"OLD-GOLD" FLOWER COLOR, THE SECOND CASE OF INDEPENDENT INHERITANCE IN OENOTHERA^{1*}

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Received October 29, 1925

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INTRODUCTION

The evening primroses display, as sharply as do any other plants or any animals, a mosaic-like combination of alternatively inherited characteristics, such as provide in most other organisms the clearest examples of typical Mendelian inheritance. A list of the more striking of these alternative characters includes: (a) tall *versus* dwarf stems; (b) red *versus* green stems; (c) red-spotted *versus* unspotted green leaves; (d) flat leaves *versus* revolute leaves; (e) narrow petals *versus* broad petals; (f) yellow petals

¹ The experiments on which this paper is based have been supported in part by grants from the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, and the ELIZABETH THOMPSON SCIENCE FUND.

* The GALTON AND MENDEL MEMORIAL FUND contributes the accompanying colored plate.

versus sulfur petals; (g) red hypanthium *versus* green hypanthium; (h) red or pink bud-cones *versus* green bud-cones; (i) long styles *versus* short styles. The first-mentioned member of each of these pairs is the dominant. In addition to these, there appear to be at least four recessive lethals paired with four corresponding dominant "vital" factors. Of all these factors and some others which are known but not here enumerated, only the short-styled form of *Oenothera brevistylis* has been found, heretofore, to be distributed in inheritance independently of the others.

The relation between the long styles of *Oenothera Lamarckiana* and the short styles of *brevistylis* may be considered a classic example of Mendelian inheritance, as this pair of contrasting characters was included by DE VRIES (1900) in the list of 11 examples cited in his original statement of the "law of disjunction of hybrids,"—one of the three announcements of the three independent rediscoveries of the Mendelian laws.

Were no lethal factors involved, each of the other character pairs mentioned above should likewise yield typical Mendelian phenomena when handled singly in the production of monohybrids; that is, allowing for errors of random sampling and deviations due to selective viability, etc., they would give uniform F_1 families of the dominant type; would split in F_2 families, following selfing of the F_1 , into 3 dominants to 1 recessive; on backcrossing to the recessive, they would give 1 : 1 ratios of dominants to recessives; and reciprocal crosses would give identical results.

The wide-spread occurrence of lethal factors has so modified this program that the *Oenotheras* have long seemed an outstanding exception to the general applicability of the Mendelian scheme of heredity.

I have shown in previous papers (SHULL 1921a, b, 1923a, b) that this disturbing effect of the lethals is due to the fact that all of the above-mentioned factors, except the pair for long and short styles, are associated together in a single linkage group (group I) in which the lethals likewise occur. I have assumed that the linkage of these factors is the result of their inclusion in a single pair of chromosomes, but recent cytological studies of CLELAND (1922, 1923, 1924, 1925, 1926) indicate the existence of a possibly alternative mechanism for the production of linkage in the *Oenotheras*, and it seems best for the present to admit the possibility that linkage may result from chromosomal cohesions (synzeuxis), though I still think that the available evidence is more favorable to the view that linkage in the *Oenotheras*, as in other organisms, is based on the inclusion of the linked genes within a single chromosome pair (monozeuxis).

This new fundamental problem as to the physical basis of linkage in the *Oenotheras* adds to the interest and importance of further genetical

studies in this group of plants, and gives special significance to any factors which may be found to lie outside the big linkage group I with which nearly all studies have heretofore been concerned.

The factor for old-gold flower color (mut. *vetaurea*) has been the first, after *brevistylis*, to fulfill this condition. One of the first families in which it appeared (2047) showed plants of the *Lamarckiana* type segregating 40 yellow : 13 old-gold. In 1922 and 1923 self-fertilized *Lamarckiana* plants in the line of direct descent from the family in which the original mutation occurred, yielded typical 3 : 1 ratios of yellow and *vetaurea*-flowered *Lamarckiana*, namely, (No. 2123) 35 Y : 8 V, and (No. 2220) 22 Y : 7 V. These 3 : 1 segregations in typical *Lamarckiana* families, have been paralleled, heretofore, only by the segregation to the short style of *brevistylis*, and show that the *vetaurea* factor is not in the same linkage group as the *Lamarckiana* lethals,—therefore, not in group I.

The fact that *brevistylis* was the only previously known character which behaved in this way, made it seem desirable to test very completely the genetical relations of the old-gold mutation. For this purpose it has been a very fortunate circumstance that the old-gold factor has no noticeable injurious effects on the reproductive processes. It is thus greatly superior to *brevistylis* for such a study, since *brevistylis*, because of the deformed and disadvantageously placed stigmas, can be used as a seed parent only with the greatest difficulty, though it produces abundant pollen, and is therefore a very satisfactory pollen parent.

The present paper gives a complete account of the breeding results with the old-gold factor, with the exception of a few recent crosses made in an attack on certain problems which are not yet ready for report.

ORIGIN OF THE OLD-GOLD MUTATION

As described in a previous paper (SHULL 1921b) the old-gold mutation (mut. *vetaurea*) manifested itself in 1921 in two families (2047 and 2052), both of which had the same individual grandparents 188 (12) × 187 (56), the parents having been two individuals, (30) and (54), in family 198. A portion of the network of cross-breedings from which these two families were derived is shown in figure 1. With exception of the year 1914, this network of descent has been continued in the same manner, with annual generations, since 1905, when it was begun with ten rosettes of *Oenothera Lamarckiana* collected at my request by Professor HUGO DE VRIES, in the field at Hilversum, Holland, from which he had secured the foundational material for his own cultures almost 20 years previously. Every plant used in this network has been a typical *Lamarckiana*, and every

family in this series of cultures has had the usual composition of *Lamarckiana* families, nearly all of the offspring in each progeny being typical *Lamarckiana*, but usually associated with a small percentage of mutant types, the majority of which have been recognizable as 15-chromosome forms. Only mut. *funifolia* (SHULL 1921b), discovered in 1918, and mut. *vetaurea*, the subject of the present paper, have been demonstrably pro-

FIGURE 1.—Five generations of the network of descent in which "old-gold" originated as a gene-mutation. The three-figure numbers are family numbers, and the numbers in parenthesis are individual numbers. The year in which each generation was grown is indicated at the top of the corresponding column. Every individual represented was a typical *Lamarckiana* except 198(54) which was a green-coned mutant. Only in families 198 and 208 was the presence of old-gold as a hidden recessive type demonstrated. The pedigree numbers of families in which old-gold plants made their appearance *de novo* are underscored. The dotted line connecting 198(30) with 2052 represents an accidental self-fertilization, which alone made it possible for a *vetaurea* plant to appear in family 2052.

duced as the result of gene mutations, and I believe that these are the only new characters in *Oenothera* which have been certainly ascertained to have been the result of gene mutations, as distinguished from the process of unmasking recessive factors which have been kept hidden because of their linkage with lethal factors.

OLD-GOLD BREEDS TRUE

The first question which presents itself, on the discovery of a new characteristic in any organism, is whether it will breed true. Experience teaches that many such new forms are due to some accident of development that produces no effect whatever on the offspring; that others are apparently the result of complex combinations of genes which by chance only rarely recombine in the grouping necessary to produce again the characteristic in question; that some produce progenies which return in part to the parental type; and that still others segregate into one or more subordinate types. In table 1 are presented the results of 21 self-fertilizations of the old-gold type (mut. *vetaurea*), and of 14 crosses between two plants of the same type. The 35 families contained a total of 1772 old-gold, and none of any other type.

As will be seen in a later section, however, old-gold may segregate a subordinate type,—but only when it is not derived from a pure yellow (or old-gold) parentage.

OLD-GOLD RECESSIVE TO YELLOW

That the old-gold factor is recessive to yellow was indicated by the splitting of the first families observed, in 3 : 1 ratios of yellow to old-gold. The numerous crosses which have been made between forms having yellow flowers and forms with old-gold flowers, have always given only yellow flowers, except, of course, when the yellow-flowered form had been rendered heterozygous by a previous cross. Table 2 shows the results of 30 such crosses, the total F_1 yield of which was 1961 yellow-flowered individuals and none of any other color.

The most important series of crosses included in this table are those which involved as one of their parents an individual in families 201 to 208, for these are the families belonging to the network of descent shown in figure 1, in which the old-gold mutation occurred. The yellow-flowered parent in each of these nine families stood in a relation of cousinship of greater or less degree of closeness to the original mut. *vetaurea*. The fact that every one of these crosses yielded only yellow-flowered offspring in the F_1 demonstrates that the *vetaurea* factor was not present in the strain, hidden, as in some other *Oenothera* mutations,—for example *nanella* and *pervirens*,—by the presence of lethal factors linked with them. This renders it certain that the *vetaurea* factor originated as a gene mutation affecting one of the two gametes whose union gave rise to one of the parents of family 198, in which family, as we have seen, several individuals were demonstrably heterozygous for the *vetaurea* factor.

TABLE 1
Families which illustrate the true-breeding of mut. vetaurea.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	NUMBER FLOWERED, ALL VETAUREA
Parents	Family				
2052(10)	21108	300	63	21.00	45
2047(40)	21109	20	1	5.00	1
2047(92)	21113	184	127	69.02	105
2047(39) }	21114	184	78	42.40	64
2047(92) }					
21108(19)	22147	300	79	26.33	3
21113(5)	22148	300	168	56.00	38
21159(63)	22216	300	123	41.00	36
21159(113)	22217	300	140	46.67	85
21108(19)	2389	300	66	22.00	44
2220(40)	2390	300	28	9.33	25
22148(69)	2391	261	119	45.59	55
2220(46) }	2393	300	124	41.33	112
22162(7) }					
22167(41)	2398	300	183	61.00	86
22216(77)	23136	300	34	11.33	34
23135(5)	2486	300	172	57.33	107
23137(92)	24101	300	50	16.67	43
23137(92) }	24102	300	212	70.67	143
23137(80) }					
23137(46) }	24103	26	10	38.46	10
23137(92) }					
23137(91) }	24104	300	70	23.33	54
23137(92) }					
2389(44) }	24105	300	94	31.33	50
23137(92) }					
23137(92) }	24112	300	85	28.33	61
23144(30) }					
23137(105)	24114	147	69	46.94	49
23137(46) }	24115	24	7	29.17	5
23137(105)					
23137(91) }	24116	300	116	38.67	57
23137(105)					
23137(46) }	24119	35	7	20.00	3
23137(80) }					
23137(91) }	24120	300	230	76.67	95
23137(80) }					
23137(91) }	24124	300	217	72.33	133
23137(63) }					
23137(91) }	24125	300	138	46.00	98
23137(76) }					
23137(91)	24126	300	56	18.67	31
23137(18)	24127	76	29	38.16	7
23137(46)	24128	154	53	34.42	15
23142(2)	24148	300	78	26.00	32
23142(2) }	24149	245	37	15.10	30
23144(30) }					
2390(5)	24150	300	54	18.00	5
2391(43)	24151	25	12	48.00	11
Total					1772

TABLE 2

Families which show the recessiveness of vetaurea in the F₁ of crosses with homozygous yellow.

PEDIGREE NUMBERS			SEEDS SOWN	NUMBER OF PLANTS SECURED	PERCENTAGE GERMINATION	NUMBER FLOWERED, ALL YELLOW
♀	Parents ♂	Family				
2047(39)	2047(42)	21111	300	154	51.33	126
2047(40)	2046(159)	21115	134	42	31.34	40
2046(159)	2047(40)	21117	300	138	46.00	111
2047(39)	20195(1)	21118	168	64	38.10	60
2047(39)	20247(47)	21119	20	3	15.00	3
2047(39)	20183(36)	21123	323	115	35.60	113
2047(39)	20202(17)	21126	85	22	25.88	20
2047(39)	201(10)	21128	78	21	26.92	16
201(13)	2047(39)	21129	139	14	10.07	11
202(28)	2047(39)	21130	175	21	12.00	20
203(33)	2047(39)	21131	269	46	17.10	38
205(17)	2047(39)	21133	141	15	10.64	11
206(30)	2047(39)	21134	300	104	34.67	90
207(34)	2047(39)	21135	300	121	40.33	85
208(39)	2047(39)	21136	154	21	13.64	17
2047(39)	208(36)	21137	300	97	32.33	84
2047(39)	2031(13)	21138	161	97	60.25	94
2031(13)	2047(39)	21139	300	134	44.67	105
22148(69)	2295(43)	2392	169	103	60.95	90
22218(34)	22162(7)	23139	300	70	23.33	64
237(23)	23137(92)	24106	300	148	49.33	129
23138(44)	23137(92)	24110	300	94	31.33	87
23138(73)	23137(92)	24111	300	106	35.33	95
237(23)	23137(105)	24117	158	61	38.61	19
23138(73)	23137(105)	24118	300	91	30.33	52
237(23)	23137(80)	24121	259	201	77.61	143
23138(73)	23137(80)	24122	300	165	55.00	87
23137(46)	23138(73)	24131	34	3	8.82	3
23138(73)	23137(46)	24132	300	167	55.67	144
23137(46)	23138(73)	24133	49	4	8.16	4
Total						1961

SEGREGATION OF OLD-GOLD FROM SELF-FERTILIZED HETEROZYGOTES

The F₂ families derived from crosses between homozygous yellow and old-gold have generally consisted of yellow-flowered and old-gold-flowered plants, in ratios which do not deviate too seriously from the 3 : 1 ratio, when the size of family is taken into account. Twenty-four families of this type are presented in table 3, and it is seen that the totals show 1005 yellow-flowered to 264 old-gold, where 3 : 1 ratios should have given 952 yellow to 317 old-gold. This very material deviation, which is paralleled

in some of the other segregations in which the old-gold factor was concerned, might be taken to indicate that, while there is no noticeable inferiority of plants possessing old-gold flowers as compared with those which have yellow-flowers, there is, nevertheless, a probable differential elimination of the old-gold type. There is a special consideration which must be taken into account, however, which may modify the stress to be laid on the deviations shown by the ratios in table 3 and in several of the other tables. The seasons of 1922 and 1923, especially the latter, were very unfavorable because of long periods of drouth, and as a result these years were marked by a failure of many plants, especially of the *Lamarckiana* type, to come to bloom.

In certain families it has been quite obvious that the old-gold type has been slightly later in coming to bloom, than the yellow-flowered type, and it is not unlikely that this slightly delayed development has been effective in decreasing the number of old-gold relatively to the yellow, when conditions were such that only a small portion of the plants secured were induced to bloom. A rather striking illustration of this is seen in families 22155 and 22160, produced by the selfing of two heterozygous sibs in family 21117. In the former, only ten plants bloomed, out of 151, and these were all yellow-flowered, while in 22160 in which 77 plants out of 134 bloomed, there were 61 yellow-flowered to 16 old-gold.

THE YELLOW SEGREGATES FROM SELF-FERTILIZED HETEROZYGOTES

The occurrence of 3 : 1 ratios following self-fertilization of *Oenothera Lamarckiana* having been previously known only in crosses of *Lamarckiana* × *brevistylis*, it seemed important to determine, experimentally, whether the dominant class had the composition which Mendelian theory required, namely, one-third homozygous yellow and two-thirds heterozygous yellow, or whether the 3-to-1 grouping was the result of some other cause or causes than the distribution of a pair of factors in such manner that equal numbers of sperms and equal numbers of eggs carried each member of the pair.

In order to determine this point, the family 2123 (see table 3 and figure 1) was made the subject of an analysis of its dominant type. Of the 35 yellow-flowered plants in this family, 34 were self-fertilized, and the resulting cultures grown in 1923. Unfortunately, there was a seven-weeks drouth in the spring of that year, in the midst of which these plants were transplanted from the greenhouse, where they had received daily irrigation, to the experimental field where the artificial application of water was not feasible. In consequence, while there were very few actual losses

by death of the plants, the development was so long retarded that most of the plants resumed the biennial habit which is normal for the species, making large rosettes but developing no stems. Very few of the rosettes lived over the following winter, and no attempt was made to flower any of them in the next season. The scanty data secured from these families are recorded in table 4. With numbers in each family so small, the results

TABLE 3
Families from self-fertilized heterozygotes, from crosses between homozygous yellow and vetaurea. See also table 4.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parent	Family				Yellow	Old-gold
198(54)	2047	164	94	57.32	42	20
208(16)	2123	300	62	20.67	35	8
2047(50)	21110	75	12	16.00	3	3
2052(11)	21158	148	22	14.87	11	3
2052(59)	21159	300	129	43.00	88	25
21115(11)	22150	260	160	61.54	65	9
21117(22)	22155	300	158	52.67	10	0
21117(62)	22160	300	134	44.67	61	16
21118(13)	22162	300	40	13.33	16	5
21118(52)	22163	300	173	57.67	85	14
21119(2)	22167	300	103	34.33	35	5
21120(4)	22170	300	73	24.33	21	11
21123(53)	22188	300	149	49.67	45	14
21126(5)	22208	300	90	30.00	50	16
21130(1)	22209	282	135	47.87	7	4
21130(20)	22210	300	144	48.00	71	15
21130(7)	22211	300	76	25.33	5	0
21139(22)	22212	139	45	32.37	10	3
22166(43)	23144	300	160	53.33	100	24
23137(94)	24129	300	45	15.00	21	8
23137(100)	24130	300	132	44.00	94	22
23139(16)	24144	300	96	32.00	49	10
23139(53)	24146	300	58	19.33	31	7
2392(30)	24152	300	111	37.00	60	22
Total					1015	264
<i>Expected (3:1)</i>					<i>959.25</i>	<i>319.75</i>

are necessarily indecisive, but they are at any rate not in disagreement with the expected results. The 34 families, if distributed as nearly as possible in a 2 : 1 ratio, would have consisted of 23 splitting and 11 non-splitting families, but actually, segregation was manifested in only 13 families and no segregation in 21. When it is observed, however, that one family had no flowering specimen, 3 had only one each, two had only two

each, and so on, and that 24 families had not more than six individuals each, it will be readily seen that a number of potentially splitting families, due alone to the errors of random sampling, will have failed to exhibit a single individual of the recessive type, and such families, consequently, must be grouped with non-splitting families. Assuming that the distri-

TABLE 4

Tests for the occurrence of homozygotes and heterozygotes in Lamarckiana family 2123, which segregated to 35 yellow and 8 vetaurea. All but one of the yellow-flowered plants were self-fertilized.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS BLOOMED	
Parent	Family				Yellow	Vetaurea
2123(1)	22111	300	84	28.00	56	9
(2)	22112	150	33	22.00	2	—
(3)	22113	300	60	20.00	7	—
(4)	22114	300	85	28.33	16	—
(5)	22115	300	32	10.67	3	2
(6)	22116	300	27	9.00	4	—
(9)	22119	300	24	8.00	10	1
(10)	22120	300	22	7.33	4	1
(11)	22121	300	84	28.00	18	—
(12)	22122	300	83	27.67	6	—
(13)	22123	300	47	15.67	4	—
(14)	22124	300	72	24.00	8	—
(15)	22125	300	77	25.67	2	1
(16)	22126	300	72	24.00	20	1
(17)	22127	300	90	30.00	1	2
(18)	22128	300	115	38.33	4	—
(19)	22129	300	121	40.33	12	2
(23)	22130	300	86	28.67	4	1
(24)	22131	300	48	16.00	5	—
(25)	22132	300	46	15.33	5	—
(26)	22133	300	56	18.67	1	—
(29)	22134	300	65	21.67	—	—
(30)	22135	300	70	23.33	1	—
(31)	22136	188	21	11.17	6	—
(33)	22137	300	57	19.00	4	—
(34)	22138	300	86	28.67	1	1
(35)	22139	300	58	19.33	10	—
(36)	22140	300	53	17.67	4	—
(37)	22141	300	58	19.33	1	—
(38)	22142	300	95	31.67	1	2
(39)	22143	300	88	29.33	5	—
(40)	22144	300	85	28.33	3	3
(42)	22145	300	73	24.33	10	1
(43)	22146	300	55	18.33	2	1

Summary: { 20 apparently non-splitting families contain 111 yellow;
14 splitting families contain 129 Y : 28 V, or 4.6 : 1.

bution of the potentially segregating and non-segregating families agrees with the expected 2 : 1 ratio, and calculating the probabilities that the recessive type will be actually included within families having the sizes indicated in table 4 as falling between zero and 6 individuals, inclusively, the theoretical grouping of the 24 families to which these specified limits apply gives 14 apparently non-splitting to 10 splitting. The actual grouping of these 24 families, as seen in the table, is 15 apparently non-splitting and 9 splitting, a very close fit to the theoretical expectation on the assumption that the gametes carrying the factor for old-gold and those carrying its dominant allelomorph are equally numerous in both eggs and sperms. The slight deficiency in the number of splitting progenies is not great enough to be significant in itself, but is in apparent harmony with a significant deficiency in the relative number of recessives as compared with dominants, which resulted from the backcrosses discussed in the next section.

YELLOW-FLOWERED HETEROZYGOTES BACKCROSSED TO OLD-GOLD

The backcrosses of the heterozygotes to the recessive, for the purpose of testing the gametic ratios, are given in table 5. The deviations from the

TABLE 5
Heterozygotes from crosses between yellow and vetaurea, backcrossed to the recessive vetaurea.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parents		Family				Yellow	Vetaurea
♀	♂						
2047(39)	2047(50)	21112	300	115	38.33	46	44
21115(11)	21108(19)	22151	82	38	46.34	17	5
21117(22)	21108(19)	22157	200	100	50.00	29	39
21117(15)	21108(19)	22158	300	73	24.33	6	5
21117(62)	21108(19)	22159	300	106	35.33	47	28
21118(13)	21108(19)	22164	300	36	12.00	6	5
21119(52)	21108(19)	22165	300	100	33.33	39	15
21119(2)	21108(19)	22168	300	87	29.00	16	9
21123(53)	21108(19)	22189	300	148	49.33	57	44
21126(5)	21108(19)	22200	300	142	47.33	39	27
21139(95)	2223(41)	22213	300	159	53.00	30	2
21139(22)	2223(41)	22214	300	113	37.67	34	20
21139(95)	2223(41)	22215	300	210	70.00	80	42
23139(16)	23144(30)	24145	300	69	23.00	27	22
23139(53)	23144(30)	24147	300	86	28.67	32	31
2392(30)	2391(43)	24153	300	204	68.00	71	58
2392(30)	23144(30)	24154	300	137	45.67	46	38
Total						622	434
Expected (1 : 1)						528	528

expected 1 : 1 ratio are considerable, but are quite irregular. There is a suggestion in some of the families in this table that there may be present in these particular families a loose linkage of the old-gold factor with a gamete lethal, but this is a subject which will require much more work, and while some experiments are already under way, bearing on this question, they are not yet ready for discussion. It is of special importance to note in this connection, that in most of those families the *vetaurea* grandparents were direct derivatives of *Oenothera Lamarckiana*, in which no gamete lethals have yet been recognized in association with factors of linkage-group I, although the occurrence of abortive pollen and abortive ovules has been repeatedly interpreted as evidence of the occurrence of lethals in this species. At any rate, if the deviations from the 1 : 1 ratio in the families listed in table 5 result from linkage with one or more gamete lethals, it is clear that these lethals are not the same lethals that so strongly dominate the manifestations of factors belonging to group I. The seventeen families listed in table 5 contained a total of 622 yellow-flowered and 434 old-gold,—a deviation from the expected ratio, 528 : 528, too great to refer to errors of sampling, but the nature of the disturbing factors must await the results of further investigation before they can be profitably discussed.

OLD-GOLD COMPLEMENTARY TO SULFUR

Another flower color, also recessive to yellow, has long been known, namely, the *sulfurea* mutation of *Oe. biennis* and of the nearly related species, *Oe. suaveolens*. Sulfur-flowered forms of these two species have been found occasionally in nature in Europe, over a period of time long antedating the beginning of genetical experiments, and they have also arisen by (crossover) mutation in these two species in experimental cultures of STOMPS (1914) and DE VRIES (1918), and once in my own cultures of *Oe. biennis*. The sulfur factor has also been introduced by DAVIS (1916) into *Oe. franciscana* by means of a cross between an *Oe. biennis sulfurea* and *Oe. franciscana*, and I have now, in turn, introduced it into the *Lamarckiana* series of forms. I have made extensive use of the sulfur factor in my genetical experiments, and have reported its close linkage with all of the factors of linkage group I (SHULL 1921a, b, 1923a, b). It has been, until recently, usually associated with one or other of the gamete lethals which control its manifestations in *Oe. biennis* and *Oe. suaveolens*.

One of the first questions which arose, on finding the new recessive flower color, *vetaurea*, was its relationship to the old recessive color, *sulfurea*, and crosses were immediately made to determine this relation-

ship. The ratio of yellow to old-gold in one of the first families containing the latter type, had suggested the possibility that old-gold might be included in the first linkage group, where sulfur had already been demonstrated to lie, and my first thought was that, like several of the eye-color factors of *Drosophila*, these two color factors were perhaps allelomorphic to each other (SHULL 1921b). This was found, however, to be a wholly erroneous guess, and another quite unexpected relationship between the two colors was discovered, for all the F_1 hybrids between these two types have had the typical yellow flowers characteristic of wild *Oenothera* species, generally. Crosses between homozygous *velaurea* and homozygous *sulfurea* are given in table 6, where it is seen that sixteen matings of this type have yielded 2026 yellow-flowered offspring, and none of any other color. I believe this is the first clear case of complementary factors which has been reported in the *Oenotheras*.

TABLE 6
Families produced by crossing together homozygous velaurea and homozygous sulfurea. See also table 10.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED ALL YELLOW
Parents		Family				
♀	♂					
2047(39)	20250(69)	21120	71	11	15.50	10
2047(39)	20241(101)	21121	300	147	49.00	141
2047(39)	20216(5)	21122	300	139	46.33	134
2047(39)	20258(75)	21124	323	127	39.32	116
20258(111)	2047(39)	21125	300	206	68.67	197
2123(8)	21254(88)	22118	300	183	61.00	91
21225(42)	21113(5)	22149	300	215	71.67	208
22167(41)	22294(49)	2399	300	34	11.33	21
22294(49)	22167(41)	23100	300	192	64.00	186
2390(5)	23141(118)	2482	300	141	47.00	137
23141(118)	2390(5)	2483	300	105	35.00	104
23141(118)	23135(5)	2487	300	148	49.33	145
23141(118)	23144(30)	2488	300	150	50.00	149
23141(118)	23147(92)	24107	300	140	46.67	140
23137(92)	23179(41)	24113	300	116	38.67	64
23141(118)	23137(80)	24123	300	216	72.00	183
Total						2026

FORMULATION OF GENETIC FACTORS FOR FLOWER COLOR

When only two flower colors, yellow and sulfur, were known in the *Oenotheras*, it was possible to formulate mnemonic symbols for the flower-color factors in two ways, either allowing *Y* to represent the yellow and

y the sulfur factor, or letting *S* be the yellow and *s* the sulfur factor. As the former represents the older method, it has commended itself to many geneticists for use except when special considerations render it inappropriate. With the finding of a second factor recessive to yellow, it becomes obvious that the *Yy* formulation is untenable, since *y*, which represents sulfur in one series of crosses, would represent old-gold in another series. Since there is no one factor for yellow, but two or more (possibly many) factors which, acting jointly, produce the yellow flower color, there is no reason for adopting the symbol *Y* as the designation of any one factor. The only alternative, if the conspicuous advantages of mnemonic symbols are to be retained, is to choose the symbol with reference to the recessive trait, as has long been the practice of the students of *Drosophila* genetics, and of some others, and as has been recommended by the Committee on Genetic Form and Nomenclature, of the AMERICAN SOCIETY OF NATURALISTS (LITTLE 1921). The advantages of choosing symbols on the basis of the recessive traits, from the very start of genetical experiments with any organism, is becoming increasingly obvious, because continued work with the given organism is almost certain to show that what appeared at first to be simple characters, are in fact the resultant of the interactions of two or more genes. Symbols must then be changed to fit the new knowledge, whereas, if the recessive trait is used as the basis of the formulation, the subsequent discovery of new recessive components of a given complex are accommodated by merely adding the new symbols required, without the need of altering the symbolization applied to previously discovered elements of the complex. Adopting, then, the symbols, *s* and *S* for the *sulfurea* factor and its dominant allelomorph, the *vetaurea* factor may be represented by *v* and its dominant allelomorph by *V*. A homozygous yellow will then have the formula (with respect only to the known factors), *SSVV*; homozygous sulfur will be *ssVV*; and homozygous old-gold will be *SSvv*.

THE ORIGIN OF GOLD-CENTER, THE FOURTH FLOWER COLOR IN *OENOTHERA*

One of the most interesting new problems which presents itself when two factors are found to be complementary to each other, is the character to be expected in the double-recessive (in this case the *vvss*) segregate. In many cases of complementary flower colors the double recessive is phenotypically indistinguishable from one of the singly recessive traits, giving rise to the phenotypic ratio 9 : 3 : 4, and it could not be foreseen in the present case whether the double recessive would be sulfur-colored, old-gold-colored, or of an entirely new color type, and if the last should

prove true, the nature of the new color type could not be predicted. Would it be a pure white, as has often proved true in other plants, or would it have some other color?

This interesting question was not decided until late in the summer of 1923, when it was recognized that a plant in family 22197, which had been on a previous occasion recorded as *vetaurea*, was really of a new color type which has since been designated "gold-center" (seg. *aurata*). A search was at once begun for other plants of this new type, and three additional plants of *aurata* were found in family 22197, one in 22171 and two in 22199 (see table 7). Unfortunately, some of the plants in these families had already passed out of bloom, so that it was impossible to check up the classification of all those plants which had been recorded as old-gold or sulfur on previous occasions. It is possible, therefore, that several other *aurata* plants should have been added to those recorded in table 7.

The gold-center flowers have the outer portion of the petals nearly as pale as in sulfur, but of a delicate creamy-white rather than the yellowish-white of *sulfurea*; the center of the flower is of about the quality of the old-gold flowers (approaching Primuline Yellow) instead of the greenish-yellow center characteristic of sulfur flowers, (see plate 1).

As the flowers fade, it becomes increasingly difficult to distinguish the several color types, but there is never any difficulty in distinguishing them when the flowers are fresh, and experience has shown that fully developed buds give decisive criteria even in the absence of fresh flowers, so that classifications can now be made with full assurance that no unavoidable mistakes are made.

On examining the families included in table 7 it is seen that in only four of them (22171, 22197, 2396 and 2397) is there a close approximation to the typical dihybrid ratio, 9 : 3 : 3 : 1. In all the other families the *sulfurea* type is either completely absent or is present in numbers very much below what would be expected if no gamete lethals had been brought into these crosses from the sulfur-flowered grandparent. In order to properly interpret these segregations, it must be kept in mind that the lethals are linked with the *Ss* factors, which are involved in the yellow and *aurata* phenotypes as well as in *sulfurea*, so that when first-chromosome gamete lethals have accompanied the *s* factor, the ratios will be modified by the complete or partial elimination of both *sulfurea* and *aurata*, and if it has accompanied the *S* factor the percentage of yellow will be reduced.

The manner in which these "gamete lethals" produce their characteristic effects is not known, and it may, in fact, turn out that the word "lethal" is in this case a misnomer. This has been recognized from the start and

LEGEND FOR PLATE 1

The four flower-color types now known in *Oenothera*. These are, in order, from upper left to lower right, yellow, old-gold, sulfur and gold-center. These figures have been engraved from autochrome photographs, and were all taken from segregates in family No. 24164 which consisted of 57 yellow : 53 old-gold, 48 sulfur : 55 gold-center (see table 16). The engraver has experienced considerable difficulty in reproducing these colors, and it should be noted that the color of the yellow type is distinctly more brilliant than here shown. The correct color being Ridgeway's citron yellow or nearly pale lemon yellow shading to about strontium yellow at the center. The color of sulfur is approximately correct, being nearly naphthaline yellow shaded to citron yellow or strontium yellow at the center. In old-gold and gold-center, the yellow of the distal portion of the petals is not quite so bright yellow as here shown, being nearer Naples yellow and cream respectively; while the warmer tone toward the center of both of these latter forms approaches primuline yellow being quite noticeably less roseate than here shown. The deviations from the correct color tones are not so great, however, as to entirely destroy the usefulness of this plate.

Yellow

Old-gold

Sulfur

Gold-center

the word has been used because of its convenience, and always with the mental reservation that the expression is in a sense figurative, and not necessarily accurately descriptive. It has been intended only to symbolize by the expression, "gamete lethals," genetic factors which make it impossible for the egg or sperm which possesses them, to function in fertilization.

If only the yellow and *vetaurea* columns of table 7 are considered, it is seen that there are here also considerable irregularities, some of which are undoubtedly due to inadequate numbers, but for others no satisfactory explanation is yet available. Only further investigations may be competent to explain the considerable deviations from the 3 : 1 ratio in the different families. The totals of these two columns agree closely with the expected 3 : 1 ratio of yellow to *vetaurea*, the summation giving 747 yellow to 229 *vetaurea*, while the theoretical distribution would be 732 yellow to 244 *vetaurea*.

TABLE 7

Families from self-fertilized heterozygotes from crosses between vetaurea and sulfurea.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED			
Parent	Family				Yellow SV	Vetaurea Sv	Sulfurea sV	Aurata sv
21120(4)	22170	300	73	24.33	21	11	2	—
21120(5)	22171	300	185	61.67	59	20	20	1
21121(24)	22176	300	76	25.33	18	1	—	—
21121(71)	22179	300	120	40.00	23	2	3	—
21122(35)	22182	300	139	46.33	38	1	—	—
21122(96)	22183	300	150	50.00	69	19	3	—
21124(29)	22191	300	106	35.33	40	20	—	—
21124(91)	22192	300	94	31.33	43	1	—	—
21125(21)	22197	300	124	41.33	40	8	5	6
21125(71)	22198	300	210	70.00	116	41	5	—
21125(170)	22199	300	253	84.33	150	60	3	2
22149(5)	2396	300	43	14.33	23	5	5	3
22149(191)	2397	300	184	61.33	80	35	20	5
23143(27)	24191	300	84	28.00	27	5	—	—

Two of the families (2396 and 2397) which gave approximately the typical dihybrid ratio, 9 : 3 : 3 : 1, are of special interest because they were derived from a cross between a strain of *Oe. franciscana sulfurea* which was thought to possess no gamete lethals and *Oe. decipiens vetaurea* which is believed to contain neither of the zygote lethals of *Oe. Lamarckiana* from which it has been indirectly derived. The near agreement of these ratios with those of typical Mendelian hybrids tends to confirm the assumptions made regarding the absence of lethals from the two P₁ plants 21225(42) and 22113(5). A more comprehensive study of this

question will be presented in a later section (page 225). Whether lethals were present in the other crosses in this table had not been predictable.

BACKCROSSES OF DIHETEROZYGOUS YELLOW, $SsVv$, TO OLD-GOLD

The discovery of the compound nature of the yellow flower color has opened up a number of new problems which will be considered in the following sections. For instance, it is important to know whether the synthesized yellow has any peculiarities of genetical behavior, as compared with the homozygous yellow of the wild type. Backcrosses to the several recessive types give the best means of testing this question. In table 8 are given the results of crossing nine diheterozygous yellow-flowered plants with a pure *Lamarckiana vetaurea* pollinator. In terms of the formulation here adopted these crosses represent the combination $SsVv \times SSvv$, and should theoretically yield 1 : 1 ratios of yellow ($SSVv + SsVv$) to *vetaurea* ($SSvv + Ss vv$). It is seen that, on the average, this expectation is closely approximated, for the totals of the nine matings consist of 337 yellow to 363 *vetaurea*, as compared with 350 of each type theoretically expected. When attention is given, however, to the individual families, it is seen at once that there are, in a number of these families, too great discrepancies between the observed and the expected ratios, to be explained by errors of random sampling. What the nature of the disturbing factors may be is not yet known.

The two *sulfurea* individuals in family 22194 may be plausibly attributed to an accident of technique which resulted in a self-fertilization of the diheterozygous mother, 21124(91).

BACKCROSSES OF DIHETEROZYGOUS YELLOW, $SsVv$, TO SULFUR

The corresponding backcrosses of the F_1 hybrids between sulfur and old-gold, to *sulfurea*, are given in table 9. These crosses may be expressed by the formula $SsVv \times ssVV$. The expectation parallels that of the preceding section, except that the 50 percent non-yellow are, in the present case, sulfur instead of old-gold. The yellow segregates should be genotypically of two sorts ($SsVV$ and $SsVv$), and the *sulfurea* segregates likewise of two genotypes ($ssVV$ and $ssVv$). If we separate out three of the eleven families in table 8 (22174, 22196 and 22202) which show rather conspicuous evidence of the effects of gamete lethals, most of the remaining families deviate but little from the expected 1 : 1 ratios, but the totals for these eight families, 496 yellow and 421 sulfur, still deviate too much from the 458.5 of each required by the 1:1 ratio.

TABLE 8
The yellow-flowered F₁ plants from crosses of vetaurea and sulfurea, backcrossed to vetaurea.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED		
♀	Parents	♂					Yellow	Vetaurea	Sulfurea
21120(4)	21108(19)	22172	(V×S)×V	190	74	38.95	5	2	-
21120(5)	21108(19)	22173	(V×S)×V	300	99	33.00	24	13	-
21171(71)	21108(19)	22180	(V×S)×V	300	131	43.67	43	9	-
21122(96)	21108(19)	22185	(V×S)×V	300	92	30.67	30	34	-
21124(91)	21108(19)	22194	(V×S)×V	300	210	70.00	40	32	2*
21124(29)	21108(19)	22195	(V×S)×V	150	48	32.00	3	6	-
21125(21)	21108(19)	22203	(S×V)×V	300	192	64.00	48	70	-
21125(71)	21108(19)	22204	(S×V)×V	300	216	72.00	66	90	-
21125(170)	21108(19)	22206	(S×V)×V	300	241	83.33	78	107	-
Totals							337	363	2*
Expected							350	350	-

* Probably the result of an accidental self-fertilization.

TESTS OF OLD-GOLD- AND SULFUR-FLOWERED SEGREGATES IN F_2
FAMILIES FROM CROSSES BETWEEN OLD-GOLD AND SULFUR

An interesting corollary of the Mendelian interpretation of the distribution of the flower-color factors here under consideration, is the inference that, whereas the sulfur and old-gold types were known heretofore only in the homozygous state, $ssVV$ and $SSvv$, respectively, the segregates having these two phenotypic characters should be divisible in each case into homozygous and heterozygous classes with respect to their genotypic constitutions, the sulfur-flowered plants being of the two genotypes, $ssVV$ and $ssVv$, and the old-gold of the two genotypes $SSvv$ and $Ssvv$.

In order to discover whether these new heterozygous *sulfurea* and *vetaurea* classes were actually present among the segregates from the crosses between old-gold and sulfur, eight *vetaurea* segregates in families 22198 and 22199 were self-fertilized and also crossed with pollen from an *Oe. franciscana sulfurea* (22294(79)), and six *sulfurea* segregates in families 22194, 22198 and 22199 were self-fertilized and at the same time crossed with pollen from an *Oe. Lamarckiana vetaurea* (2220(40)). The results of these fourteen double tests are assembled in table 10, grouped according to the demonstrated genotypes. Of the 8 *vetaurea* segregates tested, 3 proved to be homozygous ($SSvv$) yielding only *vetaurea* when selfed (the unexpected presence of 13 yellows in family 23111 being certainly due to some technical error), and when crossed with homozygous sulfur produced only yellow-flowered offspring.

The remaining 5 *vetaurea* plants tested were found to be heterozygous ($Ssvv$), splitting to *vetaurea* and *aurata* when selfed and giving yellow and *sulfurea* in approximately equal numbers when crossed with homozygous sulfur. In all of the selfings of this group of plants there was a very marked deficiency in the number of *aurata* plants, the totals showing 692 *vetaurea* to 13 *aurata*. This is interpreted as meaning that the *sulfurea* factor (s) in all of these five plants was associated with a sperm lethal. That there was no egg lethal present is shown by the fact that the crosses to sulfur gave a total of 437 yellow to 445 sulfur, a very close fit to the expected 1 : 1 ratio.

Of the five sulfur-flowered segregates tested, two proved to be homozygous ($ssVV$) (third section of table 10), giving only sulfur when selfed and only yellow when crossed with *vetaurea*, while four (fourth section of table 10) were found to be heterozygous ($ssVv$), giving both sulfur and *aurata* when selfed, and both yellow and *vetaurea* when backcrossed to *vetaurea*.

The self-fertilizations totalled 422 *sulfurea* : 94 *aurata*, or 4.5 : 1, which bears the same relation to 3 : 1 that is often seen in other cases, in which an adequate explanation is found in the assumption that the recessive is slightly less effective, physiologically, than the dominant type. The backcrosses of these same plants, however, show such an excess of *vetaurea* over yellow in all families except that derived from 22194(53), that we must assume that the inadequacy of the sulfur type is a gametic rather than a somatic one, or possibly that it is both gametic and somatic.

Notwithstanding such important discrepancies as these, it is seen that this series of tests demonstrates the occurrence of heterozygous as well as homozygous *vetaurea* and *sulfurea* individuals in the progenies of the diheterozygous yellows, and the numbers of these several types,—3 $SS'vv$: 5 $Ssvv$, and 2 $ssVV$: 4 $ssVv$,—are as close as possible to the 1 : 2 ratios expected.

GOLD-CENTER BREEDS TRUE

The finding of the new flower color, "gold-center," (seg. *aurata*) gave the basis of a new set of analyses of the flower color characters. These have not yet been carried out so extensively as have the breedings with old-gold, but they are, nevertheless, sufficiently consistent that there can be no doubt as to the validity of the conclusions to which they lead. It was assumed, at once, on the discovery of the gold-center plants, that they were the double-recessive segregates ($ssvv$) from the cross between sulfur and old-gold, and the correctness of this assumption has been demonstrated by the series of tests discussed in the last section and recorded in table 10. It was also recognized, however, that no other new character which originated as a definite segregate from a previous cross has been reported in the *Oenotheras*, and it has seemed desirable, therefore, to submit the new type to a full series of breeding tests in combination with the other color types. In table 11 it is seen that four families produced by self-fertilization of plants of the *aurata* or gold-center type yielded 489 *aurata* offspring, and none of any other color. This result agrees with what is expected of recessive types, generally, on being self-fertilized.

RECESSIVENESS OF GOLD-CENTER TO ALL OTHER COLORS

When gold-center is crossed with homozygous strains of yellow, sulfur or old-gold, it is found to be in all cases completely recessive. Table 12 gives the results of five crosses with homozygous yellow, which yielded a total of 805 yellow-flowered progeny and 1 *aurata*, the last being un-

TABLE 10

Self-fertilizations of segregates in F₂ families from crosses between vetaurea and sulfurea, and backcrosses of the same plants to sulfurea and vetaurea, respectively, to discover the several genotypes present. Arranged in four sections according to the demonstrated genotype.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED			
♀	Parents ♂	Family					Yellow	Vetaurea	Sulfurea	Aurata
22198(15)	22198(15)	23111	V×self	300	138	46.00	13*	94	—	—
22198(15)	22294(79)	23112	V×S	300	235	78.33	205	—	—	—
22199(197)	22199(197)	23127	V×self	300	234	78.00	—	226	—	—
22199(197)	22294(79)	23128	V×S	300	157	52.33	146	—	—	—
22199(238)	22199(238)	23129	V×self	300	5	1.67	—	5	—	—
22199(238)	22294(79)	23130	V×S	300	156	52.00	150	—	—	—
Totals (3 individuals)		V×self (omitting 13 yellow in 23111)					—	325	—	—
		V×S					501	—	—	—
Demonstrated genotype, <i>SSvv</i>										
22198(38)	22198(38)	23113	V×self	300	202	67.33	—	169	—	2
22198(38)	22294(79)	24114	V×S	300	254	84.67	112	—	130	—
22198(43)	22198(43)	23115	V×self	300	75	25.00	—	62	—	—
22198(43)	22294(79)	23116	V×S	300	176	58.67	75	—	83	—
22198(55)	22198(55)	23117	V×self	300	146	48.67	—	108	—	1
22198(55)	22294(79)	23118	V×S	300	171	57.00	75	—	89	—
22199(96)	22199(96)	23123	V×self	300	244	81.33	—	186	—	5
22199(96)	22294(79)	23124	V×S	300	201	67.00	112	—	75	—
22199(162)	22199(162)	23125	V×self	300	198	66.00	—	167	—	5
22199(162)	22294(79)	23126	V×S	300	135	45.00	63	—	68	—
Totals (5 individuals)		V×self					—	692	—	13
		V×S					437	—	445	—
Demonstrated genotype, <i>Ssvv</i>										
22194(8)	22194(8)	23101	S×self	300	264	88.00	—	—	127	—
22194(8)	2220(40)	23102	S×V	300	151	50.33	110	—	—	—
22199(16)	22199(16)	23131	S×self	300	95	31.67	—	—	90	—
22199(16)	2220(40)	23132	S×V	300	167	55.67	151	—	—	—
Totals (2 individuals)		S×self					—	—	217	—
		S×V					261	—	—	—
Demonstrated genotype, <i>ssVV</i>										
22194(53)	22194(53)	23103	S×self	300	190	63.33	—	—	53	8
22194(53)	2220(40)	23104	S×V	300	92	30.67	29	14	—	—
22198(20)	22198(20)	23119	S×self	300	222	74.00	—	—	128	30
22198(20)	2220(40)	23143	S×V	300	252	84.00	89	122	—	—
22198(175)	22198(175)	23120	S×self	300	232	77.33	—	—	152	25
22198(175)	2220(40)	23121	S×V	300	230	76.67	74	104	—	—
22199(91)	22199(91)	23133	S×self	300	134	44.67	—	—	89	31
22199(91)	2220(40)	23134	S×V	300	91	30.33	31	54	—	—
Totals (4 individuals)		S×self					—	—	422	94
		S×V					223	294	—	—
Demonstrated genotype, <i>ssVv</i>										

*Undoubtedly due to some technical error.

TABLE 11
Self-fertilizations of the new flower color, gold-center (*aurata*).

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED, ALL <i>aurata</i>
♀	Parents ♂	Family				
22197(94)	22197(94)	23105	300	175	58.33	139
22199(33)	22199(33)	23122	300	115	38.33	93
2396(2)	2396(2)	24155	300	176	58.67	137
23133(59)	23133(59)	24187	300	166	55.33	120
Total						489

TABLE 12
Crosses of *aurata* to homozygous yellow.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
♀	Parents ♂	Family				Yellow	<i>Aurata</i>
22197(94)	22293(76)	23107	300	65	21.67	63	—
22197(94)	2295(43)	23108	300	269	89.67	232	—
22197(94)	22166(7)	23109	300	76	25.33	70	—
22197(94)	22218(34)	23110	300	230	76.67	219	1*
22218(34)	22197(94)	23146	296	235	79.39	221	—
Total						805	1*

* Undoubtedly the result of an accidental self-pollination.

TABLE 13
Crosses of *aurata* to homozygous *vetaurea*.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED ALL <i>vetaurea</i>
♀	Parents ♂	Family				
23137(92)	23133(59)	24108	300	153	51.00	86
23133(59)	23137(92)	24109	300	198	66.00	174
23133(59)	23144(30)	24142	300	205	68.33	199
23133(59)	23135(5)	24188	300	194	64.67	178
Total						637

doubtedly the result of an accidental self-fertilization of the *aurata* mother, 22197(94). Table 13 contains the data from four crosses of *aurata* with homozygous *vetaurea*, the total progeny consisting of 637 *vetaurea* and none of any other color. Table 14 gives the corresponding results of three crosses between *aurata* and homozygous *sulfurea*, the total offspring of which consisted of 620 *sulfurea*, and none of any other color.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
♀ Parents	♂	Family				<i>Sulfurea</i>	<i>Aurata</i>
23106(16)	23106(16)	24172	300	165	55.00	90	15
<i>Expected (3:1)</i>						<i>78.75</i>	<i>26.25</i>
23105(125)	23106(16)	24173	300	155	51.67	73	38
23106(16)	23133(59)	24174	300	130	43.33	54	32
23114(125)	23113(80)	24184	300	182	60.67	78	79
23114(125)	23113(179)	24185	300	133	44.33	62	48
23114(125)	23113(192)	24186	300	182	60.67	96	51
Total						363	248
<i>Expected (1 : 1)</i>						<i>305.5</i>	<i>305.5</i>

all other cases there is a deficiency of the gold-center class. The progeny from self-fertilization shows a deviation of 11.25 ± 2.99 , and the totals from the backcrosses give a deviation of 57.5 ± 8.34 . These deviations are probably not due to lethal factors, but perhaps to some slight inferiority of the recessive type, as is so often indicated in other organisms.

THE DOUBLE-DOMINANT SEGREGATES IN DIHYBRID F_2 FAMILIES

We have seen in table 7 that families 2396 and 2397 yielded a close approximation to the 9 : 3 : 3 : 1 ratio. These two families were especially interesting because they represented a cross between *decipiens vetaurea*, believed to be free from zygote lethals, and a strain of *franciscana sulfurea*, which was assumed to have lost the gamete lethals with which the *sulfurea* factor has been usually associated. The presentation of nearly typical dihybrid ratios in the second generation of this cross seemed to confirm the assumptions regarding the freedom of the two P_1 plants from lethals. As no one else has ever reported typical dihybrid ratios in *Oenothera*, it was thought desirable to determine experimentally whether the 9 : 3 : 3 : 1 ratio in these families has the significance that it has in typical Mendelian breeding. For this purpose an analysis of the genotypes represented in the double-dominant phenotypic class, yellow, was undertaken by means of backcrosses to the double-recessive type, gold-center. The nine individuals of the double-dominant class in the theoretical 9 : 3 : 3 : 1 ratio, consist of 1 pure double-dominant ($SSVV$), 2 monoheterozygotes with respect to the first factor ($SsVV$), 2 monoheterozygotes with respect to the second factor ($SSVv$), and 4 diheterozygotes ($SsVv$). Backcrosses of plants having these several genotypic constitutions, to the double recessive ($ssvv$), should give four kinds of families in the ratio 1 : 2 : 2 : 4.

Five yellow segregates in family 2396 and nine in family 2397 were used to pollinate the flowers on sixteen different branches of a single large *aurata* segregate, 2396(2), and a seventeenth branch of the same plant was self-fertilized (see table 11). The sixteen families derived from these backcrosses were grown during the past season (1925) and the results are given in table 16. The four sections of the table correspond with the four genotypes expected on a Mendelian basis, and the fit to the theoretical ratio, 1 : 2 : 2 : 4, is as close as possible,—much closer than could reasonably be expected in a test of only 16 individuals.

When consideration is given to the ratios in the individual families, however, irregularities are seen, very much too great to be attributed to errors of sampling, though the theoretical ratios are closely approached in a number of cases. Thus, all of the ratios of the third section ($SsVV$)

agree very well indeed with the expected 1 : 1 ratio, the deviation being 11.5 and the probable error, ± 8.27 . The last ratio of section 2 is sufficiently near to the 1 : 1 ratio, and at least one family (24164) in the last section shows a very close approach to the 1 : 1 : 1 : 1 ratio, a gametic ratio that, so far as I know, has never before been reported in the *Oenotheras*. The irregularities in the other families cannot at present be accounted for, and must await the light of a fuller analytical test. The most aberrant family (24162) is of special interest because of the suggested presence of a gamete lethal cutting down the *sulfurea* and *aurata* classes. Two of the families (24163 and 24168) in the second section also suggest a rather loose linkage between a gamete lethal and the old-gold factor, reducing the old-gold class to about half the expected size. An important point to be made, however, is, that if the mentioned discrepancies are in fact the result of lethal factors, there are no indications that the lethals which affect the *vetaurea* class are the same as those which modify the *sulfurea* class. All the evidence indicates that *vetaurea* and *sulfurea* belong to different linkage groups.

INDEPENDENCE OF THE OLD-GOLD FACTOR FROM OTHER FACTORS OF THE FIRST LINKAGE GROUP

While no extensive series of tests have been made to determine the linkage or independence of the old-gold factor, *v*, and its allelomorph *V*, in relation to other factors of linkage-group I, a number of the families listed in the foregoing tables have involved other first-chromosome factors besides the sulfur factor which was specifically under consideration. For the sake of simplicity no attention has been given to these other factors in the present discussion. It is sufficient for the present to simply cite an example or two to indicate the relation of *vetaurea* to other first-chromosome factors, and to summarize the experiences to date by the statement that thus far no situation has presented itself which would suggest a linkage between old-gold and any factor included in the first linkage group.

In 1921 a cross was made between one of the original *vetaurea* plants, 2047(39), which had the form of *Lamarckiana*, tall with green-hypanthium buds, and a *rubricalyx nanella*, 20202(17), dwarf, with red hypanthia. The F_1 family (21126) consisted of 20 tall yellow-flowered plants with *rubricalyx* buds (see table 2). One of the plants of this family 21126(5), self-fertilized, produced the F_2 family 22208 listed in table 3. The tall *versus* dwarf stems and red *versus* green hypanthia are closely linked

TABLE 16

Double-dominant yellow-flowered plants in (sulfurea × vetaurea)F₂, backcrossed to the double-recessive aurata.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	FLOWER COLORS OF OFFSPRING				GENOTYPE OF YELLOW-FLOWERED PARENT PLANTS
Parents		Family				Yellow	Vesauvea	Sulfurea	Aurata	
♀	♂									
2396(2)	2396(12)	24158	300	196	65.33	159	—	—	—	SSVV (1 plant)
2396(2)	2397(8)	24163	300	140	46.67	75	43	—	—	SSVv (3 plants)
2396(2)	2397(57)	24168	300	173	57.67	90	44	—	—	
2396(2)	2397(62)	24171	300	219	73.00	97	83	—	—	
Total						262	170			
Expected (1 : 1)						216	216			
2396(2)	2396(13)	24159	300	211	70.33	86	—	90	—	SsVV (4 plants)
2396(2)	2396(40)	24161	300	140	46.67	34	—	44	—	
2396(2)	2397(50)	24165	300	249	83.00	103	—	105	—	
2396(2)	2397(60)	24169	300	177	59.00	66	—	73	—	
Total						289	312			
Expected (1 : 1)						300.5	300.5			
2396(2)	2396(1)	24156	300	144	48.00	22	20	23	14	SsVv (8 plants)
2396(2)	2396(7)	24157	300	255	85.00	46	40	54	61	
2396(2)	2396(15)	24160	300	194	64.67	39	35	49	20	
2396(2)	2396(41)	24162	300	153	51.00	70	26	4	3	
2396(2)	2397(48)	24164	300	228	76.00	57	53	48	55	
2396(2)	2397(51)	24166	300	173	57.67	46	28	30	12	
2396(2)	2397(54)	24167	300	216	72.00	43	32	54	27	
2396(2)	2397(61)	24170	291	198	68.04	38	34	54	30	
Total						361	268	316	223	
Expected (1 : 1 : 1 : 1)						292	292	292	292	
Difference						+69	—24	+24	—69	

together in the first chromosome, and as the two dominant factors, tall stems and *rubricalyx* buds, were brought into the cross from opposite sides, they segregated in the form of a typical repulsion-phase ratio, namely 1 tall with green hypanthium : 2 tall with red hypanthium : 1 dwarf with red hypanthium, the actual numbers being 14 : 39 : 13. The distribution of the flower colors was as follows:

	<i>Tall green</i>	<i>Tall red</i>	<i>Dwarf red</i>
Yellow	10	29	11
Old-gold	4	10	2

The close parallel in the two color classes is obvious. Putting all together in the form of a single ratio gives the numbers:

Observed	10 : 29 : 11 : 4 : 10 : 2
<i>Expected</i> (3 : 6 : 3 : 1 : 2 : 1)	12 : 25 : 12 : 4 : 8 : 4

The fit is remarkably close, in view of the rather small number of individuals available and the six-term phenotypic ratio.

The independence of old-gold from *rubricalyx* bud color was also shown by a cross of the same individual (21126(5)) whose self-fertilized progeny has just been considered, to a *Lamarckiana vetaurea*, 21108(19), this constituting a backcross of a plant heterozygous for yellow flowers and *rubricalyx* buds, to the double recessive with old-gold flowers and green hypanthia. The resulting family (22200) consisted of 15 *rubricalyx* yellow, 24 green-hypanthium yellow, 14 *rubricalyx vetaurea* and 13 green-hypanthium *vetaurea*, the 1 : 1 : 1 : 1 ratio expected on the basis of independence requiring 16.5 plants in each class. The value of χ^2 is 4.67 and $P=0.201$.

THE INDEPENDENCE OF OLD-GOLD AND *brevistylis*

Having now shown that the *vetaurea* factor is independent of *sulfurea*, *nanella*, *rubricalyx* and the first-chromosome lethals, there remains the question as to its relation to the second-chromosome factor for short styles, *brevistylis*.

Self-fertilizations and backcrosses of plants in which both the *vetaurea* factor *v* and the *brevistylis* factor *b*, were heterozygous have given a decisive answer to this question, as will be obvious from tables 17 and 18. The very close agreement with the 3 : 1 ratio in every color class in table 17 and with the 1 : 1 : 1 : 1 ratio in table 18, tells impressively the story of independence. The greatest deviation occurs in the *vetaurea* class in table 17 where it is 6.5 and the probable error ± 2.89 . It will be noted that the backcrosses entered in table 18 involve the same three heterozygotes whose self-fertilization progenies are given in the last three lines of

table 17. The most interesting of these families is 24139, whose parent was heterozygous not only for *vetaurea* and *brevistylis* but also for *sulfurea*. Had no "pollen lethal" been associated with the sulfur factor in linkage-group I, the sulfur-flowered segregates should have been as numerous as the old-gold segregates and there should have been also a group of *aurata* offspring as well. That the parent of this family was producing *S*- and *s*-bearing eggs in equal numbers, in agreement with Mendelian theory, and that the sulfur factor is likewise independent of *brevistylis* was shown by a backcross of 23109(23) to the double recessive *sulfurea brevistylis*, 23179(41). The resulting family (24141) consisted of:

	Yellow		Sulfur		Unclassified	
	Long-styled	<i>Brevistylis</i>	Long-styled	<i>Brevistylis</i>	Long-styled	<i>Brevistylis</i>
Observed	59	61	42	38	6	5
Expected	50	50	50	50	-	-
Difference	+ 9	+11	- 8	-12	-	-

The value of χ^2 is in this case 8.2, giving $P = .0427$. This low value of P shows that the deviation is almost certainly in part due to other causes than the errors of random sampling, but the ratio between long-styled and *brevistylis* is almost exactly 1 : 1 in each color class, and this is the criterion of independence between the color factors and the style-length factors, in which we are here specifically interested. If the ratio 59 : 61 : 42 : 38 be considered as a linkage ratio, it is seen that the crossover classes are actually slightly, though insignificantly, in excess of the non-crossover classes.

DISCUSSION

The data presented in the foregoing sections of this paper make it clear that the new flower-color factor, "old-gold," is distributed in heredity more nearly in accord with the typical Mendelian model, than any other *Oenothera* factor which has yet been studied, with exception of the *brevistylis* factor, which latter has always heretofore been the outstanding exception to the seeming perversities of the genetical situation in the *Oenotheras*.

That there are very serious deviations from the "expected" ratios in individual families must be conceded, and there is no inclination to minimize the importance of these discrepancies when I point out that the remarkable agreement with all the larger implications of the Mendelian scheme of distribution indicates that, compared with these fundamenta

TABLE 17
Segregations following self-fertilization in crosses involving *vetaurea* and *brevistylis*.

PEDIGREE NUMBERS		TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	YELLOW		<i>Vetaurea</i>		<i>Sulfurea</i>		UNCLASSIFIED	
Parent	Family					Long-styled	<i>Brevistylis</i>	Long-styled	<i>Brevistylis</i>	Long-styled	<i>Brevistylis</i>	Long-styled	<i>Brevistylis</i>
21118(13)	22162	(VL×Ybr)F ₁	300	40	13.33	14	2	3	2	—	—	—	—
21118(52)	22163	(VL×Ybr)F ₁	300	173	57.67	66	19	12	2	—	—	2	—
22166(43)	23144	(VL×Ybr)F ₂	300	160	53.33	78	22	19	5	—	—	6	2
23109(23)	24139	(AL×Ybr)F ₁	300	210	70.00	85	38	32	6	9	2	3	2
23139(16)	24144	(YL×Vbr)F ₁	300	96	32.00	38	11	7	3	—	—	—	—
23139(53)	24146	(YL×Vbr)F ₁	300	58	19.33	21	9	7	0	—	—	—	—
Totals						302	101	80	18	9	2	11	4
<i>Expected</i> (3 : 1)						302.25	100.75	73.5	24.5	8.25	2.75	11.25	3.75

TABLE 18
Backcrosses of the diheterozygote to the double recessive in crosses involving *vetaurea* and *brevistylis*.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	YELLOW		<i>Vetaurea</i>		UNCLASSIFIED	
♀	Parents ♂	Family					Long-styled	<i>Brevistylis</i>	Long-styled	<i>Brevistylis</i>	Long-styled	<i>Brevistylis</i>
23109(23)	23144(30)	24140	(AL×Ybr)F ₁ ×Vbr	100	10	10.00	2	2	1	2	1	—
23139(16)	23144(30)	23145	(YL×Vbr)F ₁ ×Vbr	300	69	23.00	18	11	9	13	1	1
23139(53)	23144(30)	24147	(YL×Vbr)F ₁ ×Vbr	300	86	28.66	15	16	18	13	2	—
Total							35	29	28	28	4	1
<i>Expected</i> (1 : 1 : 1 : 1)							30	30	30	30	—	—

agreements, the deviations of ratios in single families are of minor significance. Further intensive and extensive analyses may be expected in time to differentiate between the genetic, physiological and environmental causes of these irregularities in the ratios.

The demonstration that the old-gold factor is independent of the sulfur factor and other factors linked with sulfur in linkage-group I, and that it is also independent of *brevistylis*, the "marker" of linkage-group II, indicates that it is the first-discovered factor of linkage-group III, for which it may well serve as the "marker." That it is proper to speak of these as *linkage groups*, and not merely as independent factors, has been shown by the discovery of a second factor closely linked with old-gold in group III (SHULL 1925), and I have evidence that there is a second factor in group II, linked with *brevistylis*.

The chief importance of these new discoveries in *Oenothera*, as in purely scientific genetical experiments, generally, is in the bearing they have as indicators of the nature of the mechanism which controls the distribution of the hereditary factors. It has been demonstrated by the work of many investigators, and by evidence from several different sides, that independent inheritance (Mendelian) results from the inclusion of the genes in different pairs of chromosomes. Correlatively, the work especially of MORGAN and his students and collaborators has proven that the linkage of factors in *Drosophila* depends upon the inclusion of the linked factors in a single chromosome pair, and this interpretation has been accepted for many other organisms in which linked genes have been studied by other investigators. I have accepted the probable correctness of this same interpretation as applied to the linkage phenomena in *Oenothera*.

GATES (1908) and DAVIS (1910) have found a telosynaptic arrangement of the chromosomes in *Oenothera*, and GATES (1923) holds that this fact is an obstacle to the occurrence of partial linkage in this genus. The genetical evidence of linkage with crossing over is unequivocal, however, and every season adds new and striking examples of it in my cultures.

CLELAND'S (1922, 1923, 1924, 1925, 1926) brilliant work on the chromosome groupings in *Oenothera* has brought to light a behavior among the chromosomes, which at once suggests the possibility that the linkages in *Oenothera* might be due to the cohesion of non-homologous chromosomes, and their separation during meiosis in such manner that the maternal members of the group are drawn to one pole and the paternal members to the other pole of the spindle. CLELAND finds a marked tendency for the

alternate members of the circles of cohering chromosomes to go to the same pole. If this behavior is of general occurrence it need only be assumed that the paternal and maternal chromosomes alternate with each other in the circles, to account for the formation of gametes predominantly of two kinds, the one possessing the entire maternal complex in so far as that complex is included in the chromosomes of the circle, and the other possessing the corresponding paternal complex. Crossing over would result, then, simply through an irregularity which would reverse the usual program with respect to one or more of the pairs of chromosomes included in the circle, so that one or more maternal chromosomes would migrate to the otherwise paternal pole and *vice versa*.

CLELAND has reported an amount of irregularity in the separation of the chromosomes that would apparently account for a very much greater degree of genetic variability than has been found by students of *Oenothera* genetics, and this is one of the difficulties, perhaps, in identifying chromosomal cohesion as the mechanism of linkage in this group.

There are several other difficulties which seem to me to stand in the way of accepting the hypothesis that the linkage phenomena in *Oenothera* result from the end-to-end cohesion of non-homologous chromosomes. One of these I have already pointed out, namely, that in the first linkage group there are now known about twice as many factors as there are haploid chromosomes in *Oenothera*. It might seem necessary on this account to assume the inclusion of more than one of these factors in a single chromosome, but this is not yet a *necessary* conclusion, since all of the known differential factors belonging to group I have never been included experimentally in any single zygote, (though there is no evidence at present that all can not be so included). Not until it can be shown that more linked factors can be carried by a single gamete than half the number of chromosomes involved in the circle, can it be *certain* that two or more of the linked factors *must* lie in a single chromosome pair. CLELAND suggests that a part of the linkage may be due to inclusion of genes in the same chromosome, and part to chromosomal cohesions. If this should prove true, an important question to be determined is what criteria can be used to distinguish between these two kinds of linkage.

Another difficulty for the chromosome-cohesion hypothesis is that the numerous 15-chromosome mutants from *Oenothera Lamarckiana* are characterized by marked differences in regard to nearly all features of the plant. DE VRIES and BOEDIJN (1923) have grouped these 15-chromosome forms into seven primary groups, corresponding with the seven haploid chromosomes, and have explained them on the basis of non-disjunction

of a different chromosome for each group. If this plausible hypothesis is correct, each of the haploid chromosomes produces strikingly differential effects on many features of the plant. If the production of crossovers is due to an aberrant distribution of whole chromosomes, and each chromosome produces manifold effects, it seems reasonable to suppose that the crossover individuals would be differentiated by other characteristics besides the one which is being followed in any particular breeding problem. Except in the case of the lethal factors this has been, quite generally, not the case; as a rule the crossover individuals are quite indistinguishable from the non-crossovers, except in regard to the specific characteristic which differentiated the P_1 individuals.

The old-gold mutation appeared in a strain of *Oenothera Lamarckiana* which CLELAND (1925) has studied and has found to possess 12 chromosomes united into a circle, with one pair free. As *Lamarckiana* is demonstrably carrying, all the time, in heterozygous state, several factors of linkage-group I, it might be assumed that these linked factors are distributed among the several chromosomes of the circle, and that the *vetaurea* factor lies in the independent pair. But *Lamarckiana* has always shown independence of the factor for *brevistylis*, which is found to be likewise independent of old-gold. With only *one* independent pair of chromosomes there would be no provision for *two* independent factors. There remains the logical possibility, of course, that a *Lamarckiana* which simultaneously segregates out both old-gold and *brevistylis* might be found to possess only ten chromosomes in the circle, and two independent pairs. This is a point which needs to be investigated.

The demonstration that old-gold represents not alone an independent factor, but a member of a third linkage group (SHULL 1925), and the hint that *brevistylis* is likewise not merely an independent factor but a member of the second linkage group, suggest another line of work which may be expected to give a crucial test of the chromosome-cohesion hypothesis of linkage. Thus far, CLELAND has reported only one case (*Oe. biennis*) in which the chromosomes are grouped into more than one circle,—and in this case he assumes that, although they are grouped into two circles, they are distributed as if they were included in a single circle (CLELAND 1926). The finding of as many groups of cohering chromosomes as there are linkage groups present in any given case, would strongly support the cohesion hypothesis, if it be admitted that the circles are oriented on the spindle independently of each other. It is clear that a close coöperation between geneticist and cytologist may promptly result in a decision between the chromosome-cohesion hypothesis of linkage and the hypothe-

sis that linkage results from the inclusion of factors in a single chromosome pair. If both of these cytological phenomena give rise to linkage, there may be as many linkage groups as the number of independent chromosomes plus the number of chromosome circles that are present, but no more. In other words there could be two linkage groups only in *Lamarckiana*, two in *biennis*, five in *rubrinervis*, seven in *blandina*, and so on. There is no evidence yet available that such differences exist in the number of linkage groups in the different taxonomic forms of *Oenothera*, but this may be due merely to the fact that we are still only at the beginning of an adequate study of linkage in this group.

SUMMARY

1. The new flower color, "old-gold" (mut. *vetaurea*) made its appearance in cultures of *Oenothera Lamarckiana* in 1921.

2. Appropriate crosses have demonstrated that it resulted from a gene mutation which probably occurred two years before the new characteristic became visible.

3. The old-gold factor, *v*, is recessive to yellow, and segregates from the yellow-flowered F_1 in a manner suggestive of a typical Mendelian recessive, but with considerable variation in the ratios.

4. The dominant yellow-flowered plants in the F_2 were found to consist of homozygotes and heterozygotes in about the typical Mendelian ratio, 1 : 2.

5. Old-gold is complementary to sulfur, the F_1 plants being always wild-type yellow.

6. In the F_2 from the cross between old-gold and sulfur, a fourth flower-color, gold-center (seg. *aurata*), appeared as the double recessive.

7. These results indicate that the four flower colors now known in *Oenothera* should be represented, when in the homozygous state, by the genotypic formulae: Yellow, *SSVV*; sulfur, *ssVV*; old-gold, *SSvv*; and gold-center, *ssvv*.

8. In some of the F_2 families the sulfur and gold-center classes were strikingly modified by the action of "gamete lethals," but in four families there was a close approach to the typical Mendelian dihybrid ratio, 9 yellow : 3 sulfur : 3 old-gold : 1 gold-center.

9. Tests of old-gold and sulfur segregates in F_2 families from the cross sulfur $\times$ old-gold showed that each of these types occurred in the heterozygous condition and in the homozygous condition in about the expected 2 : 1 ratio.

10. Analysis of the yellow-flowered group in an F_2 family, showed the presence of 1 $SSVV$, 3 $SSVv$, 4 $SsVV$ and 8 $SsVv$, which is in essential agreement with the expected Mendelian ratio of 1 : 2 : 2 : 4.

11. The old-gold factor is independent in inheritance from the factor for sulfur and from the lethals and other factors associated with sulfur in linkage-group I.

12. It is also independent of *brevistylis* which has been used as the "marker" of linkage-group II.

13. It is, therefore, the first-discovered factor of linkage-group III, for which it may well serve as the "marker."

14. A brief critique is given of the hypothesis that linkage in the *Oenothera*s is based on the end-to-end fusion of non-homologous chromosomes to form circles (CLELAND).

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