Cytogenetical Studies on Ecological Races of *Mecardonia dianthera* (SW) Pennell II. Genetical and breeding behaviour

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As reported in the previous publications, (Kaul 1965, 1966, 1967) the two ecological races (viz. erect and repent forms) of *Mecardonia dianthera* though predominantly selfers are self as well as cross compatible. Also both the forms possess the same chromosome number (i.e. $n = 11$; $2n = 22$) and more or less a similar karyotype (Kaul 1965b). Hence hybridization between the two forms was thought to be possible. In the present paper, results of such an experiment are reported.

Breeding

Hybridization technique, emasculation: Since the flowers of *M. dianthera* are hermaphrodite, emasculation sufficiently in advance of dehiscence of anthers is necessary in order to cross pollinate the flowers. The anthers shed their pollen 1-2 days prior to the opening of corolla. Hence, 4.5-5 mm long buds, that would bloom after about 3 days (i.e. sufficiently prior to dehiscence of the anthers) were emasculated (Fig. 1A).

With a fine forceps, one of the larger calyx lobe was detached from the base (Fig. 1B). The corolla tube thus got exposed. By a sharp blade, an incision was given round the base of corolla taking care not to injure the ovary. A fine longitudinal cut was given to the corolla tube. The corolla was spread a little and very carefully pulled out from the flower and the epipetalous stamens with undehiscent anthers got removed alongwith the corolla. Thus the flowers got emasculated and the ovary exposed (Fig. 1C).

In all the cases, while emasculating the flowers, care was taken not to puncture the anthers. After emasculation was over and before covering the individual flower, the stigma of the emasculated flowers was carefully examined with a hand lens for the presence of any stray pollen grains. Wherever the stigma was found to be contaminated, the flower was rejected. The emasculated flowers were properly protected by covering with butter paper bags (Fig. 2 and 4). The male flowers were also similarly protected with the object of preventing foreign pollen grains from falling on them.

Crossing: In order to effect cross pollination, the pollen grains of the male parents were collected from the dehiscent anthers, in thoroughly sterilized watchglasses, by means of a fine brush. They were dusted on to the receptive

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stigma of the emasculated flower for six successive days, in morning hours, by a fine soft brush (a direct gentle rubbing of the stigma with dehiscent anthers

Figs. 4-5. 4, segregation of erect and repent forms (Some plants bagged for hybridization). Ca. $\times 1/4$. 5, repent forms (pure line). Ca. $\times 1/20$.

caused in injury to it). At the time of receptivity the stigma becomes slightly wet, swollen and fresh green. As pollination and fertilization take place the stigma turns dark green. After complete fertilization of the ovules it becomes black, shrivels up and finally falls off. Though within 4-5 days complete fertilization takes place as indicated by shriveling of stigma, the pollen were dusted on for 6 days, consecutively to ensure a complete cross pollination.

Selfing: For selfing, young unopened flower buds ranging from 3-4 mm in length were bagged

individually till seed formation took place in them. The bags were removed at the time of seed collection.

Details of breeding experiment

Erect form :

- i) One flower, each from 30 randomly selected plants of natural populations of erect form, was selfed by bagging. The seeds produced by selfed capsule were collected separately.
- ii) The progeny raised from the seeds of 28 capsules was found to be of erect plants only. The number of plants raised from the seeds formed

Figs. 1-3. 1, emasculation stages $\times 4$. A, flower bud. b. one sepal removed. C, ovary surrounded by 2 sepals after the removal of corolla tube. d, corolla with epipetalous stamens. 2, demonstrating mode of bagging in repent (1) and erect plants (2). Ca. $\times 1/9$. 3, erect and creeping plants in cultures. Ca. $\times 1/6$.

in each selfed capsule was as follows :

191, 183, 197, 169, 178, 214, 196, 217, 148, 210, 173, 192, 207, 187, 134, 206, 215, 213, 219, 197, 171, 154, 186, 172, 201, 190, 178, 201.

The above progeny was designated as AAs.

iii) The progeny raised from the seeds of remaining 2 selfed capsules exhibited a segregation into erect and repent types. These progeny counts were subjected to chi-square test as follows (see Table 1).

Table 1. Progeny ratio test (Chi-square)

Progeny ratio	Seeds of capsule no. 1		Seeds of capsule no. 2	
	Erect	: Repent	Erect	: Repent
Observed	139	38	122	47
Expected	132	44	126	42
Deviation	7	6	4	5
(Deviation) ²	49	36	16	25
χ^2 value	1.1		0.7	

Chi-square value at 0.05% level of probability for one degree of freedom is 3.841. Since the chi-square values in the table are too low, the assumed 3:1 ratio seems to be correct. The segregated repent progeny was named as aa.

One flower, each of 5 randomly selected AAs, and aa plants, was selfed and the resultant seeds of the selfed capsules were sown to observe the progeny which was as follows :

Progeny raised from	Phenotype of resulting progeny	(No. of plants got from the seeds of each capsule)
AAs ₁	Erect	179, 213, 218, 196, 184
aa	Repent	187, 153, 114, 201, 181

The progeny raised from AAs₁ and aa was labelled as AAs₂ and aas₁, respectively. 5 randomly selected aas₁ and AAs₂ plants were selfed. Direct as well as reciprocal crossing was done between the following lines :

Cross no.	Lines
A	AAs ₂ × aas ₁
B	AAs ₂ × a ₁ a ₁ s ₂
C	aas ₁ × a ₁ a ₁ s ₂

The number and phenotype of the progeny raised from the selfed and crossed lines have been set in Table 2.

Since in cross A and B, the progeny is erect, this character seems to be dominant over repent character. One capsule each of the two above got F₁ plants was selfed and the progeny segregated in ratio of 3:1::Erect:Repent, as tested by chi-square formula (Table 3).

Table 2. Progeny counts of F_1

Progeny raised from	Phenotype of resultant progeny	No. of plants got from the seeds of each capsule	
AAs ₂ (selfed) aas ₁ (selfed)	Erect	192, 201, 178, 171, 157	
	Repent	196, 113, 155, 130, 116	
		Direct	Reciprocal
Cross no. A	Erect	I. 193	174
		II. 197	203
Cross no. B	Erect	I. 186	192
		II. 189	171
Cross no. C	Repent	I. 108	110
		II. 101	102

Table 3. Progeny ratio test of F_2 generation (Chi-square)

F_2 of cross number	Total no. of plants	Observed ratio		Expected ratio (e)		Deviation Expected ratio	χ^2 value
		Erect	Repent	Erect	Repent		
A (Direct), I	169	121	48	126	42	$\frac{133}{126}$	1.13
A (Direct), II	185	136	49	138	46	$\frac{31}{138}$	0.22
A (Reciprocal), I	151	106	45	114	38	$\frac{211}{114}$	1.85
A (Reciprocal), II	149	118	31	111	37	$\frac{157}{111}$	1.41
B (Direct), I	157	112	45	117	39	$\frac{73}{117}$	0.62
B (Direct), II	165	114	51	123	41	$\frac{381}{123}$	3.09
B (Reciprocal), I	193	137	56	144	48	$\frac{241}{144}$	1.67
B (Reciprocal), II	181	127	54	135	45	$\frac{307}{135}$	2.27
C (Direct), I	129	—	129				
C (Direct), II	153	—	153				
C (Reciprocal), I	167	—	167				
D (Reciprocal), II	116	—	116				

Repent form :

i) One flower, each from 30 randomly selected plants of natural populations of repent form, was selfed by bagging. The seeds produced by selfed capsule were collected separately.

The progeny raised from the above got selfed seeds was entirely repent and the number of plants got from each selfed capsule were as follows :

174, 181, 138, 191, 118, 121, 176, 161, 132, 154, 120, 173, 136, 152, 137, 169, 163, 172, 126, 122, 153, 136, 180, 126, 116, 116, 124, 117, 160, 123.

The above progeny was designated as $a_1a_1s_1$. One flower bud each of the above five randomly selected selfed plants was again selfed, the resulting seeds of each capsule were collected separately, and sown again. The resulting progeny was found to be uniformly of repent type and number of plants was 154, 117, 140, 100, 145.

Fig. 6. Out line of the breeding experiment.

The above progeny was designated as $a_1a_1s_2$. This line was utilised for cross breeding (Cross B and C; Table 2). 5 randomly selected $a_1a_1s_2$ plants were reselfed and progeny raised from these selfed seeds was found to be of repent type only. This progeny was labelled as $a_1a_1s_3$ and number of the plants was 97, 120, 162, 103, 146.

The outline of the breeding experiment performed presently is graphically represented in Fig. 6.

Genetic explanation

Two, phenotypically different forms have been found to be growing in nature, in *M. dianthera*. (Kaul 1965, 1967a-c). One is erect and the other is repent. When erect and repent plants (that were made homozygous by selfing) were crossed, the hybrid progeny was erect. Thus, erect factor is dominant over repent factor. These erect hybrids upon selfing segregate in a ratio of 3:1::erect:repent forms, in F_2 . Since, only two phenotypic classes are produced in F_2 the F_1 hybrid appears to be a monohybrid and the allelic differences seems to lie at one locus.

Fig. 7. Genetics of breeding experiments.

Thus, if the erect character is represented by factor R and the repent by r, the erect plants may have RR or Rr factors while the repent plants will have rr factors only. It means that the erect plant can be homozygous as well as heterozygous for these characters while the repent plants are only homozygous. This assignment of the genotype, is confirmed by the following findings also:

- i) The repent plants do not segregate upon selfing. So they are homozygous for factor r.

ii) 2 erect plants, out of 30 randomly selected phenotypically erect plants, segregate in a ratio of 3:1 :: erect:repent while the rest 28 breed true for the erect character. This segregation of the 2 plants shows that they were heterozygous while the rest 28 were homozygous for the character. Thus, the factors in the erect plants can be RR or Rr and in the repent form only rr.

Based on this assumption, a genetic explanation of the breeding behaviour of the two forms of *M. dianthera* has been presented in Fig. 7.

Discussions

Ecads though represent genetically adaptive plasticity of development and ecotype is basically an environmental category, their adaptive nature is to be proved by experimentation rather than to recognise them by morphological and distributional evidence alone. Davis and Heywood (1963) have stressed to define ecotypes genetically. But majority of the studies on ecotypic differentiation on plant species are based upon transplant experimentation and habitat differentiation. No such study has been supplemented by a knowledge of cytological set up and genetical background of the ecological races.

Such a study helped in the isolation of cytotypes from ecotypes in *Artemisia vulgaris* (Kaul 1965a) and *Ageratum conyzoides* (Kaul 1965b) which otherwise in the latter species differed in morphology, ecological distribution, cytogenetics and physiology (Kaul 1965b, 1966). In *Mecardonia dianthera* such a study has yielded interesting results.

While in *A. conyzoides*, the two photoperiodic ecotypes differ in karyotype and pairing relationships etc. (Kaul 1965b), in *M. dianthera* the erect and repent forms do not seem to differ at cytological level (unpublished). By breeding experimentation, it is found that the phenotypically repent form is homozygous for recessive factors (rr). However, the phenotypically erect form may have the pure dominant (RR) or the hybrid dominant (Rr) factors for erectness and accordingly may be homozygous or heterozygous for the dominant factor (R). From culture and transplant experimentation (Kaul 1967a, b) and studies upon ecological distribution (Kaul 1967c), it is found that the repent form is an obligate calcifuge and the erect form is a facultative calcicole. Thus the erect and repent forms which are genetically adapted, heritable, ecological populations, represents the two ecotypes of the species *M. dianthera*. Since the fundamental ecological difference between the two ecotypes lies in their tolerance and adaptability to soil calcium (See Kaul 1967b), these are regarded as the edaphic ecotypes.

Origin

The origin of these two ecotypes is difficult to state at this stage. However, it may be possible that the erect ecotypes originated from the repent ecotype by the mutation of r factor to its dominant allele R. The mutant plant

because of selfing (for the plants are predominantly selfers, Kaul unpublished) segregated into progenies with RR, Rr and rr (in 1:2:1 ratio) factors. From the non-calcareous soils, plants with RR and Rr factors (morphologically erect) started migration, invasion and colonization upon calcareous soils by seed dispersal and because of a subsequent successful germination and a good growth of the resulting seedlings on the calcareous soils as was also found in culture experiments (Kaul 1967a, b).

Since most of the mutant genes are recessive, mutation of a dominant gene to recessive is easy to visualise than the reverse. Hence, the erect ecotype by mutation of the factor R to its recessive allele r, may have produced repent ecotypes after selfing of the mutant (hybrid) plant and subsequent segregation of the resulting progeny. The repent ecotype thus evolved being incapable of growing upon calcareous soils mainly because of meagre seed germination, high seedling mortality, depauperate plant growth, susceptibility to interspecific competition, and a very little viability of the resulting seeds in calcareous soils, as found by culture (Kaul 1965b, 1967b) experiments, got restricted to non-calcareous soils.

The erect ecotype though a facultative calcicole, hence, capable of growing in calcareous as well as upon non-calcareous soils, is confined in nature to moist and protected areas only. On the other hand, the repent ecotype, an obligate calcifuge, grows well upon dry and hard soils and on exposed areas (forming compact ecads therein) in addition to its luxuriant growth in moist and shady habitats. Hence, the repent ecotype exhibits greater morphological plasticity, phenotypic variability and possess a wider ecological amplitude than that of the erect ecotype.

Conclusion

Ecotypic differences recorded in outbreeders are clinal in nature for the ecotypic variations in them are continuous, following a clinal pattern and often of complex nature. Since breeding system affects gene flow from neighbouring populations (Kaul 1965b), inbreeders like *M. dianthera* produce discrete edaphic ecotypic units.

Whether the erect ecotype gave rise to the repent ecotype or vice versa ; the problem remains open for future investigations. Currently, the author is trying to induce mutations in these ecotypes. This may help to trace their origin.

Summary

Two, morphologically different forms of *Mecardonia dianthera* viz. erect and repent were found to be growing in nature. By breeding experiments it was found that the erect character (R) is dominant over repent character (r) and the allelic difference seems to lie at one locus. While the erect plants are either homozygous (RR) or heterozygous (Rr), the repent plants possess

only the homozygous recessive alleles (rr). Since the two forms differ in ecological distribution, in habitat preference (the erect form being facultative calcicole and repent form an obligate calcifuge) and genetically, these appear to be the two edaphic ecotypes of *M. dianthera*. Their probable origin is also discussed.

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