

REPRODUCTIVE VERSATILITY IN *RUBUS*

I. MORPHOLOGY AND INHERITANCE

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INTRODUCTION

THE results of breeding experiments with *Rubus* at the John Innes Horticultural Institution have been published from time to time (Crane & Darlington, 1927-32; Crane & Lawrence, 1931; Crane, 1936; Lewis, 1939). The object of the present paper is to describe anomalies of breeding behaviour in the genus which have come to light in the course of the investigations.

In *Rubus* the basic chromosome number is 7, and the species we have used range from diploids, $2n=14$, to octoploids, $2n=56$. In the indigenous European species, which we have mainly used, diploids are rare. The raspberry, *R. idaeus*, is mainly diploid; but of thirty British species of blackberries examined during the course of the investigations only one, *R. rusticanus*, the common hedgerow bramble of southern England, is diploid.

In the early years of the experiments assistance in the breeding work and chromosome determinations of species and seedlings were made by Dr A. A. Moffett, Dr M. M. Richardson and Dr A. C. Fabergé. During the past two years cytological and also embryological studies have been carried out by Dr P. T. Thomas, who describes the results of his investigations in the accompanying paper.

REPRODUCTIVE BEHAVIOUR

(a) *Diploid species*

The results we have obtained in breeding experiments with *R. rusticanus* and its varieties *inermis* and *albus* are summarized in Table I.

The dominance of the prickled character and black fruit colour in the F_1 families 25/31 and 26/31 raised from reciprocal crosses between the varieties *inermis* and *albus* shows that reproduction in these families has been strictly sexual. The F_2 and backcrossed families raised from these varieties have not yet produced flowers or fruit, and consequently only the prickled character has so far been recorded. In these families there is

TABLE I

	♂ parent chromo- some no. $2n$	Fruit colour		Prickles		Progeny chromo- some no. $2n$	
		Black	Amber	Present	Absent		
<i>R. rusticanus</i>	14	+	.	+	.	14	
<i>R. rusticanus inermis</i>	14	+	.	.	+	14	
<i>R. rusticanus albus*</i>	14	.	+	+	.	14	
Fam. no.	Parents						
25/31	<i>inermis</i> × <i>albus</i>	14	30	-	30	-	14
26/31	<i>albus</i> × <i>inermis</i>	14	30	-	30	-	14
33/35	25-5/31 × 25-4/31	14	-	-	18	12	14
35/35	25-5/31 × 25-4/31	14	-	-	145	57	14
42/36	<i>inermis</i> × 25-1/31	14	-	-	88	105	14
43/36	<i>inermis</i> × 26-6/31	14	-	-	65	111	14
44/36	<i>albus</i> × 25-17/31	14	-	-	85	-	14
45/36	<i>albus</i> × 26-5/31	14	-	-	18	-	14
11/12	<i>inermis</i> × <i>R. laciniatus</i>	28	1	-	1	-	21
1/22	<i>inermis</i> × <i>R. thyrsiger</i>	28	1	-	4	-	(3) 21 (1) 28

* For convenience this form with yellow or amber coloured fruits is referred to throughout this paper as *R. rusticanus albus*.

an excess of the prickless recessive plants, especially in the backcrossed families 42/36 and 43/36, but there is no reason to think that this is due to a departure from normal sexual reproduction. The cytology of the few plants raised from crossing *inermis* with *R. laciniatus* and *R. thyrsiger* is also in agreement with sexual reproduction. In an earlier publication (Crane & Darlington, 1927) it was shown that the exceptional plant with 28 chromosomes obtained from *inermis* crossed *thyrsiger* arose from a unreduced germ cell of the maternal parent.

We have crossed *R. rusticanus inermis* with a number of other species of *Rubus* but no viable seeds were formed.

The plants of *R. rusticanus inermis* and *albus* used in these experiments are self-sterile. The former has been repeatedly self-pollinated but no fruits or seeds formed. In family 25/31 (*inermis* × *albus*) twenty-six

plants were self-pollinated and tested for self-sterility. Fifteen plants entirely failed (self-sterile), eleven set occasional drupels (slightly self-fertile). In the reciprocal cross, family 26/31, twenty-four plants were tested. Twenty-one were self-sterile, two slightly self-fertile and one self-fertile. One example of cross-sterility was found in this family.

Rubus idaeus, $2n=14$. The data relating to this species are very considerable; they involve numerous families and several thousand individuals, and it is clear from the mode of inheritance of the various characters studied that reproduction in this species is also strictly sexual (see Crane & Lawrence, 1931; Lewis, 1939).

In both *R. rusticanus* and *R. idaeus* the occurrence and functioning of unreduced germ cells is not uncommon. For example, the Veitchberry ($2n=28$) and the mahdiberry ($2n=21$) were both raised from crossing *R. rusticanus* with *R. idaeus*, and the John Innes berry ($2n=28$) arose from non-reduction on the part of its female parent, *R. rusticanus*. The tetraploid forms of *R. idaeus* such as Belle de Fontenay, Merveille Rouge, Everbearing, Hailshamberry, etc., are autotetraploids and have probably arisen from the functioning of unreduced germ cells. These forms are comparatively recent and appear to have originated from diploids in the last eighty years. Autotriploid raspberries have also arisen from diploids in breeding experiments at Merton (Lewis, 1940). These examples demonstrate one of the methods by which polyploids originate.

Incidentally, I know of no diploid hybrid the result of hybridization between *R. rusticanus* and *R. idaeus*. It therefore seems that it is easier for fertilization to occur in conjunction with non-reduction, and possibly it is only when an unreduced germ cell, from one or both diploid parents, takes part in fertilization, or when a polyploid form of *R. idaeus* is one of the parents, that viable seeds are formed when these species are intercrossed. So far, attempts I have made to intercross these diploid species have entirely failed.

(b) *Polyploid species*

The results obtained from breeding experiments with *R. vitifolius*, the loganberry *R. loganobaccus*, and with diploid and tetraploid forms of *R. idaeus* are summarized in Table II.

The chromosome numbers ($2n=49$) of the plants in families 1/34 and 2/34 raised from reciprocal crosses between *R. vitifolius* ($2n=56$) and *R. loganobaccus* ($2n=42$) shows that they have arisen from normal sexual reproduction. Similarly, the chromosome number ($2n=35$) of the plants in family 3/34 raised from *R. vitifolius* ($2n=56$) crossed *R. idaeus* ($2n=14$)

shows that they are sexually reproduced. The morphological characters of these families detailed in Table III also agree with a strictly sexual origin.

TABLE II

Family no.	Parents			F_1				
		Sex	Chromosome no. $2n$	Sex			Chromosome no. $2n$	Mode of reproduction
				♀	♂	♂		
4/34	<i>R. vitifolius</i>	♀	56	26	5	35	56	? Sexual
	×							
2/34	<i>R. vitifolius</i>	♂	56	24	21	7	49	Sexual
	×							
1/34	Loganberry	♂	42	—	27	2	49	Sexual
	×							
3/34	<i>R. vitifolius</i>	♂	56	21	19	—	35	Sexual
	×							
	<i>R. idaeus*</i>	♂	14					
	×							
	<i>R. vitifolius</i>	♀	56					
	×							
	<i>R. idaeus*</i>	♂	28					
	×							
	<i>R. vitifolius</i>	—	—					
	×							
	<i>R. idaeus*</i>	—	—					
	×							
	<i>R. vitifolius</i>	—	—					
	×							
7/34	<i>R. idaeus*</i>	—	—	—	1	—	50	?
	×							
	<i>R. vitifolius</i>	—	—					
	×							
	<i>R. idaeus*</i>	—	—					
	×							
15/13	<i>R. vitifolius</i>	—	—	4	—	3	56	Asexual
	×							
	<i>R. idaeus*</i>	—	—					
	×							
31/37	<i>R. vitifolius</i>	—	—	1	—	—	35	?
	×							
15/13	Loganberry (selfed)	♂	42	—	53	—	42	? Sexual
31/37	7/34 (17) (selfed)	♂	42	—	45	—	? 42	? Sexual

* The diploid form of *R. idaeus* used in the experiments was the variety Superlative, and the tetraploid the variety Hailshamberry.

In family 7/34 raised from *R. vitifolius* ($2n=56$) crossed to a tetraploid form of *R. idaeus* ($2n=28$) the plants fall into two groups, with 56, and with less than 56, chromosomes. The 56 chromosome plants are obviously of asexual (maternal) origin. Morphologically they are like the maternal parent *R. vitifolius* and have the same number of chromosomes. The ten plants with 42 chromosomes are evidently sexually reproduced. They

have the chromosome number expected from the cross between an octoploid and a tetraploid, and their morphology given in Table III also supports this view. The female organs of all are well developed; six are fully hermaphrodite in structure, but in the remaining four plants the male organs are not completely developed. The three plants with 43, 44, and 50 chromosomes also appear to be of sexual origin; probably as the result of union of normal reduced germ-cells of *R. vitifolius* ($n=28$) with unbalanced germ-cells from the tetraploid male parent with extra chromosomes. The plant with 35 chromosomes shows no trace of *R. idaeus*, the male parent, and therefore appears to be of asexual origin.

Segregation of well-defined genetic characters occurs within *R. vitifolius*. In this species the flowers are prevailingly unisexual, the large majority of plants being either entirely male or entirely female (see

TABLE III

	Chromosomes		Leaves
	No.	Proportion derived from raspberry	
Raspberry × raspberry	$2x=14$	All	Five distinct leaflets
Raspberry × raspberry	$4x=28$	All	" "
<i>R. vitifolius</i> ♀ × <i>R. vitifolius</i> ♂	$8x=56$	0	Three-lobed
<i>R. vitifolius</i> × loganberry	$7x=49$	1/7	Three-lobed but more deeply incised than <i>vitifolius</i>
<i>R. vitifolius</i> × raspberry $2x$	$5x=35$	1/5	Three-lobed but more deeply incised than previous family
<i>R. vitifolius</i> × raspberry $4x$	$8x=56$	0	Three-lobed
	$6x=42$	1/3	Five distinct leaflets
Loganberry	$6x=42$	1/3	Five distinct leaflets

family 4/34, Table II); in a few plants there is a slight development towards hermaphroditism, one sex being well developed and the other developed only to a slight degree. Such flowers are not, however, functionally hermaphrodite. Variation within the species also occurs with respect to pigmentation and the amount of waxy bloom on the growth, some being pruinose, others glaucous; and in other minor respects. The asexually reproduced plants in family 7/34 show segregation of characters within the limits of the variation found in the maternal species. As shown in Table II, some have female and others male flowers. They also vary in pigmentation and waxiness.

The leaves of *R. vitifolius* are three-lobed, and, as previously described, the sexes are borne separately, some plants being male and others female. In the raspberry, both diploid and tetraploid, and in the loganberry, the leaves are pinnate, having five leaflets when fully developed; and the

flowers are hermaphrodite. As shown in Table III, in the families raised from inter-crossing these *Rubi* there is a gradation from the three-lobed leaf to the pinnate leaf with five distinct leaflets, which is directly correlated with the proportion of chromosomes derived from the pinnate-leaved raspberry.

Gustafsson (1930-S) citing Petersen (1921) states that "all American species belonging to the subgenus *Erubatus* are sexual", but the behaviour of *R. vitifolius*, a species in this subgenus, shows that this generalization is not universally valid. As shown in Table II, when this species is crossed with the loganberry or the diploid raspberry reproduction is strictly sexual, but when crossed with the tetraploid raspberry a proportion of the offspring is produced sexually and a proportion asexually. It is

TABLE IV

Family	Parents, all tetraploids, $2n=28$	Sexual	Offspring, presumed asexual on cytological grounds	Asexual
7/32	<i>R. thyrsiger</i> , selfed	—	284	—
17/32	<i>R. thyrsiger</i> × <i>R. nitidioides</i>	2	—	32
5/32	<i>R. nitidioides</i> × <i>R. thyrsiger</i>	1	—	9
15/33	<i>R. thyrsiger</i> × <i>R. calvatus</i>	5	—	6
10/32	<i>R. calvatus</i> × <i>R. thyrsiger</i>	6	—	14
16/33	<i>R. thyrsiger</i> × <i>R. procerus</i>	2	—	4
34/37	<i>R. thyrsiger</i> × <i>R. gratus</i>	5	—	33
12/32	<i>R. calvatus</i> , selfed	—	73	—
13/33	<i>R. calvatus</i> × <i>R. procerus</i>	3	—	5
9/32	<i>R. calvatus</i> × (<i>R. rusticanus</i> × <i>R. thyrsiger</i>)	2	—	6
25/35	<i>R. Schlechtendalii</i> × <i>R. procerus</i>	7	—	2
27/32	<i>R. Schlechtendalii</i> × <i>R. thyrsiger</i>	9	—	8
6/32	<i>R. nitidioides</i> , selfed	—	28	—
30/37	<i>R. nitidioides</i> × <i>R. Schlechtendalii</i>	4	—	41

therefore evident that in *R. vitifolius* the form of reproduction depends on the male used in pollination.

The breeding experiments with *R. vitifolius* and the raspberry were originally undertaken with a view to elucidating the origin of the loganberry. It may be recalled that the loganberry was originally described as a natural hybrid. The parents were believed to be the Aughinbaugh, a form of *R. vitifolius* and a raspberry (see Bailey, 1923). Subsequently, for various reasons, this came to be disputed (Darrow, 1933; Hedrick *et al.* 1925). These investigations support the originally postulated hybrid origin of the loganberry. As shown in Tables II and III the cytology, genetics and morphology of the hexaploid hybrids in the families 7/34 and 31/37 substantiates the above view. Full details will be published in a later paper.

Table IV summarizes the results with respect to sexual and non-sexual

reproduction obtained in families from crosses between a number of European species of *Rubus*. Among the different crosses made the proportion of sexually produced offspring varies considerably. In some of the selfed families, detailed in Table IV, the offspring were very uniform, but in others segregation occurred. For example in family 7/32 *R. thyrsiger* (selfed) the leaflets of the parent plant were comparatively narrow and spaced and the inflorescence very long and lax, whilst about 8 % of the selfed seedlings had much broader and overlapping leaflets and a shorter and more compact inflorescence. From the behaviour of family 7/34, described earlier in this paper, it is evident that segregation is not necessarily a criterion of sexual reproduction, consequently pending embryological investigations it is not possible to say with certainty what form of reproduction, whether sexual or non-sexual, occurs in these selfed families.

In family 7/32 three plants occurred with slender growth and small leaves. They were also comparatively infertile. It was found that these plants had lost a chromosome, their number being $2n=27$. Apparently such forms are found in nature. They correspond with the systematists' var. *microphyllus*.

In some of the crossed families the asexual offspring were uniform and identical with their female parent, but in others segregation occurred. For example, in family 17/33 *R. thyrsiger* crossed *R. nitidioides* one of the asexual plants had broad overlapping leaflets and a compact inflorescence similar to the segregates which appeared in the selfed family from *R. thyrsiger*. Another example of segregation in families asexually reproduced occurred in family 5/32, *R. nitidioides* crossed *R. thyrsiger*. One of the nine asexual plants in this family develops leaves with seven leaflets instead of the normal five leaflets of *R. nitidioides*, it also has much larger flowers and a more compact inflorescence than its maternal parent. When selfed this plant breeds true. Alternatively since such a seven-leaflet type did not appear in the selfed family raised from *R. nitidioides* it is possible that its occurrence in family 5/32 may be due to a mutation, but it seems more probable that the first view is correct.

Lidforss (1914) described many cases of false and true hybrids in F_1 families raised from interspecific crosses in *Rubus*, and states that the false hybrids, i.e. the apomictic material offspring, were all alike. However, as I have described in this paper, in some of the families we have raised segregation occurred among the apomictic offspring.

PARTHENOGENESIS

During the course of these breeding investigations with *Rubus* a number of haploid plants have occurred. For example, a hexaploid plant we obtained under the name *R. Borreri*¹ gave, when selfed, a number of plants which were triploid or approximately so, their chromosome number varying from $2n=30$ to $2n=32$. Haploids have also occurred in breeding work with certain cultivated forms of *Rubus*. The Veitchberry ($2n=28$) crossed with the raspberry ($2n=14$) and with *R. rusticanus* ($2n=14$) gave families which were mostly triploids ($2n=21$) but a small proportion were diploids ($2n=14$).

PSEUDOGAMY

In four of the species used in the experiments, which have given asexual plants when pollinated with other species, a considerable number of flowers have been emasculated and isolated and left without pollination, but in no case did fruits or seeds develop. It is therefore evident that development is pseudogamous, pollination being essential for apomictic reproduction. This is in agreement with the findings of Gustafsson (1930) and Darrow & Waldo (1933).

PARTEENOCARPY

R. procerus, when selfed, set fruit freely, but no viable seeds developed. Externally the fruits, including the endocarp, appeared to be normal, but no true seeds were formed. Self-pollination is, however, necessary for the formation of these parthenocarpic fruits, as flowers of the species when emasculated and left unpollinated entirely fail. Natural seed of this species, presumably resulting from cross-pollination, gave plants identical with the female species, *R. procerus*.

SUMMARY OF REPRODUCTIVE TYPES

The genetic results show that the types of reproduction in *Rubus* which have occurred in these experiments can be classified as follows:

- (1) Sexual reproduction (general in diploid species).
- (2) Non-reduction at meiosis on female, male or both sides (*R. rusticanus*, *R. idaeus*).

¹ This plant of *R. Borreri* was exceptional, all other plants of *R. Borreri* examined were tetraploid ($2n=28$). Presumably the hexaploid form arose from the tetraploid by non-reduction.

(3)¹ Apomixis with segregation, presumably diploid parthenogenesis (*R. vitifolius*, *R. thyrsiger*).

(4) Apomixis without segregation, presumably apospory (*R. calvatus*).

(5) Haploid parthenogenesis (*R. Borreri*, Veitchberry).

At the present stage of the investigations it is not possible to say whether the absence of segregation in class 4 is simply due to homozygosity or to exclusive aposporic reproduction which does not allow for segregation. There appears however to be a fundamental difference in the reproductive behaviour of classes 3 and 4, and this view is supported by the cytological and embryological studies of my colleague Dr P. T. Thomas as described in the following paper (pp. 122-3).

The results of the breeding experiments with diploid and polyploid species detailed in this paper are significant in view of the difficult taxonomy of *Rubus*. They show not only how new forms and species can arise, but also how they are able to maintain themselves in nature. Of the numerous species and micro-species of *Rubus*, many are evidently clones and subclones, produced by segregation or mutation, or both, and maintained by apomixis.

SUMMARY

1. The reproductive behaviour of a number of species and varieties of *Rubus* has been investigated.

2. Diploid species always behave sexually. Occasional unreduced germ-cells occur and take part in fertilization, giving rise to polyploid forms.

3. In polyploid forms and species reproduction may be entirely sexual, entirely non-sexual or partly sexual and partly non-sexual (apomictic).

4. Polyploid species vary in the degree to which apomixis is developed, and a particular species may show a variation in reproductive behaviour depending on the species used as male in cross-pollination.

5. Segregation has been found to occur within non-sexual offspring. Hence test crosses, in conjunction with cytological and embryological studies, are necessary to determine the precise mode of reproduction.

6. The results are discussed in relation to the difficult taxonomy of *Rubus*.

¹ The term apomixis is used here in the sense that there has been no intervention of the male germ-cells in reproduction. Alternatively in these cases reproduction may be by "automixis" (see Thomas, p. 123).

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