chromosomes are rather small, so it is quite likely that minor chromosome deficiencies or deletions would not be detected.

Recently, we have obtained from commercial sources two spontaneous chromosome aberrants, one of which appears to be wholly tetraploid, while the other is chimaeric, containing diploid as well as tetraploid tissues. The first named is known to be, and the other probably is, a bud sport from William Sim. Both are red-flowered. Both seem to be identical in appearance with colchicine-induced tetraploids produced by Stewart[8] and kindly shared with us. These aberrants will be subjected to genetic analysis as soon as suitable tetraploid test plants are available. Diploid test plants cannot be used so readily for this purpose because of very low seed production in crosses between tetraploids and diploids.

Summary.—These results indicate that the carnation William Sim and four mutant clones, Pink Sim, White Sim, Peppermint Sim, and Skyline Frosted Sim, all have the same genotype: *AA Ii Yy SS rr mm*. The mutant clones thus are somatic variants, probably in the nature of periclinal chimaeras, with the mutant condition on the outside, but not deep enough to affect the formation of gametes.

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*A PARTIAL MAP OF LINKAGE GROUP D IN NEUROSPORA CRASSA**

BY M. B. MITCHELL AND H. K. MITCHELL

KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY

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In connection with investigations of the genetic behavior of pyrimidine mutants in linkage group D (group IV of Barratt and Garnjobst[1]), data were obtained on linkage of other mutants in this group. This paper reports these data, together with results from additional crosses. Although these results are not entirely unambiguous, they appear to indicate the order of eleven genes located on one arm of the chromosome.

Description of Mutants.—The biochemical mutants used are, as follows: *arg* (33442), arginine;[2] *pdx* (37803), pyridoxine, not pH-sensitive;[3] *pyr 1* (263) and *pyr 2* (38502), pyrimidine, and *pyr 3* (37815), pyrimidine, temperature-sensitive;[3] *ad* (28610), adenine; *hist* (C141), histidine;[4] *pan* (34556), pantothenic acid.[3]

Of the three visible mutants, one, *co* (70007), "colonial," has been described.[2, 5] A second, *cot* (C102), "colonial," temperature-sensitive, has been described only

briefly,[6] although it has been quite useful as a genetic marker. Its growth appears normal at 25° C., but at 35° C. it is as much reduced as that of many biochemical mutants on minimal medium and is of an extreme "colonial" nature. As the temperature is lowered from 35° C., the "colonial" character becomes less extreme. A property of the mutant which facilitates scoring in certain crosses is that young hyphae, formed at 25° C., will, when put at 35° C., quickly begin to form branches, close together, all along their length.

The third mutant, *dn* (38502), dingy, has been so named because of gray lumps of material, presumably due to excess formation of microconidia, which appear in slant cultures. On agar surface the hyphae grow more slowly than wild and are bunched, crinkled, and oriented down into the medium. This mutant occurred with *pyr 2* (38502).

Two-Point Crosses.—The data in Table 1 were obtained from counts of random ascospores allowed to germinate on minimal agar in Petri dishes and incubated at the appropriate temperature (25° or 35° C.) for a time sufficient to permit the

TABLE 1

RANDOM SPORES FROM TWO-POINT CROSSES

Cross	Mutant Spores	Wild Spores	Distance (Units)	Germination (Per Cent)
co × *arg*	4,811	9	0.36	80–90
co × *pyr 3*	4,080	33	1.5	80–90
co × *pdx*	4,072	80	3.8	60–80
co × *pyr 1*	4,138	42	2.0	50–60
arg × *pyr 3**	2,125	12†	1.1	90–100
pyr 3 × *hist*	2,670	446†	29	90–100
co × *cot*	3,203	568	30	80–90
cot × *pyr 3*	2,651	376†	25	90–100
cot × *pan*	14,929	180†	2.4	90–100
cot × *ad*	2,489	53†	4.2	60–80
cot × *hist*	13,016	438†	6.4	80–90
cot × *pyr 2*	8,697	1121†	23	90–100
hist × *pyr 2*‡	2,128	320†	26	60–80
dn × *pyr 2*	2,271	43†	3.6	90–100
co × *pyr 2*	2,007	516	41	60–80

* M. B. Mitchell, T. H. Pittenger, and H. K. Mitchell, These PROCEEDINGS, **38**, 570 (1952).
† Pseudo-wilds included.
‡ F. Haas, M. B. Mitchell, B. Ames, and H. K. Mitchell, *Genetics*, **37**, 217 (1951).

difference in growth capacity between wild-type and biochemical mutants to become apparent. The figure given for each cross for the distance between genes is twice the percentage of spores which were wild with respect to both mutants.

These data indicate three groups of mutants, with the members of each group closely linked and the adjacent groups separated by distances of about 30 and 20 units. One group consists of *co*, *arg*, *pyr 1*, *pyr 3*, and *pdx;* another, of *cot*, *pan*, *hist*, and *ad;* and the third, of *dn* and *pyr 2*.

Three-Point Crosses.—The asci tabulated in Table 2 were dissected on minimal agar plates. The plates were first incubated at 25° C. and *dn* and *co* scored, then incubated for 3 or 4 hours at 35° C., after which *cot* could be scored. Segregations in these asci are consistent with the following gene order: centromere—*co*—*cot*—*dn*. From 279 asci from eight other crosses, a centromere distance of 13 units was obtained for *co*.

Table 3 gives results of counts of random spores from crosses of double mutants to single mutants. It was assumed that, with three closely linked mutants, double

crossovers, if they occurred, would be infrequent and that the gene order could be deduced from the types of recombinants appearing which were wild with respect

TABLE 2

ASCI FROM THE CROSS *co cot dn* × +++

SEGREGATION				No. ASCI	CROSSOVERS*
co cot dn	*co cot dn*	+++	+++	11	None
co cot dn	+++	*co cot dn*	+++	7	R1
co ++	*co cot dn*	+ *cot dn*	+++	16	R2
co ++	*co* ++	+ *cot dn*	+ *cot dn*	2	R2 (double)
co cot +	*co cot dn*	++ *dn*	+++	10	R3
co cot +	*co cot* +	++ *dn*	++ *dn*	1	R3 (double)
co ++	+ *cot dn*	*co cot dn*	+++	2	R1 R2
co cot dn	+ *cot dn*	*co* ++	+++	2	R1 R2
co ++	+ *cot dn*	*co* ++	+ *cot dn*	1	R1 R2 (double)
co cot dn	++ *dn*	*co cot* +	+++	3	R1 R3
co cot +	++ *dn*	*co cot dn*	+++	2	R1 R3
co ++	*co cot dn*	++ *dn*	+ *cot* +	3	R2 R3
co cot +	*co* + *dn*	+ *cot dn*	+++	2	R2 R3
co cot dn	*co* + *dn*	+ *cot* +	+++	2	R2 R3
co ++	*co cot* +	+ *cot dn*	++ *dn*	2	R2 R3
co ++	*co* + *dn*	+ *cot dn*	+ *cot* +	1	R2 (double) R3
co cot +	*co* + *dn*	+ *cot* +	++ *dn*	1	R2 R3 (double)
co ++	+ *cot* +	*co cot dn*	++ *dn*	2	R1 R2 R3
co cot +	+ *cot dn*	*co* + *dn*	+++	1	R1 R2 R3
co + *dn*	+ *cot* +	*co cot dn*	+++	1	R1 R2 R3
co cot dn	+ *cot* +	*co* + *dn*	+++	2	R1 R2 R3
				74	

* Region 1 = centromere to *co*—15 units; region 2 = *co* to *cot*—28 units; region 3 = *cot* to *dn*—23 units. Asci showing the same segregation patterns but with different arrangements of spore pairs are lumped together.

TABLE 3

RANDOM SPORES FROM THREE-POINT CROSSES

	CROSS		RECOGNIZABLE RECOMBINANTS +++	RECOGNIZABLE RECOMBINANTS *co* ++ *cot* ++	REMAINING MUTANT PHENOTYPES	GERMINATION (PER CENT)
1	*pyr 1* + *co* × + *pdx* +		2	25	4,055	60–80
2	*pyr 1* ++ × + *pdx co*		61	1	7,488	80–90
3	*pyr 1 co* + × ++ *pyr 3*		37	79	3,031	80–90
4	+ *co pyr 3* × *pyr 1* ++		20	6	2,585	80–90
5	++ *arg* × *pyr 1 co* +		8	182	8,246	90–100
6	+ *co arg* × *pyr 1* ++		130	5	3,904	60–80
7	++ *pyr 3* × *pdx co* +		55	78	4,370	50–60
8	+ *co pyr 3* × *pdx* ++		139	73	7,818	80–90
9	++ *arg* × *pdx co* +		4	45	3,853	80–90
10	+ *co arg* × *pdx* ++		45	3	2,715	60–80
11	*co* + *pyr 3* × + *arg* +		0	77	15,771	80–90
12	++ *pyr 3* × *co arg* +		53	1	10,285	80–90
13	+ *pan* + × *ad* + *cot*	(27)†	50* 0	15 9	5,118 2,991	90–100 90–100
14	+ *cot hist* × *pan* ++	(64)†	32* 3	2 4	4,077 9,702	90–100 90–100
15	+ *cot hist* × *ad* ++	(13)†	78* 27	85 49	8,315 4,562	80–90 80–90
16	++ *dn* × *cot pyr 2* +	(59)†	30	5	4,733	50–60
17	*cot* + *dn* × + *pyr 2* +	(92)†	8	29	5,728	60–80

* Pseudo-wilds included.
† Pseudo-wilds.

to both biochemical mutants involved. Thus, if two biochemical mutants, *b1* and *b2*, are on opposite sides of a visible mutant, *v*, then each of the crosses *b1v* × *b2*

and *b1* × *b2v* will give both wild and *v* progeny from single crossovers. If *b1* and *b2* are on the same side of *v*, then, from single crossovers, one of the crosses will give wild and the other *v* progeny. Which cross gives *v* and which gives wild will depend upon the order of *b1* and *b2* with respect to *v*. It may be seen, from crosses 3 through 10 in Table 3, that, by these criteria, *pyr 1* and *pdx* behave as if they are on the opposite side of *co* from *arg* and *pyr 3*. Therefore, only one type of recombinant which is wild with respect to both biochemical mutants would be expected from each of the crosses 1, 2, 11, and 12, whereas, from 1, 2, and 12, both types were found. If it is supposed that the rare recombinants from these three crosses arose from some phenomena other than single crossovers, then the data from all the twelve crosses are consistent with the order *pyr 1—pdx—co—arg—pyr 3*. Crosses 1, 2, 3, 5, 7, 9, and 11 have been repeated with different isolates of the mutants and double mutants, with essentially the same results.

A further indication that the order is *pyr 1—pdx—co—pyr 3* was obtained by observing asci from crosses 1 and 7, since among these asci were found all types which would be expected to result from single crossovers in the marked regions if the gene order is that indicated. From cross 1, among 86 asci, the following types showing recombination were found:

pyr 1 + +	*pyr 1 + co*	*+ pdx +*	*+ pdx co*	2
pyr 1 pdx +	*pyr 1 + co*	*+ + co*	*+ pdx +*	1

Among 47 asci from cross 7, the recombinant types were as follows:

+ co +	*+ + pyr 3*	*pdx co +*	*pdx + pyr 3*	4
+ + +	*+ + pyr 3*	*pdx co +*	*pdx co pyr 3*	3

A cross of *pdx co pyr 3* to wild gave results which are in agreement with this gene order, since among 3,418 spores, none were *+ co +*.

Segregations in 98 asci from a cross reported elsewhere,[3] of *pyr 1* to *pyr 3*, indicate that *pyr 1* is nearer to the centromere.

The unexpected recombinant types from crosses 1, 2, and 12 when crossed to wild appeared to be genetically pure wild or *co + +*. They are being investigated further.

Pseudo-wilds[2] from crosses which have *co* in one parent can be distinguished from true wild types on minimal plates. They grow more slowly, at least initially, and have straighter, more precisely branched hyphae. Hence pseudo-wilds have been excluded from counts from all crosses involving *co*. They were usually observed from these crosses, but rarely with a frequency greater than about one or two per thousand germinated spores. Pseudo-wilds from crosses involving *cot* and *dn* were not at first recognized as such, and in Table 1, if they occurred in these crosses, they are included with the true wilds. Later on, when crosses 13 through 17 in Table 3 were being examined, it was found that two types of wilds could be distinguished. One type resembled pseudo-wilds from *co* crosses, having straighter, more precisely branched hyphae, but the extent of growth was much more nearly that of true wilds. Samples of these isolated from crosses 13, 14, 16, 17 (and 18 and 19 below), proved to be heterocaryons composed of the two parent types in each case, and it is therefore assumed that these are pseudo-wilds. It may be seen from the counts from crosses 13, 14, and 15 that only if this type is excluded is it

possible to reach a conclusion about the gene order based on the appearance of + + + and *cot* + + recombinants. The more probable order then appears to be *ad—pan—cot—hist*, since crosses 14 and 15 gave both + + + and *cot* + +, whereas cross 13 gave only *cot* + + and pseudo-wilds. From crosses 16 and 17, with pseudo-wilds excluded, the order appears to be *cot—pyr 2—dn*, since, with this order, in cross 16, + + + could arise from single crossovers between *pyr 2* and *dn*, but *cot* + +, which is less frequent, would require a double crossover, one between *pyr 2* and *dn* and another between *cot* and *pyr 2*. In cross 17, *cot* + + could arise from single, and + + + from double, crossovers. In the same way, the order of *ad*, *cot*, and *hist* with respect to *dn* is indicated by the counts shown in the accompanying tabulation. The percentage of germination was estimated to be 50–60 in cross 18 and 60–80 in cross 19.

	Cross	+++	++ *dn*	Pseudo-wild	Other Types
18	+ *cot dn* × *ad* ++	56	4	28	3,817
19	*cot* + *dn* × + *hist* +	8	52	62	3,503

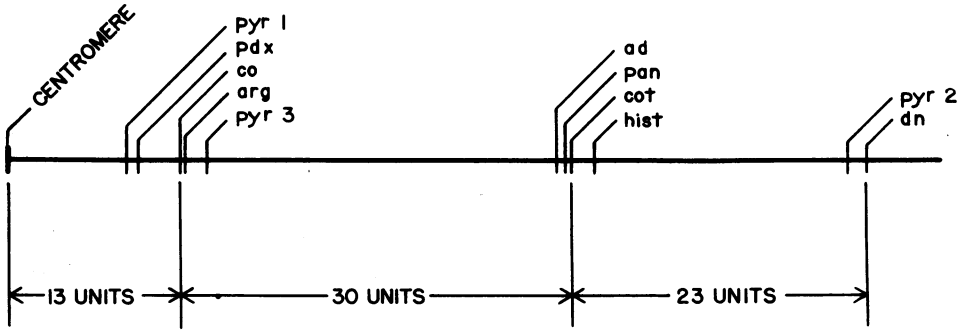

FIG. 1.—Gene order considered more probable on the basis of data presented.

The indicated gene order for all the mutants is given in Figure 1. The figures used in drawing the map to scale are not averages from all crosses from which each figure could be obtained but are derived from one or two crosses considered to be the most reliable, i.e., ones with the largest numbers of spores and the highest percentage of germination, in which wilds and pseudo-wilds were counted separately.

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