- 12. Kramer, P.J. 1983. Water relations of plants. Academic, New York.
- 13. Lakso, A.N. 1982. Precautions on the use of excised shoots for photosynthesis and water relation measurements of apple and grape leaves. HortScience 17:368-370.
- Mansfield, T.A., A.R. Wellburn, and T.J.S. Moreira. 1978. The role of abscisic acid and farnesol in the alleviation of water stress. Phil. Trans. R. Soc. London Ser. A 284:471-482

J. AMER. Soc. HORT. Sci. 113(6):914-918. 1988.

- Mizrahi, Y., S.G. Scherings, S.M. Arad, and A.E. Richmond. 1974. Aspects of the effect of ABA on the water status of barley and wheat seedlings. Physiol. Plant 31:44-50.
- 16. O'Toole, J.C. and R.T. Cruz. 1983. Genotypic variation in epicuticular wax load of rice. Crop Sci. 23:392-394.
- Shearman, R.C. 1986. Kentucky bluegrass cultivar evapotranspiration rates. HortScience 21:455–457.

The Consequences of Inbreeding on Fertility in *Vaccinium corymbosum* L.

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Additional index words. highbush blueberry, self-incompatibility

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 of 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 that promote seed abortion.

Seed set following self-pollination of blueberries, Vaccinium section Cyanococcus, varies both within and among the three predominant ploidy levels. Diploid populations consist of individuals that 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% of cross-pollinated seed production (4, 8, 15, 17). No clones have been identified that are equally cross- and self-fertile. Hexaploid (rabbiteye) cultivars, but also have much lower levels of seed set in both types of matings (8, 9).

Self-incompatibility has been proposed as the basis for selfdseed reduction in all ploidies of blueberries (2, 8, 9, 17). However, only diploid blueberry species meet one of the expectations of a true, self-incompatibility system—all clones taken from a genetically diverse population must show zero or nearzero seed set when self-pollinated. Genetic self-incompatibility generally is 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, which 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 that 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 cultivar 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 crosspollinations.

Materials and Methods

Crossing studies. The six cultivars (20-year-old plants) used in this study were growing in a complete randomized design at the Michigan Blueberry Growers Research Station, Grand Junction. Cross- and self-pollinations were made on four 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 six 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; these were removed to expose the pistil. Cross-pollinated flowers were emasculated by removing the stamens. All pollinated inflorescences 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 cultivar \times crossing treatment, without separating fruit from the four replications. Where possible, 50 or more fruit 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 six cultivars ranged from 0 to 0.19 based on pedigree analysis (10). In the case of self-

Received for publication 19 Jan. 1988. Michigan State University Agricultural Experiment Station Journal Article no. 12763. We thank John Nelson of the Michigan Blueberry Growers Assn. for this cooperation in the field. Debts are also owed to Joanne Whallon for the use of her Zeiss microscope, to Peter Callow and Carol Schumann for help in counting seeds, and to Cheryllee Finney for typing the manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

pollinations, estimates of zygotic levels of inbreeding were exactly proportional to initial F values of the seed parents (F_m). 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 six source cultivars 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 F_m 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 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 Feb. 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 fruit 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 glacial acetic acid : 3 ethanol, and stored in 70% ethanol. Tissue preparations for fluorescent microscopy involved a modification of Martin's technique (13): the samples were soaked overnight in 8 N NaOH, rinsed in several changes of distilled water (1 to 2 hr), and stained for 10 to 20 min in 0.1% aniline blue dissolved in a 0.7% potassium phosphate (tribasic) buffer, adjusted to a pH of 8.5. Pollen tube growth and ovule penetration was examined under UV light in five sample squashes from each harvest date.

Results

Effects of self- vs. cross-pollination. The effects of mating type on berry weights and seed numbers are given in Table 1. Selfing resulted in a 4% to 29% 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 the same test cultivar. Viable seed set per fruit among selfs ranged from 1.3 to about 15, well below fertility values in outcrosses, which averaged from about 9.5 to 48 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 (F_m) and seed parameters are shown in Table 2. None of the correlations were significant at the 5% level. Viable seed number per fruit was inversely correlated with F_m 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 F_m 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 = -0.59). The increased proportion of seed abortion in selfed cultivars was due primarily to the greater loss of viable 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 numbers per fruit within a cultivar (Table 1). However, this association did not always hold for comparisons among cultivars. 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 F_m . Large fruit was associated with cultivars having high F_m values (r = 0.79, selfed; and r = 0.71, outcrossed) and low fertility.

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

	F _m ^z	n	Seed per fruit									
			Fruit wt (g)		No. viable		No. aborted		Total		Aborted (%)	
Cross			x	Δ	x	Δ	x	Δ	x	Δ	x	Δ
Rubel outcrossed	0	51	0.96		22.7		25.8		48.5		56	
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	
selfed	0	51	1.16	-0.29**	15.1	-0.69**	41.0	0.83**	56.1	-0.21**	75	1.34**
Bluejay outcrossed	0.094	45	1.14		9.8		23.1		32.9		75	
selfed	0.094	66	1.09	-0.04	6.2	-0.36*	41.3	0.78**	47.5	0.44**	89	0.19**
Bluecrop outcrossed	0.110	56	2.36		26.7		29.3		56.0		57	
selfed	0.110	59	1.87	-0.21**	10.7	-0.60**	51.5	0.78**	62.2	0.11	84	0 47**
Spartan outcrossed	0.182	76	2.51		9.5		49.8		59.2		87	
selfed	0.182	81	1.91	-0.24**	1.3	-0.86**	44.0	-0.12	45.2	-0.24**	98	0.13**
Elliot outcrossed	0.188	79	2.03		43.7		21.0		64.6		34	
selfed	0.188	56	1.60	-0.21**	7.7	-0.82**	25.5	0.21*	33.2	-0.48**	77	1.26**

²Inbreeding coefficient of maternal parent.

 $^{y}\Delta$ is the proportion of change following self-pollination relative to cross-pollination; *t*-test for mean differences within a cultivar, significant at the 5%(*) and 1% (**) levels, respectively.

Table 2. Simple correlations of fruit, seed, and inbreeding parameters by pollination treatment.^z

Type of	Inbreeding, fruit, and seed parameters							
pollination	F _m y	Fruit wt	Viable	Aborted	Total			
Self								
Fruit weight (g)	0.79							
Viable seed (no.)	-0.80	-0.52						
Aborted seed (no.)	-0.08	0.51	-0.10					
Total seed (no.)	-0.28	0.25	0.35	0.90				
Aborted seed (%)	0.69	0.67	-0.87	0.57	0.15			
Cross								
Fruit weight (g)	0.71							
Viable seed (no.)	-0.23	0.05						
Aborted seed (no.)	0.44	0.60	-0.59					
Total seed (no.)	0.06	0.54	0.77	0.06				
Aborted seed (%)	0.32	0.14	- 0.96	0.76	-0.59			

²For n = 6, P < 0.05 when r = 0.81.

^yInbreeding coefficient of maternal parent.

Pollen cytology. Fluorescent microscopy revealed that both self and cross pollen reached the base of the style 2 DAP, and, at 6 DAP, both types of pollen were entering the ovules. Fig. 1 A and B 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 fertile cross-pollination vs. 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 six cultivars used in this study showed a range of viable selfed seed sets; 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 that normally are 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 noted, the zygotic levels of inbreeding resulting from bulk cross-pollinations could not be estimated with pre-

cision. Seed yield from both types of mating were highly correlated: individuals that 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 on 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 cultivars (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; our data indicate 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 postzygotic 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 (\approx 20-fold, personal observations) further supports the assumption that this class of seeds represent ovules that 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



Fig. 1. Fluorescent photomicrographs of self pollen entering ovules in 'Spartan', 6 days after pollination. 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. (A) $\times 130$; (B) $\times 180$.

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

Cross	No. viable	No. ovules/ovary	Total
	seeds/	showing tube	ovules/ovary
	fruit	penetration	penetrated (%)
Spartan selfed	2.5	18.4	26.3
Spartan x Bluejay	55.4	17.0	27.8

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% 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 postzygotic event, the magnitude of which 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 the numbers of aborted seed paralleling the steady decline in viable seed set with increasing F_m .

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-cultivar plantings with synchronous flowering periods are preferable to monocultures for maximizing fruit weight. An alternative approach is to locate and select wild genotypes that 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) that 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, before abortion. Furthermore, 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 six cultivars used in this project 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

- Aalders, L.E. and I.V. Hall. 1961. Pollen incompatibility and fruit set in lowbush blueberries. Can. J. Genet. Cytol. 3:300– 307.
- 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. USDA Yrbk. Agr. p. 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. Hort-Science 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.
- 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 styles by means of fluorescence. 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.
- Morrow, E.B. 1943. Some effects of cross-pollination versus self-pollination in the cultivated blueberry. Proc. Amer. Soc. Hort. Sci. 42:469–472.
- Orr-Ewing, A.L. 1965. Inbreeding and single crossing in Douglas-Fir. For. Sci. 11:279–290.
- 19. Seavy, S.R. and K.S. Bawa. 1986. Late-acting self-incompatibility in angiosperms. Bot. Rev. 52:195-219.
- Sorensen, F. 1969. Embryonic genetic load in coastal Douglas-Fir, Pseudotsuga menziesii var. menziesii. Amer. Nat. 103:389-398.

J. Amer. Soc. Hort. Sci. 113(6):918-924. 1988.

Electrophoretic Characterization of Diploid Plums of the Southeastern United States

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Additional index words. Prunus, electrophoresis, isozymes

Abstract. Twenty-nine Japanese-type plum clones were assayed for isozymic variability for eight enzyme systems. Glutamate dehydrogenase (GDH), leucine amino-peptidase (LAP), malate dehydrogenase (MDH), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and peroxidase (PX) showed variability among the plums surveyed. 6-phosphogluconate dehydrogenase (6PGD) and triosephosphate isomerase (TPI) were not variable. Isozymic characterization uniquely identified 38% of the clones. The remainder separated into groups of two to three clones that were distinguishable using vegetative morphological characteristics. Reported parentage of five out of nine plums examined was not consistent with their isozymic genotypes.

Electrophoresis has been used to characterize protein variability to analyze taxonomic, genetic, and evolutionary relationships of different plant population (14, 15, 19). Electrophoretically exposed genetic markers can be used in selection-monitoring procedures during introgression of new germplasm. Isozyme electrophoresis has been used for cultivar identification and for hybrid confirmation (25).

Since the first Japanese plums (*Prunus salicina* Lindl.) were introduced to the United States by Luther Burbank and others in the late 19th century, proliferation of Japanese-type plum cultivars has been rapid. Burbank released several pure *P. salicina* types, such as 'Abundance', 'Burbank', and 'Satsuma',

Received for publication 7 Dec. 1987. The computer graphics for the interpretative diagrams were done by Terry Bacon. His cooperation is gratefully acknowledged. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.