

# Influence of year and genetic factors on chilling injury susceptibility in peach (*Prunus persica* (L.) Batsch)

Pedro J. Martínez-García · Cameron P. Peace · Dan E. Parfitt · Ebenezer A. Ogundiwin · Jonathan Fresnedo-Ramírez · Abhaya M. Dandekar · Thomas M. Gradziel · Carlos H. Crisosto

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**Abstract** Chilling injury (CI) is a major physiological problem limiting consumption and export of peach and nectarine (*Prunus persica* (L.) Batsch). To clarify the genetic basis for chilling injury, inheritance of the major CI symptoms mealiness, flesh browning, flesh bleeding, and flesh leatheriness were examined over three years in two related peach progenies. In addition, genetic relationships among traits and the year-to-year variation in trait performance in these progenies were tracked. Both populations also segregated for Free-stone-Melting flesh (*F-M*) and yellow flesh. There were significant differences in CI symptoms among years. The major gene endoPG, which controls the *F-M* locus, provides resistance to mealiness in non-melting flesh fruit. Only fruit with melting flesh can develop mealiness if the tree possesses other genetic

susceptibility factors and/or experiences inducing conditions. The *F-M* locus also greatly influences susceptibility to flesh bleeding, although the physiological mechanism for this disorder is unclear and may be controlled by a different gene closely linked to endoPG. Unlike mealiness, flesh bleeding occurred primarily in non-melting flesh fruit, particularly when the fruit is white-fleshed. Flesh browning incidence was greater in mealy fruit and was not associated with flesh bleeding. Breeding for CI resistance is thus a viable long-term strategy to reduce losses in the fresh and processed peach and nectarine industries. This study is an important first step to understanding genetic control of CI symptoms in peach.

**Keywords** Breeding strategy · Chilling injury · Flesh bleeding · Flesh browning · Genetic factor · Heritability · Leatheriness · Mealiness

Pedro J. Martínez-García and Cameron P. Peace contributed equally to the preparation of this paper.

P. J. Martínez-García (✉) · D. E. Parfitt · J. Fresnedo-Ramírez · A. M. Dandekar · T. M. Gradziel · C. H. Crisosto  
Department of Plant Sciences, University of California, Davis, One Shields Ave, Davis, CA 95616, USA  
e-mail: [pjmartinezgarcia@ucdavis.edu](mailto:pjmartinezgarcia@ucdavis.edu)

C. P. Peace  
Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164, USA

E. A. Ogundiwin  
Nunhems USA, Inc, Davis, CA 95618, USA

## Introduction

Many peach (*Prunus persica* (L.) Batsch) accessions can develop a physiological disorder called chilling injury (CI) or internal breakdown (IB). This disorder, triggered by exposure to cold storage temperatures, affects several organoleptic attributes such as texture, flesh color, and juiciness (Anderson 1979; Harding and Haller 1934; Hartmann 1985; Smith 1934; Vonmollendorff and Devilliers 1988). The presence of chilling injury is a frequent source of complaints by

consumers and wholesalers of peach (Crisosto et al. 1999). Externally, fruits with chilling injury appear sound, so the problem is usually not noticed until the fruit reaches retailers and consumers (Crisosto et al. 1999). A high frequency of affected fruit reduces consumer satisfaction, limits development of long distance markets, and reduces peach consumption. The major symptoms of CI are flesh mealiness (M), flesh leatheriness (FL), flesh browning (FB), flesh bleeding (FBL), loss of flavor, and development of off-flavors (Crisosto et al. 1999). Mealiness (M) is a fruit flesh textural disorder, where affected ripe fruit have a dry, grainy feel when chewed. In simple terms, mealy fruit are dry and soft when ripe, while leathery fruit are either dry and firm when ripe or remain firm because of a failure to ripen (Luza et al. 1992; Ju et al. 2000). Desirable, non-mealy fruit are juicy with a soft, melting texture, or are juicy and firm (Crisosto et al. 1999).

On a cellular level, metabolism of the pectin component of the cell wall is altered in mealy fruit. A gel is formed as pectins in intercellular spaces absorb free water, intercellular adhesion is reduced, and cells form loose clumps rather than rupturing to release juice (Brummell et al. 2004). Flesh browning (FB) is often seen in mealy fruit, although it can also occur in the absence of mealiness (Crisosto et al. 1999; Brummell et al. 2004). Flesh browning (FB) occurs when enzymes such as polyphenol oxidase act on phenolic substrates with which they come in contact (Kader et al. 1984). Flesh bleeding (FBL) is caused by the movement of water-soluble red pigments, probably anthocyanins, through the fruit flesh during cold storage or after subsequent ripening (Lurie and Crisosto 2005). It can be present at harvest or develop as fruit ripen and senesce on the tree. The progression of M and FB symptoms is associated with reduced perception of normal flavor and with development of off-flavors (Crisosto 2002; Crisosto et al. 1999).

In most cultivars tested, flavor loss (“hidden damage”) is perceived prior to lack of juice or mealiness, and FB is generally the last visual symptom of CI damage to develop (Crisosto and Labavitch 2002; Infante et al. 2009). The documented sensory damage underscores the effect of CI complex damage on consumer preference and fruit consumption, and thus, its commercial importance. In addition, more attention should be focused on obtaining cultivars with genetic tolerance/resistance or in developing postharvest innovations to control early stages of chilling

injury (flavor loss) rather than later aspects such as FB development.

Several treatments have been attempted as a short-term solution. Controlled atmosphere (Anderson 1979; Crisosto et al. 2009; Zhou et al. 2001), warming exposures (Anderson 1979; Lill 1985; O’Reilly 1947; Zhou et al. 2001), and preconditioning (Crisosto et al. 2004; Guelfat-Reich and Ben Arie 1966) have been used commercially to mitigate CI in peach fruit (Lurie and Crisosto 2005). Among these treatments, preconditioning is widely used commercially and, when properly applied, delays CI symptom expression for 10 to 12 days, enough to improve the quality of some peach cultivars on arrival (Crisosto et al. 2009; Crisosto and Valero 2008). Unfortunately, the benefits of these treatments have been erratic, and when postharvest life has been extended, the time of extension has been too short to have a commercial impact. An early review of orchard factors affecting peach CI such as nitrogen fertilization, deficit irrigation regimes, maturity, canopy position, crop load, fruit size, environmental conditions, season, and genotypes concluded that genotype was the most important factor among them (Crisosto et al. 1997). In general, clingstone nectarine cultivars were less susceptible to CI than peach cultivars (Crisosto et al. 1999; Brovelli et al. 1998) and non-melting flesh cultivars have reduced endoPG activity and less CI than melting flesh cultivars (Lester et al. 1996).

The variation in CI susceptibility among commercial cultivars and selections when stored at either 0 or 5°C (Crisosto et al. 2008, 1999; Mitchell 1987; Cantin et al. 2010) indicated that CI is genetically controlled. Clingstone non-melting flesh (CNMF) peaches, which are primarily used in canning but are also popular in the European and South African fresh markets, are largely free of CI, although the physiological basis for this resistance has not been addressed (Brovelli et al. 1998; Crisosto et al. 1999). Melting flesh (MF) cultivars vary in susceptibility to CI, with some varieties exhibiting symptoms in all fruit after only one week of cold storage even at 0°C, while others appear resistant, withstanding six weeks of cold storage before eventually ripening and senescing (Crisosto et al. 1999). To provide a long-term solution to CI, genetic resistance to this disorder is desirable for new MF peaches destined for the fresh market. This may be achievable by phenotypic selection for resistance in breeding program progeny. However, the

inheritance of symptoms has not been quantified, and strategies for genetic improvement through breeding would be greatly aided by knowledge of the underlying mechanisms of genetic control.

The genetic basis for chilling injury has not been described in stone fruits. The inheritance of the major CI symptoms M, FB, FBL, FL, and the phenotypic correlations between those symptoms, are described here using two related peach progenies. In addition, year effect was determined for the same symptoms.

## Materials and methods

### Plant material

Two peach progenies segregating for CI symptoms were used in this study. The first, Pop-DG, was composed of 70 F<sub>1</sub> seedlings from a cross between the commercial cultivars ‘Dr. Davis’ and ‘Georgia Belle’. ‘Dr. Davis’ produces yellow, clingstone, non-melting flesh (NMF) fruit and is a major cultivar grown in California for the canning industry, while ‘Georgia Belle’ is an old cultivar producing white, freestone, MF fruit that are eaten fresh (Brooks and Olmos 1972; Okie 1998). ‘Georgia Belle’ is unusual among peach cultivars in that it is highly heterozygous, including at the *F-M* locus controlling freestone/clingstone and melting/non-melting flesh and at the *Y* locus controlling white/yellow flesh color. ‘Dr. Davis’ is homozygous recessive for these loci. ‘Georgia Belle’ is particularly susceptible to CI, while ‘Dr. Davis’ exhibits resistance to most symptoms. Pop-G, the second population of 70 seedlings, was derived by self-pollination of ‘Georgia Belle’. Crosses were made in 1998 and planted in 2000 at 2.5 × 1.8 m spacing, trained as a central leader, and maintained with standard California commercial practices, including fruit thinning. Progeny populations were evaluated at the University of California Kearney Agricultural Center at Parlier, California. Each individual genetic progeny was present twice in the experimental orchard. The two replicates of each scion grew adjacent in a row. Both populations were screened with microsatellites (SSRs) (Peace et al. 2005) to eliminate individuals resulting from outcrossing and from self-pollination in Pop-DG. Subsequently, those individuals and unviable/sick ones were removed, reducing the number of progeny to 51 and 64, respectively.

### Trait evaluation

Each parent and each individual genetic progeny were monitored for three successive years. Fifteen sound fruits per tree were harvested at commercial maturity and immediately taken to the postharvest evaluation laboratory. Each fruit was treated with a fungicide dip, 1.2 g l<sup>-1</sup> of iprodione, cold-stored under CI-inducing condition (5°C for two weeks), and then ripened at room temperature to assess CI symptom expression. The CI symptoms mealiness (M), flesh browning (FB), flesh bleeding (FBL) and flesh leatheriness (FL) were evaluated (details below). Each tree was classified as producing fruit that were either freestone or clingstone, melting or non-melting flesh, and white or yellow flesh.

After two weeks storage, fruit exhibiting fungal decay caused by brown rot (*Monilinia fructicola*) were removed. Sound fruit were sectioned into two halves and CI symptom severity determined. M and FL were assessed as described (Crisosto et al. 1999) and their presence or absence in each fruit was recorded. Although “juicy” fruit were defined as non-decayed fruit that was neither mealy nor leathery, this category was not developed because examining the separate non-juicy components M and FL provided a more relevant genetic assessment. FB was scored visually for each fruit on a scale of 1 (no flesh browning) to 6 (intense FB over most of the flesh). FBL was recorded as the presence or absence of red pigmentation spread into the mesocarp.

For all traits, data on individual fruits were recorded during each year, and from these, progeny averages were calculated. Tree values for CI symptoms were recorded as the proportion of measured fruit (%) with M, FBL, or FL, and as the average score for FB.

### Statistical analysis

Segregation ratios for qualitative traits controlled by the *Freestone (F)*, *Melting flesh (M)*, and *Flesh color (Y)* loci were tested with Chi-squared analysis. The homogeneity of variance of data was first checked using Levene’s test (Levene 1960). Effects of year (Years 1, 2, and 3) and the Mendelian loci of *F-M* (FMF = freestone melting flesh and CNMF = clingstone non-melting flesh) and *Y* (yellow flesh and white flesh) on quantitative traits were examined by two-tailed *t*-tests. Genetic and year effects on each

quantitative trait were calculated by two-factor (genotype-year for the two-week storage duration) analysis of variance (ANOVA) through the GLM procedure of Statistical Analysis System 9.1.3 (SAS Institute, 2005). Broad sense heritability was estimated (proportion of phenotypic variation given by the genotype, Falconer and Mackay 1996) for each trait. The number of fruit analyzed per individual was 30. Pearson correlation coefficients between the CI traits M, FB, FBL, and FL were performed, within progeny subsets based on *F-M* and *Y* genotypes, and combining observations across all years and both storage durations.

## Results

### Inheritance of morphological traits

Freestone/clingstone and melting/non-melting flesh segregate together as a single locus (*Freestone-Melting flesh, F-M*), with freestone and MF dominant over clingstone and NMF (Ogundiwin et al. 2009). In our populations, the two morphological traits exhibited the expected two phenotypic classes, consistent with control by a single locus with dominant gene action (Table 1). Yellow/white flesh (*Y* locus) also segregated as a single-locus trait, with white flesh dominant over yellow flesh. For both loci, segregation in Pop-DG and Pop-G was consistent with ‘Georgia Belle’ being heterozygous and ‘Dr. Davis’ being homozygous for the recessive alleles. The two loci also exhibited independent phenotypic segregation to each other: 16:13:10:12 in Pop-DG and 35:11:14:4 in Pop-G. This is not significantly different than the expected 1:1:1:1 ratio in Pop-DG and 9:3:3:1 ratio in Pop-G. In Pop-DF, four trees exhibited the nectarine

phenotype, out of the 18 individuals arising from self-pollination of ‘Dr. Davis’, confirming heterozygosity at the *G* locus for this cultivar (Bailey and French 1949). All hybrid seedlings in Pop-DG and Pop-G produced peach fruit, confirming that ‘Georgia Belle’ is homozygous for the dominant peach allele.

### Distributions of chilling injury incidence

Both populations showed considerable phenotypic variation in the incidence of chilling injury symptoms following cold storage (Fig. 1). Incidence of mealiness (M) and flesh bleeding (FBL) across all seedlings exhibited L-shaped distributions for both populations, while flesh browning (FB) presented a more Normal distribution, with greater incidence for each of these major chilling injury symptoms in Pop-G and the ‘Georgia Belle’ parent. Flesh leatheriness (FL) incidence was very low, but slightly greater in Pop-G, and the parents themselves had similar low expression of these traits. Because of the large effect of the *F-M* locus described below, distributions were recalculated considering only freestone melting flesh (FMF) samples for M and only CNMF samples for FBL (Fig. 2). Transgressive segregation was observed for M (high incidence in some FMF seedlings of Pop-G), FB (both low and high incidence), and FBL and FL (high incidence in some seedlings) (Figs. 1, 2).

### Heritability of CI symptoms

Broad-sense heritability values were above 0.50, ranging from 0.58 for FBL in Pop-DG to 0.70 for M in Pop-G (Table 2). Heritability was always greater in Pop-G than Pop-DG, and lower in the FMF and CNMF seedling subsets than for all seedlings (0.37 to 0.49). Heritability for FL was low in both populations.

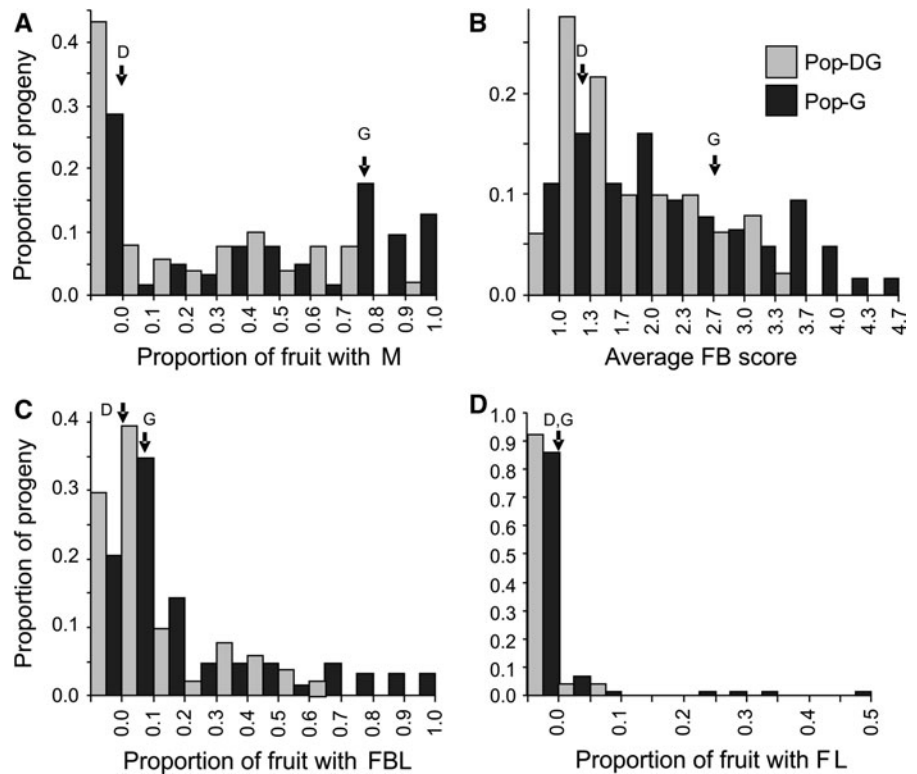
**Table 1** Inheritance of the *Freestone-Melting flesh (F-M)* and *Flesh color (Y)* loci in two segregating populations of peach

Locus	Parent genotype		Segregation (number of progeny)			
	‘Dr. Davis’	‘Georgia Belle’	Pop-DG		Pop-G	
			Ratio (1:1)	$\chi^2$	Ratio (3:1)	$\chi^2$
<i>F-M</i>	CNMF (f1f1)	FMF (Ff2)	29 <sub>Ff1</sub> : 22 <sub>f1f2</sub>	ns	45 <sub>F-</sub> : 19 <sub>f2f2</sub>	ns
<i>Y</i>	Yellow (yy)	White (Yy)	26 <sub>Yy</sub> : 25 <sub>yy</sub>	ns	49 <sub>Y-</sub> : 15 <sub>yy</sub>	ns

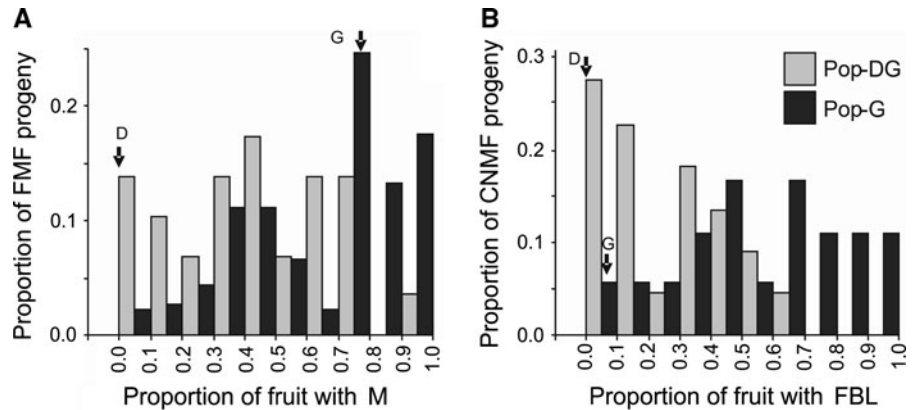
CNMF clingstone non-melting flesh, FMF freestone melting flesh, ns not significantly different to expected segregation ratio

<sup>a</sup> The fruit type of one seedling in Pop-G that did not produce fruit was predicted by the *endoPG* DNA test for *F-M* (Peace et al. 2005) and senescent leaf color for *Y* (Williamson et al. 2006)

**Fig. 1** Phenotypic distribution of chilling injury symptoms in two peach cultivars (*D* ‘Dr. Davis’; *G* ‘George Bell’) and in two peach progenies (Pop-DG and Pop-DG) averaged over three years. **a** *M* mealiness; **b** *FB* flesh browning; **c** *FBL* flesh bleeding; **d** *FL* flesh leatheriness



**Fig. 2** Phenotypic distribution of **a** mealiness in two peach cultivars (*D* ‘Dr. Davis’; *G* ‘George Bell’) and in FMF progeny only and **b** flesh bleeding in the same two cultivars and in CNMF seedlings only. *M* mealiness; *FBL* flesh bleeding; *FMF* freestone melting flesh; *CNMF* clingstone non-melting flesh



Genetic factor influences on CI incidence

The *F-M* locus was strongly associated with differences in *M*, *FBL*, and *FB* (Table 3). Mealiness (*M*) was never observed in fruit from CNMF seedlings, while FMF seedlings displayed an incidence of from 3 to 100%. For *FBL*, the association with the *F-M* locus was reversed: FMF seedlings exhibited very low incidence of this CI symptom, while CNMF progeny ranged from low (1%) to high *FBL* (100%)

(Fig. 2). Mealiness (*M*) in FMF progeny was bimodally distributed in both populations. *FBL* in CNMF seedlings was bimodal, but skewed toward low incidence in Pop-DG and high incidence in Pop-G. The distribution of *FBL* in CNMF seedlings from Pop-G was similar to that of *M* in FMF seedlings. *FB* was significantly higher in FMF than CNMF seedlings (Table 3). White-fleshed seedlings were consistently associated with a higher incidence of *FBL*, and *FL* was slightly greater for yellow flesh. Other associations

**Table 2** Means and broad-sense heritabilities for chilling injury symptoms in two peach progenies (Pop-DG,  $n = 51$  and Pop-G,  $n = 64$ ) observed after two weeks of cold storage (5°C), with 30 fruit per individual for each of three years

Parameter	M (% fruit)		FB (1–6 scale)	FBL (% fruit)		FL (% fruit)
	All	FMF		All	CNMF	
Number of years	3	3	3	3	3	3
Mean $\pm$ se						
Dr. Davis	0.00 $\pm$ 0.00	–	1.29 $\pm$ 0.06	0.00 $\pm$ 0.00	–	0.000 $\pm$ 0.000
Georgia Belle	0.76 $\pm$ 0.05	–	2.69 $\pm$ 0.12	0.07 $\pm$ 0.03	–	0.000 $\pm$ 0.000
Pop-DG	0.24 $\pm$ 0.01	0.42 $\pm$ 0.01	1.84 $\pm$ 0.02	0.12 $\pm$ 0.01	0.25 $\pm$ 0.01	0.002 $\pm$ 0.001
Pop-G	0.47 $\pm$ 0.01	0.63 $\pm$ 0.01	2.15 $\pm$ 0.02	0.21 $\pm$ 0.01	0.62 $\pm$ 0.01	0.016 $\pm$ 0.002
Heritability						
Pop-DG	0.66	0.46	0.58	0.62	0.49	0.14
Pop-G	0.70	0.49	0.68	0.65	0.37	0.31

Because of the large effect of the *F-M* locus on mealiness (M) and flesh bleeding (FBL), heritability is also shown separately for mealiness in FMF progeny and for flesh bleeding in CNMF progeny

*FB* flesh browning, *FL* flesh leatheriness

**Table 3** *F-M* and *Y* locus effects on chilling injury symptoms in two peach progenies

Factor	M (% fruit)		FB (1–6 scale)	FBL (% fruit)		FL (% fruit)
	All	FMF		All	CNMF	
Pop-DG						
<i>F-M</i> locus						
CNMF mean $\pm$ se	0.00 $\pm$ 0.00	–	1.60 $\pm$ 0.03	0.25 $\pm$ 0.01	–	0.001 $\pm$ 0.001
FMF mean $\pm$ se	0.42 $\pm$ 0.01	–	2.03 $\pm$ 0.02	0.02 $\pm$ 0.00	–	0.004 $\pm$ 0.001
Significance	****	–	****	****	–	*
<i>Y</i> locus						
Yellow mean $\pm$ se	0.22 $\pm$ 0.01	0.41 $\pm$ 0.02	1.90 $\pm$ 0.03	0.08 $\pm$ 0.01	0.15 $\pm$ 0.01	0.005 $\pm$ 0.002
White mean $\pm$ se	0.26 $\pm$ 0.01	0.43 $\pm$ 0.01	1.80 $\pm$ 0.02	0.15 $\pm$ 0.01	0.37 $\pm$ 0.02	0.000 $\pm$ 0.000
Significance	***	ns	**	****	****	**
Pop-G						
<i>F-M</i> locus						
CNMF mean $\pm$ se	0.00 $\pm$ 0.00	–	1.82 $\pm$ 0.04	0.62 $\pm$ 0.01	–	0.057 $\pm$ 0.007
FMF mean $\pm$ se	0.63 $\pm$ 0.01	–	2.28 $\pm$ 0.02	0.06 $\pm$ 0.00	–	0.002 $\pm$ 0.001
Significance	****	–	****	****	–	****
<i>Y</i> locus						
Yellow mean $\pm$ se	0.59 $\pm$ 0.02	0.76 $\pm$ 0.02	1.90 $\pm$ 0.04	0.14 $\pm$ 0.01	0.45 $\pm$ 0.03	0.023 $\pm$ 0.005
White mean $\pm$ se	0.43 $\pm$ 0.01	0.58 $\pm$ 0.01	2.24 $\pm$ 0.02	0.23 $\pm$ 0.01	0.66 $\pm$ 0.02	0.014 $\pm$ 0.002
Significance	****	****	****	****	****	*

\*, \*\*, \*\*\*, and \*\*\*\* indicate  $P < 0.05$ , 0.01, 0.001, and 0.0001, respectively

ns not significant, *M* mealiness, *FB* flesh browning, *FBL* flesh bleeding, *FL* flesh leatheriness

between the *Y* locus and CI symptoms were population-specific, with yellow fruit having lower M but higher FB in Pop-DG, while the opposite was observed in Pop-G.

Non-genetic factor influences on CI incidence

Each CI symptom except FL differed significantly in incidence among the three years of observation, with

the lowest incidences of M, FB, and FBL usually observed in Year 2 (Table 4). Despite this statistically significant effect, the proportion of phenotypic variation explained by the year was usually dwarfed by the magnitude of the genotype effect.

#### Relationships among traits

Seedlings in both populations that were susceptible to M tended to be susceptible to FB (Table 5). This relationship was stronger in the FMF subsets than in the entire progeny sets: 0.70 in Pop-DG and 0.48 in Pop-G. The large negative correlation between M and FBL ( $-0.65$  in Pop-DG and  $-0.63$  in Pop-G) in both progenies appears to be an indirect influence of the *F-M* locus, as correlations were not maintained for the FMF progeny subset in which all the variation for M susceptibility occurred. FB and FBL were not correlated, whether all progeny or just the CNMF subset were considered. FL correlated positively with M (FMF subset) and FB in Pop-DG.

Comparing the incidence of chilling injury symptoms among fruit types in both progenies provided further insights into trait relationships and major locus effects. Mealy FMF fruit tended to have higher FB

scores (averaging 2.7 in both populations) than juicy FMF fruit (1.7 in Pop-DG and 1.8 in Pop-G) or juicy CNMF fruit (1.8 in Pop-DG and 2.0 in Pop-G) (Table 6). Flesh color had little effect on FB incidence in each of these fruit types in Pop-DG, while in Pop-G, yellow fruit displayed less FB in FMF fruit (mealy or juicy) but slightly higher FB in CNMF fruit. Leathery fruit tended to have the highest FB scores, although leatheriness incidence was very low overall. The few observed FBL FMF fruit tended to have lower FB scores than non-flesh bleeding (“clear”) FMF fruit, although this association was not observed in CNMF fruit, where most FBL occurred, and FB incidence in these fruit categories was consistent across white and yellow fruit. As previously noted, FBL occurrence was much greater in CNMF fruit than FMF fruit (Table 7). In both populations, white-fleshed fruit exhibited a greater tendency for FBL, regardless of whether fruit were FMF, CNMF, juicy, mealy, or leathery. Although FBL incidence was very low in FMF fruit, mealy fruit tended to have only half the FBL incidence of juicy FMF fruit in both flesh colors. No leathery flesh bleeding FMF fruit were observed, while leathery CNMF fruit were just as likely to have FBL as juicy CNMF fruit.

**Table 4** Effect of year on chilling injury symptoms in two peach progenies

Factor	M (% fruit)		FB (1–6 scale)	FBL (% fruit)		FL (% fruit)
	All	FMF		All	CNMF	
Pop-DG						
Year						
Year 1 mean ± se	0.26 ± 0.01	0.46 ± 0.02	1.99 ± 0.03	0.09 ± 0.01	0.20 ± 0.02	0.004 ± 0.002
Year 2 mean ± se	0.21 ± 0.01	0.37 ± 0.02	1.68 ± 0.03	0.09 ± 0.01	0.19 ± 0.02	0.002 ± 0.001
Year 3 mean ± se	0.25 ± 0.01	0.44 ± 0.02	1.84 ± 0.03	0.18 ± 0.01	0.36 ± 0.02	0.001 ± 0.001
Significance	**	**	****	****	****	ns
Vp <sup>a</sup>	0.3%	0.6%	0.9%	1.6%	2.7%	0.1%
Pop-G						
Year						
Year 1 mean ± se	0.55 ± 0.01	0.69 ± 0.01	2.25 ± 0.03	0.11 ± 0.01	0.43 ± 0.03	0.042 ± 0.005
Year 2 mean ± se	0.40 ± 0.01	0.53 ± 0.02	1.95 ± 0.04	0.23 ± 0.01	0.76 ± 0.03	0.000 ± 0.000
Year 3 mean ± se	0.45 ± 0.01	0.63 ± 0.02	2.21 ± 0.04	0.29 ± 0.01	0.68 ± 0.02	0.001 ± 0.001
Significance	****	****	****	****	****	****
Vp <sup>a</sup>	0.9%	1.6%	0.9%	2.1%	5.3%	2.7%

\*, \*\*, \*\*\*, and \*\*\*\* indicate  $P < 0.05$ , 0.01, 0.001, and 0.0001, respectively

ns not significant, M mealliness, FB flesh browning, FBL flesh bleeding, FL flesh leatheriness

<sup>a</sup> Vp proportion of phenotypic variance, where the corresponding genotype Vp is the per-fruit heritability value given in Table 2

**Table 5** Pearson correlation coefficients among seedling means of chilling injury symptoms and other traits measured for one to three years (as listed in Table 5) in two peach progenies

Trait	M	M-FMF	FB	FBL	FBL-CNMF	FL
M			0.35**	−0.65***		−0.18
M-FMF			0.48***	−0.22		0.14
FB	0.43**	0.70***		−0.13	0.25	0.16
FBL	−0.63***	0.08	−0.19			0.28*
FBL-CNMF			0.15			0.18
FL	0.27	0.40*	0.35*	−0.14	−0.26	

Pop-DG is in the *bottom left diagonal* and Pop-G is in the *top right diagonal*

\*, \*\*, and \*\*\* significant at  $P < 0.05$ , 0.01, and 0.001, respectively

M mealiness, FB flesh browning, FBL flesh bleeding, FL flesh leatheriness, FMF freestone melting flesh, CNMF clingstone non-melting flesh

## Discussion

### Inheritance of qualitative fruit traits

The two loci *F-M* and *Y* segregated independently, consistent with their location on separate *Prunus* linkage groups (*F-M* at the distal end of linkage group G4, *Y* in the middle of G1: Dirlewanger et al. 2004; Ogundiwin et al. 2009). Freestone/clingstone and melting/non-melting flesh were traditionally thought to be controlled by two separate but linked loci (*F* for *Freestone* and *M* for *Melting flesh*) with epistatic interaction (Bailey and French 1949). However, an argument was later advanced for a single locus with three alleles (Monet 1989). More recently, molecular analysis of the *F-M* locus based on the same populations used here, where the two traits completely co-segregated, led to the conclusion that a single locus with two endopolygalacturonase (endoPG) genes is responsible for both traits (Peace et al. 2005). EndoPG is an enzyme that degrades cell wall pectin and is implicated in fruit softening, texture modification, and abscission in other species. It controls whether fruit are FMF, clingstone melting flesh (CMF), or CNMF (Peace et al. 2005). Both of our populations segregated into fruit types of FMF (F-allelic combinations) and CNMF. Although clingstone non-softening flesh (CNSF, *f2f2*) can be phenotypically and genotypically distinguished from CNMF (*f1f1* and *f1f2*) (Peace and Norelli 2009), for our purposes of evaluating chilling injury incidence, allelic combinations of *f1f1* ('Dr. Davis'), *f1f2* (CNMF Pop-G seedlings), and *f2f2* (CNSF Pop-G seedlings) were grouped as "CNMF". CMF was absent in these crosses because the required

*f* allele was not present in either parent. As described in detail with comparison to earlier literature, segregation in the two progeny populations described here confirmed that freestone melting flesh and white flesh color are dominant to clingstone non-melting flesh and yellow flesh color in peach (Williamson et al. 2006).

### Inheritance of CI symptoms

Mealiness (M), flesh browning (FB), and flesh bleeding (FBL) were the most pervasive symptoms of chilling injury in the two progeny populations evaluated, as also observed generally in cultivars used by the California and Chilean industries (Crisosto et al. 1999, 2008). Strong year effects are commonly observed in multi-year performance trials for peach (Etienne et al. 2002; Campos-Vargas et al. 2006; Crisosto 2002; Peace et al. 2005) and underscore the need to evaluate individual trees over multiple seasons to understand the relative effects of genotypic and phenotypic factors on CI. Although statistically significant, the effect of year was relatively minor compared to differences among seedlings for the major CI symptoms. For FB, the greater degree of incidence induced with three weeks storage instead of two weeks and the higher heritability obtained for this treatment (results not shown) indicate that genetic dissection of this trait in future studies may be better achieved with a long cold storage duration. However, M and FBL are readily induced after two weeks storage. With moderate to high heritability, all three traits were under significant genetic control, allowing for investigation of further controlling genetic factors.



**Table 6** Associations between flesh browning (FB) and other chilling injury (CI) symptoms for different fruit types in two progenies of peach, on an individual fruit basis

Mean FB score (No. fruit)			
Fruit subset	Both flesh colors	White-fleshed	Yellow-fleshed
<i>Pop-DG</i>			
All fruit	2.0 (5309)	2.0 (2769)	2.0 (2540)
FMF—all	2.1 (3003)	2.1 (1672)	2.2 (1331)
CNMF—all	1.8 (2306)	1.8 (1097)	1.9 (1209)
FMF			
Juicy	1.7 (1602)	1.6 (887)	1.7 (716)
Mealy	2.7 (1340)	2.7 (775)	2.7 (565)
Leathery	3.6 (61)	4.4 (10)	3.5 (51)
CNMF			
Juicy	1.8 (2305)	1.8 (1097)	1.9 (1208)
Leathery	4.0 (1)	– (0)	4.0 (1)
FMF			
Clear	2.2 (2964)	2.1 (1646)	2.2 (1318)
Bleeding	1.4 (39)	1.5 (26)	1.2 (13)
CNMF			
Clear	1.8 (1820)	1.7 (765)	1.9 (1055)
Bleeding	1.8 (486)	1.9 (332)	1.7 (154)
Mean FB score (No. fruit)			
Fruit subset	Both flesh colors	White	Yellow
<i>Pop-G</i>			
All fruit	2.3 (5029)	2.4 (3793)	2.0 (1236)
FMF—all	2.4 (3710)	2.5 (2775)	1.9 (935)
CNMF—all	2.0 (1319)	2.0 (1018)	2.3 (301)
FMF			
Juicy	1.8 (1328)	1.9 (1106)	1.3 (222)
Mealy	2.7 (2368)	3.0 (1660)	2.1 (708)
Leathery	2.6 (14)	2.6 (9)	2.6 (5)
CNMF			
Juicy	2.0 (1333)	2.0 (1032)	2.3 (301)
Leathery	3.1 (96)	2.2 (51)	4.5 (45)
FMF			
Clear	2.4 (3511)	2.6 (2608)	1.9 (903)
Bleeding	1.7 (199)	1.8 (167)	1.5 (32)
CNMF			
Clear	2.0 (500)	1.8 (329)	2.4 (171)
Bleeding	2.1 (819)	2.0 (689)	2.2 (130)

Unlike the major CI symptoms, flesh leatheriness (FL) occurred sporadically at low incidence and with low heritability, indicating that this trait is random rather than genetic. FL may have occurred in fruit that were harvested slightly immature before entering cold storage, the suggested physiological basis of FL

(Brovelli et al. 1998). The low incidence of FL may result from our care to harvest all fruit at full commercial maturity, even though this was complicated by genetic variation in ripening within populations and lack of industry standards specific for each seedling genotype.

**Table 7** Associations between flesh bleeding (FBL) and other CI symptoms for different fruit types in two progenies of peach, on an individual fruit basis

Mean % FBL (No. fruit)			
Fruit subset	Both flesh colors	White	Yellow
<i>Pop-DG</i>			
All fruit	10 (5263)	13 (2759)	7 (2504)
FMF—all	1 (3020)	2 (1674)	1 (1346)
CNMF—all	21 (2306)	30 (1097)	13 (1209)
FMF			
Juicy	2 (1616)	2 (887)	2 (729)
Mealy	1 (1341)	1 (775)	0 (566)
Leathery	0 (61)	0 (10)	0 (51)
CNMF			
Juicy	21 (2305)	30 (1097)	13 (1208)
Leathery	0 (1)	– (0)	0 (1)
Mean % FBL (No. fruit)			
Fruit subset	Both flesh colors	White	Yellow
<i>Pop-G</i>			
All fruit	20 (5029)	23 (3793)	13 (1236)
FMF—all	5 (3710)	6 (2775)	3 (935)
CNMF—all	62 (1333)	68 (1032)	43 (301)
FMF			
Juicy	8 (1328)	9 (1106)	5 (222)
Mealy	4 (2368)	4 (1660)	3 (708)
Leathery	0 (14)	0 (9)	0 (5)
CNMF			
Juicy	63 (1223)	68 (967)	43 (256)
Leathery	55 (96)	65 (51)	44 (45)

### Genetic factor influences on CI incidence

The *F-M* locus was identified as the largest single genetic factor conditioning M and FBL susceptibility. The frequency distributions of M in both populations were indicative of control by a major locus, where one segregating allele results in low or no incidence and the other allele allows expression of genes at other loci with segregating alleles conditioning susceptibility. The frequency distributions of FBL incidence in both populations and in comparison with the CNMF subsets were also indicative of control by the *F-M* locus.

Our results are consistent with abundant reports in the literature (reviewed in Lurie and Crisosto 2005) for a major role for endoPG in the development of M. Its incidence in Pop-DG and Pop-G indicates a qualitative genetic role when both FMF and CNMF cultivars/fruit are considered. A simple explanation is that a

functional endoPG gene (that controlling *Melting flesh* at the *F-M* locus) is a prerequisite for M development.

Clingstone non-melting flesh (CNMF) fruit lack a functional *Melting flesh* endoPG gene (Peace and Norelli 2009). This type of fruit remains firm and juicy following cold storage and ripening, although they become leathery if general ripening is disabled. The lack of M in CNMF fruit has been reported previously (Brovelli et al. 1998; Crisosto et al. 1999). Although it has not been explicitly measured in the context of the M defect, CNMF fruit are expected to have much less or negligible endoPG-related depolymerization than FMF fruit under the same storage conditions (Manganaris et al. 2006).

Beyond the prerequisite of the presence of a functional *Melting flesh* endoPG gene that confers the MF genotype, which is a defining feature of most

fresh market peaches, genetic susceptibility to M depends on the presence of alleles at other controlling loci. Occurrence of M in FMF progeny subsets was not normally distributed in either population, and heritability was moderate (0.46 and 0.49 on a per-tree basis), suggesting two to three further segregating loci with dominant action for high M and probably heterozygous in 'Georgia Belle'. Nevertheless, allelic differences at the *F-M* locus between the two populations for non-melting/non-softening may underlie the greater incidence of FBL in Pop-G (Ff2 × Ff2) than Pop-DG (f1f1 × Ff2) (Peace and Norelli 2009).

The reason for such a large *F-M* effect on FBL is not clear. Conceivably, flesh softening during the endoPG-controlled melting phase or abscission of vascular fibers from the stone in FMF fruit may retard the spread of red pigment (anthocyanin) through the flesh from the red vascular rays close to the stone, assuming that these rays are the source or conduit of the pigment. Greater FBL in CNMF fruit may be an indirect pleiotropic effect of the lack of endoPG activity. Elevated ethylene production seems to be a common feature of CNMF cultivars (Brovelli et al. 1999; Manganaris et al. 2006) and may be part of a biochemical cascade that influences the spread of pigment in CNMF fruit, but not FMF fruit. Alternatively, another polymorphic gene closely linked to the *F-M* locus may be part of the control mechanism for FBL; if so, it must lie very close to *F-M* locus since the observed QTL peak coincides with this locus (Ogundiwin et al. 2007).

FBL susceptibility in CNMF peaches is suspected to be controlled by just a few segregating, dominant genes, based on its moderate heritability, bimodal distribution, and skewing toward low (Pop-DG) or high (Pop-G) FBL incidence. 'Dr. Davis' appears to have alleles for very low FBL susceptibility, while low incidence alleles in 'Georgia Belle' are not as effective in reducing or preventing FBL or are masked by other loci.

While FB was influenced by the *F-M* locus, with severity significantly higher in FMF progeny than CNMF progeny (Table 3), juicy CNMF fruit actually showed more FB than juicy FMF fruit (Table 6). The observed effect of the *F-M* locus on FB was therefore probably indirect, through the frequent occurrence of FB in mealy FMF fruit.

In both populations, white fruit exhibited a greater tendency for FBL regardless of whether fruit were FMF, CNMF, juicy, mealy, or leathery (Table 5).

These results could be explained by the reported one minor QTL on linkage group 1 (G1) very close to the *Y* locus (Ogundiwin et al. 2007), aided by the ease of observing red pigmentation in the mesocarp of white flesh. More studies are needed to clarify the role of the *Y* locus on cold storage FBL.

#### Relationships among CI symptoms

Progeny susceptibility for each chilling injury symptom and incidence in individual fruits tended to be independent of other CI symptoms, except for M and FB. FMF seedlings susceptible to M also tended to be susceptible to FB more often than in all seedlings ( $R = 0.70$  in Pop-DG and 0.48 in Pop-G, Table 5) and mealy fruit in general tended to have more FB (Table 6). FB is associated with mealy fruit in many cultivars (Crisosto et al. 1999, 2008). Although the physiological basis for this connection is unclear, it is suspected to be related to tissue senescence and decreased membrane permeability (Lurie and Crisosto 2005).

The main association between M and FBL was created by the *F-M* locus effects on these traits. The large negative correlation between M and FBL in both populations for all seedlings was due to a lack of M, but high incidence of FBL, in CNMF fruit. Susceptibility to M in FMF seedlings was not associated with susceptibility to FBL (Table 5). However, juicy FMF fruit were twice as likely to exhibit FBL as mealy fruit (Table 7). Cell wall changes that bind free water in mealy fruit may similarly restrict the spread of water-soluble anthocyanin through the flesh. Fruit can also exhibit FBL at harvest (Lurie and Crisosto 2005), which has been observed in our two progeny populations (Williamson et al. 2006), so the FBL observed in mealy fruit may have occurred prior to cold storage.

Our observations suggest that M and FL are different and unrelated phenomena. Susceptibility of seedlings to M and its incidence in individual fruit were not consistently linked to FL. Therefore, while M and FL are both characterized by a lack of juice, FL is probably under different genetic control. Indeed, detailed physiological studies concluded that these two disorders have a different physiological basis (Ju et al. 2000). Our results are consistent with the view that M is the result of altered enzyme activity and cell wall metabolism following the disruption of normal ripening by extended cold storage, while FL is caused

by a complete suppression of normal ripening in immature fruit.

While our data suggest relationships between FL and FB or FBL, the low frequency of leathery fruit in the entire data set may have caused spurious associations. FB and FL susceptibilities were associated only in Pop-DG ( $R = 0.35$ ) (Table 5). However, leathery fruit in both populations had a high incidence of FB (Table 6). The physiological association between these two traits is not clear. Unlike FB and M, where tissue senescence may be the common link, physical membrane disruption caused by chilling may explain the association between FB and FL.

#### Breeding strategies for CI resistance

The considerable genetic variation in chilling injury susceptibility and its moderate to high heritability underscore the genetic basis of these traits. This provides an opportunity to develop new cultivars for both the fresh and canning industries that are free of chilling injury symptoms. Unfortunately for the peach industry, the effect of the *F-M* locus on M and FBL—the association of M only with FMF cultivars and FBL almost entirely within CNMF cultivars—is in the direction that limits supply of high quality fruit. Mealiness (M) in CNMF fruit used for canning is not a problem, as fruit are quickly processed after harvest and do not require long cold storage, and FBL in fresh market fruit is acceptable to consumers. Fresh market CNMF fruit is one way to avoid M (Brovelli et al. 1998), but consumers tend to prefer the melting texture (Brovelli et al. 1999). However, there is hope for creating new cultivars with genetic resistance to M in FMF fruit or FBL in CNMF fruit.

Although non-melting peaches are more susceptible to FBL, canning peach breeders routinely breed for FBL resistance to avoid FB after canning. From the present study, this breeding target appears readily achievable using the common strategy of crossing among non-melting cultivars; however, introducing MF parents may introduce higher susceptibility at other loci. Additionally, the use of parents that have considerable heterozygosity such as ‘Georgia Belle’ can result in a transgressive segregation through recombination between parents with antagonistic phenotypes (QTLs) (Rieseberg et al. 2003). A combination of superior parental alleles (complementary gene action), resulting in a phenotype exceeding the

parental value, was observed in apple (Liebhard et al. 2003).

The cling peach breeding program of Dr. Gradziel at UC Davis is pursuing the improvement of peach traits by using wide crosses with almond (*Prunus dulcis*) and other *Prunus* accessions closely related to peach. After several backcrosses, the recurrent improved cultivar will fix the desired traits and undesirable wild traits will be removed. In addition, our group is using high throughput sequencing and associated genomes analysis methods to detect single nucleotide polymorphisms (SNPs) within the parents of Pop-DG. The resulting selected and validated SNPs were used to genotype all progeny; this effort will resolve quantitative trait loci (QTLs) for chilling injury symptoms and detect additional QTLs associated with those symptoms. This information, added to the translational genomics, will make the selection process faster and more effective in cost per trait fixed, because at least three years is required per generation. Our populations contained FMF progeny with low incidence of mealiness, flesh browning, and flesh bleeding, and CNMF progeny with low incidence of flesh bleeding, low flesh browning, and zero mealiness. Thus, these traits can be mutually improved with a correct breeding strategy.

#### Conclusions

This study represents the most complete and comprehensive genetic analysis to date of chilling injury symptoms in two related peach progeny populations and contributes to our understanding of the genetic control of CI symptoms in peach. Strong genetic influences were detected for mealiness, flesh browning, and flesh bleeding, making breeding for resistance or low susceptibility to chilling injury an achievable goal. The expected significant year-to-year variation in trait performance was confirmed, but was less important than the genetic factors. Our new, integrated, and saturated SNP linkage map and high density QTL discovery will permit development of new markers to aid selection of new cultivars with low susceptibility or resistance to CI.

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