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MECHANISMS AND GENETIC FACTORS CONTROLLING SELF AND OUTCROSS FERTILITY IN VACCINIUM CORYMBOSUM L.

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MECHANISMS AND GENETIC FACTORS CONTROLLING SELF AND OUTCROSS FERTILITY IN *VACCINIUM CORYMBOSUM* L.

By

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ABSTRACT

MECHANISMS AND GENETIC FACTORS CONTROLLING SELF AND OUTCROSS FERTILITY IN VACCINIUM CORYMBOSUM L.

By

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Seed yields in the highbush blueberry, Vaccinium corymbosum L., are typically lower following self-pollination than cross-pollination. However, self-fertility varies among genotypes, ranging from complete self-sterility to individuals with high self seed set. This research was initiated to determine the developmental barriers to inbreeding in Vaccinium and the genetic factors controlling these mechanisms.

Post-zygotic seed abortion (inbreeding depression), rather than genetic self-incompatibility, was proposed as the primary factor affecting fertility in V. corymbosum. This interpretation was based on 1) growth of self pollen tubes into embryo sacs, 2) an inverse relationship between zygotic levels of inbreeding (F_z) and seed set, 3) a positive correlation between F_z and seed abortion, and 4) a strong association between self and outcross fertility. In addition, inbreeding caused a decrease in male and female fertility.

Because this species is an autotetraploid, inbreeding depression (fertility reduction) was attributed to both segregational and mutational load. That is, seed abortion may have been due to homozygosity for sub-lethal recessive

mutations at loci controlling embryogenesis or endosperm development, or a consequence of the loss of heterotic allelic interactions at these loci with inbreeding. The possible number of gene interactions at an autotetraploid locus is much greater than a diploid locus.

Variation in fertility among clones from a natural population of highbush blueberries was interpreted according to this genetic load model. Fertility among seed parents varied widely, ranging from clones that were essentially female sterile to individuals with high seed yields in both self- and cross-pollinations. Estimates of the number of lethal equivalents per zygote (embryonic genetic load) ranged from 2.2-20.4, with a population mean of 9.6, and this variability was significantly associated with individual differences in heterozygosity at 9 enzyme loci. Associations of low pollen viability, reduced female receptivity to pollen tubes, and low seed survivorship suggested a genetic load 'syndrome' affecting various components of the reproductive process in V. corymbosum.

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Guidance Committee:

The journal paper format was chosen for this thesis in accordance with departmental and university regulation. The thesis is divided into four chapter. Chapter 1 has been published in the Journal of the American Society for Horticultural Science. Chapter 2 has been accepted for publication in Heredity. Chapter 3 will be submitted to Theoretical and Applied Genetics. Chapter 4 is intended for publication in Evolution.

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INTRODUCTION

Plants exhibit a wide array of mating systems, ranging from cross-pollinating to self-pollinating to species which are characterized by both types of sexual reproduction. The mating system of a species is often considered an 'evolved' or 'evolving' trait which maintains genetic conditions favorable to its survival and propagation. When outcrossing plants are selfed, or selfers outcrossed, a disruption of the genetic status quo occurs that can have harmful effects on the fitness of the species. In self-pollinating populations, for example, recombination via outcrossing can break up coadapted gene complexes and favorable alleles which had been 'fixed' in the homozygous condition. Among outcrossers, the phenomena of inbreeding depression following self-pollination or sib-matings is well known. It is therefore not surprising to find that plants which are characterized by one type of mating system use a variety of mechanisms to prevent the opposite type from occurring.

The present study deals with one aspect of this issue, namely the means by which outcrossers avoid inbreeding. Generally, this occurs in two ways: 1) mechanisms which reduce the amount of inbred (usually self) pollen that lands

on stigmas (pre-mating barriers), or 2) mechanisms which prevent or reduce seed set following self-pollination (post-mating barriers to inbreeding). The first strategy is evident in plants which have evolved sexual dimorphisms (dioecy, monoecy), asynchronous development of floral parts (protandry, protogyny), or special floral architectures (stigma exsertion, heterostyly). The second (post-mating) kind of mechanisms, which are more characteristic of hermaphroditic plants, consist of systems for stylar recognition and 'rejection' of self pollen (genetic self-incompatibility, SI), or abortion of self-fertilized ovules.

The highbush blueberry, Vaccinium corymbosum is considered a predominantly outcrossing species with many factors - insect pollination, protogyny, and inverted flowers - promoting cross-pollination. When selfing does occur, following hand-pollination (or geitonogamy), there are additional barriers to inbred seed production. My research was primarily focused on this aspect of the mating system in V. corymbosum, with the goal of determining the mechanisms and genetic factors responsible for reduced seed set following self-pollination.

Perhaps the most salient feature of self-fertility (or conversely, self-sterility) in Vaccinium is the wide variation within and among species for this trait. Diploids are mostly self-sterile, while tetraploids and hexaploids range from zero to moderate (and occasionally high) levels of self-fertility. This variability proved crucial in

forming initial genetical hypotheses to test (under the assumption that fertility differences had a genetic basis). Of the two possible mechanisms for inbreeding avoidance following self-pollination, pre-zygotic SI or post-zygotic seed abortion, the latter is more likely to account for continuous variation in self-fertility. In SI species, all individuals are expected to have zero or near-zero self seed set. Alternatively, abortion of self-fertilized ovules, which can be considered a form of inbreeding depression caused by the 'unmasking' of embryo-lethal mutations in the homozygous condition, could easily vary from genotype to genotype, depending on the numbers of deleterious alleles carried by individuals.

Most of the experiments in this thesis were designed to test the 'inbreeding depression' model for fertility reduction in V. corymbosum. The separate chapters are presented in a chronological order, since some questions raised in prior studies were pursued in subsequent analyses. Chapter 1 is a preliminary study which looked at the cytology of self pollen tube growth and characterized self versus outcross seed set in 6 highbush blueberry cultivars. Chapter 2 investigates an issue tangential to fertility, but directly related to the discussion of inbreeding - mode of inheritance of allozyme markers in tetraploid V. corymbosum. Chapter 3 is a collection of several experiments which tested the relationship between zygotic levels of inbreeding, seed set, and seed abortion. In addition, the

effect of inbreeding on male fertility (pollen viability) and female fertility was examined. A discussion of inbreeding depression and genetic load (both mutational and segregational) as factors affecting embryo abortion (i.e. seed set) took into account a tetrasomic mode of inheritance in this species. Chapter 4 assesses variability in fertility in a natural V. corymbosum population in light of the previous genetical studies. The specific objectives were to quantify (embryonic) genetic load in the population, to correlate genetic load with average heterozygosity among individuals, and to determine the associations between gametic, gametophytic, and embryonic components of fertility.

CHAPTER 1

THE CONSEQUENCES OF INBREEDING ON FERTILITY IN *VACCINIUM CORYMBOSUM* L.

Abstract

Seed counts from self- and cross-pollinated highbush blueberry cultivars suggested that fertility in both mating systems is under similar genetic control. Viable seed set following selfing and outcrossing was inversely correlated with zygotic levels of inbreeding, and percentage seed abortion in both crosses showed a positive association with zygotic F-values. Among six genotypes, cross- and self-fertility were highly correlated. Fluorescent microscopy revealed no differences in the frequency of self and foreign pollen tube growth into ovules. Variation in self- and cross-fertility among these cultivars was attributed to differences in zygotic levels of homozygosity and cumulative expression of recessive mutations which promote seed abortion.

Introduction

Seed set following self-pollination of blueberries, Vaccinium section Cyanococcus, varies both within and among the 3 predominant ploidy levels. Diploid populations consist of individuals which are self-sterile (2,14), whereas wild collections of tetraploid V. angustifolium and hexaploid V. ashei exhibit low to moderate levels of self-fertility, (1,9). Viable seed yield following self-

pollination of tetraploid highbush cultivars, V. corymbosum, ranges from 0 to 75 percent of cross-pollinated seed production (4,8,15,17). No clones have been identified which are equally cross- and self-fertile. Hexaploid ('rabbiteye') cultivars show less variation in self-fertility than highbush cultivars, but also have much lower levels of seed set in both types of mating (8,9).

Self-incompatibility has been proposed as the basis for selfed-seed reduction in all ploidies of blueberries (2,8,9,17). However, only among diploid blueberry species is one of the expectations of a true, self-incompatibility system met - that all clones taken from a genetically diverse population show zero or near zero seed set when self-pollinated. Genetic self-incompatibility is generally defined as a mechanism for maintaining strict allogamy via a self-rejection process in which self-pollination does not result in seed set (6,12,19). Variation in self-fertility can otherwise be attributed to differences in the degree of inbreeding depression, expressed as seed abortion, that occurs when different genotypes are selfed. Among Angiosperms, inverse correlations between viable seed yield and zygotic levels of inbreeding have been documented in several genera which lack self-incompatibility barriers (3,5,19).

In this study, we measured the effects of selfing and outcrossing on seed and fruit parameters of six highbush tetraploid cultivars of V. corymbosum. Since the inbreeding

coefficient (F-value) of each variety is known (10), it was also possible to test whether or not differences in F-values among test plants could account for variable levels of seed set after self- and cross-pollinations.

Materials and Methods

Crossing Studies

The 6 cultivars (20-year-old plants) used in this study were growing in a complete randomized design at the Michigan Blueberry Growers Research Station in Grand Junction, Michigan. Both cross- and self-pollinations were made on 4 separate ramets of each cultivar in May, 1985. One hundred pollinations were made for each crossing treatment (25 per replication). The pollen used for outcrossing consisted of a bulk sample collected from all 6 cultivars in roughly equivalent amounts, gauged by using equal numbers of flowers as pollen sources. Pollinations were made on flowers in which the style was elongated but the stigma was still enclosed by the petals, which were removed to expose the pistil. Cross-pollinated flowers were emasculated by removing the stamens. All inflorescences pollinated were tagged and covered with cheesecloth, which was removed 2 weeks later when the ovaries showed signs of swelling. In July, the ripe fruit was harvested weekly and bulked for each variety x crossing treatment, without separating fruit from the 4 replications. Where possible, 50 or more fruits per treatment were weighed, and counts

were made of fully developed seed per fruit (plump tan or brown seed) and aborted seed per fruit (shrunken or flattened, usually light colored seed). Unfertilized ovules were much smaller than either of the above seed classes, and were not visible without the aid of a dissecting microscope.

Inbreeding effects on seed and fruit characters were estimated with simple correlation matrices based on treatment mean values. Inbreeding coefficients of the 6 cultivars ranged from 0 to 0.19, based on pedigree analysis (10). In the case of self-pollinations, estimates of zygotic levels of inbreeding were exactly proportional to initial F-values of the seed parents (Fm). However, zygotic inbreeding estimates following outcrossing may not have paralleled maternal F-values, since pollen bulks were used in cross-pollinations, and each of the 6 source varieties may have differed in its contribution to the bulk, in the growth rates of its pollen, and in its degree of relatedness to the seed parent. Nonetheless, since Fm is the only 'controlled' parameter in either type of mating, it was used as an estimator of the average level of inbreeding in progeny (zygotes) resulting from both self- and cross-pollinations.

Pollen Cytology

Pollen tube growth studies were carried out using fluorescent microscopy in order to determine whether or not self pollen could enter ovules normally and at the same frequency as foreign pollen. Two crosses known to represent

extremes in seed set ability, 'Spartan' selfed and 'Spartan' x 'Bluejay', were made in the greenhouse in February, 1986. The method of pollination was as described above, except that flowers were emasculated for selfs as well as outcrosses. Twenty flowers were pollinated for each cross, and from these 10 fruits per cross were harvested at maturity for seed counts. From the remaining pollinations, pistils were harvested 2 and 6 days after pollination (DAP), fixed in 1:3 glacial acetic acid:ethanol, and stored in 70 percent ethanol. Tissue preparations for fluorescent microscopy involved a modification of Martin's technique (13): the samples were soaked overnight in 8N NaOH, rinsed in several changes of distilled water (1 to 2 hours), and stained for 10 to 20 minutes in 0.1 percent aniline blue dissolved in a 0.7 percent potassium phosphate (tribasic) buffer, adjusted to a pH of 8.5. Pollen tube growth and ovule penetration was examined under UV light in 5 sample squashes from each harvest date.

Results

Effects of Self- Versus Cross-Pollination

The effects of mating type on seed numbers and berry weights are given in Table 1. Selfing resulted in a 4 to 29 percent reduction in fruit weight depending on the cultivar. Self-pollinated berries had significantly fewer viable seeds, equal or greater numbers of aborted seeds, and often a lower total seed number than cross-pollinated fruit from

Table 1. Means for seed and fruit characters following self- and cross-pollination in 6 highbush blueberry cultivars.

Cross	F_m[1]	n	Fruit weight (g)		# viable seed per fruit		# aborted seed per fruit		# total seed per fruit		% aborted seed per fruit	
			$\bar{x}$	Δ[2]	$\bar{x}$	Δ	$\bar{x}$	Δ	$\bar{x}$	Δ	$\bar{x}$	Δ
'Rubel' outcrossed	0	51	0.96		22.7		25.8		48.5		56	
'Rubel' selfed	0	50	0.82	-0.15*	11.8	-0.48**	25.7	0	37.5	-0.23*	71	0.27**
'Jersey' outcrossed	0	75	1.64		48.4		22.4		70.8		32	
'Jersey' selfed	0	51	1.16	-0.29**	15.1	-0.69**	41.0	0.83**	56.1	-0.21**	75	1.34**
'Bluejay' outcrossed	.094	45	1.14		9.76		23.1		32.9		75	
'Bluejay' selfed	.094	66	1.09	-0.04	6.23	-0.36*	41.3	0.78**	47.5	0.44**	89	0.19**
'Bluecrop' outcrossed	.110	56	2.36		26.7		29.3		56.0		57	
'Bluecrop' selfed	.110	59	1.87	-0.21**	10.7	-0.60**	51.5	0.78**	62.2	0.11	84	0.47**
'Spartan' outcrossed	.182	76	2.51		9.47		49.8		59.2		87	
'Spartan' selfed	.182	81	1.91	-0.24**	1.27	-0.86**	44.0	-0.12	45.2	-0.24**	98	0.13**
'Elliot' outcrossed	.188	79	2.03		43.7		21.0		64.6		34	
'Elliot' selfed	.188	56	1.60	-0.21**	7.7	-0.82**	25.5	0.21*	33.2	-0.48**	77	1.26**

1 Inbreeding coefficient of maternal parent.

2 Δ is the proportion change following self-pollination relative to cross-pollination.

* P<.05, t-test for significant mean differences within a cultivar.

** P<.01

the same test cultivar. Seed set per fruit among selfs ranged from 1.3 to 15.1, well below fertility values in outcrosses, which averaged 9.5 to 48.4 seeds per fruit. All cultivars showed a significant increase in the proportion of aborted seeds per fruit after selfing.

Relationships of Seed, Fruit, and Inbreeding Parameters

The correlations between maternal inbreeding coefficients (Fm) and seed parameters are shown in Table 2. None of the correlations were significant at the 5 percent level. Viable seed number per fruit was inversely correlated with Fm in both self-pollinations (r=-0.80) and cross-pollinations (r=-0.23). Mean selfed and outcrossed seed yields were positively correlated with one another (r=0.70, calculated from Table 1). These trends indicate that a high F-value in the seed parent was associated with reduced seed set in both types of mating. Conversely, cultivars with low inbreeding coefficients generally showed high levels of both self- and cross-fertility.

The proportion of aborted seeds per fruit (aborted seed divided by total seed) showed a positive correlation with Fm following selfing (r=0.69) and outcrossing (r=0.32). The nature of this response differed in the two types of matings. In self-pollinated fruit, viable and aborted seed number were almost independent (r=-0.10) while in cross-pollinations they were strongly interdependent (r=-0.60). The increased proportion of seed abortion in selfed cultivars was due primarily to the greater loss of viable

Table 2. Simple correlations of fruit, seed, and inbreeding parameters by pollination treatment.[1]

	Fm	Fruit weight	Viable seed #	Aborted seed #	Total seed #
Self-pollinations					
Fruit weight	0.79				
Viable seed #	-0.80	-0.52			
Aborted seed #	-0.08	0.51	-0.10		
Total seed #	-0.28	0.25	0.35	0.90	
% aborted seed	0.69	0.67	-0.87	0.57	0.15
Cross-pollinations					
Fruit weight	0.71				
Viable seed #	-0.23	0.05			
Aborted seed #	0.44	0.60	-0.59		
Total seed #	0.06	0.54	0.77	0.06	
% aborted seed	0.32	0.14	-0.96	0.76	-0.59

[1]For n=6, P<.05 when r=0.81.

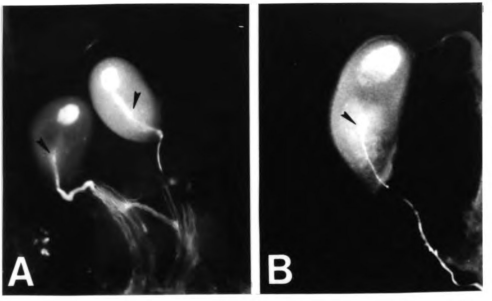
seed without a concomitant increase in the number of aborted seeds - total seed number declined with viable seed reduction (Table 2). Among cross-pollinated plants, total seed number remained fairly constant, and the two seed classes varied inversely.

Fruit weight increased with viable seed number per fruit within a cultivar (Table 1). However, this association did not always hold for comparisons among varieties. Table 2 shows that viable seed number and berry weight were negatively correlated in self-pollinations (r=-0.52), and negligibly correlated in cross-pollinations (r=0.05). In contrast, both aborted and total seed number showed positive associations with weight of selfed and outcrossed fruit (Table 2). The relationship between berry weight and maternal inbreeding level was opposite that of seed yield and Fm. Large sized fruit was associated with cultivars having high Fm values (r=0.79, selfed, and r=0.71, outcrossed) and low fertility.

Pollen Cytology

Fluorescent microscopy revealed that both self and cross pollen reached the base of the style 2 DAP, and that 6 DAP both types of pollen were entering the ovules. Figures 1A and 1B show examples of ovule penetration by self pollen tubes - the pollen tube tip appears to have entered the embryo sac in the vicinity of the egg nucleus. It was not possible to determine if fertilization had occurred. The results of a comparison of pollen tube growth in a highly

Figures 1A and 1B. Fluorescent photomicrographs of self pollen entering ovules in 'Spartan', 6 DAP. Arrows indicate position of pollen tube tips at the basal end of the embryo sac (micropylar haustorium). The large staining region in the chalazal end of the ovules is due to callose deposits in the nucellus or integument. (X130, Fig. 1A and X180, Fig. 1B).



Figures 1A and 1B.

Table 3. Comparative rates of ovule penetration by self and foreign pollen on 'Spartan', 6 DAP.

Cross	# viable seeds/ fruit	# ovules/ovary showing tube penetration	% of total ovules/ovary penetrated
'Spartan' selfed	2.50	18.4	26.3
'Spartan' x 'Bluejay'	55.4	17.0	27.8

fertile cross-pollination versus a nearly sterile self-pollination are given in Table 3. The percentage of ovules showing tube penetration 6 DAP did not differ significantly between the two crosses, although seed set in 'Spartan' x 'Bluejay' was almost 25 times greater than that in 'Spartan' selfed.

Discussion

The 6 cultivars used in this study showed a range of viable selfed seed sets, and this variation was inversely correlated with the degree of inbreeding in the seed parent ($P<0.10$). This trend suggests a system in which the reduction in seed set following self-pollination is dependent on the zygote's own genotype rather than an interaction between the pollen and maternal genotypes. Under severe forms of inbreeding such as selfing, recessive lethal or deleterious mutations which are normally carried at low frequency in the heterozygous state (genetic load) can be expressed in the homozygous condition. It is reasonable to assume that such deleterious genes exist in a normally outcrossing species such as V. corymbosum, and that expression of some of them would inhibit zygotic or early embryonic development. If the rate of embryo failure is determined by number of loci homozygous for such mutations, then the amount of seed set following self-pollination is expected to be inversely proportional to the average level of homozygosity accumulated in both the seed parent and its

developing offspring. Reduced fertility resulting from inbreeding has been documented in Medicago sativa (3), Borago officinalis (5), and several gymnosperms (18,20). In all these cases, self-pollen can enter the ovules and fertilize the egg nuclei.

Following cross-pollination, seed set and seed abortion also decreased and increased, respectively, with higher inbreeding coefficients in the seed parent. Cultivar inbreeding coefficients were not as accurate predictors of fertility in outcrosses as in selfs, but as mentioned above, the zygotic levels of inbreeding resulting from bulk cross-pollinations could not be estimated with precision. Seed yield from both types of mating were highly correlated: individuals which were least self-fertile were also generally least cross-fertile. This raises the possibility that fertility levels in both selfing and outcrossing are under similar genetic control, dependent upon parental levels of inbreeding and relatedness, and ultimately the genetic constitution of the resulting zygote. Our calculations from data presented by El-Agamy et al (8) show that a significant correlation between selfed and outcrossed seed yield also exists among four Florida highbush cultivars ($r=0.63$) and seven hexaploid varieties ($r=0.86$).

An earlier study of inbreeding depression in highbush and rabbiteye blueberries by Hellman and Moore (11) demonstrated that in crosses of varying relatedness, seed set was frequently an inverse function of progeny F-values,

whereas seed germination and seedling growth rates rarely showed a negative response to increased levels of inbreeding. Inbreeding depression may affect any stage of sporophyte development, and our data indicates that in V. corymbosum it may have a strong impact during seed development. Abortion of highly inbred embryos and survival of 'higher quality' offspring would tend to reduce the amount of inbreeding depression manifested during seed germination, seedling growth, or later stages of progeny development. In natural systems, such early acting inbreeding depression, which could increase the average fitness of seedlings produced, may play an important role in the evolution of long-lived plants (18,20).

Whether or not the shrunken and flattened seeds observed in blueberry fruits are all the result of post-zygotic abortion remains uncertain. The cytological results from one test plant showed that self pollen can enter ovules at the same frequency as foreign pollen, regardless of the fate of that pollen in producing viable offspring. Fertility differences are therefore not reflected in different pollen behavior between the time it lands on the stigma and reaches the embryo sac. It is not known if fertilization by either type of pollen is actually occurring at the same rate - however, differential fertilization by self and foreign male nuclei, once they are present in the ovule, is rare, and has been documented only in one species, Theobroma cacao (19). The large size difference between

unfertilized ovules and aborted seeds (approximately 20 fold, personal obs.) further supports the assumption that this class of seeds represent ovules which were fertilized, partially developed, and subsequently aborted.

Cultivated highbush blueberries do not appear to have a mating system involving self-incompatibility (SI). The results from this study are not consistent with the characteristics of a true incompatibility system (6) which are 1) zero selfed seed set and strict allogamy among individuals in a population, 2) maternal recognition and rejection of the haploid pollen genotype and 3) lack of correlation between selfed and outcrossed seed set. In a gametophytic SI system, for example, a self-sterile plant may be 0 to 100 percent cross fertile with other individuals, depending on their S-allele constitutions.

On the other hand, our attempt to provide an alternative genetic model - one in which variation in self-compatibility is explained in the context of inbreeding depression - has been only partially successful. Although the cytological evidence indicated that self-pollen enters the embryo sac normally, the crossing data failed to provide unequivocal support for the notion that selfed-seed failure is a post-zygotic event which magnitude depends on the zygotic level of inbreeding. The lack of significant associations between inbreeding coefficients and fertility parameters could represent experimental 'noise' rather than an inherent flaw in the hypothesis. In particular, scoring

seed types is partially unreliable, since some shrunken seeds may not have been fertilized (overestimating abortion), while in other cases, during severe inbreeding for example, embryo failure may have occurred so early in development that it was not detected (underestimating abortion). This latter situation might account for the fact that in the selfed (but not the outcrossed) cultivars there was no increase in aborted seed number paralleling the steady decline in viable seed set with increasing Fm.

This study has some potential applications for improvement of commercial blueberries. It shows, as other reports have, that cross-pollination will increase seed number and berry weight over levels achieved by self-pollination of any cultivar (7,15,16). This supports suggestions that mixed varietal plantings with synchronous flowering periods are preferable to monocultures for maximizing fruit weight. An alternative approach is to locate and select wild genotypes which are equally cross- and self-fertile, in addition to having desirable fruit characters. Surveys of a lowbush population (21 clones) and wild rabbiteye accessions (19 clones) have not revealed highly self-fertile variants (1,9). However, if fertility in polyploid blueberries is polygenically controlled, as proposed above, then variation in self-fertility is expected to be continuous, including genotypes (those with few deleterious recessives) which approach normal seed production.

Selection for high fertility *per se* will not guarantee large fruit size. This study shows that both aborted and total seed number have a greater positive effect on berry weight than does viable seed number alone. Possibly, factors such as the frequency of pollinator visitations, pollen load, and the number of fertilization events per ovary are more important for fruit development than a genotype's intrinsic seed yield capacity. Additional studies are needed to determine the time of seed abortion and proportion of fruit growth that occurs during early seed development, prior to abortion. Futhermore, berry weight is dependent on clonal differences in the ability of a developed seed to provide a hormonal stimulus to fruit growth (16). Our results reaffirm this independence of seed number and fruit size in comparisons across genotypes. Some of the larger-fruited cultivars in this study, such as 'Spartan', have relatively low seed set. Trends among the 6 cultivars in this report suggest that highbush blueberry pedigree breeding has historically involved selection for increased fruit size in cultivars despite higher levels of inbreeding and reduced overall fertility.

Literature Cited

1. Aalders, L. E. and I. V. Hall. 1961. Pollen incompatibility and fruit set in lowbush blueberries. Can. J. Genet. Cytol. 3:300-307.
2. Ballington, J. R. and G. J. Galletta. 1978. Comparative crossability of 4 diploid Vaccinium species. J. Amer. Soc. Hort. Sci. 103:554-560.
3. Busbice, T. H. 1968. Effects of inbreeding on fertility in Medicago sativa L. Crop Sci. 8:231-234.
4. Colville, F. V. 1937. Improving the wild blueberry. U.S. Dep. Agric. Yearb. Agric. 559-574.
5. Crowe, L. K. 1971. The polygenic control of outbreeding in Borago officinalis. Heredity 27:111-118.
6. de Nettancourt, D. 1977. Incompatibility in angiosperms. Springer-Verlag, Berlin.
7. Eaton, G. W. 1967. The relationship between seed number and berry weight in open-pollinated highbush blueberries. HortScience 2:14-15.
8. El-Agamy, S. Z., W. B. Sherman, and P. M. Lyrene. 1981. Fruit set and seed number from self- and cross-pollinated highbush (4X) and rabbiteye (6X) blueberries. J. Amer. Soc. Hort. Sci. 112:443-445.
9. Garvey, E. J. and P. M. Lyrene. 1987. Self-incompatibility in 19 native blueberry selections. J. Amer. Soc. Hort. Sci. 112:856-858.

10. Hancock, J. F. and J. H. Siefker. 1982. Levels of inbreeding in highbush blueberry cultivars. HortScience 17:363-366.

11. Hellman, E. W. and J. N. Moore. 1983. Effect of genetic relationship to pollinizer on fruit, seed, and seedling parameters in highbush and rabbiteye blueberries. J. Amer. Soc. Hort. Sci. 108:401-405.

12. Lewis, D. 1979. Sexual incompatibility in plants. Studies in Biology No. 110. Edward Arnold, London.

13. Martin, F. W. 1959. Staining and observing pollen tubes in the style by means of flouresence. Stain Technol. 34:125-128.

14. Meader, E. M. and G. M. Darrow. 1944. Pollination of the rabbiteye blueberry and related species. Proc. Amer. Soc. Hort. Sci. 45:267-274.

15. Meader, E. M. and G. M. Darrow. 1947. Highbush blueberry experiments. Proc. Amer. Soc. Hort. Sci. 49:196-204.

16. Moore, J. N., B. D. Reynolds, and G. R. Brown. 1972. Effects of seed number, size, and development on fruit size of cultivated blueberries. HortScience 7:268-269.

17. Morrow, E. B. 1943. Some effects of cross-pollination versus self-pollination in the cultivated blueberry. Proc. Amer. Soc. Hort. Sci. 42:469-472.

18. Orr-Ewing, A. L. 1965. Inbreeding and single crossing in Douglas-Fir. Forest Sci. 11:279-290.

19. Seavy, S. R. and K. S. Bawa. 1986. Late-acting self-incompatibility in angiosperms. Bot. Rev. 52:195-219.

20. Sorensen, F. 1969. Embryonic genetic load in coastal Douglas-Fir, Pseudotsuga menziesii var menziesii. Am. Nat. 103:389-398.

CHAPTER 2

TETRASOMIC INHERITANCE OF ISOENZYME MARKERS IN THE HIGHBUSH BLUEBERRY, *VACCINIUM CORYMBOSUM* L.

Abstract

Segregation ratios at four enzyme loci (*Mdh*-2, *Pgi*-2, 6*Pgd*-2, and *Got*-3) in *Vaccinium corymbosum* (4X) cultivars were in close agreement with the expectations of tetrasomic inheritance. Random chromosome segregation was suggested by the absence of double reduction progeny genotypes and previous reports of bivalent pairing in this species. A common species origin of autotetraploid highbush blueberry and other *Vaccinium* polyploids is proposed. Different genetic and evolutionary consequences of polysomic inheritance versus disomic inheritance are discussed with reference to the literature on polyploids.

Introduction

The subgenus of 'true' blueberries, *Vaccinium* section *Cyanococcus* (X=12), is composed of diverse and widely distributed species which form a polyploid complex of diploids, tetraploids, and hexaploids. There is currently no consensus regarding the origins of these polyploids, whether they derived from intra- or interspecific hybridization among the diploid species, and subsequently whether they are autopolyploids, allopolyploids, or segmental allopolyploids. The nature of genome amplification in the cultivated highbush blueberry, *V.*

corymbosum (4X), is of particular interest, since knowledge of chromosome pairing behavior and mode of inheritance at the tetraploid level will affect breeding strategies in addition to providing information about phylogenetic relationships within this subgenus.

Polyploidy in blueberry species has been discussed mainly on the basis of morphology, ecology, and cytology. In the monograph on eastern North American Cyanococcus species by Camp (1945), V. corymbosum was designated an allotetraploid hybrid complex. An allopolyploid origin for V. corymbosum and four related tetraploid taxa was suggested by the finding that the tetraploids as a group exhibited higher pollen stainability than a group of seven diploid species (Cockerham and Galletta, 1976). Several cytogenetic studies of meiotic pairing and disjunction in highbush cultivars revealed a preponderance of bivalent formation, which was interpreted as evidence for non-random, preferential pairing of chromosomes (Newcomer, 1941; Stushnoff and Hough, 1968; Jelenkovic and Hough, 1970). However, in these same studies and others which have examined tetraploid cultivars and related species (Jelenkovic and Harrington, 1971), multivalents and secondary associations of bivalents at metaphase I were occasionally observed, indicating that V. corymbosum may be an autopolyploid. Regular chromosome pairing in polyploids is not conclusive evidence for allopolyploidy, since many autopolyploids undergo extensive diploidization of their

genomes such that multivalent formation among homologues is restricted.

Inheritance data are the least equivocal means of distinguishing between auto-and allotetraploidy. Because of preferential pairing in allotetraploids, they exhibit independent assortment at the duplicated loci (disomic inheritance) and fixed heterozygosity where these loci are homozygous for different alleles. Segregation in autotetraploids is tetrasomic - for diagnostic heterozygous genotypes, random association of the four homologues during the first meiotic division results in a lower frequency of homozygous gametes than would be produced by an allotetraploid of the same genotype. Draper and Scott (1971) observed segregation ratios for the recessive 'albino seedling' trait in V. corymbosum and obtained results largely consistent with tetrasomy. However, they found a significant excess of heterozygotes which was attributed to some degree of preferential pairing among chromosomes bearing the marker gene, or possibly to segregation distortion caused by the lethality of the mutant albino allele.

Inheritance studies of morphological characters in Vaccinium have historically been limited by the scarcity of single gene polymorphisms (Galletta, 1975; Lyrene, 1988). Our objective was to tap an alternate source of marker genes, isoenzyme polymorphisms, for observing segregation patterns in the tetraploid highbush blueberry, V.

corymbosum. There are many precedents for this kind of analysis in plants. Electrophoretic evidence of tetrasomic inheritance has been obtained for Medicago sativa (Quiros, 1982), Solanum tuberosum (Martinez-Zapater and Oliver, 1984; Quiros and McHale, 1985), Haplopappus spinulosus (Hauber, 1986), Tolmiea menziesii (Soltis and Soltis, 1988), Heuchera micrantha (Soltis and Soltis, in press), and Heuchera grossulariifolia (Wolf et al., submitted). Among allopolyploids, disomic inheritance has been documented for duplicated enzyme loci in tetraploid Tragapogon mirus and T. miscellus (Roose and Gottlieb, 1976), triplicated loci in hexaploid Triticum aestivum (Hart, 1983), and quadruplicated loci in octaploid Fragaria X ananassa (Arulsekar et al, 1981).

Materials and Methods

Progeny were derived from crosses among four tetraploid highbush cultivars, V. corymbosum, which differed genotypically at several electrophoretic loci. These cultivars were 'Rubel', a wild plant selection, and 'Jersey', 'Bluejay', and 'Spartan', genotypes which have resulted from one to five cycles of pedigree selection beyond the initial wild accessions. Seeds were germinated and seedlings grown in a growth chamber (14 hour daylength, 15-25°C diurnal temperatures). Beginning with the 5-6 leaf stage of seedling growth, tissue was prepared for starch gel electrophoresis.

The extraction buffer was made as follows: 0.1 M K-phosphate buffer (pH 7.5), 5 mM EDTA, 10 mM dithiothreitol, 0.1% 2-mercaptoethanol, and 0.5% Tween-80. To make a stock solution of 0.1 M phosphate buffer pH 7.5, 84 ml of 0.2 M K_2HPO_4 (dibasic) was added to 16 ml of 0.2 M KH_2PO_4 (monobasic) and diluted with H_2O to a final volume of 200 ml. After dissolving the rest of the components, the extraction buffer was adjusted to pH 8.0 with 4 N KOH.

Prior to leaf protein extraction, insoluble PVPP (Sigma #P-6755) was hydrated overnight in the above extraction buffer. Seedling leaves were ground in spot plate wells containing extraction buffer (approx. 5:1, v/w, buffer: tissue ratio) plus a small amount (weighing spatula tip) of the hydrated PVPP. The crude extract resembled a wet slurry rather than a thick paste. The leaf extract was soaked onto filter paper wicks through a 35μ nylon screen. Throughout the process, all materials were maintained at 4°C.

Seven enzyme systems have been consistently resolved from blueberry leaf extractions; four of these were used in the present study. The gel and electrode buffer systems, as well as the enzyme staining protocols, were adopted from previous reports without major modifications. The staining schedules which gave best results were those of Conkle et al. (1982) for glutamate oxaloacetate transaminase (GOT) and 6-phosphogluconate dehydrogenase (6PGD), Soltis et al. (1983) for isocitrate dehydrogenase (IDH), shikimate dehydrogenase (SKDH), and phosphoglucomutase (PGM), and Vallejos (1984)

for phosphoglucose isomerase (PGI) and malate dehydrogenase (MDH). Standard gel and electrode buffers used were the morpholine-citrate system, pH 6.1 (Clayton and Tretiak, 1972) for resolving MDH, IDH, 6PGD, and SKDH, and the lithium borate, tris-citrate system, pH 8.3 (Scandalios, 1969) for resolving GOT, PGI, and PGM.

For each segregating locus, a chi-square test of fit of progeny ratios to the following genetic models was performed: monogenic inheritance (diploidy), digenic-disomic inheritance (allotetraploidy), and digenic-tetrasomic inheritance (autotetraploidy). Segregants were classed by electrophoretic phenotype, heterozygous or homozygous, rather than by genotype. This approach is more reliable since it does not require assigning gene dosages based on a visual assessment of differential band staining intensity among heterozygotes.

Results

Segregation was observed at six loci encoded by four dimeric enzymes: Mdh-1, Mdh-2, Got-3, Pgi-2, 6Pgd-1, and 6Pgd-2 (Figs. 1-3, Table 1). In all segregating crosses, one or both parents were tri-banded, diallelic heterozygotes carrying one to three copies of each allele per locus. The effect of gene dosage on electrophoretic phenotype appeared to be a multiplicity of heterozygote banding patterns. Diallelic parents and progeny exhibited either a 'balanced' tri-banded phenotype in which the two homodimers were equal

Figure 1. Tetraploid segregation patterns at two malate dehydrogenase (MDH) loci in the cross 'Bluejay' x 'Jersey'. MDH is a dimeric enzyme. Anodal direction is above. Numbers and letter superscripts at lefthand margins of photograph indicate putative loci and alleles, respectively. Bottom letters refer to parents (A and B) and representative progeny which have been assigned genotypes for illustrative purposes. In this cross, parental genotypes at the Mdh-1 locus are aaaa x aaab, and at the Mdh-2 locus the parent genotypes are abbb x abbb. For Mdh-1, the expected progeny genotypic ratio is 1 aaaa (C,E): 1 aaab (D) or a phenotypic ratio of 1 a:1 ab. The expected genotypic ratio for Mdh-2 is 1aabb (C): 2 abbb (E): 1 bbbb (D) or a phenotypic ratio of 3 ab: 1 a (digenic-disomic or digenic-tetrasomic. ID designates a putative interlocus heterodimer formed between Mdh-2^b and Mdh-3.

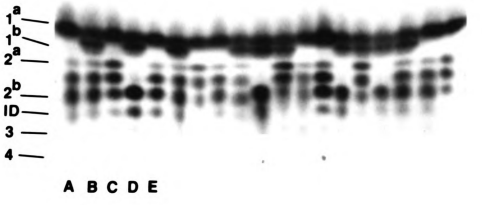


Figure 1.

Figure 2. Progeny segregation at a phosphoglucose isomerase locus, Pgi-2, in the cross 'Spartan' x 'Bluejay', parental genotypes aaab x aabb. Numbering, lettering, and gel orientation are described in Figure 1. The expected genotypic distribution among offspring is 1 aaaa (E): 5 aaab (D): 5 aabb (C): 1 abbb (F), or a phenotypic ratio of 11 ab: 1 a (digenic-tetrasomic).

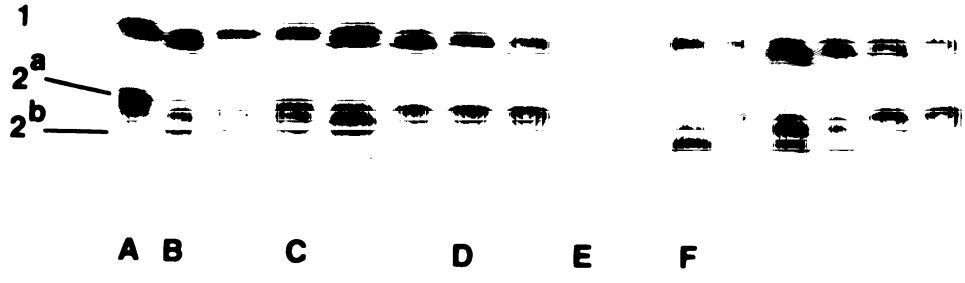


Figure 2.

Figure 3. **Progeny segregation at two 6-phosphogluconate dehydrogenase (6PGD) loci in the cross 'Spartan' x 'Rubel'. Numbering, lettering, and gel orientation are described in Figure 1. For the 6Pgd-1 locus (Parental genotypes aaaa x aaab) the expected progeny genotypic ratio is 1 aaa (C,E): 1 aaab (D,F). For 6Pgd-2, the expected tetrasomic segregation pattern is 1 bbbb (D): 5 abbb (F): 5 aabb (C): 1 aaab (E) or a phenotypic ratio of 11 ab: 1 b.**

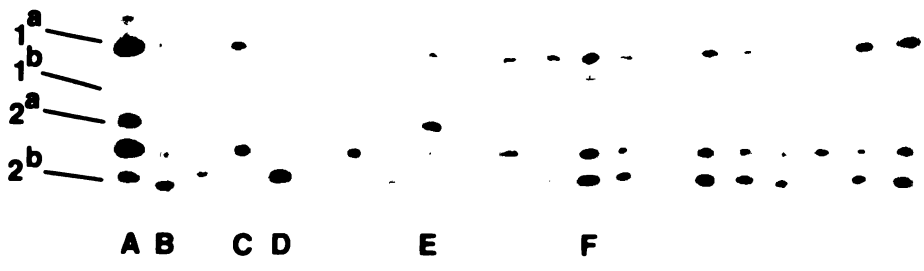


Figure 3.

in staining intensity and both less intensely stained than the heterodimer mid-band, or one of two 'unbalanced' phenotypes in which one homodimer and the heterodimer had greater staining intensity than the remaining homodimer (Figs. 1-3, A-E). None of the parent cultivars showed complex banding patterns (six or more bands per locus) indicative of tri- or tetra-allelic genotypes for dimeric proteins.

Chi-square tests for mode of inheritance in tetraploid V. corymbosum are given for four diagnostic loci in Table 1. At the 6Pgd-2, Pgi-2, and Got-3 loci, observed progeny phenotypic ratios were consistently best explained by the assumption of tetrasomic inheritance, with P values ranging from 0.25 to 0.90. For these three loci, the alternative genetic hypothesis, digenic-disomic inheritance, was statistically rejected ($P < 0.05$) in all crosses. Segregation ratios at a fourth locus, Mdh-2, provided evidence for gene duplication (i.e. digenic control) without distinguishing between disomy and tetrasomy. The 3:1 phenotypic distribution among progeny at this locus most likely results from a cross between diallelic parents which are both carrying the same allele, Mdh-2^b, in triplicate (Fig. 1). Monogenic control of Mdh-2 segregation in these crosses would result in a 1:2:1 expected phenotypic ratio that is clearly at odds with the observed distribution.

Progeny distributions of 1:1 were observed for several loci and parental combinations: 6Pgd-1 in the crosses

Table 1. Chi-square tests for mode of inheritance at four electrophoretic loci.

Locus	Parental genotypes		Progeny phenotypes			χ^2	P
			b	ab			
6*Pgd*-2	Spartan x Rubel	Exp. ratio					
	(*aabb* x *abbb*)	Disomic*	1	7		5.40	0.025
		Tetrasomic	1	11		0.18	0.70
		Obs. no.	18	226			
			a	ab	b		
	Bluejay x Jersey	Exp. ratio					
	(*aabb* x *aabb*)	Disomic	1	14	1	11.66	0.01
		Tetrasomic	1	34	1	0.27	0.85
		Obs. no.	4	172	5		
			a	ab	b		
	Spartan x Bluejay	Exp. ratio					
	(*aabb* x *aabb*)	Disomic	1	14	1	13.96	0.002
		Tetrasomic	1	34	1	2.98	0.25
		Obs. no.	1	160	4		
			a	ab			
Pgi-2	Spartan x Bluejay	Exp. ratio					
	(*aaab* x *aabb*)	Disomic	1	7		4.75	0.03
		Tetrasomic	1	11		0.24	0.65
		Obs. no.	14	183			

Table 1. (continued)

Locus	Parental genotypes		Progeny phenotypes		χ^2	P
			a	ab		
Got-3	Spartan x Rubel	Exp. ratio				
	(aaaa x aabb)	Disomic	1	3	9.88	0.002
		Tetrasomic	1	5	0.04	0.90
		Obs. no.	38	200		
			a	ab		
Mdh-2	Spartan x Bluejay	Exp. ratio				
	(abbb x abbb)	Disomic or Tetrasomic	1	3	0.26	0.60
		Obs. no.	41	137		
			a	ab		
	Bluejay x Jersey	Exp. ratio				
	(abbb x abbb)	Disomic or Tetrasomic	1	3	0.92	0.35
		Obs. no.	34	125		
			a	ab		
	Spartan x Rubel	Exp. ratio				
	(abbb x abbb)	Disomic or Tetrasomic	1	3	0.05	0.85
		Obs. no.	63	181		

*Alleles were assumed to be in a configuration that would not result in fixed heterozygosity.

'Spartan' X 'Rubel' (Fig. 3) and 'Bluejay' X 'Jersey', Got-3 in the cross 'Spartan' X 'Bluejay', Pgi-2 in the cross 'Spartan' X 'Rubel', and Mdh-1 in the cross 'Bluejay' X 'Jersey' (Fig. 1). Inheritance data from these crosses is not reported in Table 1 because the parental genotypes precluded discrimination between monogenic and digenic genetic control. Where progeny phenotypic ratios of 1:1 resulted from a homozygote X diallelic heterozygote cross, no quantitative distinction could be made between diploid and tetraploid inheritance at that locus. Six putative enzyme loci were monomorphic among the four parents: Mdh-3, Mdh-4, Got-1, Got-2, Got-4, and Pgi-1.

Discussion

Segregation ratios at four enzyme loci were in close agreement with a genetic model which included 1) tetrasomic inheritance, 2) codominant expression of alleles coding for dimeric enzymes, and 3) random chromosome segregation. In conjunction with the previous report of 'albino seedling' inheritance (Draper and Scott, 1971), our report of tetrasomic segregation of allozyme markers provides strong evidence for an autopolyploid origin of V. corymbosum. The data were concordant with several other aspects of autotetraploidy. There was no evidence of fixed heterozygosity, which is a predictable and consistently observed consequence of allopolyploidy (Roose and Gottlieb, 1976; Gottlieb, 1981; Werth et. al., 1985). Differences in

gene copy number (dosage) resulted in multiple diallelic phenoyptes at a single locus. Electrophoretic surveys of several taxa have shown that, as expected, autotetraploid individuals can carry up to four different alleles per locus (Quiros, 1982; Crawford and Smith, 1984; Soltis and Rieseberg, 1986). Tri- and tetra-allelic genotypes were not present in this study. However, a survey of a natural V. corymbosum population in Michigan has revealed multiple allelism at the Pgi-2, Pgm-2, and Mdh-1 loci (Krebs and Hancock, unpubl.).

No chromatid segregation was observed in our crosses. Double reduction events would have been detectable, for example, as aaaa progeny resulting from an abbb X abbb cross. For all segregating loci in the present study, there was at least one cross involving parental genotypes diagnostic for these exceptional events. The lack of double reduction segregants is somewhat surprising, since multivalent formation - a prerequisite for this kind of genetic non-disjunction - is reported in tetraploid highbush blueberries. One to five quadrivalents per cell were observed in clones which exhibited primarily bivalent associations (Roussi, 1966; Jelenkovic and Harrington, 1971). It is quite possible that the marker allozymes used in this analysis map to homologues which do not form multivalents. Lack of chromatid segregation in Heuchera grossulariifolia (Wolf et. al., submitted) and H. micrantha (Soltis and Soltis, in press) was attributed to localization

of enzyme loci on short chromosomes (~2 μm) with low chiasma frequencies. Multivalent formation may be similarly restricted by the small size of many Vaccinium chromosomes, which vary from 1.3 to 2.5 μm, with 10 of the 12 chromosomes less than 2 μm in length (Hall and Galletta, 1971). Regardless of whether or not they map to bivalent or multivalent-forming chromosomes, these marker allozymes may also be centromere-linked and thus restricted in crossing-over. Either scenario would result in random chromosome segregation based on the electrophoretic evidence.

Several lines of evidence indicate that there has been little genomic divergence during speciation in the Cyanococcus subgenus. Tetrasomic inheritance in V. corymbosum indicates that its diploid progenitors are probably geographic races of a common species. Structural and genetic homology of the Vaccinium genome is also evidenced by the lack of chromosome karyotype differences among diploid species (Hall and Galletta, 1971) as well as the high degree of interspecific fertility within the three predominant ploidal levels (Galletta, 1975; Ballington and Galletta, 1978). It has been noted (Lyrene and Ballington, 1986) that much of the current disagreement over species designations in this section (Camp, 1945; Vander Kloet, 1980) has its source in the confounding effects of frequent interspecific hybridization and differentiation of local races. Our report suggests that Camp's (1945) original assessment of both auto- and alloployploids in Vaccinium

section Cyanococcus was incorrect, and that the higher ploid taxa are problably polysomic polyploids like V. corymbosum.

Tetrasomic inheritance is a single-locus model for duplicate gene segregation. This feature, in contrast to the spontaneous generation of independent, duplicate loci by allotetraploidy, has notable genetic and evolutionary consequences. The adaptive success of polyploids (estimates as high as 50% of all plant taxa) is freqently attributed to the ability of gene duplication events to increase levels of heterozygosity, create novel hybrid characters, and allow structural or functional divergence in gene expression (Roose and Gottlieb, 1976; Stebbins, 1980; Tal, 1980; Gottlieb, 1982; Levin, 1983; Soltis and Rieseberg, 1986). The extent to which these phenomena occur and are maintained in populations depends largely on the polyploid organism's origins and mode of inheritance.

Increased heterozygosity has been electrophoretically documented in allopolyploids (Roose and Gottlieb, 1976; Hart, 1983; Werth et.al., 1985) and autopolyploids (Soltis and Rieseberg, 1986; Lumaret, 1986; Ness et al., in press) relative to their diploid ancestors. Enzyme multiplicity in either type of polyploid may confer biochemical versatility and genotypic 'buffering': in allopolyploids, increased heterozygosity frequently has the added advantage of resulting in novel heteromeric enzymes not found in the diverged diploid parent species (Roose and Gottlieb, 1976; Werth et.al., 1985). Electrophoretic variation in

autopolyploids is typically not unique, since it represents a subset of the diversity which already exists in the common ancestor taxon (Crawford and Smith, 1984; Soltis and Rieseberg, 1986; Ness et al., in press).

Strategies for maintaining heterozygosity differ between the two types of polyploids. In response to inbreeding, tetrasomic polyploids have a much slower approach to homozygosity than disomic polyploids (Haldane, 1930), but where autotetraploids can ultimately be fixed (four identical alleles) at single loci, multilocus allotetraploids are buffered against homozygosis by fixed heterozygosity. Mating systems are present which complement these differences. All autopolyploids, including V. corymbosum (Krebs and Hancock, 1988), maintain heterozygosity via cross-fertilization and exhibit low fertility in response to inbreeding. In contrast, most allopolyploids are self-compatible and several are predominantly self-pollinated (Mac Key, 1970).

Tetrasomy versus disomy will also affect the likelihood of duplicate gene divergence. Redundant, independently segregating loci are required to allow fixation of null alleles or alleles with altered specificity. Hence the best examples of gene silencing or divergence among polyploids are disomic organisms such as Triticum aestivum (Hart, 1983), Chenopodium quinona (Wilson et.al., 1983), or the catastomid fishes (Buth, 1983). According to Levin (1983), autopolyploids are expected to undergo duplicate gene

evolution at a much slower rate than allopolyploids, since a shift from tetrasomic to disomic segregation is required. There is no clear evidence that this process has actually occured in a polysomic polyploid. The presence of multi-allelic genotypes and gene dosage effects in polyploid species of Medicago (Quiros, 1982), Solanum (Martinez-Zapater and Oliver, 1984), Tolmiea (Soltis and Soltis, 1988), Coreopsis (Crawford and Smith, 1984), and Vaccinium (present study), is evidence that duplicate genes have maintained expression. Duplicate gene silencing was reported in an autotetraploid lily species, Urginia maritima, a conclusion which was based on the appearance of individuals homozygous for nulls at an esterase locus (Oliver et.al., 1983). However, other genotypes expressed alleles at this locus, suggesting that functional and non-functional allozymes were segregating at a single locus, and that gene silencing had not occured at the population level. Salmonid fishes provide a single putative instance of an ancient autopolyploid which has passed from tetrasomy to disomy ('residual' tetrasomic inheritance occurs at some enzyme loci, in males only) and subsequently shows divergence of duplicate gene expression (Allendorf and Thorggard, 1984; Buth, 1983).

Whether or not the subgenus Vaccinium section Cyanococcus meets these expectations of autopolyploid evolution remains to be established. Indeed, polysomic inheritance has yet to be determined in other tetraploid

and hexaploid blueberry species. Furthermore, comparative studies of diploid, tetraploid, and hexaploid populations are required to determine the genetic consequences of genome amplification in this section. Studies of isoenzyme variation offer a valuable approach to these problems. A growing body of evidence, including the identification of 'new' autopolyploids, indicates that polysomic speciation is fairly prevalent and not necessarily maladaptive (Levin, 1983; Soltis and Soltis, 1988).

Literature Cited

1. Allendorf, F.W. and Thorggaard, G.H. 1984. Tetraploidy and the evolution of Salmonid fishes. In Turner, B.J. (ed.). Evolutionary Genetics of Fishes, Plenum Press, New York, pp. 1-53.
2. Arulsekar, S., Bringhurst, R.S. and Voth, V. 1981. Inheritance of PGI and LAP isozymes in octaploid cultivated strawberries. J. Amer. Soc. Hort. Sci., 106, 679-683.
3. Ballington, J.R. and Galletta, G.J. 1978. Comparative crossability of 4 diploid Vaccinium species. J. Amer. Soc. Hort. Sci., 103, 554-560.
4. Buth, D.G. 1983. Duplicate isozyme loci in fishes: origins, distributions, phyletic consequences, and locus nomenclature. In Rattazzi, M.C., Scandalios, J.G., and Whitt, G.S. (eds.). Isozymes, Vol. 10: Genetics and Evolution, A.R. Liss, New York, pp. 381-400.
5. Camp, W.H. 1945. The North American blueberries with notes on other groups of Vacciniaceae. Brittonia, 5, 203-275.
6. Clayton, J.W. and Tretiak, D.N. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fisheries Res. Board. Can., 29, 1169-1172.
7. Cockerham, L.E. and Galletta, G.J. 1976. A survey of pollen characteristics in certain Vaccinium species. J. Amer. Soc. Hort. Sci., 101, 671-676.

8. Conkle, M.T., Hodgskiss, P.D., Hunnally, L.B. and Hunter, S.C. 1982. Starch gel electrophoresis of conifer seeds: a laboratory manual. USDA Forest Service Technical Report PSW-64, Pacific Southwest Forest and Range Experiment Station, Berkeley.

9. Crawford, D.J and Smith, E.B. 1984. Allozyme divergence and intraspecific variation in Coreopsis grandiflora (Compositae). Syst. Bot., 9, 219-225.

10. Draper, A.D. and Scott, D.H. 1971. Inheritance of albino seedlings in tetraploid highbush blueberry. J. Amer. Soc. Hort. Sci., 96, 791-792.

11. Galletta, G.J. 1975. Blueberries and cranberries. In Moore, J.N. and Janick, J. (eds.). Advances in Fruit Breeding, Purdue Univ. Press, Lafayette, pp. 154-196.

12. Gottlieb, L.D. 1981. Electrophoretic evidence and plant populations. Progress Phytochem., 7, 1-47.

13. Gottlieb, L.D. 1982. Conservation and duplication of isozymes in plants. Science, 216, 373-380.

14. Haldane, J.B.S. 1930. Theoretical genetics of autopolyploids. J. Genet., 22, 359-372.

15. Hall, S.H. and Galletta, G.J. 1971. Comparative chromosome morphology of diploid Vaccinium species. J. Amer. Soc. Hort. Sci., 96, 289-292.

16. Hart, G.E. 1983. Genetics and evolution of multilocus isozymes in hexaploid wheat. In Rattazzi, M.C., Scandalios, J.G., and Whitt, G.S. (eds.). Isozymes, Vol. 10: Genetics and Evolution, A.R. Liss, New York, pp. 365-380.

17. Hauber, D.P. 1986. Autotetraploidy in Haplopappus spinulosus hybrids: evidence from natural and synthetic tetraploids. Amer. J. Bot., 73, 1595-1606.

18. Jelenkovic, G. and Harrington, E. 1971. Nonrandom chromosome associations at diplotene and diakinesis in a tetraploid clone of Vaccinium australe Small. Can. J. Genet. Cytol., 13, 270-276.

19. Jelenkovic, G. and Hough, F. 1970. Chromosome associations in the first meiotic division in three tetraploid clones of Vaccinium corymbosum L. Can. J. Genet. Cytol., 12, 316-324.

20. Krebs, S.L. and Hancock, J.F. 1988. The consequences of inbreeding on fertility in Vaccinium corymbosum. J. Amer. Soc. Hort. Sci., 113, 914-918.

21. Levin, D.A. 1983. Polyploidy and novelty in flowering plants. Am. Nat., 122, 1-25.

22. Lumaret, R. 1986. Doubled duplication of the structural gene for cytosolic phosphoglucose isomerase in the Dactylis glomerata L. polyploid complex. Mol. Biol. Evol., 3, 499-521.

23. Lyrene, P.M. 1988. An allele for anthocyanin-deficient foliage, buds, and fruit in Vaccinium elliottii. J. Hered., 79, 80-82.

24. Lyrene, P.M. and Ballington, J.R. 1986. Wide hybridization in Vaccinium. HortSci., 21, 52-57.

25. Mac Key, J. 1970. Significance of mating systems for chromosomes and gametes in polyploids. Hereditas, 66, 165-176.

26. Martinez-Zapater, J.M. and Oliver, J.L. 1984. Genetic analysis of isozyme loci in tetraploid potatoes (Solanum tuberosum L.). Genetics, 108, 669-679.

27. Newcomer, E.H. 1941. Chromosome markers of some species and varieties of Vaccinium and related genera. Proc. Amer. Soc. Hort. Sci., 38, 468-470.

28. Ness, B. D., Soltis, D. E. and Soltis, P. S. Autopolyploidy in Heuchera micrantha (Saxifragaceae). Amer. J. Bot. In press.

29. Oliver, J.L., Martinez-Zapater, J.M., Pascual, L., Enriquez, A.M., Ruiz-Rejon, C. and Ruiz-Rejon, M. 1983. Different genome amplication mechanisms and duplicate gene expression in Liliaceae. In Rattazzi, M.C., Scandalios, J.G. and Whitt, G.S. (eds.) Isozymes, Vol. 10: Genetics and Evolution, A.R. Liss, New York, pp. 341-363.

30. Quiros, C.F. 1982. Tetrasomic inheritance for multiple alleles in alfalfa. Genetics, 101, 117-127.

31. Quiros, C.F. and McHale, N. 1985. Genetic analysis of isozyme variants in diploid and tetraploid potatoes. Genetics, 111, 131-145.

32. Roose, M.L. and Gottlieb, L.D. 1976. Genetic and biochemical consequences of polyploidy in Tragapogon. Evolution, 30, 818-830.

33. Rousi, A. 1966. Cytological observations on some species and hybrids of Vaccinium. Zuchter/Gen. Brdg. Res., 36, 352-359.

34. Scandalios, J.G. 1969. Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem. Genet., 3, 37-79.

35. Soltis, D.E., Haufler, C.H., Darrow, D.C. and Gastony, G.J. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J., 73, 9-27.

36. Soltis, D.E. and Rieseberg, L.H. 1986. Autopolyploidy in Tolmiea menziesii (Saxifragaceae). Amer. J. Bot., 73, 310-318.

37. Soltis, D.E. and Soltis, P.S. 1988. Electrophoretic evidence for tetrasomic segregation in Tolmiea menziesii (Saxifragaceae). Heredity, 60, 375-382.

38. Soltis, D.E. and Soltis, P.S. Tetrasomic inheritance in Heuchera micrantha (Saxifragaceae). J. Hered. In press.

39. Stebbins, G.L. 1980. Polyploidy in plants: Unsolved problems and prospects. In Lewis, W.H. (ed.) Polyploidy, Plenum Press, New York, pp. 495-520.

40. Stushnoff, C. and Hough, L.F. 1968. Sporogenesis and gametophyte development in 'Bluecrop' and 'Colville' highbush blueberries. Proc. Amer. Soc. Hort. Sci., 93, 242-247.

41. Tal, M. 1980. Physiology of polyploids. In Lewis, W.H. (ed.). Polyploidy, Plenum Press, New York, pp. 61-75.

42. Vallejos, C.E. 1984. Enzyme activity staining. In Tanksley, S.D. and Orton, T.J. (eds.). Isozymes in Plant Genetics and Breeding, Part A, Elsevier Science, Amsterdam, pp. 469-516.

43. Vander Kloet, S.P. 1980. The taxonomy of the highbush blueberry, Vaccinium corymbosum. Can. J. Bot., 58, 1187-1201.

44. Werth, C.H., Guttman, S.F. and Eshbaugh, W.H. 1985. Electrophoretic evidence of reticulate evolution in the Appalachian Asplenium complex. Syst. Bot., 10, 184-192.

45. Wilson, H.D., Barber, S.C. and Walters, T. 1983. Loss of duplicate gene expression in tetraploid Chenopodium. Biochem. Syst. Ecol., 11, 7-13.

46. Wolf, P.G., Soltis, P.S. and Soltis, D.E. Tetrasomic inheritance and chomosome pairing behavior in the naturally occurring autotetraploid Heuchera grossulariifolia (Saxifragaceae). Genome. Submitted.

CHAPTER 3

GENETIC LOAD AND MATING SYSTEM IN THE HIGHBUSH BLUEBERRY, *VACCINIUM CORYMBOSUM* L.

Abstract

Both cultivated and wild Vaccinium corymbosum clones exhibit wide variability in seed set following self- and cross-pollinations. In this report, a post-zygotic mechanism (embryo abortion) under polygenic control is proposed as the basis for fertility differences in this species. 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 (S1) progeny from self-fertile parents were largely self-sterile. At zygotic levels of inbreeding of $F>0.30$ there was little or no fertility, suggesting an inbreeding 'threshold' exists which effectively controls mating systems in V. corymbosum. Individuals below the threshold are facultative selfers, while those above it are obligate outcrossers. Inbreeding also caused a decrease in pollen viability, and 'inbred' x 'non-inbred' reciprocal crosses demonstrated that female fertility was reduced more rapidly by inbreeding 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 balanced polymorphisms essential for embryonic vigor). An attempt is made to address the debate over 'late-acting'

self-incompatibility systems versus 'early-acting' inbreeding depression, as it applies to post-zygotic self seed failure in highbush blueberries.

Introduction

The tetraploid highbush blueberry, Vaccinium corymbosum, is primarily an outcrossing species with features such as pendant flowers, protogyny, and insect pollinators contributing to 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 almost complete self-sterility (Ballington and Galletta, 1978; Mead and Darrow, 1944). Low to moderate levels of self-fertility, relative to outcross seed set, are characteristic of most tetraploid and hexaploid blueberry cultivars (Morrow, 1943; Meader and Darrow, 1947; El-Agamy et al., 1981; 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 previously described as 'self-incompatibility'

(Ballington and Galletta, 1978; Morrow, 1943; El-Agamy et al, 1981; Garvey and Lyrene, 1987). However, studies have only recently been initiated to determine the developmental mechanisms and genetic factors controlling these barriers to self-fertility in Vaccinium. In an earlier report on self- and cross-fertility in six highbush cultivars, we observed 1) self pollen tubes entering ovules at the same frequency as foreign pollen tubes, 2) an inverse relationship between self-seed set and zygotic inbreeding coefficients, 3) variable self-fertility among genotypes, and 4) a positive correlation between self and outcross levels of seed production (Krebs and Hancock, 1988). It was noted that these phenomena are inconsistent with those typical of a genetic self-incompatibility system, 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. We hypothesized that mating success in V. corymbosum is polygenically controlled, regulated by post-zygotic levels of inbreeding and cumulative expression of recessive mutations causing seed abortion.

In this paper we report further evidence in support of this hypothesis. Although fluorescent microscopy revealed self pollen tubes entering ovules normally (Krebs and Hancock, 1988), it was not possible by this technique to detect if fertilization had occurred. This leaves open the possibility of a 'late-acting' incompatibility reaction preventing syngamy between male nuclei and egg cells similar

to that which has been documented in Theobroma cacao (Cope, 1962). Therefore, a pollen-chase experiment was performed to determine whether reduced seed set following self-pollination of V. corymbosum is caused by pre-zygotic or post-zygotic factors. In addition, a diallel cross was made to provide an additional test of the relationship between seed set, seed abortion, and levels of inbreeding in parents and progeny (zygotes). Inbreeding coefficients (F values) could be estimated for all matings in this experiment, unlike in our previous report where cross-pollinations were made with bulked pollen samples (Krebs and Hancock, 1988). Groups of self (S1) progeny from several highbush blueberry cultivars were used to document the effects of higher levels of inbreeding on seed set (zygotic viability) and on paternal and maternal fertility (gametic viability). Since gametes from tetraploid V. corymbosum are 2X, allelic interactions resulting in heterosis or inbreeding depression can occur in both pollen and egg cells.

Estimates of inbreeding in V. corymbosum must be re-evaluated in light of new evidence that this species is an autotetraploid (Krebs and Hancock, in press). Because of tetrasomic inheritance, autotetraploids subjected to inbreeding approach homozygosity at a much slower rate (~1/3) than diploids or polyploids having disomic inheritance (Haldane, 1930; Dessureaux and Gallais, 1969). Previous calculations of inbreeding coefficients for tetraploid highbush blueberry cultivars, based on pedigree

analysis and the assumption of disomy (Hancock and Siefker, 1982), are therefore overestimates. Furthermore, it is important to note that F values in an autotetraploid species do not refer directly to homozygosity, as is the case in diploids (Busbice and Wilsie, 1966; Dessureaux and Gallais, 1969). Because autotetraploids can carry up to four different alleles per locus, inbreeding causes loss of 'higher-order' heterozygosity (tetra-allelic -> tri-allelic -> di-allelic) in addition to increasing homozygosity (di-allelic -> mono-allelic). Analysis of the effects of inbreeding on fertility in V. corymbosum must take these considerations into account.

Materials and Methods

Pollen Chase

Crosses were made on two 4-year-old ramets (vegetative clones) of the cultivar 'Spartan' in a greenhouse during April, 1988. Using the method of Moore (1964), opened flowers were removed one day, the plants were caged overnight, and all unopened flowers were removed the following day. Pollinations were thus made on opened blossoms which were of a uniform stage of development and had been emasculated just prior to mating. The plants were removed from the cages only for periodic pollinations. Two matings, 'Spartan' selfed and 'Spartan' x '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' x 'Bluejay' matings were made 2 DAF and 4 DAF, on previously unpollinated flowers to determine pistil receptivity to pollen. The pollen chase treatments were made by applying 'Bluejay' pollen to previously selfed 'Spartan' flowers at a 1, 2, 3 or 4 day interval between the two pollinations. Number of developed (plump, brown) seed per fruit was determined for ten ripe fruit from each treatment.

Diallel Cross

Four highbush blueberry cultivars were inter-crossed in all combinations, including self-pollinations, in a field experiment at Grand Junction, Michigan during the spring of 1986. The individual ramets used were 20-year-old plants grown in a completely randomized design. Twenty pollinations per mating were made on each of two randomly chosen ramets (replicates). Pollination techniques were identical to those employed in an earlier field experiment (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 (plump, brown) and aborted (shrunken, pale) seeds per fruit. An additional parameter, the percentage of aborted seeds per fruit (aborted/ aborted + developed), was arcsine-transformed to normalize the distribution. The relationships between these fertility parameters and inbreeding coefficients 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 matings (F_Z) are given in Table 1. These values were derived by pedigree analysis using the probabilistic 'identity by descent' method defined for diploids by Wright (1922) and modified for application to autotetraploids by Kempthorne (1957). Under the assumption of tetrasomic inheritance and random chromosome segregation (zero double reduction), the inbreeding coefficient of an individual I with parents X and Y is

$$F_I = 1/6\ (F_X + 4\ r_{XY} + F_Y)$$

where r_{XY} is the coancestry between the parents. From this expression it is apparent that inbreeding in offspring from autotetraploid matings can occur if the parents are related (identity between uniting gametes), or inbred and non-related (identity within 2X gametes). For our analysis, it was assumed that the initial highbush blueberry selections from which the pedigree derived were non-inbred and non-related.

S1 Progeny Fertility

1) S1 self-pollinations: groups of ten selfed (S1) progeny from three highbush cultivars, and one ramet of each parent cultivar, were self-pollinated in a greenhouse during March, 1986. The cultivars - 'Elliott', 'Jersey', and 'Bluecrop' - and their S1 progeny were 2-3 year-old plants grown in pots. Approximately 20 self-pollinations were made per individual. In addition, each of the parent cultivars

Table 1. Inbreeding coefficients of 4 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_p	0	0	.033	.043
	F_m				
Rubel	0	.167			
Jersey	0	.083	.167		
Bluejay	.033	.035	.033	.194	
Spartan	.043	.041	.038	.046	.202
		F_z			

was cross-pollinated using bulked pollen from 'Spartan', 'Rubel', and 'Bluejay' cultivars, as previously described. Self-pollinated flowers were not emasculated. Ten ripe fruit per cross were sampled from most genotypes, although some S1 progeny showed zero or very low self fruit set. To adjust for differences in fruit set, numbers of developed (plump, brown) seed were calculated on a per pollination basis rather than a per fruit basis.

2) Parent x S1 progeny reciprocal crosses: the same population of highbush cultivars and selfed offspring was used to generate 'non-inbred' x 'inbred' and 'inbred' x 'non-inbred' matings. Twenty pollinations per cross were made on 3-4 year-old potted plants in a greenhouse during March, 1987. The number of developed (plump, brown) seeds was determined for ten ripe fruit from each cross.

3) Pollen stainability: fresh pollen collected from single ramets of the three parent cultivars and S1 progeny was stained with 0.05% aniline blue in lactophenol (Stanley and Linskens, 1974) and observed under a light microscope. This stain is specific for callose deposits in microspore walls and cytoplasms. A minimum of 100 pollen tetrads per genotype were examined, and counts made of the number of tetrads showing 0/4, 1/4, 2/4, 3/4, or 4/4 microspores stained. From these data, the percentage of total stained microspores ($n \geq 400$) was calculated.

Results

Pollen Chase

Comparison of control pollinations made zero days after flowering (0 DAF) showed that out-crossed seed set (35.5 seeds per fruit) was significantly greater than self-seed set (2.0 seeds per fruit, Table 2). There was no loss of fertility with increased flower age over the five day interval (0-4 DAF) in the 'Spartan' x 'Bluejay' crosses; seed set was highest when pollinations were made 2 DAF. Seed production following treatment of previously selfed 'Spartan' flowers with 'Bluejay' chaser pollen was affected by the length of the time interval between the two pollinations. A one-day delay resulted in an average 38.3 seeds per fruit, not significantly different from the cross-pollinated controls (33.4-43.5 seeds per fruit, Table 2). A 2-4 day interval between self- and cross-pollinations caused a sharp reduction in fertility (1.9-4.8 seeds per fruit) to levels not significantly different from the self-pollinated control.

Diallel Cross

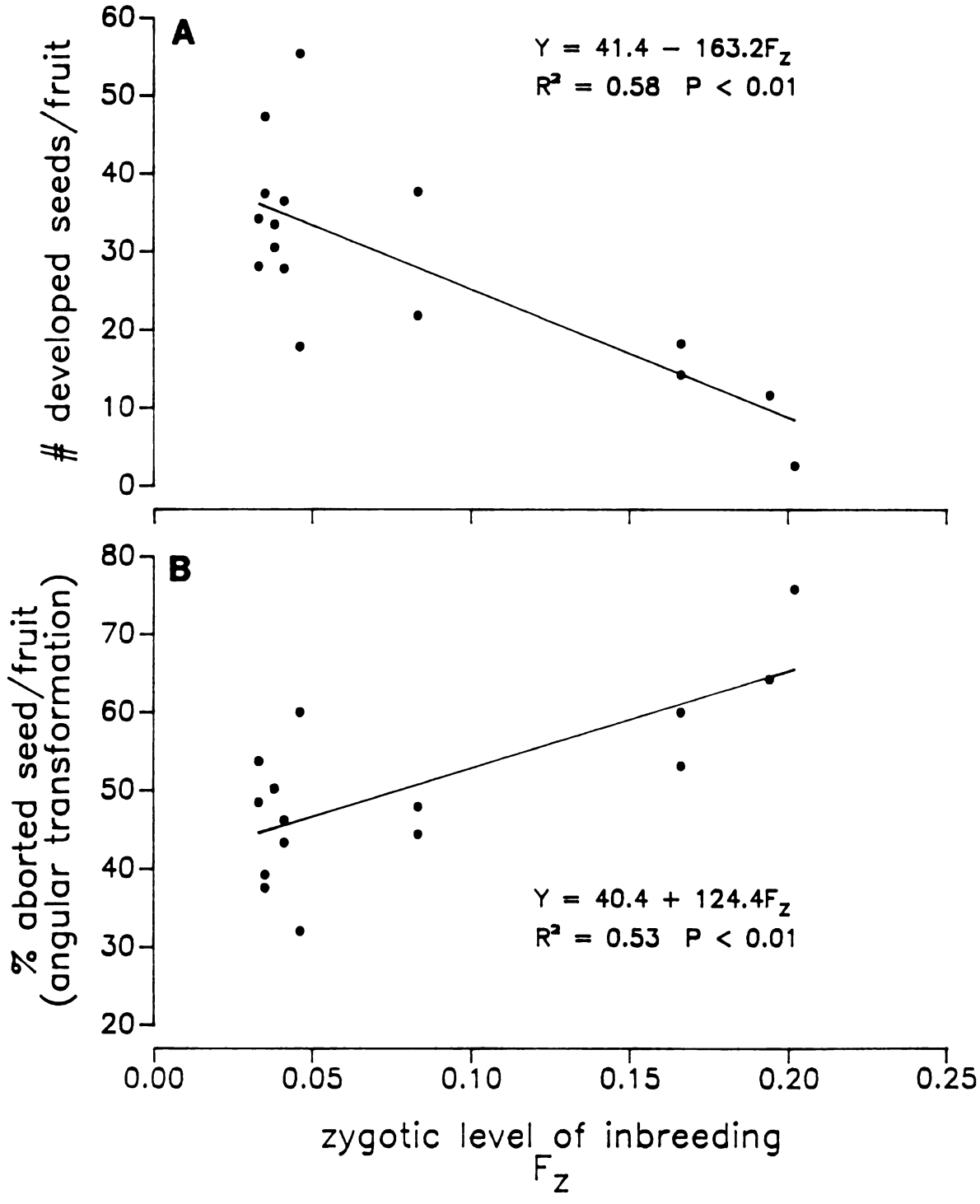
Seed yields from the diallel cross of four *V. corymbosum* cultivars are presented in Figure 1 as a function of zygotic inbreeding coefficients. A decrease in the number of developed seeds per fruit, and an increase in the proportion of aborted seeds per fruit, was associated with an increase in the level of inbreeding among progeny. Linear regressions of seed yield (Fig. 1A) and seed abortion

Table 2. Pollen chase results, showing the average seed set per fruit[1] for each pollen source, flower age, and time interval treatment.

Pollinations	Number of days after flowering (DAF) when pollination was made				
	0	1	2	3	4
Controls					
'Spartan' selfed	2.0	-	-	-	-
'Spartan' x 'Bluejay'	35.5	-	43.5	-	33.4
Treatments					
('Spartan' selfed) x 'Bluejay'	-	38.3	4.8	2.8	1.9

[1]For seed no. per fruit, Fisher's protected LSD (.05)=8.5 and (.01)=11.3.

Figures 1A and 1B. Regression of seed set (A) and seed abortion (B) on zygotic levels of inbreeding in 16 highbush blueberry matings.



Figures 1A and aB.

(Fig. 1B) on zygotic inbreeding coefficients were significant: F_z alone accounted for 58% of the variation in developed seed number and 53% of the variation in percent seed abortion. Expanding the linear regression models to include maternal (F_m) and paternal (F_p) inbreeding coefficients did not significantly increase the R^2 values.

S1 Progeny Fertility

S1 progeny showed little or no self-fertility, in contrast to their less inbred parents. The mean seed yield per self-pollination ranged from 0.2-0.7 among the 3 groups of selfed offspring, compared to a 9.5-11.1 range among parents (Table 3). For all the cultivars, there was little variability in self seed production among S1 progeny. The percentage of completely self-sterile segregants was 40%, 50% and 80% for the 'Bluecrop', 'Jersey', and 'Elliot' groups, respectively; the most self-fertile individuals in each S1 progeny group had seed set values (1.3-3.6) closer to zero than to the parental yields.

From Table 3, it is apparent that the inverse relationship between zygotic inbreeding coefficients and seed set is disproportionate - small increments in F_z have large effects on fertility. Comparing cross- and self-pollinations of the parent cultivars, an approximate 20% increase in F_z corresponded to an average 70% decline in seed yield. In the S1 generation, F_z estimates were about 30% higher than the values for parental outcrosses, with the

Table 3. Comparisons of fertility in three parent cultivars and their selfed (S1) progeny.

Cross	F_z[1]	No. developed seed per pollination[2]	Δ[3]
'Elliot' outcrossed	0.049	46.4	
'Elliot' selfed	0.203	9.5	-79.5
'Elliot S1' 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 S1' selfed	0.329	0.7 (0-3.6)	-98.3
'Jersey' outcrossed	0.051	29.9	
'Jersey' selfed	0.167	11.0	-63.2
'Jersey S1' selfed	0.305	0.4 (0-2.0)	-98.7

[1] 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.

[2] Numbers shown are mean values for 10 S1 progeny with ranges indicated in parentheses.

[3] Δ is the average percent decrease in 'non-inbred', cross-pollinated seed set.

result that, at F ≈ 0.30, self-pollinations were almost 100% non-fertile.

Paternal and Maternal Effects

Pollen stainability, which is used to estimate pollen viability, was very high (95-100%) in the three highbush cultivars studied (Table 4). A marked decrease in stainability was observed in selfed offspring of these cultivars - the proportion of stained microspores ranged from an average 79.2% in the 'Elliot' S1 progeny group to 87.7% in the 'Jersey' group. There was marked variation around these mean values. Some S1 segregants exhibited a marked reduction in pollen staining, while others had viability estimates close to the 'non-inbred', 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 staining class was never more than 4.9% with one generation of selfing (in 'Elliot' S1 progeny, Table 4), there was some segregation for this trait, ranging as much as 0-20.6% among S1 individuals.

Comparisons of seed yields in parent x S1 progeny reciprocal crosses (Table 5) indicated that inbreeding had a greater effect on female than male fertility. In all but one reciprocal cross, seed set was lower in the 'inbred' x 'non-inbred' mating than in the 'non-inbred' x 'inbred' mating. One S1 progeny, 'JRS1-10', was fertile as a male parent but set zero fruit as a female parent. Five other

Table 4. Pollen stainability of three highbush blueberry cultivars and their selfed (S1) progeny, measured as percentage of pollen tetrads completely non-viable (0/4 microspores stained) and percentage of total microspores stained.[1]

Genotype	F	# Ind. scored	% Tetrads not stained	% Stained microspores	Δ^2
Jersey	0	1	1.6	95.1	
Jersey S1	0.167	9	2.3 (0-9.0)	83.7 (69.4-96.0)	-12.0
Bluecrop	0.033	1	0	100.0	
Bluecrop S1	0.194	9	3.1 (0-8.3)	81.3 (56.2-93.5)	-18.7
Elliot	0.044	1	0	100.0	
Elliot S1	0.203	8	4.9 (0-20.6)	79.2 (41.1-96.8)	-20.8

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

[2]Δ is percentage change in S1 mean pollen viability relative to the parent cultivar.

Table 5. Comparisons of seed set in reciprocal crosses of highbush blueberry cultivars with their selfed (S1) progeny.[1]

Cross	# Developed seed per fruit	Δ[2]
EL x ELS1-5	7.7	
ELS1-5 x EL	5.1	-33.7
EL x ELS1-1	27.7	
ELS1-1 x EL	10.7	-61.4**
JR x JRS1-5	2.9	
JRS1-5 x JR	1.8	-37.9
JR x JRS1-4	15.5	
JRS1-4 x JR	3.7	-76.1**
JR x JRS1-10	4.6	
JRS1-10 x JR[3]	0	-100.0
BC x BCS1-5	13.8	
BCS1-5 x BC	6.2	-55.1**
BC x BCS1-6	8.6	
BCS1-6 x BC	11.7	+36.0
BC x BCS1-10	10.6	
BCS1-10 x BC	7.4	-30.2
BC x BCS1-9	35.5	
BCS1-9 x BC	22.3	-37.2*
BC x BCS1-7	8.9	
BCS1-7 x BC	4.6	-48.3**

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

[2]Δ is the percentage change in the S1 progeny x cultivar seed set, relative to the cultivar x S1 progeny cross, significant at the 5% (*) or 1% (**) levels (t-test).

[3]No fruit resulted from these pollinations.

progeny showed significant reductions in fecundity when acting as the seed parent, compared with their performance as pollen donors. Therefore, in most cases inbred offspring functioned better as males than females in controlled crosses. Overall fertility in some S1 progeny - 'ELSI-1' and 'BCS1-9', for example - was noticeably higher than the other progeny. A correlation of male and female fertility of individuals proved to be highly significant (r=0.84, calculated from Table 5).

Discussion

In the pollen chase experiment, pistil receptivity to pollination did not decrease over the interval studied (5 days). Other reports have documented a prolonged period of female receptivity in blueberry clones - up to 8 days from anthesis (Wood, 1962; Moore, 1964). The undiminished capacity for fertilization in flowers which were open for 5 days means that the differences in seed set reported in Table 2 are due to pollen treatment effects (source and timing of application) rather than flower age.

The results of the pollen chase experiment may be explained by post-zygotic mechanisms for self seed failure. The inability of 'Bluejay' chaser pollen to produce large numbers of seed when applied to previously self-pollinated 'Spartan' flowers (allowing for a minimum 2-day 'head start' by self pollen) could be attributed to 1) a saturating level of self-fertilization which precluded fusion of 'Spartan'

egg cells with the later-arriving 'Bluejay' pollen nuclei, and 2) subsequent abortion of most self-fertilized embryos. 'Spartan' ovaries contain about 80 ovules on average (pers. obs.). These inferences are consistent with cytological evidence from identical crosses, showing that self-pollen enters embryo sacs normally and at the same rate as foreign pollen (Krebs and Hancock, 1988).

Pollen chase and pollen mixture experiments have been previously used as evidence of post-zygotic self-sterility in Gasteria varrucosa (Sears, 1937) and Borago officinalis (Crow, 1971). It has been argued that the wrong conclusions can be drawn from this kind of experiment, since a pre-zygotic mechanism - blockage of micropyles by self pollen tubes - could also prevent fertilization by chase pollen (Seavy and Bawa, 1986). In Vaccinium, a well-defined micropyle appears to be absent, and pollen tube penetration into the ovule has been described as 'porogamous' (Stushnoff and Palser, 1969), raising the possibility of multiple paths of tube entry into the embryo sac. The fact that some viable seed is produced following self-pollination of highbush cultivars, in addition to the presence of aborted seeds which are considerably larger than unfertilized ovules, further suggests that early-arriving self pollen has a precluding rather than blocking effect on later-arriving outcross pollen.

Other instances of embryo abortion following self-fertilization have been reported among plant species.

Failure of inbred embryos to develop has been cytologically documented in many gymnosperms, particularly conifers such as Pseudotsuga menziesii (Orr-Ewing, 1957), Picea glauca (Mergon et al., 1965), and Pinus puce (Hagman and Mikkola, 1963). There is little evidence for true self-incompatibility systems in gymnosperms - not surprisingly, they lack specialized floral structures, such as pistils, which enable many angiosperms to actively 'oppose' self pollen. Foresters generally attribute variation in self-fertility within and among conifer species to differences in genetic load at the individual or population level (Franklin, 1972; Sorenson, 1969; Park and Fowler, 1984). Forced self-pollination results in the expression of deleterious or lethal alleles normally masked by heterozygosity in predominantly outcrossing species, with subsequent inbreeding depression of sporophytic vigor. Interestingly, a high proportion of genetic load in conifers is expressed during embryo development - inbreeding generally has a much more negative effect on seed set than on seed germination or seedling survival.

Aborted development of self-fertilized embryos and endosperms has also been described in some angiosperms. In addition to the above-mentioned example of Gasteria, cytological evidence for self seed abortion has been found in Liquidambar styraciflua, several Rhododendron species, Medicago sativa, Asclepias syriaca, and Lotus corniculatus (see reviews by Charlesworth, 1985; Seavy and Bawa, 1986).

In other taxa - Borago officinalis (Crowe, 1971), Ulmus americanus (Lester, 1971), Acer saccharum (Gabriel, 1966), and Gmelina arborea (Bolstad and Bawa, 1982) - post-zygotic abortion of self-fertilized ovules has been inferred from crossing studies and the presence of shrunken seeds.

Because true self-incompatibility systems are a predominant feature of angiosperm evolution, a central question concerning reproductive biology in these species, as well as the current example of V. corymbosum, is whether self seed abortion results from a late-acting (post-zygotic) incompatibility reaction or from early-acting (pregermination) inbreeding depression, as in the case of the conifers. This is a problematic area, since some of the above species exhibit characteristics of a fully developed self-incompatibility system, such as strict allogamy within a population or a uniform developmental failure of self-fertilized ovules (Charlesworth, 1985; Seavy and Bawa, 1986). A further complication arises with the suggestion that the definition of self-incompatibility be extended to include any mechanism (pre- or post-zygotic) which enables parents to discriminate among offspring and preferentially invest their resources in 'high-quality' (i.e. non-inbred) progeny (Seavy and Bawa, 1986; Uyenoyama, 1988). Conceptually, the difference between 'passive' embryo abortion based solely on the zygote's genotype (genetic load) and 'active' elimination of progeny based on maternal-zygotic interactions (self-incompatibility) is

straightforward. However, it is extremely difficult to experimentally distinguish between these two post-fertilization mechanisms.

The experimental results in this report support our earlier contention that fertility in V. corymbosum is regulated by zygotic levels of inbreeding and expression of embryonic lethal genes (Krebs and Hancock, 1988). In the diallel cross, seed yield from a mating was inversely correlated with progeny inbreeding coefficients, and the proportion of aborted seed was positively correlated with zygotic F values (F_z). Regression analysis (Fig. 1A and 1B) showed that differences in F_z accounted for a significant amount of the variation among crosses for these fertility parameters.

According to Kempthorne's definition of F for autotetraploids, two factors - relatedness between parents and parental inbreeding - determine F_z. In the diallel, levels of inbreeding in the parent cultivars (F_m and F_p, ranging from 0-0.043) had negligible effects on seed set or abortion, indicating that consanguinity is the principle factor affecting fertility in these crosses. Since considerable variation (~40%) in seed yield was unaccounted for by the regression, it is not a foolproof predictor of fertility in highbush blueberry matings. Furthermore, deviations between observed seed sets and those predicted by the regression were greater at low F_z values, which means

that the equation may be a more accurate estimator of fertility at high levels of inbreeding.

There is additional evidence supporting the major conclusion of the diallel cross - that a common genetic system is controlling seed set in both self- and cross-pollinations. Significant correlations of self- and cross-fertility have been demonstrated in V. ashei and V. corymbosum cultivars (Krebs and Hancock, 1988) and in clones from a wild Michigan population of V. corymbosum (Krebs and Hancock, unpublished). Similar associations have been observed in Medicago sativa, an autotetraploid species in which fertility is also regulated by individual genetic loads, consanguinity in matings, and embryo abortion (Busbice, 1968; Fyfe, 1957; Cooper and Brink, 1940). 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.

The diallel analysis predicts that complete self-sterility (or total seed abortion) will occur at fairly low zygotic levels of inbreeding (F_Z ranging from 0.25-0.28). This was observed when S1 progeny from three cultivars were self-pollinated (Table 3). The three parents were highly cross-fertile (F_Z=0.043 - 0.051), moderately self-fertile (F_Z=0.167 -0.203), and a high proportion of their S1 progeny were self-sterile (F_Z=0.305 - 0.336). There was little

variation within each offspring group - the most fertile S1's had self seed sets far below the parental values.

Thus the inbreeding barrier to self-fertility is established quite rapidly in V. corymbosum. Whether this is due to rapid loss of a few favorable allelic interactions, presence of a high mutational load, or epistatic interactions among a few deleterious genes - all of which could confer self-sterility at low levels of inbreeding - is discussed in more detail below. The inbreeding threshold represents a polygenic control of mating system in highbush blueberries - genotypes below the critical F value are facultative selfers, while those above the threshold are obligate outcrossers.

A genetic model based on S-alleles at a single locus (gametophytic self-incompatibility, GSI) does not explain the lost capacity for autogamy in these cultivars with one generation of selfing. Lewis (1979) proposed that the breakdown of GSI in some species by chromosome-doubling (autotetraploidy) was due to 2X pollen grains heterozygous at the S-locus, resulting in a heterodimeric incompatibility protein that was no longer 'recognized' by the stylar genotype. According to this interpretation, some selfed progeny of an autotetraploid parent carrying 2 or 3 different S-alleles would be self-sterile, while the majority are expected to be as self-fertile as the parent. The majority of S1 progeny in this report were self-sterile.

Inbreeding has a negative effect on gametic as well as zygotic viability in V. corymbosum. This is expected, since presence or absence of gene interactions in 2X pollen can cause heterosis or inbreeding depression. The average pollen stainability (% stained microspores) of S1 progeny was 12-21% below the parent cultivar values (Table 4). Variation among progeny for this trait was fairly continuous, ranging from parental levels (~100% staining) to much lower estimates of pollen viability (41-70%). Although pollen staining results in higher estimates of male fertility than those obtained by pollen germination, the two techniques result in similar relative rankings of gamete viability among Vaccinium clones (Cockerham and Galletta, 1976). The average loss and variation in pollen viability among selfed offspring can be attributed to nuclear factors, since all individuals within an S1 progeny group shared a common cytoplasm.

It is not known if ovule viability is similarly reduced by inbreeding. However, a definite maternal effect, which did not appear in the diallel cross, was observed at higher levels of inbreeding in the 'parent' x 'S1 progeny' reciprocal crosses (Table 5). The inbred individuals were generally less fertile as females than males. A significant correlation of male and female fertility among crosses ($r=0.84$, calculated from Table 5) indicated that they probably have a common genetic basis - S1 segregants

experiencing the most inbreeding depression had lowest overall fecundity.

These fertility differences in reciprocal crosses of 'inbred' and 'non-inbred' individuals also appear to be under nuclear control, since the parental and S1 progeny cytoplasms are identical. The maternal inbreeding effect might be caused by a greater impairment of gametogenesis in the ovary (affecting ovule number or ovule viability) than in the anthers. If aborted seed development is related to endosperm breakdown, maternally-carried deleterious genes would play a predominant role in determining the fate of that tissue. Alternatively, since the female parent bears most of the reproductive 'cost' of producing offspring, a reduction in available resources or disrupted allocations caused by inbreeding might result in the observed maternal effect.

In addition to these data from V. corymbosum, there is corroborating evidence from other autotetraploid species which suggests that early-acting inbreeding depression is expressed during meiotic and post-meiotic, as well as embryonic stages of development. Two generations of selfing in Kalmia polifolia (another ericaceous species) resulted in pollen sterility as well as reduced vegetative vigor (Jaynes, 1968). Inbred lines of Secale cereale exhibit chromosome breakage, pre-meiotic spindle formation, asynapsis, and a decrease in chiasma frequency (Rees, 1961). In Medicago sativa and Solanum tuberosum, production of

polyhaploids (2X progeny from parthenogenetic embryos in 4X-2X crosses) results in severe inbreeding. Polyhaploids often have regular chromosome pairings, but some individuals show meiotic abnormalities - in both cases, their pollen viability is very low and intermatings of polyhaploids are frequently sterile (Bingham and Gillies, 1971; Yeh et al., 1964).

In V. corymbosum, the large number of empty tetrads (0/4 class) produced by some S1 segregants (Table 4) suggests that inbreeding depression could be affecting chromosome behavior during or before metaphase I. However, most of the inbred progeny had a high proportion of tetrads with 2 or more stained microspores, so the average loss of pollen viability is more readily explained by disrupted post-meiotic development.

An inbreeding effect on gametophytic development might occur in two ways. An initially weak system of maternal recognition of and opposition to self-pollen could increase in potency with inbreeding, resulting in an incompatibility system that parallels inbreeding depression (Seavy and Bawa, 1986; Mulcahy and Mulcahy, 1983; Uyenoyama, 1988). However, no stigmatic, stylar, or ovarian inhibition of self pollen was observed in a relatively non-inbred clone (Krebs and Hancock, 1988), and examination of one S1 progeny revealed that self pollen tubes were entering ovules normally, 6 DAP (personal obs.).

Alternatively, inbreeding might alter pollen interactions in mixtures such that self pollen becomes decreasingly competitive. In V. corymbosum, competition favoring foreign male gametophytes was observed in the pollen chase experiment (Table 1), where 'Bluejay' pollen applied 1 day after self-pollination of 'Spartan' stigmas resulted in seed set comparable to the 'Spartan' x 'Bluejay' cross-pollinations. The stronger competitiveness of non-self gametophytes in pollen mixtures may be due to pollen x pollen rather than pollen x style interactions - in our previous cytological study, 'Spartan' and 'Bluejay' gametophytes on separate 'Spartan' pistils showed no differences in growth rates (Krebs and Hancock, 1988). There is no data available from highbush blueberries to indicate whether inbreeding further reduces the competitive ability of self pollen to reach the ovules, nor is there much information elsewhere. In Zea mays, inbreeding over seven generations had the opposite effect - increasing the proportion of self-fertilizations following pollination with a mixture of inbred (self) and non-inbred (outcross) pollen (Johnson and Mulcahy, 1978).

Our study indicates that the reduction in seed set following inbreeding of highbush blueberries is not caused by failure of gametes to fuse, but results instead from loss of heterozygosity in the fusion products (embryo and/or endosperm). The magnitude of expressed genetic load and subsequent aborted seed development depends on parental

levels of inbreeding and consanguinity. Depending on the number of different alleles at a locus, loss of heterozygosity in an autotetraploid can result in homozygosity for deleterious alleles (mutational load), fewer favorable allelic interactions per locus (segregational or heterotic load), or both phenomena.

It was noted above that small changes in zygotic levels of inbreeding in V. corymbosum were associated with dramatic reductions in seed yield. One generation of selfing (F=.167-.203 in our populations) resulted in almost 100% self-sterility. Segregational load may account for this phenomenon. The situation in V. corymbosum could parallel that of autotetraploid Medicago sativa, where large reductions in both fertility and vegetative vigor with small increments in F have been attributed to loss of multiple allelism and high-order heterotic interactions (Busbice and Wilsie, 1966; Busbice, 1968; Dessureaux and Gallais, 1969). The loss of maximal allelic interactions with one generation of selfing an autotetraploid (F=.167), for example, is much higher at tetra-allelic (83.3%) and tri-allelic (61.1%) loci than at di-allelic loci (5.5-25.0%, depending on gene copy number). Therefore, if multiple allelic loci are required for normal embryonic or endosperm vigor, loss of heterosis and subsequent seed abortion could occur very rapidly with inbreeding.

Deleterious recessive mutations kept at low frequencies in outbreeding populations are generally considered to be a

primary cause of inbreeding depression. Models based on mutation-selection equilibria predict that polyploidy should decrease the amount of inbreeding depression caused by lethal or sub-lethal mutations (Lande and Schemske, 1985; Hedrick, 1987). However, these predictions are more applicable to allopolyploids, which can fix heterozygosity and are frequently selfers, than to autopolyploids, which are predominantly outcrossers (Mac Key, 1970). Because of polysomic inheritance, alleles detrimental to fitness characters can accumulate under mutation pressure at a much higher equilibrium frequency in autopolyploids than in diploids or disomic polyploids (allopolyploids). This state of affairs is reinforced by an outcrossing mating system. While newly-derived autopolyploids are likely to have reduced expression of genetic load compared to their diploid progenitors, such genomic buffering against mutations may erode over time, resulting in older populations which exhibit strong inbreeding depression. Autopolyploidy thus represents a postponement rather than permanent reduction of expressed mutational load.

The fact that V. corymbosum is a long-lived perennial species bears directly on the issues of mutational load and inbreeding depression. Klekowski (1988) has proposed that accumulated mutation frequency in plants is a function of age, or more precisely, the number of somatic cell cycles in apical meristems. The fact that plant reproductive structures, unlike those in higher animals, are derived from

somatic cell lineages has two important consequences. First of all, loci controlling sexual reproductive characters (meiosis, gamete formation, gametophyte development, or embryogenesis) may be particularly prone to genetic decay because selection against such somatically-derived mutations does not occur during prolonged periods of vegetative growth in perennials. Secondly, soma-germline continuity allows accumulated mutational load to be sexually transmitted in plants.

Applying these ideas to V. corymbosum, the observed responses to inbreeding - decline in male and female fertility, increased seed abortion, and weak competitiveness of self pollen tubes - may reflect both accumulated genetic load and a means of reducing this load in the next generation. Sexual life cycle mechanisms which screen out defective gametes, gametophytes, or embryos are thought to act as 'developmental selection sieves' in plants to increase the frequency of viable offspring (Klekowski, 1988).

For this strategy to be most effective, aborted inbred ovules must be compensated for by viable outcross (non-inbred) seed, so that total fertility of the individual is not severely reduced. Reproductive compensation is possible in highly fecund plants which produce an 'excess' of gametes and in perennials which can compensate on a year-to-year basis as well. In wild V. corymbosum populations, fecundity is very high - an average 4000 flowers per genet, for a

range of young and old genets (Pritts and Hancock, 1985). An estimated 9-10,000 flowers are formed on 15-year-old highbush cultivars (Hancock, in press), and the number of ovules per ovary in cultivars averages about 80 (personal obs.). Thus the seed/ovule ratio in V. corymbosum is probably low, allowing selective seed abortion and subsequent compensation to occur. In an interesting synthesis, Wiens (1984) notes that seed/ovule ratios are lowest in long-lived woody perennials, which have a higher predicted mutational load than herbaceous perennials or annuals. As genetic load increases, the probability of forming a viable seed from a given ovule decreases. By the same token, increased fecundity 'buffers' against seed abortion and ensures some fertility.

Self-fertility in V. corymbosum cultivars did not appear to result from the breakdown of a gametophytic self-incompatibility system due to polyploidy. Paradoxically, most diploid blueberries exhibit strict allogamy and thus appear to have a true incompatibility system (Ballington and Galletta, 1978). Several investigators have noted that it is unusual for genetic load to 'mimic' self-incompatibility to the extent that most individuals in a population have zero or near-zero self seed set (Charlesworth, 1985; Seavy and Bawa, 1986). However, the incidence of self-sterility due to mutational load is high in some conifer species (Sorensen, 1969; Franklin, 1972; Park and Fowler, 1982), at least one angiosperm species, Liquidambar styraciflua

(Schmitt and Perry, 1964), and this phenomenon may occur in other long-lived, highly fecund taxa where 1) genetic load increases with age, 2) reproductive compensation occurs via outcrossing (see above), so that female fertility is assured, and 3) the expression of inbreeding depression early in sporophyte development (e.g. pregermination) reduces the level of genetic load expressed after seedling establishment, thereby lowering the 'cost of natural selection' at the population level (Haldane, 1957). In woody plants, any deleterious mutations or allelic combinations affecting fertility which are not selected against during reproduction (developmental selection) will persist in the progeny's perennial contribution to the gametic pool. Adult sterility lowers the reproductive capacity of the population, and represents an additional 'cost' if space and resources are limited.

There is no data available on the genetic control of self-infertility in diploid Vaccinium species. However, we observed self pollen tube growth into ovules of two V. darrowi (2X) genotypes occurring at about the same frequency as penetration by foreign pollen tubes 6 DAP - a third clone exhibited a faster rate of growth for non-self pollen (unpublished). Embryological studies of these same genotypes revealed cellular endosperm divisions within 8 days of self pollination, and continuing up to 16 DAP, at which point many flowers abscised (unpublished). Although a well-defined zygote was not observed (zygote divisions do

not generally occur until 14 DAP, Stushnoff and Palser, 1969), the presence of a fertilized zygote can be inferred from fertilization of the polar endosperm nuclei, since double fertilization is normal.

It is possible, then, that early-acting inbreeding depression, expressed as self seed abortion, is occurring at the diploid level, and that polyploidization in Vaccinium effectively reduces the genetic load (at least temporarily) and allows some degree of self-fertility. A comparable situation exists in ericaceous Kalmia species, where selfing reduced seed set 85-90% in diploid taxa, but not at all in a tetraploid species (Jayne, 1968). A severe genetic load among diploids, and subsequent buffering against mutations in polyploids, is also consistent with the observation that tetraploid blueberry species have higher average pollen fertility than diploid taxa (Cockerham and Galletta, 1976).

Self-incompatibility and early-acting inbreeding depression are not mutually exclusive mechanisms for regulating fertility and offspring quality in plants. Some researchers have suggested they may be under joint genetic control, although this generally involves a redefinition of 'classic' self-incompatibility models (see review by Seavy and Bawa, 1986). For example, in the 'heterosis model' (Mulcahy and Mulcahy, 1983), self-incompatibility is attributed to pre-zygotic expression of genetic load due to homozygosity at complementary pollen and stylar loci. Uyenoyama's (1988) explanation for the evolution of self-

incompatibility is based on a model in which incompatibility loci are linked to viability loci, the former serving as a predictor of homozygosity and inbreeding depression in the latter.

There are good reasons to believe that pre-zygotic SI and post-zygotic abortion of inbred seed may operate independently in the same individual. One example of this is Phlox drummondii, a self-incompatible species which, when bud-pollinated, exhibits variable levels of self-fertility (Levin, 1984). In cross pollinations, seed abortion in Phlox appeared to be directly associated with the level of consanguinity (as inferred from physical distance among mates). This species appears to have two complementary lines of defense against the production of inbred offspring - a SI system which provides a 'leaky' recognition of relatedness in cross-pollinations, but reduces ovule abortion, and a post-zygotic seed abortion mechanism that directly 'senses' consanguinity, but at a high reproductive cost. It would be extremely interesting to know if other species with pre-zygotic self-incompatibility systems have in addition similar post-zygotic barriers to inbreeding.

Literature Cited

1. Aalders, L.E. and I.V. Hall. 1961. Pollen incompatibility and fruit set in lowbush blueberries. Can. J. Genet. Cytol. 3:300-307.
2. Ballington, J.R. and G.J. Galletta. 1978. Comparative crossability of 4 diploid Vaccinium species. J. Amer. Soc. Hortic. Sci. 103:554-560.
3. Bingham, E.T. and C.B. Gillies. 1971. Chromosome pairing, fertility, and crossing behavior of haploids of tetraploid alfalfa, Medicago sativa L. Can. J. Genet. Cytol. 13:195-202.
4. Bolstad, P.V. and K.S. Bawa. 1982. Self-incompatibility in Gmelina arborea L. (aceae verben). Silvae Genet. 31:19-21.
5. Busbice, T.H. and C.P. Wilsie. 1966. Inbreeding depression and heterosis in autotetraploids with application to Medicago sativa L. Euphytica 15:52-67.
6. Busbice, T.H. 1968. Effects of inbreeding on fertility in Medicago sativa L. Crop Sci. 8:231-234.
7. Charlesworth, D. 1985. Distribution of diocey and self-incompatibility in angiosperms: In Greenwood, P.J., P.H. Harvey, and M. Slatkin (eds). Evolution: Essays in honor of John Maynard Smith. Cambridge Univ. Press, Cambridge, pp. 237-268.
8. Cockerham, L.E. and G.J. Galletta. 1976. A survey of pollen characteristics in certain Vaccinium species. J. Amer. Soc. Hortic. Sci. 101:671-676.

9. Cooper, D.C. and R.A. Brink. 1940. Partial self-incompatibility and the collapse of fertile ovules as factors affecting seed formation in alfalfa. J. Agric. Res. 60:453-472.

10. Cope, F.W. 1962. The mechanisms of pollen incompatibility in Theobroma cacao L. Heredity 17:157-182.

11. Crowe, L.K. 1971. The polygenic control of outbreeding in Borago officinalis. Heredity 27:111-118.

12. Dessureaux, L. and A. Gallais. 1969. Inbreeding and heterosis in autotetraploid alfalfa. I. Fertility. Can. J. Genet. Cytol. 11:706-715.

13. El-Agamy, S.Z., W.B. Sherman and P.M. Lyrene. 1981. Fruit set and seed number from self- and cross-pollinated highbush (4X) and rabbiteye (6X) blueberries. J. Amer. Soc. Hortic. Sci. 106:443-445.

14. Franklin, E.C. 1972. Genetic load in loblolly pine. Am. Nat. 106:262-265.

15. Fyfe, J.L. 1957. Relational incompatibility in diploid and tetraploid lucerne. Nature 79:591-592.

16. Gabriel, W.J. 1967. Reproductive behavior in sugar maple: self-compatibility, cross-compatibility, agamospermy, and agamocarpy. Silvae Genet. 16:165-168.

17. Garvey, E.J. and P.M. Lyrene. 1987. Self-incompatibility in 19 native blueberry selections. J. Amer. Soc. Hortic. Sci. 112:856-858.

18. Hagman, M. and L. Mikkola. 1963. Observations on cross-, self-, and inter-specific pollinations in Pinus peuce Griseb. Silvae Genet. 12:73-79.

19. Haldane, J.B.S. 1930. Theoretical genetics of autopolyploids. J. Genet. 22:359-372.

20. Haldane, J.B.S. 1957. The cost of natural selection. J. Genet. 55:511-524.

21. Hancock, J.F. and J.H. Siefker. 1982. Levels of inbreeding in highbush blueberry cultivars. HortScience 17:363-366.

22. Hancock, J.F. 1989. Why is Elliott so Productive? A comparison of yield components in six highbush blueberry cultivars. Fruit Var. J. In press.

23. Hedrick, P.W. 1987. Genetic load and the mating system in homosporous ferns. Evol. 41:1282-1289.

24. Jaynes, R.A. 1968. Self incompatibility and inbreeding depression in three laurel (Kalmia) species. J. Amer. Soc. Hortic. Sci. 93:618-622.

25. Johnson, C.M. and D.L. Mulcahy. 1978. Male gametophyte in maize: II. Pollen vigor in inbred plants. T.A.G. 52:211-215.

26. Kempthorne, O. 1957. An introduction to genetic statistics. John Wiley and Sons, New York. pp. 96-99.

27. Klekowski, E.J. 1988. Mutation, developmental selection, and plant evolution. Columbia Univ. Press, New York.

28. Krebs, S.L. and J.F. Hancock. 1988. The consequences of inbreeding on fertility in Vaccinium corymbosum L. J. Amer. Soc. Hortic. Sci. 113:914-918.

29. Krebs, S.L. and J.F. Hancock. Tetrasomic inheritance of isoenzyme markers in the highbush blueberry, Vaccinium corymbosum L. Heredity. In press.

30. Lande, R. and D.W. Schemske. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evol. 39:24-40.

31. Lester, D.T. 1971. Self-compatibility and inbreeding depression in American elm. For. Sci. 17:321-322.

32. Levin, D.A. 1984. Inbreeding depression and proximity-dependent crossing success in Phlox drummondii. Evol. 38:116-127.

33. Lewis, D. 1979. Sexual incompatibility in plants. Studies in biology No. 10. Edward Arnold, London. pp. 29-31.

34. MacKey, J. 1970. Significance of mating systems for chromosomes and gametes in polyploids. Hereditas 66:165-176.

35. Meader, E.M. and G.M. Darrow. 1944. Pollination of the rabbiteye blueberry and related species. Proc. Amer. Soc. Hortic. Sci. 45:267-274.

36. Meader, E.M. and G.M. Darrow. 1947. Highbush blueberry experiments. Proc. Amer. Soc. Hortic. Sci. 49:196-204.

37. Mergen, F., J. Burley and G.M. Furnival. 1965. Embryo and seedling development in Picea glauca (Moench.) Voss after self-, cross- and wind-pollination. Silvae Genet. 14:188-194.

38. Moore, J.N. 1964. Duration of receptivity to pollination of flowers of the highbush blueberry and the cultivated strawberry. J. Amer. Soc. Hortic. Sci. 85:295-301.

39. Morrow, E.B. 1943. Some effects of cross-pollination versus self-pollination in the cultivated blueberry. Proc. Amer. Soc. Hortic. Sci. 42:469-472.

40. Mulcahy, D.L. and G.B. Mulcahy. 1983. Gametophytic self-incompatibility reexamined. Science 220:1247-1251.

41. Orr-Ewing, A.L. 1957. A cytological study of the effects of self-pollination on Pseudotsuga menziesii (Mirb.) Franco. Silvae Genet. 6:179-185.

42. Park, Y.S. and D.P. Fowler. 1984. Inbreeding in black spruce (Picea mariana (Mill.) B.S.P.): self-fertility, genetic load, and performance. Can. J. For. Res. 14:17-21.

43. Pritts, M.P. and J.F. Hancock. 1985. Lifetime biomass partitionings and yield component relationships in the highbush blueberry, Vaccinium corymbosum (Ericaccae). Amer. J. Bot. 72:446-452.

44. Rabaey, A. and J. Luby. 1988. Fruit set in half-high blueberry genotypes following self and cross pollination. Fruit Var. J. 42:126-129.

45. Rees, H. 1961. Genotypic control of chromosome form and behavior. Bot. Rev. 27:288-318.

46. Schmitt, D. and T.O. Perry. 1964. Self-sterility in sweetgum. For. Sci. 10:302-305.

47. Sears, E.R. 1937. Self-sterility in plants. Genetics 22:130-182.

48. Seavy, S.R. and K.S. Bawa. 1986. Late-acting incompatibility in Angiosperms. Bot. Rev. 52:195-219.

49. Sorenson, F. 1969. Embryonic genetic load in coastal Douglas-fir, Pseudotsuga menziesii. Am. Nat. 103:389-398.

50. Stanley, R.G. and H.F. Linskens. 1974. Pollen: biology, biochemistry, management. Springer-Verlag, Berlin. pp. 67-86.

51. Stushnoff, C. and B.F. Palser. 1969. Embryology of five Vaccinium taxa including diploid, tetraploid, and hexaploid species or cultivars. Phytomorphology 19:312-331.

52. Uyenoyama, M.K. 1988. On the evolution of genetic incompatibility systems. III. Introduction of weak gametophytic self-incompatibility under partial inbreeding. Theor. Pop. Biol. 34:47-91.

53. Vander Kloet, S.P. and P. Cabilio. 1984. Annual variation in seed production in a population of Vaccinium corymbosum L. Bull. Torr. Bot. 111:483-488.

54. Weins, D. 1984. Ovule survivorship, broad size, life history, breeding systems, and reproductive success in plants. Oecologia 64:47-53.

55. Wood, G.W. 1962. The period of receptivity in flowers of the lowbush blueberry. Can. J. Bot. 40:685-686.

56. Wood, G.W. 1968. Self-fertility in the lowbush blueberry. Can. J. Plant. Sci. 48:431-433.

57. Wright, S. 1922. Coefficients of inbreeding and relationship. Am. Nat. 56:330-338.

58. Yeh, B.P., S.J. Peloquin and R.W. Hougas. 1964. Meiosis in Solanum tuberosum haploids and haploid-haploid F_1 hybrids. Can. J. Genet. Cytol. 6:393-402.

CHAPTER 4

ASSOCIATIONS AMONG COMPONENTS OF FERTILITY, GENETIC LOAD, AND HETEROZYGOSITY IN A NATURAL POPULATION OF *VACCINIUM CORYMBOSUM*

Abstract

Hand-pollinations of 28 wild V. corymbosum accessions from a single population resulted in lower self than outcross seed set. Fertility among seed parents varied widely, ranging from clones that were effectively female sterile to individuals with high seed yields in both matings. Self and outcross fertility were highly correlated. A genetic load model was invoked to explain these phenomena. Reduced fertility following self-pollination was attributed to homozygosity for sub- lethal mutations at loci controlling embryo development, or possibly loss of balanced polymorphisms at these loci. Similarly, clones with zero female fertility were thought to carry a large number of 'defective' genes or gene combinations resulting in seed abortion. Estimates of the number of lethal equivalents per zygote carried by individuals in this population ranged from 2.2 - 20.4, with a mean of 9.6. Embryonic genetic load was significantly correlated with heterozygosity at 9 polymorphic enzyme loci. A tendency towards low pollen viability and reduced receptivity to pollen from any source was noted in the low fertility genotypes, suggesting that gametic, gametophytic, and embryonic development are symptomatic of genetic loads during reproduction. The roles of developmental selection, differential reproductive

success, and an indeterminate mating system in reducing genetic load (i.e. the freqeuncy of 'sterility' genes) are discussed.

Introduction

A plant's reproductive success is usually measured by seed production and the relative survivorship, vigor, and fecundity of progeny. In sexually reproducing species, natural selection (mortality) can occur at any point along the continuum from zygote to meiocyte. While most plant studies have emphasized relative fitnesses in post-germination stages of sporophyte development, there is growing interest in differential viability of gametes, gametophytes, and zygotes during the reproductive cycle.

In outcrossing plant species, several aspects of gametic and embryonic development can affect both offspring number and quality. The descriptor 'quality' is used here to characterize phenotypes in terms of inbreeding depression, e.g. low quality means not vigorous. Genetic self-incompatibility (SI) systems are the best documented examples of pre-zygotic avoidance of inbreeding in angiosperms, occuring in over 3000 species of flowering plants (de Nettancourt, 1977). In plant taxa lacking SI, other mechanisms increase the probability of forming viable offspring. Competition among germinating pollen tubes (gametophytic selection) may favor the production of vigorous progeny (Mulcahy, 1974; Stephenson and Bertin,

1983). Similarly, gametic selection can result in elimination of defective pollen and egg genotypes. After fertilization occurs, low-quality embryos may be preferentially aborted (Wiens et al.,1987). Seed yield is affected by the stringency of selection during gametogenesis, gametophyte development, and embryogenesis.

Collectively, these phenomena are thought of as 'developmental selection sieves' which reduce the number of deleterious alleles or allelic combinations in the 'surviving' seed pool (Buchholz, 1922; Klekowski, 1988). Differential viability of low and high quality genotypes during reproduction thus provides an 'early acting' mechanism for reducing genetic loads in populations. Haldane (1957) noted that the negative consequences of genetic load on population fitness could be reduced by expression of that load early in offspring development (e.g.pre-emergent) rather than at later stages of the life cycle.

Developmental selection may play a particularly prominent role in regulating progeny number and quality in plant species because 1) plants carry heritable somatic mutations and 2) many plants maintain and even accumulate deleterious recessive mutations or 'balanced' allelic combinations (genetic load). Genetic loads, and subsequent inbreeding depression, are characteristic of species with life history attributes that maintain high levels of genetic variability - outcrossing mating system, long life span, high fecundity, and polyploidy, among other features

(Hamrick, 1979; Klekowski, 1988). For example, Wiens (1984) and Wiens et al. (1987) found that seed:ovule ratios were lower in perennials (50%) than in annuals (85%), and lower still in outcrossers (22%) than inbreeders (90%). The large differential between realized and potential seed set in allogamous perennials was attributed to embryo abortion rather than resource or pollen limitations. Klekowski (1988) noted that in organisms with high genetic load-bearing capacity, the probability of forming a viable offspring from a given ovule is reduced.

Viability depression during reproduction, particularly in embryo stages of development, has been documented in many plant taxa. When seed abortion increases with inbreeding, embryo mortality is generally attributed to developmental lethal or sub-lethal mutations occuring in the homozygous state. Embryonic genetic load, as well as inbreeding depression at other stages of sporophyte growth, is frequently measured as the number of lethal equivalents carried by a genotype. A lethal equivalent is defined as 'a group of mutant genes of such number that, if dispersed in different individuals, they would cause on average one death, e.g. one lethal mutant, or two mutants with with 50 percent probability of causing death, etc.' (Morton et al., 1956). Whereas as a single recessive embryo-lethal causes certain death in the homozygous state, a lethal equivalent is a mutation with a much smaller effect, so that several or many must occur simultaneously in the homozygous condition

to be lethal (Klekowski, 1988). An increase in the number of embryonic lethal equivalents carried by an individual results in greater seed abortion during inbreeding.

Conifers are good examples of organisms which frequently abort seeds following self-fertilization (Orr-Ewing, 1957; Mergen et al, 1965). In Pseudostuga menziesii, estimates of the number of lethal equivalents expressed during embryogenesis ranged from 3 to 27 in a sample of 35 trees, with a median of 10 (Sorensen, 1969). Similar studies of inbreeding in Pinus taeda (Franklin, 1972) and Larix laricina (Park and Fowler, 1982) yielded median estimates of 7 and 11 lethal equivalents per zygote, respectively. In contrast, Homo and Drosophila populations average 1 to 4 embryonic lethal equivalents (Levin, 1984 and references therein).

It has been shown that the expression of 'hidden' genetic load in conifers is age-specific, and that most of it is expressed in the embryo phase of sporophytic development. For example, 89% of offspring mortality between self-fertilization and the 6-week seedling stage in Pinus taeda was attributable to seed abortion (Franklin, 1972). In Picea marianna, the total number of lethal equivalents acting in a population from time of pollination through 6 years of age was between 6 and 8 - of this total, the population carried 5 to 7 embryonic lethal equivalents (Park and Fowler, 1984). Other inbreeding experiments on conifers also document that, while viability depression does occur in

the post-germination phase of progeny growth, the majority of genetic load is expressed prior to seed maturity (Sorensen, 1969; Koski, 1973).

Few studies have characterized the role of seed abortion in determining the reproductive success of angiosperm species. This is partly due to the pre-eminence of genetic SI systems among angiosperms, and a tendency among researchers to portray both partial and complete reduction in self seed set as incompatibility phenomena (Seavy and Bawa, 1986). However, variability for self-fertility in some angiosperm species parallels that observed in conifers, suggesting that genetic load rather than genetic SI is regulating fertility in these populations as well.

Polygenic control of fertility in Borago officinalis was demonstrated by Crowe (1971), who observed that seed set following self- and cross- pollinations was inversely proportional to parental relatedness and levels of inbreeding. Post-zygotic seed abortion was the proposed mechanism for this type of 'incompatibility reaction'. In two tetraploid perennials, Medicago sativa and Vaccinium corymbosum, a linear decline in seed set, and increase in seed abortion, occured as zygotic inbreeding coefficients (F values) increased (Busbice, 1968; Sayers and Murphy, 1966; Krebs and Hancock, in prep.). In all the above examples, a threshold level of inbreeding was observed in which a shift from self-fertility to self-sterility occured. Genetic load

also appeared to reduce pollen viability in V. corymbosum inbreds and retard pollen tube development in M. sativa.

Pollinations of Phlox drummondii, a self-incompatible annual which can be bud pollinated, revealed variable levels of self-fertility within and among populations, as well as an inverse relationship between relatedness (judged by physical proximity of parents) and crossing success (Levin, 1984). Embryonic load in 28 Phlox populations averaged 0.79 lethal equivalents per zygote. Tree species such as Liquidambar styraciflua (Schmitt and Perry, 1964), Eucalyptus regnans (Eldridge and Griffin, 1983), Ulmus americanus (Lester, 1971), and Acer saccharum (Gabriel, 1967), also exhibit variable self-fertility within populations. Although genetic control of this variability has not been determined, post-zygotic abortion appears to regulate fertility in Acer and Liquidambar.

This study assesses variation in both self- and cross-fertility in a natural population of tetraploid highbush blueberries, Vaccinium corymbosum (section Cyanococcus). While diploid Vaccinium taxa are self-sterile (Ballingtion and Galletta, 1978), low to moderate levels of self-fertility have been documented in tetraploid V. angustifolium (Aalders and Hall, 1961) and hexaploid V. ashei (Garvey and Lyrene, 1987). A single study of a natural V. corymbosum population reported wide variation in open-pollinated seed production among clones, and zero self

seed set in hand pollinations (Vander Kloet and Cabilio, 1984).

Previous genetic analysis of V. corymbosum cultivars indicated that mating success in these individuals was dependent on genetic load and levels of inbreeding in developing zygotes (Krebs and Hancock, 1988; Krebs and Hancock, in prep.). The present study of fertility in a natural highbush blueberry population was undertaken in order to 1) document variation in self- and cross-fertility among wild V. corymbosum clones 2) derive a quantitative estimate of genetic load in this population 3) determine associations between gametic, gametophytic, and zygotic components of fertility and 4) correlate genetic load with electrophoretic estimates of genetic diversity at the individual level.

Materials and Methods

Collection:The highbush blueberry, Vaccinium corymbosum, is a woody perennial which occurs throughout the northeastern and Great Lakes regions of the U.S.. It is a crown-forming, rather than rhizomatous, species and therefore sexually propagated. Several reproductive traits-pendant flowers, insect pollination, and partial to complete self-infertility - promote outcrossing in V. corymbosum. It is also an autotetraploid species (Krebs and Hancock, in press). The site used in this study was Otis Lake, Barry

Co., Michigan, a population in which annual reproductive effort is very high (Pritts and Hancock, 1985).

A single cane (ramet) from each of 40 clones (genets) was collected in May 1986. These clones varied from 5 - 30 canes per crown. No attempt was made to estimate ages of the clones. Canes were separated from the crowns along with some of the root system, cut back, potted in a peat-sand-composted soil mix, and transferred to a greenhouse. The plants were vernalized in this greenhouse from October 1986 through February 1987. In March, pollinations were made on 28 of the 40 individual canes, henceforth refered to as [daughter] clones, which produced adequate numbers of flowers for fertility comparisons.

Pollinations: Twenty self- and cross-pollinations each were made per clone. The crosses were made using standard techniques (Galletta, 1975; Krebs and Hancock, 1988). Selfed flowers were not emasculated. Cross- pollinations, following removal of the stamens, were made with a bulk of cultivated V. corymbosum pollen ('Bluecrop', 'Bluejay', and 'Jersey' cultivars). This bulk was used because of its high pollen stainability (approx. 90%) and non-relatedness to the study population - these cultivars derive from New Hampshire and New Jersey germplasm (Galletta, 1975). Therefore, the outcross pollinations conceivably estimated maximum female fertility among genotypes. Inflorescences were bagged in cheesecloth following pollination.

Fruit set, seed set, and seed germination: Three weeks after pollination, the cheesecloth coverings were removed and the number of enlarged ovaries per self- and cross-pollination was determined. From these counts, the percent self and outcross fruit set (based on 20 pollinations) was estimated for each clone.

The number of developed and aborted seeds per fruit was scored for ten ripe fruit from each mating, whenever possible. Developed seeds were relatively large, rounded, and dark brown in color. Smaller seeds that were either brown or tan, and shrunken or flattened in appearance were considered aborted. Since fruit set resulting from self- and cross- pollinations differed within and among clones (Table 1), both developed and aborted seed numbers were calculated on a per pollination rather than per fruit basis (e.g., no. developed seed/pollination = no. developed seed/fruit x proportion fruit set). Five clones which set zero fruit when selfed were assigned scores of zero developed seed per pollination (Table 2). This seemed reasonable, since parthenocarpic fruit were not observed in this V. corymbosum population - all ripe fruit contained at least one developed seed.

Directly after seed counts were made for each genotype x mating sample, the total fruit contents (aborted plus developed seed) were pooled and sown for a germination study. This was done by filling Petri dishes with moistened Bacto soil mix, spreading the seeds on the soil surface, and

covering the dishes. These containers were placed in a growth chamber set for a 15 hour daylength and a 10°/22° C diurnal temperature flux. Cumulative counts were made of the number of germinated seeds per sample over a period of 4 months. Percent germination of developed self and outcross seed was determined for 23 clones - the 5 self unfruitful genotypes were omitted from this comparison.

Relative survivorship, lethal equivalents, and relative self-fertility: Of the total number of fertilized ovules resulting from a mating, a certain proportion develop into viable seeds while the remainder abort, presumeably due to genetic defects. Following the example of Sorensen (1969) and Levin (1984), relative survivorship is defined as the proportion of developed seed per self-pollination (S) divided by the proprotion of developed seed per cross-pollination (S_1).

Relative seed survivorship (S/S_1) can be used to estimate embryonic genetic load in units of lethal equivalents. This is achieved by the following equation from Morton et al. (1956), first applied to plants by Sorensen (1969):

$$S/S_1 = e^{-(A+FB)}/e^{-A}$$
$$= e^{-FB}$$

where F is the inbreeding coefficient, A is the 'expressed' genetic load in a randomly mating population (A is estimated by S_1 at F=0), and B is the 'concealed' load per gamete, measured as lethal equivalents, which is manifested in the

homozygous condition during inbreeding (B is estimated by S at F=1/6 for a selfed autotetraploid). The number of lethal equivalents per zygote (2B) = $-12\ln(S/S_1)$ for an organism with tetrasomic inheritance.

In our study, relative survivorship values and quantitative estimates of genetic load could not be reliably obtained for the 5 clones which had zero self fruit set (Table 2). Although embryonic load is probably quite high in these individuals, based on their zero self-fertility and low cross-fertility, assigning various S values to them results in dramatic differences in load estimates. For example, if clone W6 (Table 2) is given an S value of 1%, the number of lethal equivalents per zygote is 43.6 - if it is assigned an S value of 5%, the number of embryonic lethal equivalents is 24.3. Therefore, these 5 individuals were omitted from the genetic load analysis.

Pollen staining: Fresh pollen was collected from random flowers on all 28 genotypes, stained in 0.05% aniline blue in lactophenol (Stanley and Linskens, 1974), and observed under a light microscope. Counts were made of the number stained microspores per tetrad for a minimum of 100 tetrads, and the percentage of total stained microspores was calculated for each clone.

Pollen tube growth rates: Four of the Otis Lake clones were self- and cross-pollinated in a greenhouse on the same day in February 1989. The greenhouse was illuminated (16 hour daylength) and diurnal temperatures ranged from 18°-

28° C. The genotypes used in this study (Table 4) were chosen because they represented a range of low to high levels of female fertility, as previously determined in the 1987 crossing experiment (Table 2).

Cross-pollinations were made using a frozen bulk of 'Bluecrop', 'Bluejay', and 'Jersey' pollen (similar in composition to the pollen bulk used in the 1987 fertility study). Fresh self pollen was used. Using the method of Moore (1964), flowers at a similar stage of development (recently opened) were emasculated, pollinated, and wrapped in cheesecloth. Six days after pollination (DAP), pistils from each genotype x mating treatment were fixed and prepared for flourescent microscopy as previously described (Krebs and Hancock, 1988). Eight ovaries per treatment were dissected, and counts were made of the number of ovules per ovary and the number of ovules which showed pollen tube entry into the embryo sac. Analysis of variance in the percentage of ovules entered by pollen tubes 6 DAP was performed on arcsine transformed data.

Allozyme diversity among clones: Tissue from the 28 Otis Lake clones was subjected to horizontal starch gel electrophoresis using extraction procedures, gel and electrode buffers, and staining schedules previously reported (Krebs and Hancock, in press). Optimal enzyme activity was achieved from floral buds breaking dormancy. Seven enzyme systems yielded clear and consistent banding patterns - malate dehydrogenase (MDH), isocitrate

dehydrogenase (IDH), shikimate dehydrogenase (SKDH), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and glutamate oxaloacetate transaminase (GOT).

Although polymorphisms appeared for all 7 systems, IDH and SKDH isozyme patterns proved too complex to interpret at the tetraploid level in the absence of progeny tests, and were therefore not included in the present analysis. Tetrasomic inheritance of alleles coding for 4 dimeric enzymes - MDH, 6PGD, PGI, and GOT - has been documented in V. corymbosum (Krebs and Hancock, in press). Limited analysis of PGM isozyme segregation, based on selfed progeny from several Otis Lake clones, suggested 1) a monomeric form of the functional enzyme 2) tetrasomy and 3) the presence of 2 loci. The 5 enzyme systems used in this study provided a total of 15 loci at which heterozygosity was determined for each clone.

Results

Fertility: Mean comparisons of self and outcross fertility in this V. corymbosum population are given in Table 1. Average fruit set following self-pollination (49.2%) was significantly lower than outcross fruit set (75.8%). Similarly, mean seed set per self pollination (3.5) was significantly less than outcross seed yield (14.1). Percent seed germination was approximately the same (75%) in

Table 1. Comparison of self and outcross means for fruit set, seed set and seed germination in the Otis Lake population.

Mating	Fruit set[a] (%)	No. developed seed/pollination	Developed seed[a] (% of total)	Germination[a] (%)
Self	49.2	3.5	23.4	72.9
Outcross	75.8	14.1	46.0	78.9
t-statistic	2.82**	5.23**	5.51**	0.87

[a]Mean comparison based on arcsine transformed percentages.

**Significant at the .01 level of probability.

either mating, indicating that most seeds scored as 'developed' were in fact viable.

A great deal of variation in fertility among clones was present. Individual self seed sets ranged from 0 - 23.0 per pollination, while outcross seed yields varied fron 0.4 - 33.5 per pollination (Table 2). Clones with zero or near-zero self fertility (< 1 seed per self pollination) comprised about 40% of the population. Differences in total fertility (combined self plus outcross seed yield) are graphically presented in Figure 1. The distribution was fairly continuous between genotypes with high combined female fertility (~50 seeds) and genotypes with very low total fertility as pistillate parents (< 1 seed).

Two associations between self- and cross-fertility were noted. Percent self and outcross fruit set were significantly correlated, r = 0.74, Figure 2. Seed sets following self- and cross-pollinations also showed a significant positive correlation, r = 0.72, Figure 3. Clones which were completely or nearly self-sterile generally had lower outcross seed yields than genotypes with moderate to high levels of self- fertility.

Load estimates: The percentage of developed seeds following self- pollination (S) ranged from 11.0% (clone OL7) to 58.7% (clone OL13), Table 2. For each genotype, percent seed viability was higher with outcrossing, where S_1 estimates varied between 13.5% (clone OL(2)) and 75.0% (clone OL13). S and S_1 were significantly correlated (r =

Table 2. Mean values for fertility, diversity, and genetic load parameters in a sample of 28 V. corymbosum clones from Otis Lake, Michigan. Population means are given at the bottom of the table.

Clone	No. developed seed/pollination		% developed seed		Relative survivorship	Lethal equivalents	No. alleles per locus	Percent stained
	self	outcross	self (S)	outcross (S_1)	(S/S_1)	per zygote	per individual	microspores
W6[a]	0.0	4.4	-	38.3	-	-	1.44	56.1
D2[a]	0.0	6.9	-	34.5	-	-	1.67	47.2
OL[a]	0.0	3.2	-	21.0	-	-	1.78	65.5
W7[a]	0.0	0.4	-	17.5	-	-	2.00	69.4
OL(2)[a]	0.0	0.4	-	13.5	-	-	1.78	72.7
OL5	0.1	14.7	15.6	58.6	0.27	15.9	1.89	96.9
OL11	0.1	3.6	17.6	35.0	0.51	8.2	1.89	91.7
W3	0.2	8.9	22.7	53.9	0.42	10.4	1.78	30.8
OL15	0.3	12.3	13.5	39.9	0.34	13.0	1.67	56.4
D3	0.6	15.3	26.2	65.9	0.40	11.1	2.00	95.4
OL(3)	0.8	4.0	17.6	21.2	0.83	2.2	1.75	83.9
OL17	1.0	9.1	32.3	63.6	0.51	8.2	1.67	77.0
GL	1.2	7.1	23.5	29.5	0.80	2.7	1.67	93.1
D1	2.0	8.4	26.0	54.1	0.48	8.8	1.56	75.0
OL9	2.1	21.3	23.9	58.8	0.41	10.8	2.00	77.0
OL12	2.3	12.6	12.5	38.0	0.33	13.3	2.00	82.2
OL10	3.0	25.4	22.2	63.8	0.35	12.7	1.50	93.2
SR	3.1	14.8	22.3	38.5	0.58	6.6	1.56	79.8
OL19	3.7	9.3	26.8	48.7	0.55	7.2	1.78	94.2
OL7	3.9	26.6	11.0	60.2	0.18	20.4	2.00	98.0
OL14	4.8	27.2	13.3	51.1	0.26	16.1	2.00	90.5
OL3	5.0	17.6	18.2	60.9	0.30	14.5	1.78	93.5
D4	5.5	26.5	29.4	63.7	0.46	9.3	1.78	83.9
D5	5.6	13.0	13.2	27.0	0.49	8.6	1.56	98.8
OL2	7.1	24.1	40.3	65.7	0.61	5.8	1.78	98.9
D7	7.3	14.5	20.3	33.0	0.62	5.8	1.67	96.1
OL18	15.0	33.5	31.9	56.0	0.57	6.8	1.56	100.0
OL13	23.0	30.9	58.7	75.0	0.78	2.9	1.67	97.4
$\bar{\bar{x}}$	3.5	14.1	23.4	46.0	0.48	9.6	1.76	81.9

[a]Clones which had zero self fruit set.

Figure 1. Variation in total female fertility based on hand-pollinations of 28 V. corymbosum clones from Otis Lake, Michigan.

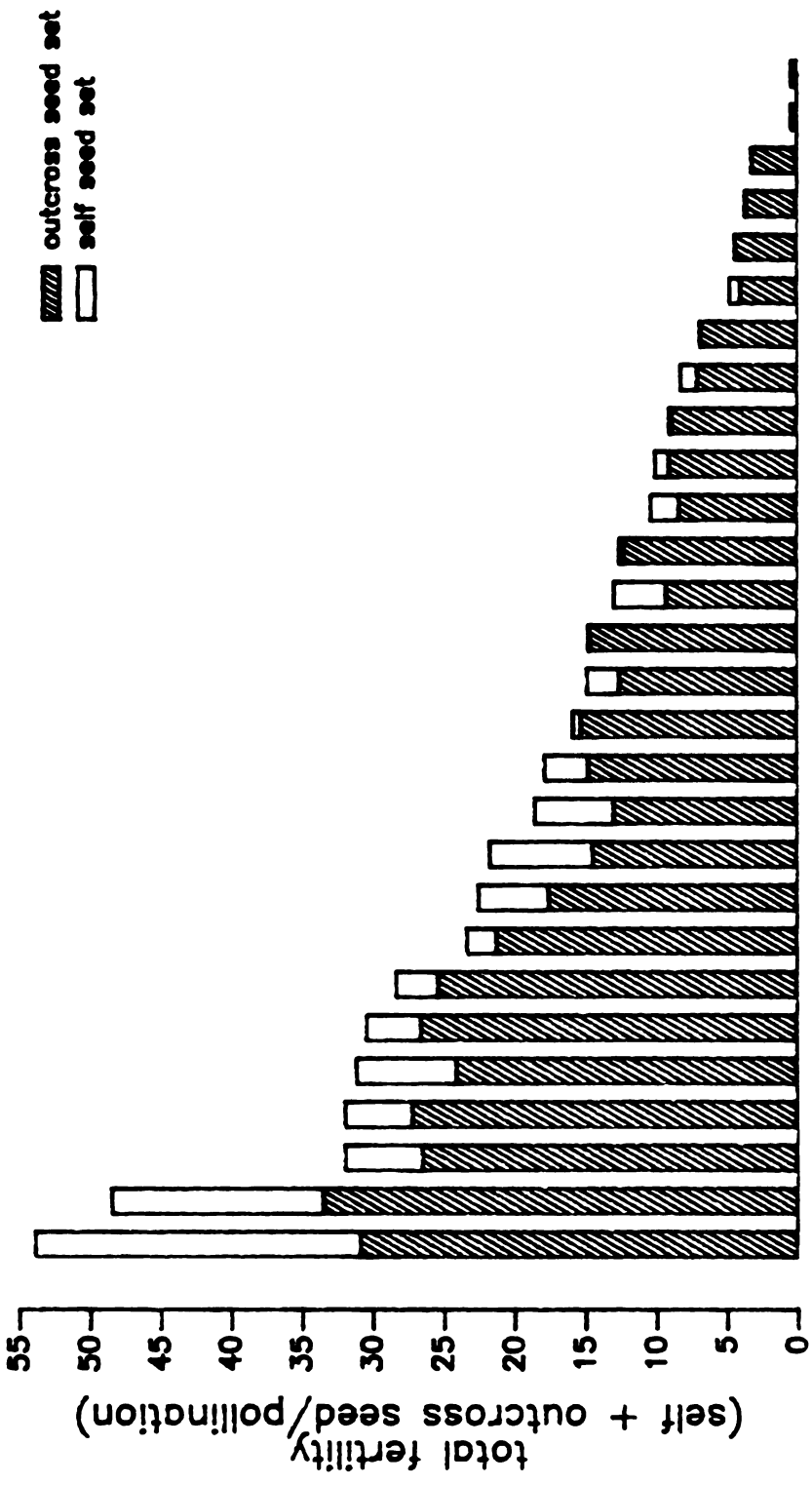

outcross seed set
self seed set
total fertility
(self + outcross seed/pollination)
55
50
45
40
35
30
25
20
15
10
5
0
clone

Figure 1.

Figure 2. Correlation of self and outcross fruit set on 28 *V. corymbosum* genotypes (arcsine transformed percentages).

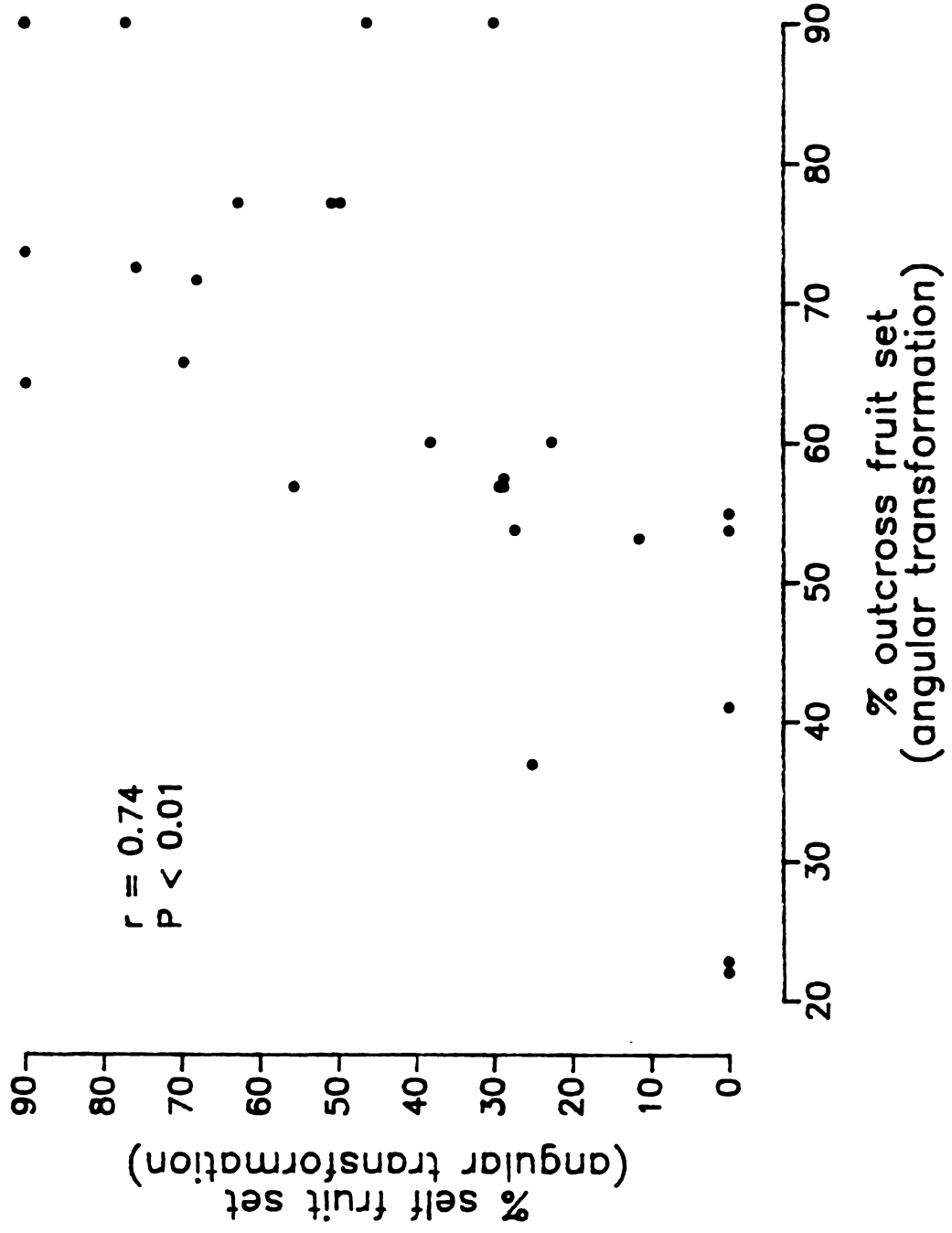

% self fruit set
(angular transformation)
90
80
70
60
50
40
30
20
10
0
r = 0.74
P < 0.01
20
30
40
50
60
70
80
90
% outcross fruit set
(angular transformation)

Figure 2.

Figure 3. **Correlation of self and outcross seed set in 28** *V. corymbosum* clones.

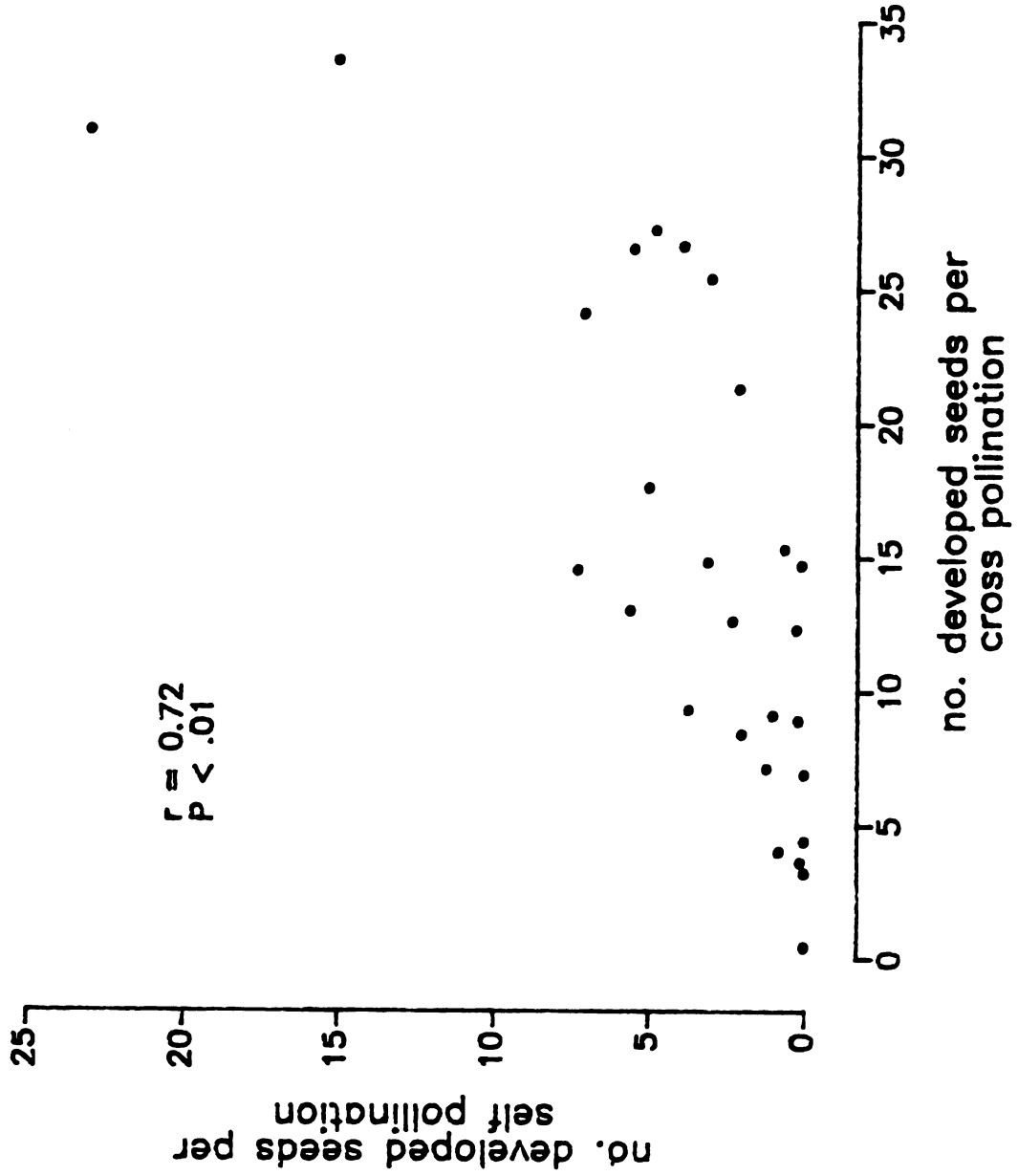

no. developed seeds per
cross pollination
0
5
10
15
20
25
30
35
r = 0.72
P < .01
no. developed seeds per
self pollination
0
5
10
15
20
25

Figure 3.

0.57, $P < 0.01$, based on 23 comparisons from Table 2). The average proportion of embryos which formed viable seeds was significantly lower in self- pollinations (23.4%) than in cross-pollinations (46.0%), Table 1. Conversely, mean self seed abortion of 76.6% was significantly higher than the mean outcross seed abortion of 54.0%.

The average survivorship of self seed relative to outcross seed was 0.48, varying from 0.18 - 0.83 among clones (Table 2). The numbers of lethal equivalents per zygote, which were derived from relative survivorship values, averaged 9.6 for the population. The range among genotypes extended from 2.2 - 20.4 embryonic lethal equivalents.

Genetic diversity: The sample population contained a great deal of genetic variability, based on electrophoretic estimates of enzyme diversity. Of the 15 loci characterized, 9 (60%) were polymorphic (Table 3). No variation was observed for Mdh-3, Mdh-4, Pgm-1, Got-1, Got-2, or Got-4. Three or more allozymes were detected at most polymorphic loci, averaging 2.1 variants over all 15 loci. Multiple allelism was observed at 2 loci, Pgi-2 and Pgm-2, where tri-allelic genotypes were present. Average heterozygosity was also highest (> 2.0) at these loci. Relatively low heterozygosity at two other loci, Pgi-1 and 6Pgd-1, was due to a high frequency for one of the 3 alleles present in the population at each of these loci.

Table 3. Summary of genetic diversity at 9 polymorphic enzyme loci, based on a sample of 28 Otis Lake highbush blueberry accessions.

Locus	No. allelic variants in population	Mean no. alleles per individual
Pgi-1	3	1.29
Pgi-2	4	2.25
Mdh-1	3	1.82
Mdh-2	3	1.50
Pgm-2	3	2.29
6Pgd-1	3	1.36
6Pgd-2	2	1.57
Got-3	2	1.82
Got-5	3	1.86
$\bar{x}$	2.1[a]	1.76

[a]Averaged over all 15 loci (9 polymorphic, 6 monomorphic).

Figure 4. Regression of embryonic load estimates on average heterozygosity in 23 V. corymbosum individuals. Numbers of alleles per locus are based on 9 polymorphic loci.

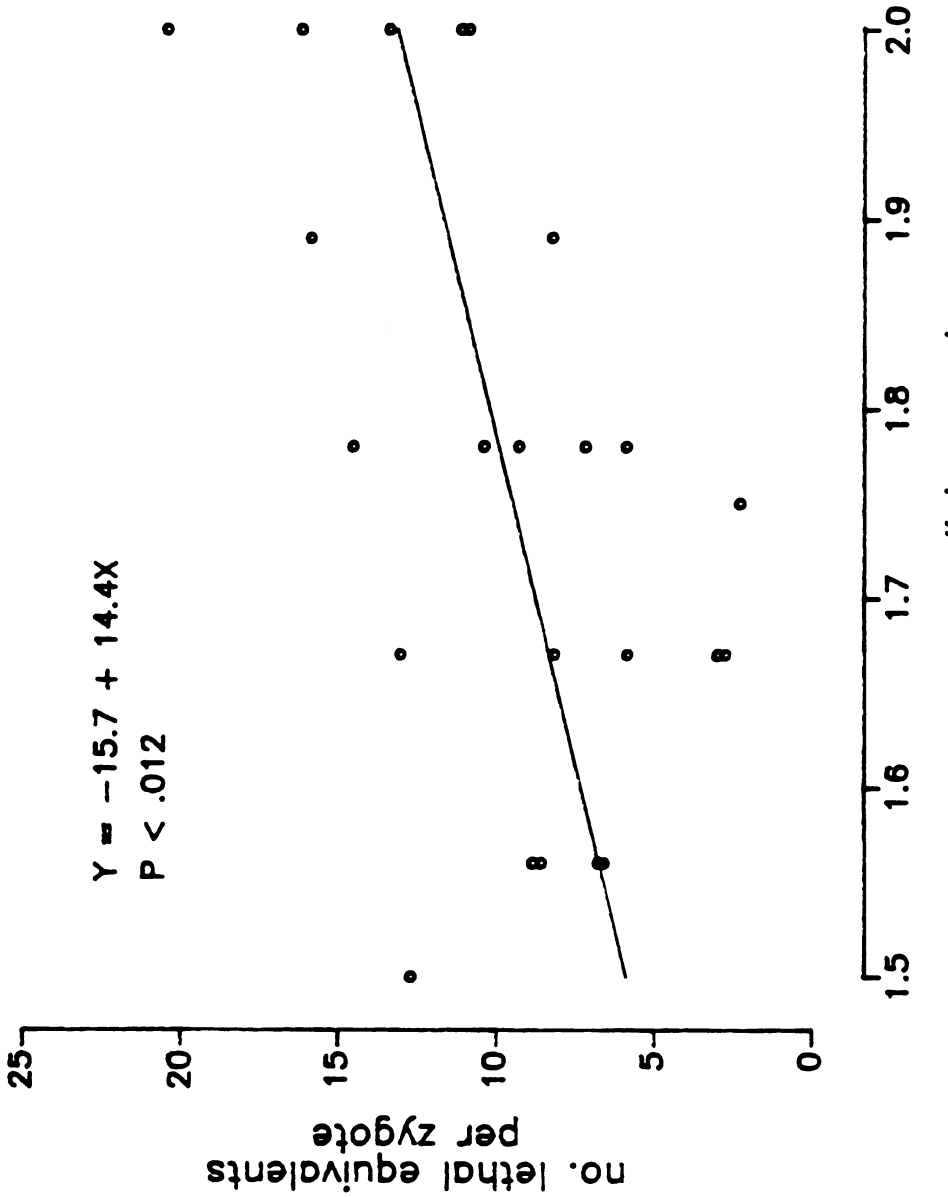

Y = −15.7 + 14.4X
P < .012
no. lethal equivalents per zygote
25
20
15
10
5
0
1.5
1.6
1.7
1.8
1.9
2.0
mean no. alleles per locus per clone

Figure 4.

Individual clones averaged 1.44 - 2.00 alleles per (polymorphic) locus, and the population mean was 1.76 (Table 2). The relationship between embryonic genetic load and heterozygosity is given as a linear regression in Figure 4. The number of lethal equivalents per zygote increased as genetic variability within clones increased. The regression coefficient was significant - differences in heterozygosity among individuals accounted for about 26% of the variation in embryonic load.

Pollen characteristics: Estimates of pollen viability, based on stainability, ranged from 30.8 - 100%, with a mean of 81.9% (Table 2). A correlation of the number of lethal equivalents per gamete (1/2 the number of embryonic lethal equivalents) with pollen viability was not significant ($r = -0.06$, based on 23 comparisons).

Growth rates for self and outcross pollen on 4 Otis Lake genotypes are given in Table 4. Also shown are previously determined estimates of seed set for each genotype x mating treatment, as well as pollen viabilities. The proportion of ovules entered by pollen tubes 6 DAP ranged from 14.7% (D2 selfed) to 79.7% (OL13 outcrossed). Analysis of variance of these data (Table 5) showed that there were significant effects of mating type, female genotype, and their interactions on pollen tube growth rates.

Self pollen tubes entered ovules at a significantly slower rate than outcross pollen in three of the 4 clones

Table 4. Growth rates of self and outcross pollen in 4 V. corymbosum clones, measured as the proportion of ovules in which pollen tubes had entered the embryo sac, 6 days after pollination.

Genotype	Mating	Viable seed/ pollination[a]	% stained microspores[a]	% ovules entered by pollen tubes[b]	
OL13	self	23.0	97	54.2	(26.0/48.0)
	outcross	30.9	87	79.7	(41.1/51.6)
GL	self	1.2	93	67.0	(26.9/40.2)
	outcross	7.1	87	61.8	(24.6/39.8)
D2	self	0	47	14.7	(9.2/62.4)
	outcross	6.9	87	35.1	(19.6/55.9)
OL	self	0	65	22.4	(11.2/49.8)
	outcross	3.2	87	38.2	(22.5/58.9)

[a]Data from other studies and presented in Table 2 (outcross pollen staining is an average of 3 cultivars used in the bulk).

[b]Percentages are followed in parentheses by the mean number of ovules penetrated divided by the mean number of ovules per ovary.

Table 5. Analysis of variance showing the effects of mating type and pistillate genotype on pollen tube growth rates into ovules (arcsine-transformed percentage data).

Sources	df	MS
Mating	1	1401.6**
Genotype	3	3007.9**
Mating x genotype	3	344.9**
Error	56	51.0

**Significant at the .01 level of probability.

studied. In the remaining genotype, GL, pollen tubes from either source were roughly equivalent in growth rates (Table 4).

Differences in pistillate genotype accounted for most of the variation in gametophyte development (Table 5). If the 4 clones are assessed in terms of their seed producing capacity, some correspondance between female fertility and receptivity to pollen is observed. The 2 low fertility individuals, OL and D2, exhibited rates of tube entry in both types of mating (14.7 - 38.2%) that were significantly lower than rates of ovule penetration (54.2 - 79.7%) by either type of pollen in the higher fertility clones (GL and OL13), Table 4. Identical cross pollinations in a common environment showed that high fertility clones were more receptive to the outcross pollen bulk than the low fertility clones.

Discussion

Embryonic genetic load and fertility: A great deal of variability in both self- and cross-fertility was observed in this V. corymbosum population. The distribution was fairly continuous between two extremes - clones that were essentially female fertile and those with high seed yields in either mating. The distribution was also skewed towards a higher frequency of self-sterile (or nearly self-sterile) than self- fertile genotypes. Average seed set per self-pollination in the Otis Lake population (3.5)was much higher

than that reported elsewhere for V. corymbosum (Vander Kloet and Cabilio, 1984) and related taxa such as V. angustifolium (Aalders and Hall, 1961) and V. ashei (Garvey and Lyrene, 1987). Highly self-fertile variants, such as clones OL13 and OL18 in our collection, were not observed in these other surveys.

Previous studies of V. corymbosum cultivars demonstrated that fertility is regulated to a large extent by seed abortion (Krebs and Hancock, 1988; Krebs and Hancock, in prep). Most notably, seed yields of cultivars were inversely correlated, and seed abortion postively correlated, with zygotic levels of inbreeding. Variability in fertility within and among clones was attributed to 1) differences in relatedness among mates and 2) differences in the amount of embryonic genetic load carried by individuals. As an outcrossing, perennial, autotetraploid species, V. corymbosum is expected to maintain considerable genetic variability (Hamrick et al., 1979), and therefore to have a large genetic load-bearing capacity. In highbush blueberries, it was hypothesized that much of this load consists of recessive mutations or allelic combinations which disrupt normal seed development and cause abortion. Inbreeding would result in the expression of embryo-lethal recessives (mutational load) or loss of allelic interactions, i.e. heterozygosity, essential to embryonic vigor (segregational load).

Several associations noted in the Otis Lake population are consistent with this genetical interpretation. In particular, the significant correlations between self and outcross fruit set, seed set, and seed survivorship indicate a common genetic control of fertility in both matings by the pistillate genotype. According to the genetic load model, seed parents with a high incidence of embryo-lethal or semi-lethal factors are expected to have zero or very low self-fertility, and low average cross fertility as well.

Estimates of genetic load in this V. corymbosum population ranged from 2.2 - 20.4 lethal equivalents per zygote, with an average of 9.6 and a median value of 8.8. The Otis Lake average value is at least twice as large as the mean number of embryonic lethal equivalents calculated for other angiosperm species by Levin (1984), and is comparable to the highest genetic loads observed among conifers (Sorensen, 1969; Franklin, 1972; Park and Fowler, 1982). The average value of 9.6 lethal equivalents per zygote in our survey is a conservative estimate because the self-unfruitful (and presumeably self-sterile) clones were omitted from the genetic load analysis. These individuals also exhibited below average outcross seed survivorship (Table 2) and thus probably carried a large number of embryo-lethal mutations.

Gametic, gametophytic, and zygotic components of fertility: The data suggest that the incidence of seed abortion is a primary cause of differential fertility among

these V. corymbosum clones. In addition, there is some evidence indicating that variable frequencies of fertilization may also affect seed sets. Fertilization frequency is determined by factors such as pollen viablity, number of pollen tubes growing into the ovary, and pollen tube growth rates. As mentioned earlier, inbreeding depression may be expressed during gametogenesis and gametophyte development. Viability depression may thus reduce the probability that a given microspore will germinate, grow down the style, and fertilize an ovule.

Flourescent microscopy of V. corymbosum ovaries 6 days after pollination revealed 1) a significantly lower frequency of ovule penetration by self pollen tubes in 3 of the 4 clones examined and 2) a tendency for low fertility genotypes to be less 'receptive' to both self and outcross pollen, i.e. show lower frequencies of tube entry into ovules than high fertility clones. Differential growth rates for self and foreign pollen in highbush blueberry cultivars were previously inferred from experiments in which removal of self-pollinated styles reduced fruit set more than removal of cross-pollinated styles (Knight and Scott, 1964). In addition, a pollen chase study demonstrated that in mixtures, outcross pollen 'outcompetes' self pollen and reaches the ovules first, even when applied to stigmas 24 hours later than self pollen (Krebs and Hancock, in preparation).

Partial inhibition of self pollen has also been observed in Medicago sativa (Cooper and Brink, 1940) and Lotus corniculatus (Wojciechowska, 1963). In a parallel study of seed set and pollen tube growth in M. sativa (Sayers and Murphy, 1966), associations between female fertility and receptivity to pollen were strikingly similar to those observed in V. corymbosum. The fact that clones with low self and outcross seed yields also exhibit reduced fertilization frequencies to some extent reflects a cause-and-effect relationship, but in both these species, the number of ovules fertilized often greatly exceeds the number of viable seeds produced (Table 4), indicating that embryo abortion is the stronger determinant of reproductive success. Nonetheless, the association suggests that both gametophytic and embryonic development are mutually affected by increased genetic loads in the pistillate parent, such that the probability of pollen tubes effecting fertilization, as well as the probability of fertilized ovules forming viable seeds, is reduced.

Is gamete viability also a function of genetic load in highbush blueberry? Pollen stainability in the Otis Lake population ranged from 30.8 - 100%, but the variability for this trait was not significantly correlated with differences in the number of lethal equivalents per gamete. However, this comparison omitted 5 self-unfruitful clones which had below average pollen stainabilities (Table 2). The correlation of self seed set with pollen viability, using

the complete data set (n=28), was significant ($r = 0.40$, $P < 0.05$, from data presented in Table 2), as was the correlation between outcross seed set and pollen viability ($r = 0.38$, $P < 0.05$). Genotypes with low overall fertility thus showed some tendency towards reduced pollen staining, suggesting that male, as well as female, fertility is symptomatic of genetic load conditions.

Additional support for this notion is provided by the observation that inbred progeny of V. corymbosum cultivars have reduced pollen viabilities (Krebs and Hancock, submitted). Also, a survey of pollen from many Vaccinium species demonstrated that differences in viability were probably due to post-meiotic abortion rather than chromosome pairing abnormalities, and that pollen from tetraploid species had higher stainability than diploid pollen (Cockerham and Galletta, 1976). Polyploidy is considered an event which reduces genetic load (Lande and Schemske, 1985; Hedrick, 1987).

Genetic load and isozyme variability: The high levels of inbreeding depression (embryo mortality) observed in the Otis Lake population are perhaps best understood when the genetic diversity of V. corymbosum is considered. Genetic load and heterozygosity are interrelated phenomena. Genetically variable species experience inbreeding depression because 1) deleterious recessive mutations are normally carried at low frequencies by such organisms, and these mutations are expressed in the homozygous condition

after inbreeding and 2) phenotypic vigor may depend on allelic interactions (balanced polymorphisms) which are lost upon inbreeding.

This general relationship between genetic load and heterozygosity is confirmed in our study, where estimates of embryonic load (number of lethal equivalents per zygote) and genetic diversity (average number of alleles per enzyme locus) at the individual level were significantly correlated. Similar associations were noted in a comparison of fern species (Hedrick, 1987) which showed that outcrossers had high genetic loads while inbreeders and mixed-mating types had low genetic loads. However, Levin (1984) did not find a significant correlation between lethal equivalents and gene diversity in his comparison of 15 Phlox populations.

It is instructive to compare intrapopulation diversity in V. corymbosum with electrophoretic variability in other species which have similar life-history characteristics (see review by Hamrick et al., 1979). Otis Lake diversity parameters such as percent polymorphic loci (P = 60), average number of alleles occuring per locus in a population (A = 2.1), or mean heterozygosity per individual (H = 0.38) have relatively high values, comparable to those estimated for other outcrossing, long- lived, and highly fecund plant species (e.g. conifers and woody dicots).

Life-history traits favoring the maintenance and accumulation of genetic variability are also conducive to

the build-up of genetic load. Accumulation of somatic mutations is probably higher in perennials than in biennials or annuals because of prolonged meristematic cell divisions (Klekowski, 1988). Loci controlling fertility traits in long- lived plants have 'neutral' fitnesses throughout most of the life cycle, and thus are particularly prone to accumulating deleterious mutations. High fecundity in plant species allows the perpetuation of balanced polymorphisms by intense selection against recombinant types (Hamrick et al., 1979). Large gametic outputs temper the damage inflicted by embryonic genetic load, since the reduced probability of an ovule forming a viable seed is offset by an increased number of ovules. Mating system theory predicts higher loads for outcrossers than inbreeders (Lande and Schemske, 1985; Hedrick, 1979). In addition to these life-history characteristics, V. corymbosum also has tetrasomic inheritance, which results in higher mutation-selection equilibrium frequencies (Bennett, 1976; but see Lande and Schemske, 1985). It is therefore not surprising that V. corymbosum and other species with similar life-histories, such as conifers, have the highest reported genetic loads.

Pregermination and postgermination inbreeding depression: Very little is known about the relative vigor and fecundity of inbred and non-inbred highbush blueberries. This is much needed information, since it addresses the question of whether or not embryo abortion acts as a genetic 'sieve' which effectively reduces the number of low-quality

offspring in the next generation. It was noted earlier that in many conifers, inbreeding depression is more severe during embryogenesis than at later stages of the life cycle, in accordance with Haldane's (1957) prediction that pregermination mortality is preferable to postgermination mortality in terms of population fitness.

Studies of Vaccinium inbreds have produced mixed results. In hexaploid V. ashei cultivars, self progeny averaged a 37% decrease in seedling survival and a 36-40% decline in 2-year-old vegetative growth, compared to outcross progeny (Lyrene, 1983). In another study, increased relatedness among parent cultivars of V. ashei and V. corymbosum was consistently associated with decreased seed set, but had little or no effect on offspring germination or 8-month-old seedling fresh weights (Hellman and Moore, 1983). No difference between self and outcross seed germination was observed in the present study. Self progeny of V. corymbosum cultivars were largely self-sterile (in contrast to their self-fertile parents), and exhibited a reduction in both male and female fertility (Krebs and Hancock, in prep.).

Based on these limited data, it is difficult to judge the relative impact of genetic load at various stages of sporophyte development in V. corymbosum. The average survivorship of self-fertilized embryos in the Otis Lake population was about 50% that of cross-fertilized embryos (a conservative estimate), indicating fairly high mortality

due to 'early-acting' inbreeding depression. However, the data from cultivars suggest that viability depression at juvenile and adult growth stages would also be expected in inbreds from this population.

N_e, mating systems, and load reduction: Muller (1932, 1964) first formulated the idea that sex is 'necessary' as a means of reducing mutational load in populations, for the simple reason that sexual reproduction produces recombinants with fewer mutations than the parents, whereas asexual reproduction does not. Differential mating success can also reduce genetic load if individuals carrying fewer deleterious alleles are able to produce more offspring and increase their genetic contribution in the next generation. This may be a means by which the frequency of 'sterility genes' (embryo lethals) among offspring in reduced in V. corymbosum populations.

Vander Kloet and Cabilio (1984) studied open-pollinated seed sets in 50 wild V. corymbosum clones over a 4 year period. The average number of seeds per fruit ranged from 2.9 - 33.7, with a mean of 11.1. These values are very similar in magnitude to those obtained in hand- pollinations on the Otis Lake genotypes. The significant finding was that 2 high fertility clones (4% of the population) produced 25% of the total seed pool each year, and that half the population (low fertility clones) accounted for less than 12% of the total annual seed yield. In our collection of highbush blueberries, the 2 highest fertility genotypes (7%

of the population) contributed 21% of the total (self plus outcross) offspring. At the other extreme, 2 clones produced essentially no seeds at all.

Effective population size (N_e) is therefore much lower than the actual number of adults in V. corymbosum populations. Reproductive success is disproportionately higher in matings involving high fertility genotypes (clones with fewer developmental mutations affecting embryogenesis). Although a small N_e creates an inbred population structure, the negative aspects of such non-random mating in V. corymbosum are probably offset by the benefits - an increased probability that offspring themselves will be highly fertile.

The differences in mating system among these Otis Lake clones points to another mechanism which reduces genetic load by restricting the transmission of sterility genes. The population consisted of self- sterile (strictly outcrossing) and self-fertile (mixed mating) genotypes. In V. corymbosum cultivars, we noted that fairly low levels of inbreeding at the autotetraploid level (F = 0.17 - 0.20) could induce self-sterility, and hence a shift in mating system from facultative selfing to obligate outcrossing (Krebs and Hancock, in prep). Apparently, an inbreeding 'threshold' exists, which can be imagined as a certain number of fertility loci homozygous for sublethal developmental mutations. Once this threshold is reached (most rapidly by selfing), further fixation of deleterious alleles results in

100% embryo abortion, and reproductive success occurs only via outcrossing and recombination.

Conclusion: We have used a genetic load model to assess fertility in a natural V. corymbosum population, and to characterize differential viability of gametes, gametophytes, and zygotes during reproduction. Associations of low pollen viability, reduced female receptivity to pollen tubes, and low seed survivorship suggested that these components of fertility are all part of a genetic load 'syndrome'. However, embryo abortion was the primary factor controlling seed set in this population.

Embryo abortion in ovaries with multiple ovules (e.g. highbush blueberries, with ~50 ovules/ovary) can conceivably result from two causes: 1) competition among different embryo genotypes in a given pistillate environment and 2) expression of developmental lethals during embryogenesis. Competition favoring more vigorous embryos is considered a form of developmental selection (Buchholz, 1922; Klekowski, 1988), which is also 'soft' selection, since relative offspring vigor is assessed and at least one genotype survives. In contrast, lethal genotypes are under 'hard' selection, since they are eliminated regardless of the population and environment in which they occur. Hard selection against defective embryos is evident in V. corymbosum, since fertility is zero (in self and some outcross matings as well) when a genetic load threshold is exceeded. In his survey of reproductive success in flowering

plants, Wiens et al.(1987) noted that both the 'relativistic' action of developmental selection and 'absolutistic' genetic load mechanisms could provide a combined means of ensuring offspring quality.

Literature Cited

1. Aalders, L.E. and I.V. Hall. 1961. Pollen incompatibility and fruit set in lowbush blueberries. Can. J. Gen. Cytol. 3: 300-307.
2. Ballington, J.R. and G.J. Galletta. 1978. Comparative crossability of 4 diploid Vaccinium species. J. Am. Soc. Hort. Sci. 103: 554-560.
3. Bennett, J.H. 1976. Expectations for inbreeding depression on self-fertilization of tetraploids. Biometrics 32: 449-452.
4. Buchholz, J.T. 1922. Developmental selection in vascular plants. Bot. Gaz. 73: 249-286.
5. Busbice, T.H. 1968. Effects of inbreeding on fertility in Medicago sativa L. Crop Sci. 8: 231-234.
6. Cockerham, L.E. and G.J. Galletta. 1976. A survey of pollen characteristics in certain Vaccinium species. J. Am. Soc. Hort. Sci. 101: 671-675.
7. Cooper, D.C. and R.A. Brink. 1940. Partial self-incompatibility and the collapse of fertile ovules as factors affection seed formation in alfalfa. J. Agric. Res. 60: 453-472.
8. Crowe, L.K. 1971. The polygenic control of outbreeding in Borago officinalis. Heredity 27: 111-118.
9. de Nettancourt, D. 1977. Incompatibitility in angiosperms. Springer- Verlag, Berlin.
10. Eldridge, K.G. and A.R. Griffin. 1983. Selfing effects in Eucalyptus regnans. Silvae Genet. 32: 216-221.

11. Franklin, E.C. 1972. Genetic load in loblolly pine. Am. Nat. 106: 262- 265.

12. Gabriel, W.J. 1967. Reproductive behavior in sugar maple: self- incompatibility, cross-compatibility, agamospermy, and agamocarpy. Silvae Genet. 16: 165-168.

13. Galletta, G.J. 1975. Blueberries and cranberries. In: Moore, J.N. and J. Janick (eds.). Advances in fruit breeding. Purdue Univ. Press, West Lafayette. pp. 159-161.

14. Garvey, E.J. and P.M. Lyrene. 1987. Self-incompatibility in 19 native blueberry selections. J. Amer. Soc. Hort. Sci. 112: 856-858.

15. Hamrick, J.L., Y.B. Linhart, and J.B. Mitton. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Sys. 10:173-200.

16. Hedrick, P.W. 1987. Genetic load and the mating system in homosporous ferns. Evolution 41: 1282-1289.

17. Hellman, E.W. and J.N. Moore. 1983. Effect of genetic relationship to pollinizer on fruit, seed, and seedling parameters in highbush and rabbiteye blueberries. J. Amer. Soc. Hort. Sci. 108: 401-405.

18. Klekowski, E.J. 1988. Mutation, developmental selection, and plant evolution. Columbia Univ. Press, New York.

19. Knight, R.J. and D.H. Scott. 1964. Effects of temperature on self- and cross-pollination and fruiting of four highbush blueberry varieties. J. Am. Soc. Hort. Sci. 85: 302-306.

20. Koski, V. 1973. On self-pollination, genetic load, and subsequent inbreeding in some conifers. Comm. Inst. For. Fenn. No. 78.

21. Krebs, S.L. and J.F. Hancock. 1988. The consequences of inbreeding on fertility in Vaccinium corymbosum. J. Am. Soc. Hort. Sci. 113:914-918.

22. Krebs, S.L. and J.F. Hancock. Tetrasomic inheritance of isoenzyme markers in the highbush blueberry, Vaccinium corymbosum. Heredity. In press.

23. Krebs, S.L. and J.F. Hancock. Genetic load and mating system in the highbush blueberry, Vaccinium corymbosum. Submitted.

24. Lande, R. and D.W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39: 24-40.

25. Lester, D.T. 1971. Self-compatibility and inbreeding depression in American elm. For. Sci. 17: 321-322.

26. Levin, D.A. 1984. Inbreeding depression and proximity-dependent crossing success in Phlox drummondii. Evolution 38: 116-127.

27. Lyrene, P.M. 1983. Inbreeding depression in rabbiteye blueberries. HortSci. 18: 226-227.

28. Mergen, F., J. Burley, and G.M. Furnival. 1965. Embryo and seedling development in Picea glauca (Moench.) Voss after self-, cross-, and wind-pollination. Silvae Genet. 14: 188-194.

29. Morton, N.E., J.F. Crow, and H.J. Muller. 1956. An estimate of mutational damage in man from consanguineous marriages. Nat. Acad. Sci., Proc. 42: 855-863.

30. Moore, J.N. 1964. Duration of receptivity to pollination of flowers of the highbush blueberry and the cultivated strawberry. J. Am. Soc. Hort. Sci. 85: 295-301.

31. Mulcahy, D.L. 1974. Correlation between speed of pollen tube growth and seedling height in Zea mays. Nature 249: 491-493.

32. Muller, H.J. 1932. Some genetic aspects of sex. Am. Nat. 66: 118-138.

33. Muller, H.J. 1964. The relation of recombination to mutational advance. Mutat. Res. 1: 2-9.

34. Orr-Ewing, A.L. 1957. A cytological study of the effects of self- pollination on Pseudostuga menziesii (Mirb.) Franco. Silvae Genet. 6: 179-185.

35. Park, Y.S. and D.P. Fowler. Effects of inbreeding and genetic variances in a natural population of tamarack (Larix laricina (Du Roi) K. Koch) in eastern Canada. Sivae Genet. 31: 21-26.

36. Park, Y.S. and D.P. Fowler. 1984. Inbreeding in black spruce (Picea mariana (Mill) B.S.P.): self-fertility, genetic load, and performance. Can. J. For. Res. 14: 17-21.

37. Pritts, M.P. and J.F. Hancock. 1985. Lifetime biomass partitionings and yield component relationships in the highbush blueberry, Vaccinium corymbosum (Ericaceae). Am. J. Bot. 72: 446-452.

38. Sayers, E.R. and R.P. Murphy. 1966. Seed set in alfalfa as related to pollen tube growth, fertilization frequency, and post-fertilization ovule abortion. Crop Sci. 6: 365-368.

39. Schmitt, D. and T.O. Perry. 1964. Self-sterility in sweetgum. For. Sci. 3: 302-305.

40. Seavy, S.R. and K.S. Bawa. 1986. Late-acting incompatibility in angiosperms. Bot. Rev. 52: 195-219.

41. Sorensen, F. 1969. Embryonic genetic load in coastal douglas fir, Pseudostuga menziesii var. menziesii. Am. Nat. 103: 389-398.

42. Stanley, R.G. and H.F. Linskens. 1974. Pollen: biology, biochemistry, management. Springer-Verlag, Berlin. pp. 67-86.

43. Stephenson, A.G. and R.I. Bertin. 1983. Male competition, female choic, and sexual selection in plants. In: Real, L.A. (ed). Pollination biology. Academic Press, New York.

44. Vander Kloet, S.P. and P. Cabilio. 1984. Annual variation in seed production in a population of Vaccinium corymbosum L. Bull. Torr. Bot. 111: 483-488.

45. Wiens, D. 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. Oecologia 64: 47-53.

46. Wiens, D., C.L. Calvin, C.A. Wilson, C.I. Davern, D. Frank, and S.R. Seavy. 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. Oecologia 71: 501-509.

47. Wojciechowska, B. 1963. Embryological studies in the genus Lotus. Part I. Fertilization and seed development following open- and self- pollination of Lotus corniculatus L. Genet. Polon. 4: 53-63.

48. Wood, G.W. 1968. Self-fertility in the lowbush blueberry. Can. J. Plant Sci. 48: 431-433.