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Early-acting inbreeding depression and reproductive success in the highbush blueberry, *Vaccinium corymbosum* L.

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Summary. Tetraploid *Vaccinium corymbosum* genotypes exhibit wide variability in seed set following self- and cross-pollinations. In this paper, a post-zygotic mechanism (seed abortion) under polygenic control is proposed as the basis for fertility differences in this species. A pollen chase experiment indicated that self-pollen tubes fertilize ovules, but are also 'outcompeted' by foreign male gametes in pollen mixtures. Matings among cultivars derived from a pedigree showed a linear decrease in seed number per fruit, and increase in seed abortion, with increasing relatedness among parents. Selfed (S_1) progeny from self-fertile parents were largely self-sterile. At zygotic levels of inbreeding of $F > 0.3$ there was little or no fertility, suggesting that an inbreeding threshold regulates reproductive success in *V. corymbosum* matings. Individuals below the threshold are facultative selfers, while those above it are obligate outcrossers. Inbreeding also caused a decrease in pollen viability, and reduced female fertility more rapidly than male fertility. These phenomena are discussed in terms of two models of genetic load: (1) mutational load – homozygosity for recessive embryo-lethal or sub-lethal mutations and (2) segregational load – loss of allelic interactions essential for embryonic vigor. Self-infertility in highbush blueberries is placed in the context of 'late-acting' self-incompatibility versus 'early-acting' inbreeding depression in angiosperms.

Key words: Mating systems – Self-incompatibility – Genetic load

Introduction

The tetraploid highbush blueberry, *Vaccinium corymbosum,* is primarily an outcrossing species with features such as pendant flowers and insect pollinators that facilitate cross-pollination. In addition, seed set is reduced following self-pollination. Numerous controlled-pollination studies have documented the extent and variability of self-fertility in *V. corymbosum* and related taxa. Diploid species exhibit near to complete self-sterility (Ballington and Galletta 1978; Meader and Darrow 1944; Vander Kloet and Lyrene 1987), whereas low to moderate levels of self-fertility are reported for higher ploid species and cultivars (Morrow 1943; Meader and Darrow 1947; El-Agamy et al. 1981; Vander Kloet and Lyrene 1987; Krebs and Hancock 1988). Natural populations of *V. corymbosum* (4x), *V. angustifolium* (4x), and *V. ashei* (6x) generally have skewed distributions, with a higher frequency of self-sterile genotypes than self fertile clones (Aalders and Hall 1961; Wood 1968; Rabaey and Luby 1988; Garvey and Lyrene 1987; Vander Kloet and Cabilio 1984).

The partial to complete failure of seed set following self-pollination of blueberries at all ploidal levels has been described as 'self-incompatibility' (Ballington and Galletta 1978; Morrow 1943; El-Agamy et al. 1981; Garvey and Lyrene 1987; Vander Kloet and Lyrene 1987). However, the developmental mechanisms and genetic factors controlling these barriers to self-fertility in *Vaccinium* have not been determined. In an earlier report on self- and cross-fertility in six tetraploid highbush cultivars, we observed variable self-fertility, an inverse relationship between self-seed set and zygotic inbreeding coefficients, and growth of self-pollen tubes into ovules at the same rate as outcross pollen tubes (Krebs and Hancock 1988). These phenomena are not characteristic of genetic self-incompatibility (SI) systems, where strict allogamy is maintained by a pre-zygotic maternal inhibition of self-pollen growth based on allelic recognition at one or a few *S*-loci (Lewis 1979). We hypothesized that

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variable self- and outcross fertility in *V. corymbosum* is a consequence of early-acting inbreeding depression (seed abortion) and is regulated by the level of zygotic inbreeding generated by a given mating.

In this paper we report additional evidence in support of the hypothesis stated above. Experiments were performed to answer the following questions (1) Does self-fertilization occur? (2) Is seed set reduced, and seed abortion increased, in response to increased inbreeding? (3) Are male and female components of fertility (prezygotic) affected by inbreeding? The latter question addresses a phenomenon unique to polyploids – the possibility of inbreeding depression (or heterosis) in gametes and gametophytes carrying two or more genomes.

Materials and methods

Pollen chase

The object of the pollen chase experiment was to determine whether early-arriving self-pollen tubes fertilize ovules, thus preventing their fertilization by later-arriving outcross gametophytes. Crosses were made on two 4-year-old ramets (vegetative replicates) of the cultivar 'Spartan' in a greenhouse during April, 1988. Using the method of Moore (1964), pollinations were made on opened flowers of a uniform stage of development that had been emasculated just prior to mating. Fresh pollen was used in all crosses (and in all experiments described below). Two matings, 'Spartan' selfed and 'Spartan' × 'Bluejay' were chosen as controls because they differ widely in seed set (Krebs and Hancock 1988). Both crosses were made zero days after flowering (0 DAF); additional 'Spartan' × 'Bluejay' matings were made 2 DAF and 4 DAF on previously unpollinated flowers to determine pistil receptivity to pollen. Pollen chase treatments were made by applying 'Bluejay' pollen to previously selfed 'Spartan' flowers at 1-, 2-, 3-, or 4-day intervals between the two pollinations. The number of developed (plump, brown) seeds per fruit was counted for ten ripe fruit per treatment.

Diallel cross

Four highbush blueberry cultivars (20-year-old plants) were mated in a complete diallel cross during a field experiment at Grand Junction/MI in the spring of 1986. Twenty pollinations per mating were made on each of two randomly chosen replicates, using techniques previously described (Krebs and Hancock 1988). Twenty ripe fruit per cross were sampled by pooling ten random fruit from each replicate, and counts were made of the number of developed and aborted (shrunken, angular, pale) seeds per fruit. The percentage of aborted seeds per fruit (aborted/aborted + developed) was calculated and arcsine-transformed to normalize the distribution. The relationships between these fertility parameters and inbreeding coefficients (F) were determined by linear regression of each parameter on F values, using the SYSTAT program. Estimates of inbreeding coefficients for paternal and maternal parents (F_p and F_m, respectively) and for zygotes produced by the diallel mating (F_z) are given in Table 1.

Calculation of inbreeding coefficients

Previous calculations of F for tetraploid highbush cultivars (Hancock and Siefker 1982), which were based on the assumption of disomic inheritance, had to be revised in light of new evidence that *V. corymbosum* is an autotetraploid species (Krebs and Hancock 1989). Inbreeding coefficients were derived by pedigree analysis using the probabilistic 'identity by descent' approach defined for autotetraploids by Kempthorne (1957). With tetrasomic inheritance and zero double reduction ($\alpha = 0$), the inbreeding coefficient of an individual X with parents A and B is

$$F_X = 1/6\ (F_A + 4r_{AB} + F_B)$$

where F_A and F_B are the inbreeding coefficients of the parents and r_{AB} is the co-ancestry value between parents. The assumption of zero or near-zero double reduction appears to be valid in light of allozyme segregation data (Krebs and Hancock 1989).

Given four alleles per locus in an autotetraploid, F is the average probability of identity for all six pair-wise combinations of alleles. The above equation indicates that inbreeding in autotetraploid matings results when the parents are related (identity between uniting gametes) and/or inbred (identity within 2x gametes). In contrast, F values in diploids are determined only by parental relatedness, not parental inbreeding. Since the starting parent cultivars in the highbush blueberry pedigree were wild plant selections from different populations (Eck and Childers 1966), they were assumed to be noninbred and nonrelated.

F values in autotetraploids do not refer directly to homozygosity (Busbice and Wilsie 1966). Organisms with tetrasomic inheritance approach homozygosity (four identical alleles per locus) at approximately 1/3 the rate of diploids (Haldane 1930; Dessureaux and Gallais 1969). Because autotetraploids can carry up to four different alleles at a single locus, inbreeding can result in loss of 'higher order' heterozygosity (tetraallelic → triallelic → diallelic) at a faster rate than subsequent increases in homozygosity (diallelic → monoallelic). The amount of homozygosity generated by inbreeding thus depends on the proportion of multiple allelic loci present in the genome.

S_1 progeny fertility

Groups of ten selfed (S_1) progeny from the cultivars 'Elliot', 'Jersey', and 'Bluecrop' were grown to anthesis in a greenhouse (2-year-old plants). In March 1986, these S_1 progeny and one 3-year-old ramet of each parent cultivar were self-pollinated (20 pollinations per individual). In addition, each of the parent cultivars was cross-pollinated using bulked pollen from 'Spartan', 'Rubel', and 'Bluejay' cultivars. Ten ripe fruit per cross were sampled from most genotypes, although some S_1 progeny showed zero or reduced self-fruit set. To adjust for these differences, numbers of developed seed were calculated on a per-pollinaton basis rather than a per-fruit basis.

Male and female fertility in S_1 progeny

Pollen stainability was used to determine the effect of inbreeding on male gamete viability. Fresh pollen from the three parent cultivars and S_1 progeny described above was stained with 0.05% aniline blue in lactophenol (Stanley and Linskens 1974) and observed under a light microscope. A minimum of 100 pollen tetrads per genotype was examined, and the percent of total stained microspores ($n > 400$) was calculated. Because pollen tetrads represent products of single meiotic events, the percentage of unstained tetrads (0/4 viable microspores) was scored as a measure of inbreeding effects during or prior to Metaphase I.

Parent × S_1 progeny reciprocal crosses were made using the three cultivars and selfed offspring described above, in order to generate 'inbred' × 'noninbred' and 'noninbred' × 'inbred' matings. The parent cultivars had F values ranging from 0 to 0.043 (Table 1), while the S_1 progeny F values averaged 0.167–0.203 (Table 3). Twenty pollinations per cross were made on 3-year-old greenhouse plants in March, 1987. The number of developed seeds was determined for ten ripe fruit from each cross.

Table 1. Inbreeding coefficients of four maternal (F_m) and paternal (F_p) parents and the zygotes (F_z) produced from selfing and intermating these parents

		Rubel	Jersey	Bluejay	Spartan
F_m	F_p	0	0	0.033	0.043
Rubel	0	0.167			
Jersey	0	0.083	0.167		
Bluejay	0.033	0.035	0.033	0.194	
Spartan	0.043	0.041	0.038	0.046	0.202
		├──────── F_z ────────┤			

Table 2. Pollen chase results, showing the average seed set per fruit[a] for each pollen source, floral age, and time interval treatment

Pollinations	No. of days after flowering (DAF) when pollination was made				
	0	1	2	3	4
Controls					
'Spartan' selfed	2.0	–	–	–	–
'Spartan' × 'Bluejay'	35.5	–	43.5	–	33.4
Treatments					
('Spartan' selfed) × 'Bluejay'	–	38.3	4.8	2.8	1.9

[a] For seed no. per fruit, Fisher's protected LSD (0.05) = 8.5 and 0.01) = 11.3

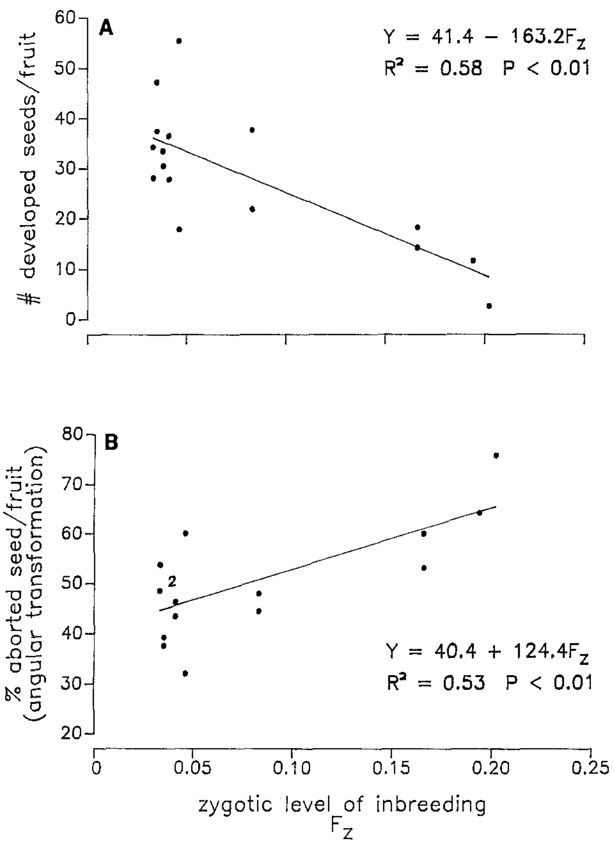

Fig. 1 A and B. Regression of **A** seed set and **B** seed abortion on zygotic levels of inbreeding in 16 highbush blueberry matings

Results

Pollen chase

Outcross seed set was significantly greater than self-seed set (35.5 versus 2.0 seeds per fruit) for control pollinations made zero days after flowering (0 DAF, Table 2). No significant change in fertility with increased flower age was detected over a 5-day interval (0–4 DAF) in 'Spartan' × 'Bluejay' matings. A 1-day delay in application of 'Bluejay' chaser pollen to previously selfed 'Spartan' flowers resulted in 38.3 seeds per fruit, which was not significantly different from the cross-pollinated control. A 2-to 4-day interval between self- and chase cross-pollinations resulted in reduced fertility (1.9–4.8 seeds per fruit) to levels not significantly different from the self-pollinated control.

Diallel cross

Seed yields from the complete diallel cross of four *V. corymbosum* cultivars are presented in Fig. 1 as a function of zygotic inbreeding coefficients (F_z). F_z values were negatively associated with seed number per fruit and positively associated with the proportion of aborted seeds per fruit. Linear regressions of seed yield (Fig. 1 A) and percent seed abortion (Fig. 1 B) on zygotic inbreeding coefficients were significant; F_z accounted for 58% of the variation in developed seed number and 53% of the variation in percent seed abortion. Expanding the linear regression model to include maternal (F_m) and paternal (F_p) inbreeding coefficients did not significantly increase the R^2 values.

S_1 progeny fertility

The mean seed set per self-pollination ranged from 0.2 to 0.7 among the three groups of selfed (S_1) progeny, compared to a 9.5–11.1 range among the parent cultivars (Table 3). There was little segregation for self-fertility within S_1 progeny groups. The percentage of completely self-sterile S_1 segregants was 40%, 50%, and 80% for the 'Bluecrop', 'Jersey', and 'Elliot' groups, respectively; the most self-fertile progeny in each group had seed-set values (1.3–3.6) closer to zero than to parental self-fertility.

From Table 3 it is apparent that severe reductions in fertility occur at low levels of zygotic inbreeding. Self-pollinations of the parent cultivars ($F_z \approx 0.2$) resulted in an average 70% decrease in seed set, relative to outcross levels, and S_1 self-pollinations ($F_z \approx 0.3$) were nearly 100% nonfertile.

Table 3. Comparisons of fertility in three parent cultivars and their selfed (S_1) progeny

Cross	F_z[a]	No. developed seed per pollination[b]	Δ[c]
'Elliot' outcrossed	0.049	46.4	
'Elliot' selfed	0.203	9.5	−79.5
'Elliot S_1' selfed	0.336	0.2 (0–1.3)	−99.6
'Bluecrop' outcrossed	0.043	42.5	
'Bluecrop' selfed	0.194	11.1	−73.9
'Bluecrop S_1' selfed	0.329	0.7 (0–3.6)	−98.3
'Jersey' outcrossed	0.051	29.2	
'Jersey' selfed	0.167	11.0	−63.2
'Jersey S_1' selfed	0.305	0.4 (0–2.0)	−98.7

[a] F_z is the estimated zygotic inbreeding coefficient generated by a mating. Cross-pollinations were made using bulked pollen from 'Spartan', 'Rubel', and 'Bluecrop' cultivars; the average of 3 F_z values generated by these pollinators is given for each outcrossed cultivar

[b] Numbers shown are mean values for ten S_1 progeny with ranges indicated in parentheses

[c] Δ is the percent decrease in seed set relative to outcross values for each parent

Table 4. Pollen stainability of three highbush blueberry cultivars and their selfed (S_1) progeny, measured as percentage of pollen tetrads completely nonviable (0/4 microspores stained) and percentage of total microspores stained[a]

Genotype	F	No. ind. scored	%Tetrads completely unstained	%Stained microspores	Δ[b]
Jersey	0	1	1.6	95.1	
Jersey S_1	0.167	9	2.3 (0–9.0)	83.7 (69.4–96.0)	−12.0
Bluecrop	0.033	1	0	100.0	
Bluecrop S_1	0.194	9	3.1 (0–8.3)	81.3 (56.2–93.5)	−18.7
Elliot	0.044	1	0	100.0	
Elliot S_1	0.203	8	4.9 (0–20.6)	79.2 (41.1–96.8)	−20.8

[a] Pollen data is presented as mean values followed by ranges in parentheses

[b] Δ is percentage change in S_1 mean pollen viability relative to the parent cultivar

Table 5. Comparisons of seed set in reciprocal crosses of highbush blueberry cultivars with their selfed (S_1) progeny[a]

Cross	No. developed seed per fruit	Δ[b]
EL × ELS1-5	7.7	
ELS1-5 × EL	5.1	− 33.7
EL × ELS1-1	27.7	
ELS1-1 × EL	10.7	− 61.4**
JR × JRS1-5	2.9	
JRS1-5 × JR	1.8	− 37.9
JR × JRS1-4	15.5	
JRS1-4 × JR	3.7	− 76.1**
JR × JRS1-10	4.6	
JRS1-10 × JR[c]	0	−100.0
BC × BCS1-5	13.8	
BCS1-5 × BC	6.2	− 55.1**
BC × BCS1-6	8.6	
BCS1-6 × BC	11.7	+ 36.0
BC × BCS1-10	10.6	
BCS1-10 × BC	7.4	− 30.2
BC × BCS1-9	35.5	
BCS1-9 × BC	22.3	− 37.2*
BC × BCS1-7	8.9	
BCS1-7 × BC	4.6	− 48.3**

[a] Cultivars were 'Elliot' (EL), 'Jersey' (JR), and 'Bluecrop' (BC)

[b] Δ is the percentage change in the S_1 progeny × cultivar seed set, relative to the cultivar × S_1 progeny cross

[c] No fruit resulted from these pollinations

*,** Significant at the 5% (*) or 1% (**) levels (t-test)

Paternal and maternal effects

Estimates of pollen viability were very high (94%–100%) in the three parent cultivars studied (Table 4). Average pollen viabilities in the corresponding S_1 progeny groups were lower, ranging from a 12% decrease in the 'Jersey' inbreds to a 21% decrease in 'Elliot' inbreds. Within each S_1 group, some segregants exhibited a marked reduction in pollen staining, while others had viability estimates comparable to the 'noninbred' parental values. Part of the decrease in percent microspores stained could be attributed to an increase in the frequency of completely empty pollen tetrads. Although the average increase in the 0/4 viability class was never greater than 4.9% with one generation of selfing (in 'Elliot' S_1 progeny, Table 4), there was some segregation for this trait, ranging as much as 0%–21% among selfed offspring.

The results of the parent × S_1 reciprocal crosses are given in Table 5. In all but one pair of reciprocals, seed set was lower when the inbred (S_1) acted as female parent, suggesting that inbreeding had a greater effect on female than male fertility. A correlation of male and female fertility in these reciprocal matings was highly significant ($r = 0.84$, calculated from Table 5).

Discussion

Post-zygotic barriers to self-fertility

In a previous study, self-pollen tubes of the cultivar 'Spartan' were observed growing into ovules at the same rate and frequency as 'Bluejay' gametophytes, but resulting in much lower seed yield (Krebs and Hancock 1988). However, it was not possible to detect whether self-fertilization had occurred using fluorescent microscopy. A late-acting incompatibility reaction preventing syngamy between self-pollen nuclei and egg cells present together in the embryo sac, similar to that which has been reported in *Theobroma cacao* (Cope 1962), remained an alternative explanation for the low self-fertility of 'Spartan'.

Crosses identical to the above study were made in the pollen chase experiment. Because pistil receptivity did not decrease over the time course examined (Table 2), fertility differences could be attributed to pollen treatment effects (sources and timing of application). A 2-to 4-day interval between selfing and application of 'Bluejay' chaser pollen resulted in low fertility, comparable to 'Spartan' self-pollinations. This result is not consistent with a SI mechanism, because pre-zygotic barriers to self pollen would have allowed fertilization of ovules by the later-arriving 'Bluejay' gametophytes (a high fertility mating). Alternatively, low seed sets in the pollen chase treatments could be attributed to post-zygotic events: (1) initial self-fertilization of ovules, which precluded later fertilization by the outcross pollen tubes, and (2) subsequent abortion of most self seeds. While it thus appears that self-pollen tubes can fertilize eggs, it was also apparent that they required an adequate 'headstart' (2-day minimum) to reach the ovules before the chaser pollen.

Pollen chase and pollen mixture studies have been used previously as evidence for post-zygotic self-sterility in *Gasteria verrucosa* (Sears 1937) and *Borago officinalis* (Crowe 1971). However, it has been argued that the wrong kind of conclusions can be drawn from these experiments, because a pre-zygotic mechanism – blockage of micropyles by self-pollen tubes – could also prevent fertilization by chaser pollen (Seavy and Bawa 1986). In *Vaccinium*, a well-defined micropyle is absent, and pollen entry into the embryo sac has been described as 'porogamous' (Stushnoff and Palser 1969), raising the possibility of multiple paths of pollen-tube entry into the ovule. The fact that self-pollination frequently results in some developed seeds in addition to aborted seeds, which are considerably larger than unfertilized ovules, further suggests that chaser pollen reaches ovules that have already been self-fertilized.

The effect of inbreeding on fertility

The diallel crosses (Fig. 1) demonstrated that seed yields of highbush blueberry cultivars decreased, and seed abortion increased, with an increase in F_z. This response was primarily due to significant mean differences between self- and outcross fertility. When selfs were omitted from the analysis, the regression line did not differ significantly from zero (data not shown). Apparently, F_z values comparable to full-sib and half-sib matings (0.08 and 0.04 in an autotetraploid) were too low to result in a detectable fertility response.

Continued selfing provided additional evidence for fertility depression with inbreeding. The diallel linear regression equations (Fig. 1) predicted that complete sterility (or total seed abortion) would occur at fairly low F_z values (0.25–0.28). This was observed in self-pollinations of S_1 progeny (Table 3). The three parent cultivars ($F=0.04-0.05$) were self-fertile ($F_z=0.17-0.20$), while their S_1 progeny ($F=0.17-0.20$) had zero or near-zero self-seed set ($F_z=0.31-0.34$). These results suggest that sterility occurs at or beyond a zygotic inbreeding threshold of $F_z=0.30$. In natural populations, a parental inbreeding threshold ($F=0.17-0.20$) may act as an internal 'switch' which influences mating systems in *V. corymbosum* – genotypes below the critical F value are facultative selfers, while those above it are obligate outcrossers.

The lost capacity for autogamy in the S_1 progeny cannot be explained by a genetic model based on *S*-alleles at a single locus (gametophytic SI). Lewis (1979) proposed that the breakdown of gametophytic SI in some species by chromosome doubling (autotetraploidy) was due to 2x pollen heterozygous at the *S*-locus, resulting in a heteromeric incompatibility protein that was no longer 'recognized' by the stylar genotype. According to this interpretation, some selfed progeny of an autotetraploid carrying two or three different *S*-alleles would be self-sterile, while the majority are expected to be as self-fertile as the parent. Most S_1 progeny in this experiment were self-sterile.

The early-acting inbreeding depression model predicts that zygote abortion will regulate the production of outbred progeny in all matings, depending on the amount of inbreeding generated. Our experiments showed the expected decline in fertility with strong inbreeding (selfing) but not in cross-pollinations among relatives. In a study of native diploid, tetraploid, and hexaploid *Vaccinium* accessions, Vander Kloet and Lyrene (1987) observed progressive decreases in seed set resulting from nonrelated, sibling, and self-pollinations for all three ploidal levels. Although self-infertility was described by them as an SI response, it clearly represents an extreme form of inbreeding depression of fertility. Similar trends have been noted in *Medicago sativa*, an autotetraploid species in which fertility is regulated by individual genetic loads, consanguinity in matings, and embryo abortion (Busbice 1968; Fyfe 1957; Cooper and Brink 1940).

A common genetic control of fertility in all blueberry matings is further suggested by significant correlations between self- and cross-fertility in *V. ashei* and *V. corymbosum* cultivars (El-Agamy et al. 1981; Krebs and Hancock 1988) and in a native Michigan population of *V. corymbosum* (S. L. Krebs and J. F. Hancock unpublished results). In a SI system, a correlation of self- and outcross fertility is not expected, since cross-compatibility can range from 0% to 100%. According to the inbreeding depression model, a genotype with a large genetic load of embryo lethal genes will have zero or near-zero self-fertility, and its *average* cross-fertility will also be reduced.

Male and female fertility

Inbreeding had a negative effect on gametic as well as zygotic viability in *V. corymbosum.* This is expected, because the loss of gene interactions in 2x gametes or gametophytes could result in viability depression. Average male fertility (pollen viability) in S_1 progeny was 12%–21% below the parental values (Table 4). Although pollen staining results in higher estimates of viability than those obtained by pollen germination, the two techniques result in similar rankings of gamete fertility among *Vaccinium* genotypes (Cockerham and Galletta 1976). The average loss and variation in pollen viability among selfed offspring can be attributed to nuclear factors, because parents and individuals forming an S_1 progeny group shared a common cytoplasm.

Inbreeding in autotetrapoloid *Secale cereale* resulted in chromosome breakage, pre-meiotic spindle formation, asynapsis, and reduced chiasma frequencies in pollen mother cells (Rees 1961). In autotetraploid *Medicago sativa* and *Solanum tuberosum*, inbred polyhaploids (2x progeny from parthenogenetic embryos in 4x – 2x crosses) frequently exhibit meiotic abnormalities (Yeh et al. 1964; Bingham and Gillies 1971). In *V. corymbosum*, the large number of empty tetrads (0/4 stained microspores) produced by some S_1 segregants (Table 4) suggests that inbreeding depression affects meiosis before or during Metaphase I in these individuals. However, most of the inbred progeny had a high proportion of tetrads with two or more stained microspores, so the average loss in viability is more readily explained by abnormal post-meiotic development. Differences in pollen viability within and among 13 *Vaccinium* species were also attributed to early post-meiotic abortions (Cockerham and Galletta 1976).

A significant maternal effect, which was not detected in the diallel mating, was observed at higher levels of inbreeding between 'parent' × 'S_1 progeny' reciprocal crosses (Table 5). Inbred individuals were less fertile as females than males. A significant correlation of male and female fertility among crosses suggested a common genetic basis – S_1 segregants experiencing the most inbreeding depression had lowest overall fecundity. Because of common cytoplasms, these reciprocal fertility differences are also under nuclear control. The maternal inbreeding effect might be caused by greater impairment of gametogenesis in the ovary (affecting ovule number or viability) than in the anthers. If aborted seed development is related to endosperm breakdown, then maternally carried deleterious genes would play a predominant role in determining the fate of that tissue. Alternatively, the female parent bears most of the reproductive 'cost' of producing offspring, and a reduction in resource availability or disrupted allocations patterns caused by inbreeding might result in the observed maternal effect.

Inbreeding may also affect gametophytic development in *V. corymbosum.* The pollen chase experiment demonstrated that 'Bluejay' pollen applied 1 day after self-pollination of 'Spartan' pistils reached the ovules first. However, gametophytic competition favoring outcross pollen-tube growth was not observed in another report (Vander Kloet and Lyrene 1987), where mixtures of self and nonself pollen resulted in seed set intermediate to self- and outcross controls, suggesting equal competitiveness. In the absence of gametophytic competition, the major reproductive factor limiting the production of inbred progeny in *V. corymbosum* is embryo abortion. Even if competition favoring outcross pollen does occur in natural populations, a high frequency of geitonogamy due to bee visitation patterns (Vander Kloet and Lyrene 1987) limits the effectiveness of gametophytic competition as a 'partial' SI mechanism.

Causes of fertility depression with inbreeding

The association between inbreeding depression and homozygosity is usually explained by two rival hypotheses. In the partial dominance hypothesis, inbreeding depression results from the fixation of recessive or partially recessive deleterious alleles at loci encoding the trait of interest (mutational load). The overdominance hypothesis attributes inbreeding depression to the loss of favorable allelic interactions at these loci (segregational or balanced load). Empirical studies of heterosis in diploid organisms indicate a stronger role for partial dominance and epistasis than for heterozygote advantage (Charlesworth and Charlesworth 1987). In an autotetraploid species, however, the potential for multiple allelism at many loci suggests that overdominance may be an equally important cause of viability depression.

Segregational load could account for the near-complete loss of fertility at $F_z \approx 0.30$ in *V. corymbosum* (the actual level of homozygosity would be less than 30%, assuming that some parental loci were tetraallelic, triallelic, and diallelic 'duplex'). A parallel phenomenon in autotetraploid *Medicago sativa*, where large reductions in fertility and vegetative vigor occur with small increments in F, has been attributed to the rapid loss of multiple-allelism and 'higher-order' heterotic interactions with partial inbreeding (Busbice and Wilsie 1966; Busbice 1968; Dessureaux and Gallais 1969). Using the notation of Busbice and Wilsie (1966), e.g., the genotypic value of a locus carrying four different alleles is the sum of 4 additive gene values plus 11 interaction values – 6 possible two-gene interactions (first order), 4 three-gene interactions (second order), and 1 four-gene interaction (third order). With one generation of selfing

($F=0.167$), approximately 83% of the third order, 67% of the second order, and 47% of the first order interactions are lost at tetra-allelic loci, without any increase in homozygosity. Therefore, if multiple-allelism at many loci is required for normal vigor (e.g., viable seed development), then loss of heterotic interactions and subsequent viability depression (e.g., embryo abortion) could occur rapidly with inbreeding Not surprisingly, much of the breeding strategy for autotetraploid crops is based on 'maximizing heterozygosity' (Bingham 1980).

Severe inbreeding depression of fertility in *V. corymbosum* may also reflect damage caused by mutational load. Models based on mutation-selection equilibria predict that polyploidy should decrease the amount of inbreeding depression caused by the fixation of lethal or sub-lethal mutations, thus allowing evolution of autogamy in allopolyploids (Hedrick 1987; Lande and Schemske 1985) and autopolyploids (Lande and Schemske 1985). However, autopolyploids may be more prone to inbreeding depression than allopolyploids because: (1) they lack the 'fixed' heterozygosity of allopolyploids, and (2) tetrasomic inheritance allows deleterious mutations to accumulate at a much higher equilibrium frequency than under disomic inheritance (Bennett 1976). A fundamental difference in the ability to provide genomic buffering against mutational load is evidenced by the fact that many allopolyploids are capable of selfing (some, such as wheat and oats, are predominantly autogamous), whereas autopolyploid species are usually cross-pollinated (Mac Key 1970).

While embryo abortion may result from segregational or mutational loads exposed by inbreeding, simpler types of gene action could account for severe fertility depression in *V. corymbosum*. One possibility is that epistasis among loci controlling embryogenesis is involved, so that homozygosity for deleterious alleles at one locus overrides the effect of viability alleles at all other loci. With epistasis, complete sterility could result at low levels of inbreeding. Another possible explanation for rapid inbreeding depression of seed set in highbush blueberries is partial dominance for embyo lethal or sub-lethal factors. The correlations between self- and outcross fertility observed in cultivars and at least one native population (see above) suggest that genes conferring sterility in self-pollinations are also being expressed in the heterozygous progeny of cross-pollinations. Klekowski (1988) observed dominant mutational load for fertility traits in ferns, and noted that such mutations can readily accumulate in long-lived plants (e.g., *Vaccinium*). This occurs because: (1) plant reproductive structures are derived from somatic cell lineages, (2) mutations accumulate as a function of age (number of somatic cell cycles), and (3) loci controlling meiosis, gametogenesis, or embryogenesis are selectively neutral throughout the prolonged periods of vegetative growth in perennials.

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