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GENETIC STUDIES IN DIOECIOUS *MELANDRIUM*. I.

SEX-LINKED AND SEX-INFLUENCED INHERITANCE IN *MELANDRIUM ALBUM* AND *MELANDRIUM DIOICUM*

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Sex-linked and sex-influenced inheritance are of interest because of their relation to the still intriguing problem of sex determination. Genes involved in the formation of the sex organs are regarded to be sex-determining genes. These genes may be present in all chromosomes including the sex-chromosomes. Other genes present in the sex-chromosomes, but not involved in sex determination, are the sex-linked genes. A mutation for narrow leaves we came across in our *M. album* material is regarded as a case of sex linkage. Also the certation effect observed in *M. album* and *M. dioicum* must have been caused by genes on the sex-chromosomes. In both cases, however, it is not altogether unlikely, that the genes, regarded as sex-linked ones, actually take part in the process of sex-determination.

Sex-determining genes might influence the effect of other genes, that are therefore called sex-influenced genes. We observed a number of such sex-influenced characters in *Melandrium*.

In *M. album*, female plants are, on the whole, larger than male plants, having larger stems and leaves. The petals, however, are larger in male plants, except in families with very broad petals. The leaves and petals are narrower in female plants than in male ones, except in families with very broad leaves and families with broad petals, where the difference in shape was no longer present. Usually, slightly more anthocyanin is formed in male plants than in females both in petals and in green parts. More glandular hairs were observed on male plants than on female ones.

Insofar the observations were made in *M. dioicum* the same results were obtained.

We regard these phenomena to be an expression of the different physiological conditions in female and in male plants, these conditions being provoked by the sex-determining genes and more favourable for vegetative growth in female than in male plants.

Introduction

Already at the beginning of this century, DE VRIES (1900), CORRENS (1900–), BATESON (1902–), BAUR (1910–), SHULL (1910–) and WINGE (1917–) made more or less extensive investigations in *Melandrium*.

Their main reason for undertaking these investigations was that shortly after the rediscovery of Mendel's laws, the genetical determination of sex and sex differences became an important topic. The dioecious *M. album* and *M. dioicum* appeared to be very suitable for this purpose. In both species the female plants have 2 X-chromosomes and 22 autosomes and the male plants an X- and a Y-chromosome and 22 autosomes.

Genes that are situated in the sex chromosomes but which are not involved in sex determination are sex-linked genes. Genes that are not involved in sex determination but whose effect is influenced by the sex-determining mechanism are called sex-influenced genes. They may be present in all chromosomes.

The first case of sex-linkage found in plants, the recessive X-linked gene *angustifolia*, was described by BAUR (1912) and SHULL (1914). WINGE (1927) proved that this gene, responsible for extremely narrow folia in *M. album*, was lethal to pollen. Thus only male plants with such narrow folia could arise.

WINGE (1931) found three other sex-linked genes: *aurea*, *abnormal*, and an inhibitor for *variegated*. He detected all three types in the generations that followed upon crosses between *M. album* and *M. dioicum*.

The gene *aurea* is a recessive gene in the X-chromosome. When it is homozygous it is lethal, therefore no *aurea* female plants are found. According to WINGE the gene is not lethal in the hemizygous condition, so that yellowish-green male plants can occur. In the presence of an autosomal inhibitor *A*, the *aurea* gene is neither lethal nor displays the yellowish-green phenotype.

The recessive gene *abnormal* is localized in the homologous parts of the X- and the Y-chromosome. Three autosomal inhibitors, *G*, *H* and *I* were found. As described by WINGE (1932), the *abnormal* plants have their calyx constantly closed, because of which the flowers never open.

The Y-chromosome contains an inhibitor for the autosomal recessive gene *variegated*, since no *variegated* male individuals occur. Moreover, three autosomal inhibitors *L*, *M* and *O* were detected.

Differences between factors in the X- and Y-chromosome active in the haploid phase were demonstrated by the experiments of CORRENS (1921, 1922, 1924, 1926). He demonstrated certation of gametes which,

as we now know, have different sex chromosomes. Pollen tubes of the gametes with an X-chromosome grow more rapidly than the pollen tubes of the gametes with a Y-chromosome.

HARTSHORNE (1963) found no certation in *M. dioicum*, but LAWRENCE (1963, 1964) demonstrated a certation effect both in *M. album* and in *M. dioicum*.

Gametes with a Y-chromosome are more resistant to storage and to alcoholic vapours than are gametes with an X-chromosome, (CORRENS, 1922, 1924).

A number of investigations in *Melandrium* are known where it is not always clear whether cases of sex-linkage or of sex-influenced inheritance are described.

SCHULZ (1890) stated that in *M. album* female plants have smaller petals than male plants.

LÖVE (1944) and BAKER (1951) observed that female plants of *M. album* have bigger flowers than male individuals, whereas in *M. dioicum* the reverse situation is met with.

LÖVE (1944) measured the length of the calyx, the length of the whole flower and the diameter of the corolla. In all cases the sizes of these parts in the pistillate plants of *M. album* and in the staminate ones of *M. dioicum* exceeded those in the other sex of the same species. LÖVE (1944) assumed this to be due to sex-linked factors. BAKER (1951) measured the length of the leaves on the fifth node on a shoot. Neither in *M. album* nor in *M. dioicum* did he find any differences in the l/w ratios between male and female plants.

According to BAKER (1951) the intensity of the petal colour is greater in male than in female flowers. LÖVE (1944) supposed some sex-linked genes for acidity in the petals. WINGE (1931) obtained some peculiar results after crossing *M. dioicum* × *M. album* and *M. album* × *M. dioicum*. In the first cross the flowers of the male plants were, on the whole, of a deeper red than the female flowers, in the second cross, however, the female flowers were more intensily coloured than the male flowers. According to WINGE this must be due to sex-linked factors.

STANFIELD (1937, 1944) demonstrated differences in the chemical composition of roots and tops between male and female individuals of *M. album*. He found the highest phosphorus and sugar content in both the vegetative and early flowering phases of female plants. In the

early flowering stage female plants showed a greater phenoloxidase activity in roots and tops than male plants.

Our investigations were undertaken because several data given in the literature seem to contradict each other. We are of opinion that it is of interest to investigate which characters are controlled by sex-linked genes and which by sex-influenced genes. Sex-influenced phenomena might give an indication about the different physiological conditions in female and in male plants which cause the development of respectively gynoecia and androecia. We therefore assume sex-influenced characters to be of importance for obtaining a better insight into the mechanism of sex determination in *M. album* and *M. dioicum*.

Material and Methods

Melandrium album (Mill.) Garcke and *M. dioicum* (L.emend.) Coss. et Germ. are two dioecious species, sometimes regarded as subspecies (LÖVE, 1944), of the Caryophyllaceae. In "Flora europaea" (TUTIN, HEYWOOD et al., 1964) the species are described as *Silene alba* (Miller) E. H. L. Krause and *Silene dioica* (L.) Clairv.

A comprehensive description of both species and their ecotypes has been given bij BAKER (1947, 1948). LÖVE (1944) and BAKER (1947) compiled a list of differential characters of both species. The most conspicuous differences between *M. album* and *M. dioicum* are found in flower characters. The white flowers of *M. album* open at night and have a greater diameter than the reddish purple flowers of *M. dioicum* which are open at daytime. The leaves of *M. album* are elliptical-lanceolate, the stem leaves of *M. dioicum* are ovate. An important difference is the overwintering system. *M. album* has a small rosette and large roots, whereas *M. dioicum* has a much bigger rosette but small roots. The leaves of *M. dioicum* are of a darker green than the leaves of *M. album*. The two species have different habitats. *M. album* is mainly found along roadsides and in open places that are or where recently cultivated, *M. dioicum* prefers a wooded environment.

We started our experiments with material collected in their original habitats. In the Netherlands seed from *M. album* was collected from a large population on a beet-field near Utrecht (R 255), along a roadside near Naarden (R 259), on an irregularly cultivated open space in the wood near Hilversum (R 460), near the dunes on the isles of Voorne (R 287) and "de Beer" (R 490), on beet-fields on Overflakkee (R 289) and on dikes on Goeree (R 290). In France, seed samples were gathered along the road Montpellier–Palavas (R 327), in Italy near La Sarre, south of Aosta at a height of 600 m. above sealevel (R 470). *M. dioicum* was collected in the Netherlands near Epe (Zuid Limburg) under hedges and on the border of wood (R 401, R 437), in bushes near Warmond (R 436), Naarden (R 260–262), Dedemsvaart (R 330), and in the dunes near Leiden (R 488). In France, *M. dioicum* was collected near Bonneval on a "Hochstauden" meadow

1900 m. above sealevel (R 471), along the brook Lenta (R 472, 1850 m.), and under shrubs (R 473, 1950 m.). In Italy, seed of *M. dioicum* was sampled near Cogne on a "Hochstauden" meadow (R 465, 1650 m.). In Switzerland, seeds were taken from plants growing in the surroundings of Klosters (R 489, 2800 m.). Professor WESTERGAARD kindly supplied us with seeds from tetraploid ♀ *M. album* with the mutated Y^1 chromosome (R 319).

Until 1961 we had no greenhouse at our disposal. The seeds were sown in sowing pans which were kept in frames in the experimental garden. After germination the seedlings were transplanted into pots of pressed soil which were subsequently planted out into the garden at the proper time. Half of the material was sown in September and after transplantation kept in frames during the winter. The seedlings were planted out into the garden in April of the following year. The second half of the material was sown in March and planted out into the field about May–June. The first group flowered about June–July, the second group during the months of July and August. This spreading of flowering facilitated the ordering of our experiments.

When in 1961 glasshouses became available, all the material was sown in a hothouse, the first group in February, the second in March. This way we got the same spreading in flowering time as before. In August just after harvesting, part of the material was sown, and planted out in October in a hothouse and an unheated glasshouse. In the former, flowering started at the end of January, in the latter in March–April. The seed harvested in the hothouse was sown immediately, and frequently a second flowering generation was obtained from it in the same year.

In our experimental garden *M. album* and *M. dioicum* were grown under identical conditions on a rather heavy clay soil in the open field. Both species grew very well.

Before crossing, male and female flowerbuds were isolated with a paperbag. When the flowers were open, the male flowers were removed from the plant and the crosses were performed by brushing the pistils with the stamens of the male flowers. After seedsetting was evident, the paperbags were removed. Each capsule was marked with a coloured thread.

Material originating from outside the experimental garden is registered with R followed by a number. Seedsamples from crosses performed in our garden are denoted by a letter, a different letter for each year, preceded by a number.

Results

SEX-LINKED INHERITANCE

A gene for narrow leaves

The recessive gene *angustifolia* (BAUR 1911, SHULL 1914) causes strong inhibition of growth in width of the leaves. WINGE (1927) demonstrated that the mutated X-chromosome with the gene *an-*

gustifolia caused sterility in the pollen containing this chromosome, no female plants with the angustifolia phenotype were found.

In our material of *M. album*, we also obtained a sex-linked gene which is responsible for the development of very narrow leaves, especially in the rosette stage. Our gene also affects the shape of the petals.

We first found three rosettes with narrow leaves in a family (76 J) selected for narrow petals. One of these plants, a male plant, came into flower. Obviously the pollen of this plant was rather sterile, since the first year pollinations did not result in seedsetting. The second year we obtained some progeny, all plants, males and females, were normal. In the next generation there were male plants with narrow leaves and male and female plants with normal leaves (Table 1). In other crosses also female plants with narrow leaves were obtained. Although phenotypic resemblance with the *angustifolia* type is striking, our gene for narrow leaves (*f*) is not necessarily identical with the gene (*b*) reported by BAUR. His gene is lethal in pollen, since only male plants have been discovered by WINGE in the progeny of an X_b Y plant. In our case males and females occur, though the fertility of the pollen both with X_f and with Y had considerably decreased.

TABLE 1

CROSSES DEMONSTRATING X-LINKED INHERITANCE OF A RECESSIVE GENE CAUSING NARROW LEAVES (n.l.) IN *M. album*

Parents	Offspring							
	Observed		Expected		Observed		Expected	
	norm.	n.l.	norm.	n.l.	norm.	n.l.	norm.	n.l.
XX (norm.) × X_fY (n.l.)	146	—	146	—	83	—	83	—
X_fX (norm.) × XY (norm.)	222	—	222	—	91	97	94	94
X_fX (norm.) × X_fY (n.l.)	23	29	26	26	15	11	13	13
X_fX_f (n.l.) × X_fY (n.l.)	—	118	—	118	—	80	—	80

The gene abnormal

WINGE described the recessive gene *abnormal*. In our material we very often came across a mutant which is probably identical with *abnormal*. The expressivity of the gene is variable. Often the plant starts flowering with rather normal flowers, but in the later flowers the petals diminish in size. Notably in male plants this leads to the

development of an inflorescence consisting of closed flowerbuds only. With the reduction in petal size the stamina become smaller and sterile. This gene seems to be very common because it frequently occurred as a disturbing inbreeding effect both in *M. album* and *M. dioicum*.

Certation

In *Melandrium*, deviations from the expected 1/1 ratio, female to male are due to different velocities in growth of the pollen tubes from gametes with an X- and gametes with a Y-chromosome, as was already demonstrated by CORRENS (1917, etc.). HARTSHORNE (1963) found no certation.

TABLE 2

SEX RATIOS OF VARIOUS CROSSES IN *Melandrium*

Type of cross		Number of families counted	Total number of plants	sex ratio (♀/♂) mean	extremes
M. album	× *M. album*	19	1871	1.5	0.9 – 2.9
M. dioicum	× *M. dioicum*	11	1129	1.6	0.5 – 2.8
M. album	× *M. dioicum*	6	913	2.3	1.6 – 3.0
M. dioicum	× *M. album*	1	119	1.2	
M. (*a.* × *d.*)	× *M.* (*a.* × *d.*)	5	395	2.6	1.2 – 4.9
M. album	× *M.* (*a.* × *d.*)	1	92	1.2	
M. (*a.* × *d.*)	× *M. album*	1	48	0.8	
M. d. × (*d.* × (*d.* × (*d.* × (*d.* × *a.*))))		5	435	2.6	1.1 – 3.3

We did find a certation effect in *M. album*, *M. dioicum* and in various crosses where different combinations of sex chromosomes of both species were involved (Table 2). The variation in sex ratios of the different families will partly be due to the uncontrolled conditions during pollination, such as temperature, humidity, etc. Moreover, the length of the style and the quality of the pollen might be important factors. It is clear, however, that male gametes with an X-chromosome succeed more often in fertilizing a female gamete than do gametes with a Y-chromosome.

Genetic differences between X-chromosomes or between Y-chromosomes may also contribute to the variation in sex ratios. That an

X-chromosome can be responsible for an extreme ratio is demonstrated by a number of crosses (Table 3). From these crosses, we obtained female offspring only, with two exceptions (113 K, 35 L). Among their ancestors the male partners of these crosses have in common the female *M. dioicum* plant R 436II 18. The Y-chromosomes in these males are from four different origins (Table 3). It is highly unlikely that these Y-chromosomes, which behave normally in other crosses, are responsible for the extreme certation effect. Neither can the female partners of the crosses of table 3 be responsible, because they are also from very different origins, namely *M. album*, *M. dioicum*, *M.d.* × *M.a.*, and a plant (5R) which is a special flower colour type selected from a F2 generation of *M.a.* × *M.d.* These data show that the X-chromosome originating from R 486II 18 must cause this remarkable result. The fairly large number of male descendants in 35 L may be explained by the low fertility of the male parent of this cross, which fertility will have the same effect as pollination has with few pollen, which also works against certation.

TABLE 3

CROSSES SHOWING AN ABNORMAL SEX RATIO CAUSED BY A SPECIAL X-CHROMOSOME PRESENT IN THE MALE PARENTS, ORIGINATING FROM THE ♀ *M. dioicum* PLANT R 436 II 18.

Family number and cross	Origin of Y-chromosome of ♂ parent	Offspring ♀	Offspring ♂
21 L *M. dioicum* × *M. dioicum*	R 436	40	—
35 L *M. (d. × a.)* × *M. (d. × a.)*	R 327	67	22
75 L *M. album* × *M. (d. × a.)*	,,	185	—
76 L *M. album* × *M. (d. × a.)*	,,	190	—
108 L 5 R *) × *M. (d. × a.)*	R 289	36	—
109 L *M. dioicum* × *M. (d. × a.)*	,,	45	—
110 L *M. dioicum* × *M. (d. × a.)*	,,	38	—
111 L *M. album* × *M. (d. × a.)*	,,	43	—
112 K *M. (d. × a.)* × *M. (d. × a.)*	R 255	92	—
113 K *M. (d. × a.)* × *M. (d. × a.)*	,,	81	1
175 K *M. (d. × a.)* × *M. (d. × a.)*	,,	116	—

*) R is a special flower colour type originally selected out of an F_2 generation of *M. album* × *M. dioicum*.

SEX-INFLUENCED INHERITANCE

Petals

Many measurements were carried out in *M. album* to investigate the variation in size and shape of the petal laminae. The length/width ratio of the petal-lobes was used as an indication of shape. The length and width of the lamina of one petal half pro plant were measured to the nearest tenth of a mm (Fig. 1). The petal halves when collected were stuck on transparent tape. Subsequently measurements were made with a Leitz binocular loupe (10 ×) leading the tape with petal halves over graph paper. Petal halves were taken because whole petals, especially broad ones could not be stuck on tape without wrinkling, which made exact measurements impossible.

When from a family at least 20 measurements pro sex were carried out, as a rule no big differences between the means of different samples of 20 measurements of the same family were found. The variation between the means is of course dependent on the heterogeneity of the material. The differences between the mean l/w ratios of different samples of one family never exceeded 5% of the largest mean l/w value observed in that family.

The significance of the differences occurring between the sexes was tested by a modified Wilcoxon test, $P > 0.05$ difference between the sexes is regarded as not significant.

TABLE 4

THE l/w RATIO ($\Sigma l/\Sigma w$), AND MEAN LENGTH AND WIDTH (IN mm) OF PETAL HALVES OF UNSELECTED *M. album* POPULATIONS

Populations		N	Σl/Σw	P	$\bar{l}$	$\bar{w}$
R 255	♀	63	2.13	0.082	12.36	5.79
	♂	72	2.05		13.38	6.53
R 327	♀	27	2.14	0.074	10.30	4.81
	♂	43	2.04		10.95	5.36
R 470	♀	36	2.46	0.032	11.61	4.71
	♂	74	2.32		12.91	5.56
R 490	♀	21	2.06	0.006	9.73	4.72
	♂	48	1.82		10.04	5.50
R 460	♀	23	1.95	0.002	13.10	6.71
	♂	22	1.70		12.89	7.59

TABLE 5

THE l/w RATIO ($\Sigma l/\Sigma w$), AND MEAN LENGTH AND WIDTH (IN mm) OF PETAL HALVES OF *M. album* FAMILIES SELECTED FOR NARROW PETALS

Generations *)		N	$\Sigma l/\Sigma w$	P	$\bar{l}$	$\bar{w}$
R 255	♀	63	2.13	0.082	12.36	5.79
	♂	72	2.05		13.38	6.53
E (4)	♀	293	2.99	< 0.001	13.03	4.35
	♂	301	2.58		13.30	5.15
F (2)	♀	51	3.19	< 0.001	13.80	4.33
	♂	55	2.61		13.45	5.16
G (3)	♀	80	3.08	< 0.001	12.93	4.20
	♂	70	2.85		13.63	4.79
H (6)	♀	225	2.95	0.004	12.35	4.19
	♂	164	2.72		13.16	4.84
J (6)	♀	246	3.11	< 0.001	12.19	3.92
	♂	249	2.83		12.22	4.31
K (5)	♀	211	3.72	< 0.001	12.83	3.45
	♂	162	3.41		12.72	3.73

*) Selection started in the population R 255 and was continued during 6 subsequent generations (E–K). Number of families raised pro generation between brackets.

TABLE 6

THE l/w RATIO ($\Sigma l/\Sigma w$), AND MEAN LENGTH AND WIDTH (IN mm) OF PETAL HALVES OF *M. album* FAMILIES SELECTED FOR BROAD PETALS

Generations *)		N	$\Sigma l/\Sigma w$	P	$\bar{l}$	$\bar{w}$
R 255	♀	63	2.13	0.082	12.36	5.79
	♂	72	2.05		13.38	6.53
E (2)	♀	64	1.79	0.061	15.36	8.60
	♂	71	1.69		15.01	8.89
F (8)	♀	207	1.75	0.065	15.99	9.11
	♂	238	1.67		14.66	8.77
G (7)	♀	212	1.49	0.534	13.68	9.19
	♂	181	1.46		13.54	9.29
H (12)	♀	335	1.48	0.439	13.54	9.13
	♂	363	1.53		13.09	8.56
J (8)	♀	354	1.43	0.671	14.90	10.39
	♂	360	1.38		14.50	10.51
K (4)	♀	98	1.30	0.545	15.26	11.76
	♂	128	1.28		14.40	11.27

*) Selection started in the population R 255 and was continued during 6 generations (E–K). Number of families raised pro generation between brackets.

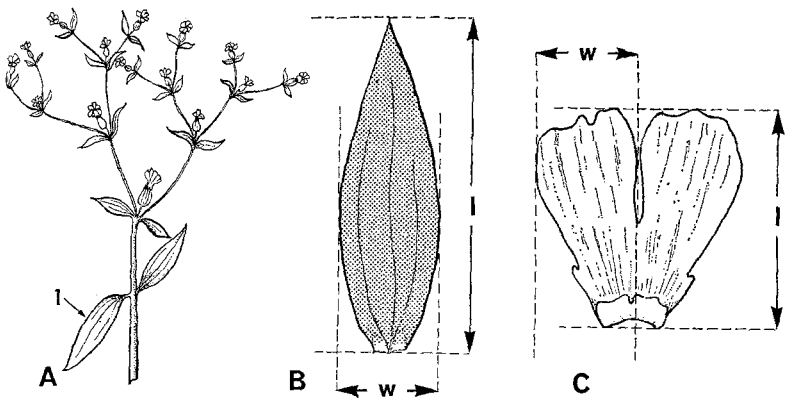

Fig. 1. Leaves on the first node beneath the inflorescence on the main stem were measured (A–1). Length and width of leaves and petal halves were determined as shown (B and C).

In table 4 data are given of measurements of some populations of *M. album*, raised in our garden from seed collected on various habitats. In this unselected material the female plants have narrower petals (higher l/w value) than the male plants of the same family.

A two-way selection program was started in population R 255. In this population individuals with the narrowest petals were crossed with each other. The petals of the resulting families were measured. In the family which on the average had the narrowest petals, again the individuals with the narrowest petals were selected for further crosses. A similar method was used for families with broad petals. These selections were done for 6 generations.

Selection for narrow petals seems to increase the difference between male and female plants (Table 5). After selection for broad petals the difference disappears (Table 6).

When we compare families with broad petals (Table 6) and families with narrow petals (Table 5) it appears that narrow petals are at the same time slightly shorter than broad ones. In families with broad petals, the female petals are larger than the male petals, but have the same l/w ratios. In families with narrow petals the petals of the female flowers are smaller than those of the male flowers, but have a higher l/w ratio than the latter, due to the stronger decrease in width. An accumulation of genes causing narrow petals thus seems to have a stronger effect in female plants than in male plants.

The data given pro generation in table 5 and table 6 are presented pro family in a graph (Fig. 2). This graph too illustrates that the effect of the genes for narrow petals, which causes a decrease especially in the width of the petals, seems to be stronger in female plants than in male ones.

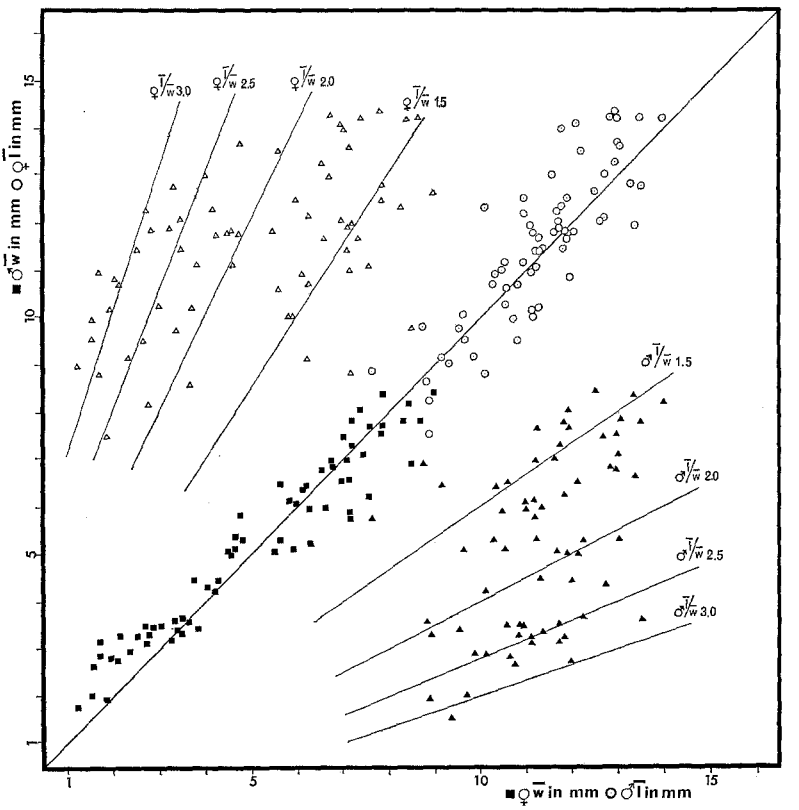

Fig. 2. The ♀ l̄, ♀ w̄, ♂ l̄ and ♂ w̄ of petal halves of a number of *M. album* families are used as coordinates in various combinations.

○ Abscissa: ♂ l̄ (in mm); ordinate: ♀ l̄ (in mm).
■ Abscissa: ♀ w̄ (in mm); ordinate: ♂ w̄ (in mm).
▲ Abscissa: ♂ l̄ (in mm); ordinate: ♂ w̄ (in mm).
△ Abscissa: ♀ w̄ (in mm); ordinate: ♀ l̄ (in mm).

Notably in families with a small w̄, the ♂ w̄ is larger than the ♀ w̄ of the same family.

Leaves

Size and shape of leaves were determined in *M. album* and *M. dioicum*. Measurements were performed in both species on unselected material and in *M. album* also on families selected for narrow leaves and on other families selected for broad leaves. The same selecting method was used as in selection for petal-shape.

Pro plant the length and width was determined in mm, of one stem leaf on the first node below the inflorescence on the main stem (Fig. 1A and B).

With regard to shape, the leaves of *M. album* display the same tendencies as the petals. In unselected material from various origins, the $\Sigma l/\Sigma w$ ratio is higher in female plants than in the male (Table 7). After selection for narrow leaves (Table 8) the difference becomes more distinct. Selection for broad leaves comprises two generations only (Table 9). This is probably the reason why the difference between the sexes still exists. The populations R 470 and R 490 (Table 7) have,

TABLE 7

THE l/w RATIO ($\Sigma l/\Sigma w$), MEAN LENGTH AND WIDTH (IN mm) OF LEAVES OF UNSELECTED *M. album* FAMILIES FROM VARIOUS ORIGINS

Original populations *)		N	$\Sigma l/\Sigma w$	P	$\bar{l}$	$\bar{w}$
R 255 (14)	♀	851	4.51	< 0.001	69.96	15.51
	♂	672	3.88		58.36	15.05
R 259 (6)	♀	317	4.24	< 0.001	75.60	17.81
	♂	290	3.43		54.00	15.73
R 289 (2)	♀	91	5.23	< 0.001	64.89	12.40
	♂	133	4.25		58.44	13.75
R 290 (2)	♀	101	3.39	< 0.001	68.68	20.91
	♂	81	2.89		60.20	22.37
R 327 (5)	♀	325	3.55	0.021	56.93	16.04
	♂	166	3.25		42.68	13.15
R 460 (2)	♀	213	4.37	< 0.001	56.60	12.94
	♂	261	3.57		43.76	12.25
R 470 (4)	♀	174	2.82	0.545	55.75	19.80
	♂	238	2.84		50.06	17.64
R 490 (0)	♀	45	2.13	0.675	60.58	28.44
	♂	42	2.14		53.95	25.17

*) Number of families between brackets.

TABLE 8

THE l/w RATIO ($\Sigma l/\Sigma w$), MEAN LENGTH AND WIDTH (IN mm) OF LEAVES OF *M. album* FAMILIES SELECTED FOR NARROW LEAVES DURING 5 (F–K) AND 3 (H–K) GENERATIONS

Original populations	Generations *)		N	$\Sigma l/\Sigma w$	P	$\bar{l}$	$\bar{w}$
R 255	F (4)	♀	156	5.18	0.005	74.34	14.35
		♂	149	4.59	.	62.46	13.60
	G (3)	♀	90	4.90	0.033	†)	
		♂	121	4.72			
	H (8)	♀	277	5.18	0.046	77.62	14.99
		♂	271	5.06		65.00	12.84
	J (5)	♀	229	7.19	< 0.001	63.89	8.89
		♂	274	6.35		55.81	8.78
	K (2)	♀	56	7.31	< 0.001	67.38	9.21
		♂	59	6.25		53.32	8.53
R 289	H (2)	♀	150	5.34	< 0.001	61.96	11.59
		♂	75	3.72		48.07	12.92
	J (3)	♀	134	6.78	< 0.001	72.89	10.75
		♂	100	4.55		60.47	13.29
	K (3)	♀	151	6.06	< 0.001	59.39	9.79
		♂	101	5.32		52.73	9.91

*) Number of families pro generation between brackets.

†) Data are lost.

TABLE 9

THE l/w RATIO ($\Sigma l/\Sigma w$), MEAN LENGTH AND WIDTH (IN mm) OF LEAVES OF *M. album* FAMILIES SELECTED OUT OF R 255 AND R 327 FOR BROAD LEAVES DURING 2 GENERATIONS

Original populations	Generations *)		N	$\Sigma l/\Sigma w$	P	$\bar{l}$	$\bar{w}$
R 255	J (2)	♀	108	3.48	0.017	83.60	24.02
		♂	129	3.11		76.55	24.62
	K (4)	♀	88	2.99	0.044	88.95	29.80
		♂	111	2.83		76.31	26.93
R 327	J (3)	♀	229	3.38	0.018	59.18	17.52
		♂	112	3.11		51.65	16.63
	K (4)	♀	168	2.92	0.027	54.30	18.58
		♂	156	2.72		45.04	16.53

*) Number of families pro generation between brackets.

TABLE 10

THE l/w RATIO ($\Sigma l/\Sigma w$), MEAN LENGTH AND WIDTH (IN mm) OF LEAVES OF *M. dioicum* POPULATIONS

Populations		N	Σl/Σw	P	$\bar{l}$	$\bar{w}$
R 191	♀	193	1.65	0.196	60.63	36.69
	♂	102	1.59		48.64	30.56
R 401	♀	105	2.31	0.072	61.62	26.70
	♂	119	2.52		51.24	20.37
R 436	♀	113	2.01	0.535	61.28	30.51
	♂	61	2.01		54.89	27.25
R 437	♀	120	2.12	0.603	55.77	26.27
	♂	146	2.13		45.92	21.57
R 488	♀	30	1.99	0.590	51.27	25.77
	♂	49	2.04		43.74	21.41
M.d. Zuid	♀	272	1.88	0.404	55.65	29.48
Limburg *)	♂	283	1.83		49.22	26.96

*) Measured on the original habitat.

TABLE 11

THE l/w RATIOS ($\Sigma l/\Sigma w$) OF LEAVES IN DIPLOID *M. album* FAMILIES WITH FEMALE PLANTS AND HERMAPHRODITES

Family		N	$\Sigma l/\Sigma w$	P
195 H (197 F)	♀ (♀)	47 (31)	4.65 (4.66) *)	0.030 (0.002)
	♀ (♂)	39 (32)	4.30 (4.27)	
196 H (104 G)	♀ (♀)	45 (77)	3.79 (3.86)	< 0.001 (0.011)
	♀ (♂)	22 (84)	3.03 (3.24)	
195 J (198 F)	♀ (♀)	37 (45)	4.75 (4.61)	< 0.001 (0.002)
	♀ (♂)	58 (45)	4.01 (4.14)	
196 J (90 H)	♀ (♀)	49 (50)	4.21 (4.24)	< 0.001 (< 0.001)
	♀ (♂)	51 (35)	3.64 (3.44)	
200 J (199 F)	♀ (♀)	60 (49)	5.35 (5.47)	< 0.001 (< 0.001)
	♀ (♂)	58 (39)	4.68 (5.08)	

*) Data between brackets show comparable ratios in families with female and male plants.

TABLE 12

THE l/w RATIOS ($\Sigma l/\Sigma w$) OF LEAVES IN TETRAPLOID *M. album* FAMILIES WITH FEMALE PLANTS AND HERMAPHRODITES AND TWO TETRAPLOID FAMILIES WITH FEMALE AND MALE PLANTS

Family		N	$\Sigma l/\Sigma w$	P
R 319	♀	31	2.93	< 0.001
	♀	49	2.24	
37 G	♀	20	2.63	< 0.001
	♀	32	2.13	
138 H	♀	33	2.70	0.0015
	♀	63	2.43	
139 H	♀	51	2.64	0.0(
	♀	83	2.35	
142 H	♀	16	3.08	< 0.001
	♀	70	2.54	
146 H	♀	37	2.61	< 0.001
	♀	82	2.17	
27 H	♀	48	2.82	< 0.001
	♂	62	2.54	
199 H	♀	57	3.55	
	♂	57	3.31	0.030

without selection, broad leaves and no differences between the Σl/Σw ratios of the sexes were observed. Also *M. dioicum* with its broad leaves displays no differences between the Σl/Σw ratios of female plants and male ones (Table 10).

The leaves of female plants of *M. album* and *M. dioicum* are on the whole larger than the leaves of male plants.

In a number of *M. album* families with female plants and hermaphrodites, the latter caused by an abnormal Y-chromosome called Y^a (NIGTEVECHT, 1966), l/w ratios of leaves were determined (Table 11). The differences between the l/w ratios of females and hermaphrodites were equal to the differences between the l/w ratios of female plants and male plants in normal families with comparable leaf shapes. In tetraploid families, female plants and hermaphrodites also showed a significant difference in l/w ratios (Table 12). In these families an abnormal Y-chromosome (Y^1) found by WESTERGAARD (1946) caused the hermaphroditism.

Anthocyanin formation

The green parts of plants of *M. album* and of *M. dioicum* are often stained reddish brown by anthocyanin. We noticed that especially *M. album* is very variable in this respect. It is known that in general, anthocyanin formation is strongly dependent on light, so that plants growing on shaded places usually have far less anthocyanin than individuals on sunny sites. However, since in the experimental garden all plants were grown in the open, the differences noted cannot have been due to such an effect.

A classification of anthocyanin content in the calyx and in the stem was made as follows:

Anthocyanin in the calyx.

0. No anthocyanin.
1. Main veins with anthocyanin only.
2. Main and side veins with anthocyanin.
3. Also between the side veins some anthocyanin.
4. Between the side veins very much anthocyanin.

Anthocyanin in the stem.

0. No anthocyanin.
1. Anthocyanin in the very first node of the stem only.
2. Anthocyanin in nearly all nodes of the stem.
3. Anthocyanin in nearly all nodes of the stem and some anthocyanin in the internodes.
4. Internodes with very much anthocyanin.

An analysis was made of a number of randomly chosen *M. album* families (Table 13). There appeared to be a difference in anthocyanin content between the sexes, male plants having more anthocyanin in calyx and stem than female plants.

As the genes for anthocyanin formation have constantly a stronger effect in male plants than in the female irrespective whether the family as a whole displays much or little anthocyanin in the green parts, we may consider these genes to follow a sex-influenced inheritance.

Also anthocyanin formation in the petals appears to be sex-influenced. The petals of *M. album* are as a rule without any anthocyanin. When some plants in a population of *M. album* resulting from introgressive hybridisation with *M. dioicum* have very faint-red coloured

TABLE 13

PLANTS OF DIPLOID (2n) AND TETRAPLOID (4n) FAMILIES OF *M. album* CLASSIFIED ACCORDING TO ANTHOCYANIN CONTENT IN CALYX AND STEM. INTENSITY CLASSES 0–4, SEE TEXT

Family		N	Numbers of plants classified to anthocyanin content in calyx					Numbers of plants classified to anthocyanin content in stem				
			0	1	2	3	4	0	1	2	3	4
17 E	♀	33	17	5	10	1	—					
2n	♂	38	—	—	11	27	—					
30 E	♀	144	38	40	61	5	—					
2n	♂	106	6	3	26	71	—					
209 G	♀	56	—	9	18	19	10	—	2	33	21	—
2n	♂	50	—	—	—	—	50	—	—	10	37	3
210 G	♀	8	2	3	2	1	—	—	—	5	3	—
2n	♂	36	—	1	13	7	15	—	1	13	20	2
171 H	♀	58	—	—	26	29	3	—	—	54	4	—
2n	♂	71	—	—	4	18	49	—	—	61	10	—
173 H	♀	48	—	5	29	9	5	—	—	46	2	—
2n	♂	63	—	—	5	35	23	—	—	60	3	—
138 H	♀	33	1	—	12	20	—	—	1	28	4	—
4n	♂	63	—	1	3	29	30	—	—	50	13	—
142 H	♀	15	—	—	4	11	—	—	—	14	1	—
4n	♂	69	—	—	1	54	14	—	—	57	12	—
146 H	♀	37	—	1	17	18	1	—	—	29	8	—
4n	♂	82	—	1	17	63	1	—	—	53	28	1

petals, these plants are mainly male plants. Possible differences in anthocyanin formation between male and female petals of *M. dioicum* cannot be demonstrated by comparing the intensities of the deep reddish purple coloured petals. In the lighter coloured offspring of *M. album* × *M. dioicum* crosses, the male petals appear to be somewhat more vividly coloured than the female petals.

We found another way of comparing anthocyanin formation in female and male petals in *Melandrium*. The pH of the cell sap of the petals appeared to fluctuate with the colour intensity, possibly due to the fact that the anthocyanins present in the material studied were acylated. The more anthocyanin is present the lower the pH. In crosses not mentioned here no independent gene for pH determination could be detected.

Measurements were performed on the pH of an extract of the petals of *M. album*, *M. dioicum*, *M. album* × *M. dioicum* and *M. dioicum* × *M. album* (Table 14). Pro family the pH of male petals was determined on a sample consisting of petals from all male plants. The pH of female petals was determined analogously. Measurements were made on fresh ground petals in doubly distilled water. A solution was made equivalent to 2 grams of petals in 30 ml distilled water.

In *M. album* no consistent difference between female and male plants was found. The pH of the extract was about 6.0 in both sexes. The same pH was found in the extract of the leaves of *M. album* and of *M. dioicum*. When anthocyanin was present in the petals the pH

TABLE 14

CORRELATION BETWEEN COLOUR INTENSITY (RANGING FROM 0 TO 5) AND PH OF PETAL EXTRACTS IN *Melandrium*

Family	Colour intensity of petals	pH		Number of plants	
		♀	♂	♀	♂
M. album					
47 J	0	6.0	5.9	20	46
131 J	0	6.0	5.9	39	36
175 J	0	5.9	6.1	35	32
269 J	0	5.8	6.0	15	87
270 J	0	6.0	6.0	22	64
M. dioicum					
222 J	5	5.2	5.1	23	20
230 J	4	5.4	5.3	10	59
M. a. × *M. d.*					
224 J	3	5.7	5.5	49	49
226 J	3	5.6	5.5	46	50
227 J	3	5.6	5.4	51	52
M. d. × *M. a.*					
212 J	3	5.5	5.3	44	53
214 J	2–3	5.8	5.5	59	58
215 J	2–3	5.8	5.5	21	14

Without anthocyanin a pH of approximately 6.0 was observed. Families with anthocyanin in the petals show a higher pH of the cell sap in the petals of the female plants than of the male plants.

of the petals was always lower than the pH of the leaves and the pH in male petals was always lower than the pH in female petals of the same family.

These data support our presumption that the genes that determine the amount of anthocyanin in the petals behave like sex-influenced genes.

Glandular hairs

In most cases *M. album* plants have glandular hairs. The density of these hairs is highest on the top of the plants. Downward a decrease in number is seen. With a decrease in number the length of the hairs and the diameter of the top cells become at the same time smaller. When no glandular hairs are found on the calyces, the whole plant will lack these hairs.

When all the plants of a family have many glandular hairs it is very difficult to distinguish different categories. In families where the glandular hairs are less abundant an analysis can be made. In R 490, R 470 and the families 55 K, 76 K and 77 K raised from R 470, on the whole, less glandular hairs were observed, than in many other families of *M. album*. They had many normal hairs instead. In these families it was possible to classify the plants according to the relative amount of glandular hairs on the calyx. The data (Table 15) show a difference between both sexes. As a rule female plants have fewer glandular hairs than male plants of the same family. This is demonstrated by

TABLE 15

PRESENCE OF GLANDULAR HAIRS ON *M. album* PLANTS OF R 470, R 490 AND OF THREE FAMILIES RAISED FROM R 470

	Percentage of plants with glandular hairs		Mean relative amount of glandular hairs on calyces of plants with these hairs *)	
	♀	♂	♀	♂
R 490	18	83	1.5	2.0
R 470	18	75	1.7	1.9
55 K	82	100	1.8	2.5
76 K	40	89	1.5	2.1
77 K	6	60	1.0	1.7

*) Estimation 0, no glandular hairs, to 3, many glandular hairs.

the different percentages of female plants and male plants with glandular hairs and also by the difference in relative amounts of glandular hairs on female and male plants with glandular hairs.

The genes for glandular hairs obviously behave differently in the two sexes. In female plants these genes have a lower expressivity than in male plants. Therefore, when the plants have a genotype for few glandular hairs the character might show up in the male plants and not in the female ones.

TABLE 16

CROSSES BETWEEN *M. album* PLANTS WITHOUT GLANDULAR HAIRS SELECTED OUT OF 77 K (TABLE 15)

Family	Glandular hairs *)									
	♀					♂				
411 M	+	−	−	−	−	1–2	1	1	+	+
412 M	−	−	−	−	−	1	+	+	−	−
413 M	−	−	−	−	−	1–2	1	−	−	−
414 M	−	−	−	−	−	1	1	1	+	+
415 M	+	−	−	−	−	+	+	+	−	−
415 MA	−	−	−	−	−	1	1	1	1	+
416 M	−	−	−	−	−	1	+	+	+	−
417 M	+	−	−	−	−	1	1	+	−	−
418 M	−	−	−	−	−	1	1	1	+	+
419 MA	−	−	−	−	−	+	+	+	+	−

*) Glandular hairs determined on calyces of 5 female and 5 male plants pro family. −, no glandular hairs; +, sometimes a glandular hair is found; 1, one row of glandular hairs on the main veins; 2, more than one row of glandular hairs on the main veins.

A number of special crosses between plants without glandular hairs (Table 16) displays the same difference in penetrance. Out of 10 families 5 individuals pro sex were observed very carefully. Among these plants 47 females were found without glandular hairs and 3 with a few, where 11 male plants had none and 39 some glandular hairs. Apparently the genes for glandular hairs which had no penetrance in the female parents of these families did have some effert in most male offspring.

These genes can therefore be considered as sex-influenced genes.

Discussion

Sex-linked genes are genetically linked to the mechanism of sex determination. Sex-influenced genes might be regarded as physiologically linked to the same mechanism.

Sex-linked genes are by definition genes that are situated in the sex chromosomes, but take no part in sex determination. We described the gene in the X-chromosome causing narrow leaves (*f*), as a sex-linked gene. However, *f* influences sex expression, since notably in male plants the fertility has decreased markedly. In fact the same holds true for the gene *abnormal*. Therefore both genes might be regarded as sex-determining factors. In the differential part of one or both sex chromosomes, genes must be present that influence the growth of the pollen tubes, thus giving rise to the certation effect. An extreme certation effect, caused by a special X-chromosome, was demonstrated by crosses that yielded female offspring only (Table 3). As there is at the moment no proof that the genes causing certation are sex-determining genes they must be called sex-linked genes.

Sex-influenced genes play no part in sex determination, but their effect is influenced by the sex-determining genes, in such a way that when the inner milieu is suitable for the development of a gynoecium only, the phenotype brought about by the sex-influenced genes is not the same as when the sex-determining mechanism leads to the development of the male organs only.

Sex-influenced inheritance leads to sex dimorphism. This dimorphism is not restricted to situations where two different genotypes are present, as in dioecious species. The monoecious *Zea mays* has male and female inflorescenses, which differ clearly from each other.

The dioecious orchid *Catasetum barbatum* produces every now and then a monoecious plant (GOEBEL, 1913). The female and male flowers differ so profoundly that before the monoecious individuals were found male plants and female were described as belonging to different genera.

The influence of the sex-determining mechanism on the sex influenced genes often extends beyond the flowers. Female plants of the dioecious species *Rubus chamaemorus* have five-lobed leaves on the vegetative shoots, and three-lobed leaves on the flowering shoots. Male plants have three-lobed leaves and single-lobed ones respectively.

Female plants in most dioecious species are larger than the male

plants (GOEBEL, 1913). *Melandrium* is no exception to this rule. In *M. album* and in *M. dioicum* female plants are bigger and have also larger leaves than male plants. The presence of less anthocyanin in females than in males might also be an indication of a more vigorous growth of females, since anthocyanin often occurs where growth has been inhibited.

On the other hand, petals of male plants are larger than petals of female plants in unselected populations (Table 4).

This seems to be in contradiction with the greater over all size of the female plants. However, in dioecious species the male petals are often larger (GOEBEL, 1913). This might be explained by assuming that the same mechanism that in the female flowers inhibits the development of the stamina, suppresses to a lesser extent the develop-

TABLE 17

THE l/w RATIO ($\Sigma l/\Sigma w$) OF LEAVES AND PETALS OF *M. album* DEMONSTRATING INDEPENDENT INHERITANCE OF SHAPE OF LEAVES AND SHAPE OF PETALS

	Leaves $\Sigma l/\Sigma w$		Petals $\Sigma l/\Sigma w$	
	♀	♂	♀	♂
No selection				
R 327	3.49	3.39	2.14	2.04
R 460	4.32	3.57	1.95	1.70
R 470	3.48	3.26	2.46	2.32
R 490	2.13	2.14	2.06	1.82
Selection for broad petals				
71 H	4.32	3.70	1.18	1.26
76 H	4.11	3.58	1.12	1.20
Selection for narrow petals				
270 F	4.16	3.64	3.49	2.54
114 G	4.29	3.68	3.30	2.90
Selection for broad petals and narrow leaves				
299 H	7.00	6.17	1.66	1.50
175 J	7.13	6.10	1.59	1.47
269 J	7.28	6.65	1.37	1.36
270 J	8.15	6.87	1.82	1.59

ment of the petals. In the discussion on sex determination (Part II) more will be said about this suppressing principle (NIGTEVECHT, 1966).

It is not clear by which physiological principle the sex determination mechanism influences the formation of glandular hairs and the shape of petals and leaves.

Obviously shape of leaves and shape of petals are not determined by the same genes (Table 17). Without selection distinct differences may exist between the l/w ratios of leaves of various populations; for instance R 460, ♀ l/w 4.32 and R 490, ♀ l/w 2.13. However, there is no difference in the l/w ratios of the petals of the same populations. By selection the discrepancy can be made even stronger. The families 71 H and 76 H are selected for broad petals, ♀ l/w 1.18 and 1.12 respectively. The families 270 F and 114 F are selected for narrow petals, ♀ l/w 3.49 and 3.30. These four families, however, do not differ in shape of leaves. A number of other families could be selected for narrow leaves and broad petals at the same time, 269 J ♀ l/w leaves 7.28, petals 1.37.

We assume therefore that different gene complexes are involved in petal shape and leaf shape determination. However, both gene complexes are influenced by the sex determination mechanism in the same direction.

The inhibition of growth in width caused by genes for narrow leaves and by other genes for narrow petals seemed to be stronger in female plants than in male ones. A series of measurements on leaves in families consisting of females and hermaphrodites demonstrated a similar difference between females and hermaphrodites (Tables 11, 12). Moreover, the calyces of the hermaphrodite plants contained clearly more anthocyanin than the calyces of the females in the respective families. So, with regard to these sex-influenced characters, males in *Melandrium* are more like hermaphrodites than females are.

These observations on sex-influenced characters might be of significance for an understanding of the physiological processes involved in sex determination, as the mechanism of sex determination expresses itself not only in the formation of the sex organs but also in the sex-influenced characters.

The results of our investigations on sex determination will be reported in part II of these "studies in dioecious *Melandrium*" (NIGTEVECHT, 1966).

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