

# Genetic Variation in Chilling Requirement in Apple Progeny

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**ABSTRACT.** Genetic variation in chilling requirement was investigated over three growth periods using clonal progenies of six apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] families derived from crosses of high and low chill requiring cultivars. Two quantitative measurements related to chilling requirement, viz., the time of initial budbreak (vegetative and reproductive) and the number of breaking buds over a specified time interval, were used as evaluation criteria. Genetic and environmental variances of the traits are presented as intra-class correlation coefficients for clones within and between families. For budbreak time, reproductive and vegetative, broad-sense heritability averaged around 75% and 69% respectively, indicating a high degree of genetic determination in this material. For budbreak number, moderate to low genetic determination was found with broad-sense heritabilities around 30%. Estimates of genetic components of variance between families were generally very low in comparison to the variance within families and predict potentially favorable responses to truncation selection on the traits within these progeny groups. Analysis of the data showed that distribution of budbreak time is typical of quantitative traits with means distributed closely around midparent values. Skewed distributions towards low budbreak number were obtained in varying degrees in all families.

Adaptedness refers to the way in which plants can survive and reproduce in specific environments (Hill et al., 1998) and is reflected by the degree to which developmental events are synchronized with the climate (Dietrichson, 1964). Many factors influence the adaptive potential of apple (*Malus sylvestris* var. *domestica*) cultivars planted around the world and for important horticultural traits, such as yield and fruit quality, it is unrealistic to expect the same level of performance in all environments. This is the main reason why cultivars bred in other countries are not always suitable for local production and why fruit tree breeding for improvement in economically important traits requires attention to traits related to adaptedness as well. Adaptedness is a complex interaction between various environmental factors and the plant. Breeding for climatic adaptation is of increasing interest among plant breeders.

Most temperate zone woody deciduous trees, including apple require a certain degree of chilling to break endodormancy before active shoot growth in the Spring, (Rodriguez and Sherman, 1985; Sorensen, 1983), a phenomenon generally referred to as the chilling requirement (CR) (Martinez et al., 1999; Sorensen, 1983). Wide variation in CR exists among cultivars, wild species, and hybrids (Hauagge and Cummins 1991a). The number of hours below 7.2 °C before budbreak occurs, is used frequently as measure of CR and expressed as cold unit (CU) accumulation (Linsley-Noakes et al., 1994; Weinberger, 1944). For example, it has been estimated that ‘Anna’, a low CR cultivar (Brooks and Olmo, 1972) needs 200 to 300 h below 7.2 °C to break bud dormancy, compared to ‘Dorsett Golden’ and ‘Golden Delicious’

apples which require 800 to 900 and 1050 to 1100 CU respectively (Hauagge and Cummins, 1991a).

Generally, CR is seen as a complex genetically determined trait, probably multigenic or, at least, partly controlled by multiple genes (Dennis, 1987; Howe et al., 2000). Inheritance studies on CR are complicated by the quantitative nature of the dormancy process and environmental factors such as temperature, photoperiod, drought, and mineral nutrient availability (Howe et al., 2000). The quantitative nature of dormancy-related traits has been demonstrated in controlled experiments on cold hardiness in Douglas fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) trees (Aitken and Adams, 1997), date of budbreak in Scottish birch (*Betula pubescens* Ehrh.) (Billington and Pelham, 1991) and the date of bud set and budbreak in Norway spruce [*Picea abies* (L.) Karst.] (Eriksson et al., 1978). Dormancy related genes controlling other characteristics such as branching pattern may also play a role in the process of budbreak and dormancy release (Hauagge and Cummins, 1991b; Howe et al., 2000).

Timing of bud set at the end of the growing season and budbreak after fulfilment of the CR is tied to climatic cycles (Howe et al., 2000) and these traits are used frequently in studies relating to dormancy and dormancy release. Time of budbreak, also described as bud flush or bud burst, marks the initiation of shoot elongation as an indicator of dormancy release and fulfilment of the CR. Selection for low CR genotypes in breeding programs is normally based on seedlings that break bud within a specified time period. Cultivars with low CR such as ‘Anna’, ‘Ein Shemer’, and ‘Schlor’ were selected using early budbreak as selection criterion, i.e., selection of the earliest seedlings in the seedling families (Oppenheimer and Slor, 1968). Classification and grading systems for adaptedness using number and distribution of budbreak were also applied previously in apple by Hauagge and Cummins (1991c) and by Denardi et al., (1988). Ratings of stages in budbreak was applied in sugar maple (*Acer saccharum* Marshall) by Kriebel and Wang (1962).

According to local observations and previous studies in the Western Cape of South Africa, the most prominent symptom of incomplete dormancy release is the absence of budbreak or a long delay in budbreak (Cook and Jacobs; 2000; Jacobs et al., 1981;

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Labuschagné et al., 2002) resulting in low numbers of buds and uneven distribution of buds on shoots. These symptoms are referred to collectively as prolonged dormancy and are found in trees in areas where the temperature requirements for normal dormancy release of prevailing commercial cultivars are not met (Cook and Jacobs, 2000; Jacobs et al., 1981).

From the aforementioned it is evident that CR can be measured and expressed in several different ways depending on the criteria used and the traits measured. The genetics of CR measured in terms of prolonged dormancy symptoms and in relation to budbreak time has not been adequately investigated. We also do not know whether families and parental genotypes vary significantly in the occurrence of these symptoms. To better assess the potential for genetic manipulation, the extent of genetic variation in the above symptoms must be better understood.

In the present study a quantitative genetic analysis was conducted of budbreak time and budbreak number in order to investigate CR in seedling apple families as indicators of their adaptedness to local growing conditions. Genetic parameters were estimated in an attempt to explain the genetic control and variability of the criteria, to assess their possible implication in breeding programs, and to explore the effectiveness of early screening for CR at a young seedling stage.

## Materials and Methods

**PLANT MATERIAL.** The progenies evaluated were derived from two sets of crosses, viz., four families involving the high CR cultivar, Golden Delicious, as one parent and two families involving the low CR cultivar, Anna, as one parent. 'Golden Delicious' was the common female parent in crosses with 'Prima', 'Summerking', 'Starking Delicious', and 'Braeburn' as male parents. 'Anna' was the common male parent in crosses with 'Austin' and 'Sharpe's Early'. 'Anna' is generally regarded as one of the lowest CR cultivars found in *Malus sylvestris* var. *domestica* and originates from an Israeli cultivar, Red Hadassiya x 'Golden Delicious' (Brooks and Olmo, 1972). 'Golden Delicious', a popular commercial cultivar, originated as a chance seedling with 'Golden Reinette' and 'Grim's Golden' as putative parents. No records are available on chilling requirement of parents we used other than for 'Anna' ( $\pm 300$  CU), 'Golden Delicious' ( $\pm 1500$  CU), 'Braeburn' ( $\pm 1100$  CU), 'Prima' ( $\pm 1100$  CU) and 'Summerred' ( $\pm 999$  CU) (Hauagge and Cummins, 1991a; Hauagge, personal communication). Seedlings (60 seedlings of 'Golden Delicious' families and 100 seedlings of 'Anna') for these trials were selected at random from adult trees planted originally in the field for fruit quality evaluation and then clonally replicated by budding on Malling 793 rootstocks.

**PLANTING DESIGN.** Clonal replicates of seedlings and parents of the same age were planted in an orchard in the Western Cape region of South Africa where temperate climatic conditions characterized by low winter chilling are normally experienced (lat. 34°S; elevation = 300 m). An orchard of 0.7 ha established in the Springs of 1997 and 1998 was used as the planting site. The prominent soil form was Glenrosa and Williamson as series. Tree spacing was 1 m within rows and 3 m between rows. Parents and progenies from the two sets of crosses were planted adjacently in two trials each consisting of seven replications in randomized blocks. Sibling seedlings within cross families were planted adjacently in progeny rows within blocks, and parents used in crosses were planted at random within their progeny rows. Orchard management was typical of commercial practice except

that no pruning or other tree growth manipulations such as winter oil treatment for breaking rest, were applied.

**DATA RECORDED.** The initial time of vegetative and reproductive budbreak of each seedling and the number of vegetative and reproductive buds breaking were recorded as criteria of winter CR. In the first two seasons, all buds were scored on the whole tree and recorded as the number of buds per 100 cm length of shoot. In the third year, four 1-year-old shoots were selected at random on each seedling and budbreak expressed as the number of buds per 100 cm length of shoot. Initial reproductive budbreak (IRB) was recorded at the first sign of flowers in the tight cluster stage, and the date of initial vegetative budbreak (IVB) at the time when leaves started to emerge from a vegetative bud. IRB and IVB were recorded weekly. The number of days for IVB and IRB was recorded from 1 Jan. onwards. During the first two seasons, the number of buds breaking was recorded 21 d after IVB, a period regarded as adequate for apple trees to express ability to overcome the state of dormancy (Faust et al., 1995; Hauagge and Cummins, 1991a). During the third season, the number of buds breaking was counted 21 d after the last seedling reached IVB. The shoots that developed on each seedling tree were assigned to the following three classes: 0.5 to 30 cm, 30 to 60 cm, and 60 to 120 cm and the number of shoots in each class was recorded for each tree. Total length of the main shoot was also recorded. Chill units were calculated according to the modified Utah equation (Richardson et al., 1974), that was regarded more suitable for local chilling conditions where negative CU values are not carried from 1 day to the next (Linsley-Noakes et al., 1994). The growing degree hours (GDH) (Anderson et al., 1986) were calculated daily as the difference between the mean and assumed base temperatures (10 °C).

Data were collected on seedlings when they were 2, 3, and 4 years of age during 1997–2000. The first records were collected during 1997 for 'Golden Delicious', and during 1998 for 'Anna' seedlings. Data were collected on all traits for a full 3-year period, except IRB in 'Golden Delicious' families, where the number of seedlings flowering during the first two seasons was insufficient so data from only 1 year were available.

**DATA ANALYSIS.** Analysis of variance (ANOVA) was performed on all measurements for each of the 'Anna' and 'Golden Delicious' families. Separate analyses were performed for each year and a joint analysis for the 3 years in order to test for year  $\times$  family interaction effects. The mean square for seedlings within families was used for the comparison between families. Where a significant year  $\times$  family interaction was found in the joint analysis, the mean square for year  $\times$  family was used as error. The analyses were performed using General Linear Model Procedures of SAS (SAS Institute, Inc., 1996) after testing for heterogeneity of variance using the Levene test (Snedecor and Cochran, 1989) and the Shapiro-Wilk test for normality (Shapiro and Wilk, 1965). Weighting appeared to be advisable and the data transformed to logs where necessary. Variance components and intraclass correlation coefficients were calculated using the SAS Variance Component Estimation Procedure (SAS Institute, Inc., 1996).

**VARIANCE STRUCTURE.** The variance structure has been discussed in terms of standard quantitative genetic principles (Falconer and Mackay, 1996) and is briefly qualified for completeness. In the present experiments the repetition of measurements was different clones of single seedlings, where 1) variance of seedling trees within families of the same cross is  $\sigma_w^2 = \sigma_g^2 + \sigma_e^2$ , and  $\sigma_g^2$  is a genetic component, generated by crossing in this case, and  $\sigma_e^2$  a component ascribable to variable environment within

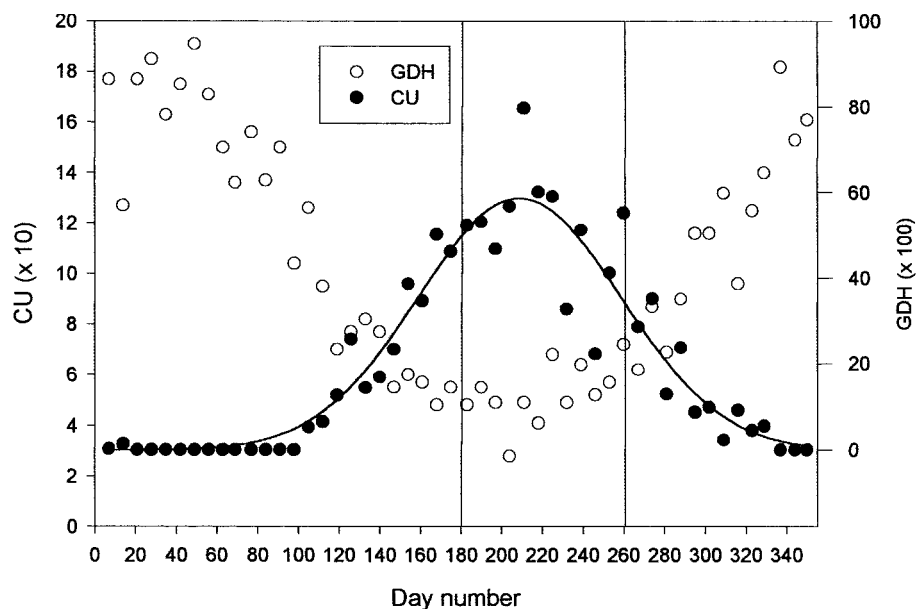


Fig. 1. Distribution of mean chill unit (CU) and growing degree hours (GDH) during four seasons in the Elgin area in South Africa (lat. 34°S; elevation = 300 m) plotted on a weekly basis (Day 1 represents 1 Jan.). Vertical lines represent the dates of the first trees that showed reproductive and vegetative budbreak in 'Anna' progenies at  $\pm 300$  CU accumulation and  $\pm 875$  CU in 'Golden Delicious' progenies.

the trial orchard, and 2) variance between families is  $\sigma^2_B = \sigma^2_G + \sigma^2_w$ , where  $\sigma^2_G$  is the genetic variance among families crosses for a given common parent, i.e., 'Golden Delicious' or 'Anna'.

Repeated measurements on clones of trees ( $n = 7$  per tree) performed per season, result in the ANOVA and expected mean squares (EMS) for estimation of components as follows:

between families (N trees per family) =  $\sigma^2_e + n\sigma^2_g + Nn\sigma^2_G$   
 between trees within families =  $\sigma^2_e + n\sigma^2_g$  (A)  
 within clones =  $\sigma^2_e$  (B).

Table 1. Means for reproductive budbreak time (days from 1 Jan.) in six apple parents and families. ANOVAs were performed separately for parents and progenies for the 'Golden Delicious' and 'Anna' groups. SDs for progeny data are included.

Parents and families	Year 1 <sup>2</sup>	Year 2 <sup>2</sup>	Year 3	Joint analysis <sup>3</sup>
Golden Delicious (GD)	---	---	298.8 b <sup>x</sup>	---
Prima	---	---	305.8 a	---
Starking Delicious	---	---	305.2 a	---
Summerking	---	---	304.8 a	---
Braeburn	---	---	289.6 c	---
GD x Prima	---	---	297.3 b	---
GD x Starking Delicious	---	---	291.8 c	---
GD x Summerking	---	---	299.0 a	---
GD x Braeburn	---	---	285.8 d	---
SD	---	---	12.7	---
Anna	219.8 c	202.8 c	188.6 c	203.7 b
Austin	257.5 b	257.2 b	252.4 b	255.7 a
Sharpe's Early	285.4 a	278.3 a	274.7 a	279.5 a
Anna x Austin	258.6 b	243.2 b	233.3 a	245.0 b
Anna x Sharpe's Early	267.8 a	253.5 a	248.2 a	256.5 a
SD	24.9	26.7	32.3	30.3

<sup>2</sup>ANOVA on 'Golden Delicious' seedlings could not be performed because of low reproductive budbreak numbers during the first 2 years of data recording.

<sup>3</sup>In cases of significant year  $\times$  family interaction, the mean square for year  $\times$  family was used as the error term in the ANOVA.

<sup>x</sup>Mean separation within columns for parents and families by LSD at  $P \leq 0.05$ .

The intraclass correlation coefficient relevant to selection between trees within families is then  $t = (\sigma^2_g)/(\sigma^2_g + \sigma^2_e)$ , estimated by  $(A - B)/A + (n - 1)B$ , with standard error (SE)

$$SE(t) = \sqrt{2[1 + (n - 1)t]^2(1 - t)^2/n(n - 1)(N - 1)}$$

The equivalent intraclass correlation coefficient relevant to selection between families is  $t = (\sigma^2_G)/(\sigma^2_G + \sigma^2_w)$ .

## Results

**WINTER CHILLING CONDITIONS.** Normal winter chilling conditions for the region were experienced during this investigation. Relatively low CU accumulation for apple production occurred during the 4 years, being 763, 897, 622, and 724 respectively, measured from the beginning of May until the end of August each year. Weekly accumulation of CU and GDH calculated from

data collected over a period of 4 years (1997–2000) is illustrated in Fig. 1.

**REPRODUCTIVE BUDBREAK TIME.** Budbreak in 'Braeburn' seedlings was significantly earlier than in the other seedlings (Table 1). 'Golden Delicious' was intermediate for IRB and 'Prima', 'Starking Delicious', and 'Summerking' were the later flowering parents. During all years of data recording, 'Anna' was the earliest flowering parent, 'Sharpe's Early' was the latest, and 'Austin' was intermediate ( $P = 0.0012$ ). A genetic basis for these differences is evident in the progenies where 'Anna'  $\times$  'Austin' seedlings showed significant early flowering compared to 'Anna'  $\times$  'Sharpe's Early' seedlings in the joint analysis over 3 years ( $P = 0.005$ ). Very low numbers of 'Golden Delicious' seedlings flowered during the second and third years after cloning. In 'Anna' families, 85.2% of seedlings flowered in the second year in comparison to 42.1% seedlings of 'Golden Delicious' crosses.

Table 2. Means for vegetative budbreak time (days from 1 Jan.) in six apple parents and families. ANOVAs were performed separately for parents and progenies for the 'Golden Delicious' and 'Anna' groups. SDs for progeny data are included.

Parents and families	Year 1	Year 2	Year 3	Joint analysis <sup>z</sup>
Golden Delicious (GD)	292.8 b <sup>y</sup>	310.1 a	297.6 c	300.2 a <sup>x</sup>
Prima	296.7 ab	283.1 c	305.2 a	295.0 a
Starking Delicious	294.0 b	297.6 b	301.5 ab	297.7 a
Summerking	299.8 a	306.7 a	298.4 bc	301.6 a
Braeburn	296.1 ab	298.8 b	292.4 d	295.8 a
GD x Prima	292.1 b	297.8 a	299.0 a	296.3 a
GD x Starking	289.0 c	290.6 c	292.0 c	290.5 b
GD x Summerking	294.6 a	293.9 b	296.7 b	295.1 a
GD x Braeburn	288.1 c	283.9 d	286.6 d	286.2 c
SD	16.4	13.5	11.3	14.0
Anna	233.8 c	213.7 c	213.3 c	220.2 c
Austin	262.8 b	250.0 b	253.3 b	255.4 b
Sharpe's Early	286.0 a	263.1 a	279.9 a	276.3 a
Anna x Austin	253.8 b	243.4 b	238.3 b	245.2 b
Anna x Sharpe's Early	262.0 a	251.9 a	251.6 a	255.2 a
SD	21.9	27.9	29.0	25.6

<sup>z</sup>In cases of significant year × family interaction the mean square for year × family was used as the error term in the ANOVA.

<sup>y</sup>Mean separation within columns for parents and families by LSD at  $P \leq 0.05$ .

Records on reproductive budbreak time in 'Golden Delicious' families were therefore analyzed for the third season only.

**VEGETATIVE BUDBREAK TIME.** 'Golden Delicious' x 'Starking Delicious' and 'Golden Delicious' x 'Braeburn' showed earlier vegetative budbreak compared to the other two 'Golden Delicious' families ( $P = 0.0007$ ) (Table 2). Parents did not differ for IVB largely due to large genotype × year interactions. The cross 'Anna' x 'Sharpe's Early' was significantly later compared to 'Anna' x 'Austin' ( $P = 0.0239$ ). This is also evident in the parental means where 'Austin' broke bud earlier than 'Sharpe's Early', but later than 'Anna' ( $P = 0.0004$ ). The succession in IVB in 'Anna' parents and families over 3 years of data recording was again consistent. The estimated phenotypic correlation between IRB and IVB was strong for the 'Anna' progeny ( $r = 0.739$ ;  $P = 0.0001$ ;  $N = 1700$ ) and for 'Golden Delicious' progeny ( $r = 0.864$ ;  $P = 0.0001$ ;  $N = 4100$ ).

**TOTAL BUDBREAK NUMBER.** Variation among individual seedlings for the number and uniformity of budbreak, and thus for the prevalence of prolonged dormancy, was of such an order that it was possible to identify trees with low and high budbreak numbers visually (Fig. 2). Low numbers of buds breaking (<10 buds per 100 cm length of shoot) were found in many 'Anna' and 'Golden Delicious' seedlings (72% and 47%, respectively). Mean budbreak number in the 'Golden Delicious' x 'Prima' family was

significantly lower than the other 'Golden Delicious' families ( $P = 0.0176$ ), indicating a genetic basis for this trait (Table 3). Although 'Golden Delicious' x 'Starking Delicious' seedlings showed high mean budbreak numbers, the joint ANOVA did not detect significant differences between 'Golden Delicious' families other than for the cross 'Golden Delicious' x 'Prima'. Parental means for budbreak number indicate that 'Prima', 'Anna',

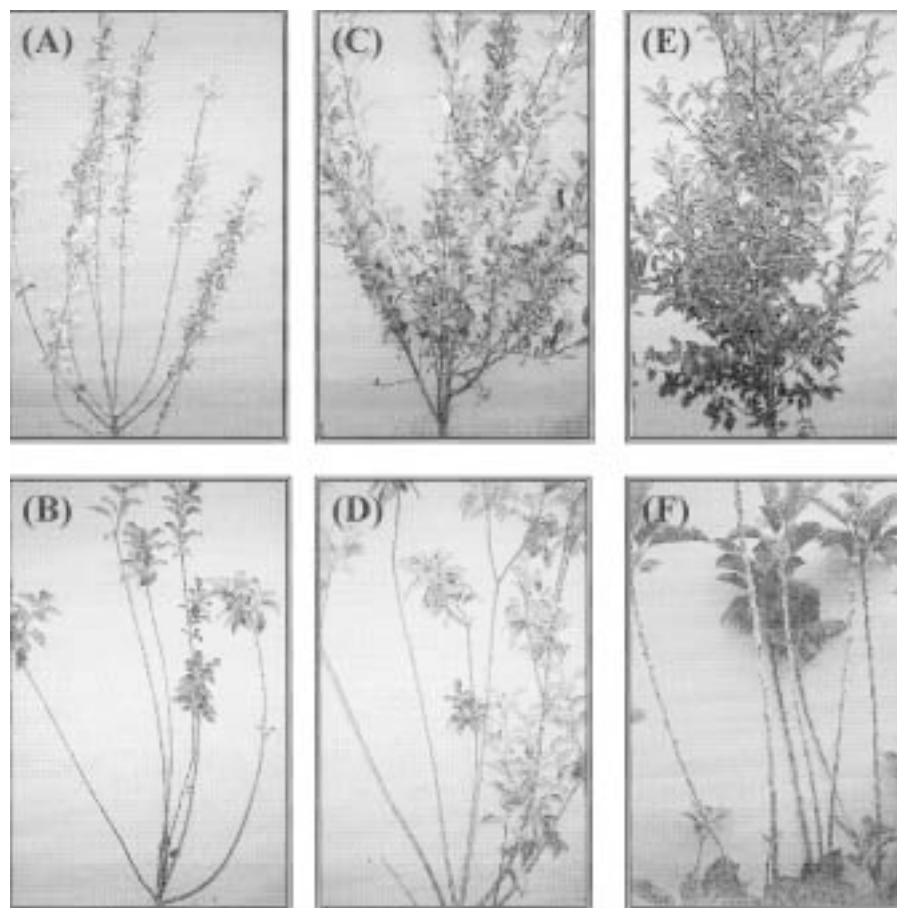


Fig. 2. 'Anna' seedlings during the first year of the experiment (1 year after budding or second growing season) with (A) high budbreak numbers and (B) low budbreak numbers. 'Anna' seedlings in third growing season with (C) high budbreak numbers and (D) low budbreak numbers. 'Golden Delicious' seedlings with (E) high and (F) low budbreak numbers during the third growing season.

Table 3. Means for total budbreak number (vegetative and reproductive) in six apple parents and families used in crosses. ANOVAs were performed separately for parents and progenies for the 'Golden Delicious' and 'Anna' groups. SDs for progeny data are included.

Parents and families	Year 1 <sup>a</sup>	Year 2 <sup>a</sup>	Year 3 <sup>a</sup>	Joint analysis <sup>a</sup>
Golden Delicious (GD)	12.7 a <sup>x</sup>	16.9 c	3.4 b	11.0 b
Prima	5.8 c	12.8 d	3.7 b	7.4 c
Starking Delicious	15.3 a	26.0 a	9.4 a	16.9 a
Summerking	15.1 a	20.7 b	3.9 b	13.2 ab
Braeburn	10.0 b	16.5 c	4.2 b	10.2 b
GD x Prima	12.4 b	14.5 b	4.0 c	10.3 b
GD x Starking	14.2 a	18.2 a	6.1 a	12.8 a
GD x Summerking	12.8 b	18.2 a	4.8 b	11.9 a
GD x Braeburn	14.0 a	18.0 a	4.9 b	12.3 a
SD	7.6	8.0	5.0	8.5
Anna	8.6 b	8.4 b	2.0 a	6.3 a
Austin	14.9 a	10.2 a	1.8 a	8.9 a
Sharpe's Early	14.6 a	8.9 ab	1.4 a	8.3 a
Anna x Austin	8.4 a	10.8 a	2.3 a	7.2 a
Anna x Sharpe's Early	8.4 a	10.5 a	2.3 a	7.0 a
SD	6.4	6.0	2.2	6.3

<sup>a</sup>Four 1-year-old shoots were selected at random on each seedling tree for bud counting.

<sup>b</sup>In cases of significant year × family interaction, the mean square for year × family was used as the error term in the ANOVA.

<sup>c</sup>Mean separation within columns for parents and families by LSD at  $P \leq 0.05$ .

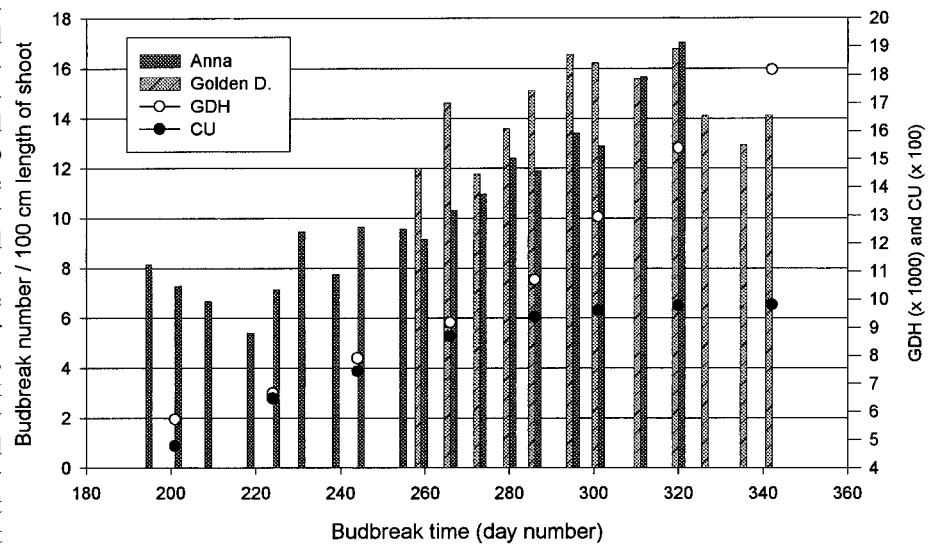
'Austin' and 'Sharpe's Early' are generally low, 'Golden Delicious' and 'Braeburn' intermediate, and 'Summerking' and 'Starking Delicious' are high (Table 3). ANOVA on parents showed that 'Prima' was significantly lower and 'Starking Delicious' significantly higher in budbreak number ( $P = 0.0051$ ) which is consistent with the above mentioned family means. 'Anna' families did not differ significantly and 'Anna' as a parent showed lower numbers compared to 'Austin' and 'Sharpe's Early' ( $P = 0.0001$ ) during the first year. According to Fig. 3 it is clear that mean budbreak number increases during the season in the 'Anna' progeny, which can be associated with CU and GDH accumulation. Correlation analyses for IVB and total budbreak number in the 'Anna' crosses was significant in year one for parents ( $r = 0.537$ ;  $P = 0.0001$ ;  $N = 142$ ) and for families ( $r = 0.316$ ;  $P = 0.0001$ ;  $N = 1371$ ); and in the second year for parents ( $r = 0.226$ ;  $P = 0.0072$ ;  $N = 140$ ) and for families ( $r = 0.311$ ;  $P = 0.0001$ ;  $N = 1344$ ). No association was found in 'Golden Delicious' crosses between budbreak time and budbreak number.

**DISTRIBUTION CURVES.** Patterns of distribution for budbreak time and number were uniform over the 3-year period. All families showed continuous distributions for IRB (Fig. 4A), IVB (Fig. 4B), and budbreak number (Fig. 4C). Only two distribution curves are shown for each trait to illustrate the tendency in 'Golden Delicious' and 'Anna' families, respectively. Within the two groups, the distribution patterns were quite consistent. Accentuated decreases in frequency of extremes and progeny means around midparents indicate possible additive gene effects for IRB and IVB. There was no evidence of segregation due to single genes of major effect for IRB and IVB. On the other hand, skewed distributions towards lower budbreak number were evident in all families, especially in the 'Anna' cross families (Fig. 4C), and might be indicative of dominant genes for low chill requirement. If one can assume that most plants originated in the tropics and that most

mutations are recessive, then high chilling should be an accumulation of recessive genes and low chilling would thus be dominant (W.B. Sherman, personal communication). The ranges for IRB in 'Anna' families (161 d) were wider than in 'Golden Delicious' families (72 d). In contrast to the IVB in 'Anna' families (142 d), 'Golden Delicious' families also showed a narrower range (96 d). Large numbers of 'Anna' seedlings showed earlier reproductive budbreak time (65% of seedlings) and vegetative budbreak time (60% of seedlings) in comparison to 'Golden Delicious'.

**GENETIC AND ENVIRONMENTAL VARIANCE COMPONENTS.** Variance component analyses summarized in Table 4 were performed on the data for each year separately. Broad-sense heritabilities and SE values were calculated from estimates of genetic and residual variance components. The IRB and IVB heritabilities calculated over a 3-year period were high (in the order of 70%),

Fig. 3. Distribution for mean budbreak number of 'Anna' progenies and 'Golden Delicious' progenies recorded over 2 years on clones of young seedling trees according to budbreak time (IVB) and accumulation of CU and GDH.



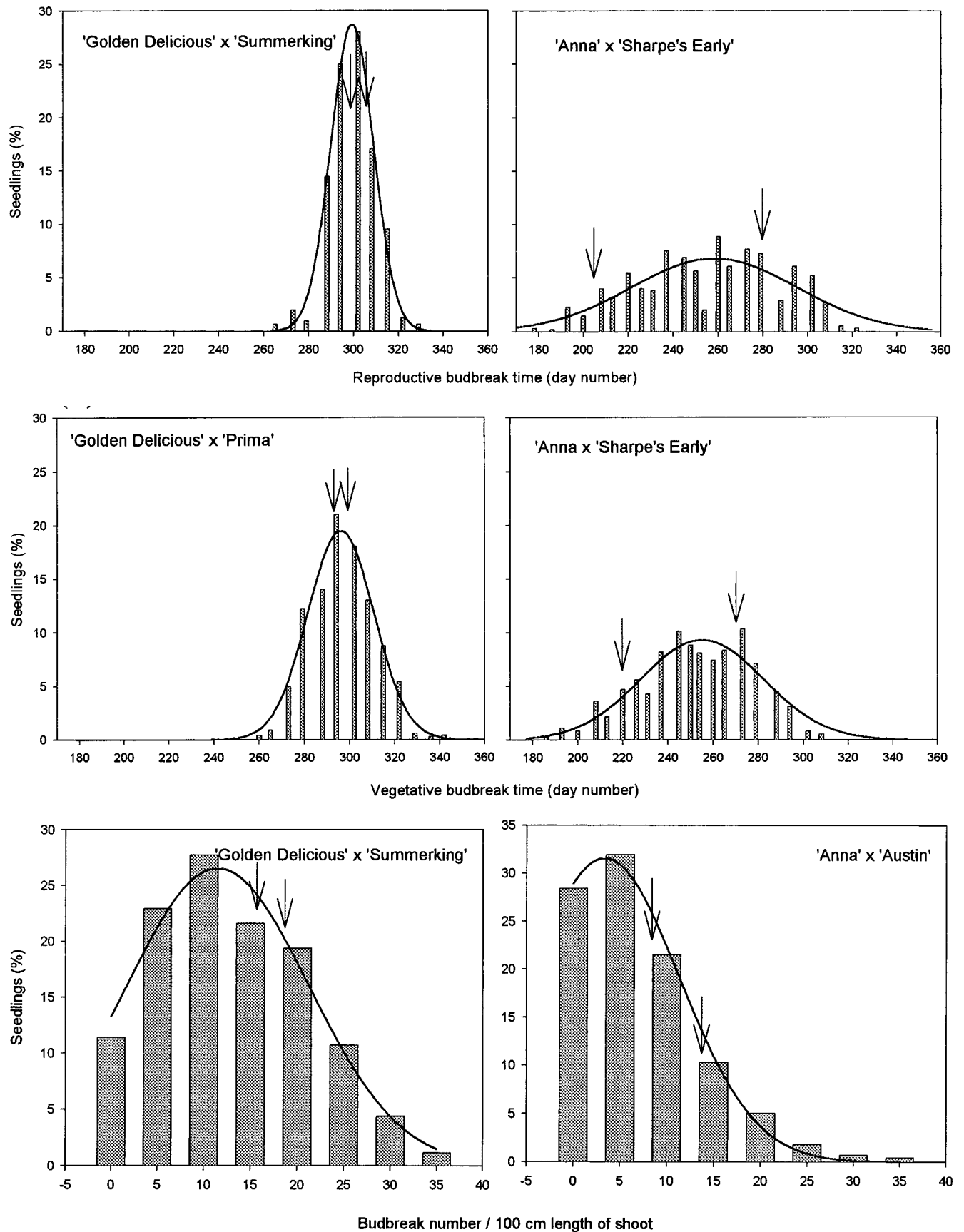


Fig. 4. Frequency distributions of (**top**) reproductive budbreak time, (**middle**) vegetative budbreak time, and (**bottom**) budbreak number based on means of seedling clones in apple families. Means of parents are indicated with arrows. The data presented were collected over a 3-year period and illustrate the tendency which was consistent in all families.

Table 4. Intraclass correlation coefficients for reproductive and vegetative budbreak time and total budbreak number in 'Anna' and 'Golden Delicious' families.

Criterion/source of variation	Golden Delicious families			Anna families		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Vegetative budbreak time						
$t_1^z$	0.001	0.003	0.183	0.002	0.003	0.001
$t_2^y$	0.615	0.574	0.655	0.686	0.824	0.827
SE <sup>x</sup> ( $t_2$ )	0.051	0.054	0.048	0.036	0.023	0.022
Reproductive budbreak time						
$t_1$	---	---	0.189	0.058	0.060	0.001
$t_2$	---	---	0.652	0.721	0.821	0.813
SE( $t_2$ )	---	---	0.048	0.032	0.023	0.024
Total budbreak number						
$t_1$	0.001	0.000	0.000 <sup>v</sup>	0.000	0.000	0.000 <sup>v</sup>
$t_2$	0.397	0.290	0.300 <sup>v</sup>	0.232	0.465	0.169 <sup>v</sup>
SE( $t_2$ )	0.058	0.056	0.056 <sup>v</sup>	0.040	0.044	0.036 <sup>v</sup>

<sup>z</sup>Intraclass correlation coefficient for between population variation:  $t_1 = (\sigma_G^2)/(\sigma_G^2 + \sigma_W^2)$ .

<sup>y</sup>Intraclass correlation coefficient for within population variation:  $t_2 = (\sigma_g^2)/(\sigma_g^2 + \sigma_e^2)$ .

<sup>x</sup>SE =  $\sqrt{2[1 + (n-1)t]^2(1-t)^2/n(n-1)(N-1)}$ .

<sup>w</sup>Variance component analyses could not be performed because of low reproductive budbreak number in 'Golden Delicious' families.

<sup>v</sup>Four 1-year-old shoots were selected at random on each tree for bud counting.

while that for budbreak number was moderate to low (30%). We have no explanation for this excepting the interpretation that budbreak number appears to be inherently more sensitive to environmental effects. Estimated broad-sense heritabilities ( $H^2$ ) were similar in the 3 years of data recording for budbreak time, but for budbreak number some difference is apparent ( $H^2 = 0.47$  in year two and  $H^2 = 0.17$  in year three). By comparison, genetic differences between families appear to be consistently small (ranging from 0% to 18%) probably attributable to the fact that families within the two groups shared one common parent. These relatively small differences between families were nevertheless statistically significant in the analyses of variance for IRB and IVB in both family groups for budbreak number in 'Golden Delicious' families. In calculations of family means as deviations from midparent values, significance was found for time of vegetative budbreak in 'Anna' crosses only. Mean gene effects, for which parents contributed by different alleles, indicate a degree of dominance towards the later parents, 'Austin' and 'Sharpe's Early'.

## Discussion

Genetic analyses of fruit traits have usually been done retrospectively from breeding program data and were often deficient in appropriate experimental design estimation of variance components and heritability. Families were usually distributed in single rows with no replication and randomization (Dicenta et al., 1993; Durel et al., 1998; Hansche et al., 1972; Tancred et al., 1995). In the present investigation the design was in accordance of what is expected for analyses of continuous traits. The trials were performed on parents and offspring grafted onto the same rootstock which gives the trees similar physiological status, combined with an experimental design allowing evaluation of genetic parameters in the six apple families. The design of the experiment allows for a partitioning of the total variance of the measurements into components between families (genetic), between seedlings within families (genetic) and within clones (environmental), and for calculation of corresponding intraclass

correlation coefficients, according to standard quantitative genetic principles (Falconer and MacKay, 1996). The intraclass correlation within clones of seedlings is then an estimate of heritability (broad sense in this case) to be used as indicator of the prospects of achieving genetic improvement by means of truncation selection between seedling clones within families. Two limitations concerning the heritability estimates need to be acknowledged. First, though collected over multiple years, the data are from a single site and thus the heritability estimates are biased upward to the extent that genotype  $\times$  location interactions would exist for the traits investigated. The multiple years of data are taken from the same trees and thus two observations on a single tree may not be independent. For these traits it seems especially important if there is the potential for cumulative effects of budbreak differences over several years. Second, the heritability estimates come from a very limited sample of apple germplasm and given the small number of crosses, the reference family for these estimates are limited only to these crosses and could probably not be generalized to all 'Anna' or all 'Golden Delicious' crosses.

Normal winter conditions for Western Cape, South Africa, were experienced during this investigation, viz., winter chilling was frequently interrupted by moderate to warm days and low CU accumulation that resulted from temperature fluctuations. Winters also differed from season to season in terms of chill and heat unit accumulation. It is clear that many of the 'Anna' seedlings needed a shorter chilling period compared to 'Golden Delicious' seedlings for dormancy release to occur. The intermediate flowering time of the parent 'Golden Delicious' in combination with three later flowering parents, 'Prima', 'Starking Delicious', and 'Summerking' resulted in nonsignificant differences between these families. The parent 'Braeburn', however, shifted the progeny mean towards earlier reproductive budbreak. The parental influence was also clear in 'Anna' progenies, with the earlier flowering seedlings from 'Anna'  $\times$  'Austin' showing more early flowering seedlings compared to the 'Anna'  $\times$  'Sharpe's Early' family. The succession in flowering time in 'Anna' parents and families was constant over the 3 years and the range was much

wider than in 'Golden Delicious' families, indicating a wide genetic variation to choose from in a selection program. 'Anna' seedlings were observed to be highly reproductive, flowering at a very young stage, indicating precocity in their adaptive response to local conditions, which is a benefit in apple cultivar development.

Means for IVB among parents of 'Golden Delicious' crosses did not differ significantly. Among the 'Golden Delicious' progenies, however, significant differences in IVB were observed where 'Starking Delicious' and 'Braeburn' induced early budbreak. Although the 'Braeburn' progeny showed early reproductive and vegetative budbreak, the period of ripening was largely extended towards the end of the cropping season compared to the other three families (data not presented). 'Golden Delicious' families showed a much later and narrower range of IVB compared to the 'Anna' families. It is possible that seedlings may differ in their heat requirement or rapidity of response to favorable conditions after their chilling requirement was satisfied (Worrall and Mergen, 1967). The duration and interrelation of chilling and heat during the premeiotic stage of bud differentiation determine whether or not this stage may proceed (Bailey and Hough, 1975). Bud exposure to warm temperatures is thus also necessary for normal budbreak (Kester et al., 1977). Wilton (2000) describes dormancy in terms of two phases: the first phase commences at or close to leaf fall and requires a period of chilling to break down growth inhibitors present during this period, and the second phase is heat driven. Onset of the second phase depends on how warm spring temperatures are. In growing conditions with warm spring weather, development of buds to budbreak will be much shorter than in cool spring conditions. The response of 'Golden Delicious' seedlings may be partly a function of accumulated heat, since the first budbreak started at a time when GDH accumulation increased during mid-September. 'Austin' was significantly earlier in IVB compared to 'Sharpe's Early' with a marked genetic influence on progeny means. The stability of family ranking for IVB indicates that families can be assessed effectively for budbreak time and that parents can be identified accordingly.

Broad-sense heritabilities calculated in this study indicate that IRB ( $H^2 = 0.75$ ) and IVB ( $H^2 = 0.69$ ) are highly heritable and that the variation between seedlings can be ascribed to genetic factors. Previous studies on apple have indicated relatively high narrow-sense heritability for the length of bud dormancy (Hauagge and Cummins, 1991b). Moderate genetic control of budbreak time has been reported for Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] ( $H^2 = 0.44$ ) (Li and Adams, 1993) and for balsam poplar (*Populus balsamifera* L.) ( $H^2 = 0.21$  to  $0.47$ ) (Farmer, 1993), and strong genetic control for trembling aspen (*Populus tremuloides* Michx.) ( $H^2 = 72$ ) (Thomas et al., 1997) and hybrid poplars [*Populus trichocarpa* (Torr. & Gray)  $\times$  *P. deltoides* Bartr.] ( $H^2 = 0.80$ ) (Howe et al., 2000). High heritabilities for budbreak time suggest that response to selection will be successful and that genetic advance is expected to be relatively rapid.

Previous studies on apple where high chilling parents were crossed with 'Anna' suggest that length of bud dormancy measured in terms of GDH accumulated from leaf fall until budbreak is ascribable to major dominant genes, modulated by minor interactive genes (Hauagge and Cummins, 1991b). Oppenheimer and Slor (1968) also found evidence that early budbreak appears to be controlled by dominant genes. From the distribution classes for CR based on leafing response in peach [*Prunus persica* (L.) Batsch (Peach group)] seedlings, Lesley (1944) suggested the presence of multiple genes with cumulative effects and absence

of dominance. Lammerts (1945) has shown that the low CR in peach based on leaf growth rate is due to accumulation of the effects of multiple genes, some recessive and cumulative. Studies in peach CR based on leaf bud activity have also suggested multiple gene control (Bowen, 1971). Continuous distributions and midparent values in the crosses in our trials indicate that additive effects of genes are probably more important than nonadditive effects in the total genetic variance of these traits.

Prolonged dormancy symptoms were common and easily observable under climatic conditions experienced during this investigation. A substantial number of seedlings exhibited severe symptoms that included bare shoots without budbreak and delay of vegetative and reproductive budbreak. These symptoms were observed in seedlings from high chill parents of the 'Golden Delicious' progenies, and also in seedlings of the low-chill 'Anna' progenies. Some seedlings of 'Anna' showed lower numbers of budbreak than seedlings of 'Golden Delicious' in contrast to previous reports where 'Anna' demonstrated total termination of dormancy and no delayed foliation (Hauagge and Cummins, 1991b). Parental means indicated that some cultivars are more inclined to lower budbreak numbers than others. The three parents used in the 'Anna' crosses, viz., 'Anna', 'Austin', and 'Sharpe's Early' generally had low budbreak number. The parent 'Prima' and its progeny were also low, similar to results obtained previously from adult trees (Labuschagné et al., 2002). 'Golden Delicious' and 'Braeburn' families were intermediate while 'Summerking' and 'Starking Delicious' families were high. 'Anna' families did not differ significantly as was observed previously in adult trees (Labuschagné et al., 2002).

Offspring of low  $\times$  low crosses tend towards low budbreak number as well as offspring of low  $\times$  intermediate crosses. Offspring of intermediate  $\times$  intermediate, and intermediate  $\times$  high crosses are intermediate. In contrast to budbreak time, broad-sense heritability of budbreak number was moderate to low ( $H^2 = 0.30$ ). Heritability estimates from parent-offspring regression for an index combining the number and distribution of budbreak under subtropical winter conditions were found to be between 0.34 and 0.37 by Hauagge and Cummins (1991b). The relatively low heritability indicates low expected response to selection for this trait and suggests the need for selection based on clonal progeny means, i.e., family selection.

It is generally accepted that vegetative budbreak time is a reflection of the chilling and heat requirement under favorable climatic conditions (Hauagge and Cummins, 1991d; Weinberger, 1944) and that genotypes showing early budbreak during the growing season, such as the cultivar Anna, are low chill requiring and, therefore, more widely adaptable. It is also expected that budbreak should be more prolific, and will occur promptly and uniformly (Hauagge and Cummins, 1991a). Thus, in mild climates budbreak time is related to bud CR (Hauagge and Cummins, 1991d; Oppenheimer & Slor, 1968). For the given level of chilling in our experiment, the response of the 'Anna' seedlings was more rapid than that of 'Golden Delicious' seedlings. Significant association between budbreak time and budbreak number was found for 'Anna' parents and progeny, but not for 'Golden Delicious' parents and progeny. Similar results have been reported previously on adult trees where a positive association between budbreak time and number was found in 'Anna' families and a negative association in 'Golden Delicious' families (Labuschagné et al., 2002). The high percentage of seedlings in 'Anna' families with low budbreak numbers compared to 'Golden Delicious', indicates that selection for early budbreak will not

automatically identify seedlings lacking prolonged dormancy symptoms. Early budbreak is probably not a useful trait to select for in climates with unfavorable fluctuating temperatures and low winter chilling conditions. Distribution of seedlings according to budbreak time and number identifies a truncation point (around day 260) at maximum CU accumulation and onset of GDH accumulation as optimal for adaptation for seedlings in the low-chill conditions of our experiment. Indirect selection using budbreak time at this point may identify seedlings with a lower tendency to prolonged dormancy symptoms.

The success of any crop improvement program depends, among other factors, on the amount of genetic variability for the trait under selection (De Souza, 1998). The substantial genetic variation within families found in the present study can most probably be explained by the fact that apples are cross-fertilizing, that within family variation is directly related to variation within cultivars, and that new genetic variation is generated by crossing and segregation. Genetic variation between families appears to be consistently small and may be explained by the fact that families within the two groups all shared one common parent. The high correlations and heritabilities obtained may seem surprising since chilling requirement is likely to be a highly complex character of which budbreak number and time are but two components and this will need further investigation.

In this study we used budbreak time and number on a continuous scale to investigate variation in chilling requirement in apple families and to determine the genetic and environmental components of variation. Trees were grown under experimental conditions where fluctuations in environmental effects could be controlled to some extent by replication and randomization. In general, the results have exposed significantly high levels of genetic variation within families, of an order indicating that the traits are amendable to genetic improvement by selection. The best strategy for producing families for early budbreak will be to cross two cultivars that have high midparent values and the simplest method to select superior individuals is based on their own performance. Clonal testing should not be necessary for selection for budbreak time, but would be advisable for the budbreak number.

The association found between budbreak time and number, and the high number of seedlings in the 'Anna' progeny with prolonged dormancy symptoms, has important practical implications for breeding programs. Budbreak number is preferred to time of budbreak as the sole criterion on the grounds that early budbreak is associated with low budbreak number under local conditions. The patterns of budbreak observed is a result of interaction between genetic and controlling environmental stimuli (CU and GDH accumulation) and not solely the direct result of inherent variation in chilling requirement as generally measured in terms of budbreak time. It is known that high temperatures gradually assume more control over bud development as trees approach completion of rest and that bud development progresses only if chilling is supplemented with temperatures favorable to growth (Brown, 1960; Kriebel and Wang, 1962; Worrall and Mergen, 1967). Although the interrelationships among the traits are not understood, the correlated response indicates that selection for early budbreak should result in negative effects on budbreak number and the occurrence of prolonged dormancy symptoms. Selection for number of buds breaking should be more difficult than selecting for time of budbreak.

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