Cytogenetic Studies in *Melandrium album*. I. An ovary formation gene located in an autosome, with special reference to its linkage relation¹

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Introduction

In 1932 I selected a modified male plant which appeared spontaneously in a culture of *Melandrium album* for the purpose of cytogenetic experiments. The pedigree culture was continued. Among the offsprings there were found a number of females with abnormal ovaries and also plants with non-lobed or entire petals; and it was found that there is a linkage relation between those two characters.

Cytological studies were mostly done with the material fixed with NAVASHIN's fluid, with pretreatment with CARNOY's 1:3 aceto-alcohol.

Figs. 1-2. Photos of imperfect female flowers showing ovaries with naked ovules. $\times 15$.
1, an ovary with the normal number of carpels, but with incomplete union of carpels.
2, an ovary with only one carpel, margin of which is incompletely closed, though the stigma and the placenta are well developed; the upper part of the stigma being removed, is not shown in the figure.

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The paraffin sections were stained with NEWTON's gentian violet; iron-alum hematoxylin was also used. All figures refer to *Melandrium album*.

Observation

1. Abnormal female plant. The ovary of the ordinary female plants shows 5 component carpels, while the abnormal females, the materials of the present study, show a variable number and various modes of the union of the carpels. They are characterized by two facts, i.e. by having a reduced number of carpels in an ovary and the incomplete fusion of the carpels (Figs. 1, 2). In the extreme case no carpel is formed, a number of naked ovules or a few, sometimes only one, rudimentary ovules being formed upon the receptacle. Also, some flowers on the abnormal female plant have almost normal ovaries, each having 5 perfect carpels and five pistils, there being only slight imperfectness in the carpelary suture, so that one can not distinguish them from the normal ones when observed from the outside of the calyx.

These two characters, the reduced number of carpels and the incomplete sutures, are probably concerned with different genes, because I noticed in my culture some plants on which all ovaries had a reduced number of carpels, usually 3, sometimes 2 or even one, while they had closed ovaries with the normal sutures. For the present however I should like to consider these genes concerning abnormal ovary as one gene *i* (imperfect) (its allele being *I*), because they seldom behave as separate genes, probably owing to their being located in one and the same chromosome.

Figs. 3-4. Photos of flowers. $\times 1$. *as*, seen from the top; *bs*, a little oblique side views. 3, *a* and *b*, one and the same male flower; petals are bilobed with deep indentation; 4, *a* and *b*, one and the same female flower with entire petals.

2. The bilobed and entire petals. The 'bilobed petal' (Fig. 3, *a* and *b*) is dominant to the entire petal (Fig. 4, *a* and *b*). I shall designate the gene concerning the entire petal as *e* (entire) and its dominant allele *E*.

3. Modified male plant. The floral structure of the modified male (so-called hermaphrodite or intersex plant) is not different from those of the normal male plants except that they have ovaries and that the calyx tube is generally slightly thicker in texture than in the normal male. In some extreme cases, however, plants have pure androecious flowers in the early flowering season, the modified male flowers in the middle season and still later the gynaeceous flowers only. Further there

are plants mostly having androeceous flowers throughout the flower season mixed with few modified male flowers. The ovaries in the modified male flowers are abnormal and the fertility in those flowers is very low, producing the fruits in the end of the season only. The shape of the fruits is irregular even when the ovary is completely closed, the carpels being of different sizes, and having small number of seeds in them (Fig. 6).

The first modified male parent in this culture gave in the offsprings true males and females as well as the modified males. But later I found a few plants which gave in the offsprings abnormal females and modified males, but none of the true males.

Figs. 5-6. The fruits. $\times 1$. 5, normal ones. To the left a fruit whose calyx is removed; to the right one with the calyx. 6, two fruits of a modified male plant; the calyx were removed to show the reduced number of the carpels, 4 carpels in the left hand one and 3 in the right hand one; they all form closed ovaries.

4. Chromosomes in the modified male plants. As BĚLAŘ (1925) already reported the chromosome constitution in the modified male plants is not different from that in the true male plants. The behaviours in their meiotic phases show no difference from each other, the 1st and the

Fig. 7. Photos of the PMCs of a modified male plant, showing the side and polar views of the equatorial plates of the 1st meiotic division. $\times 1000$.

2nd meiotic divisions occurring regularly (Fig. 7). Here I should like to mention that the X-chromosome is smaller than the Y-chromosome as was reported by BLACKBURN (1924) and confirmed by BĚLAŘ (l.c.), though according to HEITZ (1925) the relation is just opposite.

5. **Chromosomes in the somatic cells in the female plants.** In the normal as well as the abnormal female, diplosporophyte has 24 chromosomes, 11 pairs of autosomes and one pair of X-chromosomes. Microscopical examination of the structure of the chromosomes in both kinds of individuals, the normal and the abnormal female, does not show any difference from one another (Fig. 8).

Fig. 8. Camera lucida drawing of an equatorial plate of a somatic division in a pericycle cell of the root tip of an abnormal female plant. Xs, X-chromosomes. ca. $\times 1270$.

6. **Genetic experiment 1.** A female plant *Iiee* with perfect ovaries and entire petals was crossed with a male *IiEe*, with bilobed petals. The F_1 offsprings are shown in Table 1.

Table 1. The F_1 -offsprings of the cross *Ie ie* $\times$ *IE ie*.

	Female				Male*	
	Ovary perfect, petal bilobed	Ovary perfect, petal entire	Ovary imperfect, petal bilobed	Ovary imperfect, petal entire	Petal bilobed	Petal entire
Observ. no.	30	25	4	6	10	6
Theoret. no.	26.4	20.3	4.1	12.2	8	8
Deviation	+1.6	+4.7	-0.1	-6.2	+2	-2

* The modified male plants are included.

The observation number in Table 1 does not fit the expectation number for the segregation of the hybrid *Iiee* $\times$ *IiEe*, namely 3 *IE* (perfect ovary, bilobed petal) : 3 *Ie* (perfect ovary, entire petal) : 1 *iE* (imperfect ovary, bilobed petal) : 1 *ie* (imperfect ovary, entire petal). But if we consider a linkage relation between *Ii* and *Ee* the observation number fits the expectation. Then the genotype of the female plant will be *I-e i-e*, that of the male *I-E i-e*; their recombination value will be 1/4, and we have the theoretical ratio as in Table 1. The deviation under the 2nd and the 4th terms are large. This must be due to the false recognition of the imperfect ovaries for the perfect ones, as I have mentioned before about the possibility for such a mistake. The +deviation under the 2nd term and the -one under the 4th term is in accordance with the above explanation. If we take this mistake into consideration the probability of the fitness will become very high.

Experiment 2. The mother plant had imperfect ovary and bilobed petals, and the father plant was one and the same plant as was used in the experiment 1. Though the number of the offsprings was rather small, the result is shown in Table 2.

Table 2. The results of the crossing $E-i\ e-i \times I-E\ i-e$.

	Female				Male*	
	Ovary perfect, petal bilobed	Ovary perfect, petal entire	Ovary imperfect, petal bilobed	Ovary imperfect, petal entire	Petal bilobed	Petal entire
Observ. no.	8	0	2	2	1	0
Theoret. no.	5.25	0.75	3.75	2.25	0.75	0.25
Deviation	+2.75	-0.75	-1.75	-0.25	+0.25	-0.25

* The modified male plants are included.

The theoretical number was deduced under the assumption of the genotype of the female plant as $E-i\ e-i$ and that of the male plant as $I-E\ i-e$. In this table too, if we take the same mistake under the consideration in the first and 3rd terms as in the case of the experiment 1, we will have very high probability of the fitness between the observation and the expectation.

Discussion

1. **The loci of the genes Ii and Ee .** The results of the genetic experiments mentioned above show that the Ii and Ee genes are linked together with $1/4$ recombination value. Then in what chromosome are their loci situated is an important question. At present however we have no observed data which indicate their position in a definite chromosome. However, the segregation ratio shows us that they are not located in the sex chromosomes, but in an autosome. Because if the EI genes were present in the X-chromosome and ie in the Y-chromosome or vice versa, then the theoretical ratio of the segregation in the F_1 in the crossing shown in Table 1 must be one of the ratios given in Table 3.

Table 3. Theoretical ratios of the all possible cases of the segregation in the F_1 plants of $I-e\ i-e \times I-E\ i-e$ under the assumption that these genes have their loci in the sex chromosomes.

Possible kinds of genic order and constitution in the male parent*		Genic constitution of the male gametes and their ratio**		The F_1 offspring***	
X	Y	Gametes having X-chromosome	Gametes having Y-chromosome	Female plant	Male plant
EI	ei	3 EI : 1 eI	1 Bi : 3 ei	3 EI : 1 eI	1 e : 3 e
IE	ie	3 IE : 1 iE	1 Ie : 3 ie	3 IE : 1 iE	all E
Ei	eI	3 Ei : 1 IE	1 EI : 3 eI	3 EI : 1 le	all E
iE	Ie	3 iE : 1 IE	1 ie : 3 Ie	all IE	all E
ei	EI	3 ei : 1 Ei	1 eI : 3 EI	3 ei : 1 IE	1 e : 3 E
ie	IE	3 ie : 1 Ie	1 iE : 3 IE	3 ie : 1 Ie	all E
eI	Ei	3 eI : 1 EI	1 ei : 3 iE	3 iE : 1 EI	1 e : 3 E
Ie	iE	3 Ie : 1 ie	2 IE : 3 iE	all e	all E

* Under this column the order of the genic symbols, e.g. EI or IE , means the order of genes on the chromosomes from the proximal to distal end. Which gene enters into Y-chromosome after a crossing over is determined by the order of genes on the chromosome. In this table the gametic ratio was calculated under the assumption that Y-chromosome has its non-homologous part (part not homologous with X) in the distal side from the point where the crossing over occurred. If it be reverse, then the genotype for the male and female are to be reversed.

** The recombination value is $1/4$.

*** In this column, E denotes bilobed petal, e entire petal, I perfect ovary, and i imperfect ovary.

Here we see in this table that there are only two kinds of the gene combinations in either X- or Y-chromosome. Consequently under the same circumstances as are shown in Tables 1 and 2 we can not expect more than two kinds of the F_1 plants in either male or female offsprings, while we have four kinds of female offsprings in these experiments. Therefore we conclude that this linkage group is located not in the sex chromosome, but in an autosome.

The configurations of the X- and Y-chromosome pair in the meiotic prophase as we will see in the next report, show us also that there can not take place a crossing over with such a high recombination value as $1/4$.

2. The modified male plant. The dioecious form in *Melandrium album* has probably been derived from an hermaphrodite as other investigators already mentioned (CORRENS, 1913 etc.). The female plants have different cellular condition from the male plant (MANOILOV 1924, SATINA and DEMEREC 1925, YAMASAKI 1933, SINOTÔ and NAKAMURA 1939 etc.). These differences must be due directly or indirectly to their nuclear conditions. Certainly a large part of contents of the vacuoles in the cytoplasm are derived from the nucleus (YASUI, 1941). Differences in the nuclear condition here mentioned must be due to the difference between chromosome constitutions, namely the presence of the XX-chromosomes in the female and XY-chromosomes in the cell nucleus of the male plant. Certain special condition in the female plant must concern the production of the female flower in which the inhibition of the development of the anther occurs, but not that of the ovary, namely the condition does not suppress the influence of the gene *I*. On the contrary other special condition in the male plant does not suppress the development of the anthers, though it suppresses that of the ovary or the action of the gene *I*. I shall call the determination of the condition 'the primary sex determination' in which the gene *Ff* (HERTWIG, G. and P. 1922, WINGE 1931, etc.) play the role.

Though we could not yet prove the existence of the gene (or genes) which concern the development of the anther, the existence of the genes (*I* and *i*) concerning the development of the ovary suggests the presence of the former. The genes *F* and *f* are to predetermine the internal condition in the plant, and then the gene for the development of the sexual organ is to play its role, but the genes themselves exist independently in the different chromosomes as those experiments mentioned above will show: the male parent has the gene for the development of the ovary (gene *I*) and can inherit it to the female offsprings, but the gene does not play the role in the male plant itself. I should say that in the dioecious plants one condition only which leads either to the development of the anther or ovary formation occurs, while in the primary hermaphrodite

plant the condition in which the anther develops comes earlier than that for the development of the ovary or vice versa.

So-called hermaphrodite or intersex plant in *Melandrium album* is the modified male which has the anthers as well as the ovary in the flower, as many investigators already determined genetically as well as cytologically (SHULL 1910, BĚLAŘ l.c.). HERTWIG, G. and P. (l.c.) have distinguished this plant from the primary hermaphrodite as the secondary hermaphrodite. Thus the modified male plant is a mutant derivative of a male plant which is clearly shown by the presence of the XY-chromosome pair. Thus the production of this mutant, the modified male, is to be considered as due to the new formation of the 'removal gene', a gene removing the inhibition of the development of the ovary, the inhibition being due to the influence of the XY-chromosomes (the primary sex determination).

WINGE (1931) considered the genotype of the modified male (WINGE's hermaphrodite without gene *l*, SHULL's genic hermaphrodite) as X_lY ; the gene *f* in the X-chromosome being concerned in the modified male, and he gave the following formula for the segregation in the S_1 generation of the modified male: $-1 X_lX_l(\text{female}) : 2 X_lX (\text{modified male}) : 1 YY (\text{lethal})$. According to this view the gene for modified male (his *f*) must be always single in the modified male plant. But the modified male actually gave in later generation females, modified males as well as true males. Thus the modified male plant ought have had the 'removal gene' in a pair with its allele; consequently these paired genes must have their loci in an autosome pair, not in the X-chromosome.

The facts that the modified male often occurs in company with the entire petals and that it has always abnormal ovary suggest that the corresponding genes have their loci in one and the same chromosome, namely the alleles *Ee* and *Ii* are in linkage. Now I may mention that the gene now concerned is the one which acts in the removal of the inhibition of the ovary formation in the male plants, while it has nothing to do with the anther formation in the female plants, though we call it sometimes the gene for hermaphrodite.

I can not agree also with WINGE's view about the possibility of the YY plant production by selfing the modified male. According to his formula for the segregation in the S_1 generation, we must have two times of the modified males as the female plants in S_1 generation. But my results of the selfed modified males as well as of other investigators' (SHULL 1911, HERTWIG, G. and P. l.c.) show the excess of the female plants as in the case of the crossing of the female and true male as we see in CORRENS's classical experiments (CORRENS, 1918, 1921) on this plant, in which the female plant has only one kind egg cells. Finally in accordance with the results of my experiment and of other investigators, I should

suggest that the macrospores which have Y-chromosomes in the ovaries of the modified male plants are not viable or not able to form embryosac in such a degree as in that having X-chromosome, so that there is no egg cells with Y-chromosome, while there are two kinds of the male gametes as in the cases of the true male; consequently the competition between the male gametes of different constitution may occur in the pistil as in the case when the female plant was pollinated with an excess of the pollen grains, which results in the surplus production of the female plants and none of YY-plants.

Summary

1. The gene concerning the abnormal ovary formation is designated as *i* (imperfect), its dominant allele as *I* which concerns the formation of the normal ovary; the gene concerning the non-lobed or entire petal is designated as *e* (entire), and its dominant allele as *E*, which concerns the bilobed condition of the petal. These two pairs of the genes are linked together with 1/4 recombination value. The linkage group has its locus in an autosome.

2. The presence of the gene concerning the removal of the inhibition of the ovary formation in the modified male plant is suggested; and it is located in an autosome.

3. The possibility of the linkage between the removal gene with *Ii Ee* alleles is considered.

4. It is suggested that the Y-chromosome-bearing macrospore in the modified male plants is not viable, so that there is no egg cell having Y-chromosome, while either kind of microspore having Y- or X-chromosome is viable, as in the case of the true male. Therefore the ratio of the female to the male in the offsprings will be the same as that in the case of the crossing between the normal female and the true male.

5. The chromosome constitution in the nucleus of the modified male plant does not show any difference from that of the true male plants, as far as the morphological observations are concerned as BĚLAŘ maintains. The same thing can be said in regard to the chromosome constitution in the normal and abnormal female plants.

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