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The origin of polyploids *via* 2n gametes in *Vaccinium* section *Cyanococcus*

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Summary

The production of 2n pollen (pollen with the sporophytic chromosome number) was evaluated in 4x and 6x taxa of *Vaccinium* section *Cyanococcus*. Mean frequencies of 2n pollen producers were 17.1% and 8.3% in natural 4x and 6x populations, respectively. The frequency of 2n pollen producers in the 4x species ranged from 8.6% (*V. angustifolium*) to 23.8% (*V. pallidum*). Level of 2n pollen production was genotypically variable (1% to 37.4%). The widespread occurrence of 2n pollen in 2x, 4x and 6x taxa suggests that sexual polyploidization was widespread and responsible for the origin of the polyploid species found in this genus. The frequency of 2n pollen producers was not significantly different between the 4x species and their putative 2x ancestors. These results support the origin of 4x and 6x taxa as a consequence of sexual polyploidization. Polyploids derived from sexual polyploidization would be expected to have increased fitness and flexibility due to the mode of 2n pollen formation. In blueberry species the predominant mode of 2n pollen formation is genetically equivalent to a first division restitution mechanism (FDR). FDR 2n pollen transmits a high percentage of the heterozygosity and a large fraction of the epistasis from the 2x parent to the 4x offspring.

Introduction

The cultivated highbush (*Vaccinium corymbosum* L.) and lowbush (*V. angustifolium* Ait.) blueberries are polyploid species in the section *Cyanococcus* A. Gray (Ericaceae) of the genus *Vaccinium*. *Vaccinium corymbosum* has 4x and 6x cytotypes, while *V. angustifolium* is 4x. Other 4x species in this section include *V. myrsinites* Lam., *V. hirsutum* Buckley and *V. pallidum* Ait. (Vander Kloet, 1988).

There are two ways in which polyploids can arise: somatic doubling of chromsomes by endomitosis (asexual polyploidization) or by modification of the meiotic process leading to the formation of 2n gametes (sexual polyploidization). Stebbins (1950, 1971) advocated asexual polyploidization as the origin of 4x and 6x species. Conversely, Harlan & de Wet (1975) indicated that it was difficult to document somatic doubling in any species; however, as they pointed out, 2n gametes have been involved in the origin of polyploid species.

The objectives of this research were to: a) evaluate different 4x and 6x taxa for 2n pollen production and b) compare the frequency of 2n pollen producers between the 4x taxa and their putative 2x ancestors.

Materials and methods

Nine natural populations of four 4x *Vaccinium* spe-

cies and 24 individuals of 6x *Vaccinium corymbosum* were evaluated for pollen stainability and 2n pollen production during two consecutive years: 1990 and 1991. The populations, their taxonomic group, the site of collection, and the number of individuals sampled are given in Table 1.

Pollen was collected from plants that flowered in each year. Pollen from two or more flowers was sampled for each clone within each population. Pollen was stained with 1% aceto-carmine glycerol jelly and examined under the microscope (200X). In blueberry, the products of microsporogenesis, pollen, are held together (Stushnoff & Hough, 1968; Stushnoff & Palser, 1969). If meiosis is normal, a pollen tetrad is formed. Two-hundred sporads (tetrads, diads and monads) were scored per clone in each year to determine 2n pollen frequency. The production of diads or monads was considered as evidence of 2n pollen formation. The frequency of 2n pollen was estimated as follows:

$$\text{2n pollen frequency} = (2D + M)/T$$

where D is the number of diads, M is the number of monads and T the total number of pollen grains examined. Individuals with more than 1% 2n pollen production were considered 2n pollen producers.

The frequency of 2n pollen production was determined for each population and the five taxa. The standard error of the phenotypic frequency was calculated as $\sqrt{[q(1-q)/n]}$, where q is the frequency of 2n pollen producers and n is the number of individuals sampled.

The log-likelihood test, G-test (Fienberg, 1977), was used to compare the frequency distributions for 2n pollen production among different populations within a species and between different species.

Chi-square test of homogeneity was used to evaluate if the production of 2n pollen was associated with pollen stainability in populations with $\geq 20\%$ 2n pollen producers. X^2 was calculated using Yates' correction for continuity in small sample sizes (Yates, 1934).

Results

Production of 2n pollen was present in all of the 4x and 6x populations (Table 2). The frequency of 2n pollen producers ranged from 3.1% to 26.1%. Significant differences for the frequency of 2n pollen producers were found between and within populations. The annual variation in frequency of 2n pollen production by individual clones was also significant. The clone with the highest frequency of 2n pollen was NJ 88.10-7 (*V. myrsinites*), which produced 37.4% 2n pollen (Fig. 1).

The average frequency of 2n pollen producers by species is given in Table 3. There were no significant differences for the frequency of 2n pollen producers between the four 4x species. Frequencies of 2n pollen producers in 4x and 6x populations

Table 1. Collection site, location and number of individuals sampled for 2n pollen production of 4x blueberry species

Collection	Species	Location	Individuals
NC 84.6a	*V. myrsinites*	Marion Co., FL	12
NJ 88.7	*V. myrsinites*	Santa Rosa Co., FL	60
NJ 88.10[1]	*V. myrsinites*	Polk Co., FL	2
NJ 88.11	*V. myrsinites*	Highlands Co., FL	23
NJ 88.23	*V. angustifolium*	Hampden Co., MA	32
NJ 88.24	*V. angustifolium*	Chittendon Co., VT	26
NJ 88.34	*V. myrsinites*	Appling Co., GA	21
NJ 89.12	*V. pallidum*	Yell Co., AR	21
NJ 89.13-NJ 89.19[2]	6x *V. corymbosum*	Arkansas	24
NJ 91.1	4x *V. corymbosum*	Cape Cod, MA	19

[1] collected within a 2x population of *V. darrowi*. [2] 6x individuals were collected along with seven sympatric 2x populations in Arkansas.

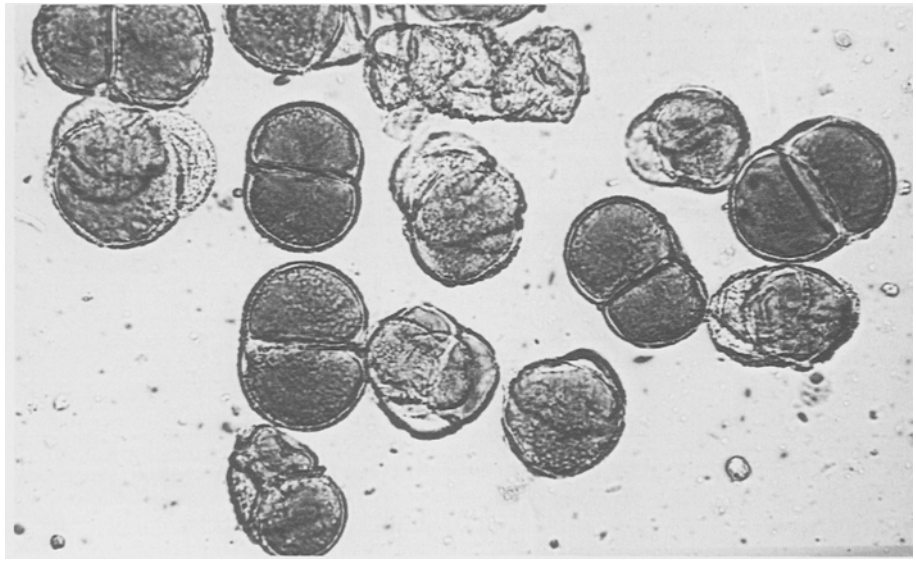

Fig. 1. High frequency of 2n pollen production in clone NJ88.10-7.

were statistically equivalent. The frequencies of 2n pollen producers were 17.1 ± 2.6 and 8.3 ± 5.6 for the 4x and 6x taxa, respectively.

The production of 2n pollen was independent of the level of pollen stainability (Table 4) in two of the populations evaluated: NJ 88.7 (*V. myrsinites*) and NJ 89.12 (*V. pallidum*). Conversely, population NJ 88.11 had an inverse relationship between pollen stainability and 2n pollen production, i.e. no 2n pollen producers had pollen stainability higher than the mean of the population (Table 4).

Table 2. Production of 2n pollen in different 4x *Vaccinium* species evaluated during two consecutive years: 1990 and 1991

Population	non 2n pollen n[1] (%)	2n pollen n (%)	Total n	Range 2n pollen production in each natural population
V. angustifolium				
NJ 88.23	31 (96.9%)	1 (3.1%)	32	1.0%
NJ 88.24	22 (84.6%)	4 (15.4%)	26	1.0– 2.8%
V. myrsinites				
NC 84.6a	10 (83.3%)	2 (16.7%)	12	1.0%
NJ 88.7	45 (75.0%)	15 (25.0%)	60	1.0–11.5%
NJ 88.10	1	1	2	37.4%
NJ 88.11	17 (73.9%)	6 (26.1%)	23	1.0–20.6%
NJ 88.34	20 (95.2%)	1 (4.8%)	21	1.0%
V. pallidum				
NJ 89.12	16 (76.2%)	5 (23.8%)	21	2.0–24.8%
V. corymbosum				
NJ 91.1	17 (89.5%)	2 (10.5%)	24	1.0– 5.7%

[1] n in the number of individuals in each class.

Table 3. Frequency of 2n pollen producers among 4x and 6x *Vaccinium* species

Species	# of populations	# individuals	% 2n pollen producers	range 2n pollen production (%)
Tetraploids				
V. pallidum	1	21	23.8 ± 9.1	2.0–24.8%
V. myrsinites	5	118	21.9 ± 3.8	1.0–37.4%
V. corymbosum 4x	1	19	10.5 ± 6.3	1.0– 5.7%
V. angustifolium	2	58	8.6 ± 3.7	1.0– 2.8%
Total	9	216	17.1 ± 2.6	
Hexaploids				
V. corymbosum 6x	1	24	8.3 ± 5.6	1.0– 2.0%

Discussion

Harlan & de Wet (1975) indicated that 2n gametes are likely to occur in crops with polyploid series. Production of 2n pollen was found in all the 4x and 6x blueberry taxa examined in this study. Interploid hybridization is typical of species with 2n gametes, since 2n gametes facilitate gene flow from 2x to 4x ploidy levels. In this way, the 'ploidy barrier' between these taxa is overcome. Tetraploid species could also arise from bilateral sexual polyploidization (2x × 2x crosses) if both 2x parent species produced 2n gametes (2n eggs and 2n pollen).

The production of 2n pollen has been reported previously in 4x and 6x blueberry species. Stughnoff & Hough (1966) reported that premature cytokinesis led to the formation of 2n pollen in the 4x highbush cultivar 'Coville'. Vorsa (1986) found the mode of 2n pollen formation in an aneuploid hy-

Table 4. Pollen stainability and 2n pollen production in three 4x populations

Population	χ^2 test for association			
NJ 88.7 (*V. myrsinites*). Year: 1991, N = 60.				
				Pollen stainability[1] (Mean = 52.6%)
		High	Low	
	2n pollen	3	7	
	non 2n pollen	28	22	$\chi^{2,2} = 1.376$ p = 0.241
NJ 88.11 (*V. myrsinites*). Year: 1990, N = 22.				
				Pollen stainability[1] (Mean = 71.8%)
		High	Low	
	2n pollen	0	4	
	non 2n pollen	5	13	$\chi^2 = 4.822^*$ p = 0.028
NJ 89.12 (*V. pallidum*). Year: 1991, N = 21.				
				Pollen stainability[1] (Mean = 82.6%)
		High	Low	
	2n pollen	3	2	
	non 2n pollen	11	4	$\chi^2 = 0.888$ p = 0.346

[1] Pollen stainability: low < mean and high > mean. [2] χ^2 calculated using Yates' correction for continuity in small sample sizes.

brid to involve three steps: desynapsis, disjunction of sister centromeres, followed by cytokinesis resulting in large diad formation. This mechanism would be genetically equivalent to first division restitution (FDR). Transmission of most of the parental heterozygosity from the 2x parent to the 4x offspring via FDR 2n pollen would be expected (Vorsa & Ortiz, 1992). FDR type mode of 2n pollen production can be valuable in *de novo* production of polyploid species where heterosis is an important component of vigor and productivity.

Cockerham & Galletta (1976) suggested that 2n pollen production was genetically controlled in blueberry. Meiotic mutants are generally inherited as recessive genes (Peloquin, 1982), and are also characterized as having variable expressivity and incomplete penetrance (McCoy, 1982). Expressivity can be affected by environmental factors and genetic background (Rao & Koduru, 1981). In potatoes the genetic system controlling desynapsis and premature cytokinesis are independent (Peloquin et al., 1990). The fact that in only one population (NJ88.11) of *V. myrsinites* 2n pollen production was associated with low pollen stainability suggests that desynapsis may not always be followed by premature cytokinesis: gene(s) involved with premature cytokinesis may be variable across populations. Thus, desynapsis, chromosome disjunction, and cytokinesis may not be controlled by the same gene(s).

The frequency of 2n pollen producers in both 2x and 4x taxa provides the evidence to support sexual polyploidization in the origin of the 4x species. The frequency of 2n pollen producers in any taxa will be equal to πp_i^x ($= p_1^x * p_2^x * \ldots * p_n^x$), where p_i is the frequency of the recessive meiotic mutant allele in the i locus controlling the production of 2n pollen, and x is the ploidy level. Therefore, the frequency of 2n pollen producers in the 4x species will be equal to or higher than in the 2x species after sexual polyploidization. In contrast, the frequency of 2n pollen producers is expected to be significantly lower in 4x than in 2x species after asexual polyploidization.

The genetic consequences of sexual versus asexual polyploidization are also different with respect to the fitness and flexibility of the newly arisen polyploid (MacKey, 1970; Watanabe et al., 1991). Asexual polyploidization results in an increased inbreeding level. Thus, in 4x blueberry where vigor and fertility have been negatively correlated with inbreeding level (Krebs & Hancock, 1988), sexual polyploidization would be advantageous. Moreover, tetraploids resulting from asexual polyploidization would lack genetic variability, and have restricted flexibility for adaptation to variable environments. Sexual polyploidization, resulting from FDR 2n gametes, would be especially advantageous in this regard.

The existence of 2n pollen production has been established in all seven 2x *Vaccinium* species (Ortiz et al., 1992). The frequency of 2n pollen producers was estimated for most of the 2x species. The production of 2n pollen in tetraploid taxa was comparable to the levels found in their putative ancestral diploids. The frequency of 2n pollen producers was 15.3 and 23.8%, in 2x and 4x *V. pallidum*, respectively, and were not significantly different (Table 3). Similarly, the frequencies were not significantly different between 2x (7.4%) and 4x (10.5%) *V. corymbosum*. These results support the origin of these 4x *Vaccinium* species through sexual polyploidization.

Vaccinium tenellum and *V. darrowii* are southeastern North American species which are considered to be the putative 2x ancestors of the sympatric 4x species *V. myrsinites* (Camp, 1945). The phenotypic frequencies for 2n pollen producers were estimated as 21.9% for *V. myrsinites*, 18.4% for *V. darrowii*, and 14.5% for *V. tenellum*. The similar phenotypic frequency estimates of the 2x and 4x species, indicate that *V. myrsinites* could have arisen through sexual polyploidization from the 2x species: *V. tenellum* and *V. darrowii*. Vorsa (unpublished data) recovered phenotypically hybrid 4x offspring in crosses of *V. darrowii* (2x) with 4x highbush cultivars, suggesting 2n egg production in *V. darrowii*, as well.

The northern 4x species *V. angustifolium* has probably been derived from two formerly sympatric 2x species: *V. myrtilloides* and *V. boreale* (Camp, 1945). The phenotypic frequencies of 2n pollen producers were estimated as 8.6%, 13.0% and 11.9% for *V. angustifolium, V. myrtilloides* and

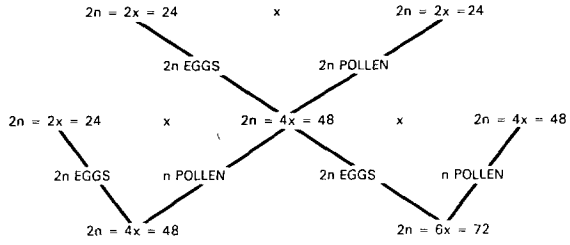

Fig. 2. Origin of 4x and 6x *Vaccinium* species through sexual polyploidization. None or very few 3x after 4x × 2x crosses due to strong '3x block'.

V. boreale, respectively. These frequency estimates were not significantly different, supporting the origin of 4x *V. angustifolium* through sexual polyploidization.

Based on this information a scheme for the origin of 4x and 6x species through sexual polyploidization is proposed (Fig. 2). In this scheme the gene flow between 2x and 4x species could occur through unilateral sexual polyploidization: 4x (n gametes) × 2x (2n gametes) crosses. In this way, the frequency of 2n pollen producers in the 4x population could also be increased. The low frequency of 3x offspring after 4x × 2x or 2x × 2x crosses indicates the presence of a strong 3x block (Sharpe & Darrow, 1959; Dweikat & Lyrene, 1988).

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