THE INHERITANCE OF CERTAIN CHARACTERS IN OENOTHERA HYBRIDS OF DIFFERENT CHROMOSOME CONFIGURATIONS¹

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INTRODUCTION

Perhaps the most significant development in the genetics of *Oenothera* in recent years is the finding of constant differences in the linkage relationships of certain characters and the association of these variations with constant differences in the chromosome configurations of the plants concerned. RENNER (1927) has shown, for example, that in one cross a character may be closely or completely linked to a second character and independent of a third character, while in another cross the first character will be independent of the second and linked to the third. CLELAND and OEHLKERS (OEHLKERS 1926, CLELAND and OEHLKERS 1929) have found that characters which are independently inherited in hybrids with pairing chromosomes are linked in hybrids in which the chromosomes are associated in large rings during meiosis.

A similar relationship between linkage of characters and association of chromosomes is found in the crosses reported in this paper.

¹ Paper from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 344.

DESCRIPTION OF TYPES

Oenothera franciscana

Oenothera franciscana Bartlett has been grown extensively by Professor DAVIS who has described this species in some detail in his paper of 1916. The characters of *franciscana* that are important in the experiments herein reported are its red bud-cones (figure 1), yellow flowers, and the *franciscana* growth-habit. CLELAND (1922) found that *franciscana* had five chromosome pairs and a circle of four chromosomes. Since then KULKARNI (1928) has reported the breaking of the circle into two pairs preceding the heterotypic metaphase. The *franciscana* plants used in the following experiments were not examined cytologically, but there is evidence (reported below) that one parent plant had the circle of four chromosomes. *Oe. franciscana* appears to be genetically homozygous and is apparently free from lethals. There are almost no empty or shrivelled pollen grains.

"Franciscana sulfurea"

The hybrid called "*franciscana sulfurea*"² arose from the cross *Oe. bien-nis* × *franciscana* (DAVIS 1916). It agrees closely with *franciscana* in growth habit, but has green bud-cones (figure 1) and sulfur-colored flowers.

FIGURE 1.

² This form has also been grown by SHULL (1923b) who obtained his material from Professor DAVIS, but Professor SHULL's present cultures must represent a secondary derivative of the parent form as his plants have all pairing chromosomes (CLELAND 1927, ILLICK 1929) and apparently have red bud-cones—"similar to *franciscana* genetically except for its sulfur-colored flowers" (ILLICK 1929, p. 614).

Fifty percent of the pollen grains are empty and shrivelled. CLELAND (1924) found a circle of twelve chromosomes and a single free pair in this form. The parents used in the following experiments were not examined cytologically, but the cytology of F₁ culture 288 (reported below) of the cross with *franciscana* and of F₁ cultures of crosses with *Oe. Hookeri* suggested that the chromosome configuration of "*franciscana sulfurea*" had become altered. One plant (650-3), a descendant of the inbred parent of culture 288, happened to be still in flower in our garden in December. This plant was examined by the aceto-carmin method and found to have two pairs of chromosomes and apparently a circle of ten (plate 1).³

When inbred, "*franciscana sulfurea*" regularly produces the parent type and a new form called sulfur-dwarf.

Sulfur-dwarf

The sulfur-dwarf segregate from "*franciscana sulfurea*" differs from the parent form in bud color and in stature. It has red bud-cones similar to those of *franciscana*. In growth-habit it differs from *franciscana* chiefly in height. The central axis of the dwarf is roughly three-quarters as long as in *franciscana*. The lateral branches are generally fewer and less erect and the leaves somewhat narrower in the dwarf than in *franciscana*. Sulfur-dwarf has been found to have almost no empty pollen grains, and all of the chromosomes pair regularly (EMERSON 1928). Sulfur-dwarf breeds true.

Splashed bud-cones

A new bud color sometimes appears in crosses between *Oe. franciscana* and "*franciscana sulfurea*." The bud-cones are distinctly red, but generally less pigmented than the red bud-cones of *franciscana*. The greatest difference is in the pattern of the pigmented area. Red bud-cones are strongly pigmented near the tip, while splashed bud-cones are always green near the tip (compare figure 1).

CROSSES BETWEEN "*FRANCISCANA SULFUREA*" AND SULFUR-DWARF

From self-pollinated "*franciscana sulfurea*," a culture of 93 plants was grown (culture 394 of 1928), in which were 90 "*franciscana sulfurea*" plants and 3 sulfur-dwarfs.⁴ The same plant pollinated by sulfur-dwarf yielded

³ The inbred descendants of 650-3 have since been examined and found definitely to have a ring of 10 chromosomes and 2 pairs.

⁴ Professor DAVIS has extensive unpublished data on the genetics of "*franciscana sulfurea*." He has often found much higher percentages of sulfur-dwarfs in progenies of selfed "*franciscana sulfurea*."

132 "*franciscana sulfurea*" plants and 9 sulfur-dwarfs (culture 397). In the reciprocal cross (culture 398) were 64 sulfur-dwarfs and no plants of the "*franciscana sulfurea*" type.

CROSSES BETWEEN "*FRANCISCANA SULFUREA*"
AND *OENOTHERA FRANCISCANA*

Reciprocal crosses between *Oenothera franciscana* and "*franciscana sulfurea*" were made in the summer of 1923, from which F₁ cultures 58 and 59 were grown the following summer. In 1927, Professor DAVIS kindly gave me seeds of similar crosses made by him in 1925. From these seeds F₁ cul-

TABLE 1

*F*₁ frequencies in reciprocal crosses between *Oenothera franciscana* and "*franciscana sulfurea*."

CROSS	CULTURE NUMBER	BUD-CONE COLOR		
		RED	GREEN	SPLASHED
<i>franciscana</i> × " <i>franciscana sulfurea</i> "	58	76	0	0
	287	461	0	0
" <i>franciscana sulfurea</i> " × <i>franciscana</i>	59	14	45	1?
	288	255	203	1?

TABLE 2

*Phenotypic frequencies in F*₂ generations from *F*₁ plants with red bud-cones.

CULTURE NUMBER	YELLOW		SULFUR		PERCENT* GERMINATION	PARENT PLANT	CHROMOSOME † CONFIGURATION
	TALL	DWARF	TALL	DWARF			
177	55	1	3	0	?	58-1	⊙ 4, 5 pairs
178	67	5	14	2	?	59-1	7 pairs
406	26	15	0	0	68	287-3	7 pairs
411	45	3	8	0	59	-4	Unknown
416	16	2	1	0	33	-6	7 pairs
456	45	15	7	1	91	288-1	7 pairs
461	45	7	2	0	72	-2	Unknown
466	51	8	4	0	85	-4	7 pairs
470	47	4	1	0	70	-10	Unknown
473	45	7	1	1	75	-49	Unknown
Total	442	67	41	4			

* The germination percentages in this and following tables were observed from earth-sown seeds.

† In this and the following tables, the symbol ⊙ is used to indicate a chromosome circle. The number following the symbol indicates the number of chromosomes in the circle.

tures 287 and 288 were grown. The pedigrees of F_2 , backcross, and F_3 generations are given in the following tables (1 to 14) in which the data obtained from these crosses are presented.

TABLE 3
Phenotypic frequencies in the backcross F_1 (red bud-cones) $\times$ sulfur-dwarf.

CULTURE NUMBER	YELLOW		SULFUR		PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
	TALL	DWARF	TALL	DWARF			
409	29	28	6	7	97	287- 3	7 pairs
414	11	15	14	15	73	- 4	Unknown
417	30	29	5	7	95	- 6	7 pairs
420	34	21	11	4	97	- 10	7 pairs
425	19	14	20	21	96	- 31	7 pairs
427	18	18	4	2	96	- 40	Unknown
428	22	19	10	14	92	- 51	Unknown
429	29	18	7	9	91	- 90	Unknown
430	18	20	10	9	97	-110	Unknown
431	20	20	7	5	75	-140	Unknown
432	30	27	7	6	98	-165	Unknown
433	31	33	3	2	96	-200	Unknown
457	25	17	5	8	83	288- 1	7 pairs
464	2	1	0	2	57	- 2	Unknown
467	26	22	14	7	93	- 4	7 pairs
471	39	18	7	4	92	- 10	Unknown
474	16	23	5	8	69	- 49	Unknown
488	13	9	7	7	52	-103	Unknown
489	16	13	5	3	53	-106	Unknown
Total	428	365	147	140			

TABLE 4
Phenotypic frequencies in the backcross sulfur-dwarf $\times F_1$ (red bud-cones).

CULTURE NUMBER	YELLOW		SULFUR		PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
	TALL	DWARF	TALL	DWARF			
410	17	10	18	5	72	287- 3	7 pairs
415	8	6	1	1	86	- 4	Unknown
418	12	15	10	15	72	- 6	7 pairs
422	8	15	8	5	48	-10	7 pairs
424	12	23	9	9	72	-19	Unknown
458	10	12	3	3	37	288- 1	7 pairs
465	12	10	7	0	73	- 2	Unknown
472	3	2	2	2	21	-10	Unknown
475	2	3	2	3	16	-49	Unknown
Total	84	96	60	43			

TABLE 5
Phenotypic frequencies in the backcross F_1 (red bud-cones) $\times$ red sulfur tall.

CULTURE NUMBER	YELLOW	SULFUR	PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
407	52	12	88	287- 3	7 pairs
412	41	18	83	- 4	Unknown
419	66	12	68	- 6	7 pairs
434	37	19	77	- 7	7 pairs
435	55	4	91	- 8	Unknown
436	49	18	92	- 9	Unknown
437	52	22	100	- 12	Unknown
438	43	23	92	- 13	Unknown
440	34	16	67	- 33	Unknown
441	22	13	47	- 34	Unknown
442	24	15	53	- 36	Unknown
443	26	15	61	- 37	Unknown
444	10	5	20	- 38	Unknown
445	36	17	71	-201	Unknown
446	20	8	37	-202	Unknown
447	38	11	67	-203	Unknown
448	12	6	24	-204	Unknown
449	31	18	67	-205	Unknown
450	34	11	61	-206	Unknown
451	27	19	63	-207	Unknown
454	30	3	79	-211	Unknown
455	20	10	44	-212	Unknown
459	52	9	84	288- 1	7 pairs
462	56	6	83	- 2	Unknown
491	48	18	66	- 81	Unknown
476	53	31	59	- 89	Unknown
477	19	10	?	- 97	Unknown
490	35	24	61	-142	Unknown
478	9	4	17	-146	Unknown
479	34	17	68	-152	Unknown
480	28	19	65	-153	Unknown
481	59	5	73	-156	Unknown
482	27	12	41	-193	Unknown
483	43	23	69	-203	Unknown
484	20	4	32	-220	Unknown
485	40	24	87	-240	Unknown
486	33	25	81	-302	Unknown
487	25	8	44	-367	Unknown
Total	1340	534			

TABLE 6

Phenotypic frequencies in the backcross red sulfur tall $\times F_1$ (red bud-cones).

CULTURE NUMBER	YELLOW	SULFUR	PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
408	44	20	92	287-3	7 pairs
413	32	17	?	-4	Unknown
460	40	23	87	288-1	7 pairs
463	23	5	65	-2	Unknown
469	51	12	87	-4	7 pairs
Total	190	77			

TABLE 7

Phenotypic frequencies in F_3 generations from F_1 (red bud-cones) F_2 red yellow tall.

CULTURE NUMBER	YELLOW		SULFUR		PARENT PLANT	CHROMOSOME CONFIGURATION
	TALL	DWARF	TALL	DWARF		
306	53	..	2	..	178- 3	Unknown
307	108	..	..	..	- 7	Unknown
308	21	0	1	1	- 9	Unknown
309	71	..	..	..	-10	Unknown
311	47	..	4	..	-16	Unknown
324	21	..	..	..	177- 1	Unknown
325	63	..	..	..	- 3	7 pairs
326	51	..	..	..	- 9	⊙ 4, 5 pairs
329	8	..	..	..	-24	⊙ 4, 5 pairs
330	66	..	..	..	-29	Unknown
331	34	..	18	..	-32	Unknown
332	43	13	..	..	-36	Unknown
333	25	..	4	..	-39	Unknown

TABLE 8

Phenotypic frequencies in F_3 generations from F_1 (red bud-cones), F_2 red sulfur tall.

CULTURE NUMBER	TALL	DWARF	PARENT PLANT
334	88	..	177-61
335	49	16	-59
336	55	19	-18
317	10	..	-83
319	15	..	-69

TABLE 9
Phenotypic frequencies in F_2 generations from F_1 plants with green bud-cones.

CULTURE	GREEN		SPLASHED YELLOW	RED		GERMI- NATION	PARENT PLANT	CHROMOSOME CONFIGURATION
	YELLOW	SULFUR		YELLOW	SULFUR			
180	20	1	1	24	0	?	59- 3	Unknown
494	23	0	7	43	0	74	288-40	Unknown
496	43	0	2	64	1	100	- 5	○ 10, 2 pairs
501	17	0	2	29	0	48	- 6	Unknown
505	18	0	1	29	0	50	- 7	Unknown
525	20	0	3	25	0	49	-20	○ 10, 2 pairs
531	1	0	0	23	0	28	-29	Unknown
534	0	0	0	3	0	4	-60	{ 2 pairs, 1 tri- valent, chain 7
Total	142	1	16	240	1			

CHROMOSOME CONFIGURATIONS OF THE HYBRIDS

Cytological examinations were made of two red-budded plants of the F_1 generations grown in 1924. Of these, one plant (58-1) had a circle of four chromosomes and five chromosome pairs, the other plant (59-1) had seven chromosome pairs. In an F_2 culture (177) from 58-1, three plants were examined. Two of these had circles of four chromosome and five pairs, the third had seven chromosome pairs.

From F_1 cultures grown in 1927, ten plants with red bud-cones were examined. One plant (287-1) proved to be a haploid (EMERSON 1929). The other plants each had seven pairs of chromosomes (plants 287-3, -5, -7, -10, -31, 288-1, -4, and -81).

Four plants with green bud-cones in the F_1 cultures of 1927 were examined cytologically. Of these, three plants had each a circle of ten chromosomes and two pairs (288-5, -20, -71), while one plant (288-60) had two chromosome pairs, a trivalent group, and a chain of seven (or eight) chromosomes. Only a few good figures were obtained from plant 288-60, but in each telophase examined there were seven chromosomes in each daughter nucleus. It will be noted in table 9 that self-pollinated seeds of plant 288-60 germinated very poorly. A recent test using about two hundred seeds of this plant gave no germination while seeds of a sister plant still germinated perfectly.

No preparations were obtained from plants with green bud-cones of F_1 culture 59.

The chromosome configurations of the various plants listed above are illustrated in plates 1 to 3.

TABLE 10

Phenotypic frequencies in the backcross F_1 (green bud-cones) $\times$ red sulfur.

CULTURE	GREEN		SPLASHED		RED		GERMI- NATION	PARENT PLANT	CHROMOSOME CONFIGURATION
	YELLOW	SULFUR	YELLOW	SULFUR	YELLOW	SULFUR			
495*	1	1	2	0	28	1	52	288- 40	Unknown
497	3	20	1	0	48	3	77	- 5	⊙ 10, 2 pairs
499*	0	18	2	0	52	3	76		
502	0	5	1	0	26	0	64	- 6	Unknown
506	1	15	1	1	47	0	69	- 7	Unknown
508*	3	20	0	1	49	4	82		
509	0	3	1	0	17	1	24	- 71	⊙ 10, 2 pairs
510	0	14	3	0	34	2	57	- 82	Unknown
511	0	6	2	1	35	0	45	- 83	Unknown
512	0	6	6	0	20	0	34	- 84	Unknown
513	0	4	2	0	23	1	34	- 91	Unknown
514	1	22	0	0	33	3	62	- 92	Unknown
515	1	17	0	1	50	0	69	- 93	Unknown
516	0	5	1	0	35	1	43	- 94	Unknown
517	0	8	0	0	16	2	27	- 99	Unknown
518*	0	11	2	0	20	1	34	-101	Unknown
519	0	6	1	0	13	0	23	-107	Unknown
520	3	6	0	0	18	1	29	-112	Unknown
521	0	2	1	0	35	2	43	-120	Unknown
522	3	7	0	0	18	1	30	-130	Unknown
523	1	9	0	0	28	2	39	-140	Unknown
526	0	2	0	0	4	0	6	- 85	Unknown
527	0	6	1	0	20	1	28	- 86	Unknown
528	0	6	1	0	26	3	37	- 87	Unknown
529*	0	6	1	0	31	1	39	- 20	⊙ 10, 2 pairs
532*	1	3	0	0	28	0	33	- 29	Unknown
535	0	4	0	0	7	0	11	- 60	{ 2 pairs, 1 tri- valent, chain 7
536	2	9	0	1	58	1	76	-319	Unknown
537	0	12	0	0	21	0	35	-356	Unknown
538	0	2	0	0	16	1	21	-143	Unknown
539	0	0	0	0	7	1	8	-144	Unknown
540	1	9	1	0	29	3	49	-154	Unknown
541	6	7	0	0	33	1	50	-259	Unknown
542	0	3	1	0	23	1	30	-286	Unknown
Total	27	274	31	5	948	41			

* A culture which was backcross to sulfur-dwarf. Since there were no dwarf segregates from hybrids with green bud-cones, these cultures are listed with the backcrosses to tall red sulfur.

288-60a

288-60b

288-60c

177-9

58-1

177-24

287-6

287-31

TABLE 11
Phenotypic frequencies in the backcross red sulfur $\times F_1$ (green bud-cones).

CULTURE NUMBER	SPLASHED YELLOW	RED		PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
		YELLOW	SULFUR			
498	5?	41	0	61	288- 5	⊙ 10, 2 pairs
500*	0	1	0	2		
503	1	39	0	91	- 6	Unknown
504*	1?	57	3	80		
507	2	38	1	42	- 7	Unknown
524*	0	65	0	66		
530*	2	17	0	58	-20	⊙ 10, 2 pairs
533*	0	39	0	40	-29	Unknown
Total	5+6?	297	4			

* A culture which was a backcross to sulfur-dwarf. Since there were no dwarf segregates from hybrids with green bud-cones, these cultures are listed with the backcrosses to tall red sulfur.

TABLE 12
Phenotypic frequencies in F_3 generations from F_1 (green bud-cones) F_2 green yellow.

CULTURE NUMBER	GREEN		SPLASHED YELLOW	RED YELLOW	PARENT PLANT
	YELLOW	SULFUR			
289	19	0	0	17	180-20
290	16	0	0	15	-25
291	20	1	2	10	-29
292	9	0	1	11	-37
293	16	1	0	17	-39
294	18	0	1?	15	-47
295	4	0	1	24	-53
Total	102	2	4+1?	109	

TABLE 13
Phenotypic frequencies in F_3 generations from F_1 (green bud-cones) F_2 red yellow.

CULTURE NUMBER	RED YELLOW	PARENT PLANT
297	125	180- 2
298	71	- 5
299	102	- 8
300	99	-12
301	14	-15
302	110	-16
303	111	-17
305	110	-52

TABLE 14

Phenotypic frequencies in cultures from self-pollinated plants with splashed bud-cones and of backcrosses to red.

CULTURE	SPLASHED	RED	PARENT
A. self-pollinated			
296	83	27	180- 1
574	36	54	291- 1
575	36	28	-31
576	38	28	292- 9
577	6	6	295-21
581	9	26	296- 7
582	8	4	-10
583	15	28	-11
584	2	36	-12
585	3	15	-15
587	10	11	-16
589	8	15	-17
591	8	11	-20
594	7	16	-33
Total	269	305	
B. splashed X red			
580	17	47	296- 7
586	18	25	-15
588	18	19	-16
590	18	21	-17
593	26	19	-21
595	1	4	-33
Total	98	135	

INTERPRETATION OF DATA

Yellow flower color has long been known to be dominant to sulfur, and the factor pair *S-s* is generally used by *Oenothera* geneticists to represent the relationship of these colors. In the crosses between *Oe. franciscana* and "*franciscana sulfurea*," there is always a deficiency in the number of functioning gametes which carry the factor *s*. The gametic ratios obtained in F_1 plants with pairing chromosomes were 2133:821 (2.6:1) for the eggs and 370:180 (2.1:1) for the pollen (tables 3, 4, 5, and 6). Gametophyte competition such as described by RENNER (1921) might explain the deficiency of the factor *s* in functioning eggs, and differential pollen-tube growth might be responsible for the deficiency of *s* in functioning sperms. Neither point was checked by direct experiment.

The dwarf stature of sulfur-dwarf is recessive to the tall stature of

franciscana and "*franciscana sulfurea*." This relationship between the two statures will hereafter be represented by the factor pair *D-d*. Gametes carrying *D* and *d* respectively are produced in approximately equal numbers in *F*₁ plants with pairing chromosomes. The observed frequencies were 575:505 (1.1:1) for the eggs, and 144:139 (1.03:1) for the sperms (tables 3 and 4).

The green bud-cones of "*franciscana sulfurea*" are dominant to red. The relationship between these bud colors will be represented by the factor pair *G_r-g_r*⁵. The factor *G_r* is always associated with a pollen lethal. The gametic ratios of this factor pair will be discussed in another connection.

Splashed bud-cones are dominant to red, but the character is not discernible in the presence of *G_r*. This relationship is shown by the presence of plants with splashed bud-cones in progenies of green-budded plants that were heterozygous for red bud-cones (tables 9, 10, 11, and 12), and by the presence of red-budded plants in progenies of plants with splashed bud-cones (table 14). No plants were found that bred true for splashed, indicating a possible association with a lethal factor. The relationship between splashed and red bud-cones will be represented by the factor pair *S_{pl}-s_{pl}*.

The chromosome complexes

Certain *F*₁ plants from crosses between *Oe. franciscana* and "*franciscana sulfurea*" have seven pairs of chromosomes. This complete pairing of chromosomes is believed to indicate that *franciscana* has seven chromosomes (functioning together as a complex) which are the homologs of seven chromosomes constituting one of the complexes of "*franciscana sulfurea*." The complexes with pairing chromosomes will be called *franciscans* in *franciscana* and *s-d-franciscans* in "*franciscana sulfurea*" as the latter carries factors for sulfur-colored flowers and dwarf stature. All functioning pollen and some of the functioning eggs of "*franciscana sulfurea*" are *s-d-franciscans* as shown by reciprocal crosses with *franciscana* and with sulfur-dwarf. Sulfur-dwarf is homozygous for this complex.

The other complex of "*franciscana sulfurea*" will be called *sulfurens*. It produces a circle of ten chromosomes and two pairs with *franciscans* and with *s-d-franciscans*, with which it must therefore have two chromosomes (and only two) in common (that is, homologous).

Oenothera franciscana may produce a second complex which forms a circle of four chromosomes with *s-d-franciscans* as found in *F*₁ plant 58-1 and some of its descendants.

⁵ The author follows RENNER (1925) in reserving the factor pair *R-r* [used by SHULL (1923a) for bud color] to denote nerve (vein) color, for which it had previously been used by HERIBERT-NILSSON. RENNER's factor *S_r* denotes a dominant red bud-color.

Linkage relationships

Oenothera "franciscana sulfurea" produces only two types of gametes, *sulfurens* (carrying factors for sulfur-colored flowers, tall stature, green bud-cones, and a pollen lethal) and *s-d-franciscans* (carrying factors for sulfur-colored flowers, dwarf stature, and red bud-cones). No recombinations of the factors for stature, bud color (red versus green) and the pollen lethal have been found in the gametes of "franciscana sulfurea" as shown by in-bred and out-cross cultures.

First generation plants with green bud-cones in the cross "*franciscana sulfurea*" $\times$ *franciscana* produce for the most part the two parental types of gametes. These gametes are *sulfurens* (carrying factors for sulfur-colored flowers, tall stature, green bud-cones, and a pollen lethal) and *franciscans* (carrying factors for yellow flowers, tall stature, and red bud-cones). No recombinations between bud color and the pollen lethal were obtained. Between bud color and flower color, however, some recombinations occurred. The observed gametic ratios were $27G_r-S:274G_r-s:979g_r-S:46g_r-s$ for the eggs, and $308g_r-S:4g_r-s$ for the sperms (tables 10 and 11). Of the eggs carrying the pollen lethal and factor for green bud-cones, 9 percent represent recombinations with the factor for yellow flowers, while 4.5 percent of the eggs carrying the factor for red bud-cones were recombinations with the factor for flower color, and but 1.28 percent of the sperms represent recombinations between these factors.

In *sulfurens* of "*franciscana sulfurea*" there is also a factor for splashed bud-cones (S_{pl}) closely associated with G_r and s . In F_1 plants with green bud-cones (*sulfurens franciscans*), 3.5 percent of the eggs carrying g_r represent recombinations with S_{pl} (gametic ratio is $989g_r-s_{pl}:36g_r-S_{pl}$, table 10) and 3.5 percent or less⁶ of the sperms carrying g_r represent recombinations with S_{pl} . In the same egg cells (carrying g_r) there was 7 percent recombination between S_{pl} and S (gametic ratio $953S_{pl}-s+s_{pl}-S:72S_{pl}-S+s_{pl}-s$).

The percentages of recombinations between these three factors might appear to be a function of a linear order of the three genes in a single chromosome. In this case the order of the genes would be:

$$\begin{array}{ccccccc} s_{pl} & (3.5) & g_r & (4.5) & s \\ \hline & \text{(region 1)} & & \text{(region 2)} & \end{array}$$

⁶ The plants in some cultures listed in table 11 were not in their best condition at the time notes were taken, and some plants listed as having splashed bud-cones may have been red but with the pigmentation poorly developed as is often the case when plants first come into flower. Plants for which the classification was difficult are listed in the tables under "splashed" and followed by a question mark (?).

The observed crossover frequencies would then be: 948 non crossovers ($s_{pl}-g_r-S$), 31 single crossovers in region 1 ($S_{pl}-g_r-S$), 41 single crossovers in region 2 ($s_{pl}-g_r-s$), and 5 double crossovers ($S_{pl}-g_r-s$) (table 10). Expressed in percentages, the frequencies of crossing over are 3.02 singles in region 1, 4.00 in region 2, and 0.49 doubles. There can thus be no interference between crossing over in regions 1 and 2.

No crosses were made in which the associations of factors for flower color ($S-s$) and for stature ($D-d$) could be studied in plants of the constitution *sulfurens*·*d-franciscans*. But since $s_{pl}-g_r-d$ are almost completely associated in *sulfurens*·*s-d-franciscans*, and since $s_{pl}-g_r-S$ are very closely associated in *sulfurens*·*franciscans*, it may fairly be concluded that s and d are actually closely associated in these hybrids.

In *franciscans*·*s-d-franciscans*, however, s and d are completely independent. In the eggs there was 47.4 percent recombination between flower color and stature (gametic ratio 568 $D-S+d-s$:512 $D-s+d-S$, table 3), while in the sperms there was 51.6 percent recombination (gametic ratio 127:156, table 4). The independence of s and d is further borne out by the segregation of F_3 cultures (tables 7 and 8).

In considering the differences in association of s and d in these crosses, it must be borne in mind that *franciscans*·*s-d-franciscans* has all pairing chromosomes, while *sulfurens*·*franciscans* and *sulfurens*·*s-d-franciscans* each have ten chromosomes in a circle. It is reasonable to suppose that the linkage of factors in the latter hybrids is due to the association of chromosomes in a circle.

Gametophyte competition

RENNER (1921) has shown that in many cases in which two dissimilar complexes are segregating in megasporogenesis, the megaspores receiving one complex have a distinct advantage over the other type of megaspore in their ability to develop into gametophytes (embryo-sacs). This superiority of the first complex may not hold when it is competing against a third complex.

Competition of this nature is to be seen in crosses involving *Oe.* "*franciscana* and *franciscana sulfurea*." In the "*franciscana sulfurea*" plant used in the crosses of 1923, the frequencies of the two types of complexes functioning as eggs were 45 *sulfurens* and 15 *s-d-franciscans* (culture 59 in table 1). In the F_1 plant 59-3, involving the same *sulfurens* complex against *franciscans*, the gametic ratio was 21 *sulfurens*:25 *franciscans* (culture 180 in table 9). In F_2 plants of culture 180, the gametic ratio was 104 *sulfurens*:114 *franciscans*. These data are interpreted as indicating that the *sulfurens*

used in 1923 had a marked superiority over *s-d-franciscans* and none over *franciscans*.

In the cross of 1925, the gametic ratio in the "*franciscana sulfurea*" plant used was 203 *sulfurens*:256 *s-d-franciscans* (culture 288 in table 1). In F_1 plants involving the same *sulfurens* against *franciscans*, the gametic ratio was 122 *sulfurens*:232 *franciscans*. In this case both *franciscans* and *s-d-franciscans* are superior to *sulfurens*, but *franciscans* is stronger than *s-d-franciscans* as in the previous case.

The *sulfurens* in the cross of 1923 may have been the original type which formed a circle of twelve with *s-d-franciscans*, while that used in 1925 had become altered to form a circle of ten. Such a difference might account for the divergent results in the crosses of 1923 and 1925.

APPLICATION OF THE BELLING HYPOTHESIS

HÅKANSSON (1928) and DARLINGTON (1929) have shown that the hypothesis (BELLING 1927) of attraction of chromosomes into circles by rearrangements (translocations) of homologous ends will give a mechanical explanation of most of the genetical and cytological phenomena of the *Oenotheras*. It is not the purpose of the present paper to review the application of BELLING's hypothesis to the *Oenotheras*, but the hypothesis offers an alternative explanation of the linkage relationships reported above which is in many respects superior to that afforded by the presence of the genes in a single chromosome.

In order to avoid the use of permanent names for the ends of the chromosomes until more information is available concerning the homologies in different species, the homologous portions of the chromosomes in the accompanying diagrams (plate 4) are indicated by differences in shading.

Figure 1 in the diagrams represents the chromosomes of an F_1 plant with green bud-cones (*sulfurens-franciscans*) of the cross "*franciscana sulfurea*" $\times$ *franciscana*. It will be noted that each chromosome part is present in the diploid condition. In pairing chromosomes, the parts have identical arrangements; but of the chromosomes in the circle, no two have the same combination of parts. The right end of any chromosome in the ring has its counterpart in the left end of the chromosome on its right, while the counterpart of the left end of the first chromosome is the right end of the chromosome to its left. It is this arrangement of homologous portions of the chromosomes, according to the BELLING hypothesis, which brings the chromosomes into the circle by the attraction between homologous parts.

Figure 2 represents the chromosomes of an F_1 plant with red bud-cones (*franciscans*·*s-d-franciscans*) in which all the chromosomes pair.

Figure 3 illustrates the method of separation of the chromosomes of figure 1 in heterotypic metaphase. CLELAND discovered that the chromosomes in a circle in *Oenothera* regularly separate by a zig-zag arrangement of the chromosomes on the metaphase plate, so that alternate chromosomes pass to the same pole. BELLING's hypothesis gives a mechanical explanation of the presence of the chromosomes of one complex in the alternate positions in the circles which necessitates the zig-zag arrangement of chromosomes on the spindle to insure the separation of homologous chromosome parts. It has long been known from genetic data that the paternal and maternal gametes separate as a whole in the reduction division of some *Oenotheras*.

The positions of the genes in the chromosomes have been chosen arbitrarily. The scheme requires that the genes be in different chromosome parts, but there is no method of determining the position of any gene in the circle with the present data.

It will be noted that the genes will appear to be completely linked even though they lie in different chromosomes, unless there are interchanges (crossovers) between homologous chromosome parts. Such crossovers are represented schematically in figures 4, 5, 6, and 7.

Crossovers of this type do not alter the chromosome configurations, but the allelomorphs present in the two complexes are exchanged. Figure 4 represents a crossover involving $G_r - g_r$ in which G_r passes into *franciscans* and becomes associated with s_{pl} and S . The same association of $G_r - s_{pl} - S$ can be obtained by crossovers involving s_{pl} and s simultaneously (figure 7), but in the latter case $G_r - s_{pl} - S$ would be in *sulfurens*.

In this scheme, percent of crossing over would not be a function of the distance between the two genes appearing as recombinations, but a function of the distances between each of the genes and the respective breaks in the chromosomes (that is, the points at which the chromosomes cease to be homologous to the members on their left and become homologous to the chromosomes on their right). Recombinations between G_r and S_{pl} should be equal to the frequency (a) of crossovers between G_r and the break in that chromosome, plus the frequency (b) of crossovers between S_{pl} and the break in that chromosome, less the frequency (ab) of simultaneous crossovers between these factors and their corresponding "breaks." Similarly, if c is the frequency of crossovers between s and the corresponding break, the number of recombinations between s and S_{pl} must be $b + c - bc$, and between s and G_r must be $a + c - ac$. Crossovers in chromosome

parts lying between any two genes have no effect on the recombinations of these genes. By substituting the observed frequencies of recombinations in each of these equations and solving for a , b , and c , one finds the following values for crossing over between the genes and the corresponding breaks: $G_r = 0.5$ percent, $S_{pl} = 3.0$ percent, and $s = 4.0$ percent.

Interference in crossing over would have little effect on the frequencies of any crossover class, if crossing over occurs in the manner just described. "Double" crossovers are comparatively rare, and the same combination of genes is obtained by a different single crossover.

In plants of the constitution *sulfurens*·*s-d-franciscans*, G_r , S_{pl} , and D have been found to be closely linked with no recombinations occurring between G_r and D , while in *sulfurens*·*franciscans* G_r , S_{pl} and s are closely linked, but in *franciscans*·*s-d-franciscans*, d and s are independent. These results are easily explained by the scheme just described if one assumes that G_r and D both lie comparatively close to the breaks in their respective chromosomes. These positions would prevent recombinations in *sulfurens*·*s-d-franciscans* in which the chromosomes are in a circle, and would permit free recombination in *franciscans*·*s-d-franciscans* in which the chromosomes pair.

Similar cases in Oenothera

Crossing over between homologous chromosomes, as described above, may be the cause of the appearance of such mutations as *biennis sulfurea* from *Oe. biennis*, *nanella* from *Oe. Lamarckiana*, and *formosa* from *Oe. pratincola* (strain E).

In the first case, *Oe. biennis* is known to be balanced for sulfur⁷ (OEHLKERS 1924), and the mutation, *biennis sulfurea*, still has the peculiar chromosome configuration of the parent form (CLELAND 1926).

The second case fulfills the same two conditions. *Lamarckiana* is balanced for *nanella* (carried in *gaudens*). I do not know if the chromosome configuration of *nanella* has been observed, but there is excellent genetic evidence that *nanella* forms *velans* and *gaudens* complexes which are identical to those of *Lamarckiana* except for the presence of the *nanella* factor in *velans* (RENNER 1925).

Oenothera pratincola (strain E) is known to be balanced for revolute leaves (COBB and BARTLETT 1919, COBB 1921). Mutation *formosa* (revolute-leaved) and *pratincola* have the same chromosome configuration

⁷ The *albicans* complex of *biennis* carries sulfur, but due to the presence of balanced zygotic lethals, *albicans* does not appear in homozygous condition, and the dominant yellow allelomorph in *rubens* masks the presence of sulfur.

(KULKARNI 1928). Thus again, the two conditions required by the method of crossing over described above are fulfilled. In this last case, however, the genetic data of Doctor BLANCHARD (COBB 1921) do not entirely agree with present predictions. Plants with the complexes of *formosa* (one, however, recovered from a cross with *pratincola*-C) but heterozygous for revolute behave in their breeding as if the factor for revolute were in a chromosome pair separate from the large complexes. No free pair is expected if the above interpretation is correct.

Similar cases in Drosophila

As a result of X-ray treatment, DOBZHANSKY and STURTEVANT (1930) have obtained reciprocal translocations between the second and third chromosomes of *Drosophila melanogaster*. While the cytology of the reduction divisions has not been completed, the somatic pairing of chromosomes in the Diptera gives a good indication of the configuration that the chromosomes must assume during reduction. In somatic cells of flies having one normal second, one normal third, and the two reciprocally translocated chromosomes, the four chromosomes often form a ring. Genetic evidence shows that alternate chromosomes of the ring pass to the same pole in the majority of cases, thus agreeing with the cytological findings for *Oenothera*.

As the paper of DOBZHANSKY and STURTEVANT includes a discussion of the relation of their findings to the problems of *Oenothera* genetics, it is necessary here only to point out that there is increasing genetical and cytological data in support of BELLING's hypothesis for ring formation. DOBZHANSKY and STURTEVANT did not collect linkage data that is completely comparable to that reported above for *Oenothera*, but it is evident that such data would be obtained if the linkage of genes in three chromosome-halves were studied together.⁸

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The studies on *Oenothera* reported above were undertaken under the direction of Professor H. H. BARTLETT and Professor B. M. DAVIS. I am

⁸ Since this paper went to press I have studied the crossing over between the four limbs of DOBZHANSKY's reciprocal translocations *A*, *B*, and *C*. All gave the predicted results and agree with the linkage observed for $S_{p1}-b_r-s$ reported above for *Oenothera*. For example: in translocation II-III B, B_1 lies to the right of the "break" in chromosome II, p^p to the left of the "break" in III and s_1 to the right. From the linkage data, the three genes might lie in a single chromosome in the order $s_1-B_1-p^p$ with 6.39 percent single crossover in region 1, 1.86 percent in region 2 and 0.40 percent double (total 2051 flies) when only 0.15 percent double would be expected if there were no interference. In the *Oenothera* cross reported above there were 0.49 percent double when only 0.17 percent would be expected with no interference.

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SUMMARY AND CONCLUSIONS

Oenothera franciscana may produce two chromosome complexes which together form a ring of four chromosomes. One of these complexes is called *franciscans*.

Hybrid "*franciscana sulfurea*" produces a complex of which all the chromosomes are homologous to those of *franciscans*, since plants composed of these two complexes give seven pairs of chromosomes. This complex of "*franciscana sulfurea*" differs from that of *franciscana* by recessive factors for dwarf stature and sulfur-colored flowers and is called *s-d-franciscans*.

The second complex of "*franciscana sulfurea*" is called *sulfurens*. *Sulfurens* forms a circle of ten chromosomes and two pairs with each of the two *franciscans*-types of complexes.

In crosses involving the three complexes just named, four factor pairs were concerned. These factors were $D-d$, $S-s$, G_r-g_r (a dominant green bud color), and $S_{pl}-s_{pl}$ (splashed bud color, dominant to red).

In *sulfurens-s-d-franciscans* with a circle of ten chromosomes, G_r , S_{pl} , and D are closely linked. In *sulfurens-franciscans* with a circle of ten chromosomes, G_r , S_{pl} , and s are closely linked. But in *franciscans-s-d-franciscans* which has all pairing chromosomes, s and d are completely independent. This relationship is believed to show that the linkage of D and s to G_r and S_{pl} in the first two hybrids is due to the association of a large number of chromosomes in a circle in each.

The interpretation that the linkage of G_r , S_{pl} , and s in *sulfurens-franciscans* is due to the presence of the three genes in one chromosome is inadequate in that it does not account for the excessive number of observed "double crossovers."

Crossing over between homologous parts of dissimilar chromosomes in the circle of ten in *sulfurens-franciscans*, however, does account for all the observed genetic behavior. Crossing over of this type presupposes the correctness of BELLING's hypothesis for the attraction of chromosomes into rings due to homologous chromosome-ends.

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