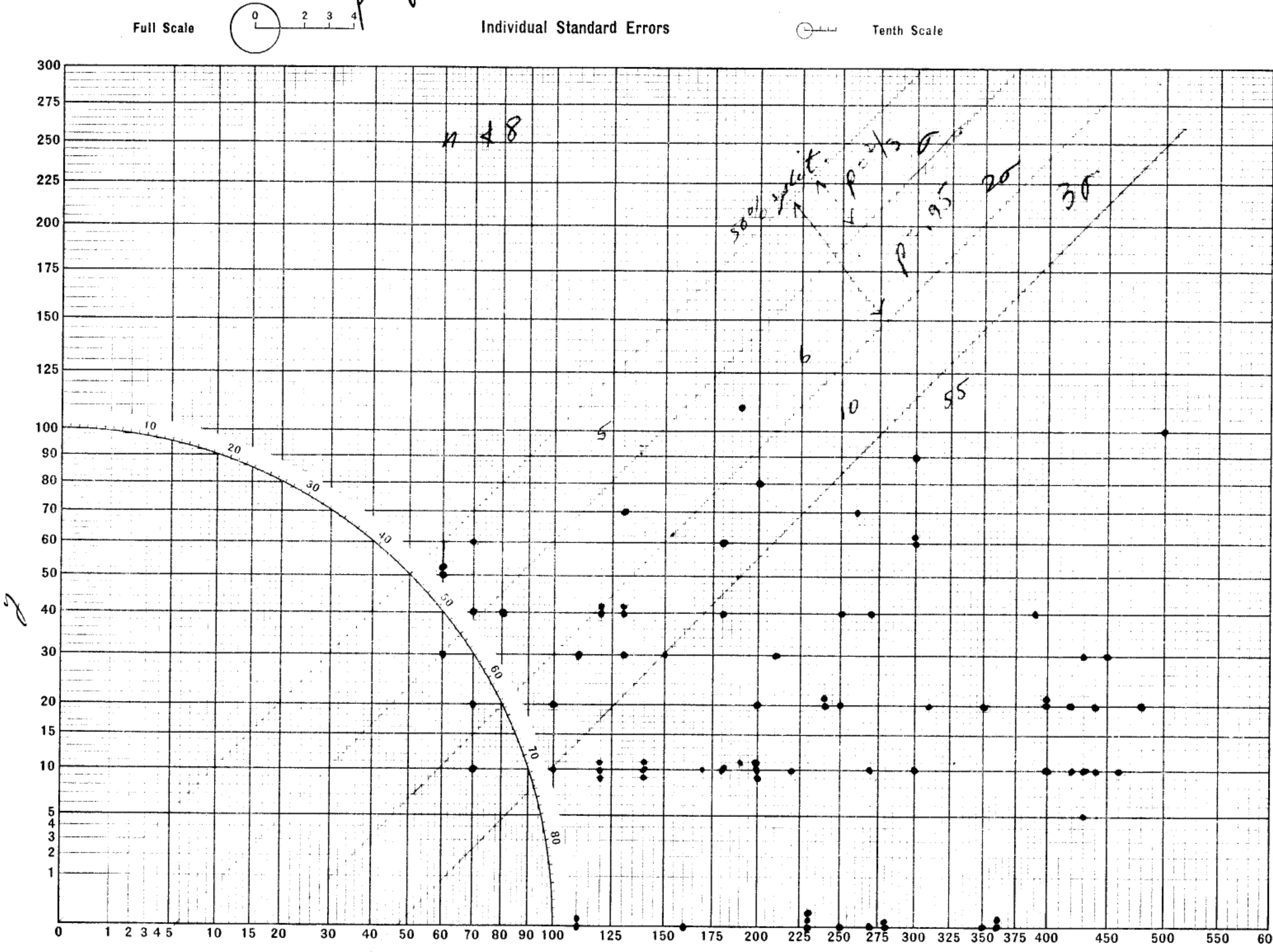
4 12

7 26

1262 1 42
2 40

3 43
4 39
9 30

0 28
0 28
1 27
2 25



Review summaries.

DATE: | REF: cf. orthoclones

1	2	3	4	5	6	7	8	9	10

1. Lines. tabulate only long (10 or more gen?) and late ones. Give statistics on others.

2. Partitions. Use scatter diagrams? on prob. paper! (Relate succ. fractions in any clone?) for $\Sigma = \pm 8$

3. Swarm-containing clones: segregation.

4. distribution of lines per clone.

5 Serum effects.

How many isolations; pedigrees?