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## ON THE GENETICS OF TRICOTYLY.

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During the embryo development of a dicotyledonous flowering plant, at the rather spherical embryo, which is not yet differentiated externally, cotyledonous primordia, from which the two cotyledons on either side of the plumula develop. This developmental process, characteristic of most dicotyledons, occasionally proceeds differently in that three or four radially symmetrically arranged cotyledonous plants appear, and correspondingly tri- or tetracotyledonous seedlings. We know from selection experiments of HUGO DE VRIES (1902) that tricotyly is hereditary. An interpretation of his findings based on our present knowledge of the gene distribution and gene action allows to conclude on the number of acting genes. However, the connection between gene and trait is only insufficiently elucidated. Because besides the realization of the characteristic takes place in a very peculiar dependence on external influences. Only the analysis of the conditions under which the tricotylyl genes unfold makes the regularity in the occurrence of the trait understandable.

### 1. DE VRIES' selection experiments and the genetic basis of tricotyledon.

DE VRIES (1902) observed in seedlings of different dicotyledons each among about 10000 seedlings 10-20 tricotyledons (~0.1 - 0.2%). Also tetracotyls and pentacotyls were also found, but extremely rarely. Finally, a small number of intermediate forms of the individual seedling types, so-called "hemi"-forms, were also found in small numbers: Dicotyledons, in which one cotyledon is split, and and tricotyledons with a split cotyledon, i.e. a precursor of the tetracotyledon. In the presentation of the experiments of DE VRIES we unite the number of cotyledons with more than three cotyledons and those of the 'hemiforms' in each case with the tricotyledons. DE VRIES succeeded in some breeds by continued selection, which consisted of up to 90% of tricotyledonous seedlings. Where at all an increase of the tricotyledon content by selection occurred, however, only few tricotyledonous individuals showed this success. In individual cases, a very strong increase in the tricotylic percentage within three generations could be observed:

1895. commercial seed .....	0.1 - 0.2%
1896. 1st generation of selection: <i>Clarkia tulchella</i> .....	6%
<i>Helichrysum compositum</i> .....	41%
<i>Papaver rhoeas</i> .....	20%
<i>Phacelia tanacetifolia</i> .....	14%
1897. 2nd generation selection: <i>Clarkia tulchella</i> .....	64%
<i>Helichrysum compositum</i> .....	51%
<i>Papaver rhoeas</i> .....	56%
<i>Phacelia tanacetifolia</i> .....	58%

The given numbers are in each case the average of several single descendants. In the third generation, an increase of the tricot percentage was no longer possible. Thereby the highest tricotyledon percentages of some progenies amounted to:

*Clarkia pulchelia* ..... 79%  
*Papaver rhoeas* ..... 75%  
*Phacelia tanacetifolia* .. 90%

These breeds with a tricot percentage of more than 50 are called DE VRIES *mediumbreeds*. With other plant species, one never gets above 20%. These are called *halfbreeds*. Here the highest percentage is reached after four generations.

Also the "*cleaned middleraces*" with 90% tricotyledons still contain dicotyledons. The selfed progeny of such dicotyledons give the same percentage of the same percentage of tri- and dicots as their tricot siblings.

Through these selection experiments, the heritability of the trait "hyperdicotyly", which we will continue to call "tricotyly" for the sake of simplicity, has been proven. It is recessive compared to dicotyly.

In order to determine the genetic basis exactly, a cross would have to be made between a tricot from a breeding with the highest tricot percentage and a dicot from a normal breeding with the highest dicot percentage would be necessary. The F<sub>2</sub>, or the backcross of the bastard with the recessive parent, the percentage of dicotyledons would allow conclusions to be drawn about the number of genes that determine the number of cotyledons. This genetic analysis is not yet carried out. Nevertheless, on the basis of the experiments of DE VRIES, *the number of genes* involved can be estimated approximately. Since only after three generations the highest percentages of tricotyledons are reached, *the trait must be polygenic*. The simplest idea is that to an actual tricotyledon gene (*t*) additional genes must be added, which determine the percentage of the *tt* is realized. In case of heterozygosity of the additional genes a lower percentage is realized than in homozygosity. Since the highest level of tricotyly is reached after three generations, the number of these additional genes is unlikely to exceed two (*Z1 Z1, Z2 Z2*). Thus, a total of about three gene pairs would be responsible for the difference between dicotyledonous and multicotyledonous.

Such a genetic situation has been clearly demonstrated for a characteristic of the flour moth, *Ephestia Kühniella* ZELLER (KÜHN & HENKE, 1929). In "glass-winged" moths, the scales on smaller or larger wing areas fall out before hatching. Thus these animals appear then in different degree glass-winged. From strains with few glass-winged individuals, just as in the case of tricotyledons, those with a higher content of glass-winged individuals can be selected: Selection for glass-wingedness also results in a maximum of 90% glass-winged + 10% normal scaled. KÜHN and HENKE were able to prove exactly that the glass-wingedness must be based on two pairs of factors, to which additional factors must be added, which determine the percentage in which the disposition for glass-wingedness is realized. Homozygous glass-winged animals (*ggkk*) have the highest percentage of glass-winged offspring, if they are also homozygous with respect to the recessive additional factors (*zz*) are homozygous. A parallel between the results of DE VRIES and the findings of KÜHN and HENKE lies also in the fact that in both cases strains with differently high content of tricotyledons or glassy-winged ones were obtained, which then proved to be constant in this certain content.

Strains with constant low content of glassy wings are characterized by homozygosity of recessive glassy wings factors (*ggkk*) and homozygosity of dominant additional factors (*ZZ*). There is nothing against symbolizing the "*halfbreeds*" of DE VRIES in an analogous way with *tt Z1Z1*, the "*middlebreeds*" with *tt z1z1*, whereby under certain circumstances *Z2Z2*, or *z2z2* are added.

We could make clear with it the genetic basis of the trikotyly. However, it remains incomprehensible why even in homozygosity of all recessive factors the dominant form still appears, and why on the one hand tricot seedlings from high percentage dicot seeds can show almost only dicots, and on the other hand dicot seedlings (from highly selected tricot strains) can show 90% tricot progeny. To understand this peculiarity, the analysis of the modifiability of tricotyly can contribute.

## 2. The modifiability of the number of cotyledons in *Petunia*.

In the *Petunia* sowings that we made annually since 1942, tricotyledonous seedlings often occurred in addition to dicotyledonous seedlings. In 25 sowings with an average of 136 seedlings, the percentage of tricot seedlings was 0.38% (3m = 0.51%). In the spring of 1946, one seeding tray was conspicuous with 20.3% tricotyledons. The seed was from the cross of two self-sterile petunias, U 228 F × W 166 K. It had been carried out in the greenhouse on November 3, 1945, that is, at a time when the petunias were producing their last last sparse flowers. The counting result was:

94 dicots..... = ~79.7%  
 2 dicotyledons with one slit cotyledon each + 19 tricotyledons + 3 tetracotyledons = ~20.3%

Reciprocal crossing had also been performed on November 3, 1945. The corresponding sowing showed *no tricotyledons*. This suggested - assuming all other conditions - to plasma effect in the determination of the cotyledons. We repeated the crosses with the same individuals in May 1946, i.e. at a time when the petunias are at their are in the best growth. The two corresponding sowings had a normal and approximately equal percentage of tricotyledons (0.55% and 0.43%). It could be assumed thereafter that the poor conditions of the late autumn were the cause of the high percentage of tricotyledons.

To test this assumption, in 1946, outgoing summer and during the fall, the reciprocal crosses were again made on the same individuals several times. Their results are summarized in Table 1. A second series of experiments with plants U 228 F and W 166 H gave the results shown in Table 2.

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Table 1.

Date.....	Cross species.....	Dicotyl.....	Tricotyl.....	Tricotyl in %:
3.8.46	U 228/F × W 166/K	146	0	0
	reciprocal	151	0	0
26.8.46	U 228/F X W 166/K	186	0	0
	reciprocal	170	0	0
13.9.46	U 228/F X W 166/K	172	0	0
	reciprocal	138	6	4,2
8.10.46	U 228/F x W 166/K	44	2	4,5
	reciprocal	81	24	22,9

Percentages higher than 2 are statistically secured against the value of "normal cultures". In the first series of experiments (Table 1), sowing from the September cross at W 166 K x U 228 F resulted in an increase in the content of tricotyledons. (Besides true tricotyledons, hemiforms occur in very small numbers, as in the DE VRIESSCHEN selection trials. We refrain from their treatment here, but will return to them later). In both series of experiments, all October values are elevated. It should be no coincidence that in both the differences of the reciprocal October crosses have the same position as the differences of the September values. The result of the experiment proves *that the bad autumn conditions influence the determination of the number of cotyledons through the general condition of the plants*. Thus, the difference between the reciprocal crosses of November 1945 (see above) becomes understandable. Experience teaches again and again that especially in autumn the individual Petunia plants can show clear differences in their general condition, which disappear only with the sprouting in the following spring. As a result of such differences, the two plants reacted differently to the autumn conditions.

When examining the relationship between external conditions and trait expression in more detail, it was first necessary to consider that autumn capsules always have fewer seeds than summer capsules (cf. in Tables 1 and 2 the number of seeds in October compared to August). So it could be the increase in the number of tricotyledons could be a consequence of the limited number of developing seeds, such that when the number of seeds is low, the single embryo is better nourished and therefore more easily forms three cotyledons. To test this possibility, we performed pollinations with little pollen under summer conditions. On 8.6.47, the stigmas of 8 flowers of W 166 K in the greenhouse were pollinated with about 200 pollen grains of U 228 F. The seed numbers per capsule were: 30, 32, 33, 36, 52, 54, 55, 73. Thus, they were still far below those of abundantly pollinated autumn capsules. The sowings showed a total of 1 tricot seedling (= 0.3%). *Thus, the formation of tricotyledons is not a consequence of the reduced seed number of the autumn capsules*. Both phenomena are independent consequences of the external conditions.

Table 2.

Date.....	Cross species.....	Dicotyl.....	Tricotyl.....	Tricotyl in %:
3.8.46	U 228/F × W 166/H	190	1	0,5
	reciprocal	178	0	0
26.8.46	U 228/F X W 166/H	172	0	0
	reciprocal	181	0	0
13.9.46	U 228/F X W 166/H	110	0	0
	reciprocal	72	1	1,4
8.10.46	U 228/F x W 166/H	58	3	4,9
	reciprocal	96	10	9,4

For further analysis of the "autumn conditions" three series of experiments were performed. In two series we tried to influence embryo development by cold, respectively short day. In both cases, only a few capsules were developed at all. They contained hardly any seeds (84, and 68 yon 8, and 10 capsules, respectively), all of which proved to be dicotyledonous. It was not necessary, however, to gain information on the influence of cold and short day by varying the conditions of these two experiments. Because the third series of experiments had a clear result: the flowers of three plants (W 166 K) were pollinated with U 228 F from 15.5.47 onwards. The plants were in the greenhouse. After 8 days, i.e. on 23.5.47, the leaves of all plants were cut off, only the bud leaflets up to a length of about 1cm remained. The plants soon stopped flowering. The last flower could be pollinated on 8.6.47. Table 3 shows the results of the corresponding sowings.

In all three plants, individual fruits with increased content of tricotyledonous seedlings appeared. In plant 2, the highest percentage of autumn capsules (22.9%, Table 1) is almost reached. Overall, the tendency of increased tricot formation towards the last flowers is evident (7, 19, 13%). Thus, it was proved that *nutritional factors modify cotyledon number*. *The „autumn conditions" influence the cotyledon number by the lack of nutrition they cause.*

However, the possibility for this modification of cotyledon number is not present in all petunias. In two large, identically designed starvation test series conducted *on other plants*, increased percentages of tricot seedlings did not occur. Apparently, plants W 166 K and U 228 F have a particular genetic basis for modifiability. However, it is unlikely that it consists in the presence of certain tricotyledons in our petunias. Self-progeny of tricotyledonous seedlings did not give increased percentages of tricotyledons in any case.

The nutritional deficiency, which modifies the number of cotyledons in *Petunia*, exerts the same effect as the tricotyledonous genes. Of the gene effects we know in many cases that they refer to quite specific individual processes. We can therefore assume that also the lack of food does not modify a multitude of reaction chains influencing embryo development, but that it acts on a very specific developmental process. This last consequence of our modifiability experiments shall be more strongly underpinned by an account of the realization of tricotyly.

Table 3.

Date of pollenization..... Dicotyl..... Tricotyl..... Tricotyl in %:

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Plant 1			
15.5.47	125	1	0,8
15.5.47	184	2	1,0
19.5.47	143	0	0
23.5.47	111	0	0
23.5.47	159	0	0
23.5.47	181	1	0,5
23.5.47	187	0	0
26.5.47	11	0	0
28.5.47	63	2	3,1
4.6.47	66	5	7,0
Plant 2			
15.5.47	23	0	0
19.5.47	159	11	6,5
19.5.47	207	1	0,5
23.5.47	221	2	0,9
25.5.47	116	4	3,3
4.6.47	89	0	0
8.6.47	78	19	19,6
Plant 3			
15.5.47	77	0	0
19.5.47	93	1	1,1
26.5.47	170	1	0,6
26.5.47	96	0	0
28.5.47	86	13	13,1
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### 3. The realization of tricotyly.

The modifiability of the number of cotyledons of *Petunia* is undoubtedly not a special case. Also in DE VRIES (1902) one can read between the lines of the same behavior of other plants. His selection experiments on *Amarantus speciosus* show it most clearly: "The mean numbers of heirs vary, in spite of steady selection, between 2 and 4 pCt, the highest mostly between 4 and 12 pCt. The year 1897 showed particularly high numbers, there occurred single individuals with 25 pCt heirs appeared. But the next year, the offspring returned to the earlier values. The alternative "di-tricotyly" is therefore partly made by the effect of genes, partly certain external conditions intervene in the realization of the trait, either in promoting or inhibiting interaction with true tricotyledons (*Amarantus*), or on the basis of a genetic basis (*Petunia*), which allows the formation of tricotyly under certain conditions. The tricotyly is realized when the internal conditions, which are decisive for the formation of the number of cotyledons, have reached or exceeded the "turning point". *The effect of the tricot genes lies in the lowering of the turnover point.* As a result, a high percentage of tricotyledons is already produced under normal conditions. *Lack of food promotes the realization of tricotyledons by shifting the internal conditions partly beyond*

*the turnover point.* According to these considerations it is obvious that in such "always turning over strains" an extraordinary dependence of the realization of the traits on external conditions must be encountered. Two other always turning over clans, namely the fasciated *Crepis-biennis*-races and the glass-winged flour moths, show the same behavior. It is now understandable why in homozygous tricot clans, consisting of 90% tricots and 10% dicots, the dicots have the same progeny composition as their tricot siblings. They are plants with pure tricot inheritance that have become dicotyledonous under modifying influences. Corresponding to them in pure dicotyledonous clades are the rarely occurring tricotyledons, whose progeny may contain extremely large numbers of dicots.

However, the always turning over tricotyledons do not consist of di- and tricotyledons alone, but also of intermediate forms. The descendants of the hemitricotylys prove that they are real variants of di- or tricotylys. The number of hemiforms always remains small. In three October-November sowings of *Petunia*, there were 2 intermediate forms in addition to 263 di- and 30 tricotyledons. A starvation trial contained 4 intermediate forms among 78 di- and 15 tricotyledons. The selection trials of DE VRIES also showed how the increase in tricotyledon content was associated with an extremely small increase in intermediate forms. In the glass-winged meal moths we find the third parallel: A maximum of normal scaled moths is followed in all cases by a minimum of glass-winged moths of low degree, which is followed by a second maximum with strongly glass-winged moths. Therefore, following KÜHN and HENKE (1929), *we can call the realization of tricotyly under different conditions a "borderline case to true alternative modifiability"*. The minimum with the hemiforms, which constitute the borderline case, is weak in the tricotyledonous clans, weaker than in the glass-winged or in the fasciated *Crepis biennis*. It remains pronounced under all circumstances, no matter how much the ratio of the two maxima with di- and tricotyledons may be shifted by accumulation of tricotyledons or by bad external conditions.

The discontinuous variability of cotyledon ratios could perhaps be explained by the fact that the intermediate forms would be kept low in number by the developmental history of the cotyledons. It would be possible that either two or three cotyledons could be created, and that the splitting of a plant into two meristem zones, leading to intermediate forms, is a peculiarity in the construction plan of the plant, which can occur only rarely. Thus, the discontinuity would not come about by a genuine reversal of the internal conditions for the trait carriers when certain external conditions are reached, but by the presence of an internal structure which excludes certain types of modifiability. We are unable to refute this second possibility in *Petunia*. However, we shall show in a later publication that the splitting of the first cotyledon meristem into several meristem zones occurs with extraordinary frequency in another plant species. According to this, at least not all plants can have an internal structure that keeps the intermediate forms of true di- and tricotyledons numerically low. Thus, nothing prevents us from considering the discontinuous variant distribution in the ratios of cotyledon numbers *as a real limiting case to the alternative modifiability*, in the sense that *we conceive the intermediate forms as the consequence of those internal conditions which rarely occur immediately at the point of turnover.*

#### 4. *The importance of the observations for phylogenetic questions.*

The occurrence of tri-, tetra-, and pentacotyledonous seedlings next to dicotyledons raises the question whether the formation of supernumerary cotyledons in dicotyledons is a phenomenon that is phylogenetically related to the polycotyly of gymnosperms. Is it possible to infer a phylogenetically primitive plant group from the clustered occurrence of such supernumerary cotyledons? This is not the case. DE VRIES found the highest percentage of tricotyledons in selection cultivars of a species belonging to the Synandreae, the second best in a species belonging to the Myrtales. If at all there is a phylogenetic connection with the gymnosperm cotyledon ratios, the genetic bases for the increase beyond dicotyly in the dicotyledons have been preserved by chance in the overall genotype, or the modifiability has been determined - also quite by chance - in different ways.

Concerning the evaluation of cotyledon characteristics, one more consequence may be referred to, which results from the observation of the *occasional occurrence of tri- or tetracotyls*. There is the opinion that the *one cotyledon of the monocotyledons* is the result of a fusion of the two cotyledons of the dicotyledons. R. von WETTSTEIN, who represents this opinion in the "Handbuch der systematischen Botanik", leads as main argument the one cotyledon of certain Polycarpicae (*Ficaria verna*), which becomes bipartite towards the tip. If this phenomenon would be only accidental, if the one cotyledon would have been lost during the transition from the dicotyledons to the monocotyledons, then one would have to find, according to R. von WETTSTEIN, *rudiments of the lost cotyledon* occasionally. However, since this is not the case, the theory of the fused monocotyledon cotyledon should be correct. Now, HUBERT WINKLER (1931) found 6 among thousands of seedlings in sowings of *Ficaria verna* that had *two forked cotyledons*. He thus believed to have found the rudiment and to have confirmed R. von WETTSTEIN's opinion. "The monocotyledons are monocotyledons" he called his thesis. According to our investigations and the multiple observations of tetracotyls, however, the explanation seems to be obvious that WINKLER did not have seedlings with one cotyledon + one rudimentary cotyledon, but "tetracotyl seedlings" in his hands, on which two each were grown together to a forked leaf. Thus, the finding of WINKLER does not speak against the opinion of R. von WETTSTEIN and we can say, if we want to follow the expression of WINKLER: The monocotyledons are syncotyl, so actually dicotyl.

### *Summary.*

The *interpretation of the selection experiments* of HUGO DE VRIES on tricot breeds shows that the trait tricotyl is based on two or three gene pairs. For the realization of the trait, first of all, a gene pair *tt* is responsible, the effect of which depends on the type of presence of the additional genes *Z1*, *Z2*, or *z1*, *z2*. *tt Z1Z1Z2Z2* individuals have the least possibility for the development of the tricotyledonous trait, *tt z1z1z2z2* individuals the greatest.

Our own studies reveal an exceptionally strong dependence of the trait difference di-trikotyl on external conditions. If embryo development proceeds under autumn conditions, 20% tricotyledons are formed compared with 0.5% under summer conditions. Defoliation of plants in summer can also produce high percentages of tricotyls. Thus, it is shown that food deficiency during embryo formation is the external factor favoring tricotyl. It causes the turnover point, at which a predisposition for tricotyl can develop, to be reached sooner than with normal nutrition. The effect of tricotyl genes can be understood as a lowering of the turnover point, so that under normal conditions the development to tricotyl is favored.

The realization of the tricotyl represents a borderline case to the genuine alternative modifiability, in that between the two maxima with di- and tricotyl also hemiforms occur. Their number always remains small, however the two maxima may be alternately increased and decreased by change of the gene basis or the external conditions.

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