

STUDIES ON THE INHERITANCE OF LEAF SHAPE
IN NICOTIANA TABACUM L.

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. DE JONG,
HOOGLEERAAR IN DE VEETEELTWETENSCHAP,
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JACOB HENDRIK VAN DER VEEN

WOORD VOORAF

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NICOTIANA TABACUM L.**

by

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GENERAL INTRODUCTION

For the past 25 years the *N. tabacum* varieties "Atropurpurea" and "Amersfoorter" have been grown in the experimental garden of the Laboratory of Genetics (Wageningen, Netherlands).

"Atropurpurea" has broad, very short-petioled leaves with an obtuse angle of venation, while "Amersfoorter" has narrow, sessile leaves with an acute angle. In view of the pronounced differences the material was thought to be suited for studying the inheritance of these leaf characters and was therefore given as a training object to students specializing in genetics. The variation in the segregating generations is at first sight rather bewildering. Moreover, the analysis is complicated by the fact that size and shape of the leaf as a whole and shape of the leafbase in particular changes regularly in passing node by node up the stem. Nevertheless it was recognized by the earlier students (for the first time by Miss S. M. HASPERS) that the main aspects of between-plant-variation could be explained on the basis of two independently segregating factor-pairs. Since 1952 the present author started a more detailed analysis in order to obtain a more systematic knowledge of the actions and interactions of the factors involved and in order to arrive at a more efficient classification in the segregating generations. The scope of the investigation was broadened by adding two other varieties to the material, viz. "Hongaars Gartenblatt", the leaves of which closely resemble those of "Atropurpurea", and "Keurhorst Elite" which has sessile leaves like "Amersfoorter" but differs from it in having a broader leafbase, a broader leafblade and a less acute angle of venation.

Several investigators studied the inheritance of leaf shape in tobacco during the past 40-50 years. Many inconsistencies, however, are met with in literature and a number of problems remained unsolved. Therefore it seems worthwhile to give a rather detailed discussion of literature (Chapter I § 13).

The presentation of my own data has been divided into two parts.

1. Chapter 1 presents the formal Mendelian analysis of the leaf characters under study.
2. Chapter 2 presents metrical data to support the conclusions advanced in chapter 1 and to give information about the (systematic) variation in shape of the full-grown leaves along the stem.

Chapter I

THE GENETICAL ANALYSIS OF THE LEAFBASE SHAPE AND OF CHARACTERS CORRELATED IN INHERITANCE TO IT

Ch 1 § 1. INTRODUCTION

Of the two *Nicotiana* species that are of economic interest *N. rustica* has long-petioled leaves, while in *N. tabacum* most varieties have sessile leaves and only a few show long-petioled or short-petioled (nearly sessile) leaves. The shape of the leafbase is not only of interest from the taxonomical point of view but is also taken into consideration in tobacco breeding as 1) the petioled condition is undesirable for several reasons and 2) among the sessile types there is much variation ranging from a short, broad base to an elongated, relatively narrow base.

Since the studies of Miss HOWARD (1913) many workers in the field of *Nicotiana tabacum* genetics focussed attention upon the inheritance of the petioled versus sessile alternative which was correctly understood by most of them to be interpretable in terms of rather simple Mendelian segregations, as contrasted with many other morphological leaf properties among which leaf size, leaf shape, proportion of midrib, etc. The discussion of literature will be postponed to the end of this chapter as it is the aim to make analytical comparisons with the results of the present investigation, to reconcile points of controversy and to venture reinterpretations of several data on the basis of my own conclusions which in this way will be generalized to the findings published so far by the different authors.

Anticipating this discussion it can be stated that the conclusions presented in literature are not always clearly consistent with each other, that some of them are rather tentative, and that in all cases considerable refinement of analysis and substantial extension (and sometimes simplification) of the conclusions could have been attained. The main causes for this can be briefly summarized as follows:

1. Incomplete classification of the phenotypes in segregating generations led to an incomplete understanding of the actions and interactions of the genes involved. In this connection it must be said that surprisingly little use was made of backcrosses. In the present study backcrosses proved to be particularly useful, not only in view of the smaller array of genotypes from "major genes" but also because of the more uniform genic background as compared with F_2 's.
2. The genes governing the leafbase shape also affect other

conspicuous aspects of the morphology of the leaf. It was more or less clearly understood by most authors that these characters behave as one complex in inheritance. However, it is not always easy to reconstruct how the components were "weighted" in classifying the phenotypes. In fact often features or complexes of features of the leaf habitus were taken into account without conclusive evidence that the correlations inferred had a fundamental genetic meaning. As a consequence often inefficient and sometimes even misleading standards for classification were established. In the present publication it will be shown that for example loci which govern certain components of leafbase shape have a large influence on leaf width. Even this being the case, leaf width is not a very reliable criterion in inferring the genotypes from the phenotypes as it is to a considerable extent dependent upon environmental agencies, residual genotype, and position of the leaf on the stem.

3. On the other hand fundamental correlations and valuable side-criteria, to be described hereafter, have been overlooked as a rule.

The present part of the investigation aims at contributing to a better understanding 1) of the so-called leafbase genes with respect to their many-sided influence on the morphological make-up of the leaf and 2) of genes that modify the expression of these genes.

Ch 1 § 2. EXPERIMENTAL METHODS

The seeds were sown in the heated greenhouse by the middle of April. Care was taken to avoid crowding in the sowing pans. After 2-3 weeks the seedlings were transplanted into frames in 5 x 5 cm. square system. As it was observed that the plantlets can be "transplanted" by earth-worms precautions against this cause of admixtures were taken. After another 2-3 weeks the plants were planted out in the garden in a 50 x 65 cm. spacing. The plants were taken as they came, in order to avoid choosing the most vigorous. Nevertheless some selection may have occurred due to slow germination of some types, but not to a considerable extent as the germination percentages were observed to be high after about 10 days.

In general the growing conditions were kept as uniform as possible, the nitrogen dose was moderate, the suckers were removed soon after they appeared and the plants were not topped. By doing so phenotypic classification of the leaf characters was facilitated.

In selfing, crossing, and seed-harvesting the appropriate methods were used and the greatest care was taken to avoid stray pollinations and seed admixtures, and to obtain well-ripened seeds. When emasculating, a small stamen or an extra sixth stamen (observed twice) must not be overlooked.

The families 53-201 to 53-208 were grown in a different way (cf. Ch 2 § 2a). With these plants a higher uniformity of environmental conditions was aimed at as metrical data had to be taken from them in connection with the studies described in Ch 2.

Ch 1 § 3. THE FOUR PARENTAL LINES

Ch 1 § 3a. *Origin and description of the parental lines*

The studies were performed on families derived from four pure lines grown in the experimental garden of the Laboratory of Genetics (Wageningen, Netherlands). The origin of these *N. tabacum* varieties could not in all cases be traced completely, as during the war 1939-1945 some of the pertaining data were lost.

1. Hongaars Gartenblatt. The name can be translated as "Hungarian Gardenleaf". It was imported from Hungary into the Netherlands in 1943/44 and added to our collection in 1948.
2. Atropurpurea. A laboratory stock since about 1930. According to HONING (1939; p. 111) it is probably identical to "*N. tabacum* purpurea" (also called "*N. tabacum* var. *macrophylla purpurea*") grown in California since 1906 as University of California Botanical Garden (U.C.B.G.) No. 06-25. This variety has been used in a good many cytogenetic investigations and is essentially the purplish-red flowered form which COMESTRETS treats as "*N. macrophylla purpurea*, hort." (cf. GOODSPEED, 1954; p. 375). U.C.B.G. No. 06-25, a photograph of which is given by GOODSPEED and CLAUSEN (1917; Fig. 1), has been used for studies on leafbase inheritance by SETCHELL et al. (1921, 1922), KELANEY (1925) and MacRAE (1941). It is now known in California as "Red Russian" (D. R. CAMERON; pers. comm.). Dr. CAMERON kindly sent me seeds of "Red Russian" and the plants were very similar to the "Atropurpurea" plants.
3. Amersfoorter. This is a collective name for a number of closely related varieties grown from of old round the Dutch town Amersfoort. One of these types was introduced into our experimental garden about 1930 and has been maintained as a laboratory stock under the name "Amersfoorter". The German variety of this name is nearly identical to our stock as can be concluded from the photographs and the description given by RAVE (1935).
4. Keurhorst Elite. A type imported from Germany during the war by a private person, who also named it. It is not excluded that this variety, grown in our garden since 1947, is a selection originating from the Forchheim tobacco institute. "Hongaars Gartenblatt" and "Keurhorst Elite" were obtained by courtesy of Ir. L.F.J.M. VAN DER VEN, Director of the "Stichting Proeftuin Tabak" (= Exp. Sta. for Homegrown Tobacco's) at Wageningen.

The following abbreviations will be used throughout this publication:

HG = Hongaars Gartenblatt
 Atr = Atropurpurea
 Am = Amersfoorter
 KE = Keurhorst Elite

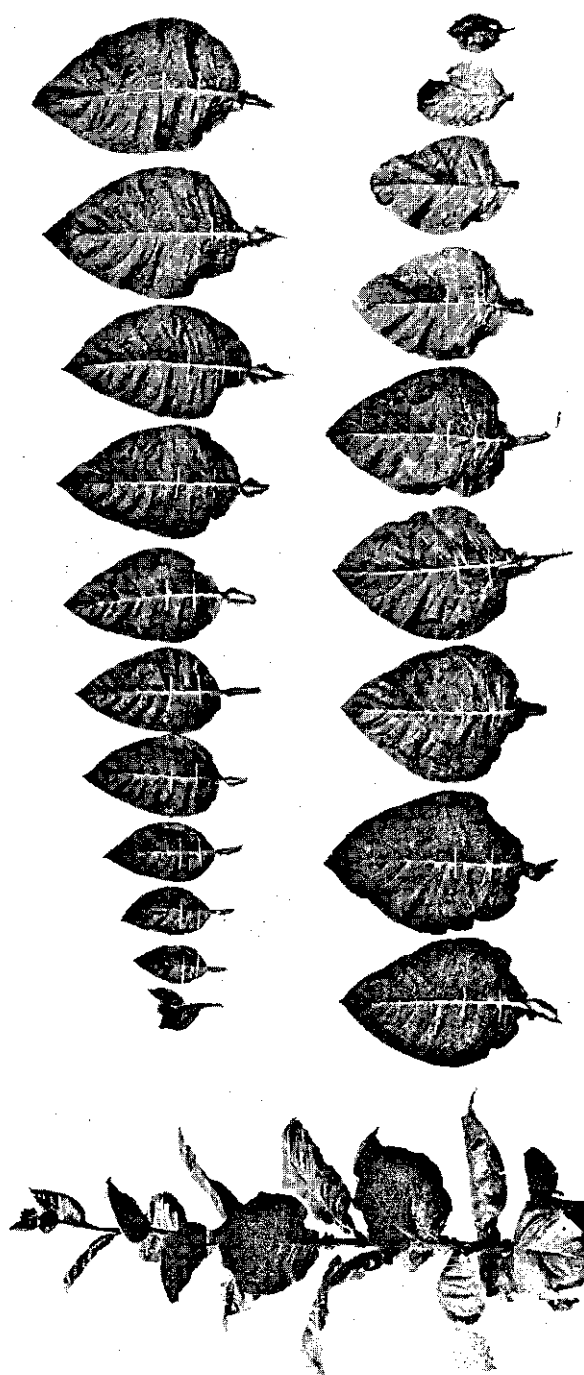


Fig. 1. Hongaars Gartenblatt (HG). A plant about the beginning of flowering together with its leaves, arranged in order from top (upper row, left) to bottom (lower row, right) of the stem. The lowest leaves were decayed by that time. The strips of paper mark the regions in which the angles of lateral veins are measured (Ch 2).

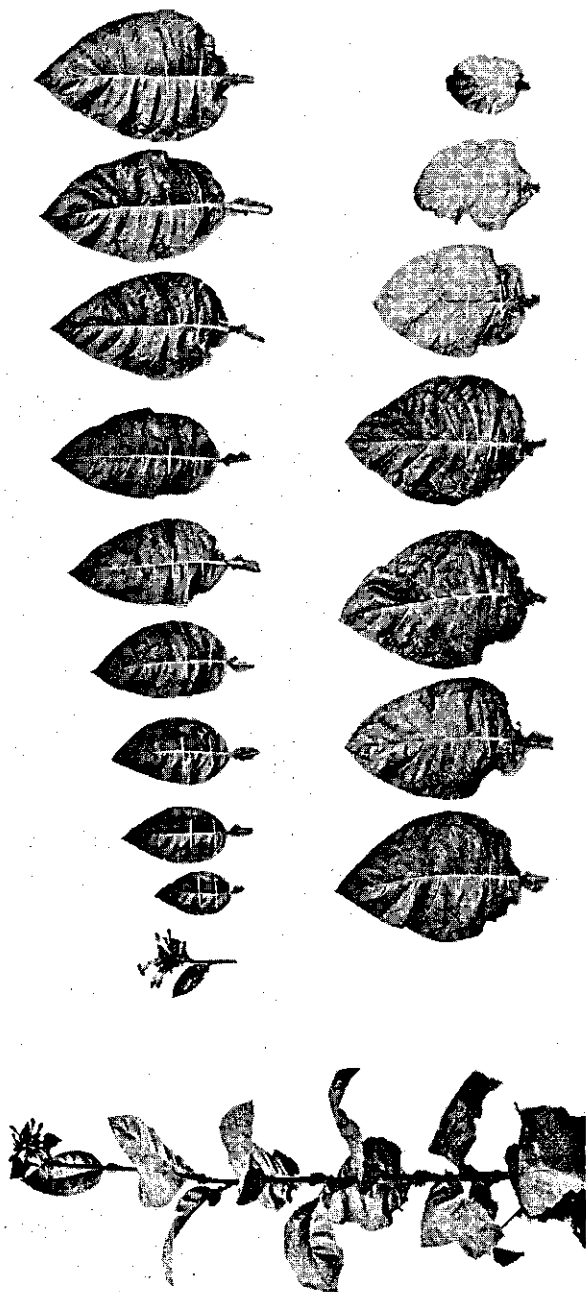


Fig. 2. *Atropurpurea* (Atr). See caption to Fig. 1.

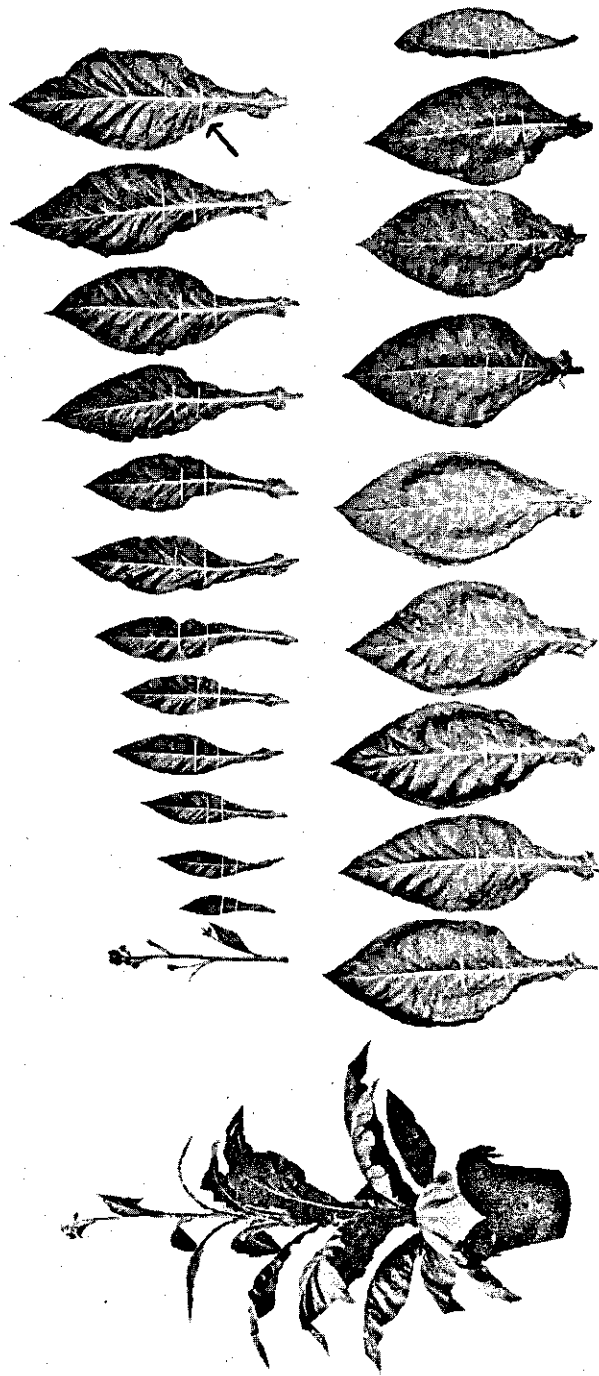


Fig. 3. Amersfoort (Am). See caption to Fig. 1. For the arrow see Ch 1 § 3a.

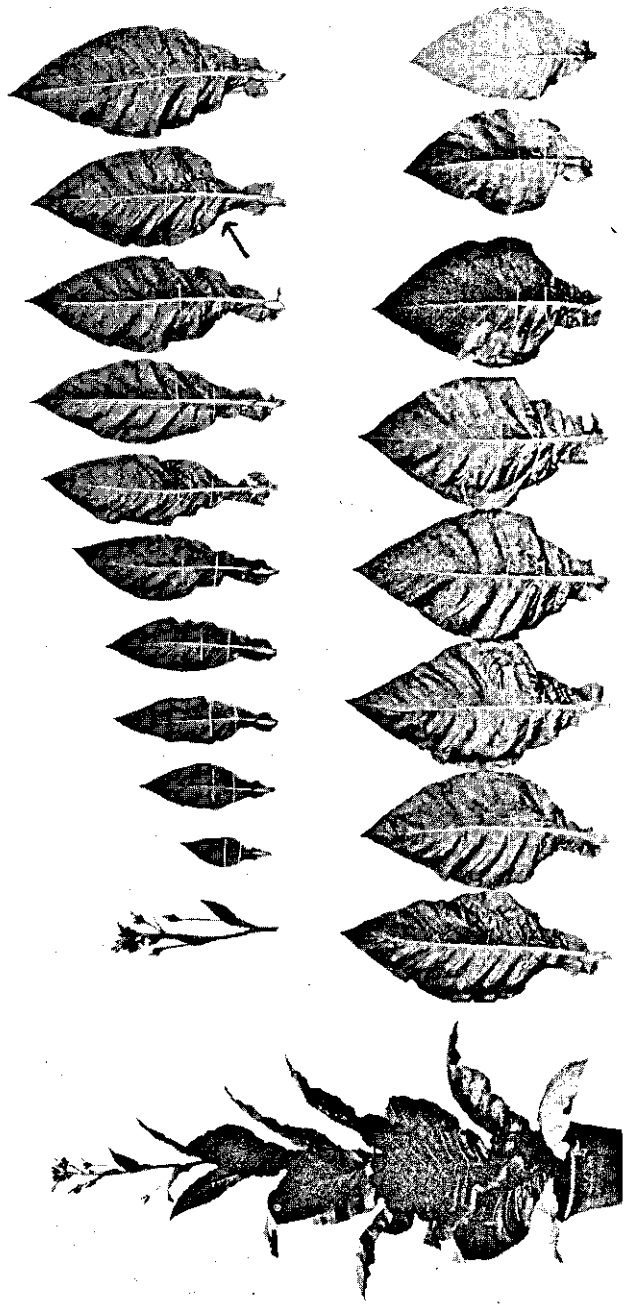


Fig. 4. Keurhorst Elite (KE). See caption to Fig. 1. For the arrow see Ch 1 § 3a.

The general habitus of the HG, Atr, Am and KE plants and full-grown leaves is illustrated by Figs. 1, 2, 3 and 4, which show the plants about the beginning of flowering along with the leaves of the same plants arranged in order from top to bottom of the stem. The characters pertinent to the present investigation are:

1. Shape of the leafbase. In the case of (long- or short-) petioled leaves the "leafbase" is taken to mean the part up to the blade. With sessile leaves one finds, in going along the margin from the region of maximum leaf width towards the stem, a more or less pronounced inward curve (*sinus*). The part below the "sinus midpoint" (cf. the arrows in Figs. 3 and 4) will be called "leafbase". The position of the "sinus midpoint" is not free from subjective interpretation on the part of the investigator, since the inward curve is sometimes weak and the leaf margin is often rather undulated in this region. Nevertheless it proved to be a very useful point of reference. Thus for example Am leaves (Fig. 3) have a more elongated leafbase than KE leaves (Fig. 4) with comparable position on the stem.
2. Angle of lateral veins. As can be seen from Figs. 1 to 4 the size of the angle varies with its position on the leaf and also with the level of insertion of the leaf (node number). A detailed presentation of the metrical data taken on this character will be given in Ch 2. For our present purpose it is best to consider the angles found on the largest leaves just below the half-way point on the midrib as the most representative.
3. Length/width ratio (L/W) of the leaf. This parameter too varies with node number and will be more thoroughly dealt with in Ch 2. The L/W value of the largest leaves on the stem will be taken as representative.
4. Number of leaves. The leaves are numbered from the cotyledons (Nos. 1 and 2) till the last leaf under the central end-flower. The number of leaves may vary considerably with environmental conditions.
5. Shape of the flowers. This character shows little response to differences in environmental conditions.
6. Flower colour.

With respect to these characters the parental lines can be briefly described as follows (cf. Figs. 1 to 5a for leaves and Fig. 5b for flowers):

1. Hongaars Gartenblatt. The leaf has a very short petiole with rather narrow wings. These wings connect the blade with the well-developed stem-clasping auricles. This type of leaf is generally called "constricted". The angle of venation is obtuse (75° - 80°), the L/W value is about 1.50 and the pink flowers have an inflated throat and a pentagonal limb contour.
2. Atropurpurea. This parental line resembles HG much, only the plants are less tall, the leaves are smaller, and the flower colour is carmine.
3. Amersfoorter. Its leaves are sessile and taper towards the stem. However, a more or less pronounced sinus (see above under "Shape of the leafbase") is nearly always present. As

will be justified on the basis of the present genetical analysis, the part below the sinus midpoint (cf. arrow in Fig. 3) which we have called the leafbase can be looked upon as a petiole with very broad wings. The laminae are to some extent decurrent along the stem. The angle of venation is acute (45° - 50°) and the L/W value is as high as 3.00 - 3.50. The pink flowers have a slender tube and throat, and an asteroid limb contour.

4. Keurhorst Elite. This sessile variety is best described in comparison with Am. The leafbase is broader and shorter, i.e. the wings are broader and the distance from the axil to the sinus midpoint (cf. arrow in Fig. 4) is shorter. The angle of venation is less acute (55° - 60°) and the leaf is broader (not shorter), giving L/W about 2.00 - 2.25. The pink flowers are less slender and have a less pointed limb contour.

For convenience the data have been arranged in Table 1.

Table 1. Comparisons between the parental lines with respect to the characters studied. Notably the data on number of leaves must be taken relative to each other in view of seasonal and other environmental influences. For further details see text.

| Parent | HG | Atr | Am | KE |
|----------------------|--------------------------------------|--------------------------------------|-----------------------|-------------------------------|
| 1. Leafbase | very short-petioled (constricted) | very short-petioled (constricted) | sessile, elongated | sessile, less elongated |
| 2. Angle of venation | 75-80 | 75-80 | 45-50 | 55-60 |
| 3. L/W ratio | ± 1.50 | ± 1.50 | 3.00-3.50 | 2.00-2.25 |
| 4. Number of leaves | 25 | 25 | 32 | 28-29 |
| 5a. Flower throat | inflated | inflated | slender | less slender |
| 5b. Limb contour | pentagonal | pentagonal | pointed (asteroid) | less pointed |
| 6. Flower colour | pink | carmine | pink | pink |

Ch 1 § 3b. *Uniformity of the parental lines*

The parents can be safely regarded as sufficiently pure lines because 1) the lines had been propagated by controlled selfings during many generations, 2) within the lines a striking simultaneity of development was observed when the plants were grown under uniform environmental conditions, and 3) under the environmental conditions prevailing in the experimental garden the full-grown plants usually resembled each other closely within each line. However, apart from easily recognized modifications such as the more luxuriant growth of edge plants, divergent growth due to diseases and damages, etc., some phenomena were observed that did not result from lack of purity either:

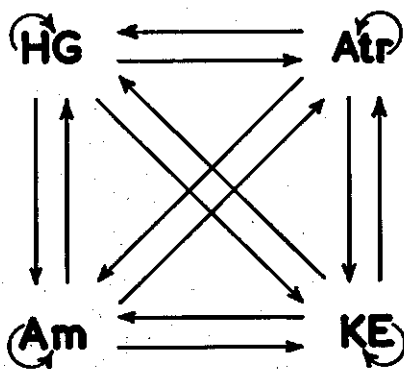
1. Atr is more susceptible to environmental fluctuations than the three other varieties. It is easily attacked by viruses and can

suffer much from temporary drought during youth growth. In 1952 it was observed that among 49 Atr plants 20 were smaller (somewhat stunted) than the others and had very dark green, thick leaves. The same phenomenon was also found, though less pronounced, in several F_1 's of Atr with other varieties. The deviants occurred in groups in the field. After selfing a number of both normal and deviating plants, no differences of this type could be found in the offspring. It is probable that the phenomenon was induced by a period of drought in the beginning of the summer.

2. In 1953 an aberrant Am seedling was found. The plant was much smaller than its sisters and its leaves were very narrow. When full-grown it proved to be sterile. Though no cytological examination was made it can be said that it was probably a spontaneous haploid like the one found by PRAKKEN (1942) in an F_3 from Am x Atr.
3. In the parental lines, the F_1 's and the segregating generations, the proportions of plants showing clockwise and counterclockwise spirality deviated not significantly from equality. No correlation with position of the seeds in the capsules could be found. Hence there is no reason to oppose ALLARD (1946) who stated: "The distribution of these two spiral alternatives so far as observed is fundamentally one of chance". In this connection it may be said that contrary to ALLARD (1942) who found in most cases 5/13 or 3/8 phyllotaxis (sometimes both on the same plant) I always found 3/8 in the present material, at least when allowance was made for torsion of the stem.

Ch 1 § 4. SURVEY OF THE FAMILIES ANALYSED

In 1950 a complete diallel set (4 parent families and 6 pairs of reciprocal F_1 's) was made, using one single plant of the lines HG, Atr, Am and KE.



Schema of the diallel set.

From the resulting 1951-plants and their sisters grown in 1952 we obtained the backcrosses to both parents, the F_2 's and some triple crosses. It may be said here that no differences between reciprocal F_1 's could be found. In connection with this, only one of each pair of reciprocal F_1 's was used to give the segregating generations, with the exception of the reciprocal pair F_2 from $HG \times Am$ and F_2 from $Am \times HG$. In the backcrosses, the F_1 was taken as the mother in all cases. A survey of the descent of the families mentioned in the text is given in Table 2.

Table 2. Descent of the families mentioned in the text. To illustrate the notation: 52-104-10 means plant No. 10 from family No. 104 of the year 1952. Families grown from sister seeds have been connected by the = sign. F_3 's and some other families have been omitted from the table. Their descent will be mentioned in the text.

| PARENT FAMILIES | | | |
|------------------------|--|--|--|
| The 4 pure line plants | | Families obtained on selfing the 4 parent plants | |
| HG : 50-1-12 | | 51-021 = 52-101 = 53-201 | |
| Atr : 50-II-8 | | 51-029 = 52-109 = 53-203 | |
| Am : 50-I-49 | | 51-033 = 52-113 = 53-204 | |
| KE : 50-2-33 | | 51-025 = 52-105 = 53-202 | |

| F ₁ and F ₂ FAMILIES | | | |
|--|--------------------------------|------------------------------------|-----------------------|
| a. Obtained from the 4 original pure line plants | | | |
| Cross | F ₁ family | No. of F ₁ plant selfed | F ₂ family |
| 1a. HG x Am | 51-024 = 52-104 = 53-205 | 10 | 53-208 = 54-006 |
| 1b. Am x HG | 51-034 | 28 | 52-129 |
| 2. HG x KE | 51-022 = 52-102 = 53-213 | 3 | 53-233 = 54-091 |
| 3. HG x Atr | 51-023 = 52-103 | 1 | 53-234 |
| 4. Atr x Am | 51-032 = 52-112 | 10 | 53-238 |
| 5. KE x Atr | 51-027 = 52-107 | 10 | 53-236 |
| 6. KE x Am | 51-028 = 52-108 | 16 5 | 52-131 53-237 |
| b. Obtained from a later pure line generation | | | |
| Cross | Parent plants | F ₁ plant selfed | F ₂ family |
| 4. Atr x Am | 52-109-4 x 52-113-2 | 54-059a-37 | 55-035 |
| 6. KE x Am | 52-105-3 x 52-113-2 | 54-060a-40 | 55-036 |

| BACKCROSS FAMILIES | | | |
|---------------------|-----------------------|------------------|--------|
| Cross | Parent plants | Backcross family | |
| 1. (HG x Am) x HG | 52-104-10 x 52-101-6 | 53-206 = 54-004 | |
| (HG x Am) x Am | 52-104-10 x 52-113-2 | 53-207 = 54-005 | |
| 2. (HG x KE) x HG | 52-102-9 x 52-101-6 | 53-219 | |
| (HG x KE) x KE | 52-102-9 x 52-105-3 | 53-220 | |
| 3. (HG x Atr) x HG | 51-023-38 x 51-021-13 | 52-137 = 53-247 | |
| (HG x Atr) x Atr | 51-023-38 x 51-029-32 | 52-139 = 53-248 | |
| 4. (Atr x Am) x Atr | 51-032-56 x 51-029-32 | 52-155 | |
| (Atr x Am) x Am | 51-032-56 x 51-033-39 | 52-156 | |
| 5. (KE x Atr) x Atr | 51-027-33 x 51-029-32 | 52-147 | |
| (KE x Atr) x KE | 52-107-2 x 52-105-3 | | 54-093 |
| 6. (KE x Am) x Am | 51-028-20 x 51-033-39 | 52-152 | |
| | 52-108-8 x 52-113-2 | | 53-232 |
| (KE x Am) x KE | 51-028-20 x 51-025-34 | 52-150 | |
| | 52-108-8 x 52-105-3 | | 53-230 |

| TRIPLE CROSSES | | | |
|-----------------|---------------------|---------------------|--|
| Cross | Parent plants | Triple cross family | |
| (KE x Am) x HG | 52-108-8 x 52-101-6 | 53-229 = 54-131 | |
| (KE x Am) x Atr | 52-108-8 x 52-109-4 | 53-231 = 54-132 | |

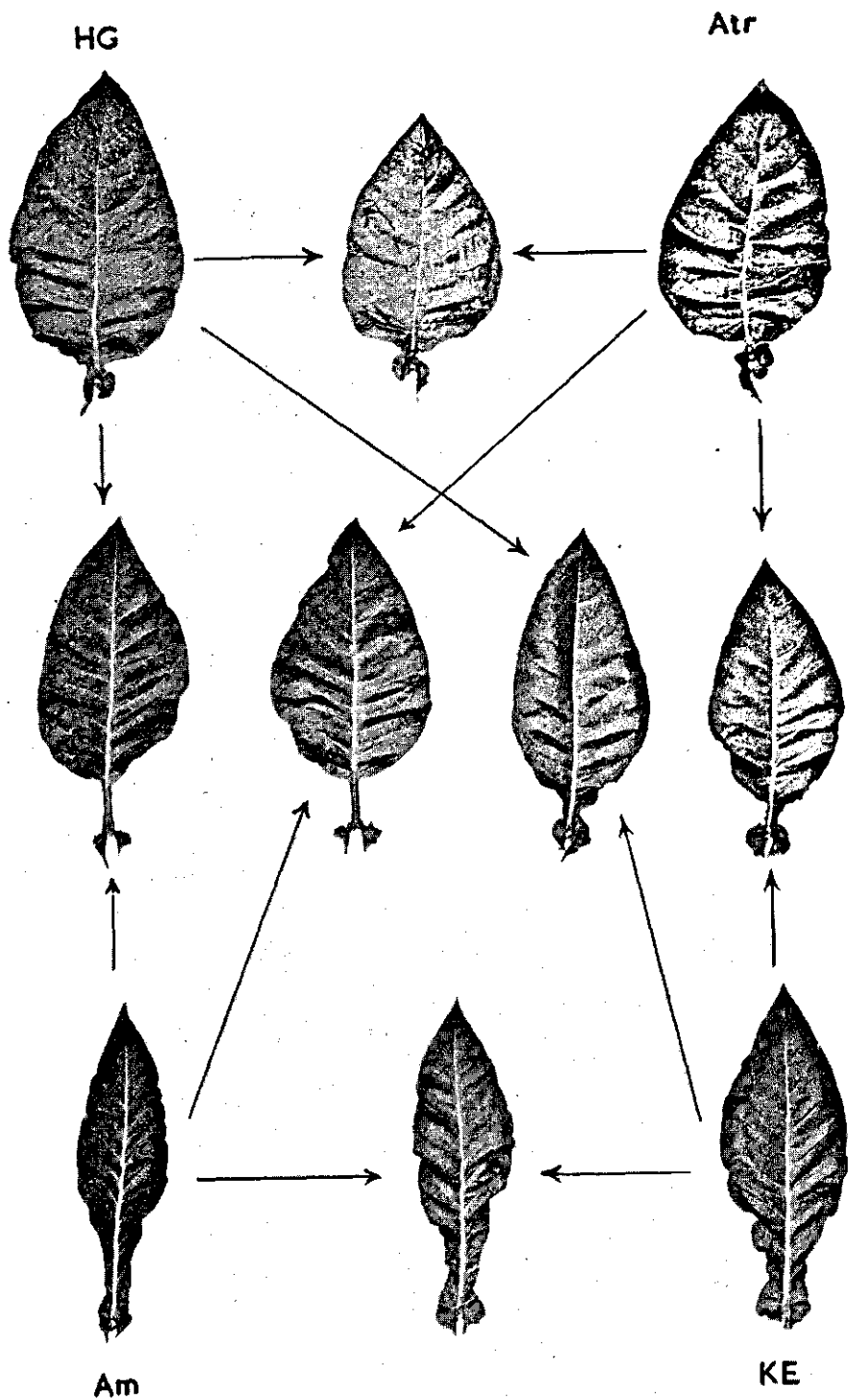


Fig.5a. Typical leaves of the 4 parental lines (corners) and of the 6 F₁ hybrids (connected to the corresponding parents by arrows).

HG = Hongaars Gartenblatt

Atr = Atropurpurea

Am = Amersfoorter

KE = Keurhorst Elite

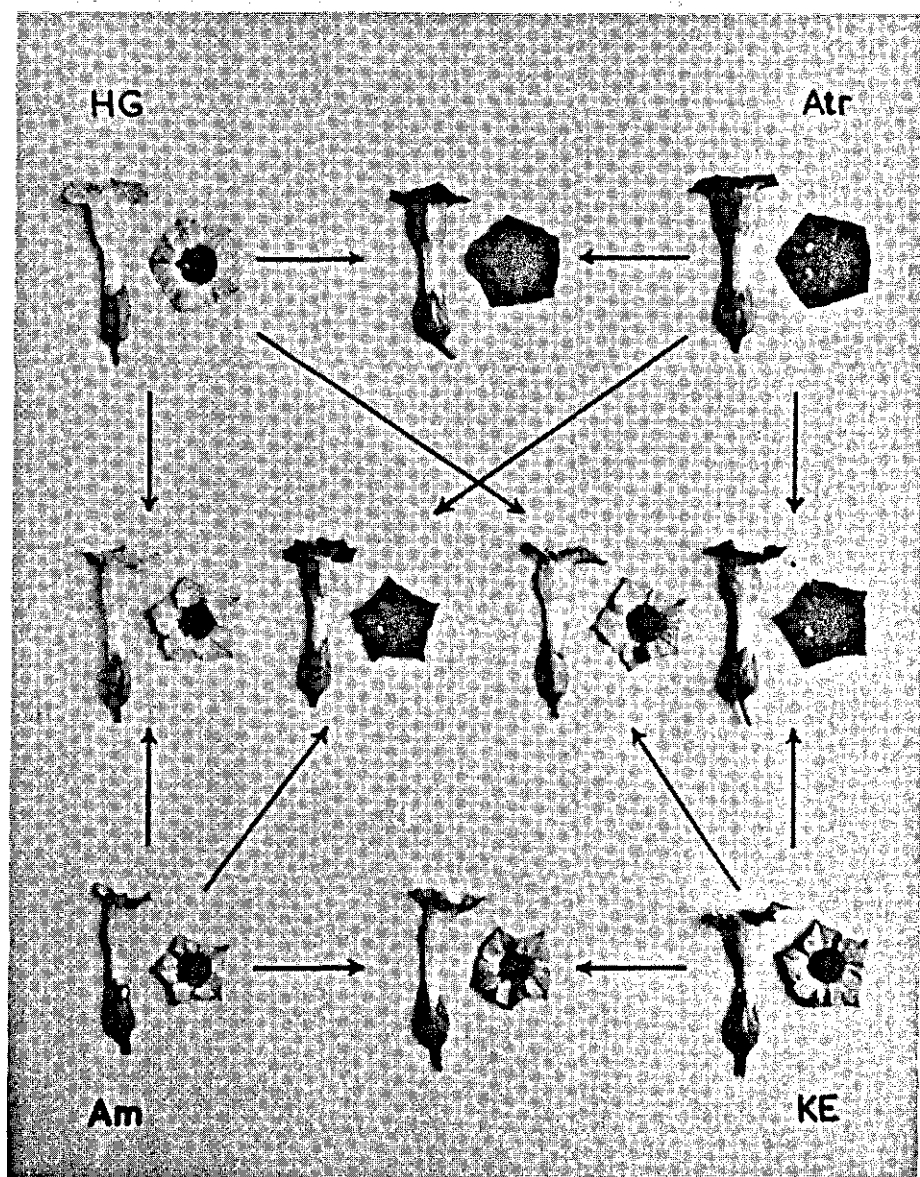


Fig. 5b. Flower pairs of the 4 parental lines (corners) and of the 6 F1 hybrids (connected to the corresponding parents by arrows). The arrangement is the same as in Fig. 5a.

Ch 1 § 5. THE SIX F₁ HYBRIDS

Typical leaves of the six F₁ hybrids are compared with those of the parents in Fig. 5a. In the same arrangement the different flower types are shown in Fig. 5b. The parents have been described in Ch 1 § 3a (cf. also Table 1).

It must be stated that no differences between reciprocal F₁'s could be observed. For angle of venation and leaf dimensions this becomes apparent from the metrical data given in Ch 2.

Regarding angle of lateral veins, L/W ratio and number of leaves all F₁'s are intermediate between the corresponding parents. The visual impression is corroborated by the metrical data presented in Ch 2. Flower shape, as described in terms of slenderness of tube and throat and of limb contour, is intermediate too. The carmine flower colour of Atr is completely dominant over the pink colour of HG, Am and KE.

With respect to leafbase shape the situation is more complicated. Referring to Fig. 5a the leafbase shape of the six F₁'s can be described as follows:

1. F₁(HG/Am) has a rather long, distinct petiole (± 8 cm.) with narrow wings (0.4 - 0.8 cm.) and its auricles are somewhat smaller than those of HG.
2. F₁(HG/KE) has a moderately short petiole with well developed wings (1.5 - 2.5 cm. at the narrowest point). HG itself has very short petioles with wings 1.0 - 2.0 cm. in width. The petiole of the F₁ is not sharply set off against the blade which merges more gradually into the wings than is the case with HG. The auricles are somewhat larger than those of HG.
3. F₁(HG/Atr) shows the same leafbase shape as its parents HG and Atr which resemble each other closely in this respect.
4. F₁(Atr/Am) closely resembles F₁(HG/Am), which is not surprising in view of the similarity between HG and Atr.
5. F₁(Atr/KE), similarly, resembles F₁(HG/KE) very much.
6. F₁(KE/Am) is sessile like its parents and intermediate between them regarding leafbase elongation, i.e. in width of leafbase and in distance from axil to sinus midpoint. The latter term has been introduced in Ch 1 § 3a (see "1. Shape of the leafbase").

The segregating generations will be discussed in the same order as the F₁'s.

| | | |
|--------|----------|--------|
| HG-Am | families | (§ 6) |
| HG-KE | families | (§ 7) |
| HG-Atr | families | (§ 8) |
| Atr-Am | families | (§ 9) |
| Atr-KE | families | (§ 10) |
| KE-Am | families | (§ 11) |

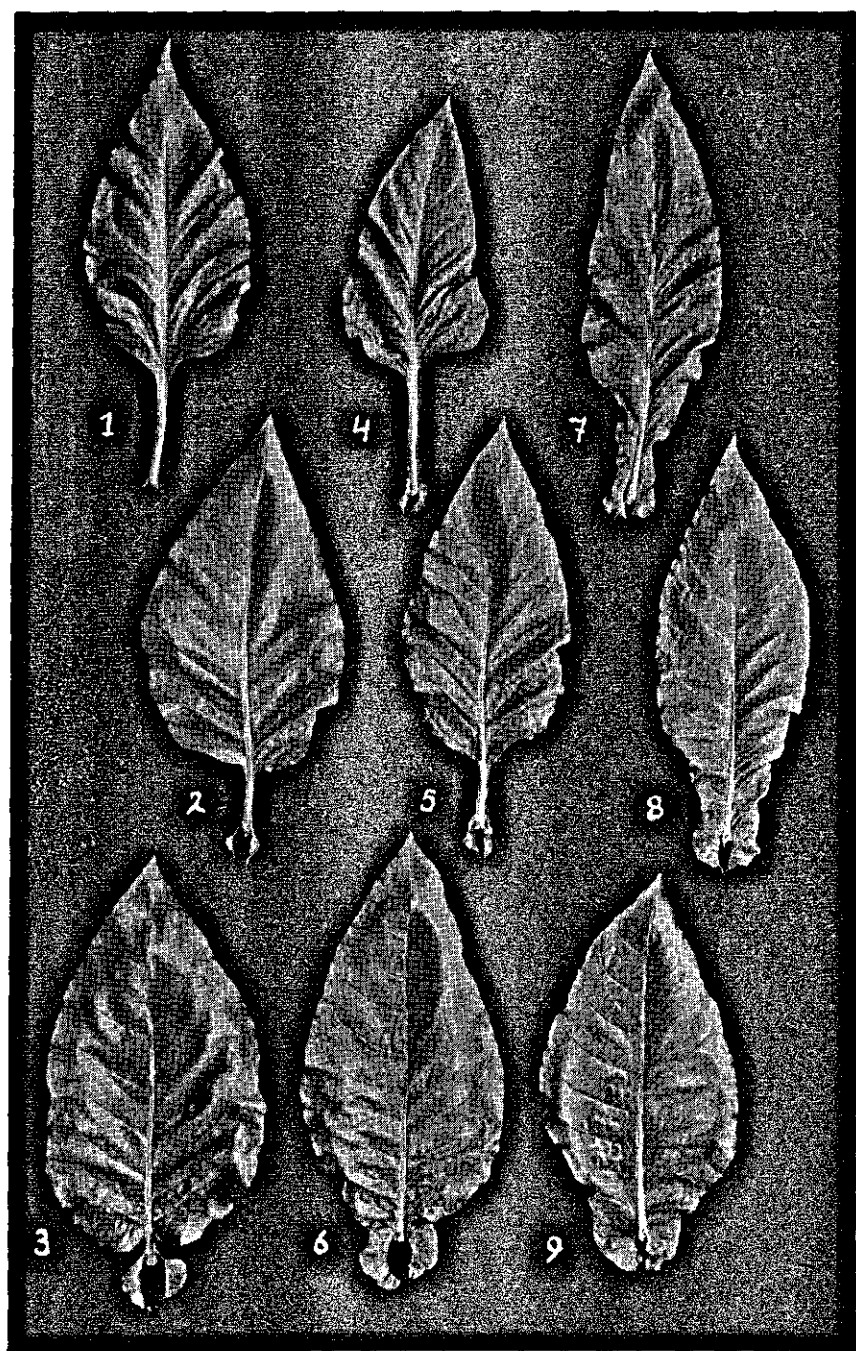


Fig. 6. The 9 "ideal" leaf types of the F_2 from the cross Hongaars Gartenblatt x Amersfoort (Fam. 53-208). In the text the types are referred to by the numbers added. All leaves have been taken from plants with the same number of leaves and from the same node number (cf. Ch 1 § 6c). The different genotypes are given at the end of Ch 1 § 6a.

Ch 1 § 6. THE HG-Am FAMILIES

For ease of presentation I will first give the results of the analysis with the help of the F_2 (§ 6a), then give an account of the analytical data (§ 6b) and finally discuss some additional aspects (§ 6c and § 6d).

Ch 1 § 6a. *Description and genetic interpretation of the F_2 types*

The F_2 has been classified into 9 phenotypic classes corresponding to the 9 genotypes of a two-factor segregation. Fig. 6 presents the 9 "ideal" types in checker-board arrangement with the four dihomozygotes (types 1, 3, 7 and 9) at the corners and the diheterozygote (type 5) in the centre. The types 3, 5 and 7 resemble HG, F_1 and Am respectively and therefore will occasionally be referred to as HG-type, F_1 -type and Am-type.

Before describing the action of the two factor-pairs with the help of Fig. 6 some remarks may be made for the sake of clearness. In tobacco literature it is customary to call types like 1, 2, 4 and 5 petiolate, types like 3 constricted and types like 6, 7, 8 and 9 sessile. This nomenclature can be used safely. However, as will become clear below, it is, for the purpose of understanding the action of the two factor-pairs, convenient to look upon the constricted type as having a very short petiole (with narrow wings) and to consider the leafbases of the sessile types as petioles with broad wings. These wings, being broad, gradually merge into the blade. In most cases, however, a distinct sinus is present, the level (midpoint) of which will be taken to indicate the length of the virtual petiole.

Turning to the three columns of Fig. 6 first, we see that in the left column (type numbers 1, 2 and 3) the types are narrowly winged to wingless, i.e. they are clearly petioled. In the right column (7, 8 and 9) the types have broad wings, i.e. they are sessile. Thus the factor-pair concerned (Br-br) has a large influence on wing width: br br (left column) versus Br Br (right column). When heterozygous (middle column) this factor-pair exhibits incomplete dominance. The direction of dominance, however, is dependent on the row involved, i.e. on the second factor-pair. This makes it sometimes difficult on the one hand to distinguish between the types 6 and 9, and on the other hand between the types 1 and 4, or 2 and 5. Further details of this will be discussed below.

The differences between the rows are generated by a factor-pair (Pt-pt) that influences 1) petiole length or leafbase elongation, 2) angle of venation, 3) leaf width, and 4) wing width. Regarding the former three characters, the top row (Pt Pt) has very long petioles (or, in the case of type 7, a very elongated leafbase), acute angles of venation and narrow blades. The bottom row (pt pt) has very short petioles (or a short leafbase), obtuse angles and broad blades. The middle row (Pt pt) is roughly intermediate in

these three respects. It should be noted that within each row the level of the sinus midpoint (sessile types) corresponds well with the length of the petioles of the neighbouring types. Before branching off from the midrib, the lateral veins grow together with it for a distance which is 0.5-1.2 cm. longer in the Pt Pt than in the pt pt types. The effect of Pt-pt on wing width must be considered within each individual column as it is superposed on the much larger effect of Br-br. For each column it can be said that the shorter the petiole, the broader the wings. Thus within the left column (br br) type 1 is wingless, type 2 has very narrow wings and type 3 has narrow wings. Within the right column (Br Br) type 7 has fairly broad wings, type 8 broad wings and type 9 very broad wings.

Within each of the three classes (rows) both angle of venation and leaf width show considerable variation (a large part of which is genetic) so that the classes overlap and the total distribution fails to show a clear trimodality (cf. Fig. 26 for angle of venation). Regarding leafbase shape, however, types of different rows are connected by relatively infrequent intermediate forms. Moreover, these intermediates can in practically all cases be assigned to the correct types by means of the device to be outlined in Ch 1 § 6c. In other words the shape of the leafbase is a better criterion for inferring the genotypes from the phenotypes than the angle of venation and the leaf width. Therefore it is justified to name the factor-pair Pt-pt after its major aspect of action on leafbase shape, i.e. its action on petiole length (or leafbase elongation). Pt Pt then stands for the long petioles of the top row and pt pt for the very short petioles of the bottom row (Fig. 6).

The major argument in choosing the symbols Pt-pt and Br-br was that they are in agreement with those adopted by CLAUSEN and CAMERON (1944) who use a constricted type (pt pt br br) as the standard type. The Californian mutant types Pt Pt and Br Br, Dr. CAMERON kindly sent me, closely resemble the present types 1 and 9 respectively. For further discussion the reader is referred to Ch 1 § 13. However, it must be said that, though it is highly probable that the factors are identical, some reserve is due, as no tests for identification have been performed yet.

The preliminary impression that the Pt-pt factor-pair, in addition to its influence on the leafbase, has a pleiotropic effect on angle of venation and leaf width, will be corroborated by the metrical data to be presented in Ch 2. Further it could be stated that in progenies from many selected plants segregation for petiole length always coincided with segregation for angle of venation and non-segregation with non-segregation, i.e. no instances of crossing-over could be found. Though with caution, angle of venation will be used as a side-criterion in inferring the Pt-pt genotypes on visual inspection of the plants.

Some remarks must be made in connection with the above-mentioned difficulties to distinguish the Br br types (middle column in Fig. 6) from the Br Br or br br types. Regarding the types 6 and 9 it can be said that in general type 6 has semi-broad

wings and that the blade is clearly set off against the base, while type 9 has very broad wings and a less distinct sinus. Type 5 differs from type 2 in having broader wings and a blade that tapers slightly towards the wings, while the petiole of type 2 is sharply set off against the blade which often curves inward in a cordate fashion. Finally type 4 has narrow wings in contrast to type 1 which, apart from inconspicuous wings near the blade and the auricles, shows a very narrow, rigid, riblike structure instead of wings.

The genotypes can be summarized as follows (arrangement conformable to Fig. 6):

| | | |
|-----------------------------------|--|-----------------------------------|
| type 1 = Pt Pt br br | type 4 = Pt Pt Br br | type 7 = Pt Pt Br Br (like Am) |
| type 2 = Pt pt br br | type 5 = Pt pt Br br (like F ₁) | type 8 = Pt pt Br Br |
| type 3 = pt pt br br (like HG) | type 6 = pt pt Br br | type 9 = pt pt Br Br |

It should be borne in mind that Br-br influences wing width, not leaf width, and that Pt-pt governs petiole length (etc.), not the petiolate versus sessile alternative.

Ch 1 § 6b. *Detailed account of the analysis*

In 1953 the F₂ from HG x Am (53-208) was grown together with the backcrosses F₁ x HG (53-206) and F₁ x Am (53-207). The families were repeated in 1954 by growing from sister-seeds 54-006, 54-004, 54-005 respectively. Of the 196 plants of the F₂ (53-208), 190 plants gave rise to an F₃ family. The smallest F₃ families were designed to consist of 12 plants. This number was reached with few exceptions, e. g. loss of 1 or 2 plants due to insect damage. In the backcrosses that are much easier to classify due to the smaller array of main genotypes and the more uniform genic background, only the few cases of doubt have been progeny-tested.

In the backcross F₁ x HG = Pt pt Br br x pt pt br br one finds the following types (arranged conformable to Fig. 6):

| | |
|--------------------------------|---|
| type 2 (Pt pt br br) | type 5 (Pt pt Br br) = F ₁ -type |
| type 3 (pt pt br br) = HG-type | type 6 (pt pt Br br) |

In the backcross F₁ x Am = Pt pt Br br x Pt Pt Br Br one meets:

| | |
|---|--------------------------------|
| type 4 (Pt Pt Br br) | type 7 (Pt Pt Br Br) = Am-type |
| type 5 (Pt pt Br br) = F ₁ -type | type 8 (Pt pt Br Br) |

Arranging the leaf types (between brackets) conformable to Fig. 6, the observed and expected frequencies and the chi square tests run as follows:

(Hg x Am) x HG

53-206

| | | | | | |
|-------|-------|----|-------|-------|-------|
| 24(2) | 25(5) | 49 | 23.75 | 23.75 | 47.50 |
| 24(3) | 22(6) | 46 | 23.75 | 23.75 | 47.50 |
| 48 | 47 | 95 | 47.50 | 47.50 | 95.00 |

$$X^2(3 \text{ d.f.}) = 0.200 \quad P = 0.95-0.90$$

54-004

| | | | | | |
|-------|-------|-----|-------|-------|-------|
| 55(2) | 46(5) | 101 | 52.5 | 52.5 | 105.0 |
| 55(3) | 54(6) | 109 | 52.5 | 52.5 | 105.0 |
| 110 | 100 | 210 | 105.0 | 105.0 | 210.0 |

$$X^2(3 \text{ d.f.}) = 1.086 \quad P = 0.80-0.70$$

(HG x Am) x Am

53-207

| | | | | | |
|-------|-------|----|-------|-------|-------|
| 18(4) | 26(7) | 44 | 23.75 | 23.75 | 47.50 |
| 24(5) | 27(8) | 51 | 23.75 | 23.75 | 47.50 |
| 42 | 53 | 95 | 47.50 | 47.50 | 95.00 |

$$X^2(3 \text{ d.f.}) = 2.053 \quad P = 0.70-0.50$$

54-005

| | | | | | |
|-------|-------|-----|--------|--------|--------|
| 57(4) | 54(7) | 111 | 54.25 | 54.25 | 108.50 |
| 52(5) | 54(8) | 106 | 54.25 | 54.25 | 108.50 |
| 109 | 108 | 217 | 108.50 | 108.50 | 217.00 |

$$X^2(3 \text{ d.f.}) = 0.235 \quad P = 0.98-0.95$$

F₂ families

53-208

| | | | | | | | |
|-------|-------|-------|-----|-------|-------|-------|--------|
| 8(1) | 21(4) | 14(7) | 43 | 12.25 | 24.50 | 12.25 | 49.00 |
| 17(2) | 50(5) | 36(8) | 103 | 24.50 | 49.00 | 24.50 | 98.00 |
| 11(3) | 30(6) | 9(9) | 50 | 12.25 | 24.50 | 12.25 | 49.00 |
| 36 | 101 | 59 | 196 | 49.00 | 98.00 | 49.00 | 196.00 |

$$\text{Segr. (Br-br)} \quad X^2(2 \text{ d.f.}) = 5.582 \quad P = 0.10-0.05$$

$$\text{Segr. (Pt-pt)} \quad X^2(2 \text{ d.f.}) = 1.010 \quad P = 0.70-0.50$$

$$\text{Both segr.} \quad X^2(8 \text{ d.f.}) = 12.163 \quad P = 0.20-0.10$$

54-006

| | | | | | | | |
|-------|-------|-------|-----|--------|---------|--------|---------|
| 18(1) | 45(4) | 27(7) | 90 | 20.875 | 41.750 | 20.875 | 83.500 |
| 36(2) | 95(5) | 42(8) | 173 | 41.750 | 83.500 | 41.750 | 167.000 |
| 16(3) | 38(6) | 17(9) | 71 | 20.875 | 41.750 | 20.875 | 83.500 |
| 70 | 178 | 86 | 334 | 83.500 | 167.000 | 83.500 | 334.000 |

$$\text{Segr. (Br-br)} \quad X^2(2 \text{ d.f.}) = 2.982 \quad P = 0.30-0.20$$

$$\text{Segr. (Pt-pt)} \quad X^2(2 \text{ d.f.}) = 2.593 \quad P = 0.30-0.20$$

$$\text{Both segr.} \quad X^2(8 \text{ d.f.}) = 7.018 \quad P = 0.70-0.50$$

Of the 190 F₃ families grown in 1954, 31 consisted of (40-)44 plants ("large families": l.f.) and 159 of (10-)12 plants ("small

families": s.f.). Summation of the F_3 frequencies within each leafbase type of the F_2 parents leads to:

Homozygotes

| | |
|---------------------------|--|
| Type 1 (2 l.f. + 4 s.f.) | All F_3 plants of type 1 as expected |
| Type 3 (3 l.f. + 8 s.f.) | All F_3 plants of type 3 as expected |
| Type 7 (3 l.f. + 11 s.f.) | All F_3 plants of type 7 as expected |
| Type 9 (2 l.f. + 7 s.f.) | All F_3 plants of type 9 as expected |

Another F_2 plant originally classified as type 9, proved to have been of the pt pt Br br genotype and has been included in the type 6 group below.

Monoheterozygotes (All F_3 families showed the monofactorial segregation that was expected)

Type 2 (4 l.f. + 12 s.f.)

| | | |
|------------|---------------|---|
| 80(1) | 75.75 | X^2 (2 d.f.) = 0.881 P = 0.70-0.50 |
| 154(2) | 151.50 | |
| 69(3) | 75.75 | |
| <u>303</u> | <u>303.00</u> | |

Type 4 (3 l.f. + 17 s.f.)

| | | | | |
|-------|--------|-------|--------|------------------------------------|
| 71(1) | 164(4) | 94(7) | 329 | X^2 (2 d.f.) = 3.219 P = 0.20 |
| 82.25 | 164.50 | 82.25 | 329.00 | |

Type 6 (5 l.f. + 24 s.f.)

| | | | | |
|--------|--------|--------|--------|---|
| 128(3) | 236(6) | 131(9) | 495 | X^2 (2 d.f.) = 1.105 P = 0.70-0.50 |
| 123.75 | 247.50 | 123.75 | 495.00 | |

Type 8 (4 l.f. + 32 s.f.)

| | | |
|------------|------------|---|
| 146(7) | 138 | X^2 (2 d.f.) = 1.565 P = 0.50-0.30 |
| 280(8) | 276 | |
| 126(9) | 138 | |
| <u>552</u> | <u>552</u> | |

Diheterozygote (All F_3 families showed bifactorial segregation as expected)

Type 5 (5 l.f. + 44 s.f.)

| | | | | | | | |
|------------|------------|------------|------------|---------------|---------------|---------------|---------------|
| 39(1) | 88(4) | 52(7) | 179 | 45.75 | 91.50 | 45.75 | 183.00 |
| 88(2) | 184(5) | 98(8) | 370 | 91.50 | 183.00 | 91.50 | 366.00 |
| 26(3) | 100(6) | 57(9) | 183 | 45.75 | 91.50 | 45.75 | 183.00 |
| <u>153</u> | <u>372</u> | <u>207</u> | <u>732</u> | <u>183.00</u> | <u>366.00</u> | <u>183.00</u> | <u>732.00</u> |

| | | |
|---------------|-------------------------|---------------|
| Segr. (Br-br) | X^2 (2 d.f.) = 8.164 | P = 0.02-0.01 |
| Segr. (Pt-pt) | X^2 (2 d.f.) = 0.131 | P = 0.95-0.90 |
| Both segr. | X^2 (8 d.f.) = 14.667 | P = 0.10-0.05 |

On pooling the three bodies of data from progenies of dihe-

terozygotes, viz. $F_2(53-208)$, $F_2(54-006)$ and F_3 's from type 5, one obtains:

| | | | | | | | |
|--------|--------|--------|------|---------|---------|---------|----------|
| 65(1) | 154(4) | 93(7) | 312 | 78.875 | 157.750 | 78.875 | 315.500 |
| 141(2) | 329(5) | 176(8) | 646 | 157.750 | 315.500 | 157.750 | 631.000 |
| 53(3) | 168(6) | 83(9) | 304 | 78.875 | 157.750 | 78.875 | 315.500 |
| 259 | 651 | 352 | 1262 | 315.500 | 631.000 | 315.500 | 1262.000 |

| | | |
|---------------|-------------------------|---------------|
| Segr. (Br-br) | X^2 (2 d.f.) = 14.975 | P < 0.001 |
| Segr. (Pt-pt) | X^2 (2 d.f.) = 0.815 | P = 0.70-0.50 |
| Both segr. | X^2 (8 d.f.) = 18.897 | P = 0.02-0.01 |

The X^2 -values of the corresponding tests of homogeneity run:

| | Segr. (Br-br) | Segr. (Pt-pt) | Both segr. |
|----------------------|---------------|---------------|------------|
| $F_2(53-208)$ | 5.582 | 1.010 | 12.163 |
| $F_2(54-006)$ | 2.982 | 2.593 | 7.018 |
| F_3 's from type 5 | 8.164 | 0.131 | 14.667 |
| Total | 16.728 | 3.734 | 33.848 |
| Pooled Families | 14.975 | 0.815 | 18.897 |
| Heterogeneity | 1.753 | 2.919 | 14.951 |
| D.f. | 3x2-2 = 4 | 3x2-2 = 4 | 3x8-8 = 16 |
| P | 0.80-0.70 | 0.70-0.50 | 0.70-0.50 |

From the latter three P-values we have no reason to reject the assumption of homogeneity between the three bodies of data. Consequently the significant deviation from the 1 : 2 : 1 ratio for Br-br, as shown by the pooled families, is generated equally by them, i.e. the deviation is a systematic one. The three groups of data also homogeneously fail to show dependence between the wing width and the petiole length frequency distribution as follows from the X^2 -values of the 3 x 3 contingency tables:

| | | |
|----------------------|-------------------------------|---------------|
| $F_2(53-208)$ | $X^2(4 \text{ d.f.}) = 4.813$ | P = 0.50-0.30 |
| $F_2(54-006)$ | $X^2(4 \text{ d.f.}) = 1.172$ | P = 0.90-0.80 |
| F_3 's from type 5 | $X^2(4 \text{ d.f.}) = 7.162$ | P = 0.20-0.10 |
| Total | 13.147 | |
| Pooled families | $X^2(4 \text{ d.f.}) = 3.518$ | P = 0.50-0.30 |
| Heterogeneity | $X^2(8 \text{ d.f.}) = 9.629$ | P = 0.30-0.20 |

This result can be taken as failing to give evidence of linkage between the two factor-pairs. In view of the disturbed segregation of the Br-br factor-pair the usual X^2 -method for the detection of linkage (MATHER, 1951) could not be used.

The cause of the disturbance of the Br-br segregation in the F_2 and the type 5 progenies is not known. The germination percentages were high, crowding in the seedpans and selection during transplantation were avoided as much as possible and only few

plants succumbed before reaching maturity. Moreover the ratios found in the backcrosses and the F_3 progenies from the types 4 and 6 were in good agreement with expectation. Two points can be taken into consideration:

1. To obtain the backcrosses the F_1 was used as the female parent. Therefore the backcross frequencies cannot be affected by differential viability or activity of the male gametes, as might be the case with the F_2 frequencies.
2. F_2 zygotes can be expected to be more subject to differential elimination due to recombination of background genes than backcross zygotes.

However, it then remains unexplained why the relative frequencies of the F_3 's from type 4 and type 6 are not affected. Finally it must be stated that faulty classification in the families from diheterozygotes cannot be involved, for the large-scale progeny test showed that in the F_2 (53-208) only one plant was classified erroneously.

In conclusion it can be said, that the data confirm the hypothesis of two unlinked factor-pairs as 1) the arrays of types in the different families are conformable to expectation, 2) no evidence of linkage is met with, and 3) the observed frequencies agree with those expected, except for the wing width frequencies in families obtained by selfing diheterozygotes. This disturbance, the cause of which is unknown, needs not prevent us from concluding to two unlinked factor-pairs.

Ch 1 § 6c. *The within-leafbase-type correlation between petiole length and number of leaves*

Before entering upon other aspects of variation round the "ideal" types, a class of modifying genes must be discussed that affect leafbase elongation to the extent of making the Pt-pt classes overlap more or less. To study this, measurements were taken on plants of F_1 (53-205), $F_1 \times HG$ (53-206), $F_1 \times Am$ (53-207) and F_2 (53-208). To reduce environmental variation the families were grown as described in Ch 2 § 2a. Leaf length was measured on the upper leaf-side along the midrib from axil to tip. Petiole length was measured from the axil to the leafblade. The number of leaves (which includes the cotyledons and the uppermost leaf under the central end-bud) is probably governed by more than two loci. Its frequency distributions are given in Table 7 (Ch 2), but in the case of the F_1 20 instead of the 10 plants given in that table will be used, the classes being 29, 30, 31 and 32 leaves with frequencies 5, 8, 6 and 1 respectively.

Petiole length is not constant from node to node (cf. Fig. 7a), nor is leaf length. From bottom to top of the stem, both leaf length and petiole length gradually increase first and then decrease, the maximum petiole length being found 1-4 nodes higher than the maximum leaf length. The petioles in the region of maximum petiole length are with visual inspection of the plants the

Table 3. Means, variances, correlation coefficients, partial corr. coeff. and levels of significance for number of leaves (1), maximum petiole length found by interpolation (2), maximum leaf length found by interpolation (3) and number of the leaf with interpolated maximum petiole length (4) in F_1 (53-205) and in type 5 from $F_1 \times HG$ (53-206), $F_1 \times Am$ (53-207) and F_2 (53-208).

At the foot of the table: mean and variance of leaf number for HG (53-201) and Am (53-204). For further details see text.

| Families | F_1 | Type 5 from $F_1 \times HG$ | Type 5 from $F_1 \times Am$ | Type 5 from F_2 |
|---|-------------------|-----------------------------------|-----------------------------------|-------------------------|
| Number of plants | 20 | 25 | 22 | 49 |
| (1) Number of leaves | | | | |
| Mean | 30.2 | 27.6 | 33.1 | 30.5 |
| Variance | 0.8 | 4.5 | 3.7 | 5.6 |
| (2) Max. petiole length (mm) by interpo- lation | | | | |
| Mean | 95.7 | 80.1 | 109.6 | 86.6 |
| Variance | 55.4 | 293.8 | 270.6 | 208.9 |
| (3) Max. leaf length (mm) by interpo- lation | | | | |
| Mean | 690.6 | 645.2 | 648.5 | 612.1 |
| r_{12} | 0.16 non-sign. | 0.86 < 0.01 | 0.80 < 0.01 | 0.70 < 0.01 |
| r_{23} | 0.29 non-sign. | 0.54 < 0.01 | 0.67 < 0.01 | 0.33 0.02-0.01 |
| r_{13} | 0.04 non-sign. | 0.42 0.05-0.02 | 0.50 0.02-0.01 | 0.19 non-sign. |
| $r_{12.3}$ | - | 0.82 < 0.01 | 0.73 < 0.01 | 0.68 < 0.01 |
| $r_{23.1}$ | - | 0.40 0.10-0.05 | 0.52 0.02-0.01 | 0.28 0.10-0.05 |
| (4) Number of the leaf corresp. to (2) | | | | |
| Mean | 23.4 | 21.0 | 23.9 | 22.2 |
| r_{14} | - | 0.88 < 0.01 | 0.82 < 0.01 | 0.92 < 0.01 |
| HG (20 plants) | | Mean = 25.6 | Variance = 0.67 | |
| Am (20 plants) | | Mean = 35.0 | Variance = 0.58 | |

most conspicuous and so contribute most to the impression of the plants leafbase shape. In order to eliminate irrelevant within-plant fluctuations, the maximum petiole length of each plant was determined by graphical interpolation as illustrated in Fig. 7b.

The correlation coefficients between leaf number and maximum petiole length were calculated for F_1 and for the F_1 type (type 5) from respectively $F_1 \times HG$, $F_1 \times Am$ and F_2 . Type 5 is the only one occurring in all these families. The data to be discussed are shown in Table 3. The levels of significance of the correlation coefficients were read from the table given by FISHER (1954; p.209). The partial correlation coefficient between two variables a and b , the fixed variable being c , is denoted by $r_{ab.c}$.

From Table 3 it appears that:

1. HG and Am diverge much in number of leaves (25.6 and 35.0 respectively). The F_1 is intermediate (30.2). Its variance equals that of the parents.
2. Both number of leaves (1) and maximum petiole length (2) have a much larger variance in type 5 (F_1 -type) from the segregating generations than in the F_1 itself. This indicates genetic segregation. Regarding maximum petiole length it implies that the character is not only influenced by the factor-pair Pt-pt but also by modifying genes.
3. In type 5 from the segregating families the number of leaves and the maximum petiole length show a high positive correlation coefficient (r_{12}). The number of leaves and the number of the leaf corresponding to the (interpolated) maximum petiole length (4) also show high positive correlation coefficients (r_{14}). So an increased number of leaves induces an upward extension of the region of increasing petiole length, which leads to a higher value for maximum petiole length. The rate of increase pro node is not much affected, as no significant correlation coefficients (not presented in Table 3) were found between number of leaves and (interpolated) petiole length of the leaves No. 14 to 17. These points have been illustrated in Fig. 7c for type 5 from the F_2 .
4. Both number of leaves and maximum petiole length are in the segregating generations positively correlated (r_{13} and r_{23}) with maximum leaf length (3). On elimination of maximum leaf length, a partial correlation coefficient ($r_{12.3}$) is found which is about as high as r_{12} . This gives further evidence for the intimate relationship suggested above between the number of leaves and the maximum petiole length.
5. On elimination of number of leaves, maximum petiole length and maximum leaf length remain positively correlated ($r_{23.1}$). For plants with equal number of leaves, differences in leaf length can be taken to reflect differences in "vigour" (whether genetically or environmentally induced). We may therefore say that variation in maximum petiole length is also brought about by variation in plant vigour.

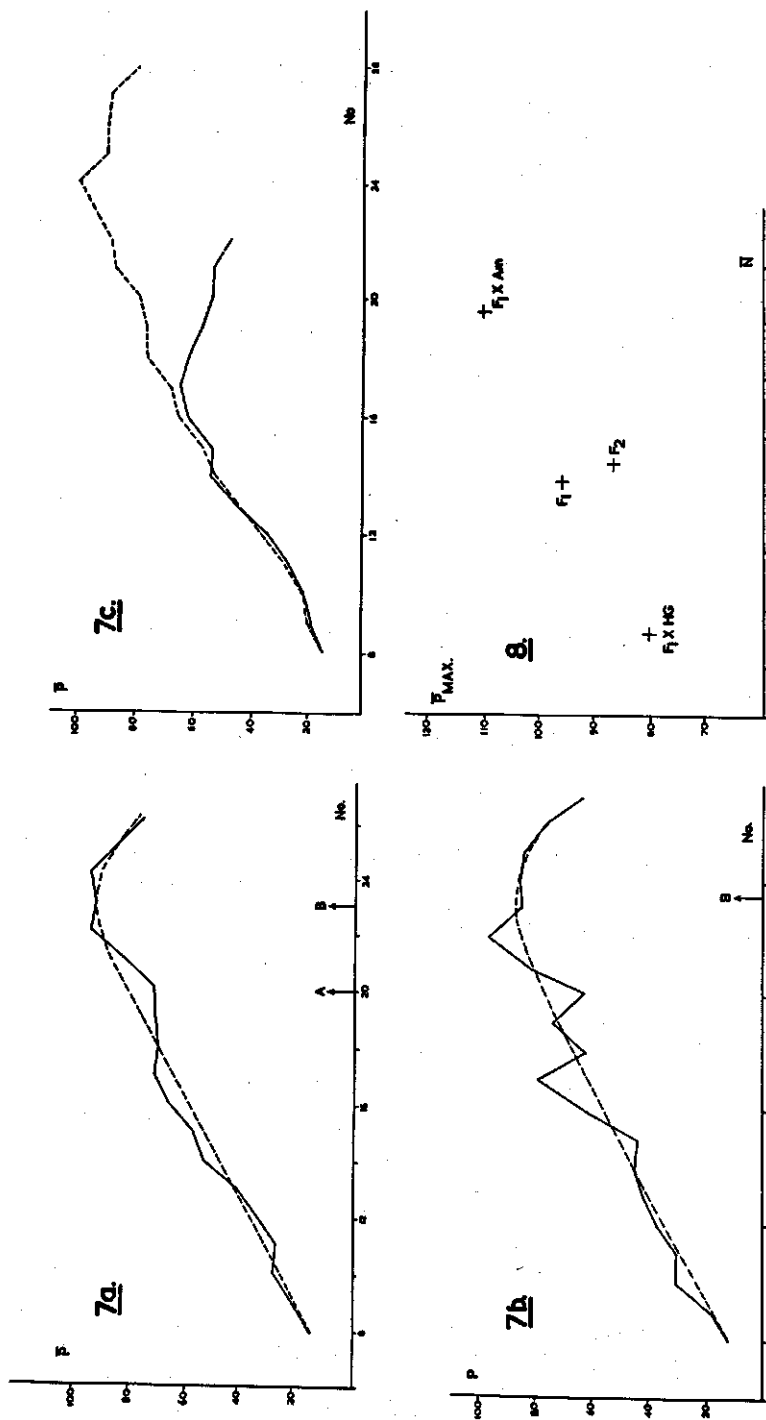


Fig. 7a. Mean petiole length (\bar{P}) in mm. of 20 plants from the F1 HG x Am (53-205) plotted against the number of leaf.

--- = interpolated trend.

A = position of maximum petiole length.

B = position of maximum petiole length from graphical adjustment.

Fig. 7b. Petiole length (\bar{P}) in mm. of the F1 plant 53-205-5 plotted against number of the leaf. See further caption to Fig. 7a.

Fig. 7c. Mean petiole length (\bar{P}) in mm. plotted against number of the leaf for 8 plants with a low number of leaves (---) and for 11

plants with a high number of leaves (---) among the type 5 plants of the F2 (53-208). For the leaf numbers of the two groups see Table 7.

Fig. 8. Mean of interpolated maxima for petiole length (\bar{P}_{max}) plotted against mean number of leaves (\bar{N}) for F1 (53-205), backcrosses

(53-206 and 53-207) and F2 (53-208).

In Fig. 8 the mean maximum petiole length is plotted against the mean leaf number for the four groups of type 5 data. The points are fairly well in a line as are the four scatter diagrams (not presented). This supplements the data for the individual families in illustrating the correlation between maximum petiole length and number of leaves. In connection with Fig. 8 it is interesting to note that the drop in mean maximum petiole length shown by the F_2 as compared with the F_1 is paralleled by a drop in mean maximum leaf length. This drop in maximum leaf length will be discussed in Ch 2 § 5f.

The correlation between maximum petiole length and number of leaves implies that 1) a large part of within-leafbase-type variation in petiole length is governed by genes for leaf number and needs for its explanation no separate system of modifiers, 2) the number of leaves is a very useful aid in inferring the leafbase genotypes from the phenotypes (see below).

Now, the correlation between maximum petiole length and number of leaves not only holds for type 5 (cf. Fig. 9) but also for the long- or rather long-petioled types 1, 2 and 4. Regarding each of the sessile types 6, 7, 8 and 9, the leafbase (axil to sinus midpoint) is clearly longer when the plants have a high number of leaves (cf. Fig. 10). Finally type 3, which normally has very short petioles, can have relatively long ones, for example type 3 plants from F_2 with as many as 34-36 leaves may reach a maximum petiole length of 3-4 cm. Within each of the nine types the wings become narrower with increasing petiole length (leafbase length) along the stem and, in connection with this, plants with longer petioles (leafbases) in the region of maximum petiole length (leafbase length) also have narrower wings in this region. It must be added that not only with petiolate but also with sessile types the leaves in the region of maximum leafbase elongation are the most conspicuous when inspecting the plants in the field and therefore contribute most to the general impression of the leafbase shape.

Before discussing the number of leaves as an aid in classifying, two points must be mentioned:

1. As will be seen in Ch 2 § 5e (cf. Table 7) the number of leaves is distributed almost wholly independently of the nine leafbase types.
2. As has been discussed in Ch 1 § 6a, the negative correlation between petiole length (leafbase length) and wing width is also found when comparing the three petiole length (leafbase length) genotypes (Pt-pt) within each of the three wing width genotypes (Br-br; columns in Fig. 6).

As an example we may take plants that are more or less intermediate in leafbase shape between type 4 and type 5. These plants prove to have either a high or a low number of leaves. When the leaf number is high the plant is a long-petioled variant of type 5, when it is low the plant is a short-petioled variant of type 4. A conspicuous difference in leaf number does not escape detection

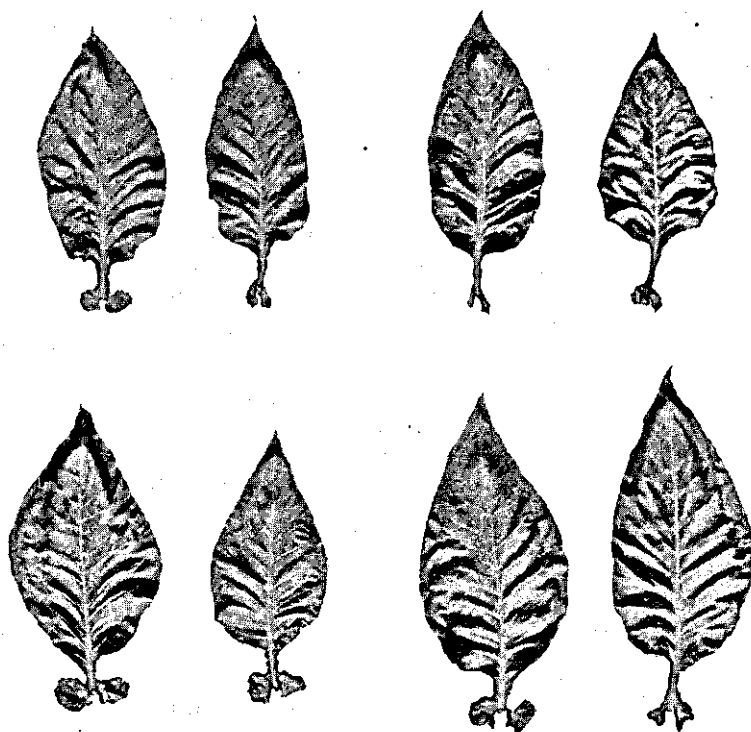


Fig. 9. 4 plants of F₂ (53-208), all type 5, each represented by 2 leaves (columns). The bottom row gives the leaves No. 19 that show but little differentiation in petiole length. The top row gives the leaves with maximum petiole length, viz. leaf No. 21, 23, 24 and 25 (left to right). The numbers of leaves of the plants are 26, 29, 31 and 33 respectively.

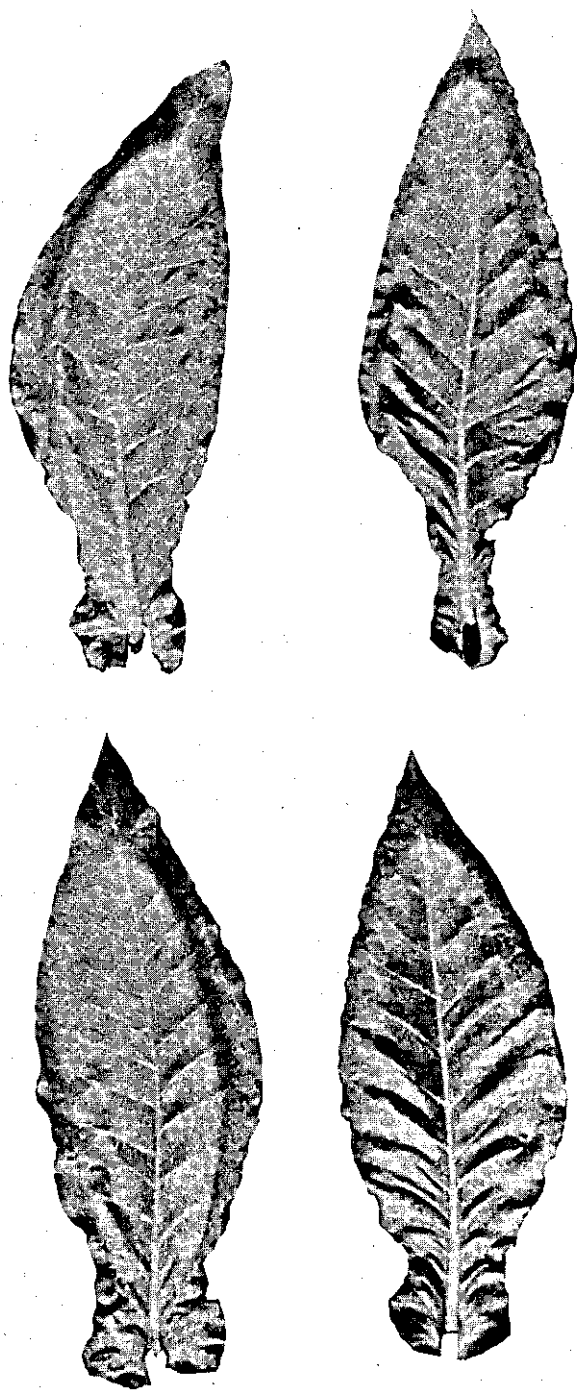


Fig. 10. 2 plants of $F_1 \times Am$ (53-207), both type 8, each represented by 2 leaves (columns). Bottom row: leaf No. 19. Top row: the leaves with maximum leafbase elongation, viz. leaf No. 23 and 26. The plants have 30 and 35 leaves respectively. In choosing these two plants for illustration the tendency has been overstressed somewhat.

in the present material, even though the bottom leaves have been withered when the plants are full-grown.

Regarding sessile types, i.e. the types most frequently met with in tobacco breeding, it is in segregating families profitable to pay attention to leaf number, notably when a large range of leaf numbers is involved (as in the present material) or when factors for leafbase elongation are operating that have a smaller effect than Pt-pt (see Ch 1 §§ 7 and 10). What appears at first sight as a more or less continuous variation can then be "stratified" into the underlying main types.

The efficiency of the procedure was tested for the $F_2(53-208)$ by growing F_3 's from 190 out of the 196 plants. None of the F_2 plants proved to have been classified erroneously with respect to its Pt-pt genotype. In classifying, conspicuous differences in plant vigour were also taken into account. Angle of venation, too, was used as a side-criterion, though with caution.

The correlation was also studied in a large number of F_3 families among which the 190 families mentioned above. The tendency towards joint fixation of the correlated deviations in leaf number and leafbase elongation was very apparent. Thus type 5 plants with a very low number of leaves and relatively short, broadly winged petioles, as are found in the backcross to HG, gave rise to progenies showing the same tendencies in all 9 types. In fact none of the F_3 families clearly showed breaking up of the correlation. This not only supports the assumption of pleiotropic action of the modifiers under discussion, but also shows that it will not be easy, if possible at all, to select at the same time for a high number of leaves and a broad leafbase within the main leafbase genotypes.

In view of the within-plant and within-type variability discussed and of other aspects of within-type variability (Ch 1 § 6d), much care must be taken in selecting for illustration a representative leaf of each type. Therefore the nine leaf types of the F_2 presented in Fig. 6 have been taken from plants with an average number of leaves (in this case 30 leaves). Moreover, the leaves had the same node number, viz. 23-24, which was in the region of maximum leaf length. Finally, deviating types (see Ch 1 § 6d) were excluded. The leaves in Fig. 6 must therefore be taken to represent the "ideal" types.

Ch 1 § 6d. *Other aspects of variation round the nine ideal types*

Additional variation of the leafbase shape is best described in connection with the individual leafbase types as some of the phenomena are more or less restricted to one or two of these types.

Type 1. Normally the petioles of this type are wingless. At times, however, plants are found on which some or all of the leaves have a slight wing development up to about half-way the petiole. When these plants are vigorous the wings can be rather pronounced, which makes it somewhat difficult to distinguish them from type 4.

However, such plants often have finger-shaped protuberances of leaf tissue, 1-2 cm. in length, at the base of the petiole. These structures, which may also be present on wingless petioles, are only found in type 1. Without referring to it in their text, GOOD-SPEED and CLAUSEN (1917; Plate 46) gave a drawing of rather pronounced protuberance formation. Fig. 11 gives an illustration of a very extreme case. The plant was an exception and the leaf itself was by far the most remarkable on the plant. On selfing this F_2 (52-129) plant, its progeny (53-208) consisted of type 1 plants with varying degrees of wing and protuberance development, though by far not so pronounced as in the parent plant. Likewise, in the case of less deviating types the phenomenon is transmitted to at least part of the descendants. Normal type 1 plants did not give rise to the deviating type. A simple Mendelian explanation could not be found from explorative crosses.

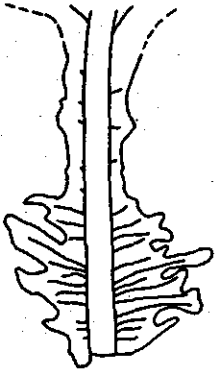


Fig. 11
Deviating type 1.
Semi-schematic.

Type 4. Both in the backcross to Am and in the F_2 , plants of this type are occasionally found, the higher leaves of which have conspicuously broad and undulated wings, while the lower leaves are more or less normal. Most of these plants are relatively vigorous in terms of leaf size. Fig. 12 shows an extreme case. The phenomenon is rather striking as 1) the higher leaves (apart from the highest) normally have longer petioles and narrower wings than the lower leaves, 2) undulated wings are not found in type 5, which has broader wings than type 4, and 3) the deviating plants were not preponderantly those with a low leaf number and short petioles. A number of the deviants were selfed and the progenies segregated into the types 1, 4 and 7. Among the segregants those of type 4 varied between normal and deviating like the parent, while those of type 1 showed varying degrees of wing and protuberance development. This indicates that both phenomena have a common cause. The underlying genetic mechanism seems complicated.

Type 2. When taking only the largest leaves into account, it may be difficult to distinguish between plants of type 5 and vigorous plants of type 2, which tend to have relatively broad wings. Fortunately the lower leaves of type 5 are always conspicuously broader-winged than those of type 2.

Type 6. It is sometimes difficult to distinguish this type from type 9. However, the highest leaves of type 6 always clearly show a constriction at the base, which is in contrast to type 9 of which all the leaves have a broad clasping base. Occasionally the veins on the basal half of the type 6 leaf show a rather acute angle, while those on the top half behave normal (cf. Fig. 12). This pattern, which also has been found in type 3, may be transmitted

to the descendants and is associated with a somewhat reduced leaf width, a rather elongated leafbase and a high number of leaves.

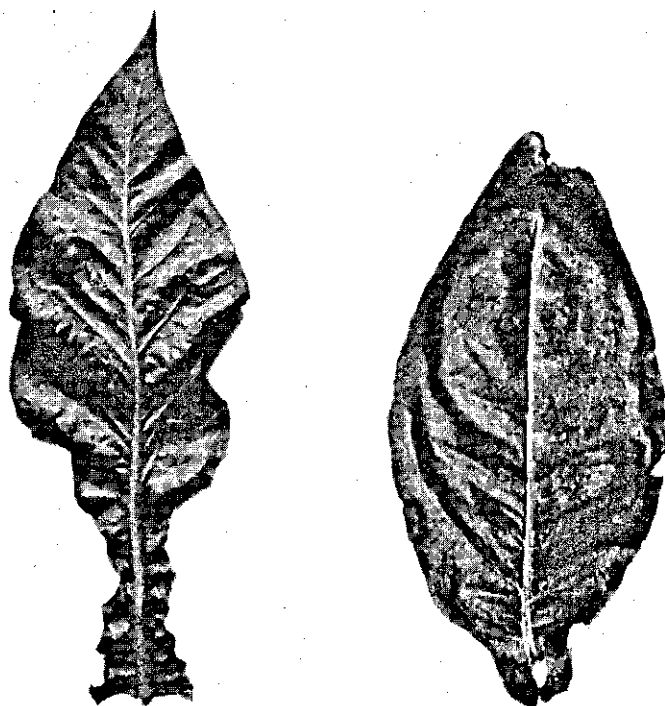


Fig. 12. Left: An extremely broad winged leaf of type 4.
Right: A type 6 leaf showing the shift in angle of venation sometimes found in this type.

From the foregoing it follows that it is often necessary to take the whole sequence of leaves into account, rather than to concentrate on specified leaves only, e. g. the largest.

Ch 1 § 6e. *Flower shape in connection with the leafbase genotypes*

The flowers of the 4 parental lines and the 6 F_1 hybrids have been compared in Fig. 5b.

In the F_2 from HG x Am three practically distinct classes of flower shape are encountered (Fig. 13):

HG-type (H). It has an inflated throat, an about pentagonal limb contour and an inflated calyx.

Am-type (A). Compared to the HG-type the tube and the throat are slender, the limb contour is asteroid (pointed lobes) and the slender calyx has pointed lobes.

F_1 -type (F). This type is more or less intermediate with respect to the components of flower shape.

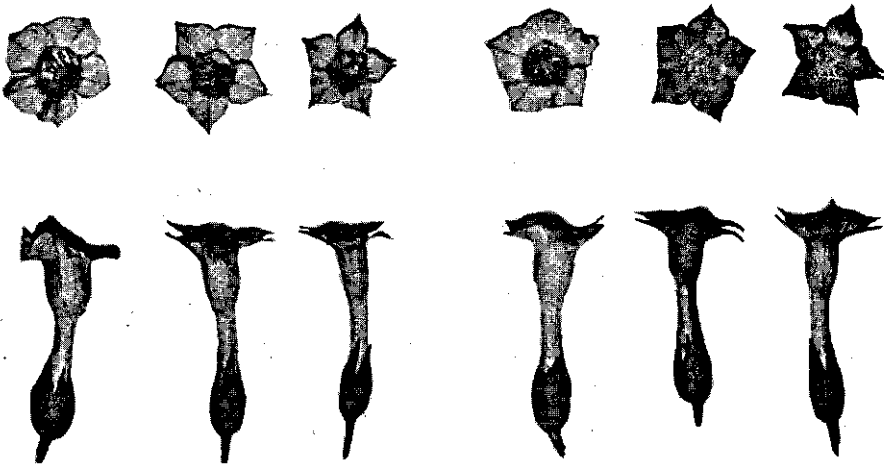


Fig. 13. From left to right: pairs of flowers (columns) of HG, F₁ and Am, and pairs of flowers of the HG-type, F₁-type and Am-type from the F₂ (53-208).

Within each of the three F₂ types and within the two types of each backcross, between-plant-variation is larger than in the isogenic families. Moreover the flower shape of the parents is not wholly recovered. Within-plant-variation is comparatively small. Flower shape is generally understood to be less susceptible to environmental factors than leaf shape. The three classes for flower shape were found to coincide with the three Pt-pt leaf shape classes. To check this, picked flowers of the F₂ and backcross plants were classified without looking at the leaf type of the plants. The results are shown in Table 4. From this table it will be seen that leaf type and flower type are highly correlated. As a rule the types 1, 4 and 7 (all Pt Pt) have Am-type flowers, the types 2, 5 and 8 (all Pt pt) have F₁-type flowers and the types 3, 6 and 9 (all pt pt) have HG-type flowers. Regarding the few exceptions that occur, it can be said that the plants with flowers intermediate between two classes (A-F and F-H) did not result from crossing-over, as appeared from their F₃ progenies; nor did the type 6 plant with F-flowers (instead of H-flowers), as its progeny had flowers varying between H and F-H.

As already mentioned (Ch 1 § 6a) leaf width is influenced considerably by the Pt-pt factor-pair. In the direction pt pt → Pt pt → Pt Pt the leaf becomes narrower. In the same direction the tube and throat become more slender and the limb contour more pointed. This is what can be expected as a flower must be looked upon as consisting of specialized leaves.

Table 4. Frequencies of flower types (H, A and F) within each leafbase type in F_2 and backcrosses (type No. between brackets). H = HG-type, F = F_1 -type and A = Am-type.

| | | | | | | |
|---|-----|------|-----|----------------------|-----|------------------------|
| F_2 (53-208) 187 plants | (1) | 6 A | (4) | 19 A 1 A-F | (7) | 12 A 2 A-F |
| | (2) | 16 F | (5) | 47 F | (8) | 1 A-F 33 F 1 F-H |
| | (3) | 11 H | (6) | 1 F 28 H | (9) | 9 H |
| $F_1 \times$ HG (53-206) 95 plants | (2) | 24 F | (5) | 25 F | | |
| | (3) | 24 H | (6) | 22 H | | |
| $F_1 \times$ Am (53-207) 95 plants | | | (4) | 18 A 1 A 1 A-F | (7) | 26 A 1 A |
| | | | (5) | 22 F | (8) | 26 F |

Ch 1 § 6f. Auricle size in connection with the leafbase genotypes

The auricles are best studied in the petioled types (1, 2, 3, 4, 5), as in the sessile types (6, 7, 8, 9) they merge into the wings (cf. Fig. 6). Their size is in several respects more or less correlated with the length of the petiole:

1. From bottom to top of the plant auricle size decreases with increasing petiole length.
2. Within each leafbase type, plants with a higher number of leaves tend to have not only longer petioles (cf. Ch 1 § 6c), but also smaller auricles in the region of maximum petiole length.
3. Auricle size decreases in the direction $pt\ pt \rightarrow Pt\ pt \rightarrow Pt\ Pt$ (cf. Fig. 6).

Further it can be stated that:

4. The Br-br factor-pair, too, influences auricle size. Within each of the Pt-pt classes the Br br plants (types 4, 5, 6) have, along with broader wings, somewhat larger auricles than the corresponding br br plants (types 1, 2, 3).
5. The more vigorous plants have larger auricles than the less vigorous ones.

These points are meant to describe the general trends only. Plants of type 1 or 4, for instance, may occur that have larger auricles than might be expected from plant vigour and number of leaves.

KADAM and RADHAKRISHNAMURTY (1953) crossed a petiolate type ($PtPt$) having very little growth of tissue at the place of the auricle (Au_0Au_0) with a "sessile" type ($ptpt$) having large auricles (au_0au_0). Both factor-pairs segregated 3 : 1. From the 43 back-

cross plants 20.93% crossing-over was calculated. For the 25 F_2 plants this percentage was 16.6. The same authors (1954) found two other loci for auricle size in small families from a different cross, viz. Au_1-au_1 and Au_2-au_2 . In this case the factors were said to interact with factors for the petiolate condition. From the present material no separate factors for auricle size could be inferred yet. In fact the situation is rather complex as appears from the different correlations mentioned.

Ch 1 § 7. THE HG-KE FAMILIES

The sessile variety KE has been described in comparison with Am in Ch 1 § 3a (cf. also Table 1). It has a shorter leafbase, broader wings, broader leaves, a less acute angle of venation and a lower number of leaves than Am. The KE leaf resembles that of type 8 from the HG-Am families, though its angle of venation is slightly more acute.

The F_1 HG x KE has been described in Ch 1 § 5 (cf. also Fig. 5a). This F_1 has a much shorter petiole, broader wings and a much more obtuse angle of venation than the F_1 HG x Am. This reflects the shorter and broader leafbase and the more obtuse angle of KE as compared with Am.

In comparing the segregating generations from HG x KE with those from HG x Am a remarkable analogy is observed, phenotypically as well as genetically. This analogy contributed much to the understanding of the genetic situation in the HG-KE families and is best discussed with the help of the 9 "ideal" F_2 types presented in Fig. 14 (arrangement conformable to Fig. 6). Again two independently segregating factor-pairs are involved, viz. a factor-pair for wing width (the three columns in Fig. 14) and a factor-pair (the three rows) that influences at the same time petiole length (or leafbase elongation), wing width (to a relatively small extent), leaf width, and angle of venation. In Ch 1 § 11 (KE-Am families) it will be shown that KE has the same factor for wing width (Br Br) as Am, while its factor for petiole length is on another locus than Pt-pt, segregating independently from it. Consequently we may put (adopting the Californian symbols Pd-pd):

HG = pt pt pd pd br br
KE = pt pt Pd Pd Br Br
Am = Pt Pt pd pd Br Br

The 9 genotypes of the present F_2 , arranged conformable to Fig. 14, are in terms of Pd-pd and Br-br (all types are pt pt):

| | | |
|-----------------------------------|---------------------------------------|-----------------------------------|
| type 1 = Pd Pd br br | type 4 = Pd Pd Br br | type 7 = Pd Pd Br Br (like KE) |
| type 2 = Pd pd br br | type 5 = Pd pd Br br (like F_1) | type 8 = Pd pd Br Br |
| type 3 = pd pd br br (like HG) | type 6 = pd pd Br br | type 9 = pd pd Br Br |

It should be noted that both in the HG-KE and the HG-Am families the types 3, 6 and 9 write pt pt pd pd.

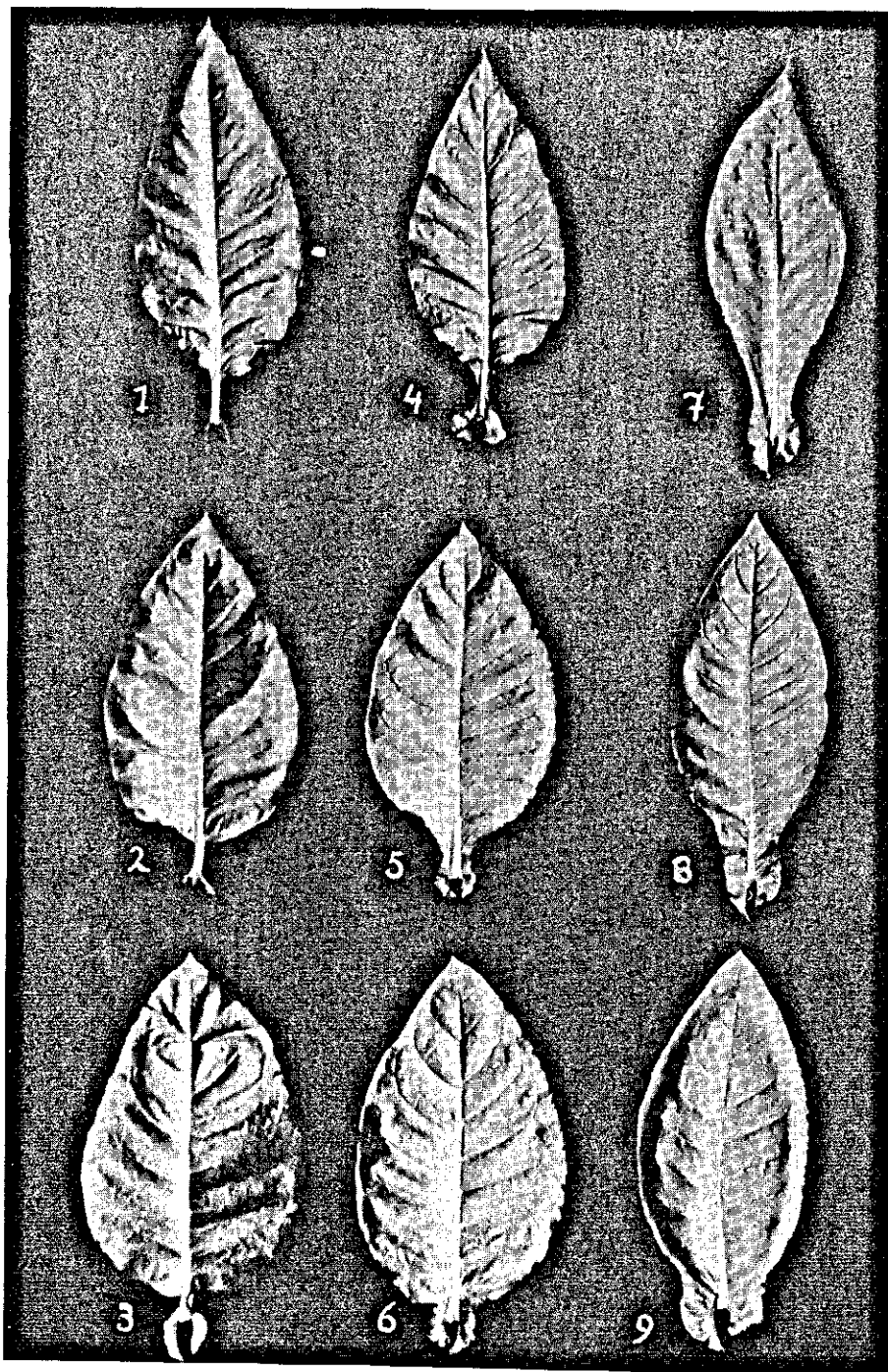


Fig.14. The 9 "ideal" types of the F₂ from the cross Hongaars Gartenblatt x Keurhorst Elite (Fam.54-091). In the text the types are referred to by the numbers added. The leaves have been taken from about the same node number when they had reached about 80% of their final length. In the case of the HG-KE material such not quite full-grown leaves show, at least on a photograph, the relevant features clearer than full-grown leaves do.

Qualitatively the 9 types of the F_2 from HG x KE (Fig. 14) compare in the same way as the 9 types of the F_2 from HG x Am (Fig. 6). Quantitatively, however, a pronounced difference is present, resulting from a quantitative difference in action between Pd-pd and Pt-pt. Regarding petiole length (or amount of leafbase elongation in the case of sessile types), acuteness of angle of venation and narrowness of the leaf, the effect of Pd-pd is much smaller than that of Pt-pt. This is reflected in the differences described above between Am (Pt Pt pd pd) and KE (pt pt Pd Pd) and those between F_1 from HG x Am (Pt pt pd pd) and F_1 from HG x KE (pt pt Pd pd). The Pd Pd types 1, 4 and 7 of the F_2 from HG x KE (Fig. 14) have petioles (leafbases) that are not only much shorter than those of the Pt Pt types 1, 4 and 7 of the F_2 from HG x Am, but are even slightly shorter (less elongated) than those of the Pt pt types 2, 5 and 8 of that F_2 (Fig. 6).

In going from pd pd to Pd Pd or from pt pt to Pt Pt the petioles (leafbases) become longer and the wings narrower, as said before. This negative correlation is also found when comparing the factors Pd and Pt. Thus not only the petioles of type 4 from the HG-KE families (Fig. 14) are shorter than those of type 4 from the HG-Am families (Fig. 6), but also the wings are broader. The same can be said when comparing the two types 5, etc.

Regarding number of leaves, KE differs less from HG than Am does (cf. Table 1). As a result the within-type variation in leafbase shape is smaller in the types from HG x KE than in those from HG x Am. In fact the "more elongated" variants of the types 3, 6 and 9 from HG x Am are not found among the corresponding types from HG x KE, which also read pt pt pd pd. However, the profit of a smaller variation in number of leaves does not compensate the disadvantage of the smaller differences in leafbase shape between the main types, i.e. classifying in the HG-KE families is more difficult than in the HG-Am families. Nevertheless leaf number again proved to be very helpful.

Angle of venation must be used with more reserve as a side-criterion than in the HG-Am families as the three classes now overlap considerably. There is no reason to reject the assumption of pleiotropic action of the Pd-pd genes (or units of crossing-over), as in F_3 's from selected plants segregation for angle of venation always coincided with segregation for petiole length (or leafbase elongation) and non-segregation with non-segregation.

The factor-pair Pd-pd affects flower shape, qualitatively in the same way as Pt-pt (Ch 1 § 6e), but quantitatively to a lesser extent. In the segregating generations the KE-type flowers (Pd Pd) have a less slender tube and throat and a less pointed limb contour and calyx lobes than the Am-type flowers (Pt Pt).

The enumeration data concerning the backcrosses are as follows (type Nos. between brackets):

| | | | | | | |
|--------------------------|-------|-------|----|-------|-------|-------|
| (HG x KE) x HG 53-219 | 11(2) | 28(5) | 39 | 17.75 | 17.75 | 35.50 |
| | 18(3) | 14(6) | 32 | 17.75 | 17.75 | 35.50 |
| | 29 | 42 | 71 | 35.50 | 35.50 | 71.00 |

$$X^2 (3 \text{ d.f.}) = 9.282 \quad P = 0.05-0.02$$

| | | | | | | |
|--------------------------|-------|-------|----|----|----|----|
| (HG x KE) x KE 53-220 | 16(4) | 17(7) | 33 | 18 | 18 | 36 |
| | 19(5) | 20(8) | 39 | 18 | 18 | 36 |
| | 35 | 37 | 72 | 36 | 36 | 72 |

$$X^2 (3 \text{ d.f.}) = 0.556 \quad P = 0.95-0.90$$

In supplement to the backcross to HG, which shows a rather high X^2 -value, a family (54-104) was raised that resulted from the cross F_1 (53-213-17) x type 3 from F_2 (53-223-174). A random sample of 74 out of 97 plants were selfed and from the progenies it appeared that 4 plants had been classified erroneously, viz. 2 plants as type 5 instead of type 6 and 2 plants as type 3 instead of type 2. The corrected frequencies are:

| | | | | | | |
|------------------------------|-------|-------|----|-------|-------|-------|
| (HG x KE) x type 3 54-104 | 21(2) | 23(5) | 44 | 24.25 | 24.25 | 48.50 |
| | 30(3) | 23(6) | 53 | 24.25 | 24.25 | 48.50 |
| | 51 | 46 | 97 | 48.50 | 48.50 | 97.00 |

$$X^2 (3 \text{ d.f.}) = 1.928 \quad P = 0.70-0.50$$

In the F_2 , notably the types 7, 8 and 9 were difficult to classify. Up to 10% of the plants may have been classified wrongly. Therefore the three frequencies have been pooled.

| | | | | | | | | |
|------------------------------|-------|-------|------|-----|--------|---------|--------|---------|
| F_2 from HG x KE 54-091 | 14(1) | 38(4) | -(7) | - | 18.625 | 37.250 | - | - |
| | 33(2) | 70(5) | -(8) | - | 37.250 | 74.500 | - | - |
| | 18(3) | 43(6) | -(9) | - | 18.625 | 37.250 | - | - |
| | 65 | 151 | 82 | 298 | 74.500 | 149.000 | 74.500 | 298.000 |

$$X^2 (6 \text{ d.f.}) = 3.584 \quad P = 0.80-0.70$$

From all these data it appears that there is no reason to reject the hypothesis of two independently segregating factor-pairs.

Ch 1 § 8. THE HG-Atr FAMILIES

Not only both parents (Ch 1 § 3) and the F_1 (Ch 1 § 5), but also all backcross and F_2 plants were of type 3 in having a "constricted" leafbase (very short petiole and narrow wings) and an obtuse angle of venation. Like the parents, all plants had flowers with an inflated throat and a pentagonal limb contour. The genetic identity of HG and Atr with respect to these characters also appears when they are crossed to Am (or KE). Therefore we can write pt pt.pd pd br br for Atr too.

The carmine flower colour of Atr (Pk Pk) is completely domi-

nant over the pink colour of HG (pk pk). In the F_2 (53-234) the frequencies 213 carmine and 68 pink were found ($X^2 = 0.096$; $P = 0.80-0.70$). This factor-pair has been mentioned by several authors (Ch 1 § 13).

Ch 1 § 9. THE Atr-Am FAMILIES

Regarding leaf shape, leaf number and flower shape Atr is similar to HG (cf. Table 1). Their genotypic identity (both are pt pt pd pd br br) has been stated in the foregoing paragraph. The F_1 Atr x Am resembles the F_1 HG x Am closely (Ch 1 § 5 and Figs. 5a and 5b).

The phenotypic and genetic properties of the segregating families of the Atr-Am material run completely parallel to those of the HG-Am material (described in Ch 1 § 6). In fact the photograph of the 9 "ideal types" from the cross between HG and Am (Fig. 6) is almost identical to a photograph that might be given for the 9 "ideal types" from the cross between Atr and Am. Regarding plant vigour the Atr-Am families show more variation than the corresponding HG-Am families and the same can be said of the Atr-KE families (Ch 1 § 10) as compared to the HG-KE families (Ch 1 § 7). In all probability this is partly or wholly a reflection of the higher susceptibility of Atr to environmental differences (cf. Ch 1 § 3b).

The frequencies in the backcrosses (4 leaf types in each) and in the F_2 (9 types) are as follows (type Nos. between brackets):

| | | | | | | |
|----------------------------|-------|-------|----|------|------|------|
| (Atr x Am) x Atr 52-155 | 13(2) | 16(5) | 29 | 12.5 | 12.5 | 25.0 |
| | 11(3) | 10(6) | 21 | 12.5 | 12.5 | 25.0 |
| | 24 | 26 | 50 | 25.0 | 25.0 | 50.0 |

$$X^2 (3 \text{ d.f.}) = 1.680 \quad P = 0.70-0.50$$

| | | | | | | |
|---------------------------|-------|-------|----|------|------|------|
| (Atr x Am) x Am 52-156 | 13(4) | 14(7) | 27 | 12.5 | 12.5 | 25.0 |
| | 10(5) | 13(8) | 23 | 12.5 | 12.5 | 25.0 |
| | 23 | 27 | 50 | 25.0 | 25.0 | 50.0 |

$$X^2 (3 \text{ d.f.}) = 0.720 \quad P = 0.90-0.80$$

| | | | | | | | | |
|----------------------------|-------|-------|-------|-----|---------|----------|---------|----------|
| F_2 (Atr x Am) 53-238 | 13(1) | 36(4) | 19(7) | 68 | 18.0625 | 36.1250 | 18.0625 | 72.2500 |
| | 30(2) | 78(5) | 43(8) | 151 | 36.1250 | 72.2500 | 36.1250 | 144.5000 |
| | 15(3) | 36(6) | 19(9) | 70 | 18.0625 | 36.1250 | 18.0625 | 72.2500 |
| | 58 | 150 | 81 | 289 | 72.2500 | 144.5000 | 72.2500 | 289.0000 |

$$X^2 (8 \text{ d.f.}) = 4.841 \quad P = 0.80-0.70$$

| | | | | | | | | |
|----------------------------|-------|-------|-------|-----|------|-------|------|-------|
| F_2 (Atr x Am) 55-035 | 9(1) | 22(4) | 13(7) | 44 | 12.5 | 25.0 | 12.5 | 50.0 |
| | 30(2) | 59(5) | 22(8) | 111 | 25.0 | 50.0 | 25.0 | 100.0 |
| | 8(3) | 29(6) | 8(9) | 45 | 12.5 | 25.0 | 12.5 | 50.0 |
| | 47 | 110 | 43 | 200 | 50.0 | 100.0 | 50.0 | 200.0 |

$$X^2 (8 \text{ d.f.}) = 8.220 \quad P = 0.50-0.30$$

There is no reason to reject the assumption of two independently segregating factor-pairs. It should be noted that on adding the two F_2 's from $Atr \times Am$, one obtains 105 br br and 384 Br . with X^2 (1 d.f.) = 3.245 and $P = 0.10-0.05$. This indicates a shortage of the br br types. A similar shortage was found in the F_2 from $HG \times Am$ (cf. Ch 1 § 6b), the cause of which remained obscure. The disturbance of the Br-br segregation does not prevent us from concluding to two independently segregating factor-pairs.

Regarding flower colour the two F_2 's gave the frequencies 361 carmine and 128 pink (expected 366.75 and 122.25).

The factor-pair for flower colour (Pk-pk) is closely linked with the factor-pair for wing width (Br-br). KELANEY (1925) was the first to describe this linkage.

The parent formulae are $Atr = Pk\ br / Pk\ br$ and
 $Am = pk\ Br / pk\ Br$

The F_2 frequencies are:

| F_2 (53-238) | br br | Br br | Br Br | |
|-----------------|-------|-------|-------|--------------------------|
| carmine (Pk .) | 58 | 136 | 10 | $p = 8.2\%$ s.e. = 1.7% |
| pink (pk pk) | 0 | 14 | 71 | |
| F_2 (55-035) | br br | Br br | Br Br | |
| carmine (Pk .) | 46 | 101 | 10 | $p = 11.3\%$ s.e. = 2.4% |
| pink (pk pk) | 1 | 9 | 33 | |

In order to apply the product-ratio method in the coupling phase the br br and Br br classes were pooled, which led to the frequencies 147, 10, 10 and 33. The recombination fractions (p) and their standard errors (s.e.) were read from the tables constructed by STEVENS (1939). The latter estimate ($p = 11.3\%$) is somewhat high as in several other F_2 and backcross families (not presented here) recombination fractions of 7% to 8% were found.

Ch 1 § 10. THE Atr -KE FAMILIES

An equally rigid parallelism as was found to exist between the Atr - Am families and the HG - Am families, is also found in comparing the Atr -KE families with the HG -KE families. The F_1 $Atr \times KE$ resembles the F_1 $HG \times KE$ closely (cf. Ch 1 § 5 and Figs. 5a and 5b). With respect to all features under discussion the 9 "ideal types" from the cross $Atr \times KE$ can be taken to be very adequately represented by the photograph of the 9 types from the cross $HG \times KE$ (Fig. 14).

The following frequencies were obtained:

| | | | | | | |
|--|-------|-------|----|----|----|----|
| (KE \times Atr) \times Atr 52-147 | 15(2) | 13(5) | 28 | 12 | 12 | 24 |
| | 9(3) | 11(6) | 20 | 12 | 12 | 24 |
| | 24 | 24 | 48 | 24 | 24 | 48 |

$$X^2 (3 \text{ d.f.}) = 1.667 \quad P = 0.70-0.50$$

| | | | | | | |
|---------------------------|-------|-------|----|-------|-------|-------|
| (KE x Atr) x KE 54-093 | 25(4) | 21(7) | 46 | 24.25 | 24.25 | 48.50 |
| | 23(5) | 28(8) | 51 | 24.25 | 24.25 | 48.50 |
| | 48 | 49 | 97 | 48.50 | 48.50 | 97.00 |

$$X^2 (3 \text{ d.f.}) = 1.103 \quad P = 0.80-0.70$$

| | | | | | | | | |
|-------------------------------------|-------|-------|-------|-----|--------|---------|--------|---------|
| F ₂ (KE x Atr) 53-236 | 15(1) | 32(4) | 10(7) | 57 | 17.125 | 34.250 | 17.125 | 68.500 |
| | 37(2) | 67(5) | 42(8) | 146 | 34.250 | 68.500 | 34.250 | 137.000 |
| | 17(3) | 39(6) | 15(9) | 71 | 17.125 | 34.250 | 17.125 | 68.500 |
| | 69 | 138 | 67 | 274 | 68.500 | 137.000 | 68.500 | 274.000 |

$$X^2 (3 \text{ d.f.}) = 6.307 \quad P = 0.70-0.50$$

The backcross to KE (54-093) segregated into 50 carmine and 47 pink. The F₂ (53-236), 243 plants of which were classified for flower colour, gave 190 carmine and 53 pink (expected 182.25 and 60.75).

The recombination fraction was calculated from:

| Backcross (54-093) | | Br br | Br Br | |
|-------------------------|--|-----------------|-------|-------------|
| carmine (Pk .) | | 47 | 3 | p = 4.1% |
| pink (pk pk) | | 1 | 46 | s.e. = 2.1% |
| F ₂ (53-236) | | br br and Br br | Br Br | |
| carmine (Pk .) | | 184 | 6 | p = 3.0% |
| pink (pk pk) | | 2 | 51 | s.e. = 1.0% |

In the F₂ the br br and Br br classes have been pooled to apply the product-ratio method in the coupling phase. STEVENS' tables for the product-ratio (1939) were used to find the recombination fraction (p) and its standard error (s.e.). The p-values are much lower than those found in the Atr-Am families (Ch 1 § 9). As both KE and Am have the same factor Br (Ch 1 § 11) the difference is presumably generated by crossing-over modifiers. It should be noted, however, that the present Atr-KE families all had the KE plasm, and the Atr-Am families the Atr plasm. Therefore the plasm may also be responsible for the difference in crossing-over percentage.

Ch 1 § 11. THE KE-Am FAMILIES

The F₁ (Ch 1 § 5 and Figs. 5a and 5b) is intermediate between its parents in all respects under discussion.

The F₁ KE x Am was selfed to give the F₂ families 52-131, 53-237 and 55-036, each consisting of 300 plants, and backcrossed to KE and Am to give respectively 100 + 75 (52-150 and 53-230) and 100 + 75 (52-152 and 53-232) backcross plants.

All plants of these families were sessile, i.e. had broad wings. In view of the large numbers of plants the absence of pe-

tioled (i. e. narrow-winged) segregants provides convincing evidence that KE and Am differ from HG (or Atr) with respect to the same locus for wing width: KE = Br Br, Am = Br Br, and HG (or Atr) = br br.

With respect to leafbase elongation (etc.) the F₂ shows transgression. On the one hand plants are found with a very short leafbase, very broad wings, broad leaves, an obtuse angle of venation and a pentagonal limb contour, thus in all respects resembling type 9 (see Fig. 6 or Fig. 14). On the other hand plants are found with an extremely long leafbase, relatively narrow wings, conspicuously narrow leaves, a very acute angle of venation and a deeply incised limb contour, thus surpassing Am in all these characters. A number of these extreme individuals were selfed and the progenies did not segregate for the characters mentioned. Now, after 4 generations of selfing, the two new lines (Fig. 15) are about as uniform as KE and Am.

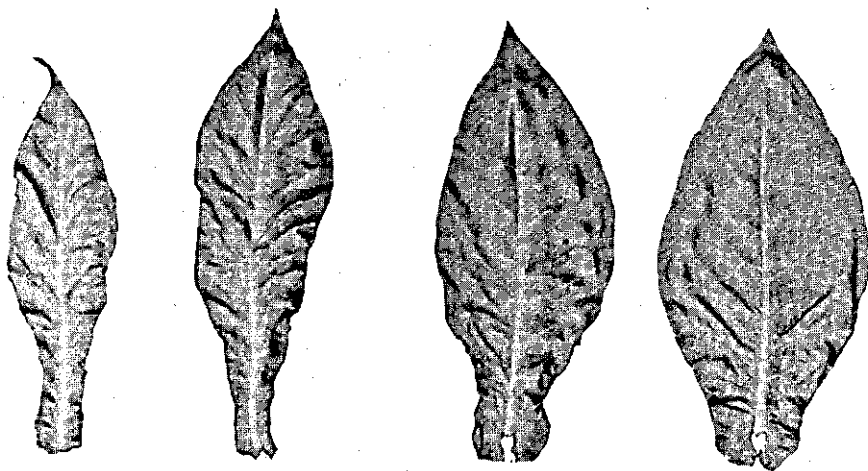


Fig. 15. Transgression homozygotes derived from the F₂ from KE x Am in comparison with Am and KE. From left to right: the types Pt Pt Pd Pd, Pt Pt pd pd (= Am), pt pt Pd Pd (= KE) and pt pt pd pd. All types are Br Br.

The transgression in the F₂ suggests that the factor for leafbase elongation (etc.) of KE (Pd ; Ch 1 §§ 7 and 10) and that of

Am (Pt ; Ch 1 §§ 6 and 9) are on different loci. In F_2 and in the backcrosses (all plants Br Br) no discrete classes could be distinguished and therefore no reliable classification could be made. As said before, the contrasts between the petiole length classes on the Br br level are much sharper than those between the classes for leafbase elongation on the Br Br level. Therefore the F_1 KE x Am was also crossed with the very short-petioled types HG and Atr, both br br. The progeny (all plants Br br) segregated into four distinct classes. Putting KE = pt pt Pd Pd and Am = Pt Pt pd pd, the F_1 writes Pt pt Pd pd and the cross F_1 x HG (or Atr) writes Pt pt Pd pd x pt pt pd pd. The four resulting types and their frequencies are:

| | (KE x Am) x HG 53-229 54-131 | | (KE x Am) x Atr 53-231 54-132 | | Total | Exp. |
|---|---------------------------------|----|----------------------------------|----|-------|--------|
| Pt pt Pd pd | 18 | 18 | 18 | 14 | 68 | 67.75 |
| Pt pt pd pd (cf. type 5 from HG x Am) | 15 | 18 | 21 | 19 | 73 | 67.75 |
| pt pt Pd pd (cf. type 5 from HG x KE) | 18 | 13 | 16 | 15 | 62 | 67.75 |
| pt pt pd pd (cf. type 6 from both crosses) | 20 | 14 | 17 | 17 | 68 | 67.75 |
| Totals | 71 | 63 | 72 | 65 | 271 | 271.00 |

The three latter types in the table are also found in families discussed earlier. The corresponding phenotypes resemble each other. The type Pt pt Pd pd surpasses type 5 from HG x Am regarding petiole length, acuteness of angle of venation, etc. The four types are shown in Fig. 16.

From the column for totals one obtains X^2 (3 d.f.) = 0.897 with $P = 0.90-0.80$. Occasionally, it was difficult to distinguish between the two Pt pt classes and between the two pt pt classes, because the factor-pair Pd-pd has a relatively small effect. However, such cases were rare. It can be concluded that we have no reason to reject the hypothesis of two unlinked factor-pairs (Pt-pt and Pd-pd) for this complex of characters. In view of the amphidiploid origin of *N. tabacum* this situation is not surprising.

It was stated earlier (Ch 1 § 6c) that within-type variation in petiole length is closely connected with variation in number of leaves. The distribution of number of leaves is largely independent of the Pt-pt and Pd-pd classes (cf. Table 7 for Pt-pt). Due to the high leaf number of Am, each of the present types pt pt pd pd (Br br) and pt pt Pd pd (Br br) has on the average a higher leaf number, and consequently longer petioles and narrower wings, than the corresponding type from the HG-KE families. As a consequence, a number of pt pt pd pd plants from (KE x Am) x HG, grown in 1952, were erroneously classified as pt pt Pd pd. This resulted into a misleading high variation in petiole length and, correlated to it, in wing width in the supposed pt pt Pd pd class.

It was even thought that KE and Am had different alleles on the wing width locus. In later years, when with the help of the number of leaves the genotypes could be inferred more correctly from the phenotypes, the assumption was rejected.

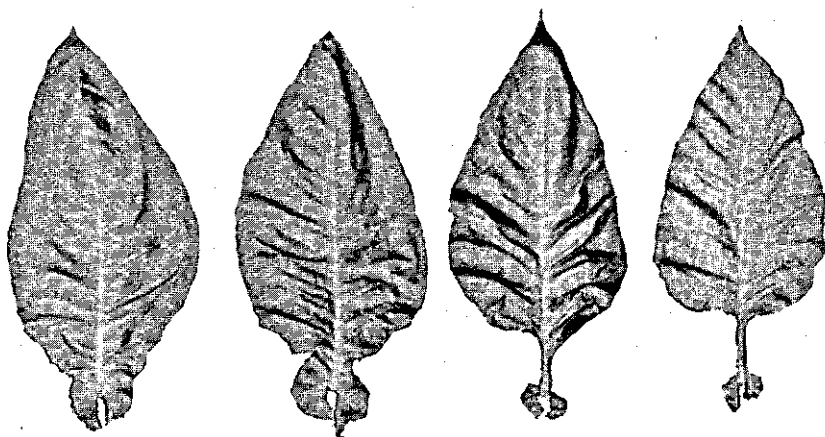


Fig. 16. From left to right: the types *pt pt pd pd*, *pt pt Pd pd*, *Pt pt pd pd* and *Pt pd Pd pd* from the triple cross (KE x Am) x HG = Fam. 53-229. All types are Br br.

Ch 1 § 12. THE IDENTIFICATION OF FACTORS FOR LEAF-BASE ELONGATION (ETC.) IN VARIETIES HAVING SESSILE LEAVES

Most of the tobacco varieties that are of economic interest have sessile leaves. In progenies from crosses like type-9 x KE, KE x Am, etc., a more or less continuous variation with respect to leafbase elongation, wing width, leaf width, angle of venation and flower shape is met with. In our material the frame of genetic variation for this complex of characters was shown to be made up by the major factors *Pt-pt* and *Pd-pd*. These factors easily escape detection, at least in the sessile types (Br Br), as in segregating families their individual expression is obscured by additional variation due to modifying genes, among which the genes for leaf number play an important role. When selecting for leaf shape properties, knowledge of the genotypes in terms of *Pt-pt* and *Pd-pd* can be useful. A device for the identification of these genes has been implicitly suggested in the foregoing paragraph and will now be described in some detail.

Let Y be the sessile (Br Br) variety to be tested. It is crossed first to some - or if necessary to all - of four Br Br varieties with known genotypes Pt Pt Pd Pd, Pt Pt pd pd, pt pt Pd Pd and pt pt pd pd. The F_1 's are then crossed to a very short-petioled (constricted) variety (pt pt pd pd br br) like HG. The resulting plants are all Br br and on this Br br level (petioled types) it will in general be comparatively easy to distinguish between segregation and non-segregation of the factors concerned, especially when we have as a basis for comparison the F_1 from Y x HG. Care must be taken, however, not to confuse segregation for Pd-pd, which factor-pair has a relatively small effect, with variation that depends on variation in leaf number. Let e.g. the variety Y write Pt Pt pd pd. Then the following genotypes are obtained:

(Y x Pt Pt Pd Pd) x HG gives Pt pt Pd pd and Pt pt pd pd

(Y x Pt Pt pd pd) x HG gives Pt pt pd pd

(Y x pt pt Pd Pd) x HG gives Pt pt Pd pd, Pt pt pd pd, pt pt Pd pd and pt pt pd pd

(Y x pt pt pd pd) x HG gives Pt pt pd pd and pt pt pd pd

The F_1 Y x HG is written Pt pt pd pd.

The cross that does not lead to segregation gives direct information about the genotype of Y, which, in terms of Pt-pt and Pd-pd, is then equal to that of the sessile tester involved. Additional confirmation is provided by the fact that the descendants of the cross involved have the same petiole length and angle of venation as the F_1 Y x HG, apart from variation due to modifying genes.

With this technique the varieties Havanna Connecticut (HC) and Havanna 2 C (H2C) have been tested. HC was imported from the U.S.A. and has been a laboratory stock since 1948. H2C is a selection of the Forchheim tobacco institute imported to the Netherlands about 1943. With respect to the characters under discussion both varieties more or less resemble KE. H2C has in the region of maximum leaf length a length/width ratio of about 2.25-2.50 and an angle of venation of about 60° . Its leafbase closely resembles that of KE. HC shows about 2.00 and 65° - 70° respectively. Its leafbase is somewhat narrower than that of KE. Both varieties made the impression of being very uniform.

Neither of the test-cross families (KE x H2C) x HG and (KE x HC) x HG did segregate for leafbase shape and angle of venation. With respect to both characters they homogeneously resembled the F_1 HG x H2C and the F_1 HG x HC respectively. The test-cross families (Am x H2C) x HG and (Am x HC) x HG, however, segregated into four types. Therefore both H2C and HC are identical to KE in having the pt pt Pd Pd genotype. This statement could not be made a priori, i.e. without making the test-crosses, because the two varieties HC and H2C diverge (cf. above), due to differences in genic background.

Ch 1 § 13. DISCUSSION OF LITERATURE

The number of characters known to segregate in a simple Mendelian way is not very large in *Nicotiana tabacum*. The most

intensively studied are flower colour and leafbase shape. For reasons outlined in Ch 1 § 1 ("Introduction") it is interesting to compare the results and conclusions presented by the different authors with those of the present investigation. The following studies will be discussed in chronological order: HOWARD (1913), SETCHELL, GOODSPEED and CLAUSEN (1921, 1922), KELANEY (1925), BOURZEV (1928), RAVE (1935), GENTSCHKEFF (1936), HONING (1923, 1927, 1939), MacRAE (1941), CLAUSEN and CAMERON (1944), KADAM and RADHAKRISHNAMURTY (1954), and KOELLE (1957).

Miss HOWARD (1913) was the first to make a systematic study on the inheritance of leaf shape in *N. tabacum*. She crossed among others the sessile varieties "Type 23" and "Type 38". The wing width of Type 23 was about 5 cm. in 1912 and about 7 cm. in 1913, that of Type 38 2.5-3 cm. in 1912 and about 4 cm. in 1913 (Table XXVIII; l. c.). As appears from Plate V and VI (l. c.) both parents had a well-marked sinus at a moderate distance from the axil, notably Type 38, the outline of which is described as "suddenly contracted". The F_1 had "... a bigger indentation than either parent" (wing width ± 3 cm. in 1913). In the F_2 petioled forms occurred "... in which the amount of the lamina on either side of the petiole is less than 0.3 cm.". There was also some indication of transgression in the other direction. Nine F_2 plants gave rise to F_3 's (p. 98 and Table XXVIII; l. c.). Of these nine, 2 plants (0.2 and 0.3 cm.) bred true, 2 plants (1.2 and 1.5 cm.) gave rise to 1 petiolate : 2 intermediate : 1 sessile, 3 plants (1.3, ? and ? cm.) had progenies showing the F_2 range of variation, and 2 plants (2.6 and 6.6 cm.) did not have petiolate descendants. The author inferred two factor-pairs for "indentation". Petiole length or leafbase elongation were not discussed. Now it is probable that both factor-pairs are comparable in action with our Br-br, 1) because it appears from Plate XXI (l. c.), giving a large number of F_2 types, that there is relatively little variation in leafbase elongation or petiole length, the leafbases corresponding roughly to the pt pt Pd Pd level, and 2) because it appears from Table XXIV (l. c.) that no substantial segregation for angle of venation occurred.

Also MacRAE (1941; see below) found two loci for wing width. It is interesting to note that Miss HOWARD (p. 99; l. c.) had the impression that "... these indentation factors are inherited ... independently of the width of the leaf", as also in our material the factor-pair Br-br influences wing width only, while the width of the leafblade is influenced by Pt-pt and Pd-pd. Moreover it is in accordance with our findings when she states (p. 93; l. c.): "All the evidence accumulated during these investigations tends to show that differences in width can be inherited quite independently of the length". The author did not discuss the influence of leaf number on leafbase length and wing width. The cross Type 23 x Type 38, however, must have led to a considerable genetic variation in leaf number, as Type 23 which has the broadest leafbase had about 20 leaves and Type 38 about 34 leaves (cf. Table X; l. c.).

This undoubtedly rendered the analysis rather difficult and may account for the fact that transgression in the direction of broad bases could not be unambiguously stated.

Miss HOWARD (l.c.) made also an attempt to analyse the inheritance of angle of venation, which was given to the nearest multiple of 5° (Tables XXII to XXIV; l.c.). In the crosses only sessile types were involved. From the cross Type 9 (50°) x Type 51 (80°) the F_1 was intermediate, the F_2 covered the whole range between the parental values, and in later generations (F_3 and F_4) not only the parent types were fixed but also types with 60° and 65° - 70° . Similar results were obtained from the cross Type 16 x Type 35. A two-factor segregation was suggested. These factors are comparable in action to our Pt-pt and Pd-pd for, as appears from the Plates I to IV (l.c.), the Types 9 and 16 had, in addition to an acute angle, very narrow leaves and the Types 51 and 35, in addition to obtuse angles, rather broad leaves. Moreover it appears from Table XXI (l.c.) that in the F_2 from Type 16 x Type 35 the angle of venation and the ratio length/width were negatively correlated, which however was not appreciated as such, although it was stated (p. 96; l.c.) that "The data of the F_3 generation clearly point to the action of several factors in producing the difference between the widths of the two leaves". The author did not discuss leafbase elongation of the four (sessile) types in connection with angle of venation. Nevertheless it seems justified to compare her Type 9 and Type 16 with our type Pt Pt Pd Pd Br Br, and her Type 51 and Type 35 with our type pt pt pd pd Br Br (cf. Fig. 15).

SETCHELL, GOODSPEED and CLAUSEN (1921, 1922) crossed *N. tabacum* var. *angustifolia*, which has a long, usually wingless petiole and an acute angle of the lateral veins, with *N. tabacum* var. *macrophylla*, which is broadly sessile and has an obtuse angle of venation (Plates 56 to 58; 1922). The F_1 had shorter petioles than "angustifolia", the petioles were definitely winged and the angle of venation was about intermediate. In the segregating generations attention was primarily focussed on the leafbase, although it appears that the total leaf shape was also taken into account in distinguishing between the "centres of variation". This was done without sufficient analysis of the pleiotropic action of the factors concerned. The inheritance of angle of venation was not discussed. Commenting on the F_2 the authors state (p. 471; 1922): "As respects leafbase characters, the segregation is so complex that no reasonable genetic analysis is possible". Therefore the F_3 and subsequent generations were studied and meanwhile a number of constant leafbase types were selected (p. 474; 1922):

1. Stenophylla derivatives. These resemble the "angustifolia" parent.
2. Latifolia derivatives. The petiole is shorter than in "angustifolia" and more or less winged.
3. Auriculata derivatives. From the description and illustration

it appears that this type is constricted (= very short petioled) like our HG and Atr.

4. Sessilifolia derivatives. These resemble the "macrophylla" parent.
5. Lanceolata derivatives. These are sessile, too, but the leaf is much elongated.
6. Lorifolia derivatives. They resemble the lanceolata type, but the leaf is still narrower ("Cow's tongue" or "Lengue de vaca").

From the segregations in F₃ and subsequent generations and in the F₂ from crosses of the derivatives to the original parents (unfortunately only the most resembling parent) the following three factor-pairs were inferred (p. 492; 1922):

1. S . versus s s. Petioled (stenophylla and latifolia derivatives and the "angustifolia" parent) versus sessile (sessilifolia derivatives and the "macrophylla" parent). This factor-pair is comparable in action to our br-Br. The authors seem to explain the shorter petiole of the F₁ (as compared to the "angustifolia" parent) in terms of the Ss condition, which is inconsistent with our findings.
2. L . versus l l. The long petiole of the stenophylla derivatives versus the shorter petiole of the latifolia derivatives. In the presence of ss (sessile) the action of this factor-pair was not distinguished.
3. A . versus a a. The broad leafbase of the "macrophylla" parent versus the constricted leafbase of the auriculata derivatives. This contrast was said to be only present with ss (sessile) and "... evidently latent when in combination with SS or Ss".

From the foregoing it is clear that the material shows much analogy to ours, but that the genetic interpretation is different. Therefore a reinterpretation will be given in terms of factor-pairs operating like Pt-pt, Pd-pd, and Br-br. In doing so two arguments are vital.

1. Two almost wingless petiolate derivatives could be fixed: stenophylla (long petioles) and latifolia (shorter petioles). The F₂ from the cross stenophylla x "angustifolia" parent did not segregate for petiole length (p. 490; 1922), while in the F₂ from latifolia x "angustifolia" parent "... the segregation was sharply into two classes: the stenophylla type of leafbase (long, naked petiole) and the latifolia type (shorter, winged petiole)", the ratio corresponding to 3 : 1 (p. 488; 1922). Among the progeny of one of the latifolia plants from the original F₂ some auriculata plants were found (p. 477; 1922), which were considered as non-petiolate. As stated above, auriculata closely resembles our HG-type (very short petioled). These points indicate that two factor-pairs for petiole length like our Pt-pt and Pd-pd are present, and that it seems not necessary to introduce the factor-pair A-a along with S-s.
2. The authors made an attempt to classify the F₂ into the classes (Table I; 1922) petiolate and non-petiolate. Comparing the frequencies 339 and 152 with the 3 S : 1 ss ratio one ob-

tains X^2 (1 d.f.) = 9.287 and $P = 0.01-0.001$. However, "... the plants were thrown into the petiole class if they were distinctly narrowed at the base, and whether naked or winged" (p. 470/471; 1922). When this means that the auriculates were included, the theoretical ratio in terms of S-s and A-a would be 13 : 3, which leads to an even higher X^2 -value. When, however, we consider a triheterozygous F_1 like Pt pt Pd pd Br br and gather all br br and Br br plants with the exception of the pt pt pd pd Br br (type 6) plants, we expect $(3/4 - 2/64) \times 491 = 353$ in a F_2 family of 491 plants. This leads to X^2 (1 d.f.) = 1.948 and $P = 0.20-0.10$.

On the basis of the foregoing considerations we suggest for the "angustifolia" parent Pt Pt Pd Pd br br instead of L L a a S S, for the "macrophylla" parent pt pt pd pd Br Br instead of l l A A s s, and for auriculata pt pt pd pd br br instead of l l a a s s. This means that A-a has been cancelled and a second factor-pair for petiole length has been introduced.

Regarding the lorifolia and extreme lanceolata type additional factors are by no means excluded. The authors say: "Their genetic relation to the other forms is, however, not well established ..." (p. 491; 1922). No ratios of occurrence in F_2 have been given, so maybe the types were exceptions.

KELANEY (1925) listed a number of varieties (furnished by CLAUSEN) in terms of leafbase shape (p. 46; l.c.) without mentioning the differences in angle of venation, which are very apparent from his photographs. As far as can be concluded from the photographs (Figs. 1 to 6; l.c.) the different types closely resemble certain types of our material and the angle of venation goes with leafbase shape in exactly the same way.

1. "Constricted". The leaves are "... sharply constricted at the base, nearly if not quite to the midrib, ...". The angle of venation is obtuse. The constricted parent *N. tabacum* var. *purpurea* probably is identical to Atr (cf. Ch 1 § 3a).
2. "Petioled". "Petioled leaves possess a distinct petiole, which is variable in length and in wing development". They even may be wingless. The angle of venation is acute.
3. "Lanceolate". These leaves are "... sessile and taper gradually towards both base and apex, ...". The angle of venation is acute. The type may be compared with Am and KE.
4. "Broad". The leaves are also sessile and have "... broad, partially-clasping basal lobes, ...". "They do not ordinarily differ sufficiently from lanceolate to permit accurate classification in mixed progenies". The angle of venation is more or less obtuse (cf. our type 9).

The author states (p. 49; l.c.): "However, though in some cases F_2 was extremely complex, it was possible to distinguish certain forms as centres of distribution and by studying F_3 progenies to arrive at a satisfactory idea of the significance of particular F_2 types".

As to the cross constricted x lanceolate, the F_1 had petioles of

moderate length with slight wing development and the F₂ was classified into 9 petioled : 6 sessile : 1 constricted. From this and other crosses KELANEY postulated aa versus AA for petioled and constricted versus lanceolate and broad; SS versus ss for petioled and lanceolate versus constricted and broad. This compares with our nine types (between brackets), arranged as in Fig. 6, as follows:

| | |
|----------------------------------|------------------------------|
| S . . a petioled (1, 2, 4, 5) | S . A A lanceolate (7, 8) |
| s s a a constricted (3) | s s A . broad (6, 9) |

It should be noted that KELANEY uses S-s and A-a in a different way from SETCHELL et al. (1922): S-s now stands for petiole length and is comparable in action to our Pt-pt (or Pd-pd), while A-a now stands for sessile versus petioled and compares with our Br-br. The interpretation given by KELANEY agrees much more with ours than that of SETCHELL et al. (l. c.), for no separate factor-pair was introduced to explain the constricted condition and it was realised that the factor-pair which generates the petioled-constricted difference also generates the lanceolate-broad contrast. In the material of KELANEY, only one factor-pair for petiole length (cf. Pt-pt) was involved, in that of SETCHELL et al. (1922) probably two (cf. Pt-pt and Pd-pd), as was argued in discussing that investigation.

Considerable refinement could have been added to the analysis, as KELANEY is aware himself:

1. "In the present investigation considerable differences in petiole length were observed, but no attempt was made to analyze them" (p. 53; l. c.). A similar remark might have been made with respect to the leafbase elongation of the lanceolates (S . A A).
2. "Wing development of the petiole and leaf shapes seemed to be independent of leaf-base types, but the point was not certainly established" (p. 53; l. c.). This preliminary impression is not consistent with our findings.
3. No use was made of the opportunity to study the inheritance of angle of venation.

The F₁ "purpurea" (constricted; carmine flowers) x "apetala" (lanceolate; pink flowers) was petioled with carmine flowers (A a P p). From the backcross data the percentage of crossing-over was calculated as 7.5%, which compares well with our findings (Ch 1 § 9). Therefore it is the more probable that A-a is identical with our Br-br and P-p with our Pk-pk.

BOURZEV (1928) studied a plant found in the progeny of certain crosses made by EGHIS. It was described as having "subpetiolate leaves" and was compared with the "auriculata" type of the American investigators. In fact its leafbase resembles that of our HG

(constricted = very short petioled) as can be seen from Plate 1 - Fig. 3 (l. c.). The plant was selfed giving Fam. No. 283, which showed a large diversity of leafbase phenotypes, "... ranging from an almost glabrous petiole to a typical broad sessile base..." (p. 89; l. c.). The plant was also crossed to a "constant subpetiole type" (Nic. Tab. No. 343), giving the Fam. Nos. 593 and 594, which also exhibited a large variation in leafbase shape.

BOURZEV seems to have focussed attention primarily on wing width and says that "... it did not appear possible to establish definite limits between the different types of leaf base on account of all the intermediate forms having been displayed..." (p. 88; l. c.). He distinguishes 5 main forms (A-E) and infers from a large number of progenies four cumulative polymeric factor-pairs for wing width.

A close examination of the many tables and drawings convinced me that the data presented can be adequately interpreted on the basis of a bifactorial segregation as found in the HG-KE families (cf. Fig. 14). The original heterozygous "subpetiole" type then is $pt\ pt\ Pd\ pd\ Br\ br$ (type 5 in Fig. 14) and the "constant subpetiole" type $pt\ pt\ pd\ pd\ br\ br$ (type 3 in Fig. 14). For this the following arguments can be given:

1. Although BOURZEV made no statements about the angle of venation, it appears from a series of 28 drawings (Plates VI to XIII; l. c.) representing the whole range of leafbase types, that the types grouped as "petioled with narrow border" all have relatively acute angles of venation, while among the broad based types both acute and obtuse angles are found except for the very broad based ones which all show an obtuse angle.
2. The leafbases of the original plant (Plate I - Fig. 3; l. c.) and the "constant subpetiole" type (Plate II - Fig. 1; l. c.) resemble each other. However, the angle of venation of the latter is obtuse, that of the former relatively acute.
3. In the progeny from the cross between the original plant and the "constant subpetiole" type, the types with very narrow and those with very broad wings are very scarce (Table 2; l. c.) as compared with the progeny obtained from selfing the original plant (Table 1; l. c.). This is not surprising when we recall that in our backcross $(HG \times KE) \times HG$ only the types 2, 3, 5 and 6 occur and not the types 1 and 9 (cf. Fig. 14).
4. Also a very broad based variety with an obtuse angle of venation (Plate I - Fig. 1) was crossed with the "constant subpetiole" variety. The leafbases of the progeny were described as "greatly shortened" and BOURZEV speaks of "... interior factors ... which apparently effect a general shortening of the leafbase" (p. 89; l. c.). This is not surprising either when we recall that in the progeny of type 3 \times type 9 the average leafbase is shorter than in that of type 3 \times type 5, the difference being generated by $Pd-pd$.

At Forchheim (Germany), RAVE (1935) studied varietal crosses and was particularly interested in the genetic basis of the petioled

condition. The varieties of main interest resembled strikingly those used in the present investigation.

1. Amersfoorter. As appears from the photograph (p. 6; l. c.) and the description (p. 3; l. c.) its type of leaf is identical to that of our laboratory stock of that name (Am).
2. Farmos. Like HG it is a Hungarian variety. The two compare well regarding the broad leafblade, the obtuse angle of venation and the constricted (= very short petioled) leafbase.
3. Alban. This Italian variety has the same type of leaf as Farmos.
4. U-stamm. A selection which resembles our KE to some extent.

The F_1 's Amersfoorter x Farmos and Amersfoorter x Alban have distinct petioles and an intermediate angle of venation (Figs. 8 and 9; l. c.). The array of phenotypes in the F_2 and the backcrosses (Figs. 10 to 21; l. c.) is similar to that found in our HG-Am families. RAVE's classification into 12 types is based on 1) presence or absence of a petiole and 2) degree of resemblance of the general leaf habitus (colour, undulation, angle of venation, elongation, etc.) to that of the parent types. His conclusion reads (p. 18; l. c.): "Für die Sorte Amersfoorter ist auf Grund von Kreuzungsanalysen der Nachweis erbracht, dass bei ihr ein oder mehrere latente Erbanlagen für Bestielung vorhanden sind. Doch tritt solches nur bei Kombination mit bestimmten Typen in Erscheinung". Thus RAVE could not arrive at a simple factorial explanation. However, in many respects the analogy between his and our material is so striking that it seems justified to infer an identical genetic situation. In the case of the long petioled plant from F_1 x Amersfoorter which on selfing gave rise to a progeny consisting of non-petioled types only (type II/c; p. 10; l. c.) some mistake seems to be involved.

There are also good reasons to infer an analogy between RAVE's Farmos (or Alban) x U-stamm and our HG (or Atr) x KE progenies.

GENTSCHKEFF (1936) used for his crosses two varieties with wingless petioles and three "sessile" (constricted included) varieties with different leafbase width. In all three crosses studied one parent was petioled, the other sessile. The F_1 's were petioled with wing width proportional to that of the sessile parent involved (Fig. 5; l. c.). From F_2 and F_3 analyses, during which attention was focussed on wing width, a polymeric factor explanation was suggested:

| Variety | Wing width in cm. (p. 197; l. c.) | Number of factor-pairs for broad base |
|--------------|--------------------------------------|--|
| Barbura | 5.20 | 3 |
| Alba | 3.32 | 2 |
| Macrophylla | 1.70 | 1 |
| Sanguinea | wingless | 0 |
| Angustifolia | wingless | 0 |

The angle of venation and the length of the petioles (or in the case of sessile types the level of the sinus) were not taken into consideration. However, the photographs and drawings give clear illustrations of these characters. The genetic relationships between the types probably not depend on factors for wing width only as will be shown by the following reinterpretation.

1. Macrophylla x Angustifolia (Fig. 4 and Tables V and VI; 1. c.). These varieties are probably related to those of the same name used by SETCHELL et al. (1921, 1922). However, GENTSCHEFF's macrophylla had a constricted instead of a broad leafbase (Fig. 5; 1. c.). In the F_2 and the F_3 , constricted (obtuse angle), narrow-winged petiolate, and long-petioled (acute angle) types were encountered. The first and third types bred true. This compares well with the monofactorial segregation for petiole length (Pt-pt) obtained by crossing type 3 and type 1 from the HG-Am families (cf. Fig. 6).
2. Angustifolia x Alba (Fig. 3 and Tables III and IV; 1. c.). The F_2 and F_3 plants ranged from wingless petioled to sessile with a rather broad base. As the length of the petioles in the petioled and the amount of leafbase elongation in the sessile types were of comparable magnitudes, and moreover all angles of venation were more or less acute, the cross can be compared with type 1 x type 7 from the HG-Am families. However, not one but two factor-pairs for wing width seem to segregate, because in the F_3 a petioled type with a fair amount of wings was fixed.
3. Sanguinea x Barbura (Fig. 2 and Tables I and II; 1. c.). The parents resemble the types 1 and 9 from the HG-Am families (Fig. 6). A number of F_2 types resembling the parents bred true, as also did some plants that were more or less constricted with an obtuse angle of venation. It cannot be decided with certainty whether in this cross a two-factor explanation is sufficient or one more factor-pair for wing width is involved as the author suggests.

HONING (1923, 1927, 1939) studied a mutant found in the variety Deli. Deli has sessile leaves with a marked sinus and a relatively narrow base (Plate I - Fig. 1; 1923). The mutant ("deformis-heterozygote") had long naked petioles. The homozygous "deformis" plants had extremely long naked petioles and much reduced leafblades, the highest leaves being even "... blosz Mittel-rippen ohne Spreite" (p. 459; 1923). The normal versus deformis factor-pair was first (1927) called N-n, but later (1939) Def-def. Hatano (Fig. 1; 1939) is a narrow-winged petioled variety differing from the sessile Deli in a factor for wing width. The F_1 (Fig. 3; 1927) had a slightly shorter petiole and somewhat broader wings than Hatano. The cross can be compared with the cross type 1 x type 7 from the HG-KE families (Fig. 14), i.e. the factor-pair for petioled versus sessile was probably studied in the presence of Pd Pd. It was first called F-f (1927). Later, when discussing comparable crosses, viz. Vorstenlanden (sessile) x Pajakombo (petiolate, narrow winged) and Vorstenlanden x Hatano, HONING called it S-s (1939).

Regarding the combined segregation (p. 6-9; 1927), the diheterozygote (Pd Pd) Def def S s had long wingless petioles like the "deformis-heterozygote". On selfing it, about 25% of the plants were "deformis" (def def) and about 50% had long wingless petioles (Def def). In the remaining group (Def Def) the hypostatic factor-pair S-s gave the segregation 1 petioled with narrow wings (S S) : 2 petioled with broader wings (S s) : 1 sessile (s s).

The factor-pair S-s is similar in action to our br-Br. Def-def influences petiole length, angle of venation, leaf width and wing width and therefore can be compared with pt-Pt, though the action of def is much more extreme than that of Pt. Its influence on wing width is very pronounced, for Def def Pd Pd s s has a long naked petiole, while Pt Pt Pd Pd Br Br (cf. our Fig. 15) is definitely sessile. Nevertheless it seems reasonable to consider S-s as a factor-pair for wing width and Def-def as a factor-pair for leafbase length, angle of venation, etc.

Of the many crosses analysed by HONING (1939) one is of particular interest, viz. *Atropurpurea* x *Vorstenlanden* 120. His *Atropurpurea* is a direct ancestor of Atr used in the present experiment. The F_1 was described as "constricted". The cross is comparable to our Atr x KE = (pt pt) pd pd br br x (pt pt) Pd Pd Br Br (cf. Fig. 14). In the F_2 452 "winged" and 217 sessile were found. HONING remarks (p. 135; 1939): "Now it is possible that in this cross some F_2 plants have been counted as sessile, which from the point of view of their genotype should be considered as winged, ...". Therefore it is quite possible that the majority of the plants resembling our type 6 was counted as sessile. This sufficiently accounts for the discrepancy from the 3 (. br) : 1(Br Br) ratio.

MacRAE (1941) studied derivatives of a synthetic trigenomatic *Nicotiana* (*N. triplex*) obtained by CLAUSEN. It originated from an unreduced gamete of the hybrid *N. tabacum* ($2n = 48$) x *N. tomentosiformis* ($2n = 24$) fertilized by pollen of *N. sylvestris* ($2n = 24$). In meiosis it exhibited 24 bivalents which is not surprising as, according to GOODSPEED (1934), there is thorough justification to assume that some progenitor of *N. tomentosa* or *N. tomentosiformis* along with some progenitor of modern *N. sylvestris* entered into the production through amphidiploidy of the modern species called *N. tabacum*. *N. triplex* was crossed with a number of *N. tabacum* monosomics. After that, a number of uniform "triplex selections" were obtained by continued selfing. These differed in leafbase shape and, as I infer from the Plates I to V (l. c.), also in angle of venation. The main forms were:

1. Constricted. Very short narrow-winged petioles and an obtuse angle of venation, like HG and Atr (pt pt pd pd br br).
2. Broad. Sessile with a broad base. The leaf presented in Plate III - Fig. 1 (l. c.) resembles our type 9 (pt pt pd pd Br Br), while those presented in Plate II (l. c.) have a somewhat less broad base and a slightly less obtuse angle of venation.
3. Petiolate. Two different types are shown in Plate IV (l. c.),

viz. 1) a type with a long naked petiole and an acute angle, like our type 1 in Fig. 6 (Pt Pt pd pd br br in the F₂ from HG x Am), and 2) a type with a naked petiole of moderate length and a less acute angle of venation, like our type 1 in Fig. 14 (pt pt Pd Pd br br in the F₂ from HG x KE).

4. Semibroad. A rather short petiole with moderately broad wings. The angle of venation is very obtuse.
5. Long-winged-petiolate. A rather long petiole with distinct wings and a rather acute angle of venation. This leaf type was selected in view of its resemblance to *N. tomentosiformis* (p. 18; l. c.).

The latter two selections are unlike any of the homozygous types mentioned in the present publication. The five triplex selections were not intercrossed but crossed to two existing *N. tabacum* varieties:

1. Constricted. This is the Californian variety "purpurea" (U. C. B. G. No. 06-25) which probably is the same as our Atr (cf. Ch 1 § 3a). It was also used as the tabacum parent of the triplex selections.
2. Lanceolate. The sessile leaves are narrower and have a longer, narrower base than "Broad". The angle of venation is less obtuse. Regarding these characters the type (Plate I - Fig. 2; l. c.) resembles KE (pt pt Pd Pd Br Br) much more than it does Am (Pt Pt pd pd Br Br). Therefore and for reasons to be mentioned below, the type is probably Pd Pd and not Pt Pt as MacRAE writes.

MacRAE discusses four factor-pairs for leafbase shape, viz. Pt-pt or better Pd-pd (see below), Br-br, Wp-wp and Pw-pw. The symbols Pt-pt, Pd-pd, and Br-br were listed a few years later by CLAUSEN and CAMERON (1944) and therefore have been adopted for my own material (cf. Ch 1 § 6a).

Though the material of MacRAE shows much analogy to that of the present author and though his genetic interpretation is in several respects in accordance with that advanced in the present study, it is worthwhile to discuss the findings of MacRAE in some length because 1) the phenotypic expression of certain genotypes is somewhat different from that in the present material, 2) the classification in the segregating generations was often inefficient and not in accordance with the genetic interpretation, and 3) genes were introduced that do not segregate in the present material, viz. Wp-wp and Pw-pw.

The factor-pairs Pd-pd and Br-br will be discussed first. From his analysis MacRAE inferred the following genotypes:

Petiolate = Pt Pt br br Lanceolate = Pt Pt Br Br

Constricted = pt pt br br Broad = pt pt Br Br

In general this is in agreement with the findings of KELANEY (1925) and with the results of the present investigation. However, in the case of MacRAE's material it is more appropriate to use Pd-pd instead of Pt-pt. The reasons for this are:

1. As stated above his lanceolate parent resembles KE (pt pt Pd Pd Br Br) much more than it does Am (Pt Pt pd pd Br Br).

2. In the HG-Am families the types 5 (Pt pt pd pd Br br) and 4 (Pt Pt pd pd Br br) are clearly petioled with narrow wings (cf. Fig. 6). In MacRAE's material the situation seems to be different. Referring to the cross constricted x lanceolate he states (p. 29; l. c.): "The heterozygous forms were not strictly petiolate. Instead they approximated the lanceolate type". The F_1 petiolate x lanceolate was not called petiolate either (p. 31; l. c.): "... lanceolate in nature characterised by a fairly broad wing definitely rugulose, ...". Now, in the F_2 from HG x KE the diheterozygote type 5 (pt pt Pd pd Br br; Fig. 14) has rather pronounced wings and in the case of a low number of leaves may even resemble type 6. Therefore it is likely that MacRAE's lanceolate parent is pt pt Pd Pd Br Br. In the following, MacRAE's Pt-pt will be replaced by Pd-pd, also in the quotations.

Though MacRAE is aware that his Pd . Br br types are less broadly winged than his Pd . Br Br types, he could not assign them to distinct phenotypic classes and decided to classify all these types as lanceolate, using the term petiolate only for leaves with almost or wholly wingless petioles. It is probable that his Br br types had somewhat broader wings than the corresponding types in the present material, as a result of a different genic background. This can also be said about other cases reported in literature: BRIEGER and FORSTER (1943), crossing a petiolate line with six sessile lines, found that petiolate was "recessive" in four of the crosses and "dominant" in the remaining two; KADAM and RADHAKRISHNAMURTY (1954) crossed a sessile type with three petiolate types and considered all three F_1 's as sessile (p. 65; l. c.).

In MacRAE's F_2 from petiolate x lanceolate (p. 31; l. c.) only petiolate and lanceolate types are distinguished and no broad and constricted ones. Therefore it is probable that also the genotype of the petiolate parent used is pt pt Pd Pd.

The F_2 from constricted x lanceolate (p. 29; l. c.) was classified into 67 lanceolate, 17 petiolate, 7 broad and 6 constricted. This means that the author did not distinguish the Pd Pd types from the Pd pd types. On the basis of 9 (Pd . Br .) : 3 (Pd . br br) : 3 (pd pd Br .) : 1 (pd pd br br) the expected frequencies are 55, 18, 18 and 6, which leads to X^2 (3 d.f.) = 9.13 and $P = 0.05-0.02$. It is possible that MacRAE classified some of the pd pd Br br plants (cf. our type 6 in Fig. 14) as lanceolate.

Turning now to the factor-pairs Wp-wp and Pw-pw, not involved in our material, Wp-wp will be discussed first.

The F_1 of the cross semibroad (pd pd br br Wp Wp) x constricted (pd pd br br wp wp) approximated semibroad and in the F_2 the ratio 3 semibroad (Wp .) : 1 constricted (wp wp) was found. Though Wp is not completely dominant the homozygous and heterozygous semibroads could not be assigned to distinct classes. Wp-wp is distinct from Br-br as it segregates independently of pk-Pk for pink versus carmine (Table 10; l. c.), while Br-br is linked to it (Table 11; l. c.). Like Br, Wp is a factor for wing width, only its effect is much smaller.

Two semibroad selections were crossed with the lanceolate parent (Pd Pd Br Br wp wp). The F₂ was classified as follows:

| | Number of loci with "dominant" allele | Exp. ratio | Observed | | Total |
|--|--|---------------|------------|------------|-------|
| | | | a. (p. 22) | b. (p. 25) | |
| Lanceolate (broad, normal and narrow) | 3 or 2 | 54 | 39 | 96 | 135 |
| Petiolate | 1 (Pd) | 3 | 7 | 9 | 16 |
| Broad | 1 (Br) | 3 | 6 | 6 | 12 |
| Semibroad | 1 (Wp) | 3 | 10 | 28 | 38 |
| Constricted | 0 | 1 | 3 | 5 | 8 |
| Totals | | 64 | 65 | 144 | 209 |

The 135 lanceolates and the 74 non-lanceolates of the combined families give, on the basis of the 54 : 10 ratio, X^2 (1 d.f.) = 61.99, which is extremely high. In my opinion, the main cause for the shortage in the lanceolate class and the excess in the semibroad class is that the supposed lanceolates Pd pd Br br wp wp (cf. type 5 in Fig. 14) and Pd pd Br br Wp wp (at least some of these) were classified as semibroad. Further it is likely that the supposed lanceolates pd pd Br Br Wp . were classified as broad, in view of both Br and Wp making the leafbase broader.

The long-winged-petiolate selection was designated as pd pd br br Pw Pw. In the F₂ from long-winged-petiolate x constricted the ratio 3 long-winged-petiolate : 1 constricted was inferred (p. 27 and p. 28; l.c.). MacRAE does not wholly reject that Pw may be identical to Wp (p. 27; l.c.) or that Pw may be a third allele of Wp-wp (p. 33; l.c.). It is the present authors opinion that the difference between constricted and long-winged-petiolate may not be monofactorial (pw pw versus Pw Pw), but bifactorial (pd pd wp wp versus Pd Pd Wp Wp). It then, of course, remains unexplained why in the F₂ from long-winged-petiolate x constricted no petiolate (Pd Pd wp wp) and semibroad (pd pd Wp Wp) types were mentioned. Nevertheless, it seems not improbable that the bifactorial interpretation is correct. The reasons for this are as follows:

1. Considering, like MacRAE does, the difference between constricted and long-winged-petiolate as monofactorial (pd pd br br pw pw versus pd pd br br Pw Pw), one sees from Plate V - Fig. 1 (l.c.) that Pw makes the petiole long, the leaf narrow and the angle of venation acute like Pd does, but that in contrast to Pd it makes the wings broader. When, however, in the new hypothesis the difference between semibroad and long-winged-petiolate is considered as monofactorial (pd pd br br Wp Wp versus Pd Pd br br Wp Wp), one sees that also upon the wings Pd acts exactly as expected on the basis of the present findings: it changes the moderately broad wings of semibroad (pd pd Wp Wp) into the narrower wings of long-winged-petiolate (Pd Pd Wp Wp).

2. Regarding the F_2 from semibroad x lanceolate MacRAE himself suggests (p. 22; l. c.): "Certain lanceolate forms in this assemblage were of a long winged petiolate type and these may have been of Wp br Pd constitution".
3. Regarding the F_2 from long-winged-petiolate x lanceolate, the classes and their frequencies can be explained as well or better on the basis of the new genetic interpretation of long-winged-petiolate. See hereafter.

MacRAE's F_2 data for long-winged-petiolate x lanceolate are:

| | Number of loci with "dominant" allele | Exp. ratio | Observed | | Total |
|-------------------|---------------------------------------|------------|------------|------------|-------|
| | | | a. (p. 27) | b. (p. 29) | |
| Lanceolate | 3 or 2 | 54 | 53 | 60 | 113 |
| Petiolate | 1 (Pd) | 3 | 5 | 7 | 12 |
| Broad | 1 (Br) | 3 | 5 | 4 | 9 |
| L. -w. -petiolate | 1 (Pw) | 3 | 18 | 47 | 65 |
| Constricted | 0 | 1 | 2 | 1 | 3 |
| Totals | | 64 | 83 | 119 | 202 |

The 113 lanceolates and the 89 non-lanceolates of the combined families give on the basis of the 54 : 10 ratio, X^2 (1 d.f.) = 124.35, which is extraordinarily high. When according to the reinterpretation of the long-winged-petiolate genotype, one writes for the cross under discussion Pd Pd br br Wp Wp x Pd Pd Br Br wp wp (instead of pd pd br br Pw Pw x Pd Pd Br Br pw pw), the 12(+3?) petiolates can be regarded as Pd Pd br br wp wp and the 65 long-winged-petiolates can be taken to represent the genotypes Pd Pd br br Wp . and Pd Pd Br br wp wp. As among the 113 lanceolates 66 were carmine (Tables 12 and 14; l. c.) and pk-Pk (for pink - carmine) is closely linked to Br-br, the large majority of the Pd Pd Br br Wp . plants must have been classified as lanceolate by MacRAE, in addition to the Pd Pd Br Br . wp plants. The 9 broads can be accounted for by assigning to them the Pd Pd Br Br Wp Wp genotype. On the basis of this reinterpretation the observed frequencies 12(+3?) petiolate, 65 long-winged-petiolate, and 122 lanceolate + broad must be compared with the ratio 1 : 5 : 10 giving the expected frequencies 12.625, 63.125 and 126.250. The agreement is very good.

CLAUSEN and CAMERON (1944) studied a complete set of 24 *N. tabacum* monosomics. These had been brought into a common genic background by using as the recurrent parent the standard variety "Purpurea" (U.C.B.G. 06-25), which according to HONING (1939; p. 111) probably is the same as *N. tabacum* atropurpurea (Atr) grown at Wageningen. Subgenomic classification of the monosomics was performed on the basis of chromosome association in 35-chromosome *sylvestris-tabacum* hybrids. They were designated A to L when the missing chromosome appeared to belong

to the *tomentosa* subgenome (12 II + 11 I in the hybrid) and M to Z (X and Y omitted) when the *sylvestris* subgenome was involved (11 II + 13 I in the hybrid).

The authors listed a number of Mendelian factors, the action of which was studied against the "Purpurea" background. The location of the factors in the chromosomes was determined by crossing to the set of monosomics.

The following four monofactorial comparisons with the standard type "Purpurea" are of special interest to us (quotations from p. 458-459; l. c.).

1. "Br-broad: broad leafbase as contrasted with the sharply constricted leafbase of the standard type. The heterozygote is intermediate, but it is most convenient to include homozygous broad and heterozygous broad in one class". Earlier (CLAUSEN; 1932 a) this factor was called Cn. It is located on chromosome P. When haplo-P contains the recessive allele br a broad leafbase is shown. Thus loss of one br-allele as well as replacement of br by Br changes constricted into "broad".
2. "pk-pink: light pink flower color as contrasted with carmine, to which it is almost completely recessive". Pk-pk is also on chromosome P. The recombination value of this and the former locus was estimated as 4.8%. Earlier CLAUSEN (1932 b) found 6% and KELANEY (1925) 7.5%. In the Atr-Am material (Ch 1 § 9) we found 8.2% and 11.3%, in the Atr-KE material (Ch 1 § 10) 4.1% and 3.0%.
3. "Pt-petioled: this factor produces a complex of differences from normal, of which the narrow, petioled leaf type is most conspicuous. Associated differences include narrow, long-pointed calyx lobes, long-pointed corolla lobes, long, narrow, pointed capsules, ... The heterozygote is somewhat intermediate, but difficult to separate accurately from the homozygote". Its location was not determined.
4. "Pd-petioloid: a petioled type with narrow, acutely pointed leaves and other associated features. Resembles petioled (q. v.) but is not so extreme either as to length of petiole or narrowing of the leaf. In combination with petioled, it produces a very extreme leaf type with a long petiole and long, very narrow, pointed blade". This factor is located on chromosome R.

As these factor-pairs are strictly comparable in action to those found in the present investigation we have for the sake of uniformity adopted these Californian symbols (cf. Ch 1 § 6a). The standard type and the mutants Pt Pt and Br Br were kindly sent to us by Dr. CAMERON under the name "Red Russian", which is closely related to "Purpurea" (cf. Ch 1 § 3a). The three types closely resemble Atr, our type 1 (Fig. 6) and type 9 (Fig. 6) respectively. Therefore, though no tests of identification have been performed yet, it is highly probable that these factors are identical to ours.

Like our type 1 the mutant Pt Pt has acute angles of venation. However, CLAUSEN and CAMERON (l. c.) did not mention this aspect of action of Pt.

The authors did not study the joint segregation of Br-br and Pt-pt (or Pd-pd), which was one of the main objects of the present investigation.* Moreover, we had the opportunity to study the effects of modifying factors as the major genes were not studied against a uniform genic background.

KADAM and RADHAKRISHNAMURTY (1954) crossed the varieties Chatham and Ialomita. Both had "sessile leaves" and the F_1 showed "... a definite constriction between the lamina and the leafbase giving a distinct appearance of a short-winged petiole..." (p. 54; l. c.). Although in the backcrosses and the F_2 the petiole plants varied in length of petiole, classification was only into sessile and petiole. The authors infer 3 complementary factor-pairs for the petioled condition. This was substantiated by 9 progenies obtained by selfing petiole plants from the backcrosses and the F_2 (Table 4; l. c.). These segregated in agreement with the ratios 27 petiole : 37 sessile (like F_2), 9 petiole : 7 sessile, or 3 petiole : 1 sessile. The inference of "complementary genes" for the petioled condition is in itself not in contrast with our conception, if we consider the constricted (= very short petioled) type as sessile like the authors did in discussing the data of MacRAE (p. 63; l. c.). Neglecting the differences in petiole length or leafbase elongation, the ratio in our F_2 then becomes 9 petiole : 7 sessile.

In addition to the three factors governing the petiole versus sessile alternative, the authors introduced a factor-pair for wing width whose influence could be ascertained only in petiole leaves. The petiole F_2 plants were "broadly classified" into a broad-winged and a narrow-winged to wingless class, with frequencies 29 and 10 respectively (Table 1; l. c.). However, as these frequencies agree also with the 2 : 1 ratio, the difference in wing development can as well be explained with the help of a factor-pair for petiole versus sessile (cf. our Fig. 14 in which the types 4 and 5 (Br br) can be called "broad-winged" and the types 1 and 2 (br br) "narrow-winged to wingless").

As the descriptions of the parents and the segregants are rather limited it is not feasible to suggest detailed alternative explanations.

* The F_1 and the F_2 from the cross between the Californian mutant types Pt Pt br br and pt pt Br Br were grown by the present author in 1957. The 9 F_2 types are in essence the same as those of Fig. 6, only the petioles (leafbases) were shorter and the wings broader. Type 1 for instance has wings of 2-3 mm. and notably the F_1 and the types 4 and 5 have relatively broad wings and short petioles. This is due to the low leaf number in the material. The leaf number is at any rate not higher than that of our Atr.

The F_2 frequencies are (types between brackets):

| | | | | | |
|-------|-------|-------|-----|-----------------------------------|-----------------|
| 17(1) | 22(4) | 11(7) | 50 | X^2 (8 d.f.) = 5.020 | $P = 0.80-0.70$ |
| 28(2) | 45(5) | 28(8) | 101 | For the Br-br segregation we find | |
| 16(3) | 21(6) | 12(9) | 49 | X^2 (2 d.f.) = 3.880 | $P = 0.20-0.10$ |
| 61 | 88 | 51 | 200 | | |

Miss KOELLE (1957) crossed at the Forchheim (Germany) tobacco institute the varieties FO (= Forchheimer Ogradowi) and Virgin D (Virginia D). FO has a very narrow leafbase, broad leaves, an obtuse angle of venation and distinct auricles. Virgin D is a "lancifolia type", i.e. the leaves are sessile and narrow with an acute angle of venation. The F_1 has long petioles and an intermediate angle.

From the description and from Fig. 1 (l.c.) it appears that FO, the F_1 and Virgin D resemble strikingly our HG, F_1 and Am respectively. From the F_2 (the only segregating generation studied) a sample of leaf types is presented in Figs. 2 to 7 (l.c.). Apart from types resembling FO, F_1 and Virgin D, types are shown that resemble our types 1 and 9 from the cross HG x Am very much. It is highly probable that both phenotypically and genetically the FO-Virgin D material is analogous to the HG-Am material.

Miss KOELLE, however, did not arrive at a classification into 9 types. She states (p. 49; l.c.): "Das Auftreten dieser langen Reihe von Bastardierungs nova, deren phänotypischen Differenzen in einander übergehen, macht die Annahme einer polyfaktoriellen Spaltung notwendig". At least four factor-pairs for leaf shape were postulated. The author recognized that the narrow leaves had acute, the broad leaves obtuse angles of venation, which is in agreement with our findings. She speaks of (p. 52; l.c.): "... der von Virgin D kommende Faktor für spitzwinkligkeit des Rippenwinkels...". For the petioled condition two complementary factor-pairs (giving 9 petioled : 7 sessile) were postulated. As the author includes the constricted types (like FO) in the sessile group, the ratio agrees with our 9 (Pt . . br; types 1, 2, 4, 5) : 7 (types 3, 6, 7, 8, 9). However, other aspects of leaf shape, notably wing width, were not recognized as depending on these two factor-pairs.

Ch 1 § 14. SUMMARIZING DISCUSSION

On the basis of the present investigation the following statements were made:

1. The factor-pairs Pt-pt and Pd-pd are pleiotropic. Qualitatively, their action is the same, but quantitatively the effect of Pt-pt is markedly larger than that of Pd-pd. Both Pt and Pd make:
 the petiole (leafbase) longer,
 the wings narrower (to a relatively small extent),
 the leafblade narrower,
 the angle of venation more acute,
 the calyx and the corolla more slender and the limb more pointed.
2. The factor-pair Br-br affects wing width to a relatively large extent. The br br leaves are petioled, the Br Br ones sessile.
3. Within each of the Pt-pt and Pd-pd genotypes maximum petiole length is positively correlated with number of leaves. Knowledge of this correlation is helpful in classifying the plants.

4. In the segregating generations the heterozygotes can be distinguished phenotypically from the homozygotes.

In the foregoing discussion of literature (Ch 1 § 13) points of agreement and of discordance with the present findings were stressed. When necessary and possible, reinterpretations were suggested in order to show that the leaf shape factors in literature are similar in action to ours. Summarizing, the following comments can be made:

1. Some authors described the leafbase phenotypes in terms of wing width only: Miss HOWARD (1913), BOURZEV (1928), GENTSCHIEFF (1936), KADAM and RADHAKRISHNAMURTY (1954). It is very likely, however, that not only typical wing width factors like our Br-br, but also factors for petiole (leafbase) length like Pt-pt and Pd-pd were involved.
 2. The presence of factors like Pt-pt and Pd-pd is apparent from the studies of SETCHELL et al. (1922), KELANEY (1925) and MacRAE (1941). CLAUSEN and CAMERON (1944), considering monofactorial differences between the very short-petioled type pt pt pd pd br br and its mutant types, described the action of Pt-pt, Pd-pd and Br-br in agreement with the conclusions from the present study.
 3. In none of the investigations, however, were the heterozygotes recognized as belonging to distinct phenotypic classes, which involved incomplete understanding of the actions and interactions of the factor-pairs. This must at least partly be due to the very little use that was made of backcrosses. In the present study these proved to be very useful for arriving at a complete classification.
 4. In some studies features of the general leaf habitus were taken into account without sufficient evidence that these characters, together with the leafbase characters, behave as one complex in inheritance. Moreover it is not always clear from the descriptions how all these characters were "weighted" in classifying the plants. The classifications given by RAVE (1935) are largely based on features of the general leaf habitus. In the present study the leafbase shape proved to be a better starting-point for revealing the pleiotropic action of Pt-pt and Pd-pd than leaf width and angle of venation, for a discrete classification can be made for leafbase shape, while leaf width and angle of venation give rise to more or less overlapping classes.
 5. Segregation for angle of venation is apparent from most of the studies. However, this character was discussed only by Miss HOWARD (1913) and Miss KOELLE (1957), though no connection with leafbase shape was mentioned by these two authors.
 6. The correlation between number of leaves and maximum petiole length (or leafbase elongation in the case of sessile leaves) escaped detection. As mentioned in Ch 1 § 6c, the petioles with maximum length are the most conspicuous on the plant.
- Thus the classifications presented in literature are far from satisfactory and the understanding of the genic actions and inter-

actions incomplete. Nevertheless it is my opinion that in most if not all cases the situation is similar to that found for the present material, i.e. that the factors involved are comparable in action to (and probably identical to) our Pt-pt, Pd-pd and Br-br.

It must be added that the existence of still other factors for leaf(base) shape has been demonstrated:

1. HONING's N-n (1927) = Def-def (1939) can in a sense be considered as a factor-pair for petiole length (etc.). The action of def is much more extreme than the combined action of Pt and Pd. It is not known whether def is a third allele on the Pt or Pd locus, or represents a different locus.
2. MacRAE (1941) found at least one more factor for wing width, viz. Wp-wp. Evidence for two wing width loci is also presented by Miss HOWARD (1913). Consequently we know not only two factor-pairs for leafbase length, etc. (Pt-pt and Pd-pd), but also two for width of the leafbase (Br-br and Wp-wp). In view of the amphidiploid origin of *N. tabacum* this situation is not surprising.

Chapter 2

ANALYSIS OF METRICAL DATA ON THE LEAF SHAPE AND
THE ANGLE OF VENATION

Ch 2 § 1. INTRODUCTION

In studying leaf shape two difficulties arise:

1. Like other organs the leaf may change shape during its growth in a more or less regular way, i.e. its shape is dependant on its size. When attention is focussed on full-grown leaves, it must be determined unambiguously when the final size is attained.
2. The shape of adult leaves may vary as a function of the level of insertion on the stem (node number): heteroblastic development. The question arises what leaves can best be taken as a basis for comparison.

The following terminology, proposed by STEPHENS (1944), will be adopted.

Growth : "The change in dimensions of an individual leaf which results from cell division and expansion".

Development : "The progressive change in fully expanded leaf shape in passing node by node up the main stem..".

This terminology was also adopted by SCHWARZ et al. (1951) who studied the leaf shape of tobacco.

In *Nicotiana tabacum*, development is continuous and therefore cannot be described by qualitative criteria (e.g. entire versus lobed leaves). Moreover a region of constant leaf shape (climax leaves) is not generally present. Consequently it is not possible to decide a priori what leaves should be taken as a basis for comparison when comparing different plants. One may take the largest leaves or leaves with a specified number. The leaves may be numbered from top to bottom of the stem like HERLAN (1931) did, or from bottom to top as is preferred in the present analysis. Like leaf shape and its components (length and width), angle of venation is not constant from node to node.

In the present biometrical analysis of these characters, graphs will be used in order to study the whole range of variation along the stem.

In Chapter 1 it was stated that the factor-pair Pt-pt, besides its effect on leafbase shape, has a considerable influence on leaf width and angle of venation. In order to arrive at a more exact analysis a large number of metrical data have been collected, which will be used to provide detailed information about the actions and interactions of the genes under consideration.

Part of the results of this analysis has been referred to in Chapter 1 to justify some of the inferences made. The metrical data on petiole length have already been presented in Ch 1 § 6c.

Ch 2 § 2. EXPERIMENTAL METHODS

Ch 2 § 2a. *Procedure of growing*

To analyse and compare leaf development in different genotypes many accurate measurements had to be taken on each plant and consequently no large numbers of plants could be studied. Therefore the environmental conditions had to be as uniform as possible.

The individual seeds were laid in the 22 x 22 cm. sowing pans in square system (1.5 x 1.5 cm.) to eliminate possible effects of crowding. On transplantation to 7-8 cm. pots, the seedlings were still kept in the heated glasshouse. After about 12 days the plantlets were placed in 22 cm. pots and transferred to the unheated glasshouse. Two weeks later they were carefully planted out on a very homogeneous experimental plot, avoiding any damage (particularly to the roots) in removing the pots. In this way loss of turgor was kept minimal and the lowest leaves remained uncovered by earth, i. e. remained accessible for measuring. The plot was surrounded by guard rows. The plants were not topped, but all suckers were removed soon after their appearance. Plants thus grown will hereafter be called "pot-plants".

Ch 2 § 2b. *Homogeneity of environmental conditions*

With the pot-plant method the parental lines and the F_1 's showed only small variation in general habitus, date of opening of the first flower, number of leaves, etc. Table 5 shows that the variation in leaf number of pot-plants is smaller and less divergent for different lines than that of non-pot-plants.

Table 5. Means and variances of leaf number in the four parental lines, grown in 1953 as pot-plants and as non-pot-plants.

| Parent | Growing method | Number of plants | Mean | Variance | F-value | P-value |
|--------|----------------|------------------|------|----------|---------|---------|
| HG | pot-plants | 20 | 25.6 | 0.67 | 2.06 | 0.05 |
| | non-pot-pl. | 24 | 27.6 | 1.38 | | |
| Atr | pot-plants | 20 | 25.2 | 0.80 | 4.70 | <0.01 |
| | non-pot-pl. | 22 | 26.0 | 3.76 | | |
| KE | pot-plants | 20 | 30.1 | 1.36 | -- | -- |
| | non-pot-pl. | 24 | 30.0 | 0.91 | | |
| Am | pot-plants | 20 | 35.0 | 0.58 | 9.40 | <0.01 |
| | non-pot-pl. | 24 | 35.5 | 4.87 | | |

In general no replicates of the different families were grown and the plants of each family were kept together. Due to the uniform environmental conditions and the relatively small size of the experimental plot (small numbers of plants were used) this was no serious drawback as will be shown when discussing reciprocal F_1 's and successive parent generations (Ch 2 § 5d).

Ch 2 § 2c. *Measurements and counts*

1. Number of leaves. The leaves are numbered from bottom to top of the stem, including the cotyledons and the last leaf under the central end-flower. This flower is occasionally absent, but its position is clearly indicated as here the stem forks into (mostly) three flower branches. As the lowest leaves have disappeared before the highest can be counted, specific leaves were marked near their tip with plastic-aluminium paint, which is weather-proof and does not damage the leaf tissues.
2. Leaf length (in mm.). It was measured on the upper side of the leaf along the midrib from axil to tip.
3. Leaf width (in mm.). This is the maximum width, measured perpendicular to the midrib. Its position cannot be determined with high accuracy and changes more or less during leaf growth.
4. Leaf shape index. The index used is the $^{10}\log$ arithm of the length/width ratio (L/W), which equals $\log L - \log W$. To simplify the calculation of averages per number of the leaf ("mean shape" = \bar{S}), the approximation $\log \bar{L} - \log \bar{W}$ was used in the case of non-segregating families. In these families the dimensions of leaves with the same number did not vary much, so that only the third decimal was affected by using the logarithms of means instead of the means of logarithms. For the highest leaves, however, this approximation could not be used, as the dimensions showed a larger variation within each family due to differences in number of leaves. Then \bar{S} was calculated as the mean of logarithms.
5. Angle of lateral veins (in degrees). Near the midrib and near the leaf margin a lateral vein is not straight. Between points about one and three inches from the midrib, its direction is rather constant. In this region the angle opening towards the leaf tip was measured on the upper side of the leaf by putting a graduated arc against the lateral vein on the side of the leaf tip and reading it at the axis of the midrib. Now the general tendency is that from a point about half-way between axil and tip, the angles of the lateral veins become more acute towards the tip, while towards the base they remain rather constant, apart from the last few that are often somewhat more acute again. It was calculated that the first four angles (two on each side of the midrib) below the half-way point vary least. Moreover when leaves with the same number taken from plants of a

non-segregating generation are compared, these four angles lead to the smallest between-plant variance. Therefore the mean of these angles can be considered as a representative value for a leaf. In Figs. 1 to 4 they have been indicated by strips of paper. By measuring two angles on each side of the midrib the effects of slight leaf asymmetry, which is often present, are eliminated.

None of these measurements were taken on leaves that were infected, damaged, or very asymmetric.

Ch 2 § 3. SURVEY OF THE FAMILIES

The families used for biometrical studies were grown as outlined in Ch 2 § 2a. They have been mentioned in Ch 1 § 4 but for convenience will be listed again.

In 1952 a complete set of diallel progenies (4 parents and 6 x 2 F₁ families), obtained in 1950 from one single plant of each of the lines HG, Atr, Am and KE, was grown together with sister families of the four parent plants. All these parent and F₁ families consisted of 10 plants each. In 1953 the following families were grown as sisters of the 1952-plants.

| | |
|--------------------------|-----------------|
| HG | 53-201 (20 pl.) |
| Atr | 53-203 (20 pl.) |
| Am | 53-204 (20 pl.) |
| KE | 53-202 (20 pl.) |
| F ₁ (HG x Am) | 53-205 (20 pl.) |

Segregating generations of the HG-Am material, obtained from single 1952-plants, were grown in 1953:

| | |
|--------------------------|------------------|
| (HG x Am) x HG | 53-206 (100 pl.) |
| (HG x Am) x Am | 53-207 (100 pl.) |
| F ₂ (HG x Am) | 53-208 (100 pl.) |

The classification in the latter three families was checked on a large scale by progeny tests (cf. Ch 1 § 6b).

Ch 2 § 4. SYMBOLS

This paragraph summarizes the symbols used throughout the following sections.

The varieties (for origin and description see Ch 1 § 3a).

| | |
|-----|------------------------|
| HG | = Hongaars Gartenblatt |
| Atr | = Atropurpurea |
| Am | = Amersfoorter |
| KE | = Keurhorst Elite |

The genotypes in the HG-Am families (cf. Ch 1 § 6a and Fig. 6).

| | | |
|-----------------|-----------------|-----------------|
| Pt Pt br br (1) | Pt Pt Br br (4) | Pt Pt Br Br (7) |
| Pt pt br br (2) | Pt pt Br br (5) | Pt pt Br Br (8) |
| pt pt br br (3) | pt pt Br br (6) | pt pt Br Br (9) |

(type numbers between brackets)

The means calculated (cf. Ch 2 § 2c).

\bar{L} = mean leaf length in mm. per number of the leaf.

\bar{W} = mean leaf width in mm. per number of the leaf.

\bar{S} = mean "leaf shape" per number of the leaf.

\bar{A} = mean angle of venation in degrees per number of the leaf.
(The value for an individual leaf is in itself the average of four specified angles; cf. Ch 2 § 2c).

It should be noted that the term "per number of the leaf" implies that, in calculating a mean, leaves were involved that had the same number on the stem. Thus for a group of plants, each leaf No. leads to a \bar{L} , a \bar{W} , a \bar{S} , and a \bar{A} value.

Ch 2 § 5. LENGTH, WIDTH, SHAPE AND ANGLE OF VENATION OF THE FULL-GROWN LEAVES

The data were obtained from leaves that had reached their final size. This was considered to be attained when no further increase in length could be found from measurements performed at regular intervals. As the lower leaves wither before the top leaves are full-grown, these final measurements were not taken on the same date.

For each of the leaf Nos. (1, 2, 3, etc.) family means (non-segregating generations) or group means (in segregating generations) were calculated. Grouping was performed according to the genotypes resulting from the segregation of Pt-pt and Br-br. Within these genotypes sub-groups were made according to number of leaves of the plants.

For each family, group or sub-group, the means for the successive leaves were plotted against the corresponding number of the leaf. The graphs thus obtained cover a large part and sometimes nearly the whole sequence of leaves on the stem so that fluctuations due to irrelevant causes can be discounted. In order to compare these "graphs of leaf development" in all possible combinations, use was made of transparent graph paper and a strong electric bulb. The analysis to be presented in the following paragraphs is based on these comparisons.

Ch 2 § 5a. *Number of leaves in parents and F_1 's*

As in the following paragraphs the number of leaves will be taken into account, the class values and class frequencies of the 4 parents and the 6 x 2 F_1 families have been summarized in Table 6.

Table 6. Class values (first line in each panel) and class frequencies (second and third line) for number of leaves in parents and F_1 families (1952; 10 plants per family). In the case of reciprocal F_1 's the type heading the column represents the female parent when the second line and the male parent when the third line is involved. In the case of the parents (leading diagonal) these two lines stand for two successive generations, both grown in 1952.

| HG | Atr | KE | Am | |
|----------------------------|----------------------------|-----------------------------------|----------------------------|-----|
| 23-24-25 1 8 1 3 6 1 | 23-24-25 3 6 1 0 8 2 | 25-26-27 2 8 0 0 7 3 | 26-27-28 1 5 4 0 2 7 | HG |
| | 24-25-26 2 7 1 3 6 1 | 25-26-27 1 6 3 0 6 4 | 27-28-29 4 5 1 8 2 0 | Atr |
| | | 27-28-29-30 0 3 6 1 3 4 3 0 | 29-30-31 3 4 3 2 8 0 | KE |
| | | | 31-32 6 4 4 6 | Am |

In each of the 4 + 6 panels of Table 6 (see caption) the difference between the two frequency distributions is very small. From the table as a whole no systematic trends appear, i. e. no indications of differences between successive parent generations or of maternal effects are present. The arrangement of the families in the field was such that the effects, if any, have not likely been obscured by counterbalancing environmental effects. Therefore the absence of differences can at the same time be taken as an illustration of the high degree of homogeneity of environmental conditions.

The four parents (diagonal in Table 6) are arranged in the order of increasing number of leaves. In all cases the F_1 is about intermediate between its parents.

Ch 2 § 5b. Comparisons between the four parental lines

The graphs of leaf development for HG, Atr, KE and Am, grown in 1952, are shown in Fig. 17a (\bar{L} = mean length and \bar{W} = mean width), Fig. 17b (\bar{S} = mean shape) and Fig. 17c (\bar{A} = mean angle of venation). In 1953, HG and Am were repeated: Fig. 18a (\bar{L} and \bar{W}), Fig. 18b (\bar{S}) and Fig. 18c (\bar{A}).

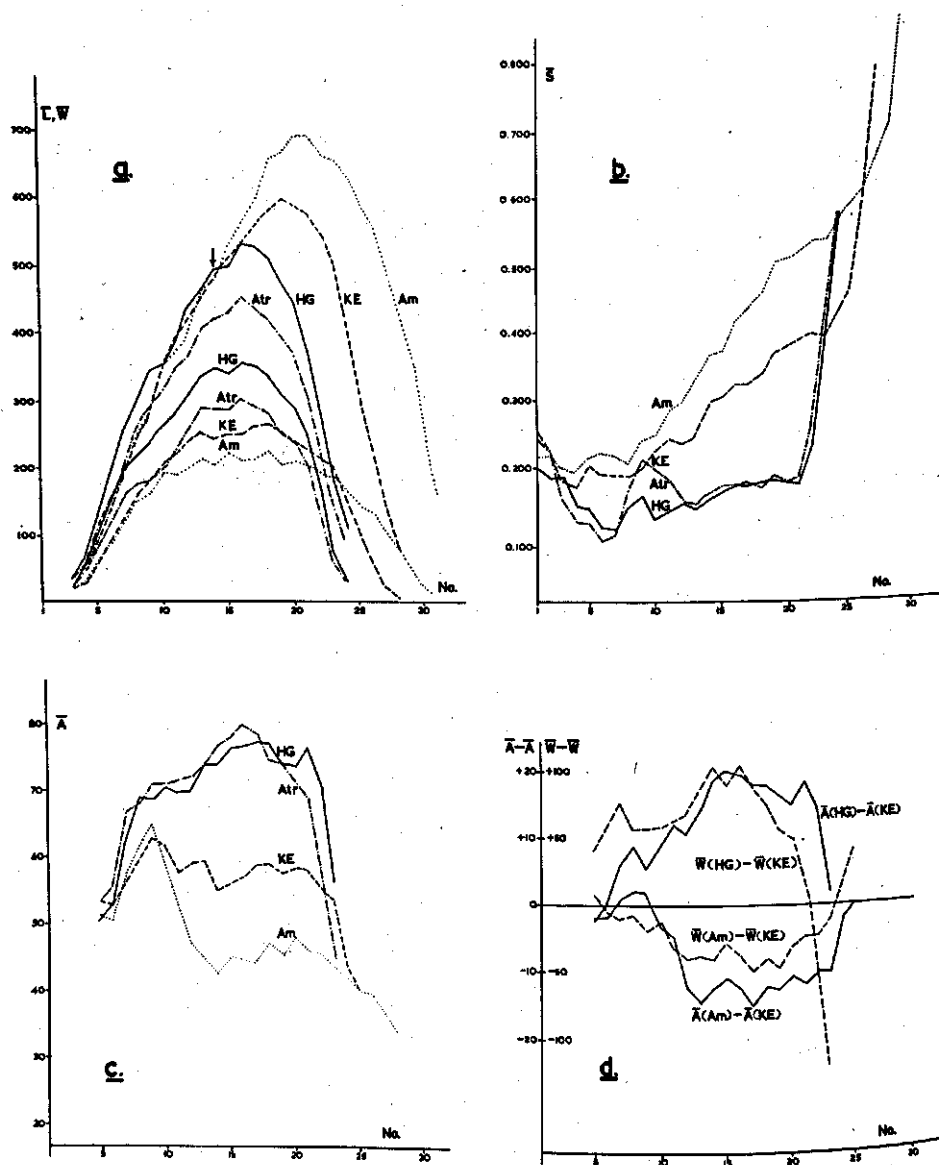


Fig. 17 (a-d). L and W (Fig. a), S (Fig. b) and A (Fig. c) plotted against No. of the leaf for HG, Atr, KE and Am. Fig. d shows for HG and Am the differences in A and W with KE. All families consisted of 10 plants.
Year: 1952. Symbols used: Ch 2 § 4.

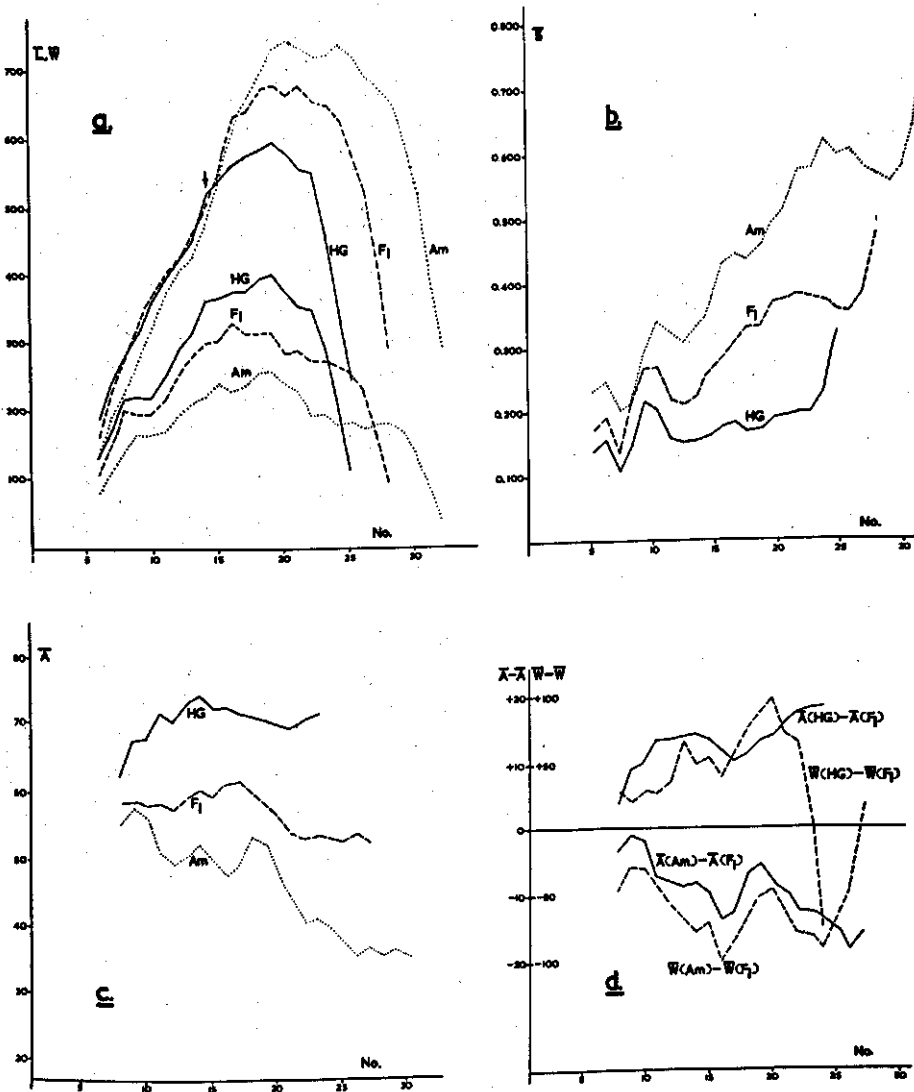


Fig. 18 (a-d). L and W (Fig. a), S (Fig. b) and A (Fig. c) plotted against No. of the leaf for HG, HG x Am and Am. Fig. d shows for HG and Am the differences in A and W with their F_1 HG x Am. The means of Figs. a and b were obtained from 10 plants, those of Figs. c and d from 20 plants.

Year: 1953. Symbols used: Ch 2 § 4

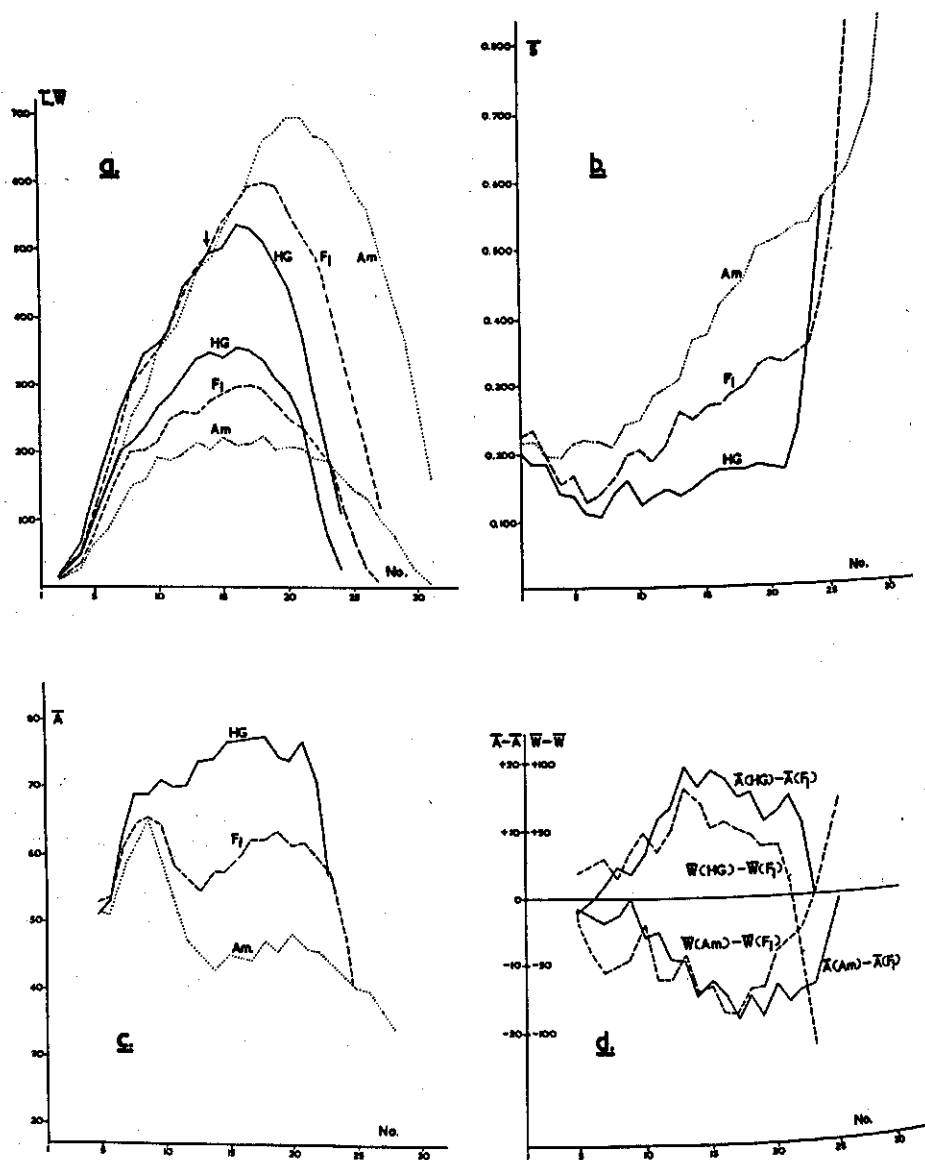


Fig. 19 (a-d). \bar{L} and \bar{W} (Fig. a), \bar{S} (Fig. b) and \bar{A} (Fig. c) plotted against No. of the leaf for HG, HG x Am and Am. Fig. d shows for HG and Am the differences in \bar{A} and \bar{W} with their F1 HG x Am. All families consisted of 10 plants. Year: 1952. Symbols used: Ch 2 § 4. Cf. Fig. 18 (a-d), which is the 1953-replicate of the present figure.

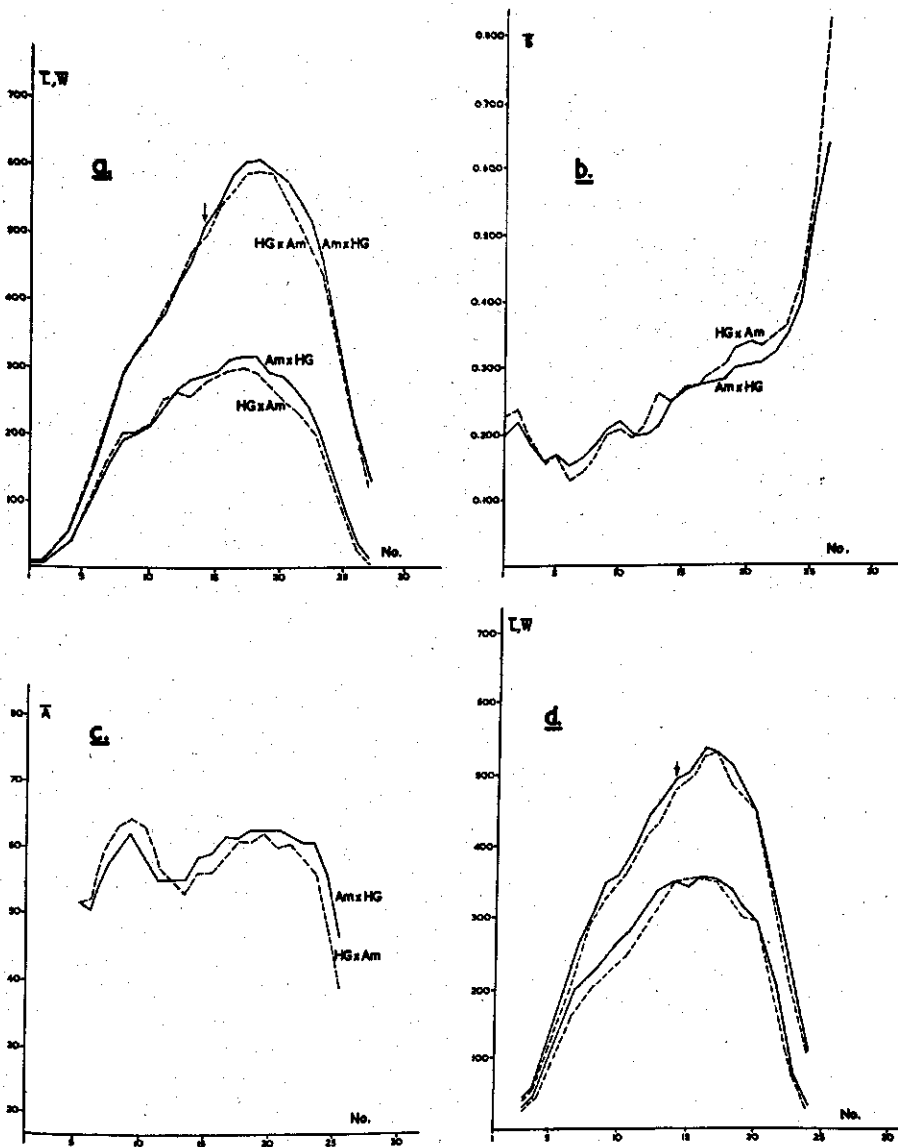


Fig. 20 (a-d). \bar{L} and \bar{W} (Fig. a), \bar{S} (Fig. b) and \bar{A} (Fig. c) plotted against No. of the leaf for the reciprocal F1's HG x Am and Am x HG. Fig. d: \bar{L} and \bar{W} plotted against No. of the leaf for two successive generations of HG, grown in the same year. All families consisted of 10 plants. Year: 1952. Symbols used: Ch 2 § 4.

L- and W-graphs (Figs. 17a and 18a).

From bottom to top of the stem both length and width of the leaves increase first and then decrease.

HG, KE and Am will be compared first. Up to leaf No. 14 (arrow) the leaves of HG are systematically longer than those of KE and Am, while at higher nodes the reverse is true. The \bar{L} -maxima are higher and also later in development, i. e. at a higher number of the leaf, in the order HG-KE-Am, i. e. in the order of increasing number of leaves of the varieties. The \bar{L} -graphs decline in a parallel way, which means that the rates of decrease in length are about equal. As regards the \bar{W} -values, those of HG are much higher than those of KE and Am, both for the lower leaves and for leaves in the region of maximum width. The \bar{W} -graphs of KE and Am diverge earlier, i. e. at a lower node, than the \bar{L} -graphs of these two varieties. The \bar{W} -maxima of HG, KE and Am occur at about the same node, which is in contrast to the \bar{L} -maxima. Moreover they are higher in the reverse order, i. e. in the order Am-KE-HG. In this order decrease in \bar{W} follows at higher rates, which contrasts to the parallel declination of the \bar{L} -graphs.

Atr is best compared with HG, which has the same leaf type and the same number of leaves. The only difference is that both length and width of Atr are systematically smaller.

S-graphs (Figs. 17b and 18b).

Regarding leaf shape HG, KE and Am diverge much, as might be expected from the opposed orders of the \bar{L} - and \bar{W} -maxima. From bottom to top of the stem the leaves of KE and Am at first remain constant in shape and then gradually become more elongated (at different rates). HG on the contrary has leaves that at first become less elongated and then remain of constant shape. Near the inflorescence the leaves of all varieties rapidly become more elongated.

The leaves of Atr have at all nodes about the same shape as those of HG. The difference therefore is only one in size.

As to the fluctuations in shape between the 7-th and 13-th leaf (notably in 1953; Fig. 18b), which correspond to "irregularities" of the \bar{W} -graphs, the only comment to be made is that during the period of rapid growth of these leaves the plants were transplanted from the glasshouse to the field.

The drop between the 23-rd and 29-th leaf shown in the \bar{S} -graph for Am in 1953 (Fig. 18b) is reflected in a region of constant leaf width (Fig. 18a). As the phenomenon did not occur in 1952 (Fig. 17b) it may be ascribed to environmental influences.

Apart from the fact that the varieties had more leaves in 1953 than in 1952, their graphs for leaf shape are essentially similar for the two years.

A-graphs (Figs. 17c and 18c).

Varietal differences in angle of venation, too, arise early in development, though not quite as early as differences in shape.

For all varieties the angles at first become more obtuse (Fig. 17c; in Fig. 18c these lowest leaves are not presented), but soon varietal differentiation follows. The angles of HG continue to become more obtuse, those of KE remain about constant and those of Am change to an increasing acuteness. For the last 2-3 leaves below the inflorescence (Fig. 17c; not presented in Fig. 18c) the angles in all varieties rapidly become more acute, which phenomenon is very pronounced in HG.

The \bar{A} -graph of Atr coincides fairly well with that of HG.

\bar{A} -graphs and \bar{W} -graphs in comparison with each other (Fig. 17d).

In the order HG-KE-Am the leaves are narrower and the angles of venation more acute.

When for each variety angle of venation and leaf width are compared from node to node, increasing leaf width may (cf. HG) or may not (cf. Am) correspond to increasing obtuseness of the angles. Therefore the relation between the two characters during development is typical of each of the varieties.

Regarding the differences in angle and in width between the varieties it can be said that both characters are affected by the differentiating genes in a way that is strikingly parallel for the whole series of leaves. This parallelism in pattern of differentiation (superposed on the developmental patterns for the individual characters) is shown in the graphs of Fig. 17d, which have been constructed as follows. KE, which has a rather constant angle of venation along the stem, has been chosen as a standard for reference. Then for \bar{A} and for \bar{W} the differences HG - KE and Am - KE have been plotted against number of the leaf. The scales have been chosen so as to facilitate the inspection of the graphs. Both for HG and Am the two graphs are seen to run strikingly parallel, though differentiation in leaf width commences somewhat earlier in development than that in angle of venation. With the highest leaves both the HG - KE and the Am - KE differences in \bar{W} change sign as, resulting from the differences in number of leaves of the varieties, the \bar{W} -graphs for HG, KE and Am cross each other (cf. Fig. 17a).

Ch 2 § 5c. *Parent- F_1 comparisons*

The graphs for HG and Am are given together with those for the F_1 HG x Am in Fig. 19a-c (1952) and in Fig. 18a-c (1953). The F_1 graphs appear to be in all respects about intermediate for all characters. The same can be said about the reciprocal cross and the other 5 x 2 F_1 families of the diallel set grown in 1952 (graphs not presented).

The F_1 HG x Am takes a similar position in relation to HG and Am as KE does (cf. Fig. 19a-c with Fig. 17a-c). Like KE, it shows a more or less constant angle of venation along the stem. When this F_1 instead of KE is taken as a standard for reference, the same rigid parallelism in pattern of relative differentiation in \bar{A} and \bar{W} is found: Fig. 18d (1953) and Fig. 19d (1952).

Ch 2 § 5d. *Reciprocal F_1 's and successive parent generations*

The graphs for the F_1 HG x Am and its reciprocal are given in Fig. 20a-c. For the four pairs of graphs a good coincidence can be stated. For each of the five other pairs of reciprocal F_1 's the coincidences of the four types of graphs are as good or even better (graphs not presented).

The agreement between the two successive generations (both grown in 1952) of each of the four parents was as good as that between reciprocal F_1 's. As an illustration of this the \bar{L} and \bar{W} graphs for the two HG families are presented in Fig. 20d.

It can be concluded that no indications are present of maternal effects or of genetic differences between successive parent generations. The small differences that do exist (Fig. 20a-d) are presumably due to environmental causes.

Ch 2 § 5e. *The influence of number of leaves in the segregating generations*

The biometrical studies on the segregating generations were confined to the cross HG x Am. In 1953 the backcrosses F_1 x HG (53-206) and F_1 x Am (53-207), and the F_2 (53-208), were grown together with HG (53-201), the F_1 HG x Am (53-205) and Am (53-204).

Within the segregating generations the different genotypes can be taken to have been planted out at random. Plants belonging to the same family were kept together. This might make between-family-comparisons less reliable. However, the environmental conditions affecting the relatively small experimental plot were very homogeneous as was illustrated in the foregoing. Therefore comparisons of this kind can be made without much risk.

The segregants were classified according to the types resulting from the segregation of Pt-pt and Br-br (4 types in each backcross and 9 in the F_2 ; Ch 1 § 6 and Fig. 6). The phenotypic classification was checked by a large number of F_3 progenies (Ch 1 § 6b). Within each of the backcross types a subdivision was made according to leaf number so as to obtain a "parent-group", an "intermediate group", and an " F_1 -group". This has been indicated in Table 7, which gives the leaf number distributions for the parents and the F_1 and for the different types in the segregating generations. Type 4 from the backcross F_1 x Am was subdivided into two groups only, in order to avoid too small group frequencies. In the F_2 , type 5 (F_1 -type) has been subdivided, into three groups.

Before discussing the influence of leaf number on leaf development it must be stated that in the segregating generations there are small differences in leaf number distribution for the different genotypes (Table 7). These differences are associated with the Pt-pt, not with the Br-br genotypes. Thus in the F_2 the means for leaf number are 30.85 for Pt Pt (types 1, 4 and 7), 30.23 for Pt pt

Table 7. Leaf number frequencies for HG (53-201), the F₁ HG x Am (53-205), Am (53-204), the four types from the backcross F₁ x HG (53-206), the four types from the backcross F₁ x Am (53-207), and the nine types from the F₂ (53-208). Grouping within the backcross types and within type 5 from F₂ is indicated by broken horizontal lines. For the different types see Fig. 6.

| Leaf number | Parents and F ₁ | | Backcross types in F ₁ x HG | | | | Backcross types in F ₁ x Am | | | | F ₂ types | | | | | | | | | | | | |
|-------------|----------------------------|-------------------------|--|----|----|----|--|------|----|----|----------------------|----|------|---|----|----|----|----|----|----|----|----|-----|
| | HG (type 3) | F ₁ (type 5) | Am (type 7) | 2 | 5 | 3 | 6 | Tot. | 4 | 7 | 5 | 8 | Tot. | 1 | 4 | 7 | 2 | 5 | 8 | 3 | 6 | 9 | Tot |
| 23 | - | | | - | - | - | 1 | 1 | | | | | | - | - | - | - | - | - | - | 1 | - | 1 |
| 24 | 1 | | | 1 | 1 | 1 | 4 | 4 | | | | | | - | - | - | - | - | 1 | - | - | 1 | |
| 25 | 4 | | | 3 | 6 | 2 | 5 | 16 | | | | | | - | 1 | - | 3 | 5 | 1 | - | 1 | 1 | |
| 26 | 4 | | | 4 | 2 | 2 | 4 | 12 | | | | | | - | - | 1 | - | 1 | - | - | - | 12 | |
| 27 | 1 | | | 3 | 1 | 7 | 2 | 13 | | | | | | - | 2 | 1 | 1 | 1 | 7 | 1 | 3 | 1 | |
| 28 | - | | | 6 | 5 | 4 | 3 | 18 | | | | | | - | - | 2 | 1 | 2 | 1 | 3 | 3 | 15 | |
| 29 | | | | 3 | 6 | 4 | 1 | 14 | | | | | 2 | 1 | 2 | 2 | 3 | 10 | 4 | 3 | 3 | 1 | |
| 30 | | 7 | | 3 | 3 | 2 | 2 | 10 | | | | | 4 | 1 | 5 | 2 | - | 6 | 7 | 1 | 8 | 2 | |
| 31 | | 2 | | 1 | - | 1 | 2 | 4 | | | | | 8 | 1 | 2 | 3 | 1 | 5 | 4 | 1 | 2 | 1 | |
| 32 | | 1 | | - | 1 | 1 | 1 | 3 | | | | | 19 | 1 | 3 | 2 | 3 | 9 | 5 | - | 3 | - | |
| 33 | | | | | | | | | | | | | | 1 | 1 | 2 | 2 | 8 | 2 | 1 | 5 | - | |
| 34 | | | | | | | | | | | | | | - | - | - | - | 2 | 3 | - | 1 | - | |
| 35 | | | 2 | | | | | | | | | | 17 | - | 4 | - | 1 | 1 | - | 1 | - | - | |
| 36 | | | 5 | | | | | | | | | | 8 | 1 | - | - | - | - | - | - | - | - | |
| 37 | | | 3 | | | | | | | | | | 2 | | | | | | | | | | |
| 38 | | | - | | | | | | | | | | 4 | | | | | | | | | | |
| Totals | 10 | 10 | 10 | 24 | 25 | 24 | 22 | 95 | 17 | 26 | 22 | 27 | 92 | 6 | 20 | 14 | 15 | 49 | 36 | 11 | 30 | 9 | 190 |

(types 2, 5 and 8) and 29.66 for pt pt (types 3, 6 and 9). In the backcross $F_1 \times Am$ one finds 34.00 for Pt Pt (types 4 and 7) and 32.96 for Pt pt (types 5 and 8). The differences 30.85-29.66 (F_2) and 34.00-32.96 ($F_1 \times Am$) are both significant at the 2% level of probability. In the backcross $F_1 \times HG$ no significant differences between means were found. The conclusion is that leaf number is distributed largely though not wholly independently of leafbase genotype.

The influence of leaf number on leaf development will be discussed with the help of Fig. 21a-c showing the \bar{L} -, \bar{W} -, \bar{S} - and \bar{A} -graphs for the three leaf number groups within type 3 (pt pt br br) from the backcross $F_1 \times HG$. Contrary to what might be expected from the comparisons between the four parent lines (Ch 2 § 5b) and from the parent- F_1 comparisons (Ch 2 § 5c), the differences in leaf number are only slightly reflected in differences between the \bar{L} -maxima and not at all in differences in number of the leaf at which the maxima are attained. The same statement holds for comparisons of this kind within all other leafbase genotypes from the backcrosses (graphs not presented) and equally for the types from the F_2 . For the latter family Fig. 21d serves as an illustration in giving the \bar{L} - and \bar{W} -graphs for the two extreme groups within type 5 (Pt pt Br br). It can be concluded that the difference in \bar{L} -maximum between the parents is not generated by the leaf number genes.

It should be noted that within type 5 from each of the backcrosses the number of leaves is positively correlated with the maximum leaf length obtained by graphical adjustment (r_{13} in Table 3; Ch 1 § 6c). However, we now see from the \bar{L} -graphs (cf. Fig. 21a) that the effect of number of leaves on maximum \bar{L} is not of a relevant magnitude. Moreover r_{13} for type 5 from the F_2 (Table 3) is not significant. In Ch 1 § 6c it was seen that the higher the number of leaves, the higher the maximum petiole length. This has been illustrated in Fig. 7c for the same two groups of type 5 plants from the F_2 (53-208) as used for the \bar{L} -graphs of Fig. 21d. The maximum mean petiole length (\bar{P}) of the group with a low number of leaves is below, that of the group with a high number of leaves above leaf No. 20. Above this leaf the two \bar{L} -graphs diverge. It cannot be decided whether the difference in \bar{P} in this region (24 mm. for leaf No. 20) is reflected in the total leaf length or not, because in proportion to the total leaf length a difference of 24 mm. is of the order of magnitude of "irrelevant" fluctuations in \bar{L} .

The \bar{L} -graphs for the leaf number groups, coinciding well below the region of maximum leaf length and rather well in this region, diverge when the phase of decrease is initiated (cf. Figs. 21a and 21d). The transition to this phase is slower when the number of leaves is higher. The \bar{W} -graphs (cf. Figs. 21a and 21d) coincide up to a higher node and the \bar{A} -graphs (cf. Fig. 21c) diverge at only a few leaves distance from the inflorescence. This means that the number of leaves affects only the higher parts of the different graphs and therefore we can combine (per No. of the leaf)

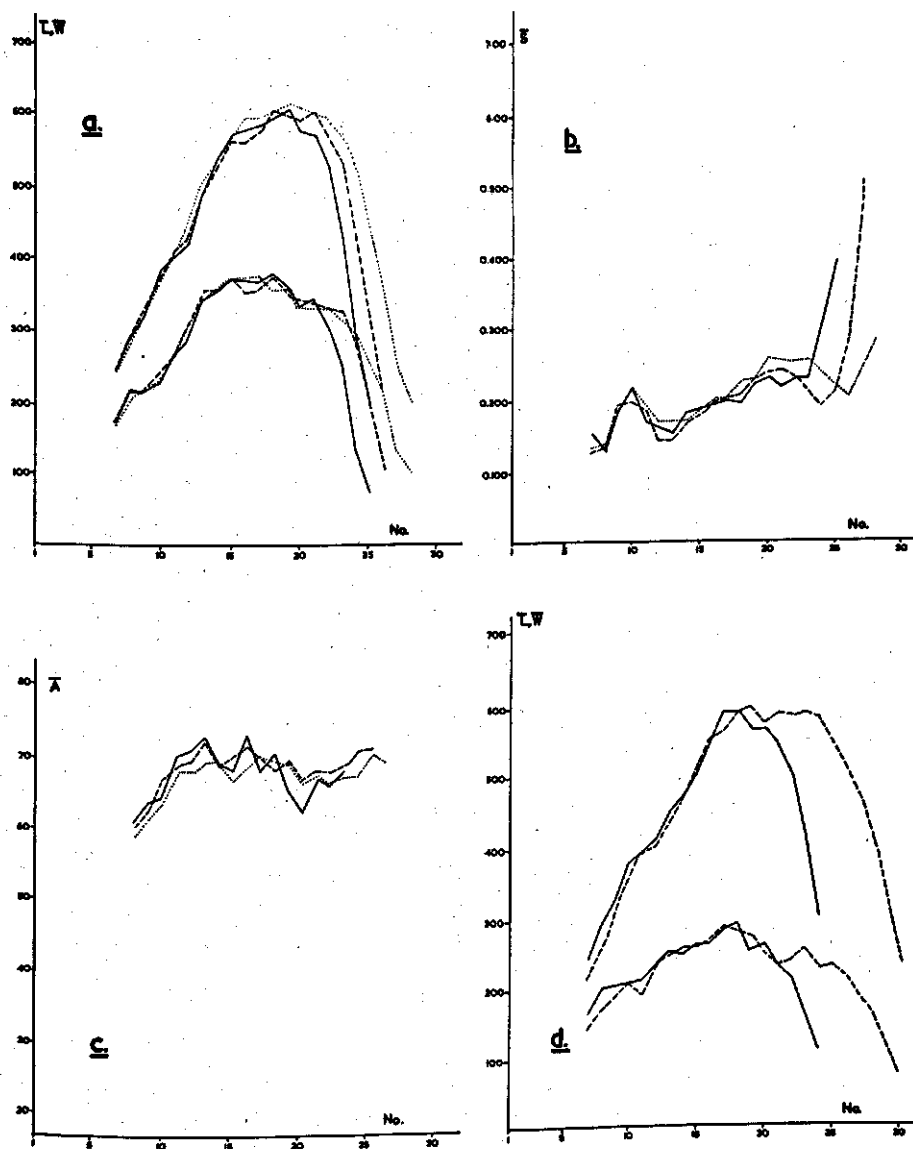


Fig. 21 (a-d). The pt pt br br (= type 3) plants from the backcross (HG x Am) x HG were grouped according to "low" (—), "intermediate" (---) and "high" (...) number of leaves (cf. Table 7). For each group \bar{L} and \bar{W} (Fig. a), \bar{S} (Fig. b) and \bar{A} (Fig. c) were plotted against No. of the leaf. Fig. d shows the \bar{L} and \bar{W} graphs for the "low" (—) and the "high" (---) group among the Pt pt Br br (= type 5) plants from the F₂ (cf. Table 7).
Year: 1953. Symbols used: Ch 2 § 4.

within each leafbase type the individual \bar{L} -data up to leaf No. 20 to 22 (region of maximum \bar{L}), the \bar{W} - and \bar{S} -data up to at least the same leaf No., and the \bar{A} -data even up to higher nodes. On the basis of the graphs thus obtained, the different types will be compared in the following paragraph.

Ch 2 § 5f. Comparisons between the leaf types in the segregating generations

\bar{L} - and \bar{W} -graphs.

The graphs of leaf development for the different genotypes, obtained by averaging the data as described above, were compared with each other within each of the segregating generations. A few of the graphs are given for illustration (Fig. 22a-b).

Considering the \bar{L} -graphs first, those of the following types were found to coincide.

| | | |
|-----------------|-------------------------------|--------------|
| $F_1 \times HG$ | : types 2 and 5 | , both Pt pt |
| | types 3 and 6 (cf. Fig. 22a), | both pt pt |
| $F_1 \times Am$ | : types 4 and 5 | , both Br br |
| | types 7 and 8 (cf. Fig. 22b), | both Br Br |
| F_2 | : types 1, 2, 4 and 5 | , Pt . . br |
| | types 3, 6 and 9 | , all pt pt |
| | types 7 and 8 | , both Br Br |

All these coincidences were very good, except the following two. Type 6 from F_2 has a \bar{L} -maximum which is somewhat lower than that of the types 3 and 9. As this tendency was not found in the backcross $F_1 \times HG$ (cf. Fig. 22a) the difference was neglected. Type 5 from $F_1 \times HG$ reaches somewhat higher values in the region of maximum \bar{L} than type 2 from this backcross. The difference was not found in the F_2 and therefore it was not considered to be very relevant.

Turning to the \bar{W} -graphs the coincidences are listed as follows.

| | | |
|-----------------|--------------------|--------------|
| $F_1 \times HG$ | : types 2 and 5 | , both Pt pt |
| | types 3 and 6 | , both pt pt |
| $F_1 \times Am$ | : types 4 and 7 | , both Pt Pt |
| | types 5 and 8 | , both Pt pt |
| F_2 | : types 1, 4 and 7 | , all Pt Pt |
| | types 2, 5 and 8 | , all Pt pt |
| | types 3, 6 and 9 | , all pt pt |

Again all these coincidences were very good, with the exception of the \bar{W} -graph of type 6, which is clearly lower than that of the other pt pt types, both in the backcross $F_1 \times HG$ (cf. Fig. 22a) and in the F_2 . This difference will be neglected because it stands alone and is not of the order of magnitude of the systematic differences (between groups of genotypes) to be discussed below (cf. the \bar{W} -graphs of the types 7 and 8 in Fig. 22a).

On the basis of all these coincidences the individual data were combined per group of genotypes. The resulting \bar{L} - and \bar{W} -graphs are presented in Fig. 23a (backcrosses) and Fig. 23b (F_2). Parts of

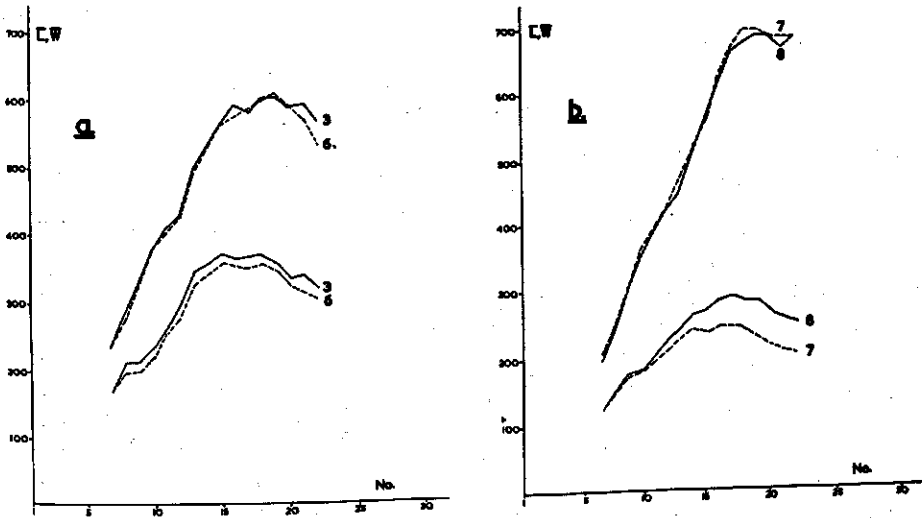


Fig. 22 (a-b). \bar{L} and \bar{W} plotted against No. of the leaf for type 3 plants (pt pt br br; —) and type 6 plants (pt pt Br br; ---) from the backcross (HG x Am) x HG.
 Fig. b: \bar{L} and \bar{W} plotted against No. of the leaf for type 7 plants (Pt Pt Br Br; ---) and type 8 plants (Pt pt Br Br; —) from the F₂.
 Year: 1953. Symbols used: Ch 2 § 4.

the parent and F₁ graphs have been added for comparison. With the help of these figures we will now discuss the influence of Pt-pt and Br-br upon leaf development.

Considering the \bar{L} -graphs first, the following comments can be made:

1. In the region of increasing leaf length the two graphs of the backcross F₁ x HG are higher than the two of the backcross F₁ x Am (Fig. 23a). This can be taken as a reflection of the fact that in this region HG has longer leaves than Am (cf. Figs. 18a and 19a).
2. In the region of maximum leaf length it is seen that the three F₂ graphs (Fig. 23b) do not diverge much, i. e. Pt-pt and Br-br have but a small influence upon maximum leaf length. The maximum becomes somewhat higher in the order short-based types (pt pt; types 3, 6, 9), long-petioled types (Pt . br br and Pt . Br br; types 1, 2, 4, 5), sessile types with elongated base (Pt . Br Br; types 7, 8). The order observed in each of the backcrosses (Fig. 23a) is consistent with the order found in the F₂. However, in the backcross F₁ x Am the two maxima re-diverge considerably. Why this is not reflected in the F₂ remains obscure. In conclusion it can be said that the large difference in maximum leaf length between HG and Am is not

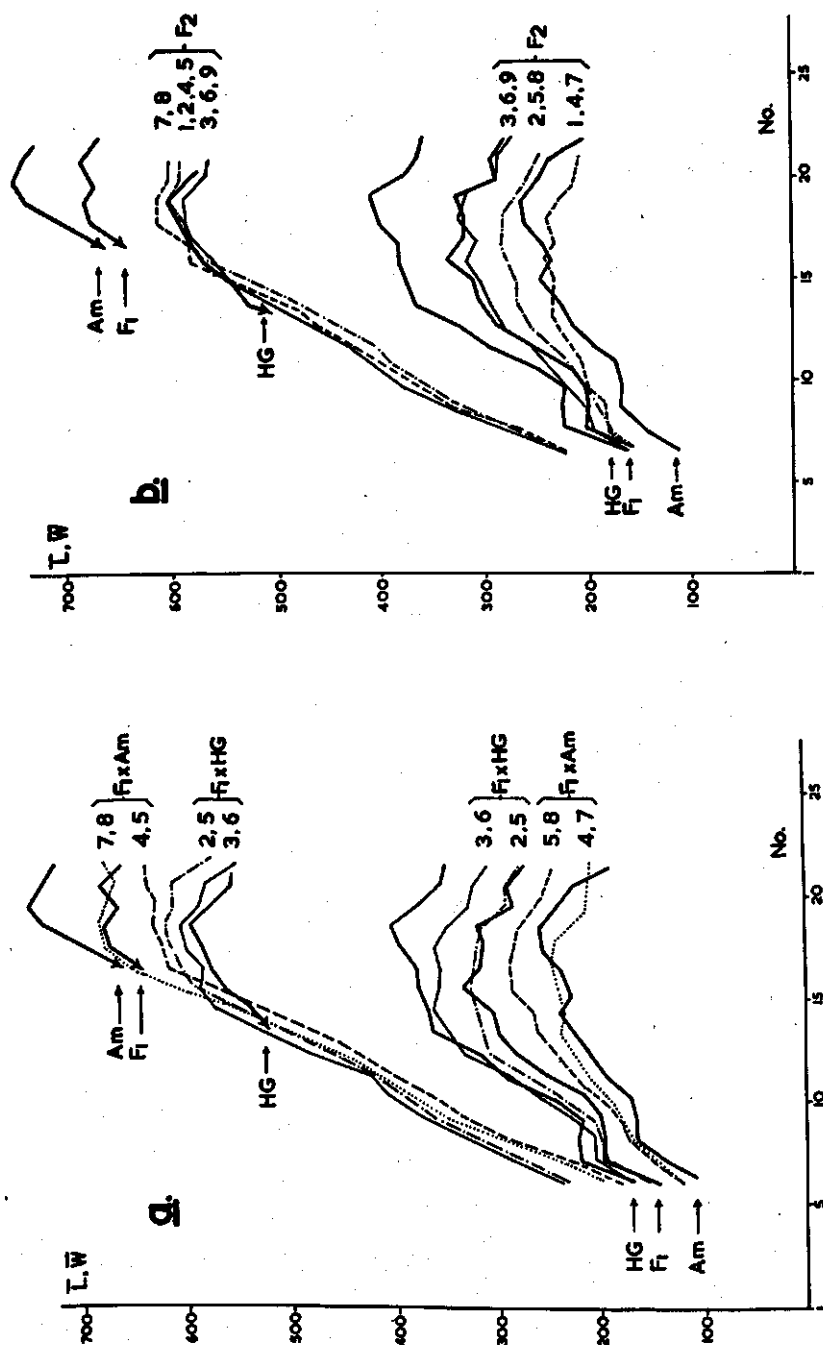


Fig. 23 (a-b). L and W plotted against No. of the leaf for backcross types (Fig. a) and F₂ types (Fig. b). Plants of different genotypes have been pooled (see text) in the way indicated by the type numbers. Graphs of the parents and the F₁ (heavy lines) have been added for comparison.
Year: 1953. Symbols used: Ch 2 § 4.

generated by Pt-pt and Br-br (nor by genes for number of leaves as shown in Ch 2 § 5b).

Therefore the differences in \bar{L} between HG and Am must have been generated mainly by genes of the genic background. Modifier segregation also appears from the \bar{L} -differences between the two backcrosses (Fig. 23a) and from the larger variance for leaf length within the F_2 types as compared with that within the non-segregating generations, as will be shown in Ch 2 § 5h.

Turning to the \bar{W} -graphs it is seen that leaf width largely depends upon the Pt-pt factor-pair: In the F_2 (Fig. 23b), the types 3, 6, 9 (pt pt) have broad leaves, the types 2, 5, 8 are intermediate and the types 1, 4, 7 have narrow leaves; in the backcrosses (Fig. 23a) the situation is analogous. Leaf width is not influenced by the factor-pair Br-br, as follows from the coinciding \bar{W} -graphs listed above, nor by genes for number of leaves as was shown in Ch 2 § 5e. However, the action of modifying genes, though not large, cannot be neglected in view of 1) the level difference between the \bar{W} -graphs for the Pt pt genotype in the two backcrosses (Fig. 23a), 2) the reduced distance between the \bar{W} -graphs within each of the segregating families as compared with that between parents and F_1 (Fig. 23a-b), and 3) the larger variance for leaf width within the F_2 types as compared with that within the non-segregating generations (Ch 2 § 5h). In the backcrosses and the F_2 the \bar{W} -graphs diverge at higher nodes than those of the parents and the F_1 (Fig. 23a-b).

An interesting phenomenon appearing from the \bar{W} - and \bar{L} -graphs of Fig. 23a-b, is the reduced maximum leaf size of the backcrosses and still more of the F_2 as compared with the average of the parents or with the F_1 which is about intermediate. It is difficult to understand what type of genic interaction is responsible for this "loss of vigour". One might suggest that the drop from F_1 to F_2 is due to loss of heterozygosity and that the parents represent homozygous genotypes that are balanced in a subtle and complex way as the outcome of selection.

\bar{S} -graphs (Fig. 24).

The mean leaf shape graphs for the four types of each of the two backcrosses, along with those for the parents and the F_1 , are presented in Fig. 24. The features observed are reflections of the behaviour of the individual \bar{L} - and \bar{W} -graphs (cf. Fig. 23a). In the backcross $F_1 \times HG$ the four \bar{S} -graphs resemble each other two by two. The difference between the two pairs is governed by Pt-pt, which has a large influence on leaf width and to a small extent also on leaf length in the region of maximum \bar{L} . The small differences between the two members in each pair may have been generated by Br-br, though the influence of this factor-pair on \bar{L} and \bar{W} was thought to be negligible. In the backcross $F_1 \times Am$, however, the four graphs all diverge. Again, Pt-pt has a large influence as a result of its action on leaf width. The action of Br-br is now rather pronounced and reflects the influence of this

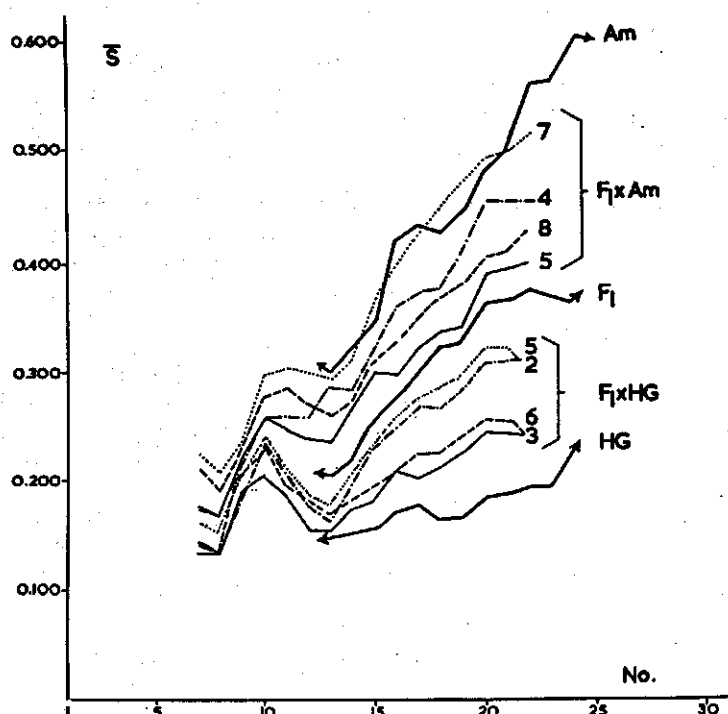


Fig. 24. \bar{S} plotted against No. of the leaf for the parents, the F_1 and the backcross types. Year: 1953. Symbols used: Ch 2 § 4.

factor-pair on leaf length, which was somewhat unexpected in this backcross.

The fluctuations between the 7-th and the 13-th leaf correspond to those shown by the parents and the F_1 (Fig. 18b). As has been said in Ch 2 § 5b the only comment to be made is that during the period of rapid growth of these leaves the plants were transplanted from the glasshouse to the field.

\bar{A} -graphs (Fig. 25a-b).

The graphs for the angle of venation of the 9 F_2 genotypes fall into three groups (Fig. 25a), generated by the factor-pair Pt-pt. In the region of differentiation, Pt Pt (types 1, 4, 7) has acute, Pt pt (types 2, 5, 8) intermediate, and pt pt (types 3, 6, 9) obtuse angles. The individual data cannot be combined per group of three types, because Br-br also influences the angle of venation. Within each of the three Pt-pt groups the angles become more obtuse in the order br br \rightarrow Br br \rightarrow Br Br. The difference between br br (—) and Br br (---) is comparatively small and tends to disappear above leaf No. 17. That between Br br (---) and Br Br

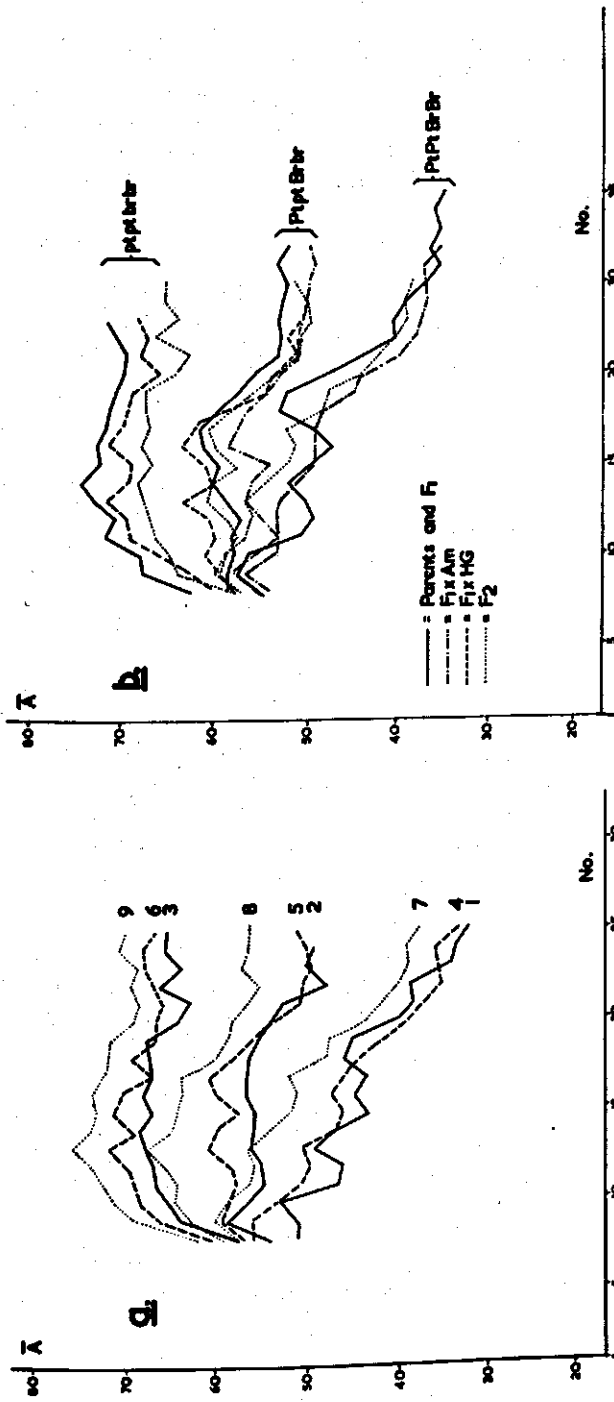


Fig. 25 (a-b). Fig. a: \bar{A} plotted against No. of the leaf for the 9 F2 (HG x Am) types.
 Fig. b: \bar{A} plotted against No. of the leaf for pt br br (type 3) plants, Pt pt Br br (type 5) plants
 and Pt Pt Br Br (type 7) plants from parent-, F1-, backcross- and F2-families.
 Year: 1953. Symbols used: Ch 2 8 4.

(...), however, is rather pronounced. The backcross graphs (not presented) compare in the same way.

Fig. 25b shows the \bar{A} -graphs for the parents and the F_1 , and for the types 3 (pt pt br br, like HG), 5 (Pt pt Br br, like F_1) and 7 (Pt Pt Br Br, like Am) from the backcrosses and the F_2 . It appears that the parental difference in angle of venation is for the greater part generated by Pt-pt and Br-br, though modifier segregation cannot be neglected, because 1) notably in the pt pt br br group the backcross graph and the F_2 graph fall short of the parent graph, and 2) the within-type variance for angle of venation (Ch 2 § 5h) is larger for the types from the segregating generations than for the parents and the F_1 .

Summarizing the conclusions of this paragraph it can be said that:

1. Pt-pt has a large influence on leaf width and on angle of venation.
2. Br-br has no influence on leaf width and affects the angle mildly.
3. Both factor-pairs have only a small effect on (maximum) leaf length. With respect to Pt-pt this implies that Pt makes the petiole longer (see e.g. the types 1, 2 and 3 in Fig. 6) without affecting the total leaf length.

Ch 2 § 5g. *Frequency distribution of angle of venation*

In addition to the \bar{A} -graphs discussed in the preceding paragraph (cf. Fig. 25a-b), Fig. 26 presents frequency polygons for the angle of venation of leaf No. 8 (commencing differentiation between the types) and leaf No. 21 (full differentiation).

Confining the discussion to leaf No. 21 and considering the total F_2 polygon first (inset; upper right corner) it is clear that no inferences can be made from it regarding the inheritance of angle of venation. When for the F_2 and the backcrosses the data are grouped according to the Pt-pt genotypes, the influence of this factor-pair becomes very apparent. The distributions slightly overlap in the backcrosses and considerably in the F_2 . This is partly due to the segregation of Br-br as can be understood from the graphs of Fig. 25a-b. Thus the tail on the right side of the polygon for Pt pt from $F_1 \times \text{Am}$ is not found in the F_1 polygon and is generated by type 8 (Pt pt Br Br). The leftward shift of the Pt Pt polygons in the F_2 and the backcross $F_1 \times \text{Am}$ as compared with the Am (Br Br) polygon is for a large part due to the presence of the br br and Br br genotypes.

Ch 2 § 5h. *Variances of leaf length, leaf width and angle of venation*

In order to obtain information about modifier segregation the variances for leaf length, leaf width and angle of venation of leaf

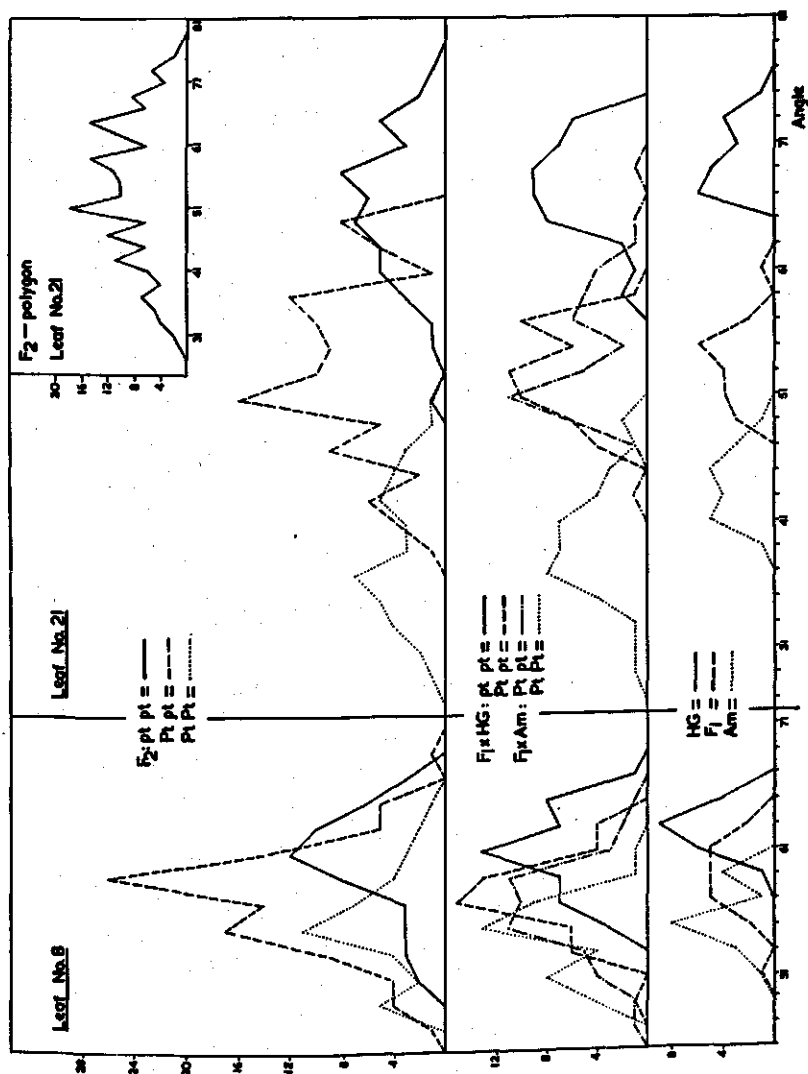


Fig. 26. Frequency polygons for angle of venation (abscissa) of leaf No. 8 (left) and leaf No. 21 (right) of parent-, F1-, backcross-, and F2-plants. The plants of the backcrosses and the F2 have been grouped according to the Pt-pt genotypes. Inset (upper right corner). The total F2 polygon for leaf No. 21. Year: 1953. Symbols used: Ch 2 § 4.

Table 8. Variances for angle of venation, leaf length and leaf width of leaf No. 18 in the parents and the F_1 , and in the parent and F_1 genotypes (in terms of Pt-pt and Br-br) from the backcrosses and the F_2 .
Number of plants between brackets.

| Character | Genotype | HG (53-201) | F ₁ (53-205) | Am (53-204) | F ₁ x HG (53-206) | F ₁ x Am (53-207) | F ₂ (53-208) |
|--------------------|---|----------------|----------------------------|----------------|---------------------------------|---------------------------------|---------------------------------------|
| Angle (degrees) | pt pt br br Pt pt Br br Pt Pt Br Br | 8.73 (20) | 13.76 (14) | 15.20 (16) | 7.01 (22) 18.69 (24) | 31.43 (16) 52.72 (19) | 48.57 (8) 51.25 (45) 47.22 (11) |
| Length (cm) | pt pt br br Pt pt Br br Pt Pt Br Br | 8.51 (20) | 11.61 (14) | 20.12 (16) | 7.40 (22) 9.00 (24) | 28.13 (16) 51.94 (19) | 36.41 (8) 53.78 (45) 54.29 (11) |
| Width (cm) | pt pt br br Pt pt Br br Pt Pt Br Br | 6.45 (20) | 6.84 (14) | 9.72 (16) | 6.62 (22) 8.40 (24) | 6.40 (16) 17.20 (19) | 47.07 (8) 24.44 (45) 12.49 (11) |

No. 18 were calculated within the parent and F_1 families, and within the parent and F_1 types from the segregating generations. The data are shown in Table 8.

The variance of each of the three characters tends to be higher for Am than for HG, the F -values being 1.74 ($P = 0.20-0.10$) for angle of venation, 2.36 ($P = \pm 0.05$) for leaf length and 1.51 ($P = \pm 0.20$) for leaf width. The comparisons between the different generations have been restricted to identical genotypes in terms of Pt-pt and Br-br. Now, apart from leaf width in the case of Pt Pt Br Br, the variances of the F_2 types are significantly larger than those of the corresponding non-segregating families. This indicates modifier segregation. In the backcross $F_1 \times$ Am the variances are larger at the 10% or 5% level of probability, with the exception of the leaf width variances. However, in the backcross $F_1 \times$ HG the variances are not larger than those of the corresponding non-segregating generations. The latter result is somewhat surprising. One might ask whether this is due to a preponderance of dominant modifiers in the HG genome, to a higher phenotypic homeostasis induced by the HG genome, or to other causes.

Ch 2 §5i. *Concluding remarks*

As length and width of the tobacco leaves change gradually from bottom to top of the stem, no region of constant size in one or the other of the two dimensions is present. Leaf shape, expressed as the logarithm of the length/width ratio, remains more or less constant for a considerable number of nodes in HG and Atr, but not in KE and Am (cf. Figs. 17b, 18b and 19b). Consequently one cannot speak of a region of constancy in leaf shape as a general phenomenon.

Therefore the plants cannot be adequately represented by one or a few leaves only. The use of "graphs of leaf development", obtained by plotting the metrical data against the number of the leaf, offers several advantages:

1. The plants are studied as entities. The trends of progressive change from leaf to leaf along the stem are fully brought to light.
2. Irrelevant fluctuations round these trends, mainly due to the fluctuating environmental conditions during growth of the successive leaves, can be discounted.
3. The levels on the stem, at which differentiation between different genotypes commences, can be determined and compared for different characters.

In the present study the graphs of leaf development proved to be a valuable tool for:

1. Comparing leaf development in different varieties, comparing reciprocal F_1 's, and studying the relation between parents and F_1 .

2. Analyzing the action of known factor-pairs, viz. Pt-pt and Br-br, in segregating generations.
3. Determining how leaf development is affected by the number of leaves.
4. Obtaining information about segregation of modifying genes.

Ch. 2 § 6. A FEW REMARKS ON GROWTH OF THE LEAVES

On the plants of the families 53-201 (HG), 53-204 (Am), 53-205 (F₁), 53-206 (F₁ x HG), 53-207 (F₁ x Am) and 53-208 (F₂), length and width of the growing leaves (Nos. 7, 8, etc.) were measured every 2-3 days as soon as they exceeded a length of 25-40 mm.

As can be seen from visual inspection of the growing plants, the difference in shape between the HG and Am leaves arises early during leaf growth. From the measurements taken it appears that length and width of the Am leaves increase at about the same rate, that is the shape of the Am leaves does not change much during growth. In the HG leaves, however, width increases at a higher rate than length, that is the HG leaves become less elongated. This means that the difference in leaf shape between HG and Am, already present before the leaves reach a length of 25-40 mm., increases during the period of growth under study.

As a means of description of the relation between leaf length (L) and leaf width (W) during growth, I used the linear relationship $\log L = \log b + k \log W$, in which k (the slope of the line) is called the "relative growth rate". In this way the "allometric technique" (HUXLEY, 1932) has been used as a tool in the study of shape in botanical objects by several investigators, among which SCHWARZ et al. (1951), who studied leaf shape in a tobacco variety resembling our KE. For each leaf, $\log L$ was plotted against $\log W$ and straight lines were fitted to the points. For HG the k -values of the successive leaves on the stem did not vary much and averaged 0.8 (a k -value less than unity implies that the leaf becomes less elongated during its growth). In the case of Am, however, the k -value was not constant from leaf to leaf and moreover the points of each leaf could not be represented by one straight line, changes in k giving rise to two and sometimes three straight line segments. Therefore Am cannot be represented by one k -value, even when we confine ourselves to one specified leaf number. As a rough estimate $k = 0.95-1.00$ might be given as an average for Am.

It was originally intended to study in the segregating generations to what extent differences in k are governed by the Pt-pt factor-pair. However, no clear-cut results could be obtained, partly owing to the fact that each plant could not be represented by a single k -value. Other aspects of leaf growth in the segregating generations are being studied.

Ch 2 § 7. DISCUSSION

In Chapter 1 the results of the Mendelian analysis of the leaf characters under study have been presented, and discussed in connection with the data in literature. The different aspects of the action of the factor-pairs Pt-pt, Pd-pd and Br-br are summarized in Ch 1 § 14 ("Summarizing discussion"). In Chapter 2 additional and more detailed information has been obtained with the help of metrical data. Combining the results given in the two chapters we can briefly describe the action of the factor-pairs on the morphology of the leaf as follows.

The action of the factor-pair Br-br is practically restricted to the leafbase, in that it differentiates between narrowly winged to wingless (petioled; br br) and broadly winged (sessile; Br Br). Br-br does not influence leaf width, its action on leaf length seems to be negligible, and it affects the angle of venation only slightly. The factor-pair Pt-pt influences a complex of characters. Pt makes the leafbase more slender (longer and narrower), the leafblade narrower, and the angle of lateral veins more acute. However, its influence on leaf length, if present at all, can be neglected, which implies that Pt-pt has a large influence on leaf-base (petiole) length without affecting the total leaf length. The factor-pair Pd-pd acts qualitatively in the same way as Pt-pt, but quantitatively its influence is smaller.

In the present paragraph some further considerations regarding the action of the factor-pairs will be advanced.

In organs, shape differences result from differences in amount and direction of cell division and cell elongation. However, shape cannot be expressed as a simple mathematical function of cell division and elongation. AVERY (1933) showed, by making ink lattices on young tobacco leaves, that the rate of increase in area was not uniform all over the leaf ("localised growth"): it was greatest in the basal half of the leaf near the midrib and fell off towards the edges and towards the tip. Growth is completed at the tip first and at the base last. The problem of how our factor-pairs affect these complicated relationships during growth falls outside the scope of the present investigation. The kind of action of the genes will be discussed from the observations on the net result: the full-grown leaf.

ANDERSON and OWNBEY (1939) who compared morphological and anatomical features of *N. alata* and *N. langsdorffii* - the former has narrower leaves, narrower bracts, longer calyx lobes and a more pointed ovary - concluded: "It seems probable that all of these correlated differences rest on a difference in the mechanism of cell elongation". The inference of a common mechanism of causation seems sound in view of the homology of the organs concerned. In itself, however, a "genetic coefficient" can only be inferred safely when it is shown by a genetic experiment that the correlations stated do not break down as a result of recombination. On the other hand the genetic experiment provides a means of tracing "genetic coefficients".

The present investigation has shown that in going from pt pt pd pd to Pt Pt Pd Pd each gene substitution makes the petiole (leafbase) longer, the wings narrower (to a relatively small extent), the leafblade narrower, and the angle of venation more acute. Qualitatively the effects of Pt-pt and Pd-pd are the same. Quantitatively the effect of Pt-pt is larger than that of Pd-pd for each of these characters. So between the genes (units of physiological action) and the character-complex there is a cumulative dosage relationship, and the dosage effect of Pt is quantitatively different from that of Pd.

Two questions now arise: 1) by what genetic coefficient can the character-complex be briefly described in terms of "more and less", and 2) referring to the "one gene - one enzyme" hypothesis, what chemical substance may (in different amounts) be produced by the genes ultimately affecting this genetic coefficient. WENT (1951) suggested to "... consider leaf form as a synthesis of two separate tendencies which are independently controlled by separate sets of growth factors: a tendency to linear development of veins, and to surface development of mesophyll". Comparing pinnately veined and palmately veined leaves he argues: "In a pinnate leaf a decrease in the amount of phyllocaline results in a narrowing of the distance between the veins, which thus make a smaller angle with the midrib. This results in a narrower leaf. Yet the veins can retain the same length if the same amount of caulocaline is available. In a palmate leaf, on the other hand, the major palmate veins retain the same angle, but a decrease in phyllocaline results in lobing". It is of interest to comment on this hypothesis in connection with the present findings.

1. Regarding the growth substances phyllocaline and caulocaline WENT gives no consistent formulations. On p. 288 (l. c.) he says that they are "... purely physiological names, that is functional, not chemical"; on p. 292 (l. c.), however, he speaks of "... the synthesis of the phyllocaline precursor and its transformation into the active material...". As long as no proof is given for the existence of the substances it is better only to speak of "amount of mesophyll" and "length of veins".
2. For pinnately veined leaves, WENT suggests a causal relationship between leaf width and angle of venation. This suggestion is supported by the pleiotropic action and the dosage relationships of Pt-pt and Pd-pd as found in the present investigation. We may say that in acting upon leaf width, wing width and angle of venation, these factor-pairs affect the genetic coefficient "amount of mesophyll". In this connection it is interesting to note that the extremely slender Pt Pt Pd Pd leaf type, notably when not quite full-grown, often has a contour that curves inwards between the lateral veins near the tip. This may correspond to the effect of reduced mesophyll growth (lobing) in palmately veined leaves. One of the examples given by WENT relates to HONING's *N. tabacum* deformis. This mutant type has extremely long, naked petioles and only a small leafblade. In the higher leaves mesophyll growth is so much

reduced that only naked midribs remain. In discussing HONING's investigations (Ch 1 § 13) it was pointed out that the gene concerned (def) is comparable in action to Pt and Pd, but that its effect is much more extreme. It is an open question by what biochemical processes the genes affect the amount of mesophyll growth, and whether this action is on an intra- or intercellular level.

3. WENT puts that length of veins and amount of mesophyll are independently controlled aspects of leaf shape. This point too finds support in the results of the present investigation. In Ch 2 § 5f it has been shown that Pt-pt, which has a large effect on the amount of mesophyll, has no influence on leaf length, i.e. on the length of the midrib. The comparison between the length of the lateral veins of the Pt Pt and pt pt leaves is somewhat difficult, as it cannot be determined with certainty what point near the leaf margin must be taken as the end-point of a lateral vein. Before branching off from the midrib the lateral veins are seen to grow together with it for some distance. This distance is longer in the Pt Pt leaves (acute angles) than in the pt pt leaves (obtuse angles). The difference is about 0.5-1.2 cm. (cf. Ch 1 § 6a). It is my impression that, when making allowance for this part of the veins, the lateral veins of the narrow Pt Pt leaves are not much shorter, if shorter at all, than those of the broad pt pt leaves.

A further point to be considered is whether and how the effect of Pt-pt and Pd-pd on the length of the leafbase (petiole) can be referred to in terms of amount of mesophyll only. This is not clear yet. In the leafbase of the sessile types (Br Br), which has been defined as the part of the leaf between axil and sinus mid-point (cf. Ch 1 § 3a), there is, in comparison to the leafblade, a reduction in tissue as a whole, in that the lamina is narrow in this part and the lateral veins rather unpronounced. Comparing the Pt-pt types on the Br Br level, it can be said that 1) a longer leafbase seems not associated with extra length of the midrib, as Pt-pt influences leafbase length without affecting the total leaf length, 2) for each type the angle of lateral veins in the leafbase is about the same as in the leafblade, that is acute in the Pt Pt type and obtuse in the pt pt type, and 3) these lateral veins are not much shorter in the relatively narrow based Pt Pt type than in the relatively broad based pt pt type. In these respects the statement that Pt-pt does not affect length of veins can be maintained. However, it remains to be settled whether or not in the leafbase of the Pt Pt type lateral veins are included, the equivalents of which in the shorter based pt pt type fall outside this region and consequently show unreduced growth. If so, Pt-pt would still affect the length of specified lateral veins. To determine what lateral veins are to be compared in the different leaf types, detailed morphological and anatomical studies on the growing and the full-grown leaves are required for establishing the exact relationships between leafblade and leafbase.

Regarding the action of Br-br a few remarks may be made. As on each Pt-pt level the distance from axil to sinus midpoint in the sessile type corresponds well with the length of the petiole in the petioled types (cf. Fig. 6), the leaf parts concerned have been regarded as equivalents in the present analysis. We may say that Br-br, the action of which is restricted to the leafbase (region of reduced tissue growth; see above), differentiates between a relatively mild reduction (Br Br; sessile types) and a pronounced reduction (br br; petioled types).

Pt-pt affects leaf shape by controlling leaf width, not leaf length. A comparable situation has been described by SIRKS (1931), who studied shape and size of *Vicia faba* leaflets. He demonstrated the existence of genes that affect the shape indices (the ratios of width, terminal length and basal length) by controlling the dimensions in a specific and independent way. The levels at which these genes act are determined by genes for leaf size, that is by genes that affect all dimensions simultaneously. SINNOTT (1935, 1936, 1937), studying fruit shape in Cucurbitaceae, did not find genes affecting one dimension without affecting the other dimensions, and introduced the conception "shape genes" as opposed to "dimensional genes". He inferred from allometric graphs (cf. Ch 2 § 6) that his shape genes primarily affect the relative growth rate (k). SINNOTT (1956) says: "There is good evidence that the inheritance of form is independent of that of size, and that what is inherited is not particular dimensions but particular growth ratios or patterns". In view of the situation in *Vicia faba* and in *N. tabacum*, however, it cannot be ignored that so-called dimensional genes should also be taken into consideration as shape determining factors.

SUMMARY

1. The present studies on the inheritance of leaf shape in *Nicotiana tabacum* have been mainly performed on F₁'s, backcrosses, F₂'s and F₃'s, derived from a complete set of crosses between four pure lines (Figs. 1 to 4): Hongaars Gartenblatt (HG), Atropurpurea (Atr), Amersfoorter (Am) and Keurhorst Elite (KE).
2. The characters studied are leafbase shape (petiole length and wing width), angle of lateral veins, leaf length, leaf width, length/width ratio, number of leaves, and flower shape.
3. The presentation has been divided into two parts. Chapter 1 deals mainly with the qualitative aspects, that is with the results obtained by visual inspection of the plants (major genes). Chapter 2 presents the analysis of metrical data on all the full-grown leaves along the stem (graphs of leaf development). Graphical comparisons in segregating generations are confined to the HG-Am material. They provide more detailed information about the action of the major genes and of modifying genes.
4. The frame of leaf (and flower) shape variation in the material depends on three independently segregating factor-pairs: Pt-pt, Pd-pd and Br-br. The parent formulae are:

| | |
|------------|---------------------|
| HG and Atr | = pt pt pd pd br br |
| Am | = Pt Pt pd pd Br Br |
| KE | = pt pt Pd Pd Br Br |

5. The F₁'s are in all respects more or less intermediate between the corresponding parents. No differences between reciprocal F₁'s have been observed.
6. The 9 F₂ types from the cross HG (or Atr) x Am and the 9 F₂ types from the cross HG (or Atr) x KE are given in Fig. 6 and Fig. 14 respectively. Types from KE x Am are presented in Fig. 15. Apart from the HG-type (type 3 in Figs. 6 and 14), the Am-type (7 in Fig. 6), the KE-type (7 in Fig. 14), and two F₁-types (5 in Figs. 6 and 14), new homozygote types are recognized, viz. pt pt pd pd Br Br (9 in Figs. 6 and 14), Pt Pt pd pd br br (1 in Fig. 6), pt pt Pd Pd br br (1 in Fig. 14), and Pt Pt Pd Pd Br Br (Fig. 15). For each factor-pair the heterozygote is more or less intermediate between the homozygotes and can be distinguished phenotypically from them.
7. The factor-pair Br-br governs wing width in that it differentiates between petioled types (br br; left column in Figs. 6 and 14) and sessile types (Br Br; right column). The length of the leafbase in the sessile types corresponds to the length of the

petiole in the petioled types (within-row comparisons in Figs. 6 and 14). Substitution of br by Br makes the angle of venation somewhat more obtuse.

8. The factor-pairs Pt-pt and Pd-pd are pleiotropic, and cumulative in action. Qualitatively their action is the same, but quantitatively the effect of Pt is markedly larger than that of Pd. When in pt pt pd pd (bottom row in Figs. 6 and 14) pt pt is substituted by Pt Pt (top row in Fig. 6) or pd pd by Pd Pd (top row in Fig. 14), the petiole (leafbase) becomes long, the wings and the leafblade narrow, the angle of venation acute, the calyx and the corolla slender, and the limb pointed. The factor-pairs have practically no influence on the total leaf length.
9. HG and Atr have a low, Am has a high, and KE has an intermediate number of leaves. Leaf number is almost wholly independent of the three main factor-pairs. In the segregating generations, differences in leaf number affect only those parts of the graphs (for leaf length, leaf width, L/W ratio and angle of venation), which cover the higher nodes. Maximum leaf length is not influenced.
10. Regarding petiole length within each of the leaf shape types, a higher number of leaves induces an upward extension of the region of increasing petiole (leafbase) length and decreasing wing width. The rate of increase per node is not affected. The high positive correlation between number of leaves and maximum petiole length must be taken into account for efficient classification of the Pt-pt and Pd-pd types.
11. A general device for the identification of factors for leafbase elongation (etc.) in sessile varieties has been discussed in Ch 1 § 12.
12. The carmine flower colour of Atr (Pk Pk) is completely dominant over pink. Pk-pk is closely linked with br-Br.
13. In Ch 1 § 13 the data presented by other authors have been discussed in connection with the present findings. When necessary and possible, reinterpretations were suggested. The conclusion is that the leaf shape factors in the literature are similar in action and presumably identical to ours.
14. In Ch 2 § 7 the action of Pt-pt and Pd-pd on leaf shape has been discussed in terms of amount of mesophyll and length of veins.

S A M E N V A T T I N G

1. Dit onderzoek naar de erfelijkheid van de bladvorm bij tabak (*Nicotiana tabacum*) werd in hoofdzaak verricht met behulp van F_1 's, terugkruisingen, F_2 's en F_3 's, voortgekomen uit een vierhoekskruising tussen de volgende lijnen (vgl. Fig. 1-4): Hongaars Gartenblatt (HG), *Atropurpurea* (Atr), Amersfoorter (Am) en Keurhorst Elite (KE).
2. De bestudeerde kenmerken zijn: vorm van de bladbasis (steellengte en vleugelbreedte), hoek van de zijnerven met de hoofdnerf, bladlengte, bladbreedte, lengte/breedte quotient, blad-aantal en bloemvorm.
3. De bespreking van het materiaal is in twee gedeelten gesplitst. In hoofdstuk 1 worden voornamelijk de resultaten behandeld, verkregen bij beoordeling van de bladtypen op het oog ("major genes"). Hoofdstuk 2 bevat de analyse van metingen verricht aan alle door de plant gevormde bladeren. De grafieken, die het verloop van de verschillende kenmerken over de plant weergeven ("graphs of leaf development"), verschaffen nadere gegevens over de werking der hoofdfactoren en over de aanwezigheid van modificerende genen.
4. De variatie in bladvorm (en bloemvorm) berust in hoofdzaak op drie onafhankelijk splitsende factorenparen: Pt-pt, Pd-pd en Br-br. De genotypen van de ouders zijn:

| | |
|-----------|---------------------|
| HG en Atr | = pt pt pd pd br br |
| Am | = Pt Pt pd pd Br Br |
| KE | = pt pt Pd Pd Br Br |

5. De F_1 's zijn in alle opzichten min of meer intermediair. Er werden geen verschillen tussen reciproke F_1 's geconstateerd.
6. De 9 F_2 -typen behorende bij de kruising HG (of Atr) x Am en de 9 F_2 -typen van de kruising HG (of Atr) x KE zijn afgebeeld in resp. Fig. 6 en Fig. 14. Typen afkomstig van de kruising KE x Am worden in Fig. 15 gegeven. Naast het HG-type (type 3 in Fig. 6 en 14), het Am-type (7 in Fig. 6), het KE-type (7 in Fig. 14) en twee F_1 -typen (5 in Fig. 6 en 5 in Fig. 14), werden de volgende nieuwe homozygote typen verkregen:
 pt pt pd pd Br Br (type 9 in Fig. 6 en 14), Pt Pt pd pd br br (1 in Fig. 6), pt pt Pd Pd br br (1 in Fig. 14) en Pt Pt Pd Pd Br Br (Fig. 15). Voor elk der drie factorenparen kan de heterozygoot phaenotypisch onderscheiden worden van de beide homozygoten.
7. Het factorenpaar Br-br beïnvloedt de vleugelbreedte: gesteelde typen (br br; linker kolom in Fig. 6 en 14) en zittende typen (Br Br; rechter kolom). De lengte van de bladbasis van het zittende type komt in elke rij overeen met de steellengte van

de gesteelde typen (Fig. 6 en 14). Br heeft een geringe, stomper makende, invloed op de nerfhoek.

8. De beide pleiotrope factorenparen Pt-pt en Pd-pd vertonen overeenkomstige werking en zijn cumulatief. De invloed van Pt is belangrijk groter dan die van Pd. Wanneer in pt pt pd pd (onderste rij in Fig. 6 en 14) pt pt vervangen wordt door Pt Pt (bovenste rij in Fig. 6) of pd pd door Pd Pd (bovenste rij in Fig. 14), dan ontstaan naar verhouding een lange bladsteel (bladbasis), een smalle vleugel, een smalle bladschijf, een scherpe nerfhoek en een slanke, spitse bloem. De invloed van beide factorenparen op de totale bladlengte valt te verwaarlozen.
9. Het bladaantal van HG en Atr is laag, dat van Am hoog en dat van KE intermediair (vgl. Table 1). Tussen de verschillende F₂-typen werden geen of slechts kleine verschillen in gemiddeld bladaantal gevonden. Uit de onder 3) genoemde grafieken blijkt, dat verschillen in bladaantal geen invloed hebben op de lengte van de bladeren tot en met het blad, waarbij de maximum lengte bereikt wordt. De grafieken voor bladbreedte en nerfhoek blijken voor een aantal daarboven volgende bladeren nog samen te vallen.
10. Bij elk der typen neemt vanaf de lagere bladeren de steellengte toe en de vleugelbreedte af. Naarmate het aantal bladeren groter is, wordt de maximum steellengte groter en pas bij een hoger bladnummer bereikt (een voortgezette, geen versnelde toename). Na het onderkennen van deze sterke, positieve correlatie tussen bladaantal en maximum steellengte was het mogelijk de verschillende Pt-pt en Pd-pd klassen volledig te scheiden.
11. In hoofdstuk 1 § 12 wordt een kruisingsschema besproken, waarmee het genotype (Pt-pt en Pd-pd) van variëteiten met zittende bladeren geïdentificeerd kan worden.
12. De donkerrode bloemkleur van Atr (Pk Pk) is volledig dominant over roze bloemkleur. Pk-pk is vrij sterk gekoppeld met br-Br.
13. In hoofdstuk 1 § 13 wordt een uitvoerige analyse, en waar nodig herinterpretatie, van de betreffende literatuur gegeven. De conclusie is, dat de daarin aan te treffen factoren voor bladvorm dezelfde werking vertonen als de hier onderzochte en daaraan hoogstwaarschijnlijk identiek zijn.
14. In hoofdstuk 2 § 7 wordt de aard van de werking van Pt-pt en Pd-pd op de bladvorm nader besproken. Zij beïnvloeden de „hoeveelheid mesophyll“, niet de „lengte der nerven“ (vgl. WENT, 1951).

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STELLINGEN

I

De genetische verschillen in bladvorm bij *Nicotiana tabacum* berusten grotendeels op een viertal factorenparen.

Dit proefschrift.

II

De invloed van de factorenparen Pt-pt en Pd-pd op de vorm van het tabaksblad komt overeen met de voorstellingen van WENT omtrent de bepaling van de bladvorm.

WENT, F.W., 1951. In "Plant growth substances" (ed. F.SKOOG), p.287-298.

Dit proefschrift.

III

Onderzoek naar de erfelijkheid van morphologische kenmerken van het tabaksblad kan niet op doeltreffende wijze aan afzonderlijke bladeren worden verricht.

Dit proefschrift.

IV

Het ontbreken van een eensluidende terminologie in de biometrische genetica leidt tot ernstige verwarring.

V

Ter verklaring van inteelt- en heterosisverschijnselen moet meer rekening gehouden worden met de betrekkingen tussen niet-allele genen.

VI

De perspectieven, die het verwekken van mutaties biedt voor de plantenveredeling, mogen de aandacht niet afleiden van de noodzaak om de bestaande genetische variatie op doeltreffender wijze ter beschikking van de veredelaar te stellen.

VII

Uit de bevindingen van EAST bij zijn *Nicotiana* onderzoek moet geconcludeerd worden, dat bij de registratie van rassen van zelfbevruchters en bij de in stand houding van deze rassen, de genoemde „constantheid van zuivere lijnen" niet als vanzelfsprekend mag worden aangenomen.

EAST, E.M., 1935. *Genetics* 20, p.443-451.

VIII

De grootheid „heritability” dient met veel reserve gehanteerd te worden, zowel gelet op de wijze waarop zij berekend wordt, als gelet op haar bruikbaarheid bij vergelijken en voorspellen.

IX

Wanneer geen afwezigheid van dominantie verondersteld wordt, luidt de formule voor de berekening van de erfelijkheidsgraad (h^2) uit de moeder-dochter correlatie: $r = a h^2$, waarin $0 \leq a \leq \frac{1}{2}$.

X

Voor een betrouwbare oogstraming zijn gegevens over de weersinvloeden op opbrengst en gehalte van suikerbieten, zoals verzameld door STUMPEL en RIETBERG, onvoldoende.

STUMPEL, J. M. H. en RIETBERG, H., 1949.
Meded. Inst. Rat. Suikerprod. 19, p. 65-89.