

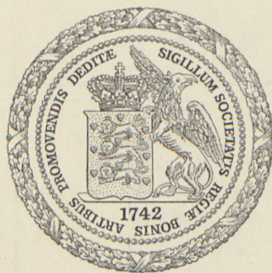
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CYTO-TAXONOMICAL STUDIES  
ON *CALAMAGROSTIS EPIGEIOS* (L.) ROTH,  
*AMMOPHILA ARENARIA* (L.) LINK, AND  
THEIR HYBRIDS (*AMMOPHILA BALTICA*  
(FLÜGGE) LINK)

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# I. Introduction.

## a. Statement of the problem.

*Ammophila baltica* (Flügge) Link (*Psamma baltica* R. and S.) is a dune grass; the main distribution is in the Baltic coastal regions. The plant has long been considered a hybrid between Wood Smallreed, *Calamagrostis epigeios* (L.) Roth and Marram, *Ammophila arenaria* (L.) Link (*Psamma arenaria* R. and S.).

In 1936, Professor C. A. JØRGENSEN called my attention to this interesting hybrid, and it was attempted to investigate the hybrid origin of *Ammophila baltica* in detail. This investigation showed that in Nature *Calamagrostis epigeios* and *Ammophila arenaria* produce no less than three different hybrids and not, as previously presumed, only one (or two, cf. p. 5).

This complicated hybridization is possible, since *Calamagrostis epigeios*, and presumably also *Ammophila arenaria*, in Nature occur in two different karyotypes, viz. a diploid with 28 chromosomes and a tetraploid with 56 chromosomes. The three hybrids arise from different combinations of these karyotypes, as appears from the diagram given below (diagram 1).

Diagram 1.

	<i>A. arenaria</i> n = 14, 2n = 28	<i>A. arenaria</i> Diploid gametes or tetraploid type, n = 28
<i>C. epigeios</i> , diploid type n = 14, 2n = 28	<i>A. baltica</i> var. <i>intermedia</i> 2n = 28	<i>A. baltica</i> var. <i>subarenaria</i> 2n = 42
<i>C. epigeios</i> tetraploid type n = 28, 2n = 56	<i>A. baltica</i> var. <i>epigeioidea</i> , 2n = 42	

It is proposed that the three hybrids should be considered varieties of *Ammophila baltica* Link under the following names: varietas *subarenaria*, var. *intermedia*, and var. *epigeioidea*. (As regards the justification of these names, cf. p. 60).

According to diagram 1, *A. baltica* var. *intermedia* ( $2n = 28$ ) arises from a crossing between the diploid karyotype of *C. epigeios* ( $2n = 28$ ) and *A. arenaria*; thus, the hybrid contains 14 chromosomes derived from *Ammophila* and 14 from *Calamagrostis*. *Ammophila baltica* var. *epigeioidea* ( $2n = 42$ ) arises from a crossing between the tetraploid karyotype of *C. epigeios* ( $2n = 56$ ) and *Ammophila arenaria* ( $2n = 28$ ); hence, it contains 28 *Calamagrostis* and 14 *Ammophila* chromosomes. The third hybrid which is called *A. baltica* var. *subarenaria* is identical with *Ammophila baltica* Link. It is also triploid ( $2n = 42$ ). According to the diagram, it is composed of 14 *Calamagrostis* chromosomes derived from the diploid karyotype, and 28 *Ammophila arenaria* chromosomes. Unfortunately, however, the author has not been able to find out definitely how the hybrid obtained the 28 *Ammophila* chromosomes. The two possibilities indicated in the diagram exist, but hitherto a tetraploid form of *Ammophila arenaria* could not be found (cf. p. 33).

The validity of the theory presented in diagram 1 has now to be proved. The theory is based on comparative morphological observations, cytological investigations, crossing experiments, and on the geographical distribution of the various types. As an introduction of this argumentation, the previous investigations on *Ammophila baltica* will be summarized in brief.

#### **b. Previous investigations on *Ammophila baltica*.**

Originally, *Ammophila baltica* was considered a genuine species. FLÜGGE (SCHRADER 1806, p. 223) termed it *Arundo baltica*. In ROEMER and SCHULTES (1817, p. 845), it is found under the name *Psamma baltica*, while LINK, who likewise interpreted it as a pure species, gave it the name *Ammophila baltica*<sup>1</sup> (LINK 1827, p. 105—106). As to other synonymes, cf. p. 61.

The theory of a hybrid origin of *Ammophila baltica* was first put forward by ROEPER (1840 and 1844). ROEPER based this theory mainly on the same arguments as held to-day, viz. (1) *Ammophila baltica* is growing in company with *Calamagrostis epigeios* and *Ammophila arenaria*. (2) The morphological characters are intermediate between the two species. (3) It is sterile. As the fourth argument of his hybrid theory, ROEPER moreover states (4) »Zu meiner grossen Freude bestätigt Professor NOLTE in Kiel meine Vermuthung durch seine reiche Erfahrung, und führt als neuen Grund für die Hybridität der *Am. baltica* an, dass sie in Holstein in zwei Formen auftritt. Bekanntlich wiederholt sich bei den Pflanzen die Erscheinung, welche das Thierreich in den Maulthieren und Mauleseln darbietet, dass, je nach der befruchtenden Art, der Bastard bald dem einen, bald dem andern seiner Erzeuger ähnlicher sieht.« (ROEPER 1844, p. 192). Although the last argument must be regarded as less

<sup>1</sup> The generic name *Ammophila* Host was established in 1807, while the generic name *Psamma* P. Beauvais originates from 1812 (cf. ASCHERSON and GRAEBNER 1902, p. 220). *Ammophila* and *Calamagrostis* are referred to two different genera by the majority of taxonomists. ASCHERSON and GRAEBNER (l. c., p. 221), however, regard *Ammophila* as a section within the genus *Calamagrostis*; by them, the species is called *Calamagrostis arenaria* Roth.

valuable to-day, the quotation is nevertheless of interest because it shows that botanists very early realized the variability of *Ammophila baltica*.

The two types mentioned by NOLTE<sup>1</sup> have later been established as two particular varieties by MARSSON (1869, p. 563—564), viz. *varietas subarenaria* Mars. and *var. subepigeios* Mars. According to MARSSON, the first one is identical with *Ammophila baltica* Link. These varieties have not been generally accepted by taxonomists. Thus, they are not mentioned in any of the Danish floral works nor in HEGI (1926). On the other hand, they were accepted by some German botanists, for instance ASCHERSON and GRAEBNER (1902, p. 220). The reason why these two varieties have not been adopted is, no doubt, that some of the characters given by MARSSON are varying and uncertain. MARSSON distinguished the varieties by means of the number of nerves of the inner pale, a character which may vary from one flower to another within the same plant (cf. PRAHL 1890, p. 251, and JUNGE 1913, p. 186—187).

The hybrid theory itself seems but slightly subjected to serious criticism. However, it has been objected that *A. baltica* may grow in localities where one of the parental species *Calamagrostis epigeios* is lacking (cf. discussion and literature in WARMING 1909, p. 61, and TILSE 1910, p. 6—7). Moreover, WARMING (l. c.) draws our attention to the peculiar fact that, in several localities, for instance the West coast of Jutland (Denmark), the two parental species may form large growths without the hybrid being present; even ROEPER observed this phenomenon.

The theory of the hybrid origin of *Ammophila baltica* is now generally adopted by taxonomists and it has been further developed by TILSE (l. c.) on the basis of anatomical investigations. As mentioned previously, the theory is fully confirmed by the present investigation.

## II. Morphology.

The morphological description is divided in three parts: first, an account of the two karyotypes of *Calamagrostis epigeios*, next, a brief description of the parental species and of the three hybrids and, finally, a more detailed investigation of the individual organs of the 5 plants, with the aim to analyse more thoroughly the interaction of the two genomes.

### a. The karyotypes of *Calamagrostis epigeios*.

As mentioned in the introduction, *Calamagrostis epigeios* in Denmark occurs in two karyotypes, one with 28 and one with 56 chromosomes (cf. the cytological

<sup>1</sup> NOLTE seems to have devoted his special interest to *Ammophila baltica*, although he himself wrote but a few lines about it (NOLTE 1826, p. 12). Through NOLTE's correspondence with HORNEMANN, *Ammophila baltica* was introduced into the Danish Flora. HORNEMANN became so much interested in the plant that he mentioned it even in three different publications from the same year. (HORNEMANN 1821, a, b, c).

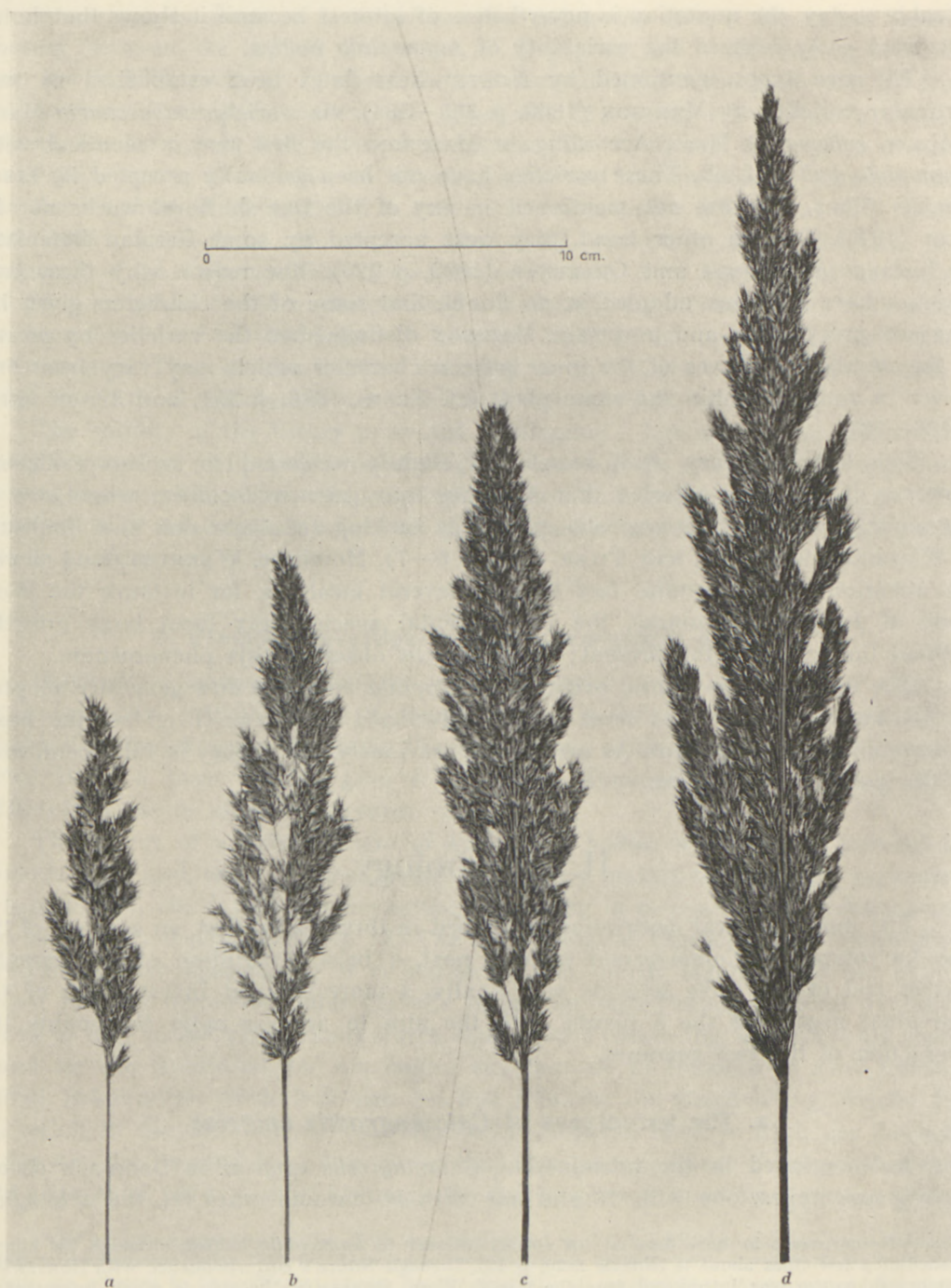


Fig. 1. Inflorescence of four diploid types of *Calamagrostis epigeios*. All from Dueodde (Bornholm).  
Leg. July 1938.  $\times 1/2$ .



Fig. 2. Inflorescence of four tetraploid types of *Calamagrostis epigeios*. All from Northern Jutland. Leg. August 1939.  $\times \frac{1}{2}$ .

section, p. 28). Obviously, it would be of the greatest interest to distinguish these two karyotypes on the basis of distinct morphological characters. In spite of careful observations during several years, it has not been possible, however, to decide on the basis of morphological characters alone whether a plant of *C. epigeios* growing in Nature belongs to the diploid

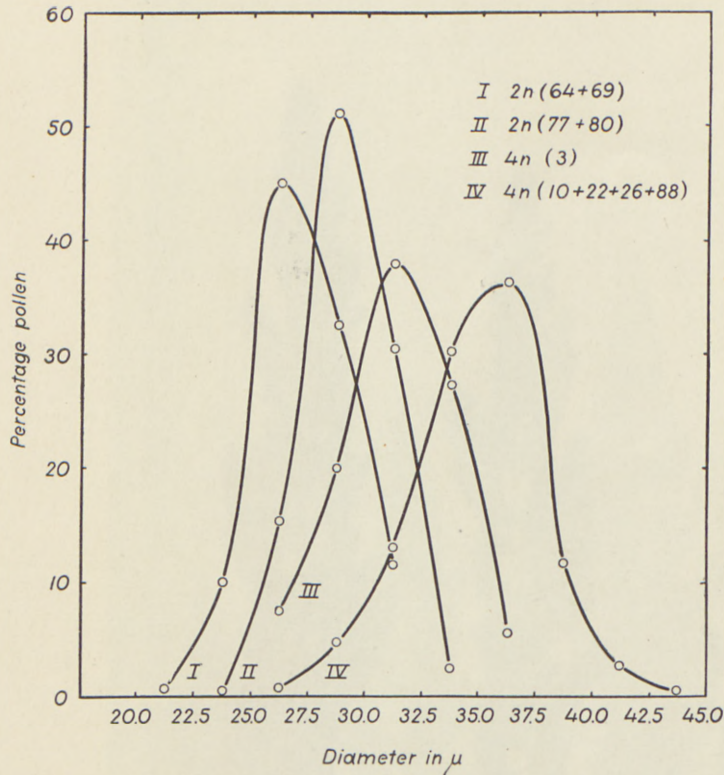


Fig. 3. Curve showing the variation in pollen diameter in diploid and tetraploid *Calamagrostis epigeios* types. Cf. table 1.

or to the tetraploid karyotype. With respect to all qualitative characters, the two forms are identical. Regarding their quantitative properties, the two karyotypes are probably different. A considerable variation within the diploid as well as within the tetraploid type is found, however, and the variations of the two types overlap to such a degree that it is impossible with any certainty to distinguish the two types in Nature on morphological features.

Figs. 1 and 2 show an example of this phenomenon. The four diploid plants in fig. 1 derive from one and the same locality (Dueodde on the Island of Bornholm); apparently, they grew under the same conditions in the outer dune region. The length of the inflorescence varies from 11 to 28 cm. and a corresponding variation is found in other quant-

itative characters, viz. the breadth of the leaves, the size of the flowers (glumes and pales), etc. The four tetraploid plants on fig. 2 (found in North Jutland) show an entirely parallel variation. The length of the inflorescence varies from 10.5 to 22 cm. (Only the four tetraploid plants have been gathered one month later, the inflorescence of the tetraploid therefore being somewhat more faded than that of the diploid.)

It will be pointed out in a later section (cf. p. 47) that in some cases this variation may be of a genotypic origin, in other cases it may be phenotypically conditioned. These pictures show that a distinction of the two karyotypes in Nature on the basis of quantitative characters is not feasible.

However, it must be emphasized that both karyotypes growing in the same place (cf. fig. 17b and c, p. 35—36), the tetraploid generally get somewhat larger flowers,



Table 1.

Species	Pedigree no.	No. of obs.	Diameter in $\mu$										Mean	P. E.
			20.0—22.5	22.5—25.0	25.0—27.5	27.5—30.0	30.0—32.5	32.5—35.0	35.0—37.5	37.5—40.0	40.0—42.5	42.5—45.0		
<i>C. epigeios</i> 4n	3	100	..	..	8	20	38	27	6	1	..	..	31.28	0.27
	10	100	..	..	1	2	8	34	40	11	3	1	35.13	0.27
	22	100	..	..	..	5	12	34	37	11	1	..	34.63	0.26
	26	100	..	..	1	6	21	30	35	6	1	..	33.98	0.27
	88	100	..	..	1	6	12	23	33	19	6	..	35.18	0.32
<i>C. epigeios</i> 2n	64	100	..	7	36	48	9	..	..	..	..	..	27.60	0.19
	69	100	1	13	54	17	15	..	..	..	..	..	26.93	0.23
	77	100	..	..	15	54	29	2	..	..	..	..	29.08	0.18
	80	100	..	1	16	49	31	3	..	..	..	..	29.11	0.19
<i>A. arenaria</i>	144	100	..	..	..	..	..	7	24	53	12	4	38.18	0.22

a more vigorous growth, broader leaves, etc. than the diploid. Thus, the variations shown in figs. 4, 7, and 8 between diploid and tetraploid *C. epigeios* are characteristic of the locality concerned. Since, however, even more conspicuous differences may be found in other localities without any correlation to the chromosome number (figs. 1 and 2), they cannot be applied taxonomically.

Since a macroscopic determination of the karyotypes was impossible, it was attempted to use the microscopic characters for a distinction of the two karyotypes. On the basis of the experience with other polyploids, the pollen size of a number of diploid and tetraploid *C. epigeios* plants was measured. The results are given in table 1.

The table shows clearly that the pollen size of the diploid plants is significantly different from that of the tetraploid plants, although the curves overlap considerably. Furthermore, the table reveals an essential difference in pollen size among the plants belonging to the diploid karyotype as well as to the tetraploid karyotype. Within the tetraploid karyotype, plant no. 3 has smaller pollen than the other four plants. (On the other hand, the pollen size of these four plants shows no significant difference). Within the diploid karyotype, the plants no. 64 and no. 69 have smaller pollen than no. 77 and no. 80. Hence, with respect to the size of the pollen, the 9 plants may be ordered in 4 groups, viz. no. 3—nos. 10, 22, 26, 88—nos. 64, 69—and nos. 77, 80. The distribution curves of these four groups are drawn in fig. 3 (cf. fig. 15, p. 31).

It should be mentioned that the plants no. 64 and no. 69 represent the comparatively slender diploid type which is shown in fig. 1 b, whereas the plants no. 77

and no. 80 represent the extremely robust diploid type shown in fig. 1 *d*. Thus, some correlation seems to exist between robust growth and larger cells.

It will also appear from the table that the difference between the tetraploid plant with the smallest pollen (no. 3) and the diploid with the largest pollen (no. 80) is considerably smaller than the difference between the smallest and the biggest tetraploid plant. In the first case, the difference amounts to  $2\mu$  only, in the latter case to nearly  $4\mu$ . Undoubtedly, the method of distinction between the two karyo-

Table 2.

Species	Pedigree	No. of obs.	Normal	Abortive
<i>C. epigeios</i> 4n . . . .	3	100	45	55
	10	100	68	32
	22	100	71	29
	26	100	67	33
	88	100	54	46
	168	100	47	53
4n total . . .	..	600	352 (59%)	248 (41%)
<i>C. epigeios</i> 2n . . . .	64	100	99	1
	69	100	88	12
	77	100	91	9
	80	100	87	13
2n total . . .	..	400	365 (91%)	35 (9%)
<i>A. arenaria</i> . . . . .	144	200	180 (90%)	20 (10%)

types by means of the pollen size has to be used with greatest caution and criticism. In the present investigation, the author has not applied this method, but the chromosome number has been determined in all cases.

The size of the stomata has often been employed for the distinction between diploid and polyploid plants. This character, however, was not very applicable in *Calamagrostis epigeios*, since the length of the stomata was found to vary considerably within one and the same leaf, so that an exact statistical comparison between different plants could not be carried out. Nevertheless, the measurements undoubtedly showed the same result as those concerning the pollen grains, the stomata of the tetraploids being somewhat larger than those of the diploids.

Moreover, it could be ascertained by measurements on the nuclei of the accessory cells of the stomata that the nuclei are larger in tetraploid plants than in diploids. Although this character so far is safe enough, this rather laborious method is of little practical value.

Finally, another pollen character, viz. the quality of the pollen, proved to be different in diploid and tetraploid *Calamagrostis epigeios*. This is demonstrated in table 2. The pollen was examined in aceto-carmin, and pollen grains which stained red were recorded as living. The tetraploid plants generally have about 60 per cent good pollen, the diploid about 90 per cent.

A decrease in pollen quality, however, may be due to other reasons than polyploidy; therefore, also this character must be considered with caution and, for a safe distinction between a diploid and a tetraploid *Calamagrostis epigeios* plant, only the estimation of the chromosome number remains.

The interesting fact that a diploid and a tetraploid karyotype of the same Linnean species, both growing in Nature, can only be distinguished cytologically but not morphologically will be discussed in detail in a later section (cf. p. 51). In the following, the description of *C. epigeios* will cover the diploid as well as the tetraploid karyotypes if nothing else is emphasized.

#### b. Description of the three *Ammophila baltica* hybrids and the parental species.

The following description refers to the pictures of inflorescence (fig. 4), leaf (figs. 5 and 6, cf. figs. 9 and 10), ligule (fig. 7), and flower (fig. 8).

##### *Ammophila arenaria* (L.) Link:

Inflorescence a spikelike panicle (a thyrse), colour pale bluish grey. Leaves bluish grey, narrow with prominent ridges on the upper surface. Leaves without prominent midrib (keel) on lower surface. Ligule long. Lower sheaths bluish grey or pale pink. Flower: inner and outer pale nearly equal. Hairs at base of flower less than  $\frac{1}{2}$  ( $\frac{1}{4}$ — $\frac{1}{3}$ ) the length of inner pale. Awn short, inserted immediately below apex of outer pale, not projecting beyond apex of outer pale.

##### *Ammophila baltica* var. *subarenaria* Mars.:

Inflorescence a loose spikelike panicle (a thyrse), colour reddish purple. Leaves bluish green, somewhat broader and with lower ridges than the latter. Prominent midrib (keel) on lower surface of leaf running along about one fifth (less than half) of length of leaf. Ligule shorter than in the latter. Lower sheaths pink-reddish purple. Flower smaller than in the latter. Inner and outer pale nearly equal. Hairs at base of flower about half the length of inner pale. Awn longer, inserted at greater distance from apex of outer pale than in the latter. About  $\frac{1}{4}$  of awn projecting beyond apex of outer pale.

##### *Ammophila baltica* var. *intermedia* var. nov.:

Inflorescence a spreading, spikelike panicle (a thyrsoïd), colour brownish purple. Leaves green, somewhat broader, with less prominent ridges than the latter. Keel of lower surface reaching to middle of leaf. Ligule shorter than in the latter. Lower sheaths dark reddish purple to scarlet. Flower smaller than in the latter. Length of inner pale about  $\frac{3}{4}$  that of outer pale. Hairs at base of flower about the length of inner pale, shorter than outer pale. Awn longer, inserted at greater distance from apex of outer pale than in the latter. About  $\frac{1}{3}$  of awn projecting beyond apex of outer pale.

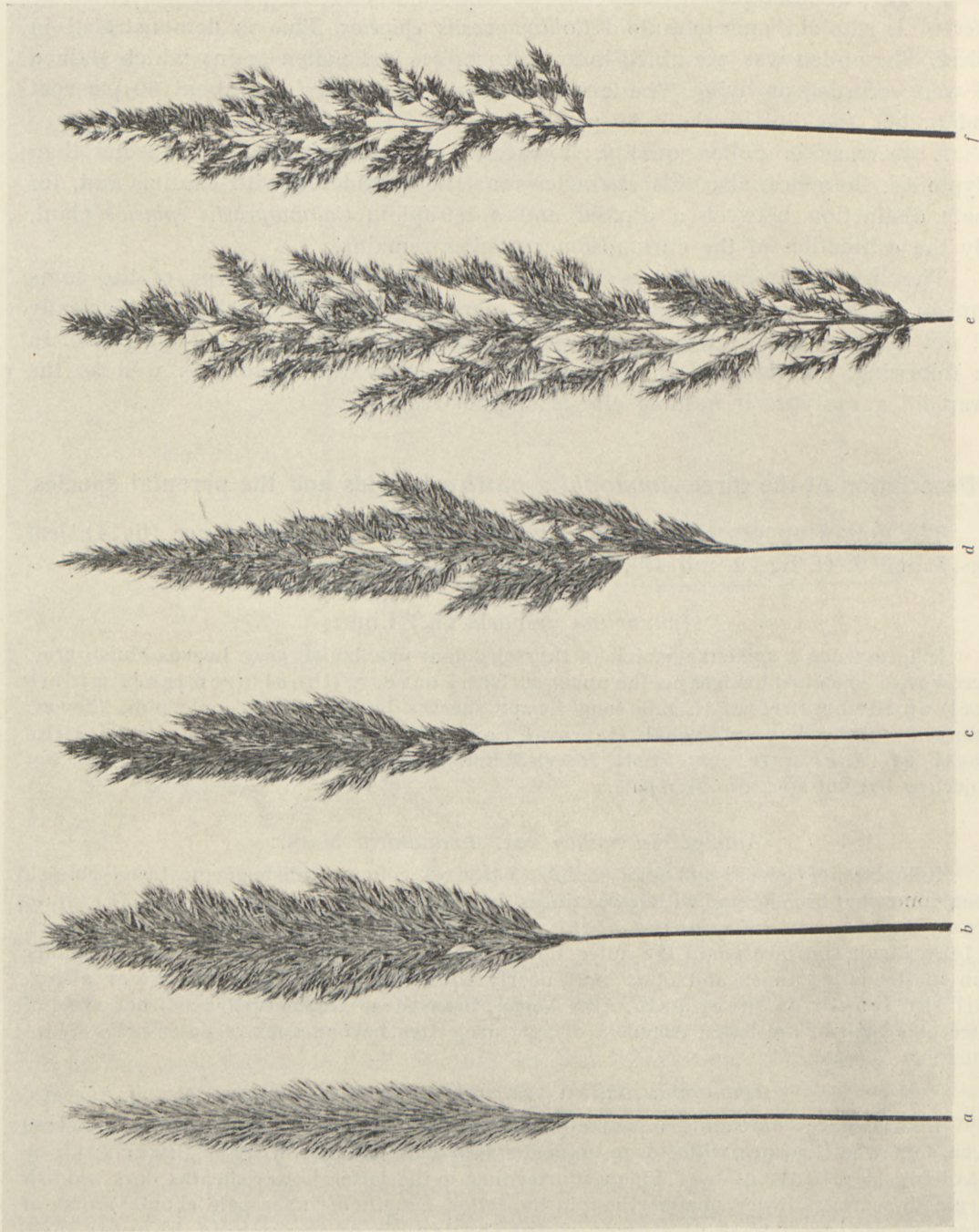


Fig. 4. Inflorescence of a: *Ammophila arenaria* ( $2n = 28$ , genome constitution AA; cp. p. 16). b: *Ammophila ballica* var. *subarenaria* ( $2n = 42$ ; AAC). c: *Ammophila ballica* var. *intermedia* ( $2n = 28$ ; AC). d: *Ammophila ballica* var. *epigeioidea* ( $2n = 42$ ; ACC). e: *Calamagrostis epigeios* tetraploid ( $2n = 56$ ; CCCC). f: *Calamagrostis epigeios* diploid ( $2n = 28$ ; CC). Leg. Villingebæk (Sealand) July 1939.  $\times 1/2$ .

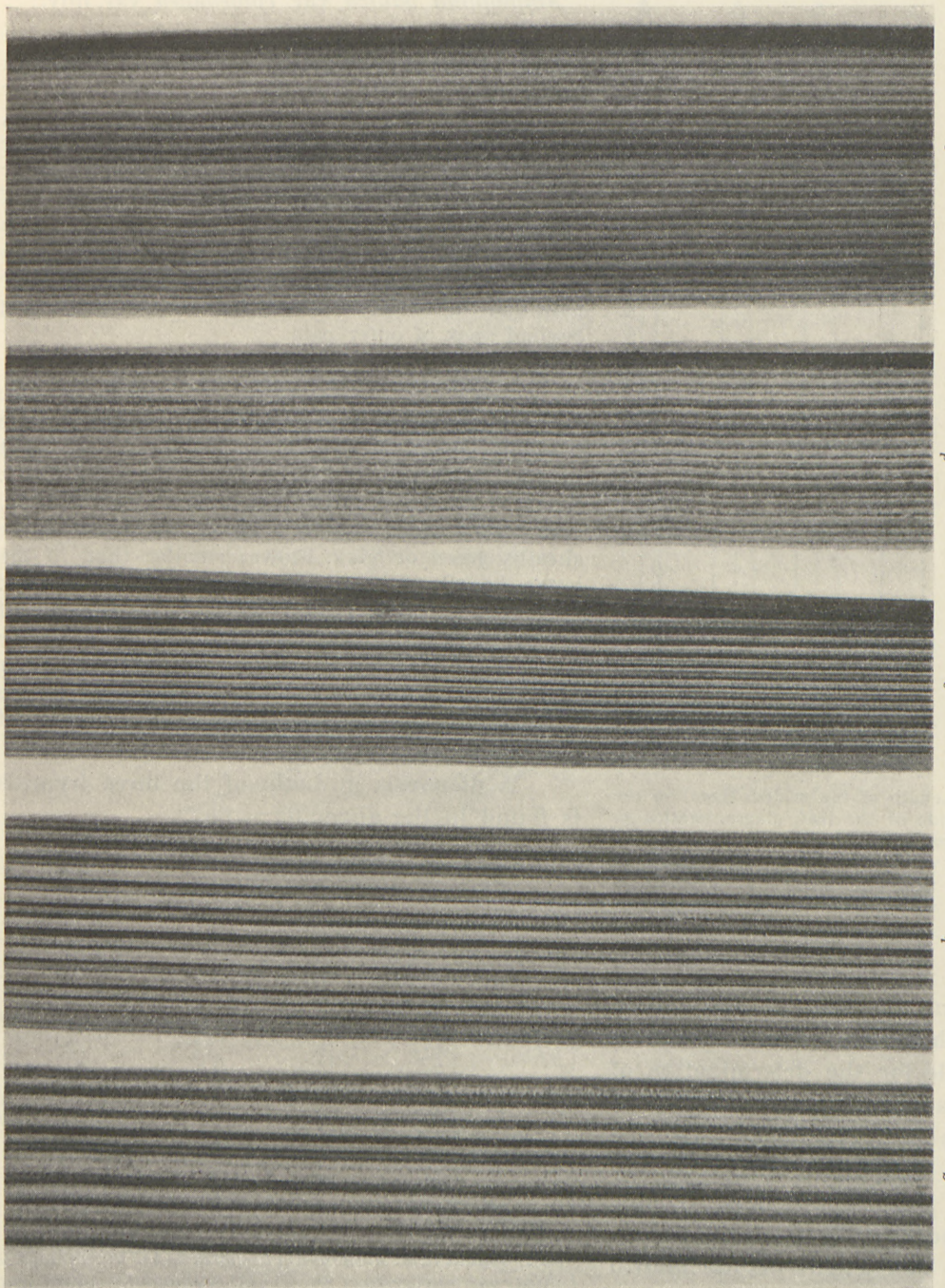


Fig. 5. Leaves of a: *Ammophila arenaria* (AA), b: *Ammophila ballica* var. *subarenaria* (AAC), c: *Ammophila ballica* var. *intermedia* (AC), d: *Ammophila ballica* var. *epigetoidea* (ACC), e: *Calamagrostis epigetos* (diploid, CC). Seen from upper side. Photo.  $\times$  ca. 2.

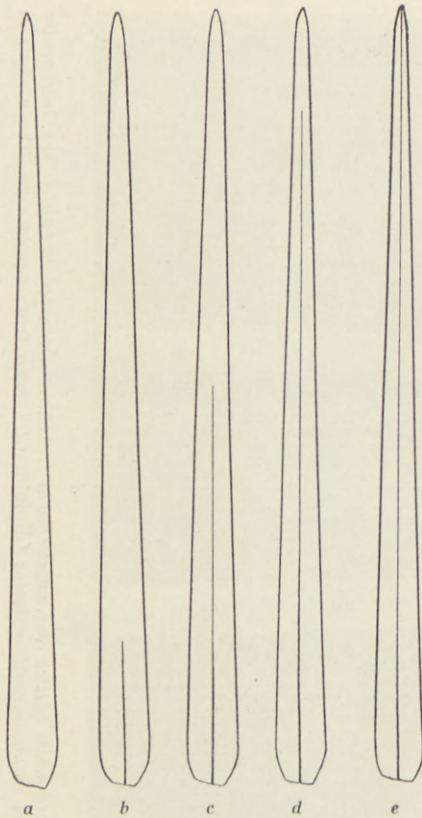


Fig. 6. Length of the midrib (keel) on the lower side of the leaf, diagrammatically. a: *Ammophila arenaria*. b: *Ammophila baltica* var. *subarenaria*. c: *Ammophila baltica* var. *intermedia*. d: *Ammophila baltica* var. *epigeioidea*. e: *Calamagrostis epigeios*.

In the above description, the characters which are most suitable for the determination of the plants are spaced. It has been shown by experience that the length of the hair relative to the inner pale is the most reliable and most readily recognized character. The length of the midrib of the lower surface of the leaf may be employed for the determ-

*Ammophila baltica* var. *epigeioidea* var. nov.:

Inflorescence a close panicle, colour brownish purple. Leaves greyish green, somewhat broader, with less prominent ridges than the latter. Keel of lower surface running further than middle of leaf, but not reaching apex of leaf. Ligule shorter than in the latter. Lower sheaths pink. Flower smaller than in the latter. Length of inner pale about  $\frac{3}{4}$  of outer pale. Hairs at base of flower longer than inner pale, about length of outer pale. Awn longer, inserted at greater distance from apex of outer pale than in the latter. Nearly half of awn projecting beyond apex of outer pale.

*Calamagrostis epigeios* (L.) Roth:

Inflorescence a panicle, colour reddish purple (in light), green (in shade). Leaves green, somewhat broader, with less prominent ridges than the latter. Keel of lower surface reaching apex of leaf. Ligule very short, shorter than in the latter. Lower sheaths green or pink. Flower smaller than in the latter. Length of inner pale about  $\frac{2}{3}$  of outer pale. Hairs at base of flower 2—2 $\frac{1}{2}$  the length of inner pale, considerably longer than outer pale. Awn inserted at about the middle of outer pale, at a greater distance from apex than in the latter. Nearly half of awn projecting beyond apex of outer pale.

A diagnosis in Latin of the three hybrids is found in the appendix p. 60.

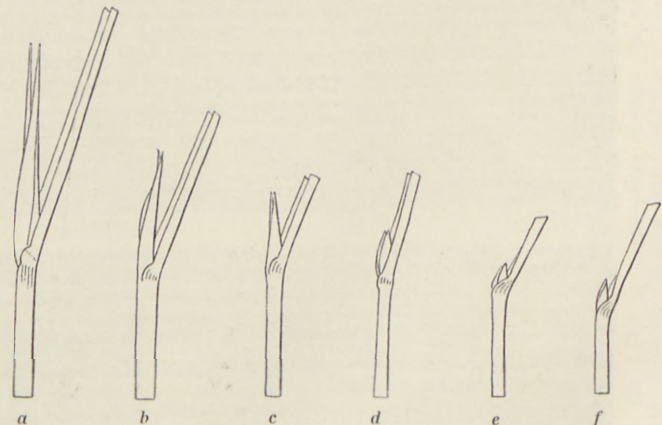


Fig. 7. Ligula in a: *Ammophila arenaria* (AA). b: *Ammophila baltica* var. *subarenaria* (ACC). c: *Ammophila baltica* var. *intermedia* (AC). d: *Ammophila baltica* var. *epigeioidea* (ACC). e: *Calamagrostis epigeios*, tetraploid (CCCC). f: *Calamagrostis epigeios*, diploid (CC). Drawing.  $\times 1$ .

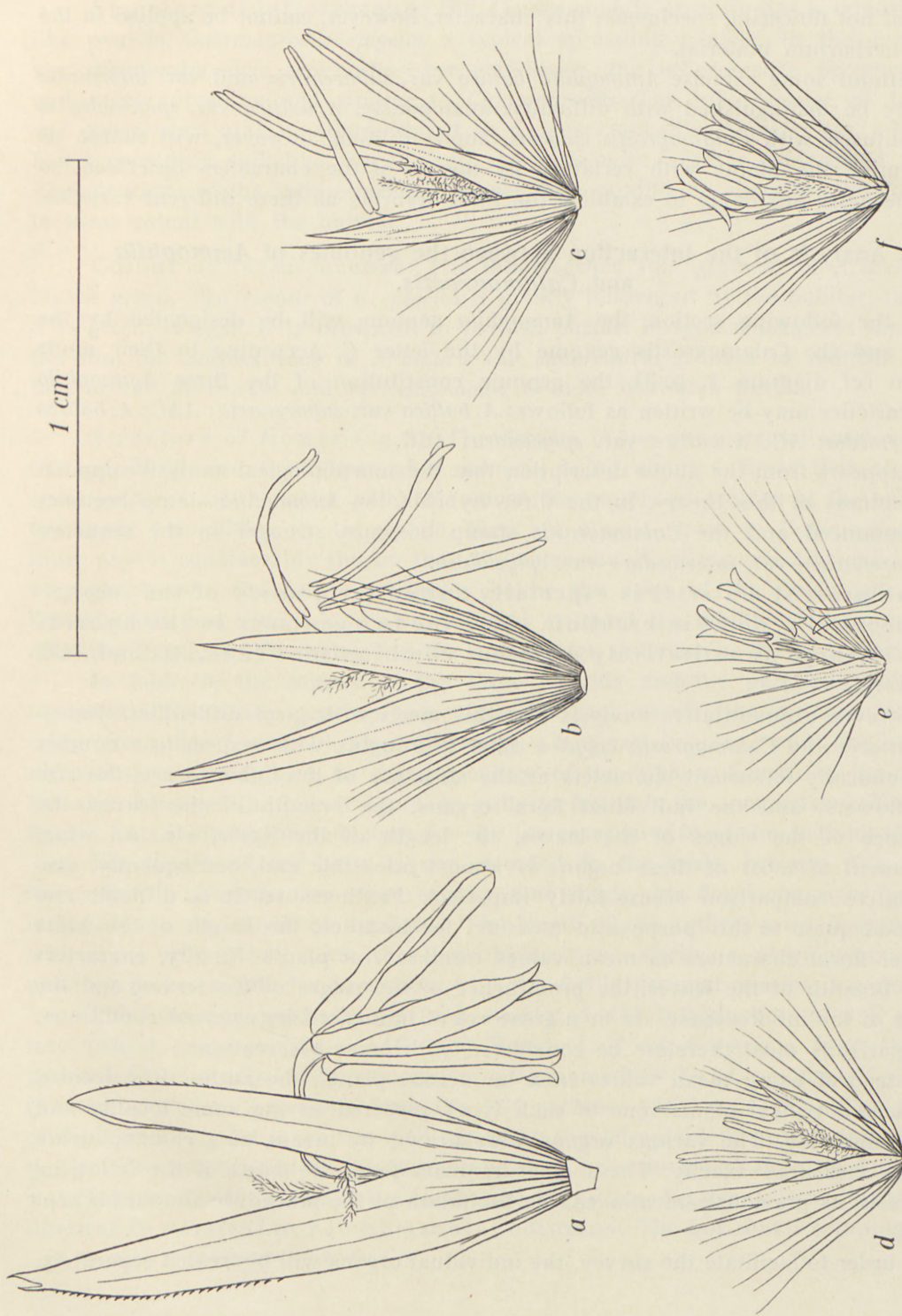


Fig. 8. Flower of a: *Ammophila arenaria* (AA). b: *Ammophila ballica* var. *subarenaria* (AAC). c: *Ammophila ballica* var. *intermedia* (AC). d: *Ammophila ballica* var. *epigeioidea* (ACC). e: *Calamagrostis epigeios*, tetraploid (CCCC). f: *Calamagrostis epigeios*, diploid (CC). Drawing.

ination of not flowering specimens; this character, however, cannot be applied in the case of herbarium material.

Without some exercise *Ammophila baltica* var. *subarenaria* and var. *intermedia* may only be distinguished with difficulty; analogously, *A. baltica* var. *epigeioidea* is often confused with *Calamagrostis epigeios*. Some training, however, will enable us to determine the plants with certainty by means of the characters described, so that it becomes justifiable to establish the three hybrids as three different varieties.

### c. Analysis of the interaction between the genomes of *Ammophila* and *Calamagrostis*.

In the following section, the *Ammophila* genome will be designated by the letter *A* and the *Calamagrostis* genome by the letter *C*. According to their mode of origin (cf. diagram 1, p. 3), the genome constitution of the three *Ammophila baltica* varieties may be written as follows: *A. baltica* var. *subarenaria*: AAC; *A. baltica* var. *intermedia*: AC; *A. baltica* var. *epigeioidea*: ACC.

It appears from the above description that the morphological analysis supports the correctness of this theory. In the three hybrids, the *Ammophila* stamp becomes less pronounced and the *Calamagrostis* stamp becomes stronger in the sequence var. *subarenaria*—var. *intermedia*—var. *epigeioidea*.

The present material is especially suited for a study of the important process of genome interaction, since the two genomes in the hybrids appear in three quantitatively different combinations (AAC, AC, and ACC, respectively).

However, a quantitative analysis like this meets with great difficulties. *Ammophila arenaria* and *Calamagrostis epigeios* differ thoroughly with respect to a number of taxonomically important characters as the structure of the inflorescence, the size of the flowers, and the individual floral organs, the breadth of the leaves, the prominence of the ridges of the leaves, the length of the ligule, etc. An exact measurement of most of these organs is almost infeasible and, consequently, also a qualitative comparison seems fairly imperfect. Furthermore, it is difficult and partly inadequate to this purpose to measure, for instance, the length of the hairs and other floral characters as mean values from several plants. Finally, characters like the breadth of the leaves, the prominence of the ridges of the leaves, and the structure of the inflorescence are to a great extent influenced by external conditions; all comparisons must therefore be considered with some reservation.

Instead of using mean values from numerous plants, the author has decided to compare 5 typical plants (one of each type) gathered in the same locality and on the same day. The various organs were drawn by means of a camera lucida and were measured exactly. These measurements form the basis of the following comparison; as previously mentioned, the limitation of the procedure should be kept in mind.

In order to facilitate the survey, the individual organs will be treated separately.



Structure of inflorescence (fig. 4): *Ammophila arenaria* has a typical spike-like panicle, *Calamagrostis epigeios* a typical spreading panicle. In the succession var. *subarenaria*—var. *intermedia*—var. *epigeioidea*, the inflorescence becomes more and more spreading and similar to *Calamagrostis*. The inflorescence of *A. baltica* var. *subarenaria* may be characterized as a dense, spikelike panicle; var. *intermedia* has a spreading, spikelike panicle, and var. *epigeioidea* a rather compressed panicle. The structure of the inflorescence, however, is a modifiable character which varies to some extent with the habitat.

Colour of inflorescence: The inflorescence (the glumes) of *A. arenaria* is bluish green. The colour of *C. epigeios* is greatly influenced by the habitat: in shade it is green, in light it is brownish to reddish purple. In typical localities exposed to the sun, the inflorescence of *A. baltica* var. *subarenaria* is next to reddish purple, that of var. *intermedia* and var. *epigeioidea* is more brownish purple.

Structure of flower (fig. 8): The flowers of the two parental species deviate first of all in size, the flower of *A. arenaria* being about 4 times that of *Calamagrostis* (length of the outer pale). Moreover, the reciprocal lengths of the outer and the inner pale differ: in *Ammophila*, they are almost equal, in *Calamagrostis*, the inner pale is considerably shorter than the outer one. Furthermore, *Ammophila* has a very short awn, inserted immediately below the apex of the outer pale, while *Calamagrostis* has a very long awn, inserted at about the middle of the outer pale. Also the lengths of the hairs and the anthers are different in the two species.

In table 3, the organs of the three hybrids and the parental species are compared. The exact measures are given for each organ (the measurements are taken from figs. 7 and 8), the differences are given in mm., and finally, these differences are calculated as the percentage of the highest figure (in the case of the awn, however, the lowest figure, in order to facilitate the comparison). The latter figures thus indicate the reduction (or the increase) in size of the individual organ.

The length of the hairs is not recorded in the table, because it is almost impossible to measure this organ exactly. One single, non-floral character has been included, viz. the length of the ligule. The length of the anthers must be considered with some reservation, since the three hybrids are sterile, a phenomenon which possibly makes a direct comparison with the fertile parental species uncertain.

The following processes are to be analyzed. (1) Introduction of a *C* genome into two *A* genomes ( $AA \rightarrow AAC$ ). (2) Removal of an *A* genome from the *AAC* genomes ( $AAC \rightarrow AC$ ). (3) Introduction of a *C* genome into the *AC* genome ( $AC \rightarrow ACC$ ). (4) Removal of an *A* genome from the *ACC* genomes ( $ACC \rightarrow CC(CC)$ ). The genome of diploid *C. epigeios* is written *CC*; regarding the tetraploid form as autopolyploid, the genome constitution of this karyotype may be written *CCCC* (cf. p. 51).

It appears from the table that the percentage figures are not constant, neither in vertical nor in horizontal columns. The horizontal columns reveal

Table 3.

Species	Chromosome number	Genome constitution	Length of outer pale, mm.			Length of inner pale, mm.			Length of anthers, mm.			Length of ligule, mm.			Length of awn, mm.		
			Difference, mm.	Difference, %		Difference, mm.	Difference, %		Difference, mm.	Difference, %		Difference, mm.	Difference, %		Difference, mm.	Difference, %	
<i>A. arenaria</i> ..	28	AA	11.7		10.3	4.9		30.0		0.5							
			3.1	26	2.4	23	0.2	4	12.5	42	0.8	160					
<i>A. baltica</i> var. <i>subarenaria</i>	42	AAC	8.6		7.9	4.7		17.5		1.3							
			2.9	34	3.4	43	1.0	21	6.5	37	0.4	31					
<i>A. baltica</i> var. <i>intermedia</i>	28	AC	5.7		4.5	3.7		11.0		1.7							
			1.3	23	1.1	24	1.2	32	4.0	36	0.4	24					
<i>A. baltica</i> var. <i>epigeioidea</i>	42	ACC	4.4		3.4	2.5		7.0		2.1							
			1.3	30	1.0	29	0.7	28	3.5	50	0.1	5					
<i>C. epigeios</i> <sup>1</sup> ..	28	CC	3.1		2.4	1.8		3.5		2.2							
	56	CCCC															

<sup>1)</sup> Only the tetraploid karyotype of *C. epigeios* was used for the comparison.

that the introduction of a *C* genome into two *A* genomes ( $AA \rightarrow AAC$ ) causes an almost equal reduction of the length of the outer and the inner pale (26 and 23 per cent). The length of the anthers, however, is hardly changed, but the length of the ligule is changed by 42 per cent. The most striking change, however, is found in the length of the awn which increases by 160 per cent. The following horizontal column shows the same phenomenon. The removal of an *A* genome thus causes a reduction in length of the outer pale by 34 per cent, but the inner pale changes considerably more (43 per cent). At the same time, the length of the anther changes by 21 per cent, that of the ligule and the awn by 37 and 31 per cent. Corresponding differences are found in the other horizontal lines, and it may be concluded that different organs of the flower respond in different ways to one and the same genome, whether an *A* or a *C* genome.

The variation in the vertical percentage figures shows that the effect of a genome depends upon the definite combination into which it is introduced. The table allows us to analyze the effect of a *C* genome introduced into two *A* genomes (the percentage figures of the upper horizontal line of the table) and the effect of a *C* genome introduced into an *AC* genome (the percentage figures in the lowest but one line). A comparison of these two columns shows that the *C* genome has an almost additive effect on the length of the outer and the inner pale and on the ligule. In the first case, the outer pale is reduced by 26 per cent, in the second case by 23 per cent; the corresponding figures of the inner pale are 23 and 24 per cent, and of the ligule they are 42 and 36 per cent, respectively). The anthers

and the awn, however, are affected in another way. If the *C* genome is introduced into two *A* genomes, the length of the anther is scarcely changed (4 per cent), but if the *C* genome is introduced into an *AC* genome, the change amounts to as much as 32 per cent. In the first case, the length of the awn increases by 160 per cent; in the second case, however, the same genome causes an alteration of the same organ by 24 per cent, only.

The *A* genome behave correspondingly. In order to facilitate the comparison, the processes are regarded as the introduction of an *A* genome partly into the combination *CC(CC)* and partly into the combination *AC* (i. e. the percentage figures of the lowest and the second line are compared). The length of the inner pale is altered considerably when the *A* genome is introduced into the *AC* combination, but less when the same genome is introduced into the *CC(CC)* combination. In the first case, the alteration amounts to 43 per cent, in the latter case to 29 per cent, only. On the other hand, the length of the ligule is altered most when the *A* genome is introduced into the *CC(CC)* genomes (50 against 37 per cent). With respect to the outer pale and the anther, a purely additive effect of the *A* genome is found, but the length of the awn is changed in the same way as that of the inner pale.

From the analysis of the percentage figures it may be concluded as follows. (1) One and the same genome has a different effect on different organs and (2) the effect of a genome on one and the same organ is not always additive; it may be a relative effect, depending on the definite combination into which it is introduced.

Table 3 does not contain the rather conspicuous character, viz. the place of insertion of the awn on the outer pale, which is almost terminal in *Ammophila*, and is nearly in the middle of the pale in *Calamagrostis*. It is difficult to describe this character in the same way as the other floral organs. The distance from the place of insertion to the apex of the outer pale may be given in percentage of the total length of the outer pale. When using this method, the following index figures are obtained. *A. arenaria* 7 per cent, *A. ballica* var. *subarenaria* 13 per cent, var. *intermedia* 22 per cent, var. *epigeioidea* 32 per cent, and *C. epigeios* about 50 per cent. The differences are 6, 9, 10, and 17, hence not even in this case the individual genome has a simple additive effect.

A further discussion of this analysis naturally leads to the problem of the specific Mendelian basis of these taxonomically important characters. Unfortunately, owing to the complete sterility of the three hybrids, knowledge of the inheritance of these differences is lacking. In the author's opinion, the demonstration of the non-additive genome effect indicates that the characters concerned are determined by polymeric, partly epistatic genes, i. e. they have a rather complex hereditary base.

Length of ligule (fig. 7): This character has already been analyzed in table 3. The figures are derived from measurements on fig. 7.

Structure of leaf (figs. 5, 9, and 10): The leaf of *Ammophila* differs from that of *Calamagrostis* in a number of characteristic structural features. The leaf of *Ammophila* (figs. 5a, 9a, and 10a) is narrow with prominent ridges on the upper surface. The leaves are conduplicate. The leaf of *Calamagrostis* (figs. 5e, 9e, and 10e) is rather broad with low ridges on the upper surface. Moreover, it is characteristic that the ridges of *Ammophila* vary in prominence while those of *Calamagrostis* are equal. The leaves are convolute.

The anatomical structure of the two leaves is thoroughly different with respect to the distribution of the photosynthetic tissue and the strengthening tissue. The leaf of *Ammophila* is of a markedly xeromorphic structure (the structure has been described previously by WARMING 1909, p. 252, and by RAUNKJÆR 1899, p. 631). Fig. 9a shows a transverse section of the leaf. The strengthening tissue is made black, the photosynthetic tissue white, the vascular bundles are hatched. In addition, fig. 10a shows details of the region surrounding the midrib. It appears from these figures that the strengthening tissue (*sc*), at least three cell layers thick, covers the whole lower side of the leaf. It continues into the ridges to the upper side of the leaf. Lateral groups of strengthening tissue are found in the large ridges from the vascular bundles to the epidermis (*sc*<sub>1</sub> in fig. 10a). Thus, the photosynthetic (*me*) tissue is confined to the sides of the ridges and to the bottom of the furrows. On the bottom of the furrows, the epidermal cells are transformed into large dead cells, the motor cells (*m* in fig. 10). Stomata (*st* in fig. 10) are only found on the upper surface of the leaf on the sides of the ridges. The upper surface of the leaf is completely covered with hairs (*h* in fig. 10). The lower surface is covered with a fairly thick cuticle (*c* in fig. 10) with an undulate surface.

The leaf of *Calamagrostis epigeios* (figs. 5e, 9e, and 10e) has a more mesomorphic structure with photosynthetic tissue and stomata both on the upper and the lower surface (cf. WARMING 1909, p. 281, and RAUNKJÆR 1899, p. 639). The strengthening tissue forms narrow bridges from the lower surface of the leaf to the vascular bundles and from the lower face of the latter into the low ribs to the epidermis of the upper face. The lateral groups of strengthening tissue found in *Ammophila* and the continuous layer of strengthening tissue in the lower face are lacking, we find but isolated groups beneath the motor cells. These groups (*sc*<sub>2</sub> in fig. 10e) consist of 3 to 8 cells of sclerenchyma. The motor cells are considerably larger than in *Ammophila*. In contrast to *Ammophila*, *Calamagrostis* shows no hairs on the upper surface of the leaf, and the cuticle is only slightly developed. Some of the epidermal cells of the lower surface of the *Calamagrostis* leaf are enlarged, transformed into papils (*p* in fig. 10e); these cannot be observed on *Ammophila*.

The details of the anatomical structure reveal still further characteristic differences between the two leaves, particularly with respect to the structure of the cells of the strengthening tissue. In *Ammophila*, this tissue consists of rather large, dead cells, the walls of which are only thickened to a small extent, the lumen being large and angular. The vascular bundles are surrounded by a layer of cells of strength-

ening tissue, the inner walls of which are thickened in the form of the letter *u*. In the leaf of *Calamagrostis*, the strengthening tissue consists of relatively small sclerenchyma cells, the walls of which are vastly thickened, so that the lumina become small and round. The middle lamella of the walls here is conspicuous, while it can only be seen with difficulty in *Ammophila* (not shown in fig. 10*a*). The cells of the strengthening tissue surrounding the vascular bundles are of the same sclerenchymatic structure as the rest of the strengthening tissue.

The pictures show how the leaves of the three *Ammophila baltica* varieties form transitions from the leaf of *Ammophila arenaria* to that of *Calamagrostis epigeios*. In the succession var. *subarenaria*—var. *intermedia*—var. *epigeioidea*, the *Ammophila* characters gradually disappear and the *Calamagrostis* characters become more conspicuous. This applies to the breadth of the leaves, the prominence of the ridges, the length and the number of the hairs, the size of the motor cells, etc.

Undoubtedly, the most characteristic feature is the change in distribution of photosynthetic and strengthening tissue. In *A. baltica* var. *subarenaria*, the continuous layer of strengthening tissue on the lower side of the leaf is still present, but it is thinner than in *Ammophila arenaria*. Moreover, the lateral strengthening tissue (*sc*) is absent in the majority of the ridges, and in *A. baltica* var. *intermedia* it is lacking completely (cf. fig. 9). In this variety, the continuous layer of strengthening tissue on the lower surface is interrupted in a number of places, where the photosynthetic tissue reaches the lower face of the epidermis. However, these breaks are not broader than a few cells (*t* in fig. 10*c*). In *A. baltica* var. *epigeioidea*, the distribution of the strengthening and the photosynthetic tissue is about the same as in *Calamagrostis epigeios*. The bridges of strengthening tissue into the ridges are broader, and the groups of sclerenchyma cells beneath the motor cells are larger in the hybrid than in *C. epigeios*. In *A. baltica* var. *epigeioidea* as in *C. epigeios*, a few stomata are found on the lower surface of the leaf (not shown in the drawing). In the two other varieties, as in *Ammophila arenaria*, the stomata are found on the upper surface of the leaf, only.

It appears from fig. 10 that the structure of the cells of the strengthening tissue is less typical. Especially, the cells of *A. baltica* var. *intermedia* (fig. 10*c*) differ to some extent from the sequence. The cells of the strengthening tissue of this variety resemble the cells of *Ammophila arenaria* to a high degree, whereas the structure of the var. *subarenaria* cells resemble that of *C. epigeios*. In view of the genome constitution of the two varieties, the opposite had to be expected. It should be remembered, however, that the structure of the cells varies considerably with the habitat.

Finally, it may be mentioned that the leaf of *Ammophila baltica* shown by WARMING (1909, p. 281) is a leaf of varietas *subarenaria*.

The colour of the lower leaf sheaths: Also this character varies to some extent with the habitat. In sunny places, the lower leaf sheaths of *Ammophila*

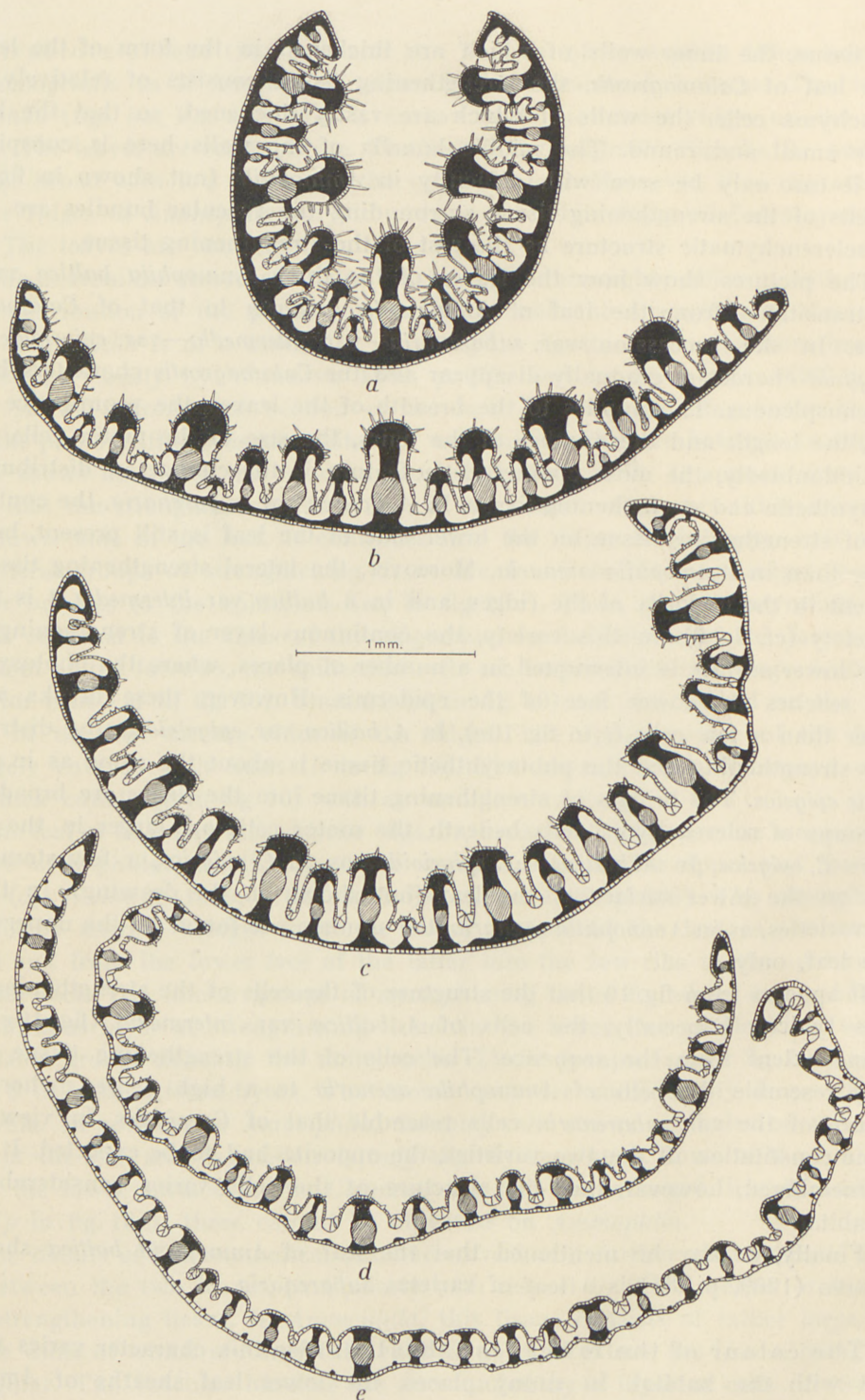


Fig. 9. Transverse sections of the leaves of a: *Ammophila arenaria* (AA). b: *Ammophila ballica* var. *subarenaria* (AAC). c: *Ammophila ballica* var. *intermedia* (AC). d: *Ammophila ballica* var. *epigeioides* (ACC). e: *Calamagrostis epigeios*, (tetraploid (CCCC). Camera lucida drawing, somewhat diagrammatically. Strengthening tissue black, photosynthetic tissue white, vascular bundles hatched.  $\times 25$ .

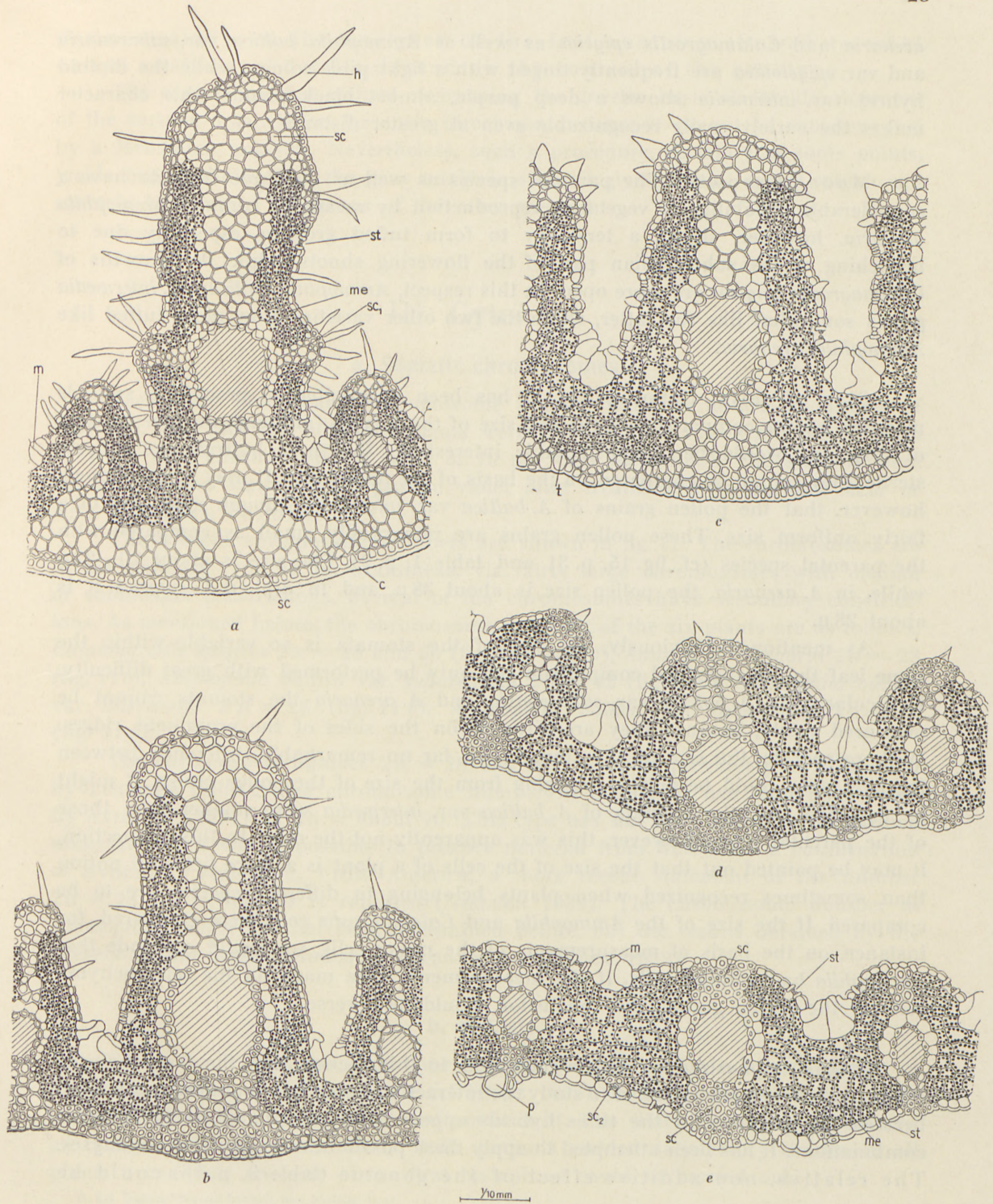


Fig. 10 a—e: The same leaves as in fig. 9. The section round the midrib drawn at a higher magnification. For explanation cf. the text.  $\times 120$ .

*arenaria* and *Calamagrostis epigeios* as well as *Ammophila ballica* var. *subarenaria* and var. *epigeioidea* are frequently tinged with a light pink colour, while the diploid hybrid var. *intermedia* shows a deep purple, almost black colour; this character makes the variety easily recognizable even at greater distance.

Mode of growth: The parental species as well as the three hybrids have a considerable capability of vegetative reproduction by means of runners. *Ammophila arenaria*, however, shows a tendency to form tufted growths especially due to branching of the subterranean part of the flowering shoots, while the growths of *Calamagrostis epigeios* are more open. In this respect, *Ammophila ballica* var. *intermedia* grows somewhat like the latter, while the two other varieties grow more tufted like *Ammophila arenaria*.

Size of cells: Unfortunately, it has been impossible to procure a suitable material for the comparison of the cell size of the 5 types, although an investigation of this kind would be of considerable interest. As the three hybrids are entirely sterile, they cannot be compared on the basis of the pollen size. It may be mentioned, however, that the pollen grains of *A. ballica* var. *intermedia*, though dead, are of a fairly uniform size. These pollen grains are remarkably small as compared with the parental species (cf. fig. 15, p. 31 and table 1, p. 9). The size is about 20–22  $\mu$  while, in *A. arenaria*, the pollen size is about 38  $\mu$ , and in diploid *C. epigeios* it is about 28  $\mu$ .

As mentioned previously, the size of the stomata is so variable within the same leaf that a statistical comparison can only be performed with great difficulty. Particularly, in *A. ballica* var. *subarenaria* and *A. arenaria* the stomata cannot be measured easily, because they are situated on the sides of the prominent ridges. With respect to the size of the stomata, so far no remarkable difference between the five types could be noticed. Judging from the size of the pollen grains, it might be expected that the stomata of *A. ballica* var. *intermedia* were smaller than those of the parental types, however, this was apparently not the case. In this connection, it may be pointed out that the size of the cells of a plant is a more fictitious notion than sometimes recognized when plants belonging to different species are to be compared. If the size of the *Ammophila* and *Calamagrostis* cells were compared, for instance, on the basis of measurements of the motor cells, we would conclude that *Ammophila* had smaller cells. If the measurements were made on the sclerenchyma cells or the pollen grains, the conclusion would be reverse.

It was emphasized in the introduction to this section that the three hybrids offer a material specially suited to study the interaction between two different genomes, because the genomes in the three hybrids appear in three quantitatively different combinations. It has been attempted to apply these possibilities to the above analyses. The relative, non-additive effect of the genome (table 3, p. 18) could be



demonstrated and the analyses of the leaf anatomy showed to what extent anatomical details depend on the control of the genotype.

The experience thus gained concerning the genetic basis of the development of the various organs is so far not a direct one, because it cannot be supplemented by a Mendelian analysis. Nevertheless, such a procedure, certainly at some points, yields better results than the gene analysis, since it gives a total aspect which can scarcely be attained by means of the other method.

### III. Cytology.

#### a. Somatic chromosomes.

Technique. The somatic chromosome number was determined on mitoses of root tips. The root tips of potted plants were fixed in LEWITSKY's fixative (8 parts of 1 per cent chromic acid : 2 parts of 10 per cent formaline). The sections were cut at  $13\mu$  and stained with gentian violet after treatment with chromic acid or with FEULGEN's Fuchsin.

The somatic chromosome numbers are shown in fig. 11. The chromosomes are of the ordinary type of the Gramineae, viz. fairly large chromosomes with median or submedian constrictions. Several of the chromosomes have secondary constrictions. As mentioned before, the chromosome numbers of the six plants are as follows. *Calamagrostis epigeios*  $2n = 28$  and  $56$  (fig. 11 a and b), *Ammophila arenaria*  $2n = 28$  (fig. 11 c), *Ammophila baltica* var. *subarenaria*  $2n = 42$  (fig. 11 d), *Ammophila baltica* var. *intermedia*  $2n = 28$  (fig. 11 e), *Ammophila baltica* var. *epigeioidea*  $2n = 42$  (fig. 11 f).

The chromosome number  $2n = 28$  has been counted previously in *A. arenaria* by TISCHLER (1934). The chromosome number of *C. epigeios* has been determined by AVDULOV (1931) as  $2n =$  about 70. This type has not been found in the Danish material and it was impossible to procure material from Russia for a confirmation of AVDULOV's statement. From America, a more recent statement of the number  $2n = 28$  for *C. epigeios* is reported (NIELSEN 1939). The chromosome number of *Ammophila baltica* has not been determined before, but the results obtained by the present author were included in MAUDE's list (MAUDE 1939).

#### b. Meiosis.

Technique. P. M. C. were partly examined in aceto-carmin smears, partly in FEULGEN smears ad modum HEITZ (cf. HEITZ 1936), in both cases after a fixation in 3 parts of alcohol : 1 part of glacial acetic acid. The slides were made permanent ad modum BUCK (cf. BUCK 1935). Both methods yielded satisfactory results, in all plants a complete analysis of the first metaphase could be carried out.

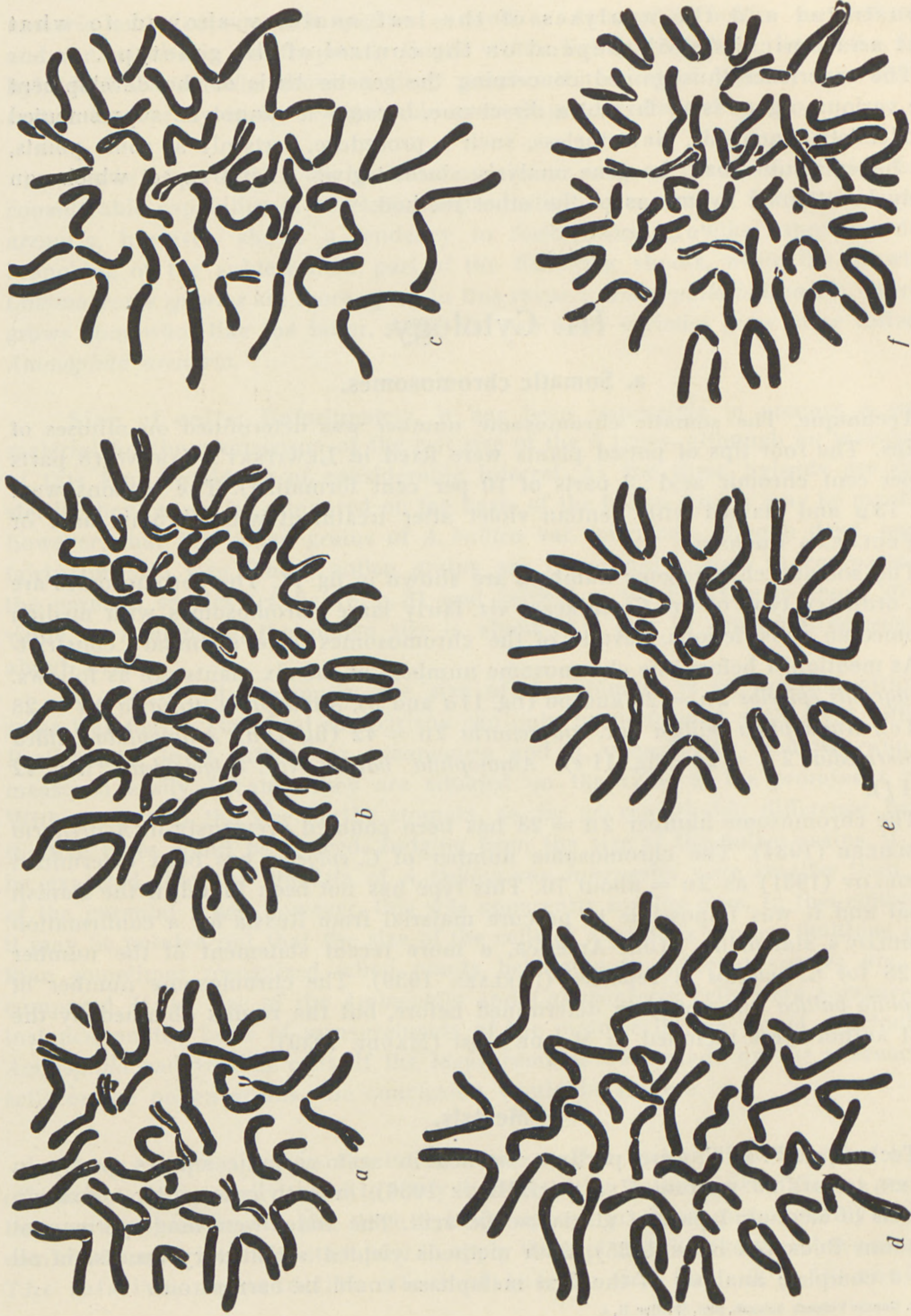


Fig. 11. Somatic chromosomes of a: *Calamagrostis epigeios*, diploid ( $2n = 28$ ), b: *Calamagrostis epigeios*, tetraploid ( $2n = 56$ ), c: *Ammophila arenaria* ( $2n = 28$ ), d: *Ammophila baltica* var. *subarenaria* ( $2n = 42$ ), e: *Ammophila baltica* var. *intermedia* ( $2n = 28$ ), f: *Ammophila baltica* var. *epigeioides* ( $2n = 42$ ).  $\times 4000$ .

*Ammophila arenaria* (fig. 12). Meiosis was examined in five plants derived from different localities. No observations were made on stages until metaphase I.

In metaphase I, 14 regular bivalents are formed in the majority of P. M. C. (fig. 12a). Some of the bivalents are associated with one or several interstitial chiasmata in both arms, a few are associated in one arm only, and occasionally, bivalents are formed which are associated by one or two terminal chiasmata only.

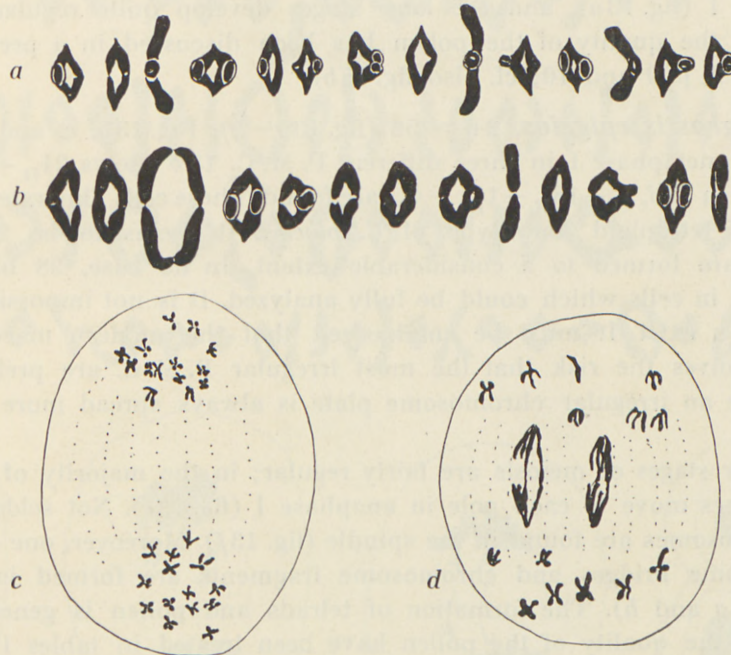


Fig. 12. Meiosis in *Ammophila arenaria*. a and b: metaphase I, c and d: anaphase I. a and b  $\times 2600$ , c and d  $\times 1800$ .

As a statistical analysis of the chiasma frequency seems to lack interest for the present problem, such an analysis has not been performed.

In all the plants examined, a few P. M. C. were observed which, instead of 14 bivalents, contained 13 bivalents and a ring or a chain of 4 chromosomes (fig. 12b). These cells constituted always much less than 1 per cent of the P. M. C. examined. It was not possible to decide, whether it were always the same four chromosomes that entered the multivalent association. The occurrence of these tetrasomes shows that *A. arenaria* is not completely stabilized, but contains one or a few reciprocal translocations. As the multivalent associations are very rare, the translocated pieces of chromosomes are probably quite short.

The course of the later stages of meiosis is generally quite normal (fig. 12c). The anaphase separation, however, may be difficult for a few chromosomes (fig. 12d), a phenomenon which may be related with the multivalent associations observed in metaphase I. Actual chromosome bridges were not observed.

The formation of tetrads and pollen takes place in a normal way. The pollen size and the quality of the pollen has already been mentioned (tables 1 and 2, p. 9 and 10; cf. also fig. 15a).

*Calamagrostis epigeios*,  $2n = 28$  (fig. 13a). In the diploid karyotype of *C. epigeios*, meiosis is quite regular in all the plants examined (4 from different localities). 14 bivalents, associated by interstitial or terminal chiasmata, are formed in metaphase I (fig. 13a), and the later stages develop quite regularly. The size variation and the quality of the pollen has been discussed in a previous section (tables 1 and 2, p. 9 and 10; cf. also fig. 15b).

*Calamagrostis epigeios*,  $2n = 56$  (fig. 13b-h). Fig. 13b, c, and d shows the association in metaphase I in three different P. M. C. 13b shows  $24_{II} + 2_{IV}$ ; in 13c,  $18_{II} + 5_{IV}$ , and in 13d,  $1_I + 18_{II} + 1_{III} + 4_{IV}$  are found. These cells illustrate the variation found in the tetraploid karyotype of *C. epigeios*. It seems to be the rule that multivalents are formed to a considerable extent. In no case, 28 bivalents have been observed in cells which could be fully analyzed. It is not impossible, however, that such cells exist. It must be emphasized that the modern maceration-smear technique involves the risk that the most irregular P. M. C. are preferred by the analysis, since an irregular chromosome plate is always spread more readily than a regular one.

The later stages of meiosis are fairly regular; in the majority of the P. M. C., 28 chromosomes move to each pole in anaphase I (fig. 13e). Not seldom, however, lagging chromosomes are found in the spindle (fig. 13f). Moreover, one or sometimes two chromosome bridges and chromosome fragments are formed in anaphase I and II (fig. 13g and h). The formation of tetrads and pollen is generally regular. The size and the quality of the pollen have been treated in tables 1 and 2 (p. 9 and 10; cf. also fig. 15c).

In a later section (cf. p. 51), these meiotic pictures will be discussed in relation to the taxonomic and phylogenetic problems involved in the two karyotypes of *Calamagrostis epigeios*.

*Ammophila baltica* var. *intermedia* (fig. 14). The first metaphase association was analyzed in several cells and a fairly great variation in the mode of association was found. Fig. 14a shows the association in five typical cells. The type of association is given in table 4.

Table 4.

Cell no.	Univalents	Bivalents	Trivalents	Tetralents
1 .....	2	13	..	..
2 .....	4	12	..	..
3 .....	5	10	1	..
4 .....	1	12	1	..
5 .....	..	12	..	1

A completely regular association (e.g. 14 bivalents) could not be observed, but P. M. C. with up to 6 univalents were found occasionally. Associations including more than four chromosomes were not seen, on the other hand, tri- and tetrasomes were observed in a great number of P. M. C.

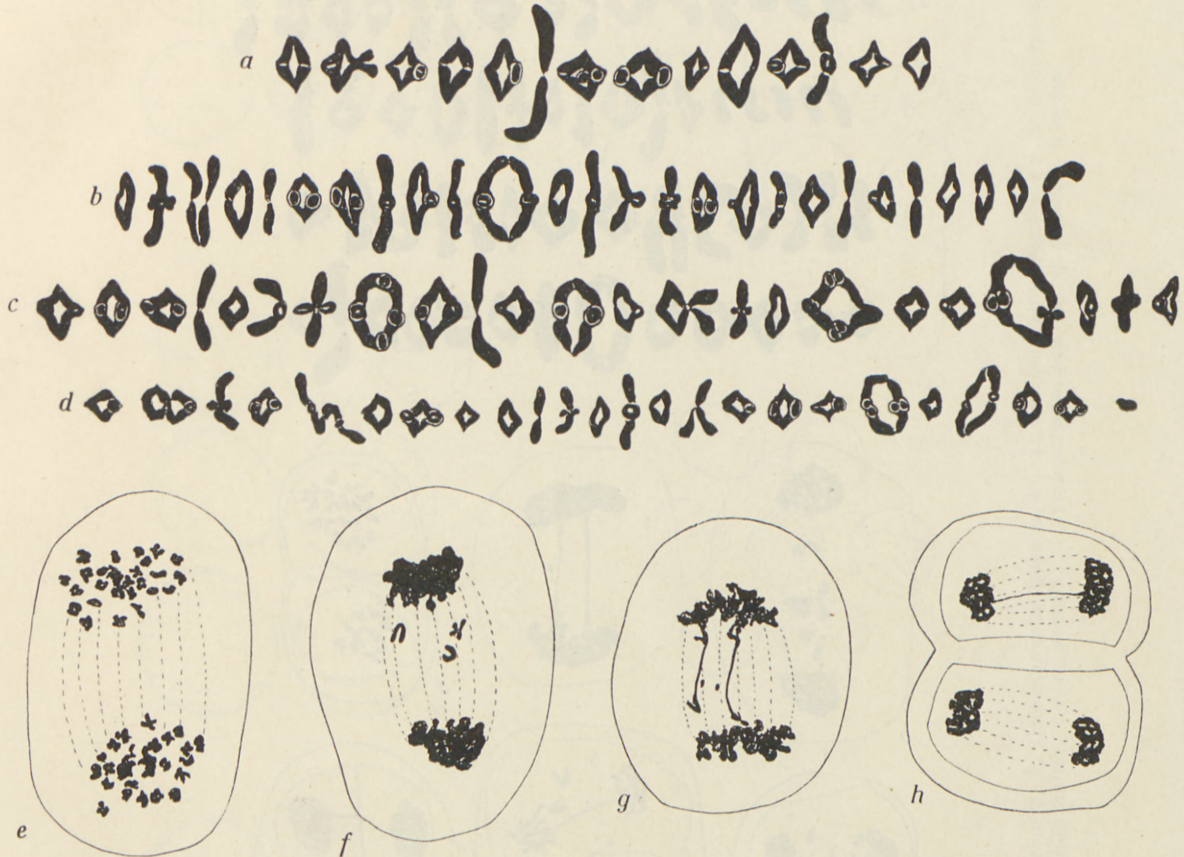


Fig. 13. Meiosis in diploid *Calamagrostis epigeios* (a) and tetraploid *Calamagrostis epigeios* (b-h). a-b-c-d: metaphase I. e-f-g: anaphase I and telophase I. h: telophase II. a-d:  $\times 2600$ ; e-h:  $\times 1800$ .

As might be expected, anaphase I is irregular, lagging chromosomes were noticed in almost every P. M. C. These lagging chromosomes seem to be univalents which divide in this stage (fig. 14b). One or two chromatin bridges and chromosome fragments occur very frequently (fig. 14c). Owing to the great number of univalents, the distribution of the chromosomes in the daughter nuclei is often irregular; in fig. 14d, 16 chromosomes may be counted in one of the plates of metaphase II. During the second division, lagging chromosomes and persistent chromatin bridges may be seen (fig. 14e, f and g). The formation of tetrads, however, is fairly regular, and nearly always four cells are formed in each pollen tetrad. The quality of the

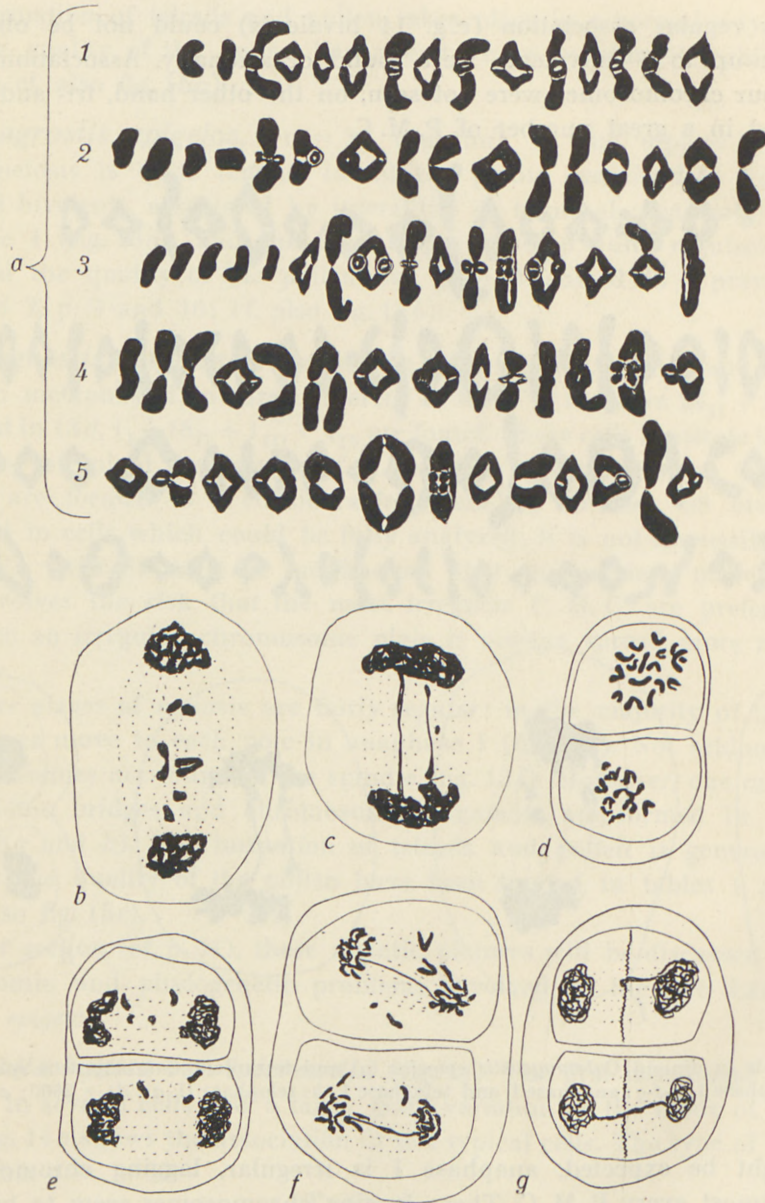


Fig. 14. Meiosis in *Ammophila ballica* var. *intermedia*. a: metaphase I. b-c: anaphase I and telophase I, d: metaphase II. e-f-g: anaphase II and telophase II. a:  $\times 2600$ , b-g:  $\times 1800$ .

pollen is very bad; considerably less than 1 per cent of the pollen grains are living (fig. 15e).

In spite of the great number of dead pollen grains, the picture of the pollen was surprisingly regular, particularly when compared with the two triploid hybrids

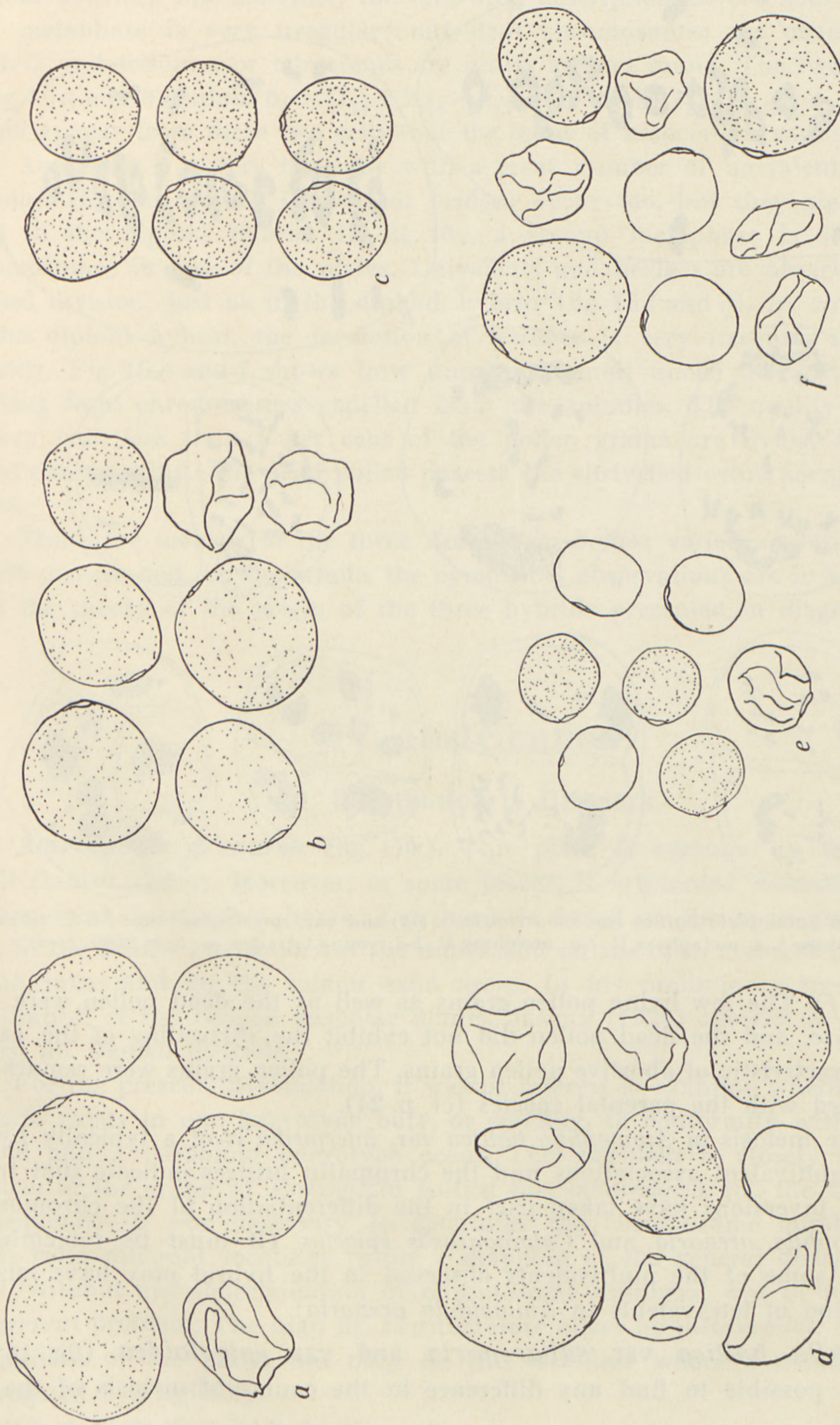


Fig. 15. Pollen grains of a: *Ammophila arenaria*. b: *Calamagrostis epigetos*, 4 n. c: *Calamagrostis epigetos*, 2 n. d: *Ammophila baltica* var. *subarenaria*. e: *Ammophila baltica* var. *intermedia*. f: *Ammophila baltica* var. *epigetoidea*.  $\times$  ca. 500.



Fig. 16. Meiosis in *Ammophila baltica* var. *subarenaria* (a-c-g) and var. *epigeioidea* (b-d-e-f-h-i). a-b: metaphase I. c-d: anaphase I. e: metaphase II. f-g: anaphase II. h-i: young tetrads. a-b:  $\times 2600$ , c-i:  $\times 1800$ .

(fig. 15d and f). The few living pollen grains as well as the dead pollen were very uniform in size, and the dead pollen did not exhibit the shrivelling of the exine otherwise characteristic of abortive pollen grains. The pollen grains were remarkably small compared with the parental species (cf. p. 24).

Thus, the meiosis in *Ammophila baltica* var. *intermedia* is of a typically hybrid nature. The multivalent associations and the chromatin bridges indicate that translocations and inversions have taken part in the differentiation of the chromosome sets of *Ammophila arenaria* and *Calamagrostis epigeios*. (It must be remembered, however, that some of the multivalents observed in the hybrid may have relation to the formation of tetravalents in *Ammophila arenaria*).

*Ammophila baltica* var. *subarenaria* and var. *epigeioidea* (fig. 16). It has not been possible to find any difference in the course of meiosis of the two



triploid hybrids, and therefore, the following description covers both of them. The first metaphase is very irregular; univalent chromosomes are observed in every P. M. C. and trisomes or tetrasomes are nearly always found. The two cells pictured in fig. 16 *a* and *b* contain  $6_I + 11_{II} + 2_{III} + 2_{IV}$  and  $12_I + 12_{II} + 1_{III} + 1_{IV}$ , respectively. It will appear from these two cells that the mode of association varies considerably.

Anaphase I is very irregular with a great number of univalents lying in the spindles (fig. 16 *c* and *d*). Chromatin bridges occur too, but they are less frequent than in the diploid hybrid. In fig. 16 *e*, a second metaphase is shown with 25 chromosomes in one of the plates. Univalents and bridges are observed during the second division, just as in the diploid hybrid (fig. 16 *f* and *g*). In contradistinction to the diploid hybrid, the formation of tetrads is very irregular in the triploid hybrids. Fig. 16 *h* and *i* shows how numerous small nuclei are formed, probably deriving from chromosomes expelled from the spindles. The quality of the pollen is very bad, less than 1 per cent of the pollen grains are living, and their size varies considerably. The dead pollen possess the shrivelled exine normal to abortive pollen.

Thus, the meiosis of the three *Ammophila baltica* varieties presents a marked hybrid picture and, on the whole, the cytological observations are in full accordance with the theory of the origin of the three hybrids presented in diagram 1.

## IV. Distribution.

### a. Distribution in Denmark.

*Ammophila arenaria* (fig. 17 *a*). This plant is common in the sand-dunes of all Danish shores. Moreover, in some places, it is planted in order to bind the shifting sand. Inside the shores, *A. arenaria* is found on the open areas of shifting sand in the heaths and moors of the dunes and on the open areas of the plantations of the dunes and on the inland sand areas. In his preliminary treatment of the distribution of the Danish vascular plants, JESSEN (1926) records *A. arenaria* from almost all Danish districts.

For the present investigation, it was of interest to examine whether *Ammophila arenaria* exists in one karyotype only, or whether, in conformity with *C. epigeios*, a diploid and a tetraploid karyotype occur. This problem is closely related to the problem of the origin of *Ammophila baltica* var. *subarenaria* (cf. the diagram, p. 3). Consequently, it was necessary to determine the chromosome number of several plants from different localities, while a mapping of the general distribution of the plant, without any determination of chromosome numbers, was of no interest for the present problem. The map fig. 17 *a* therefore offers no information on the general distribution of the plant, but only on the localities where *A. arenaria* has been investigated cytologically.

The chromosome number has been counted in 69 plants, deriving from 9 different localities. (In the majority of the cases, the chromosome number was determined on root tip mitoses fixed from potted plants). Three of these localities (in fig. 17a indicated by squares) have been examined more thoroughly, viz. (1) Ordrupnæs on Sealand (25 plants); in this locality, a single specimen of *Ammophila baltica* var. *subarenaria* is growing together with both karyotypes of *C. epigeios*. (2) Villingebæk on Sealand (10 plants), where all three *Ammophila baltica* hybrids and both karyotypes of *C. epigeios* occur. (3) A locality on the Bøtø dike on the Island of Falster (27 plants); in this locality, both karyotypes of *Calamagrostis* are found together with *A. baltica* var. *subarenaria* and var. *intermedia*.

Stress was laid upon examining as many different types as possible, but, as mentioned previously, all plants were diploid ( $2n = 28$ ) so that the author's attempt to find a tetraploid karyotype of *A. arenaria* hitherto has not been successful.

*Calamagrostis epigeios* (fig. 17b and c). According to JESSEN (l. c.), *C. epigeios* is distributed over the majority of Danish districts; especially, it is common in North and Central Jutland and also in the dunes of Bornholm and Lolland-Falster. *C. epigeios* is found partly on the shores, partly on the heaths and moors, sometimes it may even be found in the woods.

The main purpose of the present investigation is to map the distribution of the two karyotypes; mapping of the general distribution of *C. epigeios* without considering the karyotype is of little interest. Unfortunately, however, herbarium plants could not be used, because the two karyotypes cannot be distinguished morphologically (cf. p. 8). Therefore, the two maps fig. 17b and c give only very incomplete information about the distribution of the two karyotypes.

The chromosome number was counted in 83 plants. 41 were found to be diploid, whereas 42 belonged to the tetraploid karyotype. The growths on Dueodde on the Island of Bornholm were examined more thoroughly, since *C. epigeios* is extraordinarily variable in this locality (cf. fig. 1, p. 6). Here, *Ammophila baltica* var. *subarenaria* and var. *intermedia* are forming large growths, while var. *epigeioidea* could not be detected. In addition, various regions of the shores and dunes in North Jutland (Hirtshals and Svinkløv) have been thoroughly examined. Here, *C. epigeios* forms large growths in company with *A. arenaria*, while *A. baltica* var. *subarenaria* and var. *intermedia* do not occur, and the third hybrid, var. *epigeioidea*, is rare. Already WARMING (1909) drew our attention to the peculiar fact that *A. baltica* is lacking in these localities. (WARMING did not know var. *epigeioidea*).

The maps fig. 17b and c show that the tetraploid karyotype is predominant in North Jutland. 30 tetraploid plants were found, but only one diploid. On the other hand, the tetraploid karyotype seems to be lacking on Bornholm, where 24 plants examined, representing very different types, were diploid. On several other localities, however, both karyotypes occur, for instance, at Villingebæk on Sealand, Skelde wood in South Jutland, Bøtø dike on Falster, etc. The investigation is not

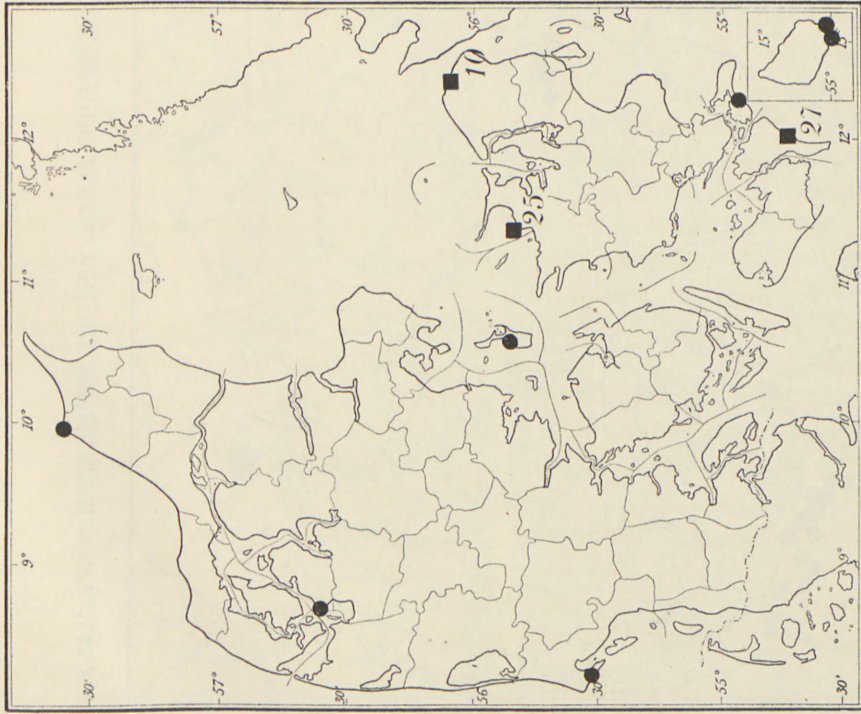


Fig. 17 a. *Ammophila arenaria*. Localities where plants have been collected for the determination of chromosome numbers. (Note: not map of distribution)

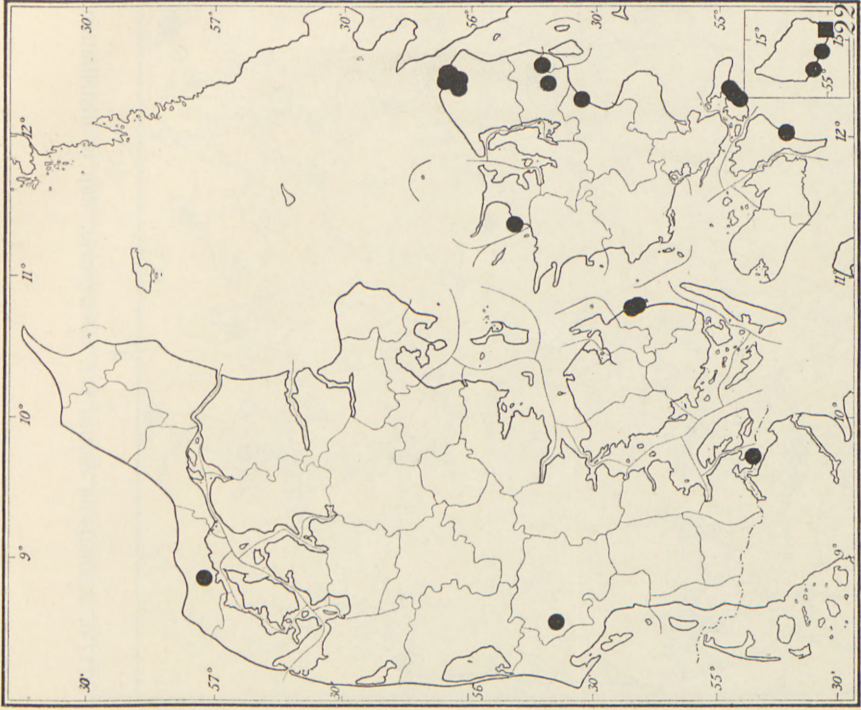


Fig. 17 b. *Calamagrostis epigeios*, diploid. Map of distribution.

A full circle: Chromosome number determined, herbarium material available.

A circle: Chromosome number not determined, herbarium material available.

A full square and a figure: Chromosome number determined on several plants from the localities concerned.

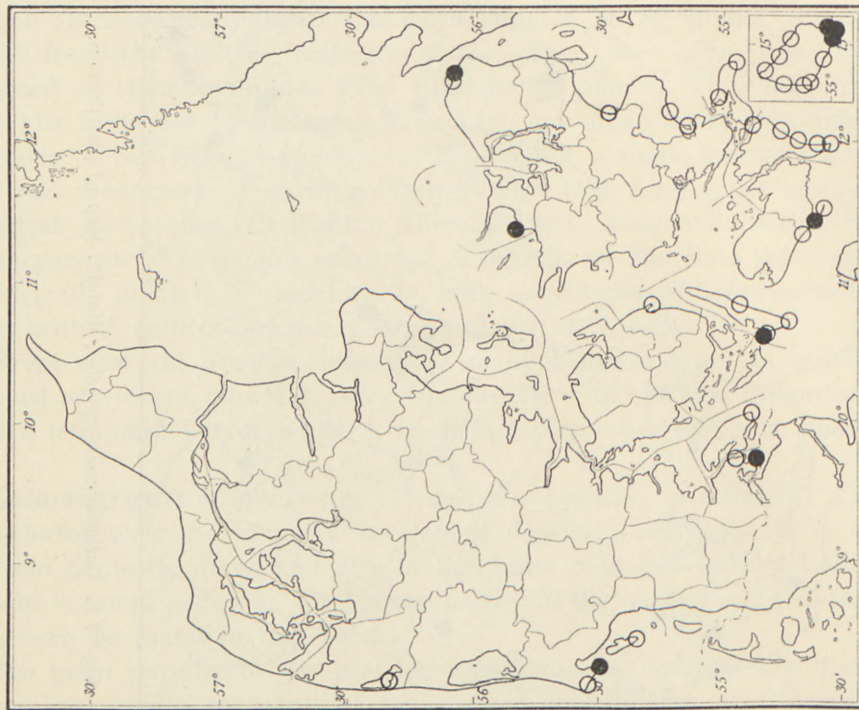


Fig. 17 c. *Calamagrostis epigeios*, tetraploid. Map of distribution.

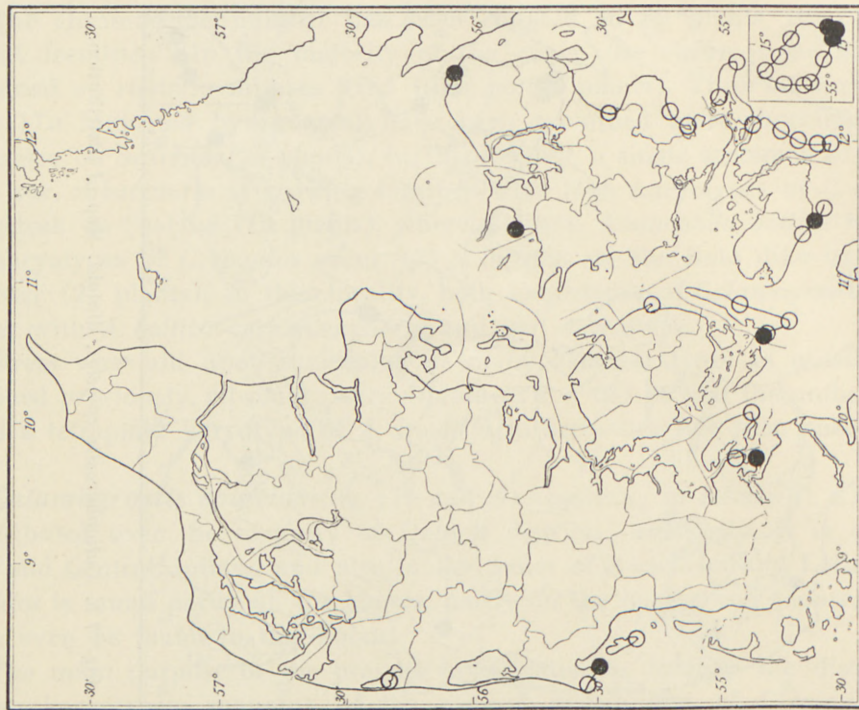


Fig. 17 d. *Ammophila ballica* var. *subarenaria*. Map of distribution.

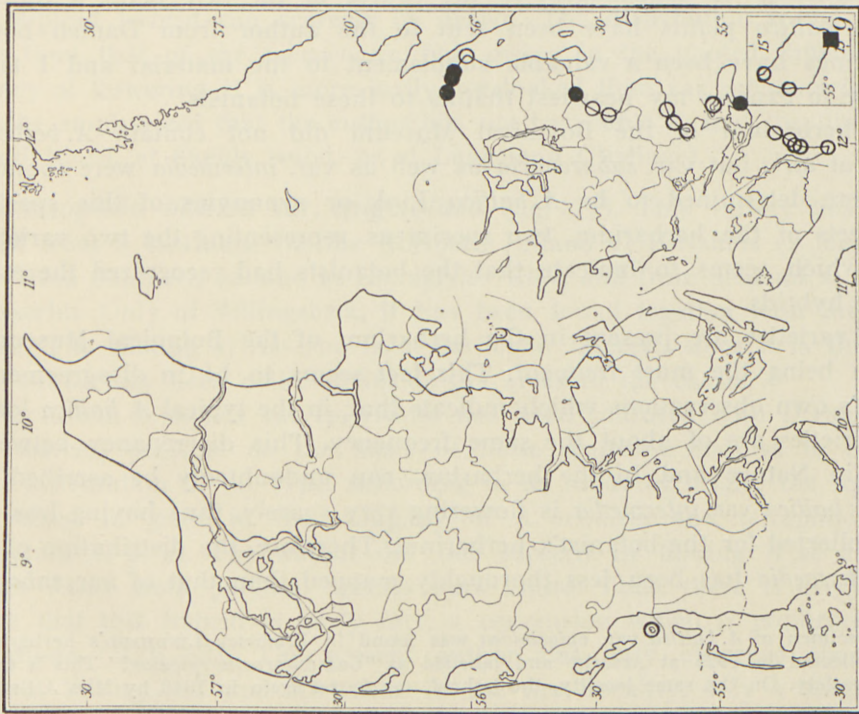


Fig. 17 e. *Ammophila ballica* var. *intermedia*. Map of distribution.

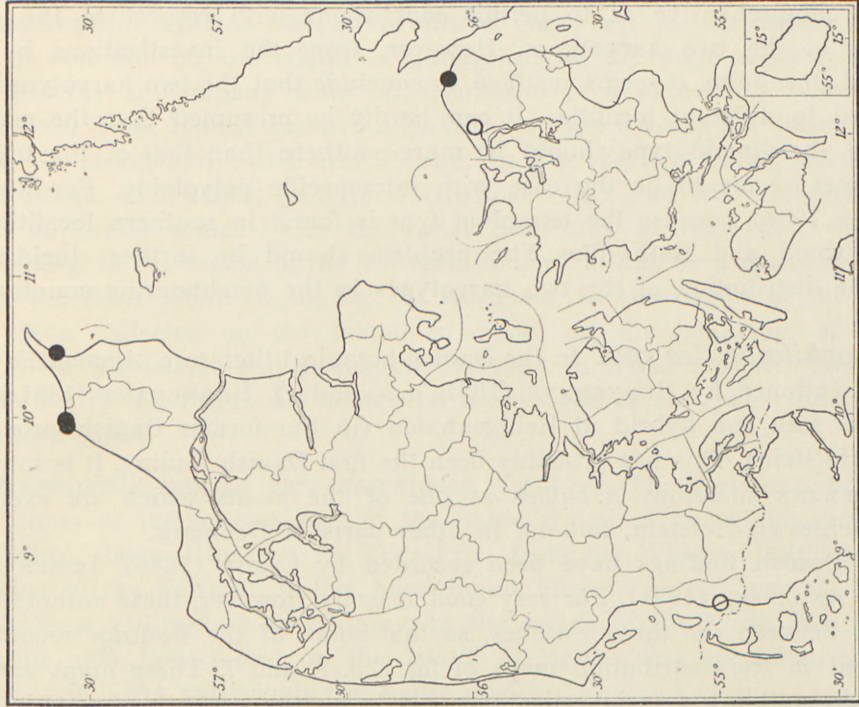


Fig. 17 f. *Ammophila ballica* var. *epigeioidea*. Map of distribution.

sufficiently comprehensive as to permit conclusions with respect to the general distribution of the two karyotypes. However, from the investigations in North Jutland and Bornholm, it seems justified to conclude that the two karyotypes might be restricted to different localities. It can hardly be presumed that the main distribution of the diploid type should be more southern than that of the tetraploid (as sometimes seems to be the case with intraspecific polyploidy. For literature, cf. TISCHLER 1934), because the tetraploid type is found in southern localities such as Skelde wood and Bøtø dike. The problem should be further elucidated by studying the distribution of the two karyotypes in the neighbouring countries.

*Ammophila baltica* coll. In the Danish botanical literature, *Ammophila baltica* was first mentioned by HORNE MANN (1821, *a*, *b*, and *c*). HORNE MANN (1821*c*) states that he has seen the hybrid at Heiligenhafen (in the former Danish province of Schleswig-Holstein). This has probably been the first Danish finding. It is interesting that HORNE MANN mentions *A. baltica* as one of the plants which are exclusively found in Schleswig-Holstein, but not in other parts of Denmark.

Later Danish findings have been recorded by LANGE (1886), JESSEN (1926), and SVEND ANDERSEN (1931). For very good reasons, however, these authors do not distinguish between the three varieties, so that some of the findings recorded are not included in the distribution maps of fig. 17*d*, *e*, and *f*. These maps are based partly on the author's own investigations, partly on the herbarium material of the Botanical Museum of Copenhagen. In the course of the last years, a number of *Ammophila baltica* plants have been sent to the author from Danish botanists. These findings have been a valuable supplement to the material and I take the opportunity to express my heartiest thanks to these botanists.

The herbarium of the Botanical Museum did not contain *A. baltica* var. *epigeioidea* at all<sup>1</sup>; but var. *subarenaria* as well as var. *intermedia* were found. Both hybrids were determined to be *A. baltica* Link or synonyms of this species. On several sheets of the herbarium, two specimens representing the two varieties are mounted, which seems to indicate that the botanists had recognized the existence of the two hybrids.

Both varieties are present in the herbarium of the Botanical Museum, var. *subarenaria* being the most frequent. This fact seems to be in disagreement with the author's own observations which indicate that, in the typical *A. baltica* localities, the two varieties are of about the same frequency. This discrepancy between the frequency in Nature and in the herbarium can undoubtedly be ascribed to the fact that *A. baltica* var. *intermedia* is flowering very sparsely, thus having less chance of being collected for the botanist's herbarium. Therefore, the distribution of *A. baltica* var. *intermedia* has been less thoroughly mapped than that of var. *subarenaria*.

<sup>1</sup> A specimen of *A. baltica* var. *epigeioidea* was found in Professor LINDHARD's herbarium. The plant was collected in 1925 at Asserbo and labelled as "*Calamagrostis epigeios*?" This is the oldest find of this variety. On the same locality, the hybrid was found again in 1940 by Miss J. GRÜNER.

*Ammophila baltica* var. *subarenaria* (fig. 17d). In the Baltic region, this variety is common on the Island of Bornholm; on the dune areas at Dueodde, it forms growths in company with var. *intermedia* of a luxuriantly scarcely seen in other places. Other typical areas of *A. baltica* are Bøtø dike on the Island of Falster, Ulfshale on Møen, and Fedet near Præstø. In the Western Baltic region, it occurs on the Island of Lolland, Langeland (Ristinge cliff), and Als, and also in South Jutland at Skelde wood. From the Great Belt, only a single find was recorded from Nyborg. In the region of the Kattegat, the hybrid has been found at Ordrupnæs and on the shore from Villingebæk to Hornbæk. In the region of the North Sea, it has been collected on the Island of Fanø, on the peninsula of Skallingen, and also on the island and peninsula of Fjand in Nissum Fjord. The specimens from the North Sea region are somewhat more vigorous and paler than those from the Baltic regions.

*Ammophila baltica* var. *intermedia* (fig. 17e). The diploid hybrid is found in the dunes of the eastern part of the Baltic region at about the same localities as described above. However, no finds exist from the Western Baltic localities. In the region of the Kattegat, it has been found only in North Sealand, where it is common from Villingebæk to Elsinore. In the region of the North Sea, this variety has so far only been found on the Island of Manø. This specimen, too, is more vigorous and looks paler than the Baltic ones.

On the basis of these findings, general conclusions concerning the distribution of the diploid hybrid can scarcely be drawn. The distribution seems to be more eastern than that of var. *subarenaria* but, owing to the quoted difference in the frequency of flowering, it is more easily overlooked than var. *subarenaria*. However, it may be emphasized that the author has not been able to find the diploid hybrid at Fjand Fjord, at Skelde wood, or at Langeland (Ristinge).

*Ammophila baltica* var. *epigeioidea* (fig. 17f). This variety has only been recorded from 6 localities, viz. the Northern Jutland at Hirtshals (2 localities), and Hulsig; from Southern Jutland at Emmerslev Klev, and from Sealand at Villingebæk and Asserbo. Only at Villingebæk, it has been found together with the two other varieties. The finding at Asserbo is of particular interest, since it is the first time that *A. baltica* has been collected inside the areas of the coasts.

No doubt, *A. baltica* var. *epigeioidea* has been confused with *C. epigeios*, and it seems scarcely possible on the basis of these scattered findings to estimate the general distribution of the type. According to its mode of origin, the hybrid might be expected to grow in the company of *A. arenaria* and tetraploid *C. epigeios* (cf. diagram 1, p. 3). The author did not succeed in finding it in a number of localities where both parental species were found. Thus, there is every reason to suppose that this hybrid is fairly rare, a conclusion which is confirmed by other observations (cf. p. 58). The present author has only observed the variety in Nature at Villingebæk and Hirtshals. In the first locality, only a single specimen

was found; at Hirtshals, where it is known to be produced in abundance by crossing every year (cf. next section), the hybrid is rare too.

According to the theory which forms the basis of the present investigation (cf. diagram 1, p. 3), *A. baltica* var. *subarenaria* and var. *intermedia* should be found where *A. arenaria* and the diploid karyotype of *C. epigeios* are growing together, whereas *A. baltica* var. *epigeioidea* accompanies *A. arenaria* and the tetraploid karyotype of *C. epigeios*. In all localities where conditions have been thoroughly examined, this assumption holds. (It must be remembered that fig. 17a gives no information regarding the general distribution of *A. arenaria*). In North Jutland (Hirtshals), where var. *epigeioidea* occurs but the other varieties are lacking, tetraploid *Calamagrostis epigeios* is common, while the diploid karyotype has not been found. On Bornholm, where the first two varieties are very common, while var. *epigeioidea* is lacking, only the diploid karyotype of *C. epigeios* has been detected. In the district of Villingebæk in North Sealand, where all three hybrids were found, both karyotypes of *C. epigeios* occur too, together with *A. arenaria*.

In some localities (for instance, Fjand in Nissum Fjord and Fedet near Præstø), *C. epigeios* has not been observed in the immediate vicinity of the growths of *A. baltica*, however, the species is known to grow in the district. In view of the great capability of dispersion of the grass pollen, it does not seem peculiar that one of the parental species may not be found in the immediate neighbourhood of the hybrid.

#### b. General distribution.

*Ammophila arenaria*. According to ASCHERSON and GRAEBNER (1902) and to HEGI (1926), this species occurs in the coastal regions of Europe except in the Arctis. Moreover, it grows in the Mediterranean region including North Africa, and also in North America.

*Calamagrostis epigeios*. According to the above authors, the main distribution is Europe, except Russia and the Northern Scandinavia, the Western, Northern, and Eastern Asia, and South Africa. As mentioned previously, the distribution of the two karyotypes is not known. KUPFFER (cf. LEHBERT 1930) determined the size of the pollen grains on Baltic specimens of *C. epigeios* to be 26—30  $\mu$ , which approximately corresponds to that of the diploid karyotype (cf. table 1, p. 9).

*Ammophila baltica*. The literature offers information concerning the distribution of the collective "species" only, while the distribution of the varieties must be studied on herbarium material. The author has examined the material from the Botanical Museum of Copenhagen, including 25 specimens, viz. 15 from Germany, 8 from Sweden, 1 from Norway (Sandø near Færder), and 1 from Lithuania. The 15 German, the Norwegian, the Lithuanian, and 6 of the Swedish specimens belonged to the variety *subarenaria*, whereas two Swedish specimens (from Ystad) belonged to the variety *intermedia*. The third hybrid, var. *epigeioidea*, was not



represented in the material. The German specimens originated from the Baltic region (Heiligenhafen, Kiel, and Eckernförde) and from the North Sea region (Amrum and Eyderstedt).

According to the literature, *A. baltica* coll. has also been found in the following countries. Germany: the dunes of the Baltic coasts and the North Sea region including all Frisian islands (cf. MARSSON 1869, PRAHL 1890, KNUTH 1895, ASCHERSON and GRAEBNER 1899 and 1902, JUNGE 1913). In the Baltic area, the hybrid was found in Lithuania and Latvia, reliable statements from Esthonia are lacking (cf. LEHMANN 1895 and STARCS 1931). In Sweden, *A. baltica* has been found in Scania, Halland, Bohuslän, Öland, and Gotland (cf. LINDMAN 1926). No findings have been recorded from Finland. In Norway, *A. baltica* has been collected in the region of the Oslo Fjord: Sandø at Færder, Tjømøy, Jomfruland (cf. BLYTT 1906 and NORDHAGEN 1940). In England, the hybrid has been found at Ross Links, Northumberland (TRIMEN 1872), and also in Norfolk (cf. BABINGTON 1904, BENTHAM and HOOKER 1920). In Holland, *A. baltica* is said to be very rare (cf. HEUKELS 1934). Reports of more southern findings from Belgium and France do not exist.

In the floral works mentioned above, *Ammophila baltica* is stated to occur in the coastal regions. In Germany, however, a single finding was made at Celle in Western Germany (SCHOLZ 1905).

In some of the German floral works, a distinction is made between *Ammophila baltica* var. *subarenaria* Mars. and var. *subepigeios* Mars. (cf. MARSSON 1869, PRAHL 1890, ASCHERSON and GRAEBNER 1899 and 1902, JUNGE 1913, and CHRISTIANSEN 1913). The first variety is identical with *A. baltica* Link. This variety is the most frequent one, while var. *subepigeios* Mars. is recorded to be rare. MARSSON found the last hybrid at Peenemünde and Zinnowitz, in Usedom and at Dars. PRAHL has not seen typical specimens himself, but he mentions a specimen from Sankt Peter near Eyderstedt (North Sea region) as being the one which corresponds best to MARSSON's description. The Eyderstedt specimen is to be found in HANSEN's herbarium (no. 1106, cf. PRAHL l. c., p. 251); it was determined by NOLTE to be *Arundo maritima* Agardh. The present author has not seen the original specimens of MARSSON's, but HANSEN's specimen from Eyderstedt is present in the herbarium of the Botanical Museum of Copenhagen. An examination of this specimen showed that it can be determined with certainty as *A. baltica* var. *subarenaria*. These facts support the view of the present author: The description of *A. baltica* var. *subepigeios* Mars. is so incomplete that it is justified to reject the name and to introduce the two new names of variety, viz. *intermedia* and *epigeioidea*.

## V. Crossing experiments.

According to diagram 1, *A. baltica* var. *intermedia* is a hybrid between diploid *Calamagrostis epigeios* and diploid *Ammophila arenaria*, and *A. baltica* var. *epigeioidea* is a hybrid between the tetraploid karyotype of *C. epigeios* and *A. arenaria*, while *A. baltica* var. *subarenaria* arises from a crossing between diploid *C. epigeios* and either a—not observed—tetraploid karyotype of *A. arenaria*, or diploid gametes of this species.

This theory can only be definitely proved if the hybrids are successfully raised experimentally, starting from the postulated parental types. The author has been able to produce the diploid hybrid var. *intermedia* and the triploid hybrid var. *epigeioidea* experimentally, whereas the experiments on synthesizing var. *subarenaria* hitherto failed.

***Ammophila baltica* var. *intermedia*.** The two parental species, diploid *C. epigeios* and *A. arenaria*, were crossed in the way that a plant of each species was placed in a greenhouse, the flowering shoots were tied together, and during the flowering period, they were shaken repeatedly. Seeds were gathered from both species and, in the spring of the following year, these seeds were sown in sterilized soil. The result of the experiment is shown in table 5.

Table 5.

Combination	No. of seeds	No. of plants	Type of offspring
<i>C. epigeios</i> 2n ♀ × <i>A. arenaria</i> ♂ . . . . .	2000 <sup>1</sup>	1500 <sup>1</sup>	{ 5 <i>C. epigeios</i> 2n, the remainder <i>A. baltica</i> v. <i>intermedia</i> 2 <i>A. baltica</i> v. <i>intermedia</i>
<i>A. arenaria</i> ♀ × <i>C. epigeios</i> 2n ♂ . . . . .	11	2	
<i>A. arenaria</i> from Hirtshals, pollinated in Nature . . . . . } <i>C. epigeios</i> 4n from Hirtshals, pollinated in Nature . . . . . }	800 <sup>1</sup>	538	{ 529 <i>A. arenaria</i> 29 <i>A. baltica</i> v. <i>epigeioidea</i> 392 <i>C. epigeios</i> 4n 8 <i>A. baltica</i> v. <i>epigeioidea</i>
	1000 <sup>1</sup>	400	
<i>A. arenaria</i> ♀ exposed to temperature shock × <i>C. epigeios</i> 2n ♂ . . . . . }	5	4	4 <i>A. baltica</i> v. <i>intermedia</i>
<i>C. epigeios</i> 4n ♀ × <i>C. epigeios</i> 2n ♂ . . . . .	24	9	<i>C. epigeios</i> 3n
<i>C. epigeios</i> 2n ♀ × <i>C. epigeios</i> 4n ♂ . . . . .	480	100	<i>C. epigeios</i> 3n

<sup>1</sup> approximately.

This table shows that the reciprocal crossing did not succeed equally well. Only 11 seeds were gathered from *A. arenaria*, but about 2000 from *C. epigeios*. From the seeds derived from *A. arenaria*, two plants arose which were not identical with the mother species. From the seeds of *C. epigeios*, a large number of deviating plants

were obtained, and 5 typical diploid *C. epigeios* plants. The deviating plants were very uniform and were identical with the two plants derived from the *A. arenaria* seeds. The chromosome number was determined on several plants which all proved to be diploid ( $2n = 28$ ). These plants got flowers in the following summer, and with respect to all floral and vegetative characters, meiosis and pollen quality, they were quite identical with the plants of *A. baltica* var. *intermedia* growing in Nature. (Fig. 18b, cf. fig. 4, p. 12).

This experiment has definitely proved that *Ammophila baltica* var. *intermedia* is a hybrid between the diploid karyotype of *C. epigeios* and *A. arenaria*.

No plasmon effect can be demonstrated in these plants, since the reciprocal hybrids are identical.

Furthermore, the experiment indicates that both parental species are self-sterile. The 5 plants of *C. epigeios* may have arisen either from self-pollination or, possibly, from pollination of other plants of *C. epigeios*, since the isolation in the greenhouse was not quite perfect.

*Ammophila baltica* var. *epigeioidea*. This hybrid has not been produced through a crossing experiment as the diploid hybrid; however, it has been raised under controlled conditions, and it is definitely proved that the plant is a hybrid between *A. arenaria* and the tetraploid karyotype of *C. epigeios*.

Ears of *A. arenaria* and *C. epigeios* were collected in the autumn of 1938 in the neighbourhood of Hirtshals (North Jutland). The plants were gathered in localities where both species were growing, so that the plants had a high chance of crossing in Nature. The plants of *C. epigeios* derived from growths of tetraploids.

It was the main purpose of this experiment to elucidate a problem propounded, for instance, by WARMING (1909), viz. the reason for the apparent lacking of *A. baltica* in a number of localities where both the assumed parental species are growing in abundance (*A. baltica* var. *subarenaria* and var. *intermedia* have not been observed in North Jutland; actually, *A. baltica* var. *epigeioidea* occurs at Hirtshals; however, when the experiment was started, the author was not aware of the existence of this hybrid). A priori, three solutions of this problem may be imagined. (1) The theory of the hybrid origin of *A. baltica* does not hold, the plant is not a hybrid between *C. epigeios* and *A. arenaria*. (2) The hybrid is produced in the localities concerned, but it does not find suitable growth conditions. (3) Special conditions hinder the crossing of the two species in the respective localities; the latter possibility might exist if one or both parental species belonged to another strain or race than that growing in the Baltic region. These three assumptions could be checked, at least partly, through the above described experiment of examining the offspring of plants pollinated in Nature.

The seeds collected at Hirtshals were sown in sterilized soil in the spring of 1939. The majority of the plants belonged to the genuine species but, in addition,



Fig. 18. a: *Ammophila arenaria*. b: *Ammophila arenaria* × *Calamagrostis epigeios*, diploid (= *Ammophila ballica* var. *intermedia*). c: *Ammophila ballica* var. *epigeioidea*; plant originating from seeds gathered on *Ammophila arenaria* plants from Hirtshals (sister plant to a). d: *Calamagrostis epigeios*, tetraploid. Cf. fig. 4. ×  $\frac{1}{2}$ .

deviating plants were found both among the seedlings of *A. arenaria* and of *C. epigeios*. (cf. table 5). These deviating plants were fairly uniform; an estimation of the chromosome number showed that they were triploid ( $2n = 42$ ) and, when flowering, they proved to be identical with the triploid *A. ballica* var. *epigeioidea* found in Nature (cf. fig. 18c and fig. 4, p. 12).

These hybrid plants arose both from seeds derived from *Ammophila arenaria* and from seeds derived from the tetraploid *C. epigeios*; this proves definitely that *A. ballica* var. *epigeioidea* in Nature arises from a crossing of these two plants.

At the same time, the experiment is decisive for our understanding of the distribution of the *A. baltica* hybrids (the problem stated above); this, however, will be discussed more thoroughly in a later section (cf. p. 58). It may, however, be emphasized here that great quantities of hybrid seeds (*A. baltica* var. *epigeioidea* seeds) are produced every year in the localities concerned; both the *Calamagrostis* and the *Ammophila arenaria* growths are very extensive, and the hybrid seeds amount to about 2 per cent of the seeds deriving from *C. epigeios* and to as much as 5 per cent of the seeds of *A. arenaria* (cf. table 5).

*Ammophila baltica* var. *subarenaria*. Seeds of *Ammophila arenaria* have been treated with colchicine in order to produce a tetraploid strain. By crossing such a strain with the diploid karyotype of *C. epigeios*, *Ammophila baltica* var. *subarenaria* should be produced. The results of the colchicine experiments, however, are not yet quite reliable.

In addition, attempts were made to produce diploid gametes of *A. arenaria* by exposing the flowers to a temperature shock simultaneously with the meiosis of the embryo sac. The treated plants were afterwards pollinated by diploid *C. epigeios*. From this crossing, 4 plants arose; they were diploid ( $2n = 28$ ) and were identical with *A. baltica* var. *intermedia* (cf. table 5, p. 42). Hence, the author did hitherto not succeed in arising *A. baltica* var. *subarenaria* experimentally.

**Triploid *Calamagrostis epigeios*.** Until the present, the author could not find triploid *C. epigeios* in Nature. For various reasons, however, it was of interest to produce this type experimentally. Therefore, a diploid and a tetraploid *C. epigeios* plant were crossed in the way described previously (cf. p. 42). This cross in both directions gave rise to triploid plants. (The chromosome number  $2n = 42$  was estimated on several plants derived from diploid as well as from tetraploid *C. epigeios*). The cross seems to be more successful if the diploid karyotype is used as the female parent (cf. table 5, p. 42). The triploid plants have not yet flowered, this is why they are not mentioned in detail in the present paper.

## VI. Other observations.

### a. Fertility.

The sterility of *A. baltica* emphasized by all previous authors has already been used by ROEPER (1844) as an argument in favour of the hybrid nature of the plant. WARMING (1909) reports that he has examined a great number of plants from Heligoland without finding a single seed. The present author has threshed a great number of plants of all three varieties (deriving from Villingebæk and the Island of Møen) with the same result. All three hybrids seem to be sterile.

It is, nevertheless, not excluded that a single seed may be formed occasionally either through self-pollination or, perhaps more probably, through pollination by

one of the parental species. Moreover, *A. baltica* is undoubtedly capable of acting as a pollen parent, since all three varieties form a few living pollen grains (cf. fig. 15, p. 31). Obviously, such back-crosses will take place from time to time in Nature (cf. the following section).

#### b. Deviating plants.

*Calamagrostis epigeios*. In Northern Jutland, *C. epigeios* now and then have very hairy lower leaf sheaths. As far as it is known, this type has not been described before in the Danish floristic literature; however, it seems to be identical with *C. epigeios* var. *paralia* Fries mentioned in HEGI (1926) and other floral works. The variety has only been demonstrated within the tetraploid karyotype.

It may be mentioned that in the experiments described on p. 43, a few *Ammophila baltica* var. *epigeioidea* plants arose with very hairy leaf sheaths. No doubt, they have arisen through a crossing between *A. arenaria* and *C. epigeios* var. *paralia* Fries which has been found at Hirtshals. However, the hairy type of *A. baltica* ("*A. baltica* var. *epigeioidea* f. *paralia*") has not yet been found in Nature.

*Ammophila baltica*. In the material collected by the author and in the herbarium material of the Botanical Museum, a few specimens of *A. baltica* were found which did not agree in all characters with any of the three varieties described. For instance, plants may be found most of whose characters are typical of *A. baltica* var. *subarenaria*, except that the awn or the hairs are longer than is normal in this variety. In other specimens whose floral organs are typical of var. *intermedia* or var. *subarenaria*, the leaves may be somewhat broader and their furrows less deep than is typical of the variety<sup>1</sup>. The latter characters may possibly be due to modifications caused by special conditions of habitat; in other cases, however, the deviating type must be genetically conditioned. Deviating plants of the last type may, a priori, arise in two ways, either through a back-cross between one of the typical varieties and one of the parental species, or by a crossing between deviating types of the parental species. The former possibility has been discussed in the previous section. With respect to the other possibility, it may be mentioned that especially *C. epigeios* is very variable; it may even vary in such essential taxonomic characters as the length or the place of insertion of the awn, the length of the hairs, etc. (Cf. HEGI 1926, ASCHERSON and GRAEBNER 1899, JUNGE 1913, p. 184, and LEHBERT 1930).

The author found these intermediate types most frequently in the dunes of Dueodde at the southern point of Bornholm. Here, the growths of *A. baltica* var. *sub-*

<sup>1</sup> One of these deviating plants is of special interest, viz. a type of *Ammophila baltica* collected by K. WIINSTEDT at Fælsted Odde in Nissum Fjord (WIINSTEDT 1914). According to the leaves, it is a specimen of var. *subarenaria*, but the panicle is more close than is typical of this variety. The size of the flower, on the other hand, agrees with var. *intermedia*. The awn is very long, as in *C. epigeios*, but it is inserted nearly at the tip of the outer pale, as in *Ammophila arenaria*; hence, it projects very much from the tip of the outer pale.

*arenaria* and var. *intermedia* are extraordinarily luxuriant, and diploid *C. epigeios* is very variable (cf. fig. 1, p. 6). The chromosome number  $2n = 28$  was determined on a few of these deviating plants from Dueodde.

### c. Ecology.

*Ammophila arenaria*. As is well-known, marram, the dominant grass in the dunes, is mainly associated with the white sand dune area. The biology of the plant has been thoroughly described (cf. WARMING 1909) and, therefore, this problem will not be dealt with here.

*Calamagrostis epigeios*. Also this plant grows in the littoral regions, particularly in moist and clayey soil. Only at Dueodde, where special conditions undoubtedly are present (cf. WARMING 1909, HAMMER PEDERSEN 1941), *Calamagrostis* is found abundantly in the sea dunes; but generally it is associated with the grey dune and particularly with the sand field (the sandy plains behind and among the grey dunes). Furthermore, it occurs frequently at banks and clayey cliffs, i. e. in areas where a formation of dunes scarcely takes place and where, consequently, *Ammophila arenaria* is generally lacking. Inside the littoral area, *C. epigeios* is found in the poor area of hill-islands in Central Jutland; in the plantations and, particularly in Northern Jutland, frequently along the highways. In Sealand, where the plant is rarer inside the littoral areas, it is found scattered on poor soil in woods.

It would be of great interest to find out whether the two karyotypes of *Calamagrostis epigeios* are ecologically different (whether they belong to different ecotypes sensu TURESSON). Indeed, different karyotypes within the same species are often ecologically different (cf. MÜNTZING 1936). Unfortunately, for several reasons this question cannot be answered easily.

It has been mentioned above that the distribution of the two karyotypes may be somewhat different, the tetraploid seeming to be dominant in Northern Jutland, while only the diploid has been detected on Bornholm. Both on Bornholm and in Northern Jutland, however, *C. epigeios* mainly grows in poor areas of sand-fields and on the roadsides, i. e. under apparently identical ecological conditions. In cases where both karyotypes were found in the same locality (Villingebæk, Skelde wood, and others, cf. fig. 17b and c, p. 35—36) they seem to grow in the same kind of soil; in this case, not either the tetraploid seems to prefer another ecological environment than the diploid.

For some years, the author has cultivated a series of diploid and tetraploid plants. In this way, some information on the nature of the variation of the *Calamagrostis* plants has been gathered, but the problem of the formation of ecotypes could not be solved. The cultivation experiments showed in some cases that the variation of *C. epigeios* is phenotypically conditioned, in other cases, it is genotypically conditioned, but the variation does not seem to have any relation to the ecology of the plant.

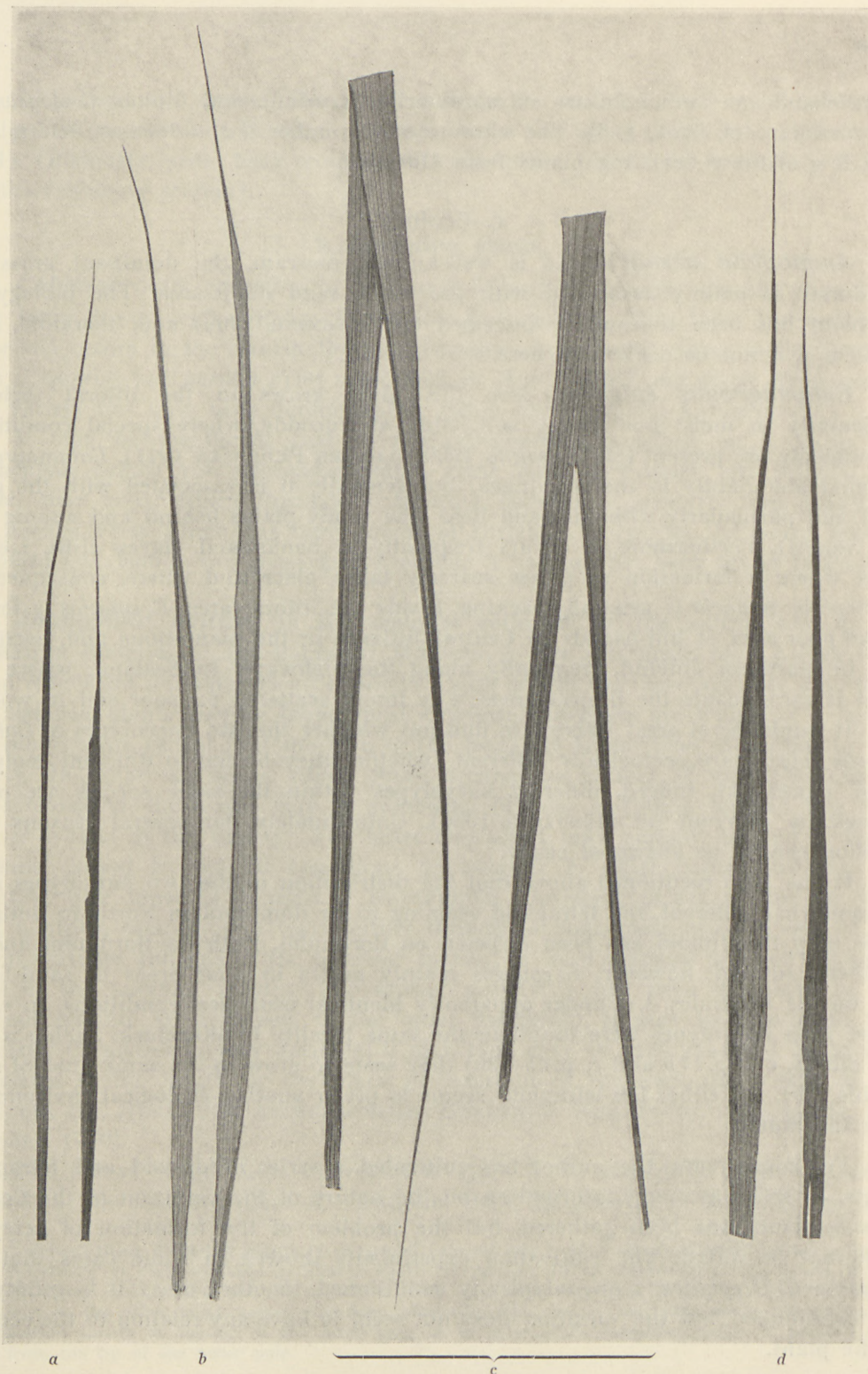


Fig. 19. Leaves of tetraploid *Calamagrostis epigeios* plants from Hirtshals. *a*: from a plant growing in a wind-swept, very exposed locality. *b*: leaves of the same plant, cultivated for one year in light. *c*: leaves of the same clon, cultivated for one year in shade. *d*: plant from another locality, growing in a sheltered place in a bushwood of *Pinus montanus*.  $\times \frac{1}{2}$ .



Fig. 19 shows an example of a phenotypically conditioned variation within the tetraploid karyotype. Narrow-leaved, slender, distorted specimens from Hirtshals (Northern Jutland), and robust, broad-leaved specimens from the same locality have developed uniformly when they were cultivated under the same conditions. Examples of the same type of variation may be found within the diploid karyotype.

On the other hand, the cultivation experiments have also yielded genotypically conditioned variations. In the sea dunes at Dueodde (Bornholm), large growths of relatively slender plants with a small, narrow bract and a red inflorescence were found (fig. 20a). Scattered over the sea dunes, moreover, a few very robust plants (seldom forming dense growths) with paler panicle and a large, broad bract were observed (fig. 20c). Both types are diploid. The difference between these types proves to be fairly constant during cultivation (fig. 20), and seems to be genotypically conditioned.

It has been mentioned previously that both karyotypes of *C. epigeios* growing in the same locality, the tetraploid generally develops more vigorously than the diploid (cf. figs. 4 and 8, p. 12 and 15). It should be emphasized that this difference is preserved in cultivated plants, hence this difference, too, seems to be genotypically conditioned.

***Ammophila baltica*.** The three varieties of *Ammophila baltica* seem to be ecologically differentiated in a way which is in fair accordance with their mode of origin.

*Ammophila baltica* var. *subarenaria* is almost exclusively associated with the shifting sand; it grows in the sea dunes, especially on their foot-hills, but, unlike *Ammophila arenaria*, it is rarely found in the actual white dune areas inside the sea dunes and almost never in the grey dunes or the sand-field areas. (WARMING who was aware of this fact assumes that the occurrence of the two plants may be explained by a greater requirement of salt of the hybrid).

*Ammophila baltica* var. *intermedia* inhabits the sea dunes, as does the preceding variety; particularly the flowering individuals are found here. However, unlike the former hybrid, it is also found in the white dunes inside the sea dunes, in the grey dunes, and in the sand-field. In the dense vegetation it rarely flowers, thus easily escaping observation. (*Ammophila arenaria*, too, flowers less in a dense vegetation).

In contrast to the two preceding varieties, *Ammophila baltica* var. *epigeioidea* has not been found in the actual areas of sand dunes. At Hirtshals, it grows in an area of sand-field at some distance from the coast and at a roadside. At Asserbo, it was found in a sand-field 2—3 km from the coast. At Villingebæk and Emmerslev, the hybrid grows on a shingle beach at the foot of a clayey cliff in an area where no dunes are found. Thus, the hybrid is associated with the same localities as *Calamagrostis epigeios*, but it seems to require more particular conditions than does the parental species.

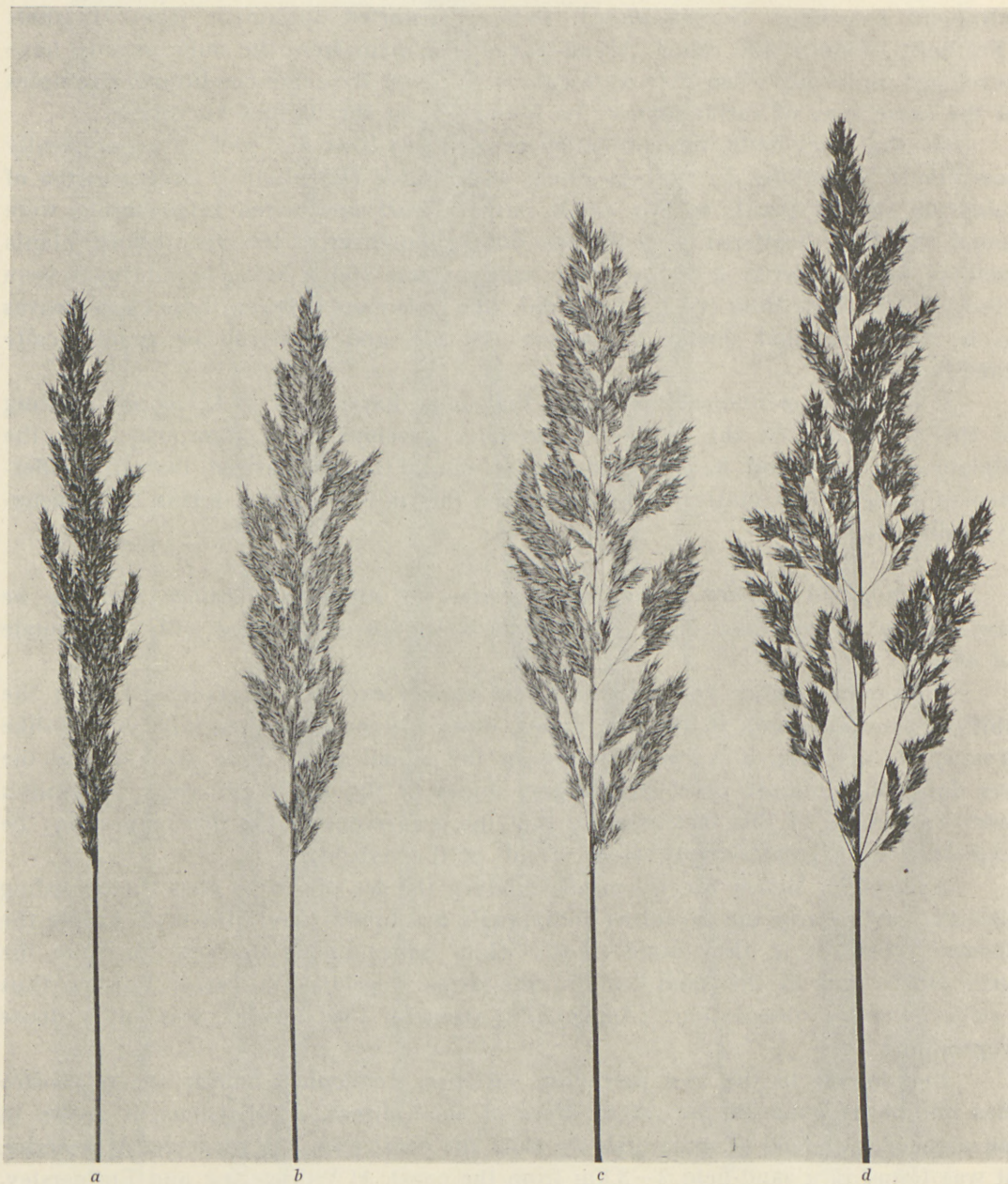


Fig. 20. Inflorescence of diploid *Calamagrostis epigeios* from the sea dune at Dueodde (Bornholm). *a* and *c*: a slender and a robust type (cf. fig. 1), collected in Nature, *b* and *d*: the same specimens, after being cultivated for one year under the same conditions.  $\times 1/2$ .

In the experimental field of the Laboratory of Genetics, all three hybrids have been cultivated in loam during several years. Like both karyotypes of *Calamagrostis epigeios*, but in contradistinction to *Ammophila arenaria*, the three hybrids thrive excellently in this soil and they attain an amazing luxuriance.

## VII. Discussion.

The aim of the present investigations is to elucidate various problems concerned with the origin of *Ammophila ballica*. During this work, the two karyotypes of *Calamagrostis epigeios* were detected. This evokes the problem of the taxonomical and genetical relationship between the diploid and the tetraploid karyotype, the origin of the tetraploid type, etc. Correspondingly, the discussion naturally falls in two sections, these problems being dealt with in the first part; subsequently, the main subject, *Ammophila ballica*, will be discussed.

### a. The karyotypes of *Calamagrostis epigeios*.

The following facts regarding the two karyotypes of *C. epigeios* may be summarized.

(1) In Nature, the two karyotypes cannot be distinguished by means of macroscopic characters. Only an estimation of the chromosome number or, less reliable, a measurement of the pollen grains enables us to decide whether a given plant belongs to the diploid or to the tetraploid karyotype. However, this does not mean that the two types are quite identical; when growing in the same locality, the tetraploid is generally more robust, more broad-leaved and large-flowered than the diploid. Due to the great variability of *C. epigeios*, exactly the same quantitative differences may be found between plants belonging to the same (diploid or tetraploid) karyotype. In some cases, these differences are phenotypically, in other cases they are genotypically conditioned.

(2) On the whole, the distribution of the two karyotypes coincides. On the Island of Bornholm, only the diploid type has been found, while the tetraploid is dominant in Northern Jutland.

(3) No ecological differentiation between the two karyotypes could be demonstrated.

(4) The reduction division is somewhat irregular in the tetraploid karyotype, multivalent associations being fairly frequent. The pollen fertility of the tetraploid type is decreased.

(5) The two karyotypes are able to cross reciprocally, the fertility being poor, however, when the tetraploid is used as female parent. The behaviour of the triploid offspring plants has not yet been studied, but it is assumed that the

fertility has decreased. Hence, in Nature the two karyotypes are probably to some extent mutually isolated by a barrier of sterility.

Some of the facts mentioned above are not in full accordance with the general behaviour of intraspecific polyploids. As is well known, the occurrence of different chromosome numbers within the same Linnean species (intraspecific polyploidy) has been described repeatedly during recent years, the first case being *Oenothera Lamarckiana* Gigas (GATES 1909). The subject has been dealt with in a survey by MÜNTZING (1936), and the general conclusions of this work have scarcely been modified by later observations.

According to MÜNTZING, intraspecific polyploids are morphologically different, the differences generally being of a quantitative character. MÜNTZING points at the parallelism between the morphology of the experimentally produced autopolyploids and the intraspecific polyploids growing in Nature. The polyploids growing in Nature, just as the experimental autopolyploids, are generally more vigorous, their flowers are larger, their leaves are broader, etc. than those of the diploid forms. Generally, the differences are so conspicuous that the polyploids can be recognized in Nature without difficulty. MÜNTZING states explicitly as follows. "Not a single case is known in which it has been demonstrated that the races are morphologically identical" (p. 269)<sup>1</sup>. This holds also for the karyotypes of *Calamagrostis epigeios* when growing in the same locality; nevertheless, due to the great variability of the plants, the karyotypes cannot be distinguished in Nature. Moreover, intraspecific polyploids will generally have a different geographical distribution and (or) they will be ecologically differentiated.

Although experimental evidence seems to be lacking in most cases, it is generally assumed that intraspecific polyploids are mutually isolated by a barrier of sterility. When hybrids are produced at all, the  $F_1$  plants are sterile as a rule or, at any rate, the offspring generation is very variable. Also in this respect the intraspecific polyploids resemble the experimental autopolyploids (MÜNTZING 1936).

With respect to geographical distribution, ecology, and mutual isolation, the karyotypes of *Calamagrostis epigeios* seem to be more closely related than is generally the case (cf., however, SKOVSTED 1941).

Intraspecific polyploids involve difficult problems for the botanist and some confliction exists between the modern, genetically orientated and the older, morphologically orientated taxonomists.

Many intraspecific chromosome races are mutually isolated by a barrier of sterility. According to modern geneticists, isolation is an important criterion for the distinction of species (cf. MÜNTZING 1936, DOBZHANSKY 1937, NANNFELDT 1938, and DARLINGTON 1939). In recent years, intraspecific polyploids have therefore often been established as distinct species (*Empetrum nigrum*—*hermaphroditum*, *Dac-*

<sup>1</sup> However, in a recent paper, SKOVSTED (1941) describes an interesting case of intraspecific polyploidy in *Hibiscus Trionum*. There, a diploid type with 28 chromosomes and a tetraploid type with 56 chromosomes seem to be identical.

*tylis glomerata*—*Aschersoniana*, etc.). On the other hand, morphologically orientated taxonomists will often consider the morphological differentiation of the intraspecific polyploids insufficient to permit a species distinction. In fact, before the chromosome constitution was known, many intraspecific polyploids were regarded as distinct varieties by the older botanists, and many taxonomists are still unwilling to consider them as more than varieties.

In *Calamagrostis epigeios*, a taxonomical distinction of the two karyotypes would not be practicable at all, although they may be at least partly isolated, because it is impossible to distinguish the two types in Nature by means of any morphological character. Consequently, in the present paper the completely neutral, cytologically defined designation "karyotype" was preferred. Even the word chromosome race is to be avoided, since the term race ought to be associated with types which are either morphologically or, at any rate, geographically or ecologically differentiated.

In fact, the close relationship between the two karyotypes allows us to speak of an example of "crypto-species" formation. DARLINGTON (1939) denotes types which are cytologically (or genetically) isolated from the main species without being morphologically differentiated from it as "cryptic-species". The best-known case of formation of cryptic species is the so-called "race-formation" in *Drosophila pseudo-obscura* (cf. DOBZHANSKY 1937). Here too, morphologically identical types (cf., however, DOBZHANSKY and MATHER 1939) of the same species are cytologically different and, at least partly, mutually isolated by a (cytological or genetical) barrier of sterility. In *Drosophila pseudo-obscura*, the various "races" have a different geographical distribution and they are ecologically different.

In *Drosophila pseudo-obscura*, the lack of morphological differentiation between the different karyotypes may be explained by the fact that the essential mechanism of cytological differentiation is chromosome inversions, since inversions rarely will have any phenotypical effect. In this species, we encounter an example of race formation *in statu nascendi*, as emphasized by DOBZHANSKY. The same applies, no doubt, to other less thoroughly examined cases of "crypto-species" formation (cf. DARLINGTON 1939, p. 127).

In *Calamagrostis epigeios*, the close morphological relationship between the two karyotypes is more peculiar, since a doubling of the chromosome number generally involves considerable morphological changes. Hence, the problem arises, whether this lacking morphological differentiation is a primary phenomenon or whether it is a secondary character acquired through selection. This question is closely associated with the problem of the origin of the tetraploid karyotype. In the author's opinion, none of these questions can be answered at the time being but, since it should be possible to elucidate the problems experimentally, some lines of the discussion may be drawn even now.

With respect to the origin of the tetraploid type, two possibilities exist, viz. (1) The tetraploid karyotype is an autopolyploid, arisen from a chromosome doub-

ling of the diploid *Calamagrostis epigeios*. (2) The tetraploid is an allopolyploid, arisen by a chromosome doubling of a diploid species hybrid<sup>1</sup> between *C. epigeios* and another (unknown) species of *Calamagrostis*. For the distinction between these two possibilities, we have a morphological and a cytological criterion; however, both must be used with considerable caution and criticism.

Regarding the morphological criterion: If a polyploid plant growing in Nature is differentiated from the diploid type mainly by quantitative characters, i. e. if the differences are of the same quality as those between experimental autopolyploids and their diploids, the polyploid type will generally be classified as an autopolyploid type of the diploid. If, however, the polyploid type lacks the gigas stamp characteristic of experimental autopolyploids, it will generally be considered to be of allopolyploid origin, particularly if it is differentiated from the diploid types by qualitative characters. (It must, however, be remembered that numerous experimentally produced allopolyploids show a marked gigas habit).

We may discuss whether the tetraploid karyotype of *Calamagrostis epigeios* should be characterized as a gigas type, since in some localities it may develop some gigas like characteristics (cf. figs. 4 and 8). In the author's opinion, however, the tetraploid karyotype as a whole cannot be characterized as a gigas type, neither in the macroscopic (cf. figs. 1 and 2) nor in the microscopic characters, although there is some difference with respect to the size of the pollen grains (cf. table 1). If this view is adopted, the lacking gigas stamp in tetraploid *Calam-*

<sup>1</sup> The terms autopolyploid and allopolyploid were originally defined by KIHARA and ONO (1926), but this definition has been interpreted in various ways (cf. FAGERLIND 1937, p. 391—396, MÜNTZING 1936, and DARLINGTON 1937). The basis of these interpretations is the fact that KIHARA and ONO defined the conceptions auto- and allopolyploids according to the mode of origin. ("Unter Autopolyploidie versteht man die Verdopplung desselben Chromosomensatzes, unter Allopolyploidie die durch das Zustandekommen verschiedener Chromosomensätze auf dem Wege der Bastardierung erfolgte Chromosomenvermehrung") and, according to the type of meiosis ("Hier (bei Autopolyploidie) gibt es bei der Reduktionsteilung sowohl Allosyndese wie Autosyndese — bei der Reduktionsteilung allopolyploider Kerne gibt es ausschliesslich Allosyndese"). Particularly, the cytological part of this definition caused confusion and has been the subject of interpretation.

However, these definitions were made at a time (1926) when the knowledge of the meiosis of polyploids was much more fragmentary than it is at the time being. Moreover, the possibilities for employing the mode of chromosome association in metaphase I as a criterion of the relationship and descent of polyploids were judged far more optimistically than to-day. The observations of the last decade, however, have shattered this optimism, first, because the study of meiosis in experimental polyploids has yielded a less clear picture than was to be expected (cf. p. 55); next, because the recent demonstrations of association and chiasma formation between non-homologous chromosome sections (cf. LEVAN 1942) may alter the very basis of the interpretation of chromosome association.

No doubt, the question of the value of an analysis of chiasma frequency and mode of association for the elucidation of the origin and the relationship of polyploids must be taken up for critical revision in the years to come. Therefore, it seems unfortunate to attach the greatest importance to the cytological part of KIHARA and ONO's definition and to classify the polyploids mainly on the basis of the type of meiosis (DARLINGTON) or on the basis of the type of meiosis in the—postulated and in many cases unknown—diploid parental form (FAGERLIND).

Obviously, every sharp classification of the polyploids growing in Nature is to some extent fictitious. On the other hand, the definition given above which classifies the polyploids exclusively on the basis of the presumed mode of origin (in accordance with the first part of KIHARA and ONO's definition) will afford a practical basis of discussion in most cases. Moreover, the discussion of the origin and the classification of the polyploids growing in Nature will to an increasing extent be based upon experiences gained from experimental polyploids, where the above definitions can be used without difficulty and where the cytological definition will only cause further confusion.

*agrostis epigeios* may be interpreted as indicia of an allopolyploid origin. However, the lacking quantitative differentiation between the diploid and the tetraploid karyotype are against this interpretation. We cannot imagine that a polyploid species hybrid—even between closely allied species—should become identical with one of the parental species in all taxonomic characters. Hence, in spite of the lack of *gigas habitus*, it seems most likely to presume an autopolyploid origin of the tetraploid *Calamagrostis epigeios*.

If the above view of an autopolyploid origin of the tetraploid *Calamagrostis epigeios* is adopted as a working hypothesis, it should be possible to elucidate experimentally the problem whether the lacking *gigas habitus* is a primary or a secondary phenomenon. By means of colchicine treatment, autotetraploid plants of various diploid *Calamagrostis epigeios* types should easily be produced. If such experimentally produced plants bear as little of the *gigas* stamp as do the tetraploids in Nature, there will be reason to assume that the lacking *gigas habitus* of the latter is a primary phenomenon. If, on the other hand, the experimental tetraploids develop the *gigas habitus* otherwise characteristic of autopolyploids, the lacking *gigas* stamp of the polyploids *Calamagrostis* plants growing in Nature should probably be regarded as a secondary phenomenon acquired through the process of natural selection. In connection with the latter possibility, the important investigations of WETTSTEIN (1938) on *Bryum Corrensii* should be mentioned. WETTSTEIN showed that in the course of rather few generations, an experimentally produced polyploid type may lose the *gigas* stamp—with respect to macroscopic as well as microscopic characters—through selection. At the same time, the plant acquires a normal fertility. This observation makes it quite clear that we must not conclude from lacking *gigas* stamp to allopolyploid origin, because the plant species growing in Nature are the result of a long process of selection, the individual links of which are generally unknown and unreproducible.

In order to elucidate the above problems, the author has started experiments on raising tetraploids of various diploid *C. epigeios* strains through colchicine treatment. The results of these experiments are not yet available.

The question whether a polyploid growing in Nature is of autopolyploid or allopolyploid origin may also be discussed on the basis of a cytological criterion, i. e. the behaviour of the chromosomes during the reduction division. The formation of multivalents in metaphase I is generally interpreted as indicia of autopolyploid origin, whereas a regular formation of bivalents is interpreted as indicia of allopolyploid origin (cf. MÜNTZING 1936). This criterion should be used still more cautiously than the morphological criterion. The observations of the last decades on meiosis in experimental autopolyploids and allopolyploids revealed that although the number of multivalents is high in the majority of the autopolyploids (for instance tetraploid *Datura stramonium*, BELLING and BLAKESLEE 1924), some of them may show an almost regular formation of bivalents (for instance tetraploid *Solanum nigrum*, JØRGENSEN 1928). On the other hand, a number of allopolyploids

have been raised which show a fairly high number of multivalents (for instance *Primula kewensis* with an average of 2.5 tetrasomes per cell, UPCOTT 1939). According to the cytological criterion alone, a tetraploid *Solanum nigrum* found in Nature, without its origin being known, should consequently be classified as an allopolyploid, while, conversely, the allopolyploid *Primula kewensis* under similar conditions would probably be classified as an autopolyploid.

In the tetraploid *Calamagrostis epigeios*, up to 5 tetrasomes are formed in the first metaphase (cf. p. 28). According to the general interpretation, this fact suggests an autopolyploid origin. On the other hand, the occasional occurrence of chromatin bridges and fragments, indicating that the plants contain inverted chromosome segments, show that the chromosomes in some sections are structurally differentiated. This may very well be interpreted as an indicia of allopolyploid origin. No decisive importance can, however, be attached to this criterion, since chromatin bridges also may occur in experimentally raised autotetraploids (for instance *Melandrium album*, WESTERGAARD 1940).

The behaviour of the chromosomes during meiosis in the tetraploid *Calamagrostis epigeios* shows primarily that the plant is not completely stabilized; whether the observed irregularities indicate an autopolyploid or an allopolyploid origin cannot be decided. However, the problem may be further elucidated when experimentally raised autotetraploids are available.

It appears from the above discussion that the question of the origin of a polyploid type growing in Nature—and hence bearing the stamp of selection—can hardly be solved. An autopolyploid origin of the tetraploid *Calamagrostis epigeios* is assumed merely because of the lacking quantitative differentiation.

#### b. The *Ammophila baltica* problem.

According to diagram 1 (p. 3), *Ammophila baltica* var. *intermedia* ( $2n = 28$ ) is a hybrid between diploid *Calamagrostis epigeios* and *Ammophila arenaria*, while *Ammophila baltica* var. *epigeioidea* ( $2n = 42$ ) is a hybrid between tetraploid *C. epigeios* and *A. arenaria*. The correctness of this interpretation may now be considered proved, after both hybrids have been produced under controlled conditions by means of the postulated parental types (cf. chapter V, p. 42).

The third hybrid, *Ammophila baltica* var. *subarenaria*, is triploid like var. *epigeioidea*. According to the diagram, this hybrid is composed of two sets of *Ammophila arenaria* chromosomes ( $14 + 14$ ) and one set of *Calamagrostis* chromosomes (14). In this case, the theory could not be proved directly, since the author did not succeed in raising this hybrid experimentally. The indirect proofs, viz. the morphological and cytological relationship of the plant to the parental species and to the two other hybrids are so convincing that the plant must be regarded as a hybrid between *A. arenaria* and *C. epigeios*, constituted as stated above.



However, it seems difficult to imagine in which way *Ammophila baltica* var. *subarenaria* obtained the two sets of *Ammophila arenaria* chromosomes. Either a tetraploid form of *Ammophila arenaria* exists, corresponding to the tetraploid *C. epigeios*, or the diploid *A. arenaria* occasionally forms diploid gametes; finally, a third possibility might be taken into consideration, viz. that the hybrid may be formed by a back-cross, i. e. a fertilization between diploid gametes of *Ammophila baltica* var. *intermedia* (genome constitution AC) and normal haploid gametes of *Ammophila arenaria* (A).

Since a tetraploid strain of *Ammophila arenaria* has not yet been detected, the second possibility may perhaps seem most probable but, on the other hand, it would be extremely difficult to prove definitely that a given plant in casu tetraploid *Ammophila arenaria* does not exist; particularly, if it resembles the diploid *Ammophila arenaria* as closely as the tetraploid karyotype of *C. epigeios* resembles the diploid type. Obviously, these problems would be further elucidated if a tetraploid strain of *Ammophila arenaria* could be raised experimentally; as mentioned above, such experiments have already been undertaken.

It seems almost unique to find three well-defined hybrids between two Linnean species growing in Nature, in some cases even in one and the same locality. ØSTERGREN (1940) described two spontaneous hybrids between *Agropyrum junceum* and *Agropyrum repens*, one with  $2n = 35$ , the other with  $2n = 49$ . When otherwise polymorphous hybrids are described in the literature, more or less stabilized forms of back-crosses between the  $F_1$  and the parental species seem to be involved.

Therefore, it may be of interest to point at some of the factors which have made the rather complicated hybridization between these two grasses possible. The main point is that *Calamagrostis epigeios* (and possibly *Ammophila arenaria*, too) includes different karyotypes. Furthermore, the different types grow in the same localities, they are flowering at the same time, and they are capable of hybridizing (no doubt, the chance of hybridization is favoured by the fact that the parental species are self-sterile, cf. p. 43). Last not least, the hybrids are able to thrive in competition with the parental species. In this connection, the capacity of vegetative formation by runners, which the hybrids have inherited from both parental species, must be kept in mind. This capacity, which enables the hybrids to form large uniform growths although they cannot reproduce sexually, must be considered the cause that *Ammophila baltica* from the beginning was regarded as a genuine species.

It is scarcely accidental that such a complicated hybridization is found within the Gramineae, because intraspecific polyploidy is widely distributed within this family and species hybrids occur rather frequently in Nature. Hence, similar cases will presumably be found (cf. the paper by ØSTERGREN quoted above). In this connection, it seems worth mentioning some polymorphous grass hybrids described by ASCHERSON and GRAEBNER (1902), JUNGE (1913), and LEHBERT (1913, particularly the species of *Calamagrostis*). JUNGE (1913, p. 183) describes three hybrids

between *Calamagrostis lanceolata* and *C. arundinacea* (= *C. Hartmaniana* Fries), i. e. (1) var. *Heidenreichii* Grütter; this hybrid is closely allied to *C. lanceolata*; (2) var. *intermedia* Friedrich, and finally, (3) var. *superarundinacea* Torges which is most closely allied to *C. arundinacea*. A cytological examination of these and of similar hybrids and their parental species would be an interesting subject.

It should now be possible to discuss the problem of the peculiar distribution of *Ammophila baltica* on a new basis. The restricted distribution of the hybrid compared with that of the parental species has already been the subject of discussions and, previously, it has been used as an argument against the theory of a hybrid origin of *Ammophila baltica* (cf. WARMING 1909, p. 61 and TILSE 1910). If the theory of a hybrid origin is accepted, two possibilities may a priori be imagined to explain the restricted distribution of the three varieties. Either the parental species are not capable of crossing everywhere where they are growing together, or the hybrids are actually formed in every place where the parental species occur together; but having more particular ecological requirements than the parental species, the hybrids will only be able to succeed in certain localities. According to our present experience, undoubtedly the ecological requirements of the plants restrict the distribution, while the chance of formation is of negligible importance, only.

Primarily, examinations of offspring plants of *Ammophila arenaria* and *Calamagrostis epigeios* plants pollinated in Nature (cf. table 5, p. 42) have proved that *Ammophila baltica* var. *epigeioidea* is formed in abundance in the locality concerned (Hirtshals). Nevertheless, here the hybrid is very rare and in other localities, where *Ammophila arenaria* and tetraploid *Calamagrostis epigeios* are growing together (for instance Skelde wood, the Bøtø dike, and other Baltic localities, cf. fig. 17c and f), the hybrid is completely lacking. The only possible explanation is that the hybrid is able to succeed only under very particular conditions.

Secondarily, the crossing experiments (cf. table 5, p. 42) have shown that, at least under experimental conditions, *Ammophila arenaria* and diploid *Calamagrostis epigeios* cross readily and, no doubt, this will be the case in Nature too. Hence, it is reasonable to assume that the hybrid is formed at every place where the parental species are growing together. Nevertheless, the distribution of the diploid hybrid is mainly confined to the Eastern Baltic regions (cf. fig. 17e), whereas diploid *Calamagrostis epigeios* (and *Ammophila arenaria*) is found in the Western Baltic regions too. Also here, it is most likely that the ecological requirements of the plants are responsible for the restricted distribution.

Finally, regarding the third hybrid, *Ammophila baltica* var. *subarenaria*, a similar view may be held. Even if we do not know exactly how the hybrid is formed, the chance of its formation must be the same, wherever *Ammophila arenaria* and diploid *Calamagrostis epigeios* are found together. Also this hybrid, like the diploid one, is lacking in numerous localities, especially in Jutland where both parental species are found.

It is impossible to define exactly the ecological factors which restrict the distribution of the hybrids compared with the parental species. The fact that *Ammophila baltica* var. *subarenaria* has been found as far to the north as the region of the Oslo Fjord suggests that edaphic rather than climatic factors must be taken into account.

In the above discussion, it has been supposed that in each individual locality, the hybrids have arisen through crossing. It is not impossible, however, that the hybrids may spread vegetatively over some distance. For instance, it is very probable that fragments of runners may spread along the coasts by the aid of the sea. It is actually known that *Ammophila arenaria* is able to spread in this way (cf. SER-NANDER 1901). Especially *Ammophila baltica* var. *subarenaria* should be able to spread in the same way, because this hybrid usually grows close to the sea, at the foot of the sea dunes.

Moreover, the hybrids may be spread artificially, since *Ammophila baltica* has been planted analogously to *Ammophila arenaria* in order to bind the shifting sand. (Already in 1806, SCHRADER, and in Denmark HORNEMANN (1821) highly recommend the use of *Ammophila baltica* for this purpose). Finally, *Ammophila arenaria* seeds have been exported from Denmark to a considerable extent during some years, particularly to the countries oversea. Thereby, seeds of the hybrids may have been spread at great distances.

At the start of the present investigation, it was in fact expected that *Ammophila baltica* would prove to be an amphidiploid hybrid between the two parental species, parallel to *Spartina Townsendii* (cf. HUSKINS 1930). Such a hybrid ("*Calammodiplophila baltica*") would have the chromosome number  $2n = 56$ ; morphologically, it would probably be closely related to *Ammophila baltica* var. *intermedia*. Moreover, the amphidiploid hybrid would be expected to be fertile and to yield a constant offspring.

It is very surprising indeed that the amphidiploid hybrid has not been found, since the chance of its formation should be fairly high in localities where tetraploid *Calamagrostis epigeios* and *Ammophila arenaria* are growing together. Actually, this chance should be of the same order of magnitude as the chance of the formation of *Ammophila baltica* var. *subarenaria* in localities where the diploid karyotype of *C. epigeios* is growing in the company of *Ammophila arenaria*. Nevertheless, the triploid sterile hybrid is common in numerous localities, while the constant hybrid has not been found at all. Hence, it seems most likely that the constant hybrid is actually formed in Nature, but that, unlike the three other hybrids, it is unable to succeed. For the further elucidation of these problems, attempts are made to raise the amphidiploid hybrid experimentally by means of colchicine treatment of the diploid hybrid *Ammophila baltica* var. *intermedia*. These experiments are not yet finished.

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## VIII. Appendix. Nomenclature and diagnosis in Latin.

In accordance with the interpretation prevailing in Danish botanical literature, the author has decided to refer *Ammophila arenaria* and *Calamagrostis epigeios* to two different genera; this is in contradistinction to German botanists who generally refer both species to the genera *Calamagrostis* (cf. ASCHERSON and GRAEBNER 1898). It must be admitted that the present investigation reveals a very close relationship of the two species so that the German interpretation appears most reasonable. Nevertheless, it seems justifiable to retain the distinction of genera in view of the remarkable difference in structure of the inflorescence. In the taxonomic literature of Gramineae, great importance was always attached to this character.

With respect to the designations of the varieties, it appears most reasonable to retain MARSSON'S name of variety, viz. *subarenaria* for the triploid hybrid which comprises two sets of *Ammophila* and one set of *Calamagrostis* chromosomes. The triploid hybrid entirely corresponds to the older descriptions and pictures of *Ammophila baltica* Link, and MARSSON explicitly states that var. *subarenaria* is identical with the classical *A. baltica*.

On the other hand, the second variety established by MARSSON, viz. var. *subepigeios*, is difficult to identify. MARSSON describes the variety as follows: "spiculis minoribus; glumis aequalibus; arista infra fissuram apicis palea inf. inserta; palea submembranacea tertia parte palea inf. brevior 2 nervia nervis lateralibus oblitteratis". As emphasized by PRAHL (1890), these characters are not reliable, the nervation particularly being a highly variable character. In other respects, this description covers the diploid hybrid (genome constitution AC) as well as the triploid hybrid which comprises one set of *Ammophila* and two sets of *Calamagrostis* chromosomes (ACC). Consequently, it seems to be permitted to reject the name *subepigeios* and to introduce the two new names var. *intermedia* for the diploid hybrid and var. *epigeioidea* for the triploid hybrid. (As a further argument for abandoning MARSSON'S variety name, reference is made to the specimen of *Ammophila baltica* from Eyderstedt mentioned on p. 41).

As the descriptions of *Ammophila baltica* given by the previous authors do not seem to include var. *epigeioidea*, a supplementary description of *Ammophila baltica* sensu lato will be given.

*Ammophila baltica* s.l.:

Folia convoluta, supra plus minusve dense et breviter scabro-pilosa, subtus ad basin vel fere ad apicem carinata. Panicula coarctata-thyrsoidea. Palea inferior dorso inter medium et emarginationem apicis arista plus minusve brevi instructa. Pili spiculae  $1\frac{1}{2}$  ad  $\frac{1}{2}$  longitudinis paleae superioris aequantes.

Hence, the varieties of *Ammophila baltica* may be identified by means of the following descriptions.

*Ammophila baltica* var. *subarenaria* Mars. (*Arundo baltica* FLÜGGE apud SCHRADER 1806; *Psamma baltica* ROEMER et SCHULTES 1817; *Ammophila baltica* LINK 1827, *Calamagrostis baltica* HARTMAN 1832, *Calamagrostis epigeios* × *Ammophila arenaria* ROEPER 1840, 1844; *Ammophila baltica* var. *subarenaria* MARSSON 1869):

Folia ca.  $\frac{1}{5}$  longitudinis ad basin solum carinata. Palea superior inferiorque fere aequilongae. Pili spiculae  $\frac{1}{2}$  longitudinis paleae superioris aequantes. — Specimen typicum in Villingebæk (Selandia) collectum in Mus. Hort. bot. Hafniensi.

*Ammophila baltica* var. *intermedia* var. nov.:

Folia ad medium carinata. Palea superior ca.  $\frac{3}{4}$  longitudinis paleae inferioris aequans. Pili spiculae palea superiorque aequilonga. — Specimen originale in Villingebæk (Selandia) collectum in Mus. Hort. bot. Hafniensi.

*Ammophila baltica* var. *epigeioidea* var. nov.:

Folia fere usque ad apicem carinata. Palea superior ca.  $\frac{3}{4}$  longitudinis paleae inferioris aequans. Pili spiculae palea inferiorque aequilonga. — Specimen originale in Villingebæk (Selandia) collectum in Mus. Hort. bot. Hafniensi.

## IX. Summary.

1. The two species of grasses, *Ammophila* (*Psamma*) *arenaria* (L.) Link and *Calamagrostis epigeios* (L.) Roth, form three different hybrids. These hybrids are sterile but propagate vegetatively by means of runners.

The three hybrids are called *Ammophila baltica* var. *subarenaria* Mars., var. *intermedia* var. nov., and var. *epigeioidea* var. nov. *Ammophila baltica* var. *subarenaria* Mars. is identical with *Ammophila baltica* Link which for a long time was considered a hybrid between *Ammophila arenaria* and *Calamagrostis epigeios* (ROEPER 1840).

*Ammophila baltica* var. *intermedia* ( $2n = 28$ ) arises through a crossing between *Ammophila arenaria* ( $2n = 28$ ) and a diploid karyotype of *Calamagrostis epigeios*. The genome constitution of the hybrid is AC, since it is composed of one *Ammophila* genome (A) and one *Calamagrostis* genome (C). *Ammophila baltica* var. *epigeioidea* ( $2n = 42$ ) is a hybrid between *Ammophila arenaria* ( $2n = 28$ ) and a tetraploid karyotype of *Calamagrostis epigeios* ( $2n = 56$ ). Hence, the genome constitution of this hybrid is ACC. *Ammophila baltica* var. *subarenaria* contains two sets of

*Ammophila arenaria* and one set of *Calamagrostis epigeios* chromosomes (genome constitution AAC). The hybrid must arise through a crossing between the diploid karyotype of *Calamagrostis epigeios* ( $2n = 28$ ) and either diploid gametes ( $n = 28$ ) of diploid *Ammophila arenaria* or a—undetected—tetraploid karyotype ( $2n = 56$ ) of this species.

The proofs of the existence and the constitution of the three hybrids are based on morphological (chapter II) and cytological (chapter III) studies, on the geographical distribution of the various types (chapter IV), and on crossing experiments (chapter V).

2. In the three hybrids, the structure of the flower and the anatomy of the leaves have been specially studied in order to analyse the interaction between the *Ammophila* and *Calamagrostis* genomes in the three combinations AAC—AC—ACC. This analysis showed that one and the same genome has a different effect on the various organs of the flower, and that the effect of the genome is a relative one, depending upon the constitution of the genotype into which it is introduced.

3. In *Ammophila arenaria* meiosis is fairly regular; however, a ring of four chromosomes is occasionally formed. In the three hybrids meiosis is very irregular; univalents, bivalents and a few multivalents are formed in the diploid as well as in the two triploid hybrids. Chromatin bridges and fragments are frequently found in anaphase I. The three hybrids are almost pollen sterile.

4. The distribution of the three hybrids is somewhat different (fig. 17). Moreover, the three varieties are to some extent ecologically different (chapter VIc).

5. *Ammophila baltica* var. *intermedia* has been produced experimentally by crossing *Ammophila arenaria* and diploid *Calamagrostis epigeios*. By sowing seeds of *Ammophila arenaria* and tetraploid *Calamagrostis epigeios* collected from localities where both species are growing together, it could be demonstrated that *Ammophila baltica* var. *epigeioidea* in such localities arises with a high frequency in Nature (table 5, p. 42).

6. The evidence of the origin of the three hybrids and the possibilities of finding an amphidiploid hybrid ( $2n = 56$ ) between *Ammophila arenaria* and *Calamagrostis epigeios* are discussed. Experiments on producing tetraploid *Ammophila arenaria*, tetraploid *Calamagrostis epigeios*, and the amphidiploid hybrid experimentally are under progress.

7. The diploid and the tetraploid karyotype of *Calamagrostis epigeios* cannot be distinguished in Nature by means of any macroscopic characters, because both karyotypes comprise a great number of types which exhibit an almost parallel quantitative variation (chapter IIa). In some cases this variation is genotypically, in other cases phenotypically conditioned (chapter VIc).

The pollen grains of the tetraploids are somewhat larger than those of the diploids. Also this character shows considerable variation within both karyotypes (table 1, p. 9).

The distribution of the two karyotypes in Denmark, on the whole, coincides; in several localities, both karyotypes have been found. Only the diploid type, however, has been found on the Island of Bornholm, while the tetraploid is most frequent in Northern Jutland. It has not been possible to find any ecological differences between the two types (chapter VIc).

In diploid *Calamagrostis epigeios*, meiosis is quite regular: in the tetraploid type up to 5 multivalents are formed in metaphase I. In anaphase I, chromatin bridges and fragments are occasionally seen. The quality of the pollen of the tetraploids is decreased.

The phylogenetic and taxonomic relationship of the two karyotypes of *Calamagrostis epigeios* is discussed (chapter VIIIa). It is pointed out that the tetraploid type is presumably an autopolyploid form of the diploid. The nature of the close relationship between the two karyotypes is discussed, and it is stated that they represent an instance of "crypto-species" formation sensu DARLINGTON, since they seem to be to some extent mutually isolated in Nature. (Triploids have not been detected in Nature, but they have been produced experimentally).

In connection with these problems, the definitions of the conceptions autopolyploid and allopolyploid and the possibilities for deciding, whether polyploids growing in Nature are auto- or allopolyploids, are discussed.

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