



This is the **accepted version** of the article:

Cantin, Celia M.; Wang, Xin-Wei; Almira, María; [et al.]. «Inheritance and QTL analysis of chilling and heat requirements for flowering in an interspecific almond x peach (Texas x Earlygold) F2 population». Euphytica, Vol. 216, issue 3 (March 2020), art. 51. DOI 10.1007/s10681-020-02588-9

This version is avaible at https://ddd.uab.cat/record/230980 under the terms of the $\textcircled{C}\space{0.1}{$



This is a post-peer-review, pre-copyedit version of an article published in Euphytica.Thefinalauthenticatedversionisavailableonlineat:https://doi.org/10.1007/s10681-020-02588-9

Document downloaded from:



1	Inheritance and QTL analysis of chilling and heat requirements for flowering in an
2	interspecific almond x peach (Texas x Earlygold) F2 population
3	Celia M. Cantin ^{1,2,3} , Xin-Wei Wang ⁴ , María Almira ⁵ , Pere Arús ^{1,6} , Iban Eduardo ^{1,6} *
4	
5	¹ Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Barcelona, Spain
6	² Aragon Agency for Research and Development (ARAID), E-50018 Zaragoza, Spain
7	³ Agrifood Research and Technology Centre of Aragón (CITA)-Agrifood Institute of
8	Aragon (IA2). Avda. Montañana 930. 50059, Zaragoza, Spain.
9	⁴ Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences,
10	Zhengzhou 450009, P.R.China
11	⁵ Universidad Autonoma de Barcelona, Campus UAB, Cerdanyola del Vallès (Bellaterra),
12	08193 Barcelona, Spain
13	⁶ Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Campus
14	UAB, Bellaterra, Barcelona, Spain
15	* corresponding author: <u>iban.eduardo@irta.cat</u> Tel. +34 935 636 600
16	
17	Abstract
18	Blooming in temperate fruit species is triggered by chilling and heat requirements (CR and
19	HR), with a wide range of requirements within the same species. CR for flower bud dormancy
20	release has become a limiting factor for geographical adaptation of fruit trees in warmer
21	regions. The present study investigated the genetic basis of CR and HR to break dormancy and
22	flowering time (FT) in an almond x peach F ₂ progeny. FT, HR and CR were evaluated over two
23	consecutive years (2015/2016 and 2016/2017). Seven out of the eight identified quantitative

trait loci (QTLs) were found in both periods of analysis. They affected eight traits, and included

25	a consistent QTL for breaking dormancy, CR and HR. Two of them, affecting FT and HR for
26	FT (GDHF), colocalized in G1, and the remaining QTLs, affecting chilling and heat
27	requirements, both influenced by dormancy breaking (DB), were located in G6. These results
28	indicate that factors not related to DB affect flowering time in this population. Implications of
29	the results in peach breeding are discussed.
30	
31	Keywords: chilling requirements, heat requirements, peach, flowering.
32	
33	Introduction
34	Flowering, an essential and complex developmental process in plants (Castède et al., 2015)
35	, is regulated by a number of external signals and internal elements (Hanke et al., 2007). Its
36	correct completion is fundamental for the commercial production of seeds and fruits (Zhang
37	and Taylor, 2011).
38	In fruit tree orchards, there must be synchronization between flowering phenology and
39	climatic conditions (Castède et al., 2015). As flowering is crucial for sexual reproduction, buds
40	of perennial species in temperate regions become dormant (cease growth) during the winter
41	months to survive. Endodormancy requires a certain amount of chilling for the transition to
42	ecodormancy, whereas ecodormancy, requires a certain amount of heat to start the flowering
43	process (Castède et al., 2015). Consequently, dormancy and flowering are linked, and breeders
44	must select cultivars whose CR and flowering time match local climatic conditions (Bielenberg
45	et al., 2015). However, it has been demonstrated that global warming can advance or delay
46	flowering and/or fruiting of temperate fruit trees (Heide, 1993; Ramirez and Kallarackal, 2015;
47	Rivero et al., 2016; Woznicki, et al. 2019), having an unknown and undesired effect on the
48	productivity of fruit crop species. Globally, the temperature has increased by approximately

0.6°C over the past 100 years (<u>Walther, 2002</u>). The forecast is for this trend to continue, with
studies already having observed the decrease in winter chill and the resulting changes in
phenological events (<u>Menzel et al., 2005; Menzel et al., 2006</u>).

The *Prunus* genus, within the Rosaceae family, is characterized by species that grow in 52 areas with well-marked seasons and are adapted to survive cold winters and dry summers 53 (Dirlewanger et al., 2012). Various models have been proposed to measure the accumulation of 54 CR in deciduous fruit-growing areas (Alburguerque et al., 2008). The effect and genetic basis 55 of HR on flowering is not vet well understood and studies on this topic are scarce. Previous 56 studies in Prunus have suggested that CR has a stronger effect on flowering time than HR 57 (Couvillon and Erez, 1985; Campoy et al., 2012; Alburquerque et al., 2008; Okie and 58 Blackburn, 2011; Sanchez-Perez et al., 2012). In this genus, as in most woody perennials, the 59 physiology and biochemistry of the flowering process is poorly understood (Dirlewanger et al., 60 2012; Woznicki et al., 2019). Recent reports suggest that intrinsic and environmental signaling 61 interact and dynamically affect the extent of bud dormancy (Castède et al., 2015; Woznicki et 62 al., 2019). Peach (Prunus persica (L.) Batsch) is an economically important species that 63 provides an excellent system for the genetic analysis of CR and FT due to the ample variation 64 for both traits among peach cultivars (CR between 50 to 1,050 h) (Zhebentyayeva et al., 2014). 65 66 Both CR and FT are inherited as quantitative traits (Fan et al., 2010; Hauagge and Cummins, 1991), but their molecular regulation is not yet fully understood. Some quantitative trait loci 67 (QTLs) associated with FT have been found in Prunus species (reviewed in Salazar et al., 68 2014): almond (Sanchez-Perez et al., 2012; Silva et al., 2005); peach (Dirlewanger et al., 2012; 69 Bielenberg et al., 2015); almond x peach (Donoso et al., 2015); apricot (Campoy et al., 2013; 70 Dirlewanger et al., 2012; Kitamura et al., 2018); sweet cherry (Dirlewanger et al., 2012; Calle 71 et al., 2020) and sour cherry (Wang et al., 2000). The separate effect of CR and HR on FT has 72

only been reported in a few studies in peach (Fan et al., 2010), apricot (Olukolu et al., 2009)
and cherry (Castède et al. 2014). Since these traits can only be evaluated 2-3 years after seed
germination in most *Prunus*, the identification of genetic markers linked with CR, HR and FT
would be a valuable tool to select genotypes at the seedling stage and make the breeding
process much more efficient (Bielenberg et al., 2015).

In this work, we studied an F₂ c n o q p f " z " r g c e j " r t q i g p { " * V z G. " \div G c t n { iveloped at IRTAf agd used as the *Prunus* reference map (Dirlewanger et al., 2004), for which a high-density linkage map was available (Donoso et al. 2015). The main goals were to study the inheritance of CR, HR and FT, and to test the existing chill unit (CU) models in Gimenells, Lleida (Spain) (latitude 0°23'E /longitude 41°39'N), with a temperate semi-arid climate, to identify which of them fits best with the climatic conditions in one of the major areas of peach and almond production in the world.

85

86 Materials and methods

87 *Plant material*

The *Prunus* reference interspecific almond x peach F₂ progeny (T x E), obtained by selfing c " j { d t k f " k p f k x k f w c n " * ÷ O D " 3 0 5 9 Ø + " h t q o " c " e t q u u (Donoso et al. 2015), and its parents was used for this study (Tables S1 and S2). From the original progeny of 111 hybrids, we phenotyped the 72 trees that were still alive. Trees of T x E are at the IRTA Experimental Station of Lleida in Gimenells (Spain) grafted on ÷ I c t p g o Ø " (Felipe 2009) rootstocks. Standard agricultural practices were applied.

94

95 Phenotyping

The parents, hybrid and TxE offspring were evaluated over two seasons (2015/2016 and 96 97 2016/2017, that we refer to as 2016 and 2017, respectively) following a forcing protocol widely used in temperate fruit trees (Campoy et al. 2011). Traits phenotyped were chilling 98 requirement (CR), flowering time (FT), and heat requirement (HR). Three one-year-old 99 100 fruiting branches for each individual were randomly collected once a week from November 101 1st until chilling requirements were reached. At least 30 flower buds were collected from 102 the three branches for each sampling date. The bases of the branches were placed in water 103 in a growth chamber at 25°C, under white fluorescent tubes with a 16 h:8 h, light:dark photoperiod to force floral bud break (Ruiz et al., 2007; Sánchez-Pérez et al., 2012). After 7 104 105 d, the phenological stage of the flower buds was observed. The date of dormancy breaking 106 (DB) was established when 50% of flower buds were at phenological growth stage 53 according to the international Biologische Bundesanstalt, Bundessortenamt et CHemische 107 108 Industrie (BBCH) scale (Meier et al., 1994; Alburguerque et al., 2008). Three chill models were then used to calculate chilling accumulation from October 1st until dormancy release, 109 corresponding to the CR. The flowering time (FT) was scored as the number of Julian days 110 111 when 50% of flowers were open. Also, the length of the period between DB and FT was calculated as the numbg t " q h " f c { u " d g v JD, ging reprient lof Julian" Days). For v u " * 112 each genotype, the whole tree was observed by the same person every 1 or 2 days during 113 the flowering period. A scheme of the traits evaluated can be found in Figure 1. 114

115

116 Weather data

Hourly temperatures from October 1st to flowering time for both years were obtained from
the Gimenells weather station of the Generalitat de Catalunya, in the same area as the studied
population (https://ruralcat.gencat.cat).

121 Chilling and forcing models

For this study, we used the Chill Hours (CH), Utah (CU) and Dynamic (CP) Models. The 122 Chill Hours Model (CH) (Weinberger, 1950) is the oldest and simplest model, which considers 123 all hours with temperatures between 0 and 7, C as effective for chill accumulation. The Utah 124 125 Model (Richardson et al., 1974), which measures chill in Chill Units (CU), contains a weighted function attributing chilling efficiencies to different temperature ranges, including negative 126 contributions by high temperatures, and it is particularly used in cooler areas of temperate 127 zones (Dennis et al., 2003). The Dynamic Model (Erez and Couvillon, 1987) was developed 128 for warmer areas. It considers that dormancy cessation occurs in two steps, the first being 129 130 reversible and the second irreversible, and CR are calculated as chill portions (CP). It adopts a 131 process-based concept of chill accumulation: an intermediate chill product is first formed through bud exposure to low temperatures, and once a critical amount of this intermediate has 132 133 accumulated, it is transformed into a Chill Portion (CP). The CP is then retained until the end of the chilling period (Erez and Fishman, 1998). As with the Utah model, temperatures have 134 135 different effects on dormancy, but the temperature ranges differ in the two models 136 (Alburquerque et al., 2008; Byrne, 2003).

To describe heat accumulation during the later stages of tree dormancy, we used the model proposed by Richardson et al. (1974), which calculates Growing Degree Hours (GDH) between dormancy release and flowering date. According to this model, heat builds up when hourly temperatures are between 4.5°C and 36°C (at different rates depending on the maximum temperature), with maximum accumulation at an optimal temperature (25°C). Additionally, we also calculated GDH between 1st October and flowering date (GDHF).

144 Chilling and heat requirements

Chilling and heat accumulation were calculated for the two consecutive dormancy seasons 145 (2016 and 2017) with the hourly temperatures measured in the field. Chill was computed 146 147 according to the Chill Hours (CH), Utah (CU) and Dynamic (CP) models and heat according to the GDH Model. Correlations between chilling and heating requirements and blooming were 148 determined using Partial Least Squares (PLS) regression (Luedeling and Gassner, 2012). Since 149 heat cannot have an effect after bloom, the flowering time was considered as the end of the 150 forcing period. CR and HR were estimated as the sum of all daily chill and heat accumulated 151 152 during the chilling and forcing periods. Heat accumulation was calculated as the number of GDH from DB to FT, and the length of this period of heat ae e wo wn c v k q p " y c u " e c n e 153 and used as a complement to GDH. Annual heat accumulation up to the date of flowering was 154 155 also recorded (GDHF).

156

157 *QTL analysis*

For QTL analysis, we used the TxE genetic map described by Donoso et al. (2015), which 158 was constructed using 1,948 molecular markers (SNPs and SSRs), covering a total genetic 159 160 distance of 472.1 cM. The interval mapping method with the MapQTL 6.0 software package (Van Ooijen et al. 2009) was used for QTL analysis of the phenotyped traits. QTLs were 161 considered consistent when the LOD \times 3.0 in both seasons, or with a LOD \times 3.0 one year and 162 $LOD \times 2.0$ the other year. QTLs were considered as major QTLs when they explained more 163 than 20% of phenotypic variation in both years of study (Tanksley, 1993). QTL positions were 164 drawn using the MapChart 2.1 software (Voorrips 2002). 165

Gene action was estimated following the guidelines of Tanksley (1993) with the ratio, d/a, 166 167 between the additive, where a = (A - B)/2, and dominance d = H - [(A + B)/2] effects, with H, A and B the average phenotypic values of the heterozygous, almond homozygous and peach 168 homozygous genotypes, respectively. Based on the d/a ratio, OTLs were classified as 169 170 underdominant (d/a Ö 1.25; U), dominant for the peach allele (-1.25 Ö d/a Ö -0.75; DP), partially dominant for the peach allele (-0.75 $\ddot{O} d/a \ddot{O}$ -0.25; PD), additive (-0.25 $\ddot{O} d/a \ddot{O}$ 0.25; 171 A), partially dominant for the almond allele (0.25 $\ddot{O} d/a \ddot{O} 0.75$; AD), dominant for the almond 172 173 allele (0.75 $\ddot{O}d/a$ $\ddot{O}1.25$; DA) and overdominant (d/a > 1.25; O).

174

175 **Results**

176 *Temperature and chilling accumulation*

Maximum and minimum daily temperatures during the consecutive years studied in Gimenells (Lleida) are shown in Supplementary Fig. S1. Higher maximum and minimum temperatures were registered during the winter in 2016, meaning a warmer winter compared to the following year. However, warmer maximum temperatures were registered at the end of the winter in 2017, reaching 25°C at the beginning of March.

182 Chilling accumulation was very similar over the winter in both seasons (Fig. 3) using the 183 CU model and the CP model, but the accumulated CH was higher in 2017. Spearman 184 correlations between models were very high for both seasons (Fig. 4).

185

186 *Chill requirements to break dormancy*

187 The range of CR for dormancy breaking (DB) for the parental lines [\div OD " 3(Θ I)5 9 ø

188 \div V g z(T) and \div G c t n (E)] syars between 42 and 64 CP. Of these, \div Ec t n { had the f ø

lowest CR (42 and 45 CP for consecutive years), whereas the requirements for H (60 and 59

190 CP) and \exists Tg z c(54 and 64 CP) were similar. There was transgressive segregation for CR, with 191 values from 33 to 71 CP (579 - 1434 CU; 484 - 1327 CH) averaged for two years (Table 1). CR 192 data for the F₂ population showed normal distributions in the two years (Fig. 4), with CR 193 skewed in both yearsto high CR. Data for each individual is given in supplementary material 194 (Table S1 and S2).

In most genotypes, CR were similar for both years evaluated, showing high correlations between them (Table 1). The date of DB was, in general, earlier in 2016 than in 2017, with the earliest on 16th Dec and 14th Dec, whereas the latest was on 4th Feb and 2nd Feb (2016 and 2017, respectively).

199

200 Heat requirements for flowering

The range of HR for flowering among the parents was 3,575 to 6,170 GDH, whereas in the 201 segregating population it was 2,681 to 7,105 GDH (averaged for the two years) (Figure 4). ÷ O D " 202 3 0 5 Hadøthe lowest HR of the parents, and was similar to $\pm Tg z c whereas \pm Ec t n \{ had n f ø$ 203 the highest. Within the segregating population, most genotypes had similar HR for both 204 consecutive years, showing a high correlation ($r^2 = 0.78$) between years (Table 1). Date of 205 flowering (FT) was, in general, earlier in 2016 than in 2017, with the exception of only three 206 genotypes. The parental lines (÷ O D 3 0 5 9 ø . " ÷ V g z c showed everyfsithilar GFT in n { i q n : 207 both years. Correlation between years for FT was lower than for other traits ($r^2 = 0.39$) (Table 208 1). The earliest FT was on 21st Feb and 3rd March, and the latest 15th and 12th March (2016 and 209 2017, respectively). The number of days between DB to FT in the segregating population 210 (JD), which is the period for heat accumulation, ranged from 27 to 77 days (averaged for the 211 two years), and there was a high correlation between both seasons ($r^2 = 0.86$). HR showed a 212

bimodal distribution in 2017, whereas it was a single peak in 2016 (Fig. 4). There were single
peaks for FT in both years, although it was slightly skewed to late bloom in 2017.

215

216 *Correlations between traits*

Highly u k i p k h k e c p v " e q t t g n c v k q p u " * T " xhřee2n0odel8 . " r " Ö used to estimate chilling accumulation for both seasons (Supplementary Table S1). Similarly, correlations between DB and these three models were very high * T " \times " 2 0 ; ; ... " r " Ö Correlations between seasons were very high for most of the traits (r² = 0.78-0.89), with lower values for FT and GDHF (r² = 0.39 and r² = 0.47, respectively) (Table 1). Flowering time (FT) showed a high correlation with GDHF. Correlation between FT and CR and HR traits was low for both years of study.

Heat requirements (GDH) were highly and negatively correlated with CR and DB (Table 1 and Fig. 5), which means that the lower the CR, the higher the HR. As expected, HR was highly correlated with the number of days bg v y g g p " F D " c p f " H V " * L F + . " c p f of days for heat accumulation was highly and negatively correlated with CR and DB.

228

229 QTL analysis

Data from the eight traits under study (DB, CH, CP, CU, FT, GDH, GDHF, L)Fwere used
for QTL analysis. One consistent QTL per trait was identified (Table 2). QTLs were located in

G1 for FT and GDHF, and in G6 for DB, the CR models (CH, CU and CP), GDH and L F(Fig. S2).

For dormancy breaking (DB), the LOD for the QTL from G6 was 3.4 in 2016 and 3.4 in 2017 and explained 18.4 and 18.1 % of the phenotypic variance, respectively. The homozygote for the peach allele increased the date of DB by 15 days compared to the almond homozygote in 2016 and by almost two days in 2017. In 2017, while both homozygous classes had similar values, there was an increase of 13 days for the heterozygous individuals. Very similar results were obtained for L F = c n v jLQDwfor jhe'QTI j ig G6 was less than 2.0 (1.8) in 2017 and therefore was not considered.

241 For CR data similar results were obtained with the three models, although the most significant QTLs were obtained using the dynamic model, while the least was for the Utah 242 model. For the dynamic model, a QTL was identified at the proximal end of G6 with a LOD 243 244 score of 3.4 in 2016 and 3.2 in 2017, explaining, respectively, 18.4% and 17.4 % of the phenotypic variance. The confidence interval for G6 QTLs spans the genomic region Pp06: 0-245 246 4.001.078 where 702 genes have been annotated. In 2016, the individuals with the homozygous peach allele needed 10 CP more to reach DB than those with the homozygous almond allele. In 247 2017, this difference was only four CP, but 10 with heterozygous individuals. 248

In the same region of G6 we c n u q "kfgpvkhkgf" c "S ViN 20h6)qt "I F J 249 explaining between 16.8% and 19.9% of the phenotypic variance respectively. For FT we 250 identified a OTL at the beginning of G1 in 2017 (LOD 3.9; $R^2 = 21\%$), but with a LOD score of 251 2.2 in 2016 ($R^2 = 11.5\%$). The confidence interval spans the genomic region Pp01:0-252 10.521.046 bp, where 1584 have been annotated. The peach allele in homozygosis increased 253 the FT by five days in 2016 and three in 2017, compared to the homozygous almond allele. In 254 the same region, we also detected a QTL for GDHF in both years, with LOD values of 3.9 and 255 3.1 respectively. 256

257

258 **Discussion**

259 Phenotypic data of chilling and heat requirements

260 It is usually assumed that almond flowers before peach. It is interesting to note that in our 261 case, \div V g z x late of lowering almond cultivar and \div G c t n, {an early filowering peach, flower at the same time even though they have different CR and HR. The CR of the progeny 262 are in the range of those reported for peach and almond from other Mediterranean areas 263 264 (Benmoussa et al., 2017; Campoy et al., 2012; Ruiz et al., 2007). However, it must be noted that TxE is an interspecific population, and therefore results are not fully comparable to single 265 species populations studies. The range observed in the TxE population exemplifies the 266 difficulties for growing certain peach and almond cultivars in warm regions where annual chill 267 accumulation is decreasing due to global warming. A large variation in the HR within the 268 269 studied progeny was also found.

The performance of chill accumulation models vary in different climate conditions, as observed in peach (<u>Balandier et al., 1993; Erez et al., 1990; Erez et al., 2000; Perez et al., 2008</u>) and other *Prunus* species (<u>Alburquerque et al., 2003; Alburquerque et al., 2008</u>; Egea et al., 2003; Ruiz et al., 2007).

In Lleida (Spain) the CR calculated using the three models were very well correlated and 274 with DB, and therefore could be used for the calculation of CR in this climatic area, as for 275 peach (Fan et al., 2010) and apricot (Campoy et al., 2012) in colder climates. This correlation 276 could be due to the lack of long periods of warm and fluctuating temperatures, so that chilling 277 accumulation based on different models all steadily increased in a similar way through the two 278 seasons. Substantial differences among the many models used have been observed in moderate 279 mild climates (Erez et al., 1990; Erez et al., 2000) since some of them, such as the Utah model, 280 281 were developed in a cold area and are not appropriate for warmer areas.

The variability of chill accumulation between years was lower with the Dynamic model than when the calculations were done with the Utah and the hours-below 7°C models. This may be explained by the homogenizing effect of the Dynamic model, which takes into account the
synergistic effect between moderate and low temperatures for breaking dormancy (Fishman et
<u>al., 1987</u>). Other authors have previously reported similar results in apricot (Campoy et al.,
<u>2012</u>; <u>Ruiz et al., 2007</u>), suggesting that the Dynamic model is optimal for the climatic
conditions of Lleida and other areas of the Ebro Valley in Northern Spain.

289

290 *Correlations among endodormancy and ecodormancy traits*

291 Vjg" jkij" pgicvkxg" eqt tangel 6GDHvvks. 6CRs (CH, 1CP; anvch CTU) dgvyg indicate that genotypes with lower CR required a longer period of heat accumulation to bloom. 292 This has been also found in peach (Li et al., 2016) and apricot (Campoy et al., 2012). Li et al. 293 294 (2016) reported a decrease of 16 days per 200 accumulated CHs, up to a threshold of approx. 950 CHs. Overall, these results suggest that in cultivars with low CRs, GDH accumulation just 295 296 after CR fulfillment is less effective than in cultivars with higher CRs, and therefore they need more time to accomplish their HR. 297 A similar result was found for the correlation between DB and CR (CH, CU, and CP) 298 299 against HR (GDH). Similar high correlations have been found in peach (Fan et al., 2010; Li et al., 2016; Pawasut et al., 2004; Scorza and Okie, 1990) and apricot (Ruiz et al., 2007). We 300 found a high level of variability among the progeny regarding HR, which disagrees with 301 Linsley-Noakes and Allan (1994) who reported no differences in HRs between three nectarine 302 cultivars with different CR. These results suggest the existence of different heat requirements 303 304 among genotypes and a major genetic contribution in the control of this trait, or that this trait is 305 not being measured accurately because the physiological base is not yet well understood. The first hypothesis is in line with the model for Douglas fir (Harrington et al., 2010), which 306

307 proposes a variable threshold for the efficiency of chill and heat temperatures. However, other

authors have reported contradictory results (<u>Couvillon and Erez, 1985</u>; <u>Guerriero et al., 2006</u>;
<u>Kotowski et al., 1980</u>), which may be due to the different climate of the cited studies. There is
no consensus in the literature about whether there is a clear relationship between CR and HR.

No significant correlation was found between HR (GDH) and FT, in agreement with other 311 312 authors in peach (Fan et al., 2010) and apricot (Campoy et al., 2012). This result indicates that GDH is not as important as CR for determining flowering time. However, a high correlation 313 was found between FT and GDHF which could indicate the importance of warm temperature 314 before dormancy breaking on the flower bud formation and development, as reported 315 previously for plum (Woznicki et al., 2019) The fact that $\pm c t n \{ is a how \ GR \ cultivar \}$ 316 317 might also explain this result. Also Li et al. (2016) found that both the days to full bloom date and HR were negatively correlated with CH, which may indicate that less accumulated CHs 318 could lengthen the days to full bloom date and increase the heat requirement. Together, the 319 320 results indicate that CR is a major factor determining flowering time, although not the only one. Indeed, it is unclear in the literature whether heat accumulation for floral or vegetative bud 321 break starts before or after the release of endodormancy. Recent reports have shown a positive 322 correlation between August-September temperature and the amount and time of flowering in 323 the following spring on plum (Døving 2009; Woznicki et al., 2019) and sweet cherry (Døving 324 325 et al., 2011). We also found a low correlation between FT and CR. This is contrary to what has been shown by other authors in peach and almond populations (Castède et al., 2014; Sánchez-326 Pérez et al., 2012; Fan et al., 2010). However, there is no previous literature on CR for 327 328 interspecific populations. We believe that this distortion to the expected results is due to the existence of two different species in the progeny. Indeed, in the Figure 3 can be observed that 329 vjg" cnoqpf" ÷ Vgzcuø" cpf" vjg" rgcej" ÷ GE hasn { i qnf 330 significant lower CR than T. 331

333 *Genetic control of endodormancy and ecodormancy traits*

We have identified one genomic region controlling endodormancy and ecodormancy traits 334 (CR and GDH) located in G6 and another one controlling FT in G1, that have not been 335 336 previously identified in other *Prunus* populations. The interspecific nature of the TxE population might explain some of the differences observed with previous data obtained in 337 single species mapping populations. Data for GDHF, a trait highly correlated to FT, also 338 detected a OTL in G1 co-locating with that of FT, indicating either that GDHF is not related to 339 HR but a different way of measuring FT, or that warm temperatures during the winter 340 dormancy period, and not only after the fulfillment of the CR, are important for determining 341 342 FT. The latter hypothesis would support the high negative correlation, observed in this and other studies, between CR and HR (Fan et al., 2010; Pawasut et al., 2004; Ruiz et al., 2007; 343 344 Scorza and Okie, 1990), since the longer the period for chill accumulation, the higher amount of GDHF likely to be accumulated. 345

Consistent QTLs across years for CR, HR and FT have been described in various Prunus 346 progenies. In peach, Fan et al. (2010) identified QTLs for CR, HR and FT at the latter end of 347 G1, where the every gene (Evg) maps (Bielenberg et al. 2008), and for CR and FT in G4 348 and G7, where QTLs for FT have been found in various Prunus crops (Dirlewanger et al. 349 2012).In similar positions of G1 and G4, QTLs for CR and/or FT have been detected by 350 Bielenberg et al. (2015) in peach, by Sánchez-Pérez et al. (2012) in almond and by Quilot et al. 351 352 (2004) in an advanced backcross between P. persica cultivars and P. Davidiana. For sweet cherry, a consistent QTL in G4 for CR and FT has also been detected by Castède et al. (2014). 353 In all cases, the QTLs for CR coincided in map position with those of FT and produced effects 354 of similar magnitude and gene action, suggesting CR as a major cause for the FT phenotype. 355

Where HR was studied (Fan et al. 2010, Castède et al. 2014, Sánchez Pérez et al 2012), the 356 357 QTLs were detected at the same positions as those of CR and FT, or were not consistent over the two years and had effects generally opposite to those of the QTLs detected for CR. This 358 suggests that HR is a minor or irrelevant factor in the determination of FT, that its measurement 359 360 as GDH is inefficient, or both. Our results support these observations as we found that HR and CR detected the same QTL in both years studied. On the other hand, we did not find common 361 QTLs for CR and FT, indicating that FT was mainly determined by factors other than those we 362 measured. A possible explanation for this is that the TxE offspring, from the cross between two 363 cultivars from different species with low CR, had a lower level of variation for CR than other 364 mapping populations studied. In the cold-winter conditions of Lleida, chilling requirements 365 366 could have been rapidly met, resulting in a narrower distribution of variability that the parameters used measured with low efficiency. In agreement with our results, a QTL for FT 367 was previously found in G1 in the TxE progeny in 2012 and 2013 (Donoso et al. 2016). Here, 368 we also identified a peak with LOD of 2.9 in 2016 for FT in G6 but it was not considered as an 369 stable QTL as it had a LOD<2 in 2017 (results not shown). This does not discount that the CR 370 may be involved in FT variability, with apparently minor effects, although the population size 371 used could have been insufficient to detect them with a significant threshold. 372

373

374 Conclusions

All the models for the estimation of CR (Utah, Dynamic and Hours-below 7°) worked well for the area of study, characterized by short but cold winters, with warm falls and springs. However, the Dynamic model seems the most appropriate as it reduced the year-to-year variation observed in the population. The results indicate that, although CR appears to have a more important role than HR in determining flowering time, neither factor had a major effect on this trait under the conditions of this research. For HR, the warm temperatures during
endodormacy (not only after endodormancy release) may have also influenced flowering time.
In summary, our data supports FT as a quantitatively inherited character with a strong genotype
x environment component that is affected by both chilling and heat requirements. The observed
variation in the CRs within the population studied highlights the importance and feasibility of
breeding for low CRs in a new scenario of low chill accumulation due to global warming.

387 Acknowledgements

This project was supported in part by funding from the Spanish Ministry of Economy and Competitiveness (MINECO/FEDER projects AGL2015-68329-R and RTA2015-00050-00-00, Severo Ochoa Program for Centres of Excellence in R&D 201-2019 SEV-2015-0533) and from the CERCA Programme-Generalitat de Catalunya.

393 Tables

Table 1. Pearson's correlation coefficients for seasons 2016 and 2017 between dormancy break (DB), flowering time (FT), chilling requirements for DB [Chill Hours (CH), Chill Units (CU) and Chill Portions (CP)] and HR for blooming [Total Growing Degree hours from 1st October to FT (GDHF), Growing Degree Hours (GDH) and number of days (JD) from DB to FT]. R g c t ucorrelation coefficients between seasons 2016 and 2017 are indicated in the diagonal.

		2015-2016										
		DB	СН	CU	СР	FT	GDHF	GDH	VJD			
	DB	0.89	0.87	0.88	0.89	0.21	0.16	-0.81	-0.80			
	СН	0.90	0.89	0.89	0.90	0.22	0.16	-0.81	-0.8			
117	CU	0.86	0.84	0.85	0.86	0.21	0.16	-0.78	-0.83			
-20	СР	0.87	0.86	0.87	0.88	0.22	0.16	-0.80	-0.84			
016	FT	-0.11	-0.07	-0.10	-0.11	0.39	0.54	0.38	0.25			
5	GDHF	-0.29	-0.26	-0.28	-0.28	0.32	0.47	0.50	0.40			
	GDH	-0.88	-0.84	-0.87	-0.83	-0.13	-0.05	0.84	0.82			
	ΛJD	-0.87	-0.84	-0.86	-0.87	-0.06	-0.02	0.83	0.86			

400

401 Xcnwgu" kp" dqnf" ctg" ukipkhkecpv" cv" r Ö 202230

Table 2. Summary of consistent QTLs identified with the TxE map including trait name,
QTL names, LOD score of the maximum peak, position of the maximum peak, closest
marker, and parameters of percentage of explained phenotypic variance (R²), additivity (a),
dominance/additivity (d/a) and inferred gene action (GA). DB, dormancy break; FT,
flowering time; CH, chill hours; CU, chill units; CP, chill portions; GDHF, growing degree
hours to flowering= "LF." p wodgt "qh" f c { u " ht qo " FD" vq " HV0

Trait	QTL name	LG	Position (c	M)Closest marker	LOD	R^2	a ^a	d∕a [⊳]	GA ^c
DB-2015	qDB6	G6	2,4	SNP_IGA_61384	3,4	18,4	-7,5	-0,7	PD
DB-2016	qDB6	G6	2,4	SNP_IGA_61384	3,3	18,1	-0,9	-14,6	U
CH-2015	qCH6	G6	2,4	SNP_IGA_61384	3,2	17,3	-95,9	-0,6	PD
CH-2016	qCH6	G6	2,4	SNP_IGA_61384	3,2	17,4	-31,4	-7,0	U
CU-2015	qCU6	G6	2,4	SNP_IGA_61384	3,2	17,2	-129,0	-0,7	PD
CU-2016	qCU6	G6	2,4	SNP_IGA_61384	2,9	16,1	-2,0	-81,3	U
CP-2015	qCP6	G6	2,4	SNP_IGA_61384	3,4	18,4	-5,5	-0,7	PD
CP-2016	qCP6	G6	2,4	SNP_IGA_61384	3,2	17,4	-0,3	-26,9	U
FT-2015	qFT1	G1	9,8	SNP_IGA_2325:	2,2	11,5	-2,5	0,4	AD
FT-2016	qFT1	G1	1,4	SNP_IGA_2006	3,9	21	-1,7	0,2	А
GDHF-2015	qGDHF1	G1	5	SNP_IGA_1052(3,9	19,9	-324,8	0,1	А
GDHF-2016	qGDHF1	G1	1,9	SNP_IGA_2670	3,1	16,8	-144,3	0,1	А
GDH-2015	qGDH6	G6	2,4	SNP_IGA_61384	3	16,3	452,4	-1,2	DP
GDH-2016	qGDH6	G6	2,4	SNP_IGA_61384	3,5	19,9	82,8	-13,6	U
ΔJD-2015	q∆JD6	G6	3	SNP_IGA_61275	3,6	18,9	5,8	-1,1	DP

410 ^aAdditive effects: a = (A - B)/2, where A and B are the average phenotypic values for the

- 411 homozygotes of the almond and peach alleles, respectively.
- 412 ^bDominance d = H [(A + B)]

413 Gene action. U underdominance for peach aller partial dominance for peach

414 allele, A additivityAD partial domiance for almond allele



Fig. 1. Scheme of the different traits used in this study. CR: Chilling requirement; HR: Heat
requirements; CH: Chill hours; CU: Chill units; CP: Chill portions; DB: Dormancy break;
FT: Flowering time; GDH: Growing Degree Hours; GDHF: Growing Degree Hours to
flowering; L Frumber of Julian days between DB and FT.



Fig. 2. Progression of chill accumulation in the period October-April in 2016 and 2017 in
Gimenells (Lleida). Results are expressed in Chill Units (Utah model) (upper), Chill Portions
(Dynamic model) (centre) and Chill Hours (hours below 7°C) (lower).



Fig.3. R-squared regression among CR for breaking dormancy estimated by the Utah, Dynamic and Chill hours models in Gimenells (Lleida, Spain) for 2016 and 2017. All correlations were significant (p < 0.01).



Fig. 4. Distribution of phenological traits in the TxE population: chilling requirements (CR) in chill portions, heat requirements (HR) in growing degree hours (GDH), and flowering times (FT) as \div f c { " q h " v j g "the{parenttal dintes" are indicated by qurtows (T, \div Texasø, E, \div Earlygoldø, H, \div MB 1.37ø).



Fig. 5. Linear regression (R-squared) between chilling requirements (CR) and heat
requirements in the TxE population for 2015-2016 (above) and 2016-2017 (below). CR are
expressed in chill portions (Dynamic model), chill units (Utah model) and chill hours (hours
below 7°C). Heat requirements (HR) are expressed in growing degree hours (GDH).

443 SUPLEMENTARY DATA

- 444 Table S1. Date for dormancy breaking and chilling accumulation (in chill hours, chill units and
- 445 chill portions) for all the genotypes in the population TxE for the two consecutive years
- studied. Yearly values, mean values (ave) and coefficient of variation (cv) are shown.

Genotype	e BD		СН			CU				СР				
	2015-2016	2016-2017	2015-2016	2016-2017	ave	cv	2015-2016	2016-2017	ave	cv	2015-2016	2016-2017	ave	cv
1	11-Jan	2-Jan	814	930	872	82.0	1018	936	977	58.3	51	48	50	2.4
3	4-Jan	2-Jan	763	930	847	118.1	914	936	925	15.6	46	48	47	1.1
5	19-Jan	2-Feb	1039	1488	1264	317.5	1319	1345	1332	18.4	65	70	67	3.1
6	24-Dec	24-Dec	582	714	648	93.3	680	806	743	89.1	38	43	40	3.5
10	4-Jan	11-Jan	763	1140	952	266.6	914	970	942	40.0	46	53	50	5.0
11	21-Dec	19-Dec	528	594	561	46.7	618	728	1207	77.4	36	39	37	2.4
12	4-Feb 21 Jan	2-Feb 9 Jap	1104	1488	1025	2/1.5	1429	1345	1060	59.0 157.0	50	70 51	55	0.6
14	21-Jan	9-Jan	972	1098	956	200.8	1018	958	988	42.8	59	51	55	0.3
15	21-Dec	24-Dec	528	714	621	131.5	618	806	712	132.9	36	43	39	5.1
17	21-Jan	11-Jan	972	1140	1056	118.8	1180	970	1075	148.1	59	53	56	3.8
20	11-Jan	2-Jan	814	930	872	82.0	1018	936	977	58.3	51	48	50	2.4
21	24-Dec	2-Jan	582	930	756	246.1	680	936	808	180.7	38	48	43	7.0
22	29-Jan	2-Feb	1039	1488	1264	317.5	1319	1345	1332	18.4	65	70	67	3.1
23	15-Jan	2-Jan	864	930	897	46.7	1084	936	1010	104.7	54	48	51	4.4
25	11-Jan	2-Jan	814	930	872	82.0	1018	936	977	58.3	51	48	50	2.4
30	21-Jan	9-Jan	972	1098	1035	89.1	1180	958	1069	157.0	59	51	55	5.6
31	21-Jan	11-Jan	972	1140	1056	118.8	1180	9/0	10/5	148.1	59	53	56	3.8
27	29-Jdli 15 Jan	2-Feb 9 Jap	1039	1488	1204	165 5	109/	1545	1021	20.4	54	70 51	52	3.1
39	4-Feb	2-Feh	1104	1488	1296	271 5	1429	1345	1387	59.0	69	70	69	0.6
40	4-Jan	2-Jan	763	930	847	118.1	914	936	925	15.6	46	48	47	1.1
41	11-Jan	11-Jan	814	1140	977	230.5	1018	970	994	33.9	51	53	52	1.5
43	11-Jan	11-Jan	814	1140	977	230.5	1018	970	994	33.9	51	53	52	1.5
44	29-Jan	26-Jan	1039	1400	1220	255.3	1319	1206	1262	80.3	65	64	64	0.9
46	21-Jan	2-Feb	972	1488	1230	364.9	1180	1345	1262	117.0	59	70	64	7.6
47	29-Jan	2-Feb	1039	1488	1264	317.5	1319	1345	1332	18.4	65	70	67	3.1
49	29-Jan	26-Jan	1039	1400	1220	255.3	1319	1206	1262	80.3	65	64	64	0.9
53	21-Jan	2-Feb	972	1488	1230	364.9	1180	1345	1262	117.0	59	70	64	7.6
55	29-Jan A-Eob	2-Feb 2-Feb	1039	1488	1204	317.5 271.5	1/120	1345	1332	18.4	69	70	60	3.1
59	24-Dec	2-rep 24-Dec	582	714	648	93.3	680	806	743	89.1	38	43	40	3.5
61	11-Jan	11-Jan	814	1140	977	230.5	1018	970	994	33.9	51	-5	52	1.5
63	29-Jan	26-Jan	1039	1400	1220	255.3	1319	1206	1262	80.3	65	64	64	0.9
69	4-Jan	24-Dec	763	714	739	34.6	914	806	860	76.0	46	43	45	2.5
72	15-Jan	2-Jan	864	930	897	46.7	1084	936	1010	104.7	54	48	51	4.4
73	15-Jan	26-Jan	864	1400	1132	379.0	1084	1206	1145	86.3	54	64	59	6.9
74	4-Jan	11-Jan	763	1140	952	266.6	914	970	942	40.0	46	53	50	5.0
83	16-Dec	24-Dec	452	714	583	185.3	524	806	665	199.8		43	37	7.8
84	21-Jan	2-Feb	972	1488	1230	364.9	1180	1345	1262	117.0	59	70	64	7.6
85	29-Jan	2-FeD	1039	1488	1264	317.5	1319	1345	1332	18.4	20	/0	6/	3.1
90	24-Dec 11-Jan	2-Jdf1 11-Jan	582	930	/50 077	240.1	1018	930	808	33.0	38	48	43 52	7.0
95	2-Nov	2-Feb	1165	1488	1327	230.3	1523	1345	1434	125.5	73	70	71	2.7
97	29-Jan	2-Feb	1039	1488	1264	317.5	1319	1345	1332	18.4	65	70	67	3.1
98	4-Jan	24-Dec	763	714	739	34.6	914	806	860	76.0	46	43	45	2.5
100	16-Dec	16-Dec	452	515	484	44.5	524	634	579	78.1	32	34	33	1.8
105	4-Jan	2-Jan	763	930	847	118.1	914	936	925	15.6	46	48	47	1.1
106	29-Jan	2-Feb	1039	1488	1264	317.5	1319	1345	1332	18.4	65	70	67	3.1
108	16-Dec	16-Dec	452	515	484	44.5	524	634	579	78.1	32	34	33	1.8
117	11-Jan	11-Jan	814	1140	977	230.5	1018	970	994	33.9	51	53	52	1.5
118	21-Jan	11-Jan	972	1140	1056	118.8	1180	970	1075	148.1	59	53	56	3.8
120	16-Dec	10-Dec	9/2	504	1020	100 /	1180	9/0 970	1012	148.1	59	23	9C 25	3.ð 5.0
122	4-lan	2-lan	452	930	847	118 1	914	936	925	15.6	52 46	39 48	47	11
125	15-Jan	26-Jan	864	1400	1132	379.0	1084	1206	1145	86.3		-,0 64	59	6.9
133	4-Jan	2-Jan	763	930	847	118.1	914	936	925	15.6	46	48	47	1.1
150	4-Feb	26-Jan	1104	1400	1252	209.3	1429	1206	1317	157.7	69	64	66	3.5
152	15-Jan	11-Jan	864	1140	1002	195.2	1084	970	1027	80.3	54	53	54	0.5
153	21-Jan	9-Jan	972	1098	1035	89.1	1180	958	1069	157.0	59	51	55	5.6
165	29-Jan	2-Feb	1039	1488	1264	317.5	1319	1345	1332	18.4	65	70	67	3.1
166	4-Feb	2-Feb	1104	1488	1296	271.5	1429	1345	1387	59.0	69	70	69	0.6
172	4-Feb	26-Jan	1104	1400	1252	209.3	1429	1206	1317	157.7	69	64	66	3.5
194	21-Jan	9-Jan	972	1098	1035	89.1	1180	958	1069	157.0	59	51	55	5.6
202	24-Dec	1/I-Dec	814 دەع	1140 515	5/0	230.5	1018	970	994	33.9 22 ⊑	51	53	36	1.5 2 E
202	11-lan	2-lan	814	930	872	82.0	1018	936	977	58 3	51	34 48	50	2.5
211	24-Dec	2-Jan	582	930	756	246.1	680	936	808	180.7	38	_+0 48	43	7.0
218	11-Jan	11-Jan	814	1140	977	230.5	1018	970	994	33.9	51	53	52	1.5
226	21-Jan	9-Jan	972	1098	1035	89.1	1180	958	1069	157.0	59	51	55	5.6
239	4-Jan	24-Dec	763	714	739	34.6	914	806	860	76.0	46	43	45	2.5
241	29-Jan	26-Jan	1039	1400	1220	255.3	1319	1206	1262	80.3	65	64	64	0.9

- 449 Table S2. Heat requirements in growing degree hours (GDH), flowering dates (FT) and days
- 450 from dormancy breaking to FT (JD) for all the genotypes in the population TxE for the two
- 451 consecutive years studied. Yearly values, mean values (ave) and coefficient of variation (cv)
- 452 are shown. Only genotypes with two years measurements are shown.

Genotype	F	Т		GDH			ΔJD				
	2015-2016	2016-2017	2015-2016	2016-2017	Ave	CV	2015-2016	2016-2017	Ave	CV	
1	5-Mar	11-Mar	5034	6641	5837	1137	54	69	62	10.6	
3	3-Mar	9-Mar	5855	6508	6181	462	59	67	63	5.7	
5	7-Mar	11-Mar	3937	3783	3860	109	38	38	38	0.0	
6	1-Mar	11-Mar	6194	7139	6667	668	68	78	73	7.1	
10	4-Mar	9-Mar	5987	5444	5716	384	60	58	59	1.4	
11	3-Mar	9-Mar	6613	7349	6981	521	73	81	77	5.7	
12	25-Feb	3-Mar	2329	3099	2714	544	21	35	28	9.9	
14	27-Feb	7-Mar	3829	5658	4743	1293	37	58	48	14.8	
15	24-Feb	3-Mar	3991	5253	4622	892	44	54	49	7.1	
16	23-Feb	5-Mar	5656	6705	6181	742	64	72	68	5.7	
17	3-Mar	8-Mar	4375	5361	4868	697	42	57	50	10.6	
20	3-Mar	7-Mar	4816	6362	5589	1093	52	65	59	9.2	
21	24-Feb	3-Mar	5663	5956	5810	207	62	61	62	0.7	
23	10-Mar	10-Mar	5156	6553	5855	988	55	67	61	8.5	
25	24-Feb	7-Mar	3991	6362	5176	1676	44	65	55	14.8	
30	8-Mar	10-Mar	4807	5849	5328	737	47	60	54	9.2	
31	28-Feb	9-Mar	3885	5444	4664	1103	38	57	48	13.4	
34	3-Mar	9-Mar	3588	3650	3619	44	34	35	35	0.7	
37	3-Mar	9-Mar	4591	5804	5197	858	48	59	54	7.8	
39	3-Mar	3-Mar	2999	3099	3049	71	28	30	29	1.4	
40	1-Mar	7-Mar	5561	6362	5961	566	57	65	61	5.7	
41	25-Feb	5-Mar	4146	5144	4645	/06	45	54	50	6.4	
43	25-Feb	6-Mar	4146	5230	4688	766	45	55	50	7.1	
44	6-Mar	10-Mar	3874	4253	4063	268	37	43	40	4.2	
46	27-Feb	10-Mar	3829	3695	3/62	94	37	36	3/	0.7	
4/	3-Mar	6-Mar	3588	3436	3512	108	34	33	34	0.7	
49	5-Mar	10-Mar	3806	4512	4159	499	36	43	40	4.9	
53	29-Feb	6-IVlar	3976	3436	3706	382	39	33	36	4.2	
55	5-Mar	8-Mar	3806	3567	3686	169	36	35	36	0.7	
56	29-Feb	8-Mar	3347	3567	3457	155	25	35	30	7.1	
59	5-IVIar	10-IVIar	6706	7051	6878	244	/2	/6	/4	2.8	
61	25-Feb	7-IVlar	4146	5298	4/22	814	45	55	50	/.1	
63	12-Mar	12-Mar	4338	4/03	4520	258	43	45	44	1.4	
69	26-Feb	10-IVIar	5294	7051	61/2	1242	53	76	65	10.3	
72	27-Feb	5-Ivlar	4045	6208	5126	1529	43	62	53	13.4	
/3	21-Feb	8-IVIar	3321	4383	3852	/52	3/	41	39	2.8	
74	1-IVIar	10-Ivlar	2501	5490	5525	51	57	58	58	0.7	
84	10-IVIar	10-IVIar	3588	3695	3642	76	50	36	43	9.9	
01	5-IVIdi	9-IVIdi	500	5050	2019	44	54	55	55	0.7	
91	6-IVIar	10-Ivlar	5102	5490 2218	5290	2/4	55		5/ 20	2.1	
93	2 Mar	4-Iviai	Z144 E4E6	7400	6472	1/29	24	76	20	4.9	
90 105	22 Eob	12 Mar	5450	6744	6265	1430		70	50	14.1	
105	22-FED	12-Iviai	2467	2210	20202	555 E21	49	21	23	14.1	
100	1-Ividi 1-Mar	4-iviai 5-Mar	6710	7/00	2045 7105	5/5	52	80	52 79	2.2	
117	22_Eab	J-Iviai A-Mar	2050	5012	1102	243 Q1E	0/	50	70 70	2.0	
110	6-Mar	10_Mar	2029	5/00	5075	526	45	50	40 50	0.1	
120	Q_Mar	10-Mar	4001	5450	51/9	200	43	50	52	9.2 7 9	
120	21-Eah	10-Mar	4007 6199	7/82	6832	403	47	J0 Q1	76	7.0 7.9	
122	7-Mar	11-Mar	6204	66/1	6422	300	62	68	66	25	
120	7-Mar	10-Mar	6204	/512	5252	1106	53	/12	18	6.4	
120	/-Mar	10-Mar	5987	6553	6270	/00	52 60		64	1 9	
153	- War	20-Iviai 8-Mar	/501	5361	1076	544	18	56	52	4.9 5.7	
152	3-Mar	12-Mar	4331	5372	4970	705	40	62	52	1/ 1	
155	J-Mar	4-Mar	3720	2012	3460	255		21	22	2.0	
165	- Ivial 25-Feh	8-Mar	2720	3567	2948	875	21	31	28	9.2	
172	11-Mar	Δ_Mar	2329	2015	3259	020	21	20	20	0.2	
10/	1-Mar	10-Mar	2000	550/	4793	1006		60	50	14 1	
100	⊥-ivial <u>∕</u> _Mar	Q_Mar	1001	5/1/1	5106	251	-40 52	57	50	2 8	
202	Ivial 25-Feh	5-Mar	5,210	7156	6487	946	53	57 80	72	12.0	
202	1-Mar	7-Mar	/522	6363	54/12	1201	50	65	58	10.6	
211	29-Feh	10-Mar	6089	6552	6321	328	67	67	67	0.0	
215	25-Feh	5-Mar	4146	5144	4645	706	07 	54	50	6.4	
210	29-Feh	3-Mar	3976	5252	4614	903	20	54	47	10.6	
220	15-Mar	11-Mar	6791	7139	6965	246	72	77	75	35	
233	10-Mar	3-Mar	4154	3915	4034	169	, г Д?	37	40	35	
271	TO IMIDI	5 14101	41,74	3313	FUJT	109	42	57	-10	5.5	



458 Fig. S1. Maximum and minimum daily temperatures registered in the period October-April in459 2016 and 2017 in Gimenells (Lleida).







466 **REFERENCES**

467Alburquerque, N., Burgos, L., Egea, J. 2003. Apricot flower bud development and abscission
related to chilling, irrigation and type of shoots. Scientia Horticulturae 98, 265-276.

469Alburquerque, N., Garcia-Montiel, F., Carrillo, A., Burgos, L. 2008. Chilling and heat
requirements of sweet cherry cultivars and the relationship between altitude and the
probability of satisfying the chill requirements. Environmental and Experimental Botany
64, 162-170.

473Balandier, P., Bonhomme, M., Rageau, R., Capitan, F., Parisot, E. 1993. Leaf bud
endodormancy release in peach trees. Evaluation of temperature models in temperate and
tropical climates. Agricultural and Forest Meteorology 67, 95-113.

476Benmoussa, H., Ghrab, M., Ben Mimoun, M., Luedeling, E. 2017. Chilling and heat
requirements for local and foreign almond (Prunus dulcis Mill.) cultivars in a warm
Mediterranean location based on 30 years of phenology records. Agricultural and Forest
Meteorology 239, 34-46.

480Bielenberg, D.G., Wang, Y., Li, Z., Zhebentyayeva, T., Fan, S., Reighard, G. L., Scorzar, R.,
481 Abbott, A. G. 2008. Sequencing and annotation of the evergrowing locus in peach [*Prunus*482 *persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate
483 genes for regulation of terminal bud formation. Tree Genet Genomes 4(3), 495-507.
484Bielenberg, D.G., Rauh, B., Fan, S.H., Gasic, K., Abbott, A.G., Reighard, G.L., Okie, W.R.,
485 Wells, C.E. 2015. Genotyping by sequencing for SNP-based linkage map construction and
486 QTL analysis of chilling requirement and bloom date in peach *Prunus persica* (L.) Batsch.

487 Plos One 10(10).

488Byrne, D.H. 2003. Breeding peach and nectarines for mild-winter climate areas: State of the art
and future directions, In: Marra, F.P., Sottile, F. (Eds.), First Mediterranean Peach
Symposium. Paruzzo Prontostampa, Agrigento, Italy, pp. 102-112.

491Calle, A., Cai, L., Iezzoni, A., Wünsch, A. 2020. Genetic dissection of bloom time in low
chilling sweet cherry (*Prunus avium* L.) using a multi-family QTL approach. Front. Plant
Sci. 10:1647.

494Campoy, J.A., Ruiz, D., Allderman, L., Cook, N., Egea, J. 2012. The fulfilment of chilling
requirements and the adaptation of apricot (*Prunus armeniaca* L.) in warm winter climates:
An approach in Murcia (Spain) and the Western Cape (South Africa). European Journal of
Agronomy 37, 43-55.

498Campoy, J.A., Ruiz, D., Egea, J., Rees, D.J.G., Celton, J.M., Martínez-Gómez, P. 2011.
Inheritance of flowering time in apricot (*Prunus armeniaca* L.) and analysis of linked
quantitative trait loci (QTLs) using simple sequence repeat (SSR) markers. Plant Molecular
Biology Reporter 29, 404-410.

502Campoy, J.A., Ruiz, D., Nortes, M.D., Egea, J. 2013. Temperature efficiency for dormancy
release in apricot varies when applied at different amounts of chill accumulation. Plant
Biology 15, 28-35.

505Castède, S., Campoy, J.A., Le Dantec, L., Quero-Garcia, J., Barreneche, T., Wenden, B., 506 Dirlewanger, E. 2015. Mapping of candidate genes involved in bud dormancy and 507 flowering time in sweet cherry (*Prunus avium*). Plos One 10 (11): e0143250.

508Castède, S., Campoy, J.A., Quero-Garcia, J., Le Dantec, L., Lafargue, M., Barreneche, T., 509 Wenden, B., Dirlewanger, E. 2014. Genetic determinism of phenological traits highly

510 affected by climate change in Prunus avium: flowering date dissected into chilling and heat

511 requirements. New Phytologist 202, 703-715.

512Couvillon, G.A., Erez, A. 1985. Influence of prolonged exposure to chilling temperatures on

513 bud break and heat requirement for bloom of several fruit species. Journal of the American

514 Society for Horticultural Science 110, 47-50.

515Dennis, F.G. 2003. Problems in standardizing methods for evaluating the chilling requirements

516 for the breaking of dormancy in buds of woody plants. J q t v U e k g p e g " 5 : < " 5 6 9 5 7

517Dirlewanger, E., Graziano, E., Joobeur, T., Garriga-Calderé, F., Cosson, P., Howad, W., Arús,

518 P. 2004. Comparative mapping and marker-assisted selection in *Rosaceae* fruit crops.

519 Proceedings of the National Academy of Sciences 101, 9891-9896.

520Dirlewanger, E., Quero-Garcia, J., Le Dantec, L., Lambert, P., Ruiz, D., Dondini, L., Illa, E.,

521 Quilot-Turion, B., Audergon, J.M., Tartarini, S., Letourmy, P., Arús, P. 2012. Comparison

of the genetic determinism of two key phenological traits, flowering and maturity dates, in
three Prunus species: peach, apricot and sweet cherry. Heredity 109, 280-292.

524Donoso, J.M., Eduardo, I., Picañol, R., Batlle, I., Howad, W., Aranzana, M.J., Arús, P. 2015.

High-density mapping suggests cytoplasmic male sterility with two restorer genes inalmond x peach progenies. Horticulture Research 2, 15016.

527Donoso, J.M., Picañol, R., Serra, O., Howad, W., Alegre, S., Arús, P., Eduardo, I. 2016.

528 Exploring almond genetic variability useful for peach improvement: mapping major genes

and QTLs in two interspecific almond x peach populations. Molecular Breeding 36 (16).

530Døving, A. 2009. Modelling plum (*Prunus domestica*) yeild in Norway. Europ. J. Hortic. Sci.531 74, 254-259.

532Døving, A. 2011. Plant science and biotechnology in Norway. Modelling sweet cherry (Prunus
avium) fruit yield in Norway. In: Netsby, R. (ed.) Europ. J. Plant Sci. Biotech. 5 (Special
Issue 1), 62-66.

535Egea, J., Ortega, E., Martínez-Gómez, P., Dicenta, F. 2003. Chilling and heat requirements of

almond cultivars for flowering. Environmental and Experimental Botany 50, 79-85.

537Erez, A., Couvillon, G.A. 1987. Characterization of the influence of moderate temperatures on

538 t g u v " e q o r n g v k q p " k p " r g c e j 0 " L " C o " U q e " J q t v k e " U e
539Erez, A., Fishmann, S. 1998. The dynamic model for chilling evaluation in peach buds. Proc

540 6 v j " K p v " R g c e j " U { o r . " C e v c " J q t v " 6 8 7 < 7 2 9 7 3 2 0

541Erez, A., Fishman, S., Linsleynoakes, G.C. 1990. The dynamic model for rest completion inpeach buds. Acta Horticultura 276, 18.

543Erez, A., Yablowitz, Z., Korcinski, R. 2000. Temperature and chemical effects on competing

sinks in peach bud break, In: Bodson, M. (Ed.), Proceedings of the Xxv International

545 Horticultural Congress, Pt 4: Culture Techniques with Special Emphasis on Environmental

546 Implications Chemical, Physical and Biological Means of Regulating Crop Growth in547 Vegetables and Fruits, pp. 51-58.

548Fan, S., Bielenberg, D.G., Zhebentyayeva, T.N., Reighard, G.L., Okie, W.R., Holland, D.,

Abbott, A.G. 2010. Mapping quantitative trait loci associated with chilling requirement,

heat requirement and bloom date in peach (*Prunus persica*). New Phytologist 185, 917-930.

551Hgnkrg"CL" * 422; + "õHgnkpgoö."õIctpgoö."cpf"õOq

552 HortScience 44(1):196-197.Fishman, S., Erez, A., Couvillon, G.A. 1987. The temperature-

553 dependence of dormancy breaking in plants - Computer simulation of processes studied

under controlled temperatures. Journal of Theoretical Biology 126, 309-321.

555Guerriero, R., Monteleone, P., Viti, R. 2006. Evaluation of end of dormancy in several apricot

556 cultivars according to different methodological approaches, In: Audergon, J.M. (Ed.),

557 Proceedings of the Xiith Ishs Symposium on Apricot Culture and Decline, Vols 1 and 2,

558 pp. 99.

559Hanke, M.-V., Flachowsky, H., Peil, A., Hättasch, C. 2007. No flower no fruitô genetic
potentials to trigger flowering in fruit trees. Genes Genomics 1, 1-20.

561Harrington, C.A., Gould, P.J., St Clair, J.B. 2010. Modeling the effects of winter environment

on dormancy release of Douglas-fir. Forest Ecology and Management 259, 798-808.

563Hauagge, R., Cummins, J.N. 1991. Genetics of length of dormancy period in malus vegetative
buds. Journal of the American Society for Horticultural Science 116, 121-126.

565Heide, O.M. 1993. Daylength and thermal time responses of budburst during dormancy release566 in some northern deciduous trees. Physiol. Plant. 88, 5316540.

567Kitamura, Y., Habu, T., Yamane, H., Nishiyama, S., Kajita, K., Sobue, T., Kawai, T.,
Numaguchi, K., Nakazaki, T., Kitajima, A., Tao, R. 2018. Identification of QTLs
controlling chilling and heat requirements for dormancy release and bud break in Japanese
apricot (*Prunus mume*). Tree Genetics & Genomes 14, 33.

571Kotowski, S.J., Bailey, C.H., Hough, L.F. 1980. Estimate of chilling requirements of apricot
selections. Hortscience 15, 395-395.

573Li, Y., Fang, W.C., Zhu, G.R., Cao, K., Chen, C.W., Wang, X.W., Wang, L.R. 2016.
Accumulated chilling hours during endodormancy impact blooming and fruit shape
development in peach (*Prunus persica* L.). Journal of Integrative Agriculture 15, 12671274.

- 577Luedeling, E., Gassner, A. 2012. Partial Least Squares Regression for analyzing walnut 578 phenology in California. Agric For Meteorol. 158, 43-52.
- 579 Meier, U., Graf, H., Hack, H., Hess, M., Kennel, W., Klose, R., Mappes, D., Seipp, D.,
- 580 Stauss, R., Streif, J., Boom, T. van den. 1994. Phenological growth stages of pome fruits
- 581 (Malus domestica Borkh. and Pyrus communis L.), stone fruits (Prunus species), currants
- (Ribes species) and strawberry (Fragaria \times ananassa Duch.). Nachrichtenblatt des Dtsch.

- Pflanzenschutzdienstesv 46, 1416153.Menzel, A., Estrella, N., Testka, A. 2005.
 Temperature response rates from long-term phenological records. Climate Research 30, 2128.
- 586Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kubler, K., Bissolli,
- 587 P., Braslavska, O., Briede, A., Chmielewski, F.M., Crepinsek, Z., Curnel, Y., Dahl, A.,
- 588 Defila, C., Donnelly, A., Filella, Y., Jatcza, K., Mage, F., Mestre, A., Nordli, O., Penuelas,
- 589 J., Pirinen, P., Remisova, V., Scheifinger, H., Striz, M., Susnik, A., Van Vliet, A.J.H.,
- 590 Wielgolaski, F.E., Zach, S., Zust, A. 2006. European phenological response to climate
- change matches the warming pattern. Global Change Biology 12, 1969-1976.
- 592Okie, W.R., Blackburn, B. 2011. Increasing Chilling Reduces Heat Requirement for Floral593 Budbreak in Peach. Hortscience 46, 245-252.
- 594Olukolu, B.A., Trainin, T., Fan, S., Kole, C., Bielenberg, D.G., Reighard, G.L., Abbott, A.G.,
- Holland, D. 2009. Genetic linkage mapping for molecular dissection of chilling
 requirement and budbreak in apricot (*Prunus armeniaca* L.). Genome 52, 819-828.
- 597Pawasut, A., Fujishige, N., Yamane, K., Yamaki, Y., Honjo, H. 2004. Relationships between 598 chilling and heat requirement for flowering in ornamental peaches. Journal of the Japanese
- 599 Society for Horticultural Science 73, 519-523.
- 600Pérez, F.J., Ormeno N, J., Reynaert, B., Rubio, S. 2008. Use of the dynamic model for the
 assessment of winter chilling in a temperate and a subtropical climatic zone of Chile.
 Chilean Journal of Agricultural Research 68, 198-206.
- 603Quilot, B., Wu, B.H., Kervella, J., Genard, M., Foulongne, M., Moreau, K. 2004. QTL analysis
 604 of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild
- relative species *P. davidiana*. Theoretical and Applied Genetics 109, 884-897.

606Ramirez, F., Kallarackal, J. 2015. The effect of increasing temperature on phenology, In:

- 607 Ramírez, F., Kallarackal, J. (Eds.), Responses of Fruit Trees to Golbal Climate Change.
- 608 Springer Internatiaonal Publishing, pp. 11-13.
- 609Richardson, E.A., Seeley, S.D., Walker, D.R. 1974. A model for estimating the completion of
- 610 t g u v "h q t " \div T g f j c x g p ø " c p f " \div G n d g t v c ø "r g c e j "v t g g
- 611Rivero, R., Sønsteby, A., Heide, O.M., Måge, F., Remberg, S.F. 2016. Flowering phenology
- and the interrelations between phenological stages in apple trees (*Malus domestica* Borkh.)
- as influenced by the Nordic climate. Acta Agri. Scand. Sect. B. Soil Plant Sci. 67, 2786283.
- 614Ruiz, D., Campoy, J.A., Egea, J. 2007. Chilling and heat requirements of apricot cultivars for
- flowering. Environmental and Experimental Botany 61, 254-263.
- 616Salazar, J.A., Ruiz, D., Campoy, J.A., Sánchez-Pérez, R., Crisosto, C.H., Martínez-García, P.J.,
- Blenda, A., Jung, S., Main, D., Martínez-Gómez, P., Rubio, M. 2014. Quantitative Trait
- Loci (QTL) and Mendelian Trait Loci (MTL) Analysis in Prunus: A Breeding Perspectiveand Beyond. Plant Mol. Biol. Report. 32, 1-18.
- 620Sánchez-Pérez, R., Dicenta, F., Martínez-Gómez, P. 2012. Inheritance of chilling and heat
 requirements for flowering in almond and QTL analysis. Tree Genetics & Genomes 8, 379389.
- 623Scorza, R., Okie, W.R. 1990. Peaches (Prunus). Acta Horticulturae 290, 177-231.
- 624Silva, C., Garcia-Mas, J., Sánchez, A.M., Arús, P., Oliveira, M. 2005. Looking into flowering
 time in almond (*Prunus dulcis* (Mill) D. A. Webb): the candidate gene approach.
 Theoretical and Applied Genetics 110, 959-968.
- 627Tanksley, S. 1993. Mapping Polygenes. Annu. Rev. Genet. 27, 205-233.
- 628Van Ooijen, J. W. 2009. MapQTL 6.0. Software for the Mapping of Quantitative Trait Loci in
- 629 Experimental Populations. Wageningen: Kyazma, B.V.

630Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and631 QTLs. J. Hered. 93(1):77-78.

632Walther, G.R. 2002. Weakening of climatic constraints with global warming and its633 consequences for evergreen broad-leaved species. Folia Geobotanica 37, 129-139.

634Wang, D., Karle, R., Iezzoni, A.F. 2000. QTL analysis of flower and fruit traits in sour cherry.

- 635 Theoretical and Applied Genetics 100, 535-544.
- 636 Weinberger, J.H. 1950. Chilling requirements of peach varieties. Proceedings of the American637 Society for Horticultural Science 56, 122-128.
- 638Woznicki, T.L., Heide, O.M., Sønsteby, A., Måge, F., Remberg, S.F. (2019) Climate warming
- 639 enhances flower formation, earliness of blooming and fruit size in plum (*Prunus domestica*
- 640 L.) in the cool Nordic environment. Sci Hortic (Amsterdam) 257. Zhang, J., Taylor, C.,
- 2011. The dynamic model provides the best description of the chill process on 'Sirora'
 pistachio trees in Australia. Hortscience 46, 420-425.
- 643Zhebentyayeva, T.N., Fan, S.H., Chandra, A., Bielenberg, D.G., Reighard, G.L., Okie, W.R.,
 644 Abbott, A.G. 2014. Dissection of chilling requirement and bloom date QTLs in peach using
 645 a whole genome sequencing of sibling trees from an F2 mapping population. Tree Genetics
 646 & Genomes 10, 35-51.