NUCLEOLAR SUPPRESSION, ITS INHERITANCE AND ASSOCIATION WITH TAXONOMY AND SEX IN THE GENUS *RIBES*

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1. INTRODUCTION

NAVASHIN (1928) used the word "amphiplasty" to denote morphological changes occurring in chromosomes following interspecific hybridisation. Changes affecting individual chromosomes of the complement were termed "differential amphiplasty", and this phenomenon, involving the consistent disappearance of the satellite of one of the parental nucleolar chromosomes, was described in species hybrids of *Crepis* in a series of papers by Navashin and his co-workers and others (Navashin, 1927, 1928, 1934; Babcock and Navashin, 1930; Avery, 1930; Hollingshead, 1930). Interspecies hybrids of several other genera have since been found to show a similar differential amphiplasty, notably *Salix* (Wilkinson, 1941, 1944, *Solanum* (Yeh and Peloquin, 1965) and *Ribes* (Keep, 1960, 1962a, 1965; Tun, 1960, 1961). In *Crepis*, differential amphiplasty is not associated with sterility (Navashin, 1934) or phylogeny, and it occurs equally in reciprocal crosses; it is as marked in polyploids as in diploids (Babcock and Navashin, 1930). In polyploid hybrids involving *Ribes nigrum* L. (black currant) and *R. grossularia* L. (gooseberry) differential amphiplasty is less marked than in the diploid F_1 , the normally "suppressed" black currant satellite being evident in some cells. The satellite of the *R. nigrum* nucleolar chromosome reappears on segregation, as does that of *Crepis* (Navashin, 1934; Keep, 1962a).

In *Salix* (Wilkinson, 1944) and in black currant $\times$ gooseberry hybrids (Keep, 1962a) the number of satellites at metaphase and number of nucleoli per cell at late telophase in hybrids showing amphiplasty are identical. However, in interphase cells of black currant $\times$ gooseberry hybrids, one or more small nucleoli up to the number of black currant satellite chromosomes present may occur, indicating a reduced capacity to form nucleoli later in the cell division cycle. For convenience, the term "nucleolar suppression" will be used to cover all degrees of inequality in nucleolar formation by parent satellite chromosomes, as assessed on relative nucleolar size and/or number in hybrids. Further data on interspecies hybrids of *Ribes*, showing an association between the degree of nucleolar suppression and taxonomy, evidence bearing on the inheritance of nucleolar suppression and the occurrence of sex-associated nucleolar size differences in the dioecious species *R. orientale* Desf., are reported in this paper.

2. MATERIALS AND METHODS

A number of species hybrids raised or acquired for the furtherance of the *Ribes* breeding programme at East Malling were classified into four

nucleolar "suppression grades" following microscopic examination of root-tip nucleoli stained in aceto-carmin (Keep, 1962a). In grading, since the relative sizes of interphase nucleoli sometimes varied considerably

TABLE 1
Nucleolar suppression in Ribes species hybrids

Subgenus and section of parents	Parentage	No. of plants "suppression grade"			
		"3"	"2"	"1"	"0"
$R_5 \times R_5$	<i>R. nigrum</i> $\times$ <i>R. dikuscha</i>	—	—	2	—
	<i>R. nigrum</i> $\times$ <i>R. bracteosum</i>	—	—	2	—
$H_1 \times H_1$	<i>R. grossularia</i> $\times$ <i>R. alpestre</i>	—	—	1	3
	<i>R. grossularia</i> $\times$ <i>R. burejense</i>	—	—	1	—
	<i>R. grossularia</i> $\times$ <i>R. cynosbati</i>	—	—	1	—
	<i>R. grossularia</i> $\times$ <i>R. grossularioides</i>	—	—	—	2
	<i>R. grossularia</i> $\times$ <i>R. inerme</i>	—	1	1	—
	<i>R. grossularia</i> $\times$ <i>R. leptanthum</i>	—	1	—	—
	<i>R. grossularia</i> $\times$ <i>R. niveum</i>	—	1	—	—
	<i>R. grossularia</i> $\times$ <i>R. non-scriptum</i>	—	—	2	—
	<i>R. grossularia</i> $\times$ <i>R. oxyacanthoides</i>	—	—	3	—
	<i>R. grossularia</i> $\times$ <i>R. parishii</i>	—	—	—	1
	<i>R. grossularia</i> $\times$ <i>R. pinetorum</i>	—	—	1	—
	<i>R. grossularia</i> $\times$ <i>R. stenocarpum</i>	—	1	—	—
	<i>R. grossularia</i> $\times$ <i>R. watsonianum</i>	—	1	1	—
Totals	Intrasectional hybrids	0	5	15	6
$R_5 \times R_2$	<i>R. nigrum</i> $\times$ <i>R. aureum</i>	—	—	1	—
$R_5 \times R_3$	<i>R. nigrum</i> $\times$ <i>R. glutinosum albidum</i>	1	—	—	—
	<i>R. nigrum</i> $\times$ <i>R. sanguineum</i>	—	1	—	—
	<i>R. sanguineum</i> $\times$ <i>R. nigrum</i>	1	—	—	—
$R_5 \times R_4$	<i>R. nigrum</i> $\times$ <i>R. cereum</i>	4	—	—	—
$R_5 \times R_7$	<i>R. nigrum</i> $\times$ <i>R. longeracemosum</i>	—	—	1	—
Totals	Intrasubgeneric hybrids	6	1	2	0
$R_5 \times H_1$	<i>R. nigrum</i> $\times$ <i>R. grossularia</i>	5	—	—	—
	<i>R. grossularia</i> $\times$ <i>R. nigrum</i>	2	—	—	—
	<i>R. nigrum</i> $\times$ <i>R. divaricatum</i>	2	—	—	—
$B_1 \times R_5$	<i>R. orientale</i> $\times$ <i>R. nigrum</i>	1	1	—	—
$H_1 \times R_3$	<i>R. grossularia</i> $\times$ <i>R. sanguineum</i>	—	1	—	—
$B_1 \times H_1$	<i>R. orientale</i> $\times$ <i>R. grossularia</i>	5	—	—	—
Totals	Intersubgeneric hybrids	15	2	0	0

B_1 = Subgenus Berisia Spach., Section Euberisia Jancz.

H_1 = Subgenus Grossularia Rich., Section Eugrossularia Engl.

R_2 = Subgenus Ribesia Berl., Section Symphocalyx Berl.

R_3 = Subgenus Ribesia Berl., Section Calobotrya Spach.

R_4 = Subgenus Ribesia Berl., Section Cerophyllum Spach.

R_5 = Subgenus Ribesia Berl., Section Eucoreosma Jancz.

R_7 = Subgenus Ribesia Berl., Section Ribesia Berl. emend. Jancz.

from cell to cell, approximately five cells from each of four or five root tips were examined per plant. Grade "0" indicates that nucleoli appeared approximately equal in all cells examined; grade "1" indicates a slight but noticeable size difference in some cells but that in many others the

nucleoli appeared equal; grade "2" denotes a size difference, sometimes marked, in a majority of cells; and grade "3" indicates marked nucleolar suppression in all cells, cells with two nucleoli sometimes being rare in this class. On this system, several black currant and gooseberry cultivars have been graded "0" or "1".

To obtain a more accurate picture of nucleolar suppression in certain progenies, camera lucida drawings were made, at a magnification of $\times 3160$ on heavy Croxley Script paper, of interphase nucleoli of five comparable cells from each of four root tips per plant. Each drawing was carefully cut out and weighed to 0.1 mg. on a Townson and Mercer torsion balance. It was considered that the weights so obtained would provide an adequate measure of relative nucleolar sizes.

3. NUCLEOLAR SUPPRESSION AND TAXONOMY

The "suppression" grades of 52 hybrid plants, representing 25 different species combinations and grouped according to the taxonomic classification of their parents (Rehder, 1954), are included in table 1.

Average suppression grades for intrasectional, intrasubgeneric and inter-subgeneric hybrids were 0.96, 2.44 and 2.88 respectively, strongly suggesting an association between the extent of nucleolar suppression and degree of parental differentiation.

4. INHERITANCE OF NUCLEOLAR SUPPRESSION

Three small back-cross progenies derived from crossing interspecific hybrids graded "3" for nucleolar suppression to black currants were graded on relative sizes of root tip nucleoli as already described.

TABLE 2
Nucleolar suppression in first back-cross progenies

Family	Parentage	No. of seedlings suppression grade				
		"4"	"3"	"2"	"1"	"0"
B902	$\times$ B-1 <i>R. "carrierei"</i> (<i>R. nigrum</i> $\times$ <i>R. glutinosum albidum</i>) $\times$ <i>R. nigrum</i>	—	6	2	5	—
B905	(<i>R. nigrum</i> $\times$ <i>R. cereum</i>) $\times$ <i>R. nigrum</i>	—	2	—	3	—
B1111	<i>R. nigrum</i> $\times$ (<i>R. nigrum</i> $\times$ <i>R. sanguinem</i>)	2	12	15	10	—

Segregation in both progenies, B902 and B905, in which the F_1 hybrids were the seed parents, appeared to be discontinuous, with approximately equal peaks at grades "3" and "1". Although numbers are far too small to be conclusive this suggests that in these progenies nucleolar suppression was segregating more or less as a unit and was probably under major gene control. In Family B1111, however, in which *R. nigrum* was the seed parent, segregation for nucleolar suppression appeared to be more or less continuous, with a peak at grade "2". Two exceptional seedlings in which only one and three cells out of five and six root tips, respectively

contained two nucleoli (which were very unequal), were graded "4", and represented a more marked nucleolar suppression than their F_1 parent.

To obtain a more accurate picture of nucleolar suppression in this progeny, weights of nucleolar drawings were obtained as already described. The mean ratios per cell per plant of weight-of-larger/weight-of-smaller nucleolus (nucleolar suppression ratio, NSR) are shown in fig. 1. The results in general are similar to those obtained by eye grading (table 2), there being a marked peak around NSRs of 1.5 to 2.5 (grades "1" and "2") and a long "tail" of 11 seedlings (grade "3") showing increasingly marked nucleolar suppression. The two seedlings graded "4" in table 2 are not represented in fig. 1.

Within plants in Family B1111, nucleolar sizes were often markedly variable, standard errors per plant for the larger nucleolus in a cell ranging from 0.091 to 0.860, for the smaller from 0.059 to 0.541, and for the NSR from 0.052 to 4.200. There was no correlation between NSRs and standard errors for large or for small nucleoli, but in general, means and standard errors per plant of NSRs increased in parallel. Thus, plants with NSRs of 10.87, 10.04, 7.23, 6.49 and 5.99 had standard errors of 3.79, 4.20, 1.81, 1.91 and 2.45 respectively, while plants with NSRs of 3.08, 2.06, 1.69 and 1.26 had standard errors of 0.48, 0.15, 0.19 and 0.09 respectively.

5. NUCLEOLAR SIZE AND SEX IN *R. orientale* Desf.

Inspection of shoot and root-tip squashes from a male and female accession, respectively, of *R. orientale* showed that the two nucleoli in interphase cells of the female were usually approximately equal in size whereas those of the male were markedly unequal. Examination of root-tip squashes of five female and eight male seedlings from crossing these two accessions showed that the male plants again had greater size differences between their nucleoli than the females, although this difference was less marked in some plants than in the male parent.

To obtain a more accurate estimate of relative nucleolar sizes, root tips were taken from comparable clonally propagated material of four female and five male seedlings and of their female parent. Camera lucida drawings of nucleoli were made at a magnification of $\times 3040$, cut out and weighed as already described.

The average ratio weight-of-larger/weight-of-smaller nucleolus per cell in the five male plants was 1.570, with a range of 1.285 to 1.773; that of the females was 1.276, with a range of 1.163 to 1.359. Although one of the male plants (not included in the initial examination) came within the female range of ratios, an analysis of variance showed that the males as a group had significantly higher ($P < 0.05$) ratios than the females; there was also significant ($P < 0.01$) variation within each sex. The average ratios for four of these plants (two male and two female) which had been included in the initial examination were 1.773 and 1.585, and 1.182 and 1.308 respectively. Clearly, examination without actual measurement is adequate to classify into two groups based on differences of this magnitude. Thus, a total of ten male and seven female seedlings in the F_1 progeny were found to differ consistently according to sex in nucleolar size ratios, with one atypical male plant showing a ratio within the female range.

FIG. 1.—Frequency distribution of mean nucleolar suppression ratios in Family B1111 (*R. nigrum* × *R. sanguineum*).

6. DISCUSSION

Although the taxonomic relationships of *Ribes* species reflect rather accurately the degree of fertility shown by their F_1 hybrids (Nilsson, 1955; Keep, 1962b), and "distant" hybrids appear to be those most likely to show nucleolar suppression (table 1), this phenomenon in *Ribes* as in *Crepis* (Navashin, 1934) is not necessarily associated with sterility. Thus, hybrids of *R. nigrum* with *R. glutinosum albidum*, *R. sanguineum* and *R. cereum* graded "2" or "3" for nucleolar suppression (table 1), are all sufficiently fertile to back-cross to *R. nigrum*. On the other hand, the totally sterile hybrid *R. nigrum* $\times$ *R. longeracemosum* shows very little nucleolar suppression (grade "1"). Nevertheless, no fully fertile *Ribes* hybrid showing marked nucleolar suppression has yet been found.

Keep (1962a) noted that in the *Ribes* hybrids then under investigation plant vigour did not appear to be affected by the much reduced functioning of one of the parental nucleolar organisers. Similarly, in the F_1 hybrids described in this paper and in segregating back-cross progenies, vigour is not associated with full functioning of both organisers. This fact is of interest in the light of present theories on nucleolar function. The increasing evidence that nucleoli are involved in ribosomal RNA (rRNA) synthesis has been summarised by, amongst others, McConkey and Hopkins (1964) and Perry (1967). This evidence would suggest that the rRNA and possibly protein (Perry, 1967) of the ribosomes in hybrids showing marked nucleolar suppression is formed very largely in the nucleolus of the non-suppressed parent. In first back-cross hybrids, this means that approximately 75 per cent. of recurrent parent chromatin can collaborate effectively with ribosomes whose rRNA is manufactured mostly in the nucleolus of the donor parent. This suggests either that rRNA specificities are of little importance in general protein synthesis, or else that, at least in genera in which nucleolar suppression is widespread, the rRNAs of different species are similar. In this connection, Matsuda and Siegel (1967) showed that rRNAs from four plant species of four different families were remarkably similar to each other in hybridisation behaviour with several different plant DNAs.

The assumption that nucleolar suppression indicates the presence of the donor parent's nucleolar organiser only, is not justified on present evidence, and further work on later back-cross generations will be needed to determine if, in fact, the alien organiser is also the sole suppressing agent. The wide spread in NSRs in Family B1111 (fig. 1) suggests that other factors can have a considerable modifying effect. In this progeny, the recurrent parent, *R. nigrum*, was the female, in contrast to Families B902 and B905, in which the F_1 parent was the female and in which there appeared to be a sharper division into suppressed and non-suppressed seedlings (table 2). The preponderance of seedlings showing little nucleolar suppression in B1111 may be due to the presence of pure black currant apomicts which cannot be distinguished with certainty from hybrids; the majority of seedlings were, however, certainly true hybrids.

The association of a higher nucleolar suppression ratio with maleness in *R. orientale* suggests that the nucleolar chromosome may be involved in some way in sex differentiation. The occurrence of one male plant out of 11 with a ratio in the female range may indicate crossing over between a male-

determining gene and the nucleolar organiser; further investigations are needed to determine the frequency of such exceptional male plants. The frequency with which nucleolar chromosomes are also sex chromosomes in insects and bryophytes was pointed out by Gates (1939). Similarly, the nucleolar chromosome is thought to be the sex chromosome in a number of higher plants such as *Ginkgo biloba* (Lee, 1954; Pollock, 1957), *Cycas pectinata* (Abraham and Mathew, 1962), *Spinacia oleracea* (Dressler, 1958), *Coccinia indica* (Bhaduri and Bose, 1947) and *Asparagus officinalis* (Thuesen, 1960). In some of these species sex differences are associated with the presence or absence of a satellite or the number and size of nucleoli. Thus, in *Ginkgo biloba*, Pollock (1957) found four satellited chromosomes and two, three or four nucleoli in resting nuclei of female trees, and only three satellited chromosomes and one, two or three nucleoli in resting nuclei of male trees. Ho (1963) was unable to confirm Pollock's findings, although sex differences in satellite number were described by Lee (1954); possibly karyologically distinct races of *Ginkgo* exist. In *Cycas pectinata* the male has only one satellited chromosome (Abraham and Mathew, 1962), while in one strain of *Spinacia oleracea* (Dressler, 1958) the Y chromosome of male plants bears a satellite in addition to the secondary constriction found also in the X. The Y₂ chromosome, but not the X, of one race of *Rumex hastatulus* is satellited (Smith, 1969). In young pollen grains of *Cannabis sativa*, the average nucleolar volume of XX (monoecious) plants was 130 per cent. compared with the 100 per cent. of XY (male) plants, and the frequency distribution of nucleolar volumes for male plants showed a tendency towards a double peak, whereas XX types showed only a single peak (Herich, 1961).

Jensen (1940) pointed out that nearly all reported cases of sex chromosomes in plants relate to species which are members of genera which also contain monoecious or hermaphrodite species, suggesting a recent origin for the unisexual condition; also many species with "apparent" sex chromosomes show evidence of recent hybrid origin in their meiotic behaviour. In discussing the origin of dioecy in plants, Darlington (1937) and Lewis (1942) suggested that the genes primarily determining sex must be associated sooner or later with a structural change such as an inversion. This would suppress crossing-over, which is a necessary condition for the accumulation of groups of sex-differentiating genes. The inverted region of the chromosome would develop into the differential segment. It seems probable that differential amphiplasty in species hybrids, in which the satellite thread of the nucleolar chromosome remains condensed, might prove an effective barrier to crossing-over within the nucleolar organiser region. In the first back-cross Family B902 (*R. glutinosum albidum* × *R. nigrum*²), not only did segregation for nucleolar suppression occur (table 2) but also eight out of ten seedlings which reached the flowering stage failed to produce pollen, although all seedlings tested proved female fertile, and the remaining two showed 92 per cent. and 56 per cent. of apparently viable pollen respectively. Although Lewis (1942) does not consider gynodioecy to be a transitional stage in the development of dioecy, gynodioecy has not been reported in *Ribes* species, whereas all of the numerous species of the subgenus *Berisia* are dioecious. In the case of Family B902 absence of pollen was not invariably associated with presence or absence of nucleolar suppression, but such hybridisation between hermaphrodites in nature, resulting at once in male sterility and, probably, a barrier to

crossing-over in a segment of the nucleolar chromosome, might well lead to the ultimate establishment of full dioecy, the sexes being differentiated, perhaps, by relative nucleolar volumes. The genus *Salix* may represent a later stage in the evolution of dioecy following hybridisation. Jensen (1940) disagrees with the normal classification of *Salix* as a strictly dioecious genus, since numerous instances of perfect flowers and all gradations between this state and monoecy and dioecy occur, and considers the genus is not yet fully sexually stabilised. It is probably significant that in a genus in which all species are more or less dioecious, all interspecific hybrids investigated have shown differential amphiplasty, and the species themselves show evidence of a hybrid origin also involving amphiplasty (Wilkinson, 1944).

Pazourkova (1964) and Kuznetsova *et al.* (1966) report the presence of sex chromatin in male (XY_1Y_2 , Kihara and Ono, 1925) plants of *Rumex acetosa*, as does Žuk (1969) in male plants of *R. thyrsiflorus*, and Pazourkova found "nuclear bodies" in leaves of *Ginkgo biloba* and roots of *Melandrium album* (XX female, XY male, Blackburn, 1923; Winge, 1923) which could correspond to sex chromatin, although they were not confined to one sex. Choudhuri (1969) showed that the DNA replication sequence in sex chromosomes of *M. album* was similar to that of mammalian sex chromosomes, one arm of the X chromosome in the female and part of the Y being late replicating. In *Rumex thyrsiflorus*, both heterochromatic Y chromosomes, but not the euchromatic X, are late replicating (Žuk, 1969). Sex chromatin has not yet been seen in dioecious *Ribes* species, nor heteropycnotic bodies in species hybrids showing differential amphiplasty. However, an analogy could perhaps be drawn between the suppression of nucleolar function in species hybrids and the inactivation of one, or part of one, of the X chromosomes in mammals (Lyon, 1961, 1962). Although normally random inactivation of the paternal or maternal X occurs in man and mouse, Hamerton *et al.* (1969) showed that in the female mule (horse $\times$ donkey), the paternal X preferentially shows late labelling and is presumably also preferentially inactivated, since the product of the paternally derived glucose-6-phosphate dehydrogenase allele, which is X-linked, is only weakly represented. This makes the parallel between behaviour of sex and of nucleolar chromosomes even closer: consistent inactivation of the same (or part of the same) X chromosome in a female hybrid mammal and of the same parental nucleolar organiser in some plant hybrids; the presence of a differential segment in sex chromosomes and of a condensed satellite thread in plant hybrids; and dosage compensation in certain X-linked genes (data summarised by Thompson, 1965) and sometimes in production of nucleolar material (McClintock, 1934; Beerman, 1960; Longwell and Svihla, 1960; Brown and Gurdon, 1964).

7. SUMMARY

1. Marked differences in the mean sizes of the two interphase nucleoli of root-tip cells were observed in 29 out of 52 interspecific *Ribes* hybrids. This size inequality, considered to be due to the partial suppression of the nucleolar organising capacity of one of the parental satellite chromosomes, tended to increase with increasing parental differentiation.

2. Three back-cross progenies of which *R. nigrum* was the recurrent parent, segregated for nucleolar suppression, a range from little or no

suppression to suppression equal to or exceeding that found in the F_1 parents being observed.

3. In a progeny of the dioecious species *R. orientale*, the mean ratio per cell of larger/smaller nucleolus was significantly higher in male plants than in females.

4. The frequency with which nucleolar chromosomes are also sex chromosomes in insects, bryophytes and higher plants is discussed. It is suggested that nucleolar suppression in interspecific hybrids may be one pathway to the establishment of dioecy.

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