Breeding Systems in tetraploid Rubus species

By G. J. DOWRICK

Wye College, University of London, nr. Ashford, Kent

1. INTRODUCTION

It is now well established that the genus *Rubus* is particularly versatile in its reproductive behaviour. Thus diploid species are entirely sexual while many of the polyploid species are either facultative or obligate apomicts. Some polyploids are sub-sexual while others produce seed only after automixis (i.e. fusion of haploid nuclei within the embryo-sac). The extensive studies of Lidforss (1914), Gustafsson (1943, 1946) and Einset (1947, 1951) have shown conclusively that apomictic forms are pseudogamous and these authors have suggested that apomictic embryo-sacs are either aposporous or diplosporous in origin. Aposporic embryo-sacs are formed following a series of mitotic divisions; diplosporic sacs arise after restitution at the first division of meiosis, during which there is a limited amount of crossing over. The genetic consequences of the two processes are different but both result in the formation of unreduced embryo-sacs.

Evidence for the formation of such embryo-sacs in *Rubus* has been based largely on the maternal-like appearance of seedlings from a large number of interspecific crosses and from chromosome counts of seedlings. Whilst such evidence is sufficient to substantiate the apomictic nature of the species it is not conclusive proof of either apospory or diplospory. Proof could come only from cytological observations. Recently there have been reports that some obligate apomicts, e.g. *R. procerus*, produce only reduced, sexual embryo-sacs (Markarian & Olmo, 1959). Such species could not therefore be aposporous, as has previously been assumed.

In this account the breeding behaviour of three tetraploid *Rubus* species ($2n = 28$) will be considered and a comparison made with the diploids *R. tomentosus* and *R. ulmifolius*. One of these tetraploid species, *R. caesius* is an obligate apomict producing, so it is claimed, only unreduced aposporic and diplosporic embryo-sacs (Lidforss, 1914). Another, *R. laciniatus* is facultatively apomictic and might therefore be expected to produce both sexual and either aposporic or diplosporic embryo-sacs. The third species, *R. calvatus* has not been investigated previously. Evidence will be presented to show that the breeding behaviour of these species is not dependent on the formation of unreduced embryo-sacs. Thus apomixis in these three species is not a consequence of a breakdown in meiosis.

2. MATERIALS AND METHODS

Chromosome counts were made from root-tips fixed in acetic alcohol after pretreatment with α bromo-naphthalene for 2 hours. The roots were stained by the

R

feulgen technique for 3 hours after hydrolysis in N. HCl at 60°C. for 20 min. Phase contrast microscopy was used.

For embryological studies flowers, with sepals and petals removed, were fixed in Newcomer's fixative—6 pts. isopropyl alcohol, 3 pts. propionic acid, 1 pt. ether, 1 pt. acetone and 1 pt. dioxan. After fixation they were left in 70% alcohol for at least two weeks prior to embedding in wax. Before microtoming the wax blocks were immersed in water for 24 hours—this greatly facilitated ribboning. Sections were cut at 10–22μ, according to the age of the embryo-sac and stained in crystal violet.

The following species were used as pollinating parents in the crosses to be described.

2x	3x	4x	5x
R. tomentosus ulmifolius	R. crataegifolius	R. caesius calvatus laciniatus	R. thyrsoideus

| 6x	7x	8x	
R. fragrans	R. dasyphylloides	R. platyphyllus	

3. EMBRYO-SAC DEVELOPMENT

Carpels, in the five species studied, contain two anatropous, crassinucellate, unitegmic ovules, one above the other. The upper ovule invariably collapsed during development and will not be considered further. In all five species some ovules aborted at various stages but the proportion varied in different flowers of any one species.

Embryo-sac development in the three tetraploid species is identical not only to each other but also to the two diploid species.

Details of the general development of the nucellus, integuments etc. were found to correspond with those reported previously by Pratt & Einset (1955). Frequently more than one, and usually two, megaspore mother cells were present. Their nuclei were always large, stained densely and were easily distinguished. Only one normally developed to maturity, the others degenerating as in Fig. 1a. Various stages of prophase of meiosis were seen. Chromosome pairing was regular, usually resulting in seven bivalents in diploid species and fourteen in the tetraploids. In the latter however, univalents and associations of more than two chromosomes were occasionally seen (Plate 1d). Bivalents had either one or two chiasmata. The metaphase plate was small, frequently occupying only a small proportion of the cell diameter, consequently the bivalents tended to be bunched together. This, together with the short anaphase spindle, occasionally resulted in abnormal chromosome separation.

The second meiotic division followed a normal course resulting in the formation of a linear tetrad. No evidence for the formation of T-shaped tetrads, as seen by
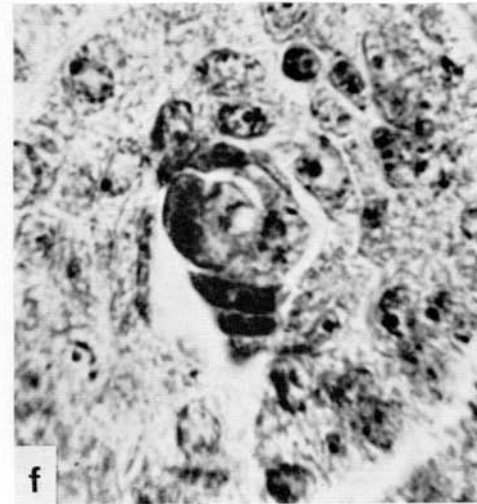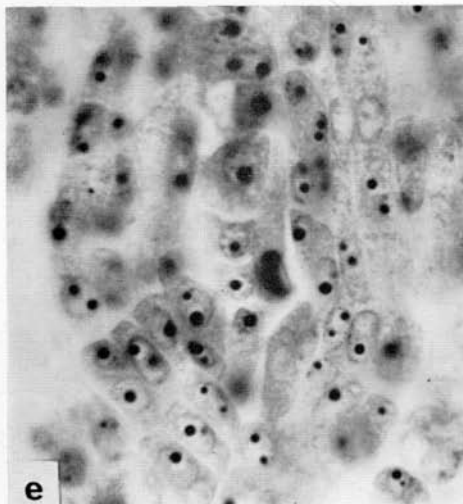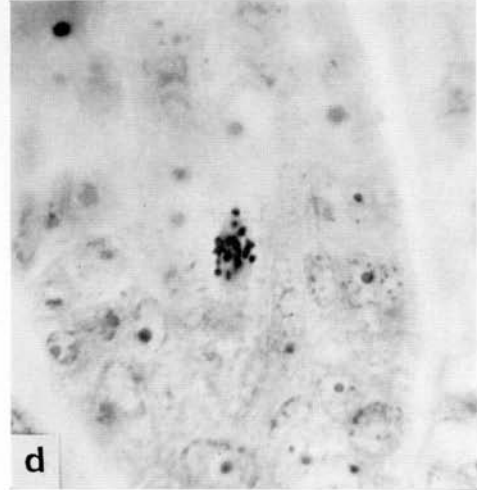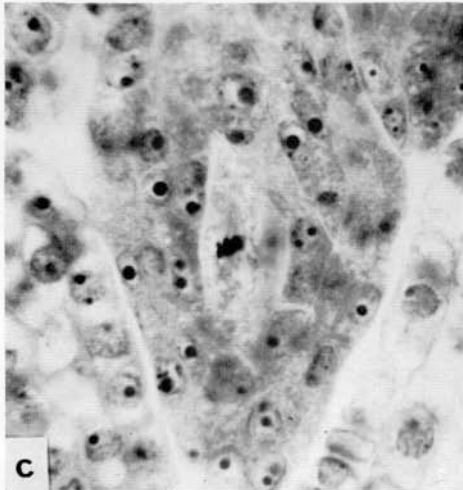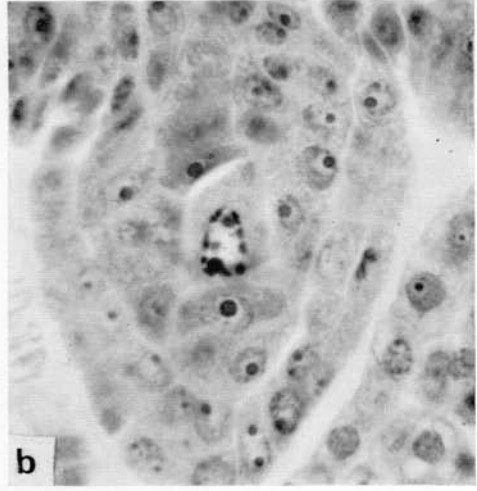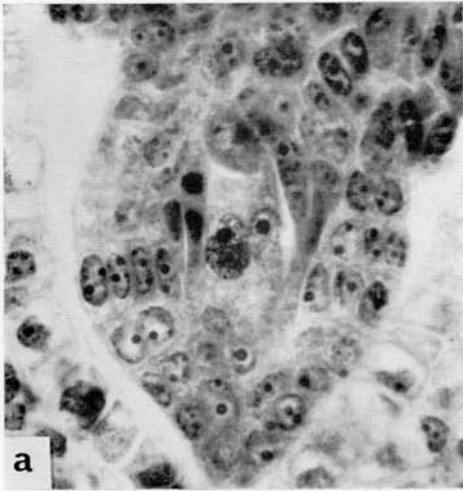
Megasporogenesis. (a) and (b) *R. caesius*, zygotene and diakinesis; (c) and (d) *R. laciniatus* M.I.; (e) *R. caesius*, early tetrad; (f) *R. laciniatus*, late tetrad. × 500.

G. J. DOWRICK
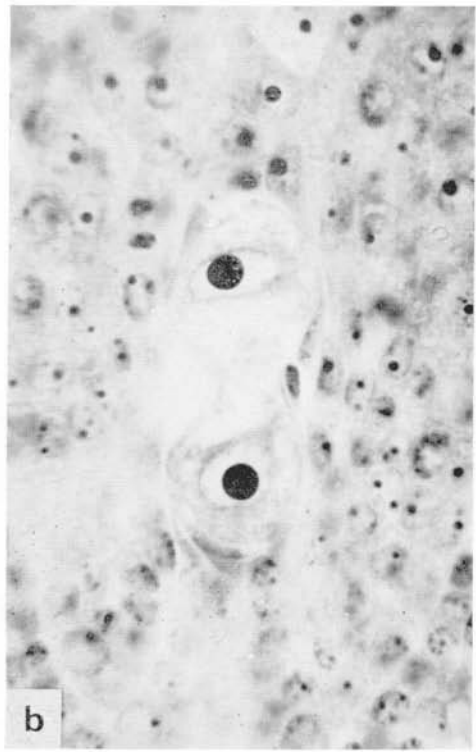
Embryo-sac formation. (*a*) *R. laciniatus*, prophase of 1st mitotic division showing reduced chromosome number; (*b*), (*c*), (*d*) *R. caesius* binucleate E.S.; anaphase of 2nd mitotic division; mature E.S. × 500.

G. J. DOWRICK

Pratt & Einset, was found. In all five species four megaspores of reduced chromosome number were therefore formed after a normal meiosis (Fig. 1 and Plate 1).

The embryo-sac itself was formed from the chalazal megaspore which regularly underwent three mitotic divisions to give rise to an eight nucleate embryo-sac of the *Polygonum* type (Fig. 2h). Details of all stages of development were found, with the exception of the third mitotic division (Fig. 2 and Plate II).
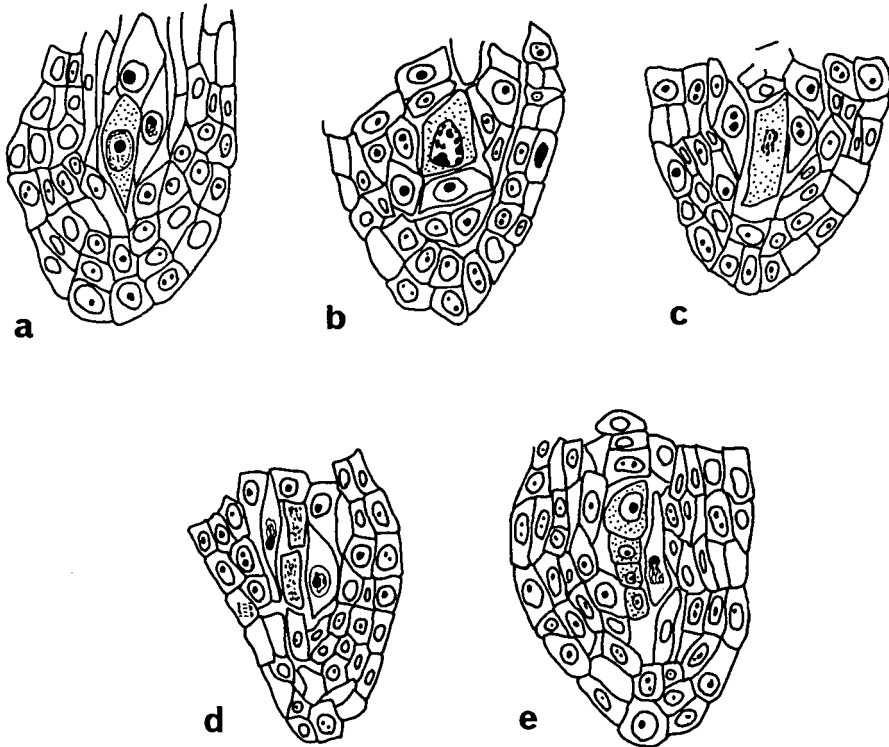

Fig. 1. Megasporogenesis in *R. caesius*. (*a*) Megaspore mother cell surrounded by other degenerating M.M.Cs.; (*b*) Diakinesis; (*c*) M.I.; (*d*) A II; (*e*) Tetrad with chalazal cell enlarging. × 500.

The antipodal cells of the mature embryo-sac usually degenerate prior to pollination. The cells at the mycropylor end, and in particular the egg cell, become highly vacuolated. The two polar nuclei, in the centre of the embryo-sac, remain separate. Though they may lie close to each other there was no evidence that they fused prior to fertilization. It is suggested that fusion does not in fact occur. The two polar nuclei and the second generative nucleus of the pollen grain enter mitosis together and have a common metaphase plate so that, in a diploid species, two triploid endosperm nuclei are formed. A single primary endosperm nucleus is not formed. This process is similar to that previously reported in multinucleate pollen grains of *Pyrus communis* (var. Beurré Bedford) (Dowrick, 1959).
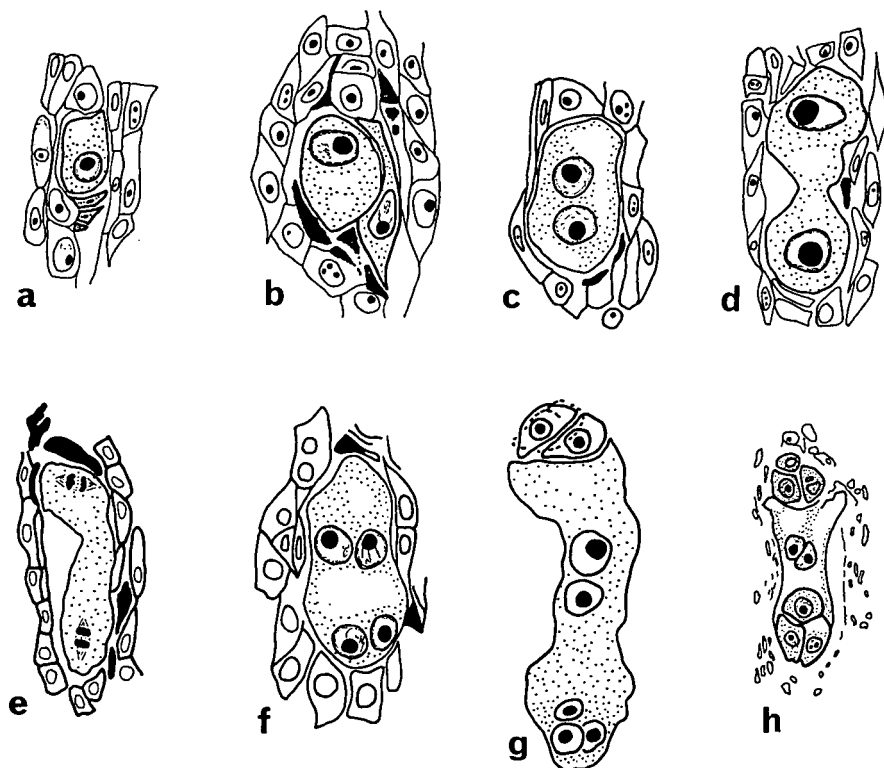
Fig. 2. Embryo-sac formation in *R. caesius*. (*a*) Tetrad; (*b*) enlarging chalazal mega-spore; (*c*) and (*d*) binucleate E.S.; (*e*) anaphase of 2nd mitotic division; (*f*) 4-nucleate E.S.; (*g*) and (*h*) mature 8-nucleate E.S. (*a*)–(*g*) × 550, (*h*) × 400.

Embryo development proceeds, first by the formation of a short pro-embryo of 3–4 cells with subsequent rapid division of the apical cell. Endosperm development, which is at first non-cellular, occurs concurrently.

4. INTERSPECIFIC HYBRIDS

Crosses have been made in which the three tetraploid species were used as the female parents. Various species, differing in degrees of ploidy, were used as polli-nating parents. Details of the crosses and results obtained are shown in Tables 1 and 2.

It will be seen that the breeding behaviour of the three species is not identical. With the exception of two haploids all seedlings from *R. caesius* and *R. calvatus* are tetraploid and phenotypically identical to the maternal species. *R. caesius* has previously been described as an obligate apomict forming only aposporic or diplo-sporic embryo-sacs. However, the breeding behaviour of these two species must be explained solely in terms of sexual, reduced embryo-sacs.

The progeny from *R. laciniatus* show considerable variation in both chromosome

Table 1. *Chromosome numbers in progenies of seedlings from* R. laciniatus

| | Nos. of seedlings | | | | | | | | | | | |
	2n=14	2n=21	2n=25	2n=27	2n=28	2n=29	2n=30	2n=35	2n=42	Total	% reduced	% fertilized
R. laciniatus♀ ×												
R. tomentosus♂ (2n=14)		11			18			12		41	26·8	56·1
R. ulmifolius♂ (2n14)		21	2	1	13		1	8		46	45·7	63·0
R. calvatus♂ (2n=28)	1				22*				3	26	23·0	30·8
self	1			1	21	1			1	25	?	?
R. thyrsoideus♂ (2n=35)					8			2		10	0	20
R. fragrans♂					4					4	0	0
R. dasyphylloides♂ (2n=49)					11					11	0	0
R. platyphyllus♂ (2n=56)					20					20	0	0
	2	32	2	2	117	1	1	22	4	183		

* Includes five probable sexuals.

Table 2. *Chromosome numbers in progenies of seedlings from* R. caesius *and* R. calvatus

	Numbers of seedlings			
	2n = 14	2n = 28		2n = 28
R. caesius♀ ×			R. calvatus♀ ×	
R. ulmifolius♂	2	12	R. ulmifolius♂	19
R. laciniatus♂		20		
self		20	self	20
R. thyrsoideus♂		9	R. thyrsoideus♂	7
R. fragrans♂		7		
R. dasyphylloides♂		7	R. dasyphylloides♂	12

number and phenotype. After pollination with the two diploid species of *R. tomentosus* and *R. ulmifolius* 26·8% and 45·7% of triploid sexual progeny were produced respectively. After pollination with the tetraploid species *R. calvatus* there was only 19·2% of sexual progeny. No sexual offspring were formed when the pollinating parent had more than $4x$ chromosomes. Conversely apomictic progeny increased from 28·3% when *R. ulmifolius* was used as the pollinating parent to 100% when higher polyploids were used.

Three other types of progeny were also formed. After pollination with the two diploid species 29·2% and 17·4% of unreduced 'sexual' seedlings where $2n = 35$ were produced. These have twenty-eight *R. laciniatus* chromosomes and seven from the male parent. Unreduced sexual progeny, where $2n = 42 (28 + 14)$, were also found after pollination with a tetraploid male parent. That these seedlings were in fact hybrids could be determined phenotypically. The *laciniatus* (cut-leaf) character is phenotypically recessive in the triploid and hexaploid hybrids but relatively more dominant in the pentaploid where the proportion of *laciniatus* chromosomes is greater. There could be no doubt that they are sexual hybrids.

Two haploid seedlings with fourteen chromosomes were found. There were also six aneuploids of unknown origin.

Since only reduced sexual embryo-sacs are formed the origin of these different classes of progeny is of interest. The normal sexual seedlings are the products of reduced egg cells and pollen grains while the two haploid seedlings presumably arose parthenogenetically from reduced egg cells.

The origin of the tetraploid *laciniatus*-like progeny and of the pentaploid and hexaploid seedlings is more difficult to understand. It is clear, however, that the tetraploids must have arisen by the doubling of the chromosome number of the egg prior to embryo formation. This could occur in one of two ways, either by restitution at the first division of the egg cell or by automixis, i.e. by 'fusion' of nuclei within the embryo-sac. The pentaploid and hexaploid sexual progeny must have arisen from reduced egg cells which became diploidized and subsequently fertilized.

The progeny from *R. caesius* and *R. calvatus* are, with the exception of two haploids, all tetraploid and maternal in phenotype. Since, in these species too, there is no evidence of either apospory or diplospory they also must be the result of diploidization. The haploid seedlings are again probably parthogenetic. The behaviour of

these two species is identical to that of *R. laciniatus* when the latter is pollinated with high polyploids. The differences in breeding behaviour between the three species can be interpreted in terms of the ability of normal, sexual egg cells to behave differently under different conditions.

A further type of progeny, termed 'sub-sexual' has been reported previously by a number of authors. Sub-sexual progeny have been described as having the same chromosome number as the female parent and as being phenotypically similar but not identical to it. It has been suggested that they could arise after restitution of nuclei from diplosporous egg cells which have undergone crossing over during meiotic prophase. As a result the seedlings would be genetically different and consequently phenotypically different from the maternal parent. Such plants are not found in the present study and there was no cytological evidence to support such a theory. If sub-sexual progeny do occur they might originate from occasional allo-syndetic pairing and crossing over between the two different chromosome complexes which probably make up these tetraploid species.

5. DISCUSSION

There are many reports of reproductive versatility in other species of *Rubus*. Amongst these one of the most interesting was found by Crane & Thomas (1939) in *R. vitifolius* ($2n = 56$). This species, which produces only sexual progeny when pollinated with *R. idaeus* ($2n = 14$), produces a mixture of sexual and apomictic progeny when pollinated with a tetraploid form ($2n = 28$) of the same species. Thus, as with *R. laciniatus*, the proportion of apomictic progeny increases with increase in ploidy of the pollinating parent. The breeding behaviour of *R. vitifolius* is most easily explained if it is assumed that, like *R. laciniatus*, only reduced egg cells are formed and that variation in the progeny results from different developmental pathways of the egg cells. The absence of apomictic seedlings in the diploid cross suggests that diploidization occurs as a result of restitution at the first division of the egg cell rather than by automixis. If automixis were responsible it might be expected that pentaploid seedlings would also have been found. The only report of automixis in *Rubus* (Thomas, 1940) was inferred and not observed.

From a cross between *R. nitidioides* ($2n = 28$) × *R. thyrsiger* ($2n = 28$) Crane & Thomas (1939) were able to distinguish three classes of progeny. Since these classes were similar to those found after crossing *R. laciniatus* × *R. calvatus* it would seem likely that this species too produces only sexual reduced egg cells.

Einset (1951), in his extensive survey of American blackberries, found different chromosome numbers in progenies from various tetraploid species. In a total of 631 seedlings 43 were haploid. The large number of such seedlings was unexpected and, as Einset suggests, must have arisen as a result of the parthogenetic development of reduced eggs. In Einset's experiments all the pollinating parents had, with one exception, at least $4x$ chromosomes and it is impossible to estimate the proportion of the remaining 588 seedlings which are of hybrid origin. It would seem

probable, however, that at least twenty-two could be classified as 'unreduced sexuals' i.e. resulting from diploidization followed by fertilization. Thus the same classes of progeny are found as in crosses involving *R. laciniatus*. It is therefore unnecessary to assume, as did Einset, that 'two types of egg cell are formed: reduced, which require fertilization, and unreduced which develop autonomously'.

Markarian & Olmo (1959) were unable to find any cytological evidence in support of the theory that unreduced embryo-sacs are formed in *R. procerus*, a facultative apomict. All embryo-sacs were sexual and reduced. Markarian & Olmo suggested that the presence of sexual embryo-sacs in their clone of *R. procerus* might be due to environmental effects. Similarly Christen (1950) was unable to find either aposporic or diplosporic embryo-sacs in *R. caesius* and Christen concluded that the form of *R. caesius* with which he was working must have been genetically different from that considered by Lidforss (1914) who described this species as an obligate apomict. The present study shows that it is unnecessary to make such assumptions.

The cytological evidence presented by previous authors in support of apospory and diplospory in tetraploid *Rubus* species is not convincing. The evidence presented above shows that the breeding behaviour of these tetraploid species can be explained simply in terms of one, i.e. normal, sexual, type of embryo-sac.

In other, unrelated genera restitution can also be an important method of restoring the maternal chromosome number. Thus Redinger (1938) reports that *Petunia nyctaginiflora* when pollinated with either *Salpiglossis variabilis* or *Nicotiana tabacum* produces only homozygous *P. nyctaginiflora* seedlings, as a result of di-ploidization of the egg cell. Similarly *Primula japonica* × *P. chungensis* gives rise only to maternal-like homozygous seedlings (Ernst, 1951). *Tradescantia* and *Trillium* species similarly form maternal-like seedlings from reduced egg cells by 'double division of the chromosomes in metaphase' (Jeffrey, 1948).

Aalders (1964) describes a further example of reproductive versatility in *Rubus*. It was found that the raspberry *R. idaeus*, a normal sexual diploid species, when used as the female parent in crosses with *R. canadensis* ($2n = 3x = 21$) produced twenty-six seedlings of which twenty-five were diploid and raspberry like with no evidence of *R. canadensis* in their genotype or phenotype. It is highly probable that in this cross too normal reduced egg cells have become diploidized and given rise to maternal-like progeny.

There is therefore a considerable body of evidence that the breeding behaviour of many species of *Rubus* depends on only one type of embryo-sac and that this is sexual in origin.

One of the genetical implications of the breeding system here suggested is that the tetraploid species of *Rubus* are homozygous. Since however they are probably amphidiploids, as fourteen bivalents are formed regularly at meiosis, there will be homozygosity only between chromosomes of each of the two complexes which col-lectively constitute the tetraploid. There will be heterozygosity as a result of the separation of the two complexes. Crossing over, although it occurs normally, will not result in segregation since diploidization restores the full maternal chromosome complement.

SUMMARY

1. The breeding behaviour of the three tetraploid *Rubus* species *R. caesius*, *R. calvatus* and *R. laciniatus* $(2n = 28)$ has been investigated.

2. Megaspore mother cells of all three species always undergo a normal meiosis and embryo-sac formation is of the *Polygonum* type. Egg cells have fourteen chromosomes.

3. There is no evidence for the production of either aposporic or diplosporic embryo-sacs as has previously been assumed.

4. The proportion of sexual and apomictic progeny differs in the three species and, in *R. laciniatus*, varies according to the chromosome number of the pollinating parent.

5. The apomictic progeny are produced by diploidization of the reduced egg cells. These diploidized egg cells can subsequently be fertilized in *R. laciniatus*.

6. The versatility in the breeding behaviour of these species is explained on the basis that only one type of embryo-sac is formed and that the developmental behaviour of the egg cell is conditioned by the chromosome number of the pollinating parent. Apomixis in these species is not a consequence of a breakdown of meiosis.

REFERENCES

AALDERS, L. E. (1964). Production of Maternal-type Parents through crosses to Apomictic Species. *Nature, Lond.* **204**, 101–2.

CRANE, M. B. & THOMAS, P. T. (1939). Reproductive versatility in *Rubus. Proc. 7th Int. Gen. Cong. Edin.* **91**.

CHRISTEN, H. R. (1950). Untersuchungen uber die Embryologie pseudogamer und sexueller Rubusarten. *Ber. schweiz. bot. Ges.* **60**, 153–98.

DOWRICK, G. J. (1959). Abnormal Gametogenesis and Embryo Abortion in the Pear variety Beurré Bedford (*Pyrus communis*). *Z. indukt. Abstamm.- u VererbLehre.* **89**, 80–93.

EINSET, J. (1951). Apomixis in American polyploid blackberries. *Am. J. Bot.* **38**, 768–72.

EINSET, J. (1947). Chromosome studies in *Rubus. Gentes Herb.* **7**, 181–92.

ERNST, A. (1951). 'Natural Hybrids' nach interspezifischen Bestäubungen in der Gattung *Primula. Arch. Julius Klaus-Stift Vererb-Forsch* **25**, 135–236.

GUSTAFSSON, A. (1943). The genesis of the European blackberry flora. *Acta. Univ. lund.* **54**, 1–199.

GUSTAFSSON, A. (1946). Apomixis in Higher plants. Part 1. The mechanism of apomixis. *Acta. Univ. lund.* N.F. Avid. 42.

JEFFREY, E. C. (1948). Hormones in relation to parthenogenesis. *Genetics* **33**, 615.

LIDFORSS, B. (1914). Resumé seiner Arbeiten über *Rubus. Ind. Abst. Vererb.* **12**, 1–13.

MARKARIAN, D. & OLMO, H. P. (1959). Cytogenetics of *Rubus*. 1. Reproductive behaviour of *R. procerus* Muell. *J. Hered.* **50**, 131–36.

PRATT, C. & EINSET, J. (1955). Development of the embryo-sac in some American blackberries. *Am. J. Bot.,* **42**, 637–45.

PRATT, C., EINSET, J. & CLAUSEN, R. T. (1958). Embryology, breeding behaviour and morphological characteristics of apomictic triploid *Rubus idaeus* L. *Bull. Torrey. bot. Club,* **85**, 242–54.

REDINGER, K. (1938). Uber die Entslehung diploider Embryonen aus unblfruchteten, mit gattungsfrundum Pollen bestaubten. Samenaulagen von *Petunia nyctaginifiora. Biol. Zbl.* **58**, 142–51.

THOMAS, P. T. (1940). Reproductive versatility in *Rubus* II, The chromosomes and development. *J. Genet.* **40**, 119–28.