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Genetic analysis of freezing tolerance in blueberry (*Vaccinium* section *Cyanococcus*)

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Abstract An understanding of the genetic control of freezing tolerance (FT) in woody perennials is important for the effective selection and development of plants with a broader climatic adaptation. This study was undertaken to examine the inheritance and gene action of FT in segregating populations of a woody perennial blueberry (*Vaccinium*, section *Cyanococcus*). Two backcross populations were derived from interspecific hybrids of the diploid species *Vaccinium darrowi and Vaccinium caesariense*, which are widely divergent in their FT. The bud FTs of uniformly cold acclimated plants of parental, F_1, and two backcross populations were evaluated with a laboratory controlled freeze-thaw regime, followed by a visual assessment of injury. FT (LT_{50}) was defined as the temperature causing 50% of the flower buds to be injured. Data indicate that the two parents were homozygous for genes for low or high FT. Freezing-tolerance values of the parental and F_1 populations indicate that freeze-sensitivity is a partially dominant trait. Results from reciprocal crosses revealed that there was no significant maternal influence on freezing tolerance. Parental phenotypes were fully recovered in 40–42 plants of each testcross population, suggesting that FT is determined by relatively few genes. The degree of dominance and an analysis of generation means revealed that FT in blueberry is controlled largely by additive gene effects and, to a lesser degree, by dominance gene effects. Testing of various genetic models indicated that FT inheritance can be adequately explained by a simple additive-dominance model; however, two epistatic models involving additive-additive and dominance-dominance interactions also fit the data.

Key words Cold hardiness · Blueberry · Inheritance · Gene action · Woody perennials

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Introduction

Winter survival of temperate-zone woody perennials is dependent on two phenological events: (1) the onset of endodormancy during the fall, and (2) an ability to increase freeze-tolerance upon exposure to low non-freezing temperatures (i.e., a change from a freeze-susceptible to a freeze-resistant state – a process called cold acclimation). Once plants are in a dormant state, exposure to a chilling period is required for floral and vegetative budbreak in the following spring. Chilling requirement and ecodormancy prevents growth from occurring during periodic warm spells during winter, and thus helps synchronize plant growth with the prevalence of favorable environmental conditions. Cold acclimation, on the other hand, enables plants to survive the sub-freezing temperatures present during winter. Due to the process of cold acclimation, plants that would be killed by temperatures slightly below 0°C during summer and early fall may survive temperatures as low as –196°C during winter (Sutinen et al. 1992). The extent to which a particular species can acclimate is largely genetically determined (Pellett 1998).

Although some studies have tried to determine the genetic control of freezing tolerance (FT) in herbaceous plants, few studies have addressed the inheritance and gene action (dominant, recessive, additive, epistatic) of FT in woody plants. Studies of overwintering cereal crops suggest that FT is a complex, quantitatively inher-

ited trait with additive and dominance components and that frost sensitivity is partially dominant (Sutka 1981). Similar results have been observed in studies of potato (Stone et al. 1993). The paucity of information on the genetics of FT in woody plants are due, in part, to: (1) long periods of juvenility prior to becoming reproductive, (2) heterozygosity of various traits in parental populations; and often (3) ability to tolerate little inbreeding (i.e., true F_2 or backcross populations segregating for FT cannot be easily generated for some crops). Despite these restraints, genetic studies, although limited in number, suggest that the gene action for FT in woody plants such as apple (Watkins and Spangelo 1970; Fejer 1976) and *Eucalyptus* (Tibbits et al. 1991) is inherited in an additive manner.

An understanding of the gene action of FT is also restricted by the difficulty in obtaining reliable estimates of a plant's FT. Often, breeders rely on the field estimates of "winter survival/winter injury". Winter survival in the field is influenced by multiple factors which, in turn, are influenced by uncontrollable environmental parameters (Hummel et al. 1982). For example, ultimate winter survival of overwintering plants is a function of factors such as fall-timing of growth cessation, FT at the cold acclimation state, tolerance to fluctuating temperature in late winter and early spring, chilling requirement, wind desiccation, snow cover etc., and thus may exhibit annual variation (Fowler and Gusta 1979; Fear et al. 1985). Whereas the evaluation of winter survival in the field may be important for breeding populations, it is often problematic to draw conclusions about the genetics of FT at a cold-acclimated state (one component of winter survival) based on the data on winter survival (a trait with several components). One such study dealing with the heritability of winter injury in a woody perennial found large general combining ability by year interaction effects, and confounding effects of two traits measured in field conditions, namely growth cessation and winter injury (Fear et al. 1985). The latter authors concluded that screening for cold hardiness under controlled conditions would be desirable, and no conclusions were attempted regarding additive, dominance and epistatic effects.

Blueberry (*Vaccinium* section *Cyanococcus*), a small-statured woody perennial, is an important small-fruit crop in the U.S. In a recent survey of blueberry research in the U.S., lack of FT and susceptibility to spring frosts were identified as the most important genetic limitations of current cultivars (Moore 1993). One of the goals of USDA blueberry breeding programs is to develop cultivars with an FT suitable for northern regions to broaden the climatic adaptation of blueberries. Realization of this goal would be facilitated by an understanding of the genetic control of FT in blueberry. It would be very useful, for example, to be able to predict the outcome of cold-hardy×cold-tender crosses and to describe the variability among segregating progeny. Prediction is important because it influences the breeding strategy (i.e., the size and number of generations) needed to develop cultivars with broader climatic adaptation. On a more basic level, the information about the gene action of FT would add to the knowledge of genetic systems controlling physiological traits of adaptive significance in woody plants.

The present study was initiated to examine the mode of inheritance and to determine the gene action of FT in blueberry. We employed two diploid species, largely divergent in their FT, and F_1 and backcross progenies derived from a cross between diploid parent plants to examine the inheritance of FT in the cold-acclimated state. FT distributions in these populations were used to determine the inheritance of FT in a generation-means analysis (i.e., additive, dominance, epistatic gene action). In addition, we were able to make a preliminary assessment of the number of genes that control FT in diploid blueberry and the presence of maternal inheritance based on the segregation of backcross populations.

Materials and methods

Plant material

Two backcross populations of blueberry derived from interspecific hybrids of the wild diploid species *Vaccinium darrowi* and *Vaccinium caesariense* were employed in this study. The *V. darrowi* (*drw*) plant, Fla4B, a clone originating from Florida (USDA Plant Hardiness Zone 9), was used as the female parent. Fla4B requires about 300 chill units for bud break, is relatively less freeze-tolerant, and is evergreen. The *V. caesariense* (*csr*) parent, W85–20, was collected from New Jersey (zone 6 to 7). It requires about 1300 chill-units and is significantly more freeze-tolerant relative to Fla4B. It is noteworthy that, in the present study, we are concerned with the FT of buds in a cold-acclimated state. We had previously determined that the FT of flower buds of Fla4B and W85–20, given a uniform cold-acclimation treatment and using a controlled freeze-thaw protocol (see 'Determination of Freezing Tolerance' below), was about –12°C and –21°C, respectively.

True F_2 or backcross diploid populations are not easily generated for blueberries due to self-sterility (Ballington and Galletta 1978) and inbreeding depression (Krebs and Hancock 1988). Consequently, three Fla4B×W85–20 F_1s were crossed with another *drw* clone, NJ88 13–15 (a wild selection from Florida), and another *csr* clone, W85–23 (another wild collection from New Jersey), to generate the testcross populations. Bud FTs of testcross parents NJ88 13–15 and W85–23 were –13°C and –21°C, respectively. These crosses resulted in *drw* and *csr* testcross populations of 40 and 42 plants, respectively. Although F_1s were mainly used as female parents in these crosses, some reciprocal crosses were made in which W85–23 (*csr* testcross parent) was used as a female parent. Hereafter, these testcross progenies will be referred to as backcrosses.

The *drw* and *csr* parent plants, as well as intraspecific hybrids and other clones belonging to the same species as the parents, were evaluated for FT to determine if parental populations were fixed for FT. For example, in addition to Fla4B and NJ88 13–15, *drw* clone US799 (a wild selection from Florida kindly provided by A. Draper) and two Fla4B×US799 hybrids were also included in the *drw* parent population. Similarly, the *csr* parent population comprised W85–20, W85–23, and six W85–20×W85–23 hybrids. Individual plants of parental, F_1, and testcross populations were vegetatively propagated to generate 3–7 clones of each plant, using hardwood cuttings, and then maintained in the greenhouse.

Determination of freezing tolerance

Four-to-five year-old potted plants (3–7 clones of each individual plant) were transferred from the greenhouse to a cold room in No-

vember, and were cold-acclimated by exposure to 4°C for 4 weeks at a photoperiod of 10/14 h (day/night) with a 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon-flux density (Arora et al. 1997). Longer exposure at 4°C does not result in further increase in blueberry bud-hardiness (Arora et al. 1997). Five- to six-cm-long shoots with 3–8 attached floral buds were excised, representing all the clones belonging to each individual plant, and then subjected to the freeze-thaw protocol as described by Arora et al. (1997). The freeze-thaw test consisted of placing three shoots/treatment temperature from each plant in test tubes (one shoot/tube; three replicates) with 0.5 ml of H_20, and subjecting them to controlled freezing in a glycol bath (model 2325; Forma Scientific, Marietta, Ohio). Ice nucleation was initiated at –1°C, samples were allowed to equilibrate for 2 h, and further cooled at 0.5°C/30 min down to –4°C, 1°C/30 min down to –8°C, and 2°C/30 min thereafter to obtain treatment temperatures ranging from –6°C to –24°C at 2°C increments. These treatment temperatures were chosen to represent 0–100% injury to blueberry buds in a laboratory freeze-thaw test (Arora et al. 1997). Bud temperature was monitored by copper-constantan thermocouples (TT-T-30) attached to a thermometer (DP465, Omega Engineering, Stamford, Conn., USA). Samples were allowed to thaw overnight at 4°C followed by a 24-h incubation at 23°C. Subsequently, the buds were dissected and observed for visual browning of the ovaries in individual florets (Fear and Lawson 1987; Flinn and Ashworth 1994). Buds were rated for 0–100% browning and the mean FT (LT_{50}) was defined as the temperature causing 50% injury (browning).

Bud FT data were collected for parents, F_1s, and backcross populations for 2 years (1995 and 1996). It was observed that, although relative mean FT values for respective populations were similar in both years, on average, absolute LT_{50} values for 1996 data were slightly larger. To standardize these values, data from 1996 were adjusted to the data from 1995. The yearly differences between the *csr* parent, the *drw* parent and the F_1 populations were averaged and 1.7°C was subtracted from all the 1996 values. Thereafter, data from 1995 and 1996 were averaged and used to estimate the FT from parental, F_1, and backcross populations.

Analysis of gene action

The degree of dominance (Falconer 1989) was determined for the cross between the two parental species. It was calculated as the deviation of the F_1 from the mid-parent (*h*), divided by the departure of the less-hardy parent (*drw*) from the mid-parent (*d*). In this scheme, a value of zero for the degree of dominance (*h/d*) indicates variance due to completely additive effects; whereas *h/d*=1 indicates complete dominance.

Joint scaling tests (Mather and Jinks 1982) were used to determine the gene action of FT in the analyses of generation means. These tests use means and variances for different generations of a particular cross to estimate values for the genetic components and the nonallelic (epistatic) interactions. These estimates are used to fit various genetic models to the data. The theoretical basis of the joint scaling test is a linear model. For the hardiness data, the mean freeze-tolerance (X) of a generation can be described by the following linear equation:

$$X=m+d+h+i+j+l$$

where *m*=mean of all possible homozygotes, *d*=additive component, *h*=dominance component, *i*=additive x additive component, *j*=additive x dominance component, and *l*=dominance×dominance component. The genetic components measured are an estimate of the net effect of all the loci at which the parents differ for the measured characteristic (Mather and Jinks 1982). The coefficients of these components (for various populations) employed in our study were as described by Mather and Jinks (1982), and have also been used in other genetic studies (Chen and Line (1995). Means and variances of the five populations [*csr* parent (P_1), *drw* parent (P_2), F_1, *csr* backcross (BC_1, F_1×*csr*), and *drw* backcross (BC_2, F_1×*drw*)] were employed to test a total of 18 genetic models that included components *md*, *mh*, or *mdh* in combination with one or more of the epistatic parameters (*i*, *j*, or *l*) in an analysis of generation means (Beaver and Mosjidis 1988). A chi-square test was used to determine the goodness of fit of each model. The standard error of the estimated genetic parameters of models with a 0.05, or greater, chi-square probability was calculated. Student *t*-tests were performed to determine the significance of the estimated genetic parameters, and genetic components estimated to be different from zero at $P<0.05$ were considered to contribute significantly to the model. We accepted the models that had a chi-square probability of 0.05 or greater and had components that were all significant.

Differences in reciprocal crosses involving backcross populations were used to test for cytoplasmic inheritance. The generation means of the backcross generations of two sets of reciprocal crosses that involved the *csr* parent were compared with one-way ANOVA. Reciprocal crosses were considered different if $P<0.05$.

Results

Distributions of freezing tolerance

Values for mean FT (LT_{50}) for parental, F_1, and the two backcross populations are presented in Table 1. The LT_{50s} of the *csr* and *drw* parents were –21.0°C and –13.5°C, respectively. The mean FT (–14.7°C) of *drw*×*csr* F_1 population of nine individuals was closer to the mean of the *drw* parent than to the mean of the *csr* parent. The F_1×*csr* population (BC_1) had a mean LT_{50} of –17.9°C, whereas the F_1×*drw* backcross population (BC_2) had a mean LT_{50} of –13.5°C.

The freezing-tolerance distributions of the two backcross progenies are shown in Fig. 1. The data indicate that both backcross populations exhibited continuous variation for FT values. In each population, with few exceptions, most of the individuals were distributed between the parental values; however, we observed some progeny with the parental phenotypes. In addition, the distributions for the backcross populations were skewed towards the recurrent parent.

Genetic analysis of freezing tolerance – model testing

Freeze-tolerance data were used to test a total of 18 genetic models that included the components *md*, *mh*, or *mdh* in combination with one or two of the epistatic, nonallelic parameters (*i*, *j*, or *l*). Based on chi-square goodness of fit tests, 8 out of the 18 models tested fit the data (P=0.15–0.99 for chi-square values) (Table 2). Of these eight models, only three – *mdh*, *mdi*, and *mdl* – had components that were all significant, based on student *t* -tests. The additive component (*d*) was significant at $P<0.01$ in *t* -tests for each of these three models. Dominance (*h*) or epistatic components (*i* or *l*) were significant at $P<0.05$ in individual models. In the other five models no parameter was significant at $P<0.05$, and thus these models were not considered acceptable. Based on the estimates of the genetic parameters as calculated by joint scaling tests (Table 3) and the coefficients described by Mather and Jinks (1982) for various genetic parameters, the three acceptable models were used to calculate the expected mean FT for each generation (Table 4).

Table 1 Means for the freezing tolerance (LT_{50}) of five blueberry populations

Generations	Sample size (*n*)	LT_{50} (°C) Means±SE
P1: *V. caesariense*[a]	8	–21.0±0.33
BC_1: F_1×*V. caesariense* (W85–23)[b]	42	–17.9±0.36
F_1: *V. darrowi*×*V. caesariense*[c]	9	–14.7±0.33
BC_2: F_1×*V. darrowi* (NJ88)	40	–14.1±0.38
P_2: *V. darrowi*[d]	5	–13.5±0.81

[a] W85–20, W85–23, and six W85–20×W85–23 progeny
[b] Included reciprocal crosses, i.e., [(Fla 4B×W85–20)×W85–23] and vice versa
[c] Fla4B×W85–20
[d] Fla4B, NJ88, US799, and two Fla4B×US799 progeny

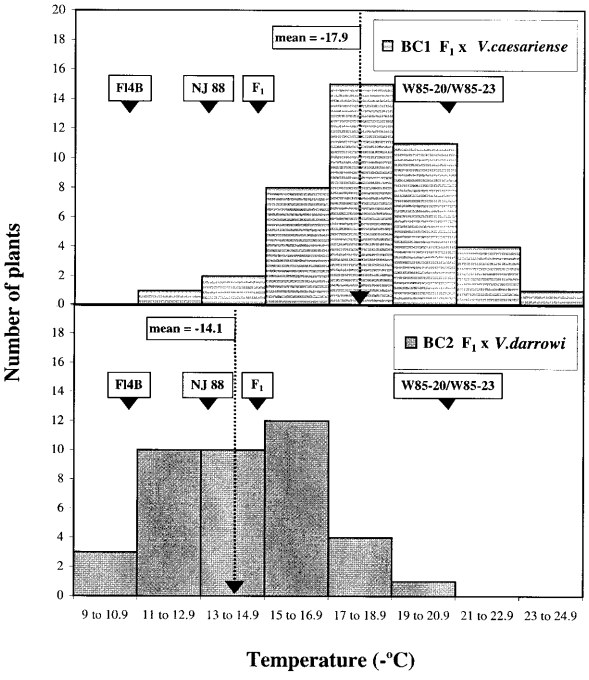

Fig. 1 Bud freezing tolerance (LT_{50}) distributions of the two blueberry populations. The mean LT_{50} for the two populations, the parents of the original cross (Fla4B and W85–20), the backcross parents (NJ88 and W85–23), and the F_1 populations are indicated at the top of each graph

Differences in reciprocal generation means were used to test for the occurrence of cytoplasmic (maternal) inheritance. In tests of the two sets of reciprocal crosses generated by crossing two different F_1s with W85–23 or vice-versa, there were no significant differences among populations based on a one-way ANOVA (P=0.23–0.33; data not shown). The first set of reciprocals included 14 (F_1×W85–23) and 5 (W85–23×F_1) plants, whereas the second set comprised 9 (F_1×W85–23) and 5 (W85–23×F_1) plants.

Table 2 Chi-square goodness of fit test and probability of the fit of a cross between *V. darrowi* and *V. caesariense* using eight different models, and the fit of the individual genetic components for models with an acceptable chi-square fit

Model[a]	Chi-square value	P[b]	Component fit
m [*d*] [*h*]	0.02	0.99	[d]** [h]*
m [*d*] [*h*] [*i*]	0.01	0.92	[d]
m [*d*] [*h*] [*j*]	0.01	0.96	[d]
m [*d*] [*h*] [*l*]	0.01	0.92	[d]
m [*d*] [*i*]	3.13	0.21	[d]** [i]*
m [*d*] [*l*]	2.31	0.32	[d]** [l]*
m [*d*] [*i*] [*l*]	0.01	0.91	[d]
m [*d*] [*j*] [*l*]	2.10	0.15	[d]

[a] *m*=estimate of the mean of all possible homozygotes, *d*=additive component, *h*=dominance component, *i*=additive-additive interaction, *j*=additive-dominance interaction, and *l*=dominance-dominance interaction
[b] The model was considered to fit if P<0.05, *=significant at P<0.05;**=significant at P<0.01

Discussion

The populations derived from interspecific hybrids of the blueberry species *V. darrowi* and *V. caesariense* are well-suited for investigating the mode of inheritance and gene action of freezing tolerance. First, these two diploid species are cross-compatible (i.e., homologous chromosomes from different species of blueberry can pair and segregate normally) (Krebs and Hancock 1992; Hokanson and Hancock 1993). The use of cultivated tetraploid blueberry for genetic studies is complicated by polysomic inheritance (N. Vorsa, unpublished results). Previous studies involving the heritability of winter injury in blueberry employed diallel-crossing designs with polyploid material (Fear et al. 1985). Since one of the main assumptions employed by this design is disomic inheritance (Hayman 1954; Baker 1978), the interpretation of genetic effects with the genotypes exhibiting tetrasomic inheritance is problematic. Second, *V. darrowi* and *V. caesariense* are highly divergent in their FT at a cold-acclimated state, and therefore gene action and the inheritance of FT can be evaluated for segregating backcross populations.

Screening method

In this study, the use of laboratory controlled freeze-thaw tests allowed for the precise assessment of FT at a cold-acclimated state for each genotype examined. Field-survival tests lack the precision needed to discern small yet significant differences in FT among progenies of a cross. In the field, there is a myriad of uncontrollable macro- and micro-climatic factors that influence the acquisition and the ultimate degree of FT in plant tissues (Palta 1991). For example, in a field study of winter-survival in *Rhododendron*, bud damage on a single plant ranged from 0 to 100% in one season (Gilkey 1996). In contrast,

Table 3 Estimates of the genetic parameters of three different genetic models used to describe freezing tolerance in a cross between *V. darrowi* and *V. caesariense*. Values are given as mean±SE

Genetic parameter	Model m[d][h]	Model m[d][i]	Model m[d][l]
m (mean value)	17.25±0.33	15.11±0.24	16.81±0.27
[d] (additive gene effect)	–3.80±0.32	–3.72±0.33	–4.02±0.30
[h] (dominance gene effect)	–2.52±0.51	–	–
[i] (additive-additive interaction)	–	2.31±0.50	–
[l] (dominance-dominance interaction)	–	–	–2.19±0.46

Table 4 Observed (determined by freeze-thaw tests) and expected (based on various models) mean freezing tolerance (–°C, but listed as absolute values) for parental, F_1, and backcross generations of a cross between *V. darrowi* and *V. caesariense*

Generation[a]	Observed mean±SE	Model *m*[*d*][*h*][b] expected mean±SE	Model *m*[*d*][*i*][b] expected mean±SE	Model *m*[*d*][*l*][b] expected mean±SE
P_1	21.0±0.33	21.06	21.13	20.84
BC_1	17.9±0.36	17.89	17.54	18.28
F_1	14.7±0.33	14.73	15.11	14.62
BC_2	14.1±0.38	14.09	13.82	14.25
P_2	13.5±0.81	13.45	13.69	12.78

[a] P_1=parent 1; P_2=parent 2; F_1=P_2×P_1; BC_1=backcross to P_1; BC_2=backcross to P_2
[b] *m*=mean of all homozygotes, *d*=additive component, *h*=dominance component, *i*=additive-additive interaction, and *l*=dominance-dominance interaction. The expected means are calculated from the estimated genetic parameters×coefficients for genetic components from Table 1

we exposed all the plants in the laboratory to an identical cold-acclimation regime and used laboratory controlled freeze-thaw tests to determine bud FT. This allowed a precise determination of the progeny FT. Data from other research support the notion that measurement of the FT of excised plant tissues in the laboratory is a valid method to estimate the field performance of whole plants. For example, the freezing tolerance of leaf discs based on laboratory freeze-thaw tests has been shown to closely agree with the relative survival of five *Rhododendron* cultivars (Lim et al. 1998a) and *Eucalyptus* populations (Tibbits et al. 1991) in natural frosts. Teutonico et al. (1993) reported a strong correlation (r=0.82 to 0.85) between laboratory and field-generated estimates of FT among nine rapeseed cultivars. Similarly, controlled freeze-thaw stress of leaf discs from 35 broadleaf evergreen species resulted in visual laboratory estimates of FT that corresponded well with field measurements (Johnson and Hirsh 1995).

Inheritance of freezing tolerance

The mean LT_{50} of F_1 (–14.7°C) was closer to the FT of the cold-sensitive, *drw* parent (–13.5°C) than to that of the cold hardy, *csr* parent (–21.0°C) (Table 1). This would suggest that freeze-sensitivity (as opposed to freeze-tolerance) may be a partially dominant trait in blueberry. Our results are analogous to those of Sutka (1981), who reported frost sensitivity in wheat to be partially dominant. He also noted that frost-sensitive varieties of wheat had the largest number of dominant genes, while frost-resistant varieties had the highest proportion of recessive genes. In a recent study of the genetic control of the components of freezing tolerance, Stone et al. (1993) noted that non-acclimated FT and cold-acclimation ability in *Solanum* species were partially recessive.

The uniformity of FT observed among parental and F_1 populations (Table 1) in our study is indicative of non-segregating (homogeneous) parental populations. For example, FT distributions for the two parental populations ranged from –19.8°C to –21.3°C (*csr* parent) and –12°C to –14.3°C (*drw* parent). FT distributions of F_1 population ranged from –14.8°C to –16.0°C. Uniformity of FT distributions in these populations suggests that the genes for FT are fixed in the parents. Since parent plants representing each species come from populations that are only adapted to a specific climatic zone, the genes for traits of adaptive significance (e.g., FT), would be expected to be fixed in each species for their respective environment. Our results support this idea.

A continuous distribution of the variation of FT in backcross populations (Fig. 1) suggests that FT is a quantitative trait under oligo- or multi-genic control, an interpretation consistent with reports from other genera (Rudolph and Nienstaedt 1962; Stone et al. 1993; Teutonico et al. 1995; Lim et al. 1998 b; Pellett 1998). In our study, a few individuals in each of the two backcross populations had LT_{50} values similar to the parental phenotypes. Recovery of parental phenotypes in relatively small backcross populations (i.e., 40 and 42 plants) suggests that FT in blueberry may be controlled by relatively few genes. Research in other genera, such as *Solanum* (Stone et al. 1993) and *Rhododendron* (Lim et al. 1998 b), has suggested a similar oligo-genic control of FT. The prospect of few genes controlling FT makes genetic mapping and "tagging" of FT genes a feasible objective. The availability of individuals segregating for the capacity to acquire FT upon exposure to low temperatures can be used to test the importance of biochemical/molecular changes associated with cold acclimation in woody plants, and should enable us to select for these characteristics in developing freeze-tolerant plants.

Although most individuals of backcross progenies had an LT_{50} ranging within the backcross parental values, a few individuals were either hardier than the hardy parent or less hardy than the cold-sensitive parent (Fig. 1), suggesting transgressive segregation. Thus, species adapted to a particular hardiness zone may have genes that are useful for another zone. A study by Hummel et al. (1982) on the inheritance of photoperiodically induced cold acclimation in intra-specific, latitudinal ecotypes of *Cornus sericea* indicated that, although acclimation responses of the F_2 ranged between parental extremes, a few transgressive segregants were also noted. It has been suggested that the selection of transgressive segregants generated via sib-mating of F_1s that are derived from parents with divergent FTs, could be used as an approach to develop cold-hardy germplasm of fruit crops, including blueberry (Stushnoff et al. 1983; Pellett 1998).

Unilateral maternal influence on the inheritance of FT has been implicated in breeding strategies for improving plant cold hardiness (Stushnoff 1972). We therefore evaluated the role of the maternal effect on the inheritance of FT in blueberry. A comparison of the mean LT_{50} of reciprocal generations derived from F_1×W85–23, or vice-versa, revealed no significant differences between the populations, and thus suggested no maternal (cytoplasmic) influence on FT. Conflicting reports exist in the literature regarding the maternal effect on FT. For example, Dorsey and Bushnell (1925) first reported a positive maternal effect on FT in *Prunus*. They observed that using *Prunus americana* (a hardier species than *Prunus salicina*) as a pistillate parent resulted in 80% of the inter-specific seedlings being hardy compared to only 45% when *P. salicina* was the female parent. Based on this, the authors suggested that a hardier female parent should be used in inter-specific crosses of *Prunus*. On the other hand, Quamme (1978) detected no reciprocal differences in the FT of progenies of apple cultivars with different FT levels. Similar results were also obtained by Hummel et al. (1982) in a study of the inheritance of photoperiodically induced cold acclimation in *C. sericea*.

Gene action of freezing tolerance

We employed the degree of dominance, as described by Falconer (1989), and joint scaling tests in the analysis of generation means to determine FT gene action. In our study, the value of h/d (–2.54/3.78)=–0.67 suggests that either freeze-sensitivity is partially dominant or freeze-tolerance is partially recessive. Our results are in accordance with reports of herbaceous and woody plant genera , namely *Solanum* (Stone et al. 1993), wheat (Sutka 1981) and *Eucalyptus* (Tibbits et al. 1991), in which freeze-sensitivity was observed to be a partially dominant trait.

Results obtained from joint scaling tests indicated that FT in blueberry can be explained by three genetic models: a simple additive-dominance model (*mdh*) and two other models (*mdi* and *mdl*) which, in addition to an additive (*d*) component, include epistatic components, (i.e., additive×additive and dominance×dominance interactions) (Table 2). It is important to note that the joint scaling test estimates nuclear genetic components and is valid only when there is no cytoplasmic inheritance involved (Chen and Line 1995), as our results indicate.

In the additive-dominance model (*mdh*), a negative value for the *h* (dominance gene effect) parameter (Table 3) indicates that FT is partially recessive (or freeze-sensitivity is partially dominant), a conclusion also drawn from our calculations of the degree of dominance. The additive component (*d*) was significant at $P<0.01$ in all three models that fit the data (*mdh*, *mdi*, and *mdl*), while remaining components (*h*, *i*, and *l*) were significant at $P<0.05$ (Table 2). Moreover, whereas both genetic parameters (*d* and *h*) contributed significantly to the additive-dominance model, the magnitude of *d* (additive-gene effect) was greater than that of *h* (dominance-gene effect) (Table 3). Thus, the additive component is a major contributor to FT in blueberry. A more significant contribution of additive gene action in FT, rather than dominance or epistatic components, may support the possibility of selecting for freezing tolerance (i.e., the additive genetic component reflects the selectable breeding value of genes, whereas dominance and epistasis are intra-locus and inter-loci interactions that are not passed on to offspring).

Predominant additive gene action has also been observed in other studies. Watkins and Spangelo (1970) concluded that dominance and epistasis were not major factors in the FT of *Malus* sp., but that low temperature-induced bud damage was controlled by additive gene action. Our results are also analogous to those of Sutka (1981) in a study of wheat. Similar to our observations, Sutka (1981) noted that an additive-dominance model could explain the genetic control of FT. He also noted that the magnitude of the additive component was higher than that of the dominance component. Stone et al. (1993) reported similar results in *Solanum*. In another study, the heritability of FT in interspecific F_1 hybrid families of 27 *Eucalyptus* species as estimated from mid-parent regressions (r=0.94) suggested that a large additive genetic component controlled the FT in inter-specific hybrids (Tibbits et al. 1991).

Based on the estimates of gene action and the segregation of FT in backcross populations, we propose that FT in blueberry is a partially recessive trait, and controlled by relatively few major genes. Its inheritance can be explained most simply by an additive-dominance model. However, since epistatic models, *mdi* and *mdl*, also fit the data, minor or modifier genes and epistasis may also contribute to genetic control of FT in blueberry. This information should be useful in future studies aimed at elucidating the biochemical/molecular-genetic basis of FT in woody plants, and in incorporating freezing-stress resistance into future blueberry cultivars.

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