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**Botany.** --- "*Variability of segregation in the hybrid*". By Dr. J. A. HONING. (Communicated by Prof. F. A. F. C. Went).

(Communicated in the meeting of November 25, 1916.)

Most botanists investigating heredity prefer to employ annual plants and endeavour to force biennials to flower in their first year in order to obtain seed, as was done by nearly all investigators of *Oenothera*. Perennials, to say nothing of trees, often require several years before their seedlings bloom, and sometimes they produce but few seeds, so that their use has naturally been less in favour.

The flowering season of annuals is brief, a few months only, and the seed obtained by self pollination of annual hybrids from different fruits is generally sown mixed, on the assumption that the segregation ratios are constant, so that for the ratios of the phenotypes it does not matter much whether seed has been collected from the first fruits or from those matured a month later. Probably this assumption is correct in many cases, perhaps in most, but not in all, as ZEDERBAUER <sup>1)</sup> has shown for *Pisum*.

There is no reason whatever to assume that *Pisum* is unique in this respect and further examples will doubtless be found. The best chance of finding clear cases will be, for annuals, among those with a long flowering season, and further among those perennials, of which one and the same individual can be studied for some years in succession. A tropical climate enabling one to collect seed almost throughout the year, would then be advantageous.

In order to ascertain whether independent Mendelian segregation can take place simultaneously with respect to a number of factors, larger than that of the chromosomes, I crossed in 1913 a variety of *Canna glauca* with one of *C. indica*. The number of genes in which these two differ was — and remains, unknown; it was certainly larger than three, the number of chromosomes in *Canna indica* according to WIEGAND <sup>2)</sup> and certainly larger than eight, the actual number of chromosomes, which was already indicated by KOERNICKE <sup>3)</sup>. A brief description of the two species will make this clear.

<sup>1)</sup> ZEDERBAUER, E., Zeitliche Verschiedenwertigkeit der Merkmale bei *Pisum sativum* Ztschr. f. Pflanzenzüchtung II p. 1—26, 1914.

<sup>2)</sup> Bot. Gaz. T. 30, 1900.

<sup>3)</sup> Ber. d. d. bot. Ges. XXI. 1903, p. 66 See also Rec. d. trav. bot. Néerl. XII, 1915, p. 23.

Organ	<i>Canna indica</i>	<i>Canna glauca</i>
Stem	<i>tall</i> , about 2 m. stool with few stalks	low about 1 m. stool with many stalks
Leaves	short about 39 cm. <i>broad</i> , about 16 cm. with <i>broad red margin</i> shiny	<i>long</i> about 50 cm. narrow, about 12 cm. green <i>dull</i> , on account of a layer of wax
Staminodes	two dark <i>red</i>  short, average 59 m.m. narrow, 8—12 m.m., average 10—8	<i>three</i> pale yellow with a few pink spots <i>long</i> , average 83 m.m. <i>broad</i> 13—20 m.m., aver- age 16—2
Ovary	<i>red</i>	green
Seeds	small round <i>uniformly black</i>	<i>large</i> <i>oblong</i> brown with black speckles

The two plants used for crossing were both F<sub>2</sub> individuals, obtained by repeated self pollination and were similar to their F<sub>1</sub> and P. After repeated failures a single ripe fruit was obtained from the cross *glauca* × *indica*; it contained two seeds, one of which failed, so that the entire F<sub>1</sub> consisted of a single individual, since the reverse crossing was unsuccessful. This one individual was tall, had long, fairly broad leaves, with a red margin and a covering of wax, somewhat orange-red flowers with 3 long, broad staminodes, red ovaries and large, long, black seeds. The dominant characters are printed in italics in the above comparison.

Of the F<sub>2</sub> 1168 seedlings have so far been obtained, of which 867 after artificial pollination and 301 after free pollination, no other *Canna*'s grew in the neighbourhood. Of these 1168 plants a fairly large number died before flowering, so that for many of the characters accurate ratios have perhaps not been found. The deviations from the numbers to be expected in an independent Mendelian segregation are in some cases, however, so considerable, that they cannot possibly be reduced to them, not even on the assumption, that all the dead individuals belonged to the type or types of which there was a shortage. For the present we must say, therefore, that there was hardly any evidence if at all of an independent Mendelian segregation, as will appear from the following discussion of some of the characters.

### The red leaf margin.

It was shown in a previous paper <sup>1)</sup> that the difference between the variety of *Canna indica* with a red leaf margin and that with entirely green leaves is one of three hereditary factors. The observed numbers of plants with and without red margin, viz. 95 red as against 127 green and 83 with red edge as against 112 green, agreed very well with the ratio 27:37 which require, for 222 and 195 individuals respectively 93.6 red:128.3 green and 82.3 red:112.7 green. In addition, however, the ratios 3:1 (45 red:17 green) and 9:7 (29 red:22 green) were found among the offspring of individuals, which must be represented genotypically by AaBbCc, and this points to the coupling of all three factors or at least of two of them. Furthermore all kinds of ratios were observed which defy explanation, as for instance, 63 red:9 green.

The F<sub>2</sub> of the cross *C. glauca* × *indica* with red leaf margin were sown in seven batches. Segregation according to three independent characters, therefore according to 27:37, did not occur, but twice the ratio 9:7 was observed with very slight deviations (nos. 5 and 6) and in three batches (nos. 3, 4 and 7) the ratio 1:1 was unmistakable, in the remaining two lots the ratio approximates most closely to 9:7, but still differs from it rather considerably. From these two taken together the deviation is small (nos. 1 and 2) See table I.

TABLE I. Segregation of F<sub>2</sub> into individuals with and without a red leaf margin

Sowing	Date	Number of seeds	Number of seedlings	With red margin	Green	Theoretically by segregation according to 9:7
1	3-9-'14	200	158	83	75	127,1:98,9 (actually 126:100)
2	28-7-'15	92	68	43	25	
3	19-8-'15	223	202	101	101	
4	29-9-'15	75	60	30	30	
5	15-12-'15	260	233	132	101	131,1:101,9
6	30-3-'16	267	232	129	103	130,5:101,5
7	17-5-'16	263	215	107	108	
Total . . . .		1380	1168	625	543	

<sup>1)</sup> HONING, J. A., Kreuzungsversuche mit *Canna*-Varietäten. Rec. d. trav. bot. Néerl. Vol. XII, p 1-26, 1915.

The seeds of sowings 2 and 5 were obtained after free pollination at a time when no other *Canna's* were in flower.

Here therefore the offspring of a single individual is split according to different ratios, whilst there is some suggestion of periodicity. Accidental variations are pretty well excluded on account of the considerable numbers employed. The mean error for segregation according to 9:7 is for 1000 individuals 0.2510 per 16 <sup>1)</sup>. Here with 1168 plants, it is 0.4384. For the separate sowings, such as those which separated into 101:101 and 107:108, the differences from the mean errors are much larger still.

There is yet another objection. In the crossing of varieties of *C. indica* it was found that one of the factors for the red leaf margin, C, might be separately visible <sup>2)</sup>, and indeed in a segregation according to 27:37 exactly as required by the theory, in  $37 - 16 = 21$  of the 64 individuals. Such plants with a very narrow red margin were, however, always present in too small a number when the segregation deviated from 27:37. In the cross with *C. glauca* this shortage extends so far that among the 543 green seedlings *not a single one* was found to have a narrow red leaf margin.

For the sowings segregating according to 9:7 on the other hand an explanation is not readily available, for C may be completely coupled to A or B. The ratio 9:7 also points to the complete joining of two of the three factors.

In the cases of segregation according to 1:1 we are *not* concerned with a mixture, formed by segregation according to two different ratios (viz. 9:7 and 27:37) for in that case some of the green seedlings would nevertheless have shown at a later stage that they possessed the factor C.

If we adhere to the assumption that, in this case also, C is completely coupled to A and B, we cannot attribute the displacement of the ratio red:green in favour of green to the coupling of A and B, for in that case the number of individuals with red leaf margin would be increased; we may, however, attribute the changed ratio to repulsion. This repulsion would not even have to go so far as to cause the ratio red:green to approximate to 1:1, for

$$AB - (Ab + aB + ab) = (2n^2 + 1) - [(n^2 - 1) + (n^2 - 1) + 1] = 2.$$

As soon as  $n$ , half the sum of the numbers expressing the ratio of the gametes, is 5, 6 or more, the difference 2 is, proportionally, very small. But then also the  $Ab$  or  $aB$  individuals, amounting to

<sup>1)</sup> JOHANNSEN, W., Elemente der exakten Erblichkeitslehre.

<sup>2)</sup> l.c. b. 18.

nearly 25% of the total. would, with C, have a narrow red leaf margin, and this phaenotype is absent.

The total absence of plants which do not at the same time have both the factors A and B, but have C and ought to be able to show that factor separately also excludes any explanation based on imperfect coupling or repulsion of C with respect to A and B.

Three possibilities still remain: *a.* part of the germ cells die off; *b.* a factor might come into play which prevented the manifestation of C and was itself dependent on the presence of A and B, *c.* with complete coupling of C to A and B unilateral reduplication might occur, as HERIBERT-NILSSON <sup>1)</sup> postulates in some cases for the factor for red leaf veins in *Oenothera Lamarckiana*. Such reduplication would then occur, not, as is supposed in *Oenothera*, in the germ cells which possess this factor, but rather in those which are deficient in the factor C.

Of course the F<sub>2</sub> must be crossed back with the recessive form, for this character therefore with *C. glauca*, and this will take much time. Moreover the same variability in the segregation ratios may be expected as in self-pollination, so that the question will probably not be cleared up much by this.

That the confusion of the Mendelian segregation involving a large number of factors need have no permanent effect on the offspring, is shown by the ratios of some of the F<sub>3</sub> numbers.

TABLE II. Segregation in F<sub>3</sub> for the character of red leaf margin.

F <sub>2</sub> No.	Number of seeds	Number of seedlings	With red margin	Green	Ratio red : green	Theory
1	70	48	36	12	3 : 1	36 : 12
9	67	49	28	21	9 : 7	27,6 : 21,4

#### Wax on the leaves.

Whether a definite wax layer is present on the leaves, as in *C. glauca*, or whether it is absent, cannot be determined in young seedlings. Not until 1—1½ months after planting out does the chance of error become small, but even then doubtful individuals remain, which are best judged by subsequently formed shoots.

The number of factors for wax is still unknown; it will most likely become evident in F<sub>3</sub>, but then only for these numbers of

<sup>1)</sup> HERIBERT-NILSSON, N, Die Spaltungserscheinungen der *Oenothera Lamarckiana*, 1916.

TABLE III. Segregation of F2 into individuals with and without wax layer.

Sowing	Number of plants with wax; of these			Number of plants without wax; of these			Ratios			
	red	green	total	red	green	total	wax: no wax	r:gr	r:gr; originally	r:gr; without wax
1	29	27	56	6	2	8	7,00	1,21	1,11	3,00
2	24	15	39	11	4	15	2,60	1,84	1,72	2,75
3	75	76	151	22	9	31	4,87	1,14	1,00	2,44
4	22	23	45	2	5	7	6,43	0,86	1,00	0,40
5	104	75	179	10	7	17	10,53	1,39	1,31	1,43
6	92	73	165	14	9	23	7,17	1,29	1,25	1,56
7	72	49	121	17	10	27	4,48	1,51	1,01	1,70
Total ...	418	338	756	82	46	128	5,91	1,30	1,15	1,78

which the F2 is considerably less heterozygotic for the other factors than the F1. Clearly, in any case, there was no independent Mendelian segregation for the factors of the wax layer and the ratios in the various batches sown showed even greater divergence than those of red and green. (See tables III).

There is an appreciable repulsion between the factors for the red leaf margin and those for the wax layer. This is best seen by observing how many red and green individuals there are without wax. The number of red ones is then found (except in the fourth sowing) to be the larger, sometimes 2—3 times as large and on the average 1,78 as large as that of the green, whereas the ratio red to green was originally 1,15:1 and was not displaced, through the slightly larger mortality of the green individuals, beyond the ratio 1,30:1.

#### The number of staminodes.

*C. indica* has two staminodes. In a few flowers, however, an indication of a third is found in the shape of a red filament, generally not longer than a few millimeters.

*C. glauca* has always three staminodes. The F1 of the crossing has 3 and the vast majority of F2 also 3.

The number of plants with 2 or 2—3 staminodes varies rather considerably in the different sowings, the last three furnishing many

more than the first four. For plants with two and three staminodes differences in the external conditions might be the cause, but for those with only two this is less likely.

TABLE IV The number of staminodes of the F<sub>2</sub>

Sowing	Number of staminodes			
	three	three to four	three to two	two
1	56	6	1	1
2	27	4	2	0
3	131	3	6	0
4	44	0	4	1
5	157	8	9	4
6	113	3	10	1
7	67	6	6	2
Total . . . . .	595	30	38	9

In a discussion of the colour of the flowers it will be shown that, even apart from the fairly large variation of these figures, no independent Mendelian segregation occurred, since in the first four sowings the individuals with two and with two or three staminodes were out of proportion more numerous among the plants with yellow than among those with red flowers.

#### The length and breadth of the staminodes.

These differ considerably in *C. indica* and *C. glauca*. In the former species the length varied from 45—69 mm. and the average was 59,297 mm. calculated from 482 specimens. The breadth was 8—14 mm., the average of 480 flowers 10,808 mm. For *C. glauca* these figures were 70—97 mm. with an average of 87,076 mm. for 435 measurements, and 13—20, average 16,235 mm.

The F<sub>1</sub> had staminodes of length 70—89 mm. average 82,661 mm. and breadth 14—18, average 16,524 mm. The staminodes were therefore a trifle shorter than those of the parent with longest staminodes; there is no appreciable difference in breadth. In comparison with *C. glauca* the extent of variation is very limited, which is no doubt explained by the fact that the F<sub>1</sub> consisted of a single



TABLE V. Length of staminodes.

Species or hybrid	Number of flowers	Limits of variation in mm.	Average	Standard-deviation
<i>C. indica</i>	482	45—69	59,297	3,225
<i>C. glauca</i>	435	70—97	87,076	4,383
F 1	454	70—89	82,661	2,438
F 2, sowing 1	2752	57—95	76,346	6,467
" " 3	620 *)	57—97	76,732	7,097
" " 4	975 **)	56—100	77,277	8,206
" total	4956	56—100	76,449	7,076

TABLE VI. Breadth of staminodes.

Species or hybrid	Number of flowers	Limits of variation in mm.	Average	Standard-deviation
<i>C. indica</i>	480	8—14	10,808	0,728
<i>C. glauca</i>	434	13—20	16,235	1,334
F 1	452	14—18	16,524	0,696
F 2, sowing 1	2748	8—21	14,508	2,050
" " 3	620 *)	8—22	14,429	2,541
" " 4	975 **)	9—21	15,135	2,236
" total	4949	8—23	14,637	2,240

individual, whereas 30 *indica*- and 14 *glauca*-plants were measured.

The figures of F2 are found in tables V and VI. As long as the number of factors has not been determined, the dimensions of the staminodes would not have any importance, if large and small flowers were uniformly distributed between the plants with green leaves and those with red leaf margin. This is, however, not the case. The shortest as well as the longest flowers are found among the green leaved individuals, which therefore have a larger variability.

In table VII is given the number of plants having an average length of the flower of 62—63, 64—65 mm. etc. The limits of variability are for the plants with red leaf margin 66—90 mm., for the green ones 62—96. The difference, 10 mm. is not so very great, but on the other hand the difference in the numbers of

\*) 10 flowers per plant.

\*\*) 25 flowers per plant.

TABLE VII. Relation between average length of staminodes and colour of leaves.

Average length in mm.	Plants with red leaf margin. Flowers red.	Leaves green		Total
		Flowers red	Flowers yellow	
62-63	—	1	1	2
64-65	—	—	—	—
66-67	3	2	6	11
68-69	2	2	1	5
	(5)	(5)	(8)	(18)
70-71	2	5	0	7
72-73	10	7	2	19
74-75	13	3	3	19
76-77	12	2	6	20
78-79	7	3	4	14
80-81	5	3	6	14
82-83	5	1	5	11
84-85	11	2	2	15
86-87	7	2	3	12
88-89	1	—	2	3
90-91	1	—	—	1
92-93	—	1	—	1
94-95	—	1	—	1
96-97	—	—	1	1
Total . . . .	79	35	42	156

individuals at the two extremes is considerable. There are 18 plants having staminodes of an average length less than 70 mm. and of these only 5 have a red leaf margin. This ratio of red : green, viz. 5 : 13 or 1 : 2.6, differs from the ratio of total reds : total greens, which is 79 : 77 or practically 1 : 1. For plants with an average staminodal length of more than 87 mm. which is that of *C. glauca*, the ratio red margin : green is 2 : 5 = 1 : 2.5 which likewise deviates. We must add that the 5 individuals all had light coloured flowers, viz. 3 yellow and 2 pale red.

Furthermore the large number of small-flowered plants among those with yellow flowers is remarkable, 8 of the 42 or 19,0% show an average of less than 70 mm. For the plants with red flowers the numbers were 10 out of 114 or 8.8%. Among the grandparents it was just the opposite, the small flowers being red and the large ones yellow. If the red-flowered plants are further separated into those with red leaf margin and those without, the green ones comprise in proportion more than double the number of small flowered individuals, namely 5 out of 35 or 14,3% as against 5 out of 79 with red margin, or 6,3%

#### Colour of the flower

According to the intensity of the red in the flowers of the F<sub>2</sub> five or six different tints may be distinguished. The yellow varies less and not more than three shades can be clearly recognized. Between these there are a number of orange colours, so that the determination of the number of factors for red will not be easy. (See table VIII).

The proportion of the numbers of plants with red and yellow flowers varies from 2.1 · 1 to 4.79 · 1 and the same proportion for the green leaved individuals from 0.35 · 1 to 1.29 · 1, differences which are so great that an independent Mendelian segregation cannot be

TABLE VIII The proportion of the number of plants.  
A) with red flowers and red leaf margin.  
B) with red flowers and green leaves  
C) with yellow flowers and green leaves.

Sowing	Number of plants			Proportions	
	A	B	C	A · B : C	red : yellow
1	34	15	15	2,27 · 1 · 1	3,27 : 1
2	22	5	6	3,67 : 0,83 · 1	4,50 : 1
3	76	19	45	1,69 : 0,42 : 1	2,11 : 1
4	24	10	15	1,60 : 0,67 : 1	2,27 : 1
5	108	18	52	2,08 · 0,35 · 1	2,42 : 1
6	79	23	25	3,16 : 0,92 · 1	4,08 : 1
7	49	18	14	3,50 : 1,29 : 1	4,79 : 1
Total . . . .	392	108	172	2,28 : 0,63 : 1	2,79 : 1

recognized for the separate sowings, and at most only for the total and non-red (yellow) flowers.

If the fourth and fifth columns of table IV for the number of staminodes is split in the same way as in table VIII, according to the colour of leaves and flowers, it is found that the first four sowings produced twice as many plants with yellow flowers having 2 or 2—3 staminodes as plants with red flowers, although the latter are two and a half times as numerous (205 as against 81).

TABLE IX. The proportionally large number of plants with yellow flowers having 2 or 2—3 staminodes.

Sowing	Three to two staminodes			Two staminodes			Total number	
	A	B	C	A	B	C	A + B	C
1	1	—	—	—	—	1	49	15
2	2	—	4	—	—	—	95	45
3	1	—	1	—	—	—	27	6
4	1	—	3	—	—	1	34	15
(Sum)	5	0	8	0	0	2	205	81
5	5	1	3	2	—	2	126	52
6	6	2	2	1	—	—	102	25
7	3	—	3	1	—	1	67	14
(Sum)	14	3	8	4	0	3	295	91

Hence there is a tendency towards coupling between the factors for red flowers and those for 3 staminodes, especially clearly among the plants with red flowers and green leaves (B), which in table IX hardly occur among the last three sowings; the latter deviate considerably from the first four, in which such flowers are entirely wanting. It is among the non-red (yellow) individuals that most plants are found to be wholly or partially recessive for the characters of the staminode number.

Summarizing we may conclude for the F<sub>2</sub> offspring of the cross *Canna glauca* × *C. indica*, in which more hereditary factors were brought together than the number of chromosomes that 1) for the factors of the red leaf margin, for the layer of wax in the leaves,

and for the number, the length and the colour of the staminodes, the proportions of the phenotypes differ widely in the different sowings, in spite of the fact that the F1 consisted of a single individual; so that the segregation of the hybrids is variable;

2) that in none of the sowings the segregation ratios correspond to those which may be expected from an independent Mendelian segregation.

**Chemistry.** — “*In-, mono- and divariant equilibria*”. XII. By Prof. F. A. H. SCHREINEMAKERS.

(Communicated in the meeting of November 26, 1916).

19. *Ternary systems with two indifferent phases.*

In communication II we have seen that in ternary systems three types of  $P, T$ -diagram [fig. 2 (II), 4 (II) and 6 (II)] exist. When, however, two indifferent phases occur in the invariant point, then, as we shall see further, four types of  $P, T$ -diagram exist.

When in the invariant point two indifferent phases occur, then consequently there are three singular phases, they are represented by three points, situated on a straight line. In the types of concentration-diagram of figs. 1, 3, 5 and 7 the indifferent phases are represented by  $A$  and  $B$ , the singular phases by  $C$ ,  $D$  and  $E$ .

In figs. 1 and 3  $A$  and  $B$  are situated on the same side, in figs. 5 and 7 on different sides of the line  $CDE$ .

In fig. 1 the prolongation of the line  $AB$  intersects the prolongation of the line  $EDC$ , in fig. 3 the prolongation of  $AB$  intersects the line  $CDE$  in a point between  $C$  and  $D$ . [Of course the type of concentration-diagram of fig. 3 remains unchanged, when the point of intersection was situated between  $D$  and  $E$ ].

In fig. 5 the point of intersection of  $AB$  and  $CDE$  is situated on the line  $CDE$ , in fig. 7, however, on the prolongation of the line  $CDE$ .

Of course a type of  $P, T$ -diagram belongs to each of the four types of concentration-diagram, they are represented in the figs. 2, 4, 6 and 8. We find in each of these diagrams:

the three singular curves:

$$(M) = C + D + E$$

$$(A) = B + C + D + E = B + (M)$$

$$(B) = A + C + D + E = A + (M)$$