Fig. 9. Visum album. From a section parallel to the surface, treated with sulphuric acid. The cuticular ridges which are seen in Fig. 8 in relation to the cell-wall net, have been loosened by the action of the sulphuric acid. The "ligaments of articulation" are dissolved in the sulphuric acid; *a* and *b* are in normal position, at *c* the ridges have fallen outwards.

Fig. 10. Rhipsalis Cassytha. From a median longitudinal section through the stem. The stoma in transverse section, the guardcells roughly halved. The section has been somewhat distorted in cutting and preparing, so that the slit, especially in the middle and below, is wider than in the intact plant.

Fig. 11. Rhipsalis Cassytha. From a section parallel to the surface. The level is that of the central slit.

Fig. 12. Rhipsalis Cassytha. From a section parallel to the surface. The level is above the guard-cells, the outer slit is seen

Fig. 13. Rhipsalis Cassytha. From a section parallel to the surface, placed in an inverted position on the slide. The level is below the guard-cells, the canal-shaped internal air space is seen, which is closed by the two subsidiary cells.

Fig. 14. Rhipsalis Cassytha. From a transverse section through the stem. The stoma in longitudinal section parallel to the slit, in a direction roughly corresponding to line *e* in Fig. 1, the preparation is orientated in a corresponding manner to the preparation of Viscum drawn in Fig. 8.

Leiden, December 1913. *Botanical Laboratory.*

Botany. — "*The explanation of an apparent exception to* MENDEL'S *law of segregation.*" By Miss TINE TAMMES. (Communicated by Prof. J. W. MOLL).

(Communicated in the meeting of February 28, 1914).

In experiments on hybridisation in recent years various cases have been observed in which the numerical proportion of different forms occurring in the second generation does not agree with what might be expected according to MENDEL'S law. Among these there are very many with respect to which there is no reason to assume that this law does not apply, and in the greater number of these cases it has been possible to show the causes of the discrepancy. These causes have been found to be of two kinds. Firstly, there may be deviations which are only the results of mistakes or wrong hypotheses on the part of the observer. Secondly, there are cases in which the deviations are due to the plant itself. The sources of error belonging to the first class are chiefly as follows.

1. It may happen that the deviation is the result of making too few observations.

2. The observer may have wrongly estimated the difference in the number of factors of the *P*-forms, so that the numerical proportion he expects, is wrong.

3. Characters have to be dealt with, which cannot be seen at the same moment in different individuals. If the observations in such a case are extended over too short a period of time, then wrong results are obtained.

4. In consequence of a strongly fluctuating variability of characters the observer has failed to distinguish the different genotypes with sufficient sharpness.

The known cases in which the cause of the deviation lies in the plant itself, are the following.

1. There are fewer gametes of a particular kind formed than ought to arise. The deviation then already arises in the formation of the sexual-products.

2. The union of some kinds of gametes comes about with more difficulty than that of others. The deviation then lies in the fertilisation-process.

3. Certain combinations of gametes are less capable of life than others, so that the young individuals die off before the character can be observed.

4. The different genes may be coupled or may repel one another.

Of all the various phenomena here enumerated examples are known. BAUR [1]) and especially PLATE [2]) give a survey of them in their textbooks.

The case, which I wish to deal with here, belongs to the second group, in which the cause of the deviation lies in the plant itself. The nature of this cause will be made clear below.

The phenomenon appeared on the crossing of a white- and a blue-flowering variety of *Linum usitatissimum* and related to the colour of the flower. The blue-flowering form was a variety obtained from Egypt, and has been described by me [3]) previously and named Egyptian flax. The seed of the variety with white flowers was obtained from Messrs. VILMORIN-ANDRIEUX of Paris. Pure lines of both varieties were used for the investigation and the reciprocal crossings were carried out in 1908 and in 1911. The whole number

1) E. BAUR, Vererbungslehre. 1911, p. 116.

2) L. PLATE, Vererbungslehre. 1913, p. 194.

3) Der Flachsstengel, eine statistisch anatomische Monographie. Verh. v. d. Holl Maatsch. d. Wetensch. Haarlem, Verz 3, Deel VI, Stuk 4, 1907, p. 22.

Das Verhalten fluktuierend variierender Merkmale bei der Bastardierung Rec. d. Trav bot. Néerl. Vol. 8, 1911, p. 206.

of crossings amounted to 20; the first generation consisted of 30 plants, which all had blue flowers. In different years further cultures of the 2nd, 3rd, and 4th generation were grown. The following table gives a survey of the observations on both reciprocal crossings, since the two sets agree. Circumstances prevented the second generation from being cultivated in 1910; it was obtained for the first time in 1911.

	White	Blue	Ratio per 4 indiv.	Deviation
F_2 1911	134	482	0.871 : 3.129	∓ 0.129
„ 1912	146	481	0.931 : 3.069	∓ 0.069
„ 1913	18	87	0.686 : 3.314	∓ 0.314
„ „	69	291	0.767 : 3.233	∓ 0.233
„ „	9	39	0.750 : 3.250	∓ 0.250
„ „	14	44	0.966 : 3.034	∓ 0.034
„ „	34	130	0.829 : 3.171	∓ 0.171
F_3 1912	113	493	0.745 : 3.255	∓ 0.255
„ 1913	91	311	0 905 : 3.095	∓ 0 095
F_4 1913	172	748	0.748 : 3.252	∓ 0.252
Total	800	3106	0.819 : 3.181	∓ 0.181

In the second and third vertical column the number of white- and of blue-flowering plants in each culture is given. It is seen from this, that the proportion of these two deviates more or less from the ratio 1 : 3. In order to make clear the extent of the deviation the ratio for each culture is reckoned per four individuals. The figures obtained are given in the fourth column and in that following it the deviations of these from the theoretical ratio 1 : 3.

In the second place, the table shows that the deviation in all cases is in the same direction. The number of white-flowering plants is always less than was to be expected, or as we may also consider it, the number of blue-flowering ones is greater. Since here however the former case obtains, as will be shown later, I shall indicate the deviation in future as a deficiency of white-flowering plants. Further it is seen from the table that the deviations, with the exception of one small culture, are considerable. For the whole number of observations on 800 white and 3106 blue ones the deviation calculated for these figures amounts to ∓ 0.181. The mean error for this number is 0.027; the deviation is therefore about 6.5 times greater. This shows that the deviation from the ratio cannot be ascribed to chance, but that a definite cause must exist. The question is now what this cause is. Of the four causes mentioned above, which result from errors or wrong suppositions, three do not here come into consideration. With regard to the first, the number of observations is very great, with regard to the second the flowering of all of the plants was observed and with regard to the third cause fluctuating variability plays no role here. The only remaining cause therefore is a wrong hypothesis as to the number of factors in which the P-forms differ. For it is possible that the numerical ratio to be expected is not 1 : 3, and that therefore we have not to deal with a monohybrid crossing or with a polyhybrid behaving as a monohybrid, but that here several factors occur, which cause the blue colour of the flower. The number of gametes in which the factors for blue are wholly absent will then be relatively smaller and white-flowering plants will arise in the second generation in smaller proportion than in monohybrid crossing. In the case in which the number of factors for blue amounts to two, each by itself producing the colour, the proportion of white and blue in F_2 is 1 : 15, whilst with three factors for blue the proportion is already 1 : 63. These proportions differ so much from the observed ones, that the cause must be different. Also when it is assumed that the blue colour is caused by still more factors, which separately or in definite groups can produce this colour, ratios are obtained which do not in the least agree with that which was observed. If indeed the existence is assumed of a very great number of factors which only produce blue when combined in a certain way, a ratio may be arrived at which sufficiently agrees with the given one. Such an assumption would only have a reasonable basis, when the phenomenon could not at all be explained in an other way. I have found, however, that we are not dealing here with complicated relations of factors, but that there are two other causes, which together produce the deficiency of white-flowering plants.

The first concerns the germinating-power of the seed.

I have repeatedly noticed that the seed of white-flowering varieties has a germinating-power inferior to that of the blue ones, i.e. a relatively smaller number of seeds of the former germinate. This difference existed also between the two forms which I crossed. The seed of Egyptian blue flax, which I used for my cultures, had very good power of germination; almost all its seed came up, as the following figures show. Out of 706 seeds 701 germinated and developed to plants, thus only 0.71 % failed. The white-flowering flax, on the other hand, germinated badly and a number of seedlings died at a very early stage. 682 seeds yielded 601 plants, that is a loss of 11.9 %. Now there are obtained from the F_1 plants and the heterozygous plants of the following generation both seeds producing white-flowering plants and seeds producing blue ones. If then a higher percentage of this seed than of the blue flax does not germinate, then it may safely be assumed that the loss almost entirely arises through the failure of the seeds of the white-flowering plants. I have not traced the proportion between the number of seeds sown and the number of plants obtained for all the cultures of the whole 3906 plants to be found in the table, but only for about the half. From these it was found that 1916 seeds yielded 1858 plants. There was therefore for 1858 plants obtained a loss of 58. That will amount to 122 for the 3906 plants reckoning all the cultures together. Since of the 3906 plants 3106 had blue flowers and since we saw that among the blue P-form the 701 plants obtained, 5 seeds did not germinate, we may assume that in this case out of the 122 seeds which did not germinate, there were 22 which should have given blue-flowering plants and consequently 100 which should have given the white-flowering ones. In this it is assumed that heterozygotes behave as homozygous blues. I shall return to this point later.

Now we may inquire whether the number of 100 thus found is sufficient to explain the whole deficiency of white-flowering plants. 3106 blue-flowering plants were observed, to which belong 22 seeds which did not germinate. We may therefore assume, that among the seeds which were sown, there were 3128 of them with a predisposition towards the blue colour of the flower. Theoretically $^1/_3$ of this number, that is $1042\,^2/_3$, or in round number 1043 seeds should have given white-flowering plants.

Only 800 are found, however. The deficiency amounts therefore to 243. Now we saw that by reason of the inferior germinating-power of the seed 100 white-flowering plants will be wanting. This is, however, much less than the observed deficiency, so much

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less that the aberrant ratio of the number cannot be explained by this means alone; some other cause must also be present which acts in the same direction.

I have indeed succeeded in demonstrating this second cause of the deficiency of white-flowering plants. It is connected with the average number of seeds which are formed in the fruit of flax. The flax-fruit may contain a maximum of ten seeds. The average number, however is distinctly less, and in the white variety it is, as I repeatedly observed, in general still smaller than in the blue one.

Now when the average number of seeds in the fruits of the heterozygotes, which contain both seeds of white- and of blue-flowering plants, is smaller than in the blue *P*-form, then the assumption is plausible, that this is caused, by the formation of relatively fewer seeds of white-flowering plants. If this seed is sown a progeny arises with a deficiency of white-flowering plants.

The investigation was however not so simple in this case. In contradistinction to the foregoing it was found that in the crossed varieties the fruits of the white flax had on the average even a greater number of seeds than those of the blue Egyptian flax. In 330 fruits of the white flax the number of seeds amounted to 2412, an average of 7.31, while in 219 fruits of the Egyptian flax, there were 824 seeds, an average of 3.76. In the white variety the average number of seeds, is therefore almost twice as great as in the blue.

Now the seed of Egyptian flax is, however, much larger than that of the white flax and comparisons of different varieties had already convinced me before that the average number of seeds is closely connected with the size of the seed and in such a way that in varieties with large seeds the average number is in general smaller than in varieties with small seeds. It is therefore possible, that in the white flax there is indeed a tendency to produce a smaller number of seeds than the average number of the Egyptian flax, but that this tendency is not revealed at all, because the difference in size of the seed of the two varieties is accompanied by a much greater difference in number in the opposite direction. In order to make this out it is necessary therefore to eliminate the influence of size. This is indeed possible in the case under consideration. My earlier investigations [1]) have shown that the difference in size between the seed of Egyptian flax and that of *Linum angustifolium* is caused by several factors. Consequently there arise in the second generation forms differing in the size of the seed, intermediate between that of

[1]) Das Verhalten fluktuierend variierender Merkmale bei der Bastardierung. Rec. d. Trav. bot. Néerl. Vol. 8, 1911, p. 212.

the two P-forms. The majority however of the F_2 plants show the mean type. This also holds for the crossing of Egyptian with white-flowering flax. Here also F_2 shows, so far as size of seed is concerned, a continuous series of forms in which the mean is most strongly represented. It was therefore not difficult to find in F_2 and F_3 a certain number of plants which agreed in size of seed. Among these were blue-flowering ones which from investigation of their progeny were found to be homozygous for the colour of the flower and in this therefore were equal to the blue P-forms. At the same time there were also white-flowering plants in this lot. These are always homozygous and did not therefore require to be cultivated further. Now since the size of the seed in these forms was the same, they could independently of this point be compared as to their average number of seeds.

An investigation of these plants gave the following results. 1100 fruits from 94 homozygous blue-flowering plants of F_2 and F_3 were examined. These yielded 6468 seeds, an average of 5.88. 71 white-flowering plants from F_2 and F_3 yielded in 800 fruits 4112 seeds, an average of 5.14. These two mean values are intermediate between those of the P-forms, in connection with the size of the seed which is also intermediate. But it further results that the white-flowering plants have a smaller number of seeds in the fruits than the blue-flowering ones, namely as 12.6% less than the blue ones. This difference cannot, when the large number of the observations is taken into account, be ascribed to chance.

A second cause of the aberrant numerical proportion is in this way demonstrated. The question now remains to what extent the deficiency in white-flowering plants can be explained by this means.

As stated above, out of the whole number of cultures 1043 white-flowering plants might be expected theoretically. The difference in the average number of seeds formed between the white and the blue varieties amounted to 12.6%, i.e., for every 100 seeds of white-flowering plants 12.6 will be wanting. For 1043 seeds this would amount to $10.43 \times 12.6 = 131$, from which a corresponding deficiency in white-flowering plants will arise.

By reason of both these causes together a deficiency of $100 + 131 = 231$ white-flowering plants can be explained. The deficiency actually amounts to 243 plants. The difference between these two values is insignificant. It may therefore be considered proved that the lesser germinating-power and the lesser number of seeds per fruit of the white-flowering variety are the causes of the aberrant numerical proportion. Although the deficiency is not wholly accounted for by the two causes, yet I do not believe that it is necessary to postulate a third cause for the absence of white-flowering plants.

In the foregoing it has been assumed in all calculations that the heterozygous blues behave as homozygous. I came to this conclusion because of an investigation of the progeny of blue-flowering F_2 individuals. Normally $^1/_3$ of the blue-flowering F_2 plants are homozygous and $^2/_3$ heterozygous. If, however, heterozygotism were to exercise an influence, it would be noticeable in the number of seeds and in their germinating-power. In proportion to the seeds which are homozygous blue for the colour of flower there should be from hybrid plants a smaller number of heterozygotes formed and this seed should moreover have less germinating-power. The result would be that in the succeeding generation too few heterozygous blue-flowering plants occurred, which would be evident from the investigation of the progeny.

This inquiry now showed that out of 43 F_2 plants, 13 were homozygous and 30 heterozygous. Instead of a deficiency in heterozygotes this number is even greater than it should be theoretically. Although the figures are small, nevertheless I think that it may be concluded, that the heterozygous blue plants behave in the same way as homozygous ones.

As well as in the crossing between the blue Egyptian flax and the white flax described above, I observed similar aberrant numerical proportion in some other crossings between white- and blue-flowering varieties. In the crossing between VILMORIN's white flax mentioned above and the blue flax commonly cultivated in Holland 318 white-flowering and 1312 blue-flowering plants were obtained in F_2, that is in the proportion 0.78 : 3.22. The deviation here observed, ∓ 0.22 is even slightly greater than that found in the previous crossing. The deviation here must also consist in a deficiency of white-flowering plants, brought about by the causes mentioned above. This is clear from the following. In 211 fruits of the common blue flax the number of seeds amounted to 1851, an average of 8.78; whilst the average for the white flax was seen to be 7.31. The blue flax has therefore a higher average number of seeds in the fruit. These two values are directly comparable because the size of the seed is about the same. In connection with this, the average number of seeds in the fruits of F_1 lies between these two values and amounts to 8.38. In these fruits therefore, in proportion a somewhat smaller number of seeds is produced for white-flowering plants than for blue-flowering ones. The germinating-power also shows a difference capable of explaining a deficiency in white-flowering plants, because the common blue flax that was used in my experiments, germinates very well. Less than 1 °/₀ fails, as in the blue Egyptian flax, whilst the white flax, as has been said, shows a loss of 11,9 °/₀.

Besides the white flax from the firm of VILMORIN still another white variety was crossed with the two blue forms mentioned. This was cultivated as a pure line from a white-flowering form grown in the province of Friesland.

By crossing this white with the blue Egyptian flax there were obtained in F_2 60 white-flowering and 214 blue-flowering plants, that is in the proportion of 0.876 : 3.124 with a deviation of $\mp$ 0.124.

The crossing between the last mentioned white flax and the common blue one gave 30 white- and 104 blue-flowering individuals, a proportion of 0.895 : 3.105 with a deviation of $\mp$ 0.105.

In both cases a deficiency in white-flowering plants appeared in F_2. Although the number of observations is not great, I think it may nevertheless be concluded that we are here dealing with the same phenomenon, because in comparison with the blue-flowering varieties this white form also has a lower average number of seeds and an inferior germinating-power. The differences are however not so great as with the white flax obtained from VILMORIN.

The question now arises how the lesser number of seeds and the lesser germinating-power of the seeds of the white-flowering variety are caused. With respect to the number of seeds it is possible that the cause lies in the number of gametes formed. Normally in F_1 as many gametes without the factor for blue should be formed as those possessing this factor. Should there however be fewer gametes formed in which the factor for blue is absent, then there will be after fertilisation not only a relatively smaller number of homozygous whites but also fewer heterozygous ones and F_2 will necessarily have a deficiency in heterozygous blues as well as in whites.

As I have shown, this is not the case, there is here no deficiency in heterozygous plants. Therefore the cause does not lie in a differing number of the two kinds of gametes and can only be sought in phenomena at or after fertilisation. It may be that the union of two gametes, both devoid of the factor for blue, happens less easily so that in some cases no fertilisation takes place. Or it is also possible that the two gametes do unite and that a zygote is produced, but that the embryo already dies off in the first stages. In both cases there will be a deficiency of seed for white-flowering plants. Something of this kind was observed by CORRENS [1]) on crossing a black-sugar maize *Zea Mais* var. *coeruleodulcis* KCKE. and a common white maize "Popcorn", *Zea Mais* var. *leucoceras* ALEF. Here a deficiency of sacchariferous granules occurred CORRENS showed by crossing the F_1-hybrid again with the sugar-containing *P*-form, that the formation of an unequal number of the different gametes was not the cause of the phenomenon. He comes therefore to the conclusion that the deficiency arises because the gametes-combination of two sacchariferous ones happens less easily.

By crossing the varieties of flax a further deficiency was caused through the seed of the white forms having less germinating-power. It either does not germinate at all, the embryo has then already died in the seed, or the seedlings die off early. The latter agrees with the case observed by BAUR [2]) in cultivating the aurea-variety of *Antirrhinum majus*. From this form, which is heterozygous, there are produced green- and aurea-plants in the proportion of 1 : 2, because the yellow individuals formed at the same time die off very young.

It is clear from the inferior germinating-power of the seed of the white varieties of flax that the gametes-combination of white with white has less vitality. This suggests that very probably the smaller number of seeds is also wholly or partly to be ascribed to the same cause. I have not been able to ascertain whether seed is wanting because fertilisation does not take place at all. It can however be said in general, that the two phenomena, the smaller number of seeds and their inferior germinating-power, which in the foregoing have been always considered separately, are only the result of a single cause, namely the smaller vitality of the gametes-combination of white with white. Only because the death of certain individuals as a result of this cause may take place at different stages of development, two different phenomena can be observed separately.

I will finally add a few words on a point resulting from these observations. It is found that the number of seeds and their germinating-power, therefore the vitality of the gametes-combination, is connected with the colour of the flowers produced from such seed, that is with the presence or absence of the factor for that colour.

[1]) C. CORRENS, Scheinbare Ausnahmen von der MENDEL'schen Spaltungsregel für Bastarde. Ber. d. d. bot. Ges. Bd. XX, 1902, p. 159.

[2]) E BAUR, Untersuchungen über die Erblichkeitsverhältnisse einer nur in Bastardform lebensfähigen Sippe von *Antirrhinum majus*. Ber. d. d. bot. Ges. Bd. 25, 1907, p. 442.

The presence or absence of this factor is even more intimately connected with the number of the seeds and their germinating-power than the nature of the mother-plant which produces the seeds. For seeds with the colour-factor and seeds without it are formed in F_1 by the same plant, even in the same fruit and yet the number and the germinating-power of those without the colour-factor is less. We see therefore that the difference between the white and blue varieties of flax, so far as concerns the number of seeds and their germinating-power, is unconnected with the difference in nutritional relationships between the plants, but only with the presence or absence in the gametes of a factor for the colour of the flower.

The following is a summary of results.

In crossing white- and blue-flowering varieties of *Linum usitatissimum* there are formed in the second and following generations white and blue individuals in numerical proportions which are not in agreement with those to be expected in accordance with MENDEL's law of segregation.

In all cases there is according to the proportion 1 : 3 a deficiency in white-flowering plants.

The deficiency arises from two causes which act in the same direction: 1. By F_2 and by the heterozygotes of the following generation there is formed a relatively too small number of seeds which will yield white-flowering plants. 2. The germinating-power of the seed, that yields white-flowering plants is less than that of the seed which produces blue-flowering ones. The smaller number of seeds which yield white-flowering plants and the inferior germinating-power of these seeds are both the result of the lower vitality of the combination of two gametes both devoid of the factor for the blue colour.

The vitality of the gametes-combination and with it the average number of seeds per fruit and the germinating-power of the seed are more closely connected with the presence or absence in the gametes of the factor for the floral colour than with the nature of the plant, which produces the seed.

Groningen, 9 January 1914. *Botanical Laboratory.*