1 **Inheritance and QTL analysis of chilling and heat requirements for flowering in an**

2 **interspecific almond x peach (Texas x Earlygold) F2 population**

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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 F2 progeny. FT, HR and CR were evaluated over two

23 consecutive years (2015/2016 and 2016/2017). Seven out of the eight identified quantitative

24 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

2

49   0.6ºC over the past 100 years (Walther, 2002). The forecast is for this trend to continue, with

50   studies already having observed the decrease in winter chill and the resulting changes in

51   phenological events (Menzel et al., 2005; Menzel et al., 2006).

52   The *Prunus* genus, within the Rosaceae family, is characterized by species that grow in

53   areas with well-marked seasons and are adapted to survive cold winters and dry summers

54   (Dirlewanger et al., 2012). Various models have been proposed to measure the accumulation of

55   CR in deciduous fruit-growing areas (Alburquerque et al., 2008). The effect and genetic basis

56   of HR on flowering is not yet well understood and studies on this topic are scarce. Previous

57   studies in *Prunus* have suggested that CR has a stronger effect on flowering time than HR

58   (Couvillon and Erez, 1985; Campoy et al., 2012; Alburquerque et al., 2008; Okie and

59   Blackburn, 2011; Sanchez-Perez et al., 2012). In this genus, as in most woody perennials, the

60   physiology and biochemistry of the flowering process is poorly understood (Dirlewanger et al.,

61   2012; Woznicki et al., 2019). Recent reports suggest that intrinsic and environmental signaling

62   interact and dynamically affect the extent of bud dormancy (Castède et al., 2015; Woznicki et

63   al., 2019). Peach (*Prunus persica* (L.) Batsch) is an economically important species that

64   provides an excellent system for the genetic analysis of CR and FT due to the ample variation

65   for both traits among peach cultivars (CR between 50 to 1,050 h) (Zhebentyayeva et al., 2014).

66   Both CR and FT are inherited as quantitative traits (Fan et al., 2010; Hauagge and Cummins,

67   1991), but their molecular regulation is not yet fully understood. Some quantitative trait loci

68   (QTLs) associated with FT have been found in *Prunus* species (reviewed in Salazar et al.,

69   2014): almond (Sanchez-Perez et al., 2012; Silva et al., 2005); peach (Dirlewanger et al., 2012;

70   Bielenberg et al., 2015); almond x peach (Donoso et al., 2015); apricot (Campoy et al., 2013;

71   Dirlewanger et al., 2012; Kitamura et al., 2018); sweet cherry (Dirlewanger et al., 2012; Calle

72   et al., 2020) and sour cherry (Wang et al., 2000). The separate effect of CR and HR on FT has

73 only been reported in a few studies in peach (Fan et al., 2010), apricot (Olukolu et al., 2009)

74 and cherry (Castède et al. 2014). Since these traits can only be evaluated 2-3 years after seed

75 germination in most *Prunus*, the identification of genetic markers linked with CR, HR and FT

76 would be a valuable tool to select genotypes at the seedling stage and make the breeding

77 process much more efficient (Bielenberg et al., 2015).

78   In this work, we studied an F_2 almond x peach progeny (TxE, almond 'Texas' x peach

79 'Earlygold') developed at IRTA and used as the *Prunus* reference map (Dirlewanger et al.,

80 2004), for which a high-density linkage map was available (Donoso et al. 2015). The main

81 goals were to study the inheritance of CR, HR and FT, and to test the existing chill unit (CU)

82 models in Gimenells, Lleida (Spain) (latitude 0°23'E /longitude 41°39'N), with a temperate

83 semi-arid climate, to identify which of them fits best with the climatic conditions in one of the

84 major areas of peach and almond production in the world.

85

86 **Materials and methods**

87 *Plant material*

88   The *Prunus* reference interspecific almond x peach F_2 progeny (T x E), obtained by selfing

89 a hybrid individual ('MB 1.37') from a cross between almond 'Texas' and peach 'Earlygold'

90 (Donoso et al. 2015), and its parents was used for this study (Tables S1 and S2). From the

91 original progeny of 111 hybrids, we phenotyped the 72 trees that were still alive. Trees of T x E

92 are at the IRTA Experimental Station of Lleida in Gimenells (Spain) grafted on 'Garnem'

93 (Felipe 2009) rootstocks. Standard agricultural practices were applied.

94

95 *Phenotyping*

96   The parents, hybrid and TxE offspring were evaluated over two seasons (2015/2016 and

97   2016/2017, that we refer to as 2016 and 2017, respectively) following a forcing protocol

98   widely used in temperate fruit trees (Campoy et al. 2011). Traits phenotyped were chilling

99   requirement (CR), flowering time (FT), and heat requirement (HR). Three one-year-old

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

104   photoperiod to force floral bud break (Ruiz et al., 2007; Sánchez-Pérez et al., 2012). After 7

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

107   according to the international Biologische Bundesanstalt, Bundessortenamt et CHemische

108   Industrie (BBCH) scale (Meier et al., 1994; Alburquerque et al., 2008). Three chill models

109   were then used to calculate chilling accumulation from October 1st until dormancy release,

110   corresponding to the CR. The flowering time (FT) was scored as the number of Julian days

111   when 50% of flowers were open. Also, the length of the period between DB and FT was

112   calculated as the number of days between both events (ΔJD, increment of Julian Days). For

113   each genotype, the whole tree was observed by the same person every 1 or 2 days during

114   the flowering period. A scheme of the traits evaluated can be found in Figure 1.

115

116   *Weather data*

117   Hourly temperatures from October 1st to flowering time for both years were obtained from

118   the Gimenells weather station of the Generalitat de Catalunya, in the same area as the studied

119   population (https://ruralcat.gencat.cat).

120

Chilling and forcing models

For this study, we used the Chill Hours (CH), Utah (CU) and Dynamic (CP) Models. The Chill Hours Model (CH) (Weinberger, 1950) is the oldest and simplest model, which considers all hours with temperatures between 0 and 7°C as effective for chill accumulation. The Utah 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 contributions by high temperatures, and it is particularly used in cooler areas of temperate zones (Dennis et al., 2003). The Dynamic Model (Erez and Couvillon, 1987) was developed for warmer areas. It considers that dormancy cessation occurs in two steps, the first being reversible and the second irreversible, and CR are calculated as chill portions (CP). It adopts a 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 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 different effects on dormancy, but the temperature ranges differ in the two models (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 4.5ºC and 36ºC (at different rates depending on the maximum temperature), with maximum accumulation at an optimal temperature (25ºC). Additionally, we also calculated GDH between 1st October and flowering date (GDHF).

Chilling and heat requirements

Chilling and heat accumulation were calculated for the two consecutive dormancy seasons (2016 and 2017) with the hourly temperatures measured in the field. Chill was computed 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 determined using Partial Least Squares (PLS) regression (Luedeling and Gassner, 2012). Since heat cannot have an effect after bloom, the flowering time was considered as the end of the forcing period. CR and HR were estimated as the sum of all daily chill and heat accumulated 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 accumulation was calculated (ΔJD) and used as a complement to GDH. Annual heat accumulation up to the date of flowering was also recorded (GDHF).

QTL analysis

For QTL analysis, we used the TxE genetic map described by Donoso et al. (2015), which was constructed using 1,948 molecular markers (SNPs and SSRs), covering a total genetic 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 considered consistent when the LOD $\geq$ 3.0 in both seasons, or with a LOD $\geq$ 3.0 one year and LOD $\geq$ 2.0 the other year. QTLs were considered as major QTLs when they explained more than 20% of phenotypic variation in both years of study (Tanksley, 1993). QTL positions were drawn using the MapChart 2.1 software (Voorrips 2002).

7

166   Gene action was estimated following the guidelines of Tanksley (1993) with the ratio, d/a,

167   between the additive, where $a = (A - B)/2$, and dominance $d = H - [(A + B)/2]$ effects, with H,

168   A and B the average phenotypic values of the heterozygous, almond homozygous and peach

169   homozygous genotypes, respectively. Based on the d/a ratio, QTLs were classified as

170   underdominant ($d/a \leq 1.25$; U), dominant for the peach allele ($-1.25 \leq d/a \leq -0.75$; DP),

171   partially dominant for the peach allele ($-0.75 \leq d/a \leq -0.25$; PD), additive ($-0.25 \leq d/a \leq 0.25$;

172   A), partially dominant for the almond allele ($0.25 \leq d/a \leq 0.75$; AD), dominant for the almond

173   allele ($0.75 \leq d/a \leq 1.25$; DA) and overdominant ($d/a > 1.25$; O).

174

175   **Results**

176   *Temperature and chilling accumulation*

177   Maximum and minimum daily temperatures during the consecutive years studied in

178   Gimenells (Lleida) are shown in Supplementary Fig. S1. Higher maximum and minimum

179   temperatures were registered during the winter in 2016, meaning a warmer winter compared to

180   the following year. However, warmer maximum temperatures were registered at the end of the

181   winter in 2017, reaching 25ºC 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 ['MB 1.37' (H),

188   'Texas' (T) and 'Earlygold' (E)] was between 42 and 64 CP. Of these, 'Earlygold' had the

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

190   CP) and 'Texas' (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 F_2 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).

195   In most genotypes, CR were similar for both years evaluated, showing high correlations

196   between them (Table 1). The date of DB was, in general, earlier in 2016 than in 2017, with the

197   earliest on 16th Dec and 14th Dec, whereas the latest was on 4th Feb and 2nd Feb (2016 and

198   2017, respectively).

199

200   *Heat requirements for flowering*

201   The range of HR for flowering among the parents was 3,575 to 6,170 GDH, whereas in the

202   segregating population it was 2,681 to 7,105 GDH (averaged for the two years) (Figure 4). 'MB

203   1.37', had the lowest HR of the parents, and was similar to 'Texas', whereas 'Earlygold' had

204   the highest. Within the segregating population, most genotypes had similar HR for both

205   consecutive years, showing a high correlation (r^2 = 0.78) between years (Table 1). Date of

206   flowering (FT) was, in general, earlier in 2016 than in 2017, with the exception of only three

207   genotypes. The parental lines ('MB1.37', 'Texas' and 'Earlygold') showed very similar FT in

208   both years. Correlation between years for FT was lower than for other traits (r^2 = 0.39) (Table

209   1). The earliest FT was on 21st Feb and 3rd March, and the latest 15th and 12th March (2016 and

210   2017, respectively). The number of days between DB to FT in the segregating population

211   (ΔJD), which is the period for heat accumulation, ranged from 27 to 77 days (averaged for the

212   two years), and there was a high correlation between both seasons (r^2 = 0.86). HR showed a

213 bimodal distribution in 2017, whereas it was a single peak in 2016 (Fig. 4). There were single

214 peaks for FT in both years, although it was slightly skewed to late bloom in 2017.

215

216 *Correlations between traits*

217 Highly significant correlations (R ≥ 0.96, p ≤ 0.01) were found between the three models

218 used to estimate chilling accumulation for both seasons (Supplementary Table S1). Similarly,

219 correlations between DB and these three models were very high (R ≥ 0.99, p ≤ 0.01).

220 Correlations between seasons were very high for most of the traits (r^2 = 0.78-0.89), with lower

221 values for FT and GDHF (r^2 = 0.39 and r^2 = 0.47, respectively) (Table 1). Flowering time (FT)

222 showed a high correlation with GDHF. Correlation between FT and CR and HR traits was low

223 for both years of study.

224 Heat requirements (GDH) were highly and negatively correlated with CR and DB (Table 1

225 and Fig. 5), which means that the lower the CR, the higher the HR. As expected, HR was

226 highly correlated with the number of days between DB and FT (ΔJD), and therefore the number

227 of days for heat accumulation was highly and negatively correlated with CR and DB.

228

229 *QTL analysis*

230 Data from the eight traits under study (DB, CH, CP, CU, FT, GDH, GDHF, ΔJD) were used

231 for QTL analysis. One consistent QTL per trait was identified (Table 2). QTLs were located in

232 G1 for FT and GDHF, and in G6 for DB, the CR models (CH, CU and CP), GDH and ΔJD

233 (Fig. S2).

234 For dormancy breaking (DB), the LOD for the QTL from G6 was 3.4 in 2016 and 3.4 in

235 2017 and explained 18.4 and 18.1 % of the phenotypic variance, respectively. The homozygote

236 for the peach allele increased the date of DB by 15 days compared to the almond homozygote

10

237 in 2016 and by almost two days in 2017. In 2017, while both homozygous classes had similar

238 values, there was an increase of 13 days for the heterozygous individuals. Very similar results

239 were obtained for ΔJD although the LOD for the QTL in G6 was less than 2.0 (1.8) in 2017 and

240 therefore was not considered.

241 For CR data similar results were obtained with the three models, although the most

242 significant QTLs were obtained using the dynamic model, while the least was for the Utah

243 model. For the dynamic model, a QTL was identified at the proximal end of G6 with a LOD

244 score of 3.4 in 2016 and 3.2 in 2017, explaining, respectively, 18.4% and 17.4 % of the

245 phenotypic variance. The confidence interval for G6 QTLs spans the genomic region Pp06: 0-

246 4.001.078 where 702 genes have been annotated. In 2016, the individuals with the homozygous

247 peach allele needed 10 CP more to reach DB than those with the homozygous almond allele. In

248 2017, this difference was only four CP, but 10 with heterozygous individuals.

249 In the same region of G6 we also identified a QTL for GDH and ΔJD (only in 2016)

250 explaining between 16.8% and 19.9% of the phenotypic variance respectively. For FT we

251 identified a QTL at the beginning of G1 in 2017 (LOD 3.9; $R^2 = 21\%$), but with a LOD score of

252 2.2 in 2016 ($R^2 = 11.5\%$). The confidence interval spans the genomic region Pp01:0-

253 10.521.046 bp, where 1584 have been annotated. The peach allele in homozygosis increased

254 the FT by five days in 2016 and three in 2017, compared to the homozygous almond allele. In

255 the same region, we also detected a QTL for GDHF in both years, with LOD values of 3.9 and

256 3.1 respectively.

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, 'Texas', a late flowering almond cultivar and 'Earlygold', an early flowering peach,

262   flower at the same time even though they have different CR and HR. The CR of the progeny

263   are in the range of those reported for peach and almond from other Mediterranean areas

264   (Benmoussa et al., 2017; Campoy et al., 2012; Ruiz et al., 2007). However, it must be noted

265   that TxE is an interspecific population, and therefore results are not fully comparable to single

266   species populations studies. The range observed in the TxE population exemplifies the

267   difficulties for growing certain peach and almond cultivars in warm regions where annual chill

268   accumulation is decreasing due to global warming. A large variation in the HR within the

269   studied progeny was also found.

270   The performance of chill accumulation models vary in different climate conditions, as

271   observed in peach (Balandier et al., 1993; Erez et al., 1990; Erez et al., 2000; Perez et al., 2008)

272   and other *Prunus* species (Alburquerque et al., 2003; Alburquerque et al., 2008; Egea et al.,

273   2003; Ruiz et al., 2007).

274   In Lleida (Spain) the CR calculated using the three models were very well correlated and

275   with DB, and therefore could be used for the calculation of CR in this climatic area, as for

276   peach (Fan et al., 2010) and apricot (Campoy et al., 2012) in colder climates. This correlation

277   could be due to the lack of long periods of warm and fluctuating temperatures, so that chilling

278   accumulation based on different models all steadily increased in a similar way through the two

279   seasons. Substantial differences among the many models used have been observed in moderate

280   mild climates (Erez et al., 1990; Erez et al., 2000) since some of them, such as the Utah model,

281   were developed in a cold area and are not appropriate for warmer areas.

282   The variability of chill accumulation between years was lower with the Dynamic model

283   than when the calculations were done with the Utah and the hours-below 7ºC models. This may

12

284 be explained by the homogenizing effect of the Dynamic model, which takes into account the

285 synergistic effect between moderate and low temperatures for breaking dormancy (Fishman et

286 al., 1987). Other authors have previously reported similar results in apricot (Campoy et al.,

287 2012; Ruiz et al., 2007), suggesting that the Dynamic model is optimal for the climatic

288 conditions of Lleida and other areas of the Ebro Valley in Northern Spain.

289

290 *Correlations among endodormancy and ecodormancy traits*

291 The high negative correlations found between ΔJD and GDH vs. CR (CH, CP and CU)

292 indicate that genotypes with lower CR required a longer period of heat accumulation to bloom.

293 This has been also found in peach (Li et al., 2016) and apricot (Campoy et al., 2012). Li et al.

294 (2016) reported a decrease of 16 days per 200 accumulated CHs, up to a threshold of approx.

295 950 CHs. Overall, these results suggest that in cultivars with low CRs, GDH accumulation just

296 after CR fulfillment is less effectivethan in cultivars with higher CRs, and therefore they need

297 more time to accomplish their HR.

298 A similar result was found for the correlation between DB and CR (CH, CU, and CP)

299 against HR (GDH). Similar high correlations have been found in peach (Fan et al., 2010; Li et

300 al., 2016; Pawasut et al., 2004; Scorza and Okie, 1990) and apricot (Ruiz et al., 2007). We

301 found a high level of variability among the progeny regarding HR, which disagrees with

302 Linsley-Noakes and Allan (1994) who reported no differences in HRs between three nectarine

303 cultivars with different CR. These results suggest the existence of different heat requirements

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

306 first hypothesis is in line with the model for Douglas fir (Harrington et al., 2010), which

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

authors have reported contradictory results (Couvillon and Erez, 1985; Guerriero et al., 2006; Kotowski et al., 1980), 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 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 was found between FT and GDHF which could indicate the importance of warm temperature before dormancy breaking on the flower bud formation and development, as reported previously for plum (Woznicki et al., 2019) The fact that 'Earlygold' is a low CR cultivar 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 could lengthen the days to full bloom date and increase the heat requirement. Together, the 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 break starts before or after the release of endodormancy. Recent reports have shown a positive correlation between August-September temperature and the amount and time of flowering in the following spring on plum (Døving 2009; Woznicki et al., 2019) and sweet cherry (Døving 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-Pérez et al., 2012; Fan et al., 2010). However, there is no previous literature on CR for 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 the almond 'Texas' and the peach 'Earlygold' bloom at the same time even though E has significant lower CR than T.

Genetic control of endodormancy and ecodormancy traits

We have identified one genomic region controlling endodormancy and ecodormancy traits (CR and GDH) located in G6 and another one controlling FT in G1, that have not been 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 single species mapping populations. Data for GDHF, a trait highly correlated to FT, also detected a QTL in G1 co-locating with that of FT, indicating either that GDHF is not related to HR but a different way of measuring FT, or that warm temperatures during the winter dormancy period, and not only after the fulfillment of the CR, are important for determining 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; Scorza and Okie, 1990), since the longer the period for chill accumulation, the higher amount of GDHF likely to be accumulated.

Consistent QTLs across years for CR, HR and FT have been described in various *Prunus* progenies. In peach, Fan et al. (2010) identified QTLs for CR, HR and FT at the latter end of G1, where the evergrowing gene (*Evg*) maps (Bielenberg et al. 2008), and for CR and FT in G4 and G7, where QTLs for FT have been found in various *Prunus* crops (Dirlewanger et al. 2012).In similar positions of G1 and G4, QTLs for CR and/or FT have been detected by Bielenberg et al. (2015) in peach, by Sánchez-Pérez et al. (2012) in almond and by Quilot et al. (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). In all cases, the QTLs for CR coincided in map position with those of FT and produced effects of similar magnitude and gene action, suggesting CR as a major cause for the FT phenotype.

356 Where HR was studied (Fan et al. 2010, Castède et al. 2014, Sánchez Pérez et al 2012), the

357 QTLs were detected at the same positions as those of CR and FT, or were not consistent over

358 the two years and had effects generally opposite to those of the QTLs detected for CR. This

359 suggests that HR is a minor or irrelevant factor in the determination of FT, that its measurement

360 as GDH is inefficient, or both. Our results support these observations as we found that HR and

361 CR detected the same QTL in both years studied. On the other hand, we did not find common

362 QTLs for CR and FT, indicating that FT was mainly determined by factors other than those we

363 measured. A possible explanation for this is that the TxE offspring, from the cross between two

364 cultivars from different species with low CR, had a lower level of variation for CR than other

365 mapping populations studied. In the cold-winter conditions of Lleida, chilling requirements

366 could have been rapidly met, resulting in a narrower distribution of variability that the

367 parameters used measured with low efficiency. In agreement with our results, a QTL for FT

368 was previously found in G1 in the TxE progeny in 2012 and 2013 (Donoso et al. 2016). Here,

369 we also identified a peak with LOD of 2.9 in 2016 for FT in G6 but it was not considered as an

370 stable QTL as it had a LOD<2 in 2017 (results not shown). This does not discount that the CR

371 may be involved in FT variability, with apparently minor effects, although the population size

372 used could have been insufficient to detect them with a significant threshold.

373

374 **Conclusions**

375   All the models for the estimation of CR (Utah, Dynamic and Hours-below 7º) worked well

376 for the area of study, characterized by short but cold winters, with warm falls and springs.

377 However, the Dynamic model seems the most appropriate as it reduced the year-to-year

378 variation observed in the population. The results indicate that, although CR appears to have a

379 more important role than HR in determining flowering time, neither factor had a major effect

16

380 on this trait under the conditions of this research. For HR, the warm temperatures during

381 endodormacy (not only after endodormancy release) may have also influenced flowering time.

382 In summary, our data supports FT as a quantitatively inherited character with a strong genotype

383 x environment component that is affected by both chilling and heat requirements. The observed

384 variation in the CRs within the population studied highlights the importance and feasibility of

385 breeding for low CRs in a new scenario of low chill accumulation due to global warming.

386

392

Tables

394 Table 1. Pearson´s correlation coefficients for seasons 2016 and 2017 between dormancy

395 break (DB), flowering time (FT), chilling requirements for DB [Chill Hours (CH), Chill

396 Units (CU) and Chill Portions (CP)] and HR for blooming [Total Growing Degree hours

397 from 1st October to FT (GDHF), Growing Degree Hours (GDH) and number of days (ΔJD)

398 from DB to FT]. Pearson's correlation coefficients between seasons 2016 and 2017 are

399 indicated in the diagonal.

		2015-2016							
		DB	CH	CU	CP	FT	GDHF	GDH	ΛJD
2016-2017	DB	**0.89**	**0.87**	**0.88**	**0.89**	0.21	0.16	**-0.81**	**-0.86**
	CH	**0.90**	**0.89**	**0.89**	**0.90**	0.22	0.16	**-0.81**	**-0.87**
	CU	**0.86**	**0.84**	**0.85**	**0.86**	0.21	0.16	**-0.78**	**-0.83**
	CP	**0.87**	**0.86**	**0.87**	**0.88**	0.22	0.16	**-0.80**	**-0.84**
	FT	-0.11	-0.07	-0.10	-0.11	**0.39**	**0.54**	0.38	0.25
	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 Values in bold are significant at p≤0.001.

402

403 Table 2. Summary of consistent QTLs identified with the TxE map including trait name,

404 QTL names, LOD score of the maximum peak, position of the maximum peak, closest

405 marker, and parameters of percentage of explained phenotypic variance (R^2), additivity (a),

406 dominance/additivity (d/a) and inferred gene action (GA). DB, dormancy break; FT,

407 flowering time; CH, chill hours; CU, chill units; CP, chill portions; GDHF, growing degree

408 hours to flowering; ΔJD, number of days from DB to FT.

Trait	QTL name	LG	Position (cM)	Closest marker	LOD	R^2	a[a]	d/a[b]	GA[c]
DB-2015	qDB6	G6	2,4	SNP_IGA_6138⸱	3,4	18,4	-7,5	-0,7	PD
DB-2016	qDB6	G6	2,4	SNP_IGA_6138⸱	3,3	18,1	-0,9	-14,6	U
CH-2015	qCH6	G6	2,4	SNP_IGA_6138⸱	3,2	17,3	-95,9	-0,6	PD
CH-2016	qCH6	G6	2,4	SNP_IGA_6138⸱	3,2	17,4	-31,4	-7,0	U
CU-2015	qCU6	G6	2,4	SNP_IGA_6138⸱	3,2	17,2	-129,0	-0,7	PD
CU-2016	qCU6	G6	2,4	SNP_IGA_6138⸱	2,9	16,1	-2,0	-81,3	U
CP-2015	qCP6	G6	2,4	SNP_IGA_6138⸱	3,4	18,4	-5,5	-0,7	PD
CP-2016	qCP6	G6	2,4	SNP_IGA_6138⸱	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	A
GDHF-2015	qGDHF1	G1	5	SNP_IGA_1052(	3,9	19,9	-324,8	0,1	A
GDHF-2016	qGDHF1	G1	1,9	SNP_IGA_2670	3,1	16,8	-144,3	0,1	A
GDH-2015	qGDH6	G6	2,4	SNP_IGA_6138⸱	3	16,3	452,4	-1,2	DP
GDH-2016	qGDH6	G6	2,4	SNP_IGA_6138⸱	3,5	19,9	82,8	-13,6	U
ΔJD-2015	qΔJD6	G6	3	SNP_IGA_6127!	3,6	18,9	5,8	-1,1	DP

409

410 [a]Additive 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 [b]Dominance d = H - [(A + B)]

413 [c]Gene action. U *underdominance*, DP *dominance for peach allele*, PD *partial dominance for peach*

414 *allele*, A *additivity*, AD *partial dominance for almond allele*.

415

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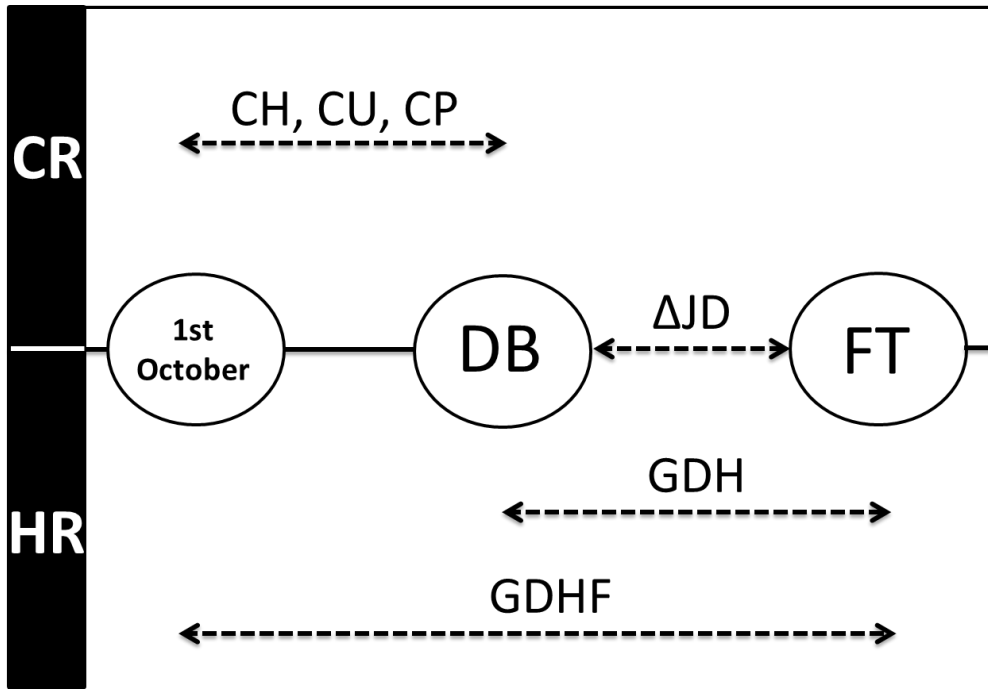
Fig. 1. Scheme of the different traits used in this study. CR: Chilling requirement; HR: Heat requirements; CH: Chill hours; CU: Chill units; CP: Chill portions; DB: Dormancy break; FT: Flowering time; GDH: Growing Degree Hours; GDHF: Growing Degree Hours to flowering; ΔJD number of Julian days between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

423
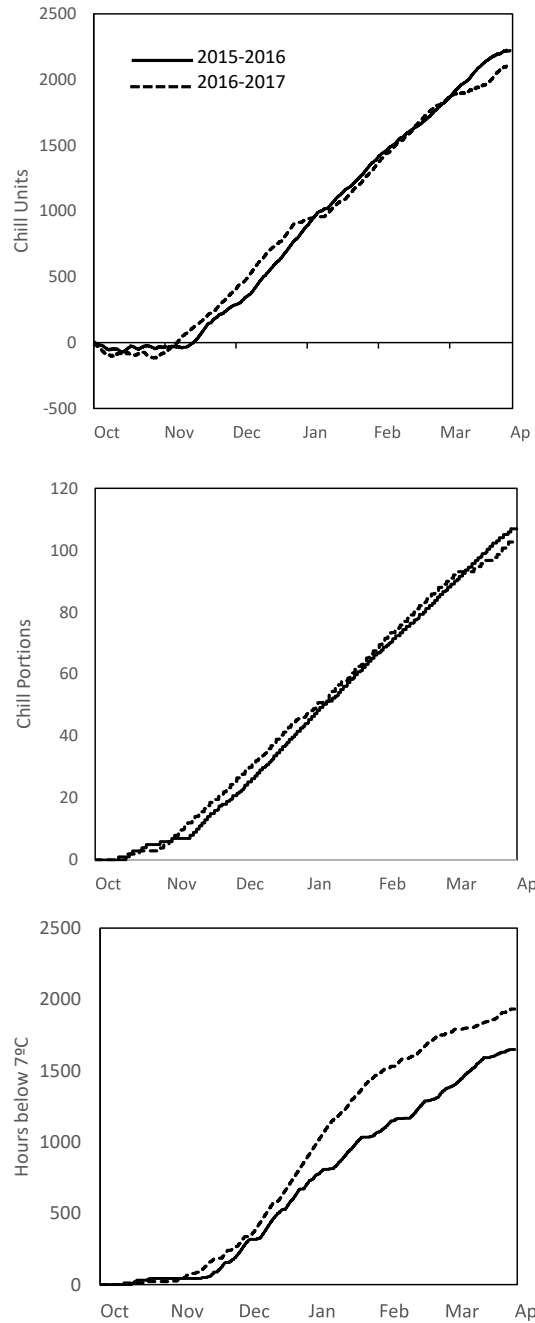
Fig. 2. Progression of chill accumulation in the period October-April in 2016 and 2017 in Gimenells (Lleida). Results are expressed in Chill Units (Utah model) (upper), Chill Portions (Dynamic model) (centre) and Chill Hours (hours below 7ºC) (lower).

427

21
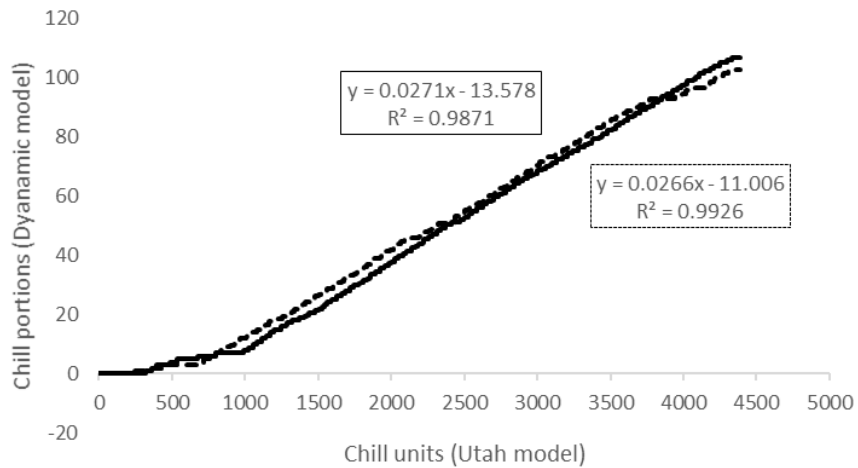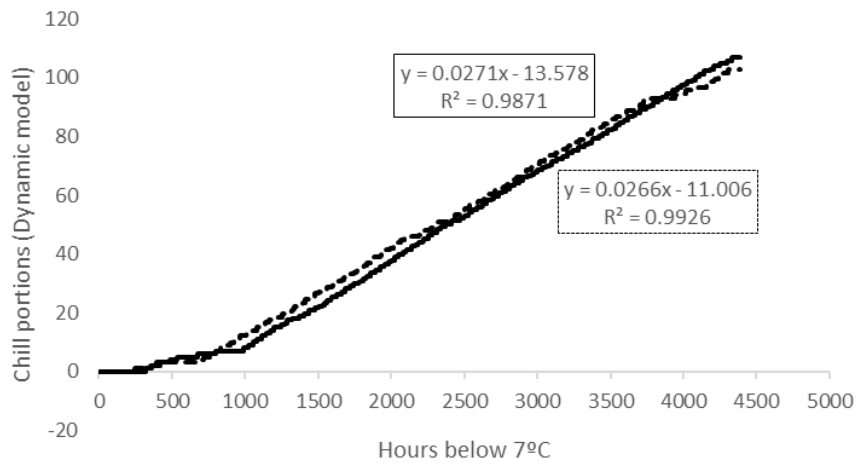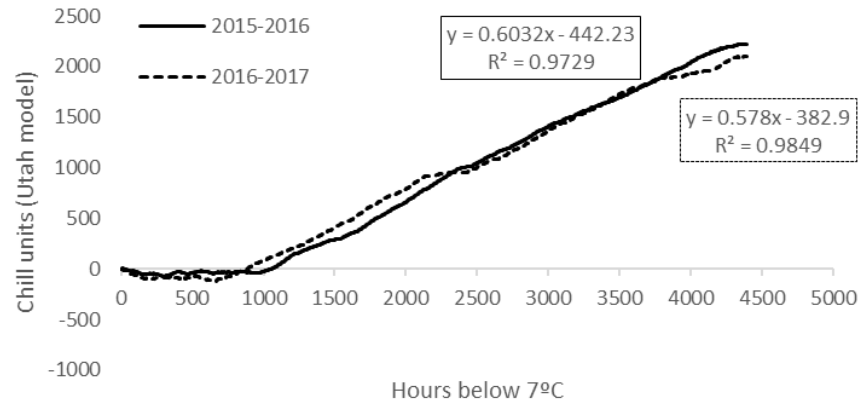
428

429 Fig.3. R-squared regression among CR for breaking dormancy estimated by the Utah, Dynamic

430 and Chill hours models in Gimenells (Lleida, Spain) for 2016 and 2017. All correlations were

431 significant ($p<0.01$).

432
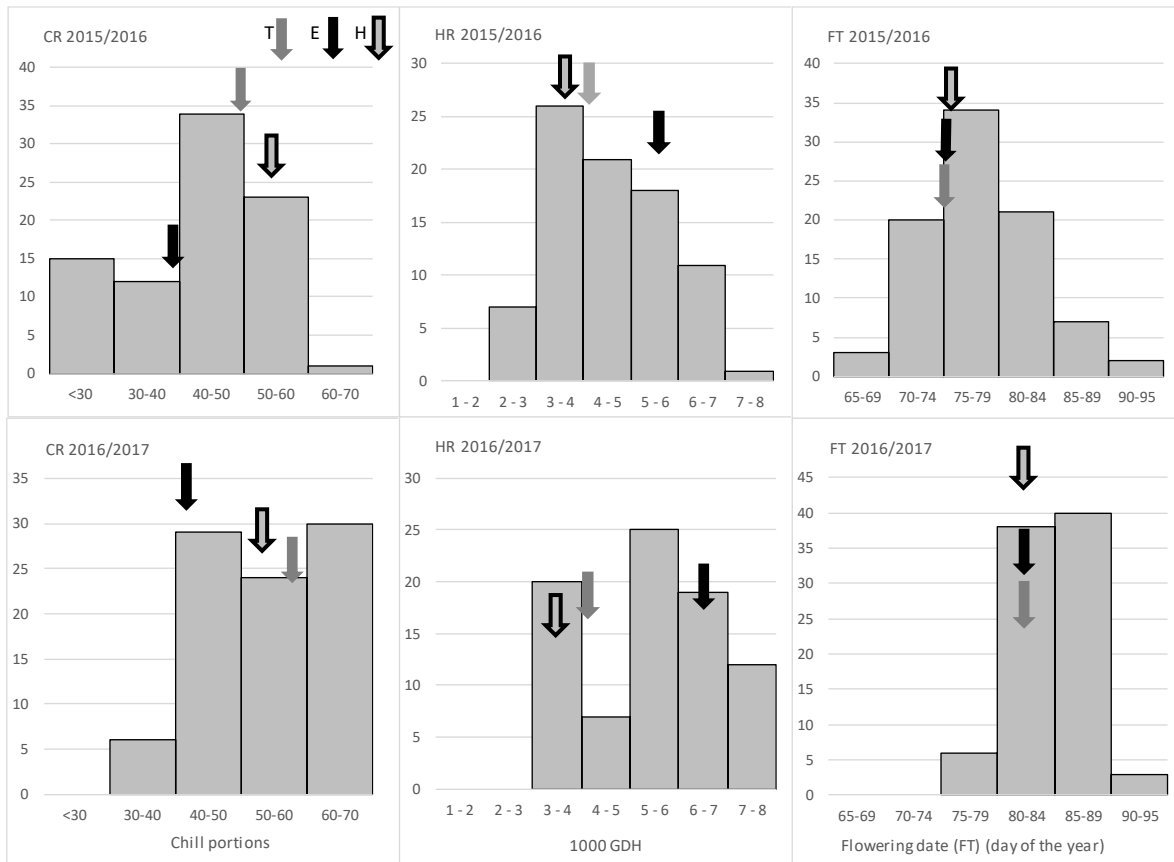
Fig. 4. Distribution of phenological traits in the TxE population: chilling requirements (CR) in chill portions, heat requirements (HR) in growing degree hours (GDH), and flowering times (FT) as 'day of the year'. Data for the parental lines are indicated by arrows (T, 'Texas'; E, 'Earlygold'; H, 'MB 1.37').
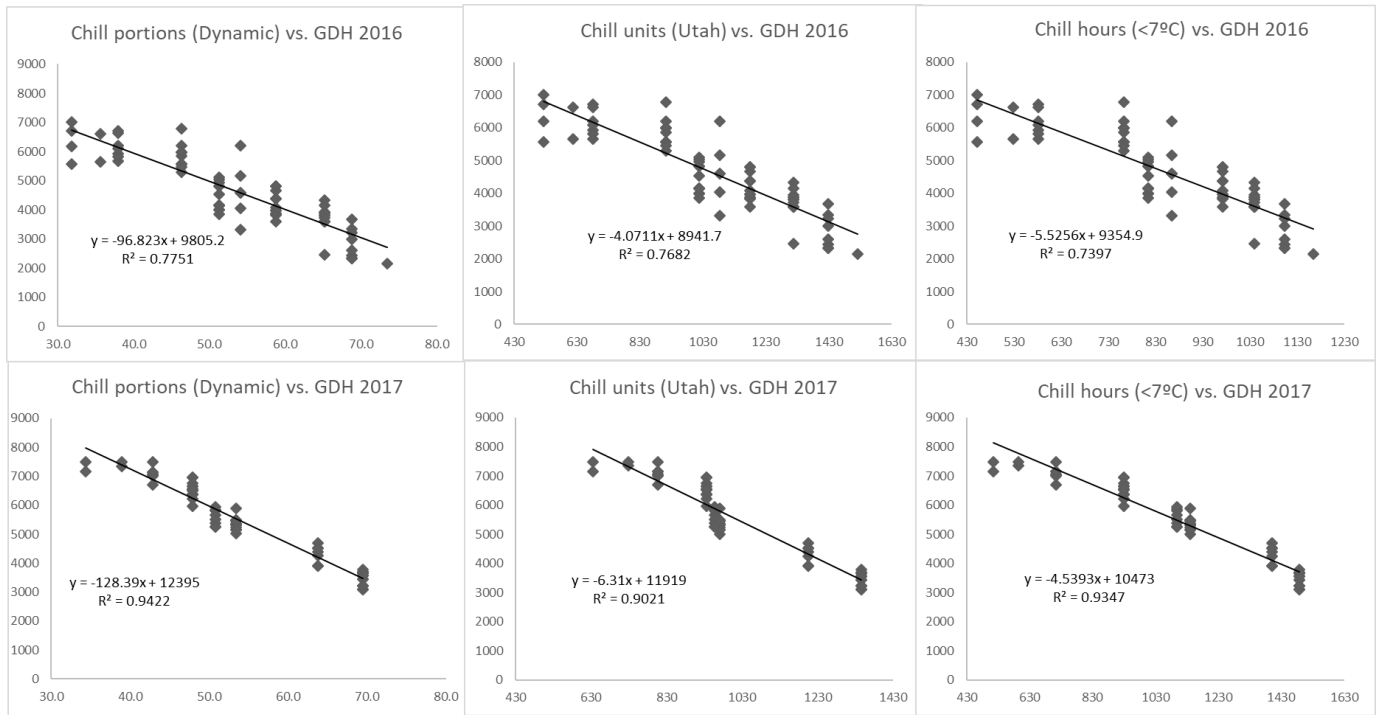
Fig. 5. Linear regression (R-squared) between chilling requirements (CR) and heat requirements in the TxE population for 2015-2016 (above) and 2016-2017 (below). CR are expressed in chill portions (Dynamic model), chill units (Utah model) and chill hours (hours below 7ºC). Heat requirements (HR) are 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

446 studied. Yearly values, mean values (ave) and coefficient of variation (cv) are shown.

447

| Genotype	BD		CH				CU				CP			
	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	673	77.4	36	39	37	2.4
12	4-Feb	2-Feb	1104	1488	1296	271.5	1429	1345	1387	59.0	69	70	69	0.6
14	21-Jan	9-Jan	972	1098	1035	89.1	1180	958	1069	157.0	59	51	55	5.6
15	11-Jan	9-Jan	814	1098	956	200.8	1018	958	988	42.8	51	51	51	0.3
16	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	970	1075	148.1	59	53	56	3.8
34	29-Jan	2-Feb	1039	1488	1264	317.5	1319	1345	1332	18.4	65	70	67	3.1
37	15-Jan	9-Jan	864	1098	981	165.5	1084	958	1021	89.1	54	51	52	2.3
39	4-Feb	2-Feb	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	2-Feb	1039	1488	1264	317.5	1319	1345	1332	18.4	65	70	67	3.1
56	4-Feb	2-Feb	1104	1488	1296	271.5	1429	1345	1387	59.0	69	70	69	0.6
59	24-Dec	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	53	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-Feb	1039	1488	1264	317.5	1319	1345	1332	18.4	65	70	67	3.1
90	24-Dec	2-Jan	582	930	756	246.1	680	936	808	180.7	38	48	43	7.0
91	11-Jan	11-Jan	814	1140	977	230.5	1018	970	994	33.9	51	53	52	1.5
95	2-Nov	2-Feb	1165	1488	1327	228.4	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	21-Jan	11-Jan	972	1140	1056	118.8	1180	970	1075	148.1	59	53	56	3.8
122	16-Dec	19-Dec	452	594	523	100.4	524	728	626	144.2	32	39	35	5.0
125	4-Jan	2-Jan	763	930	847	118.1	914	936	925	15.6	46	48	47	1.1
128	15-Jan	26-Jan	864	1400	1132	379.0	1084	1206	1145	86.3	54	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
199	11-Jan	11-Jan	814	1140	977	230.5	1018	970	994	33.9	51	53	52	1.5
202	24-Dec	14-Dec	582	515	549	47.4	680	634	657	32.5	38	34	36	2.5
211	11-Jan	2-Jan	814	930	872	82.0	1018	936	977	58.3	51	48	50	2.4
215	24-Dec	2-Jan	582	930	756	246.1	680	936	808	180.7	38	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 (ΔJD) 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.

453

| Genotype	FT		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	706	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	3762	94	37	36	37	0.7
47	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-Mar	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-Mar	10-Mar	6706	7051	6878	244	72	76	74	2.8
61	25-Feb	7-Mar	4146	5298	4722	814	45	55	50	7.1
63	12-Mar	12-Mar	4338	4703	4520	258	43	45	44	1.4
69	26-Feb	10-Mar	5294	7051	6172	1242	53	76	65	16.3
72	27-Feb	5-Mar	4045	6208	5126	1529	43	62	53	13.4
73	21-Feb	8-Mar	3321	4383	3852	752	37	41	39	2.8
74	1-Mar	10-Mar	5561	5490	5525	51	57	58	58	0.7
84	10-Mar	10-Mar	3588	3695	3642	76	50	36	43	9.9
85	3-Mar	9-Mar	3588	3650	3619	44	34	35	35	0.7
91	6-Mar	10-Mar	5102	5490	5296	274	55	58	57	2.1
95	6-Mar	4-Mar	2144	3218	2681	759	24	31	28	4.9
98	3-Mar	10-Mar	5456	7490	6473	1438	59	76	68	12.0
105	22-Feb	12-Mar	5987	6744	6365	535	49	69	59	14.1
106	1-Mar	4-Mar	2467	3218	2843	531	32	31	32	0.7
108	1-Mar	5-Mar	6719	7490	7105	545	76	80	78	2.8
117	23-Feb	4-Mar	3859	5012	4436	815	43	53	48	7.1
118	6-Mar	10-Mar	4661	5490	5075	586	45	58	52	9.2
120	8-Mar	10-Mar	4807	5490	5148	483	47	58	53	7.8
122	24-Feb	10-Mar	6188	7483	6835	915	70	81	76	7.8
125	7-Mar	11-Mar	6204	6641	6422	309	63	68	66	3.5
128	7-Mar	10-Mar	6204	4512	5358	1196	52	43	48	6.4
133	4-Mar	10-Mar	5987	6553	6270	400	60	67	64	4.9
152	3-Mar	8-Mar	4591	5361	4976	544	48	56	52	5.7
153	3-Mar	12-Mar	4375	5372	4873	705	42	62	52	14.1
165	4-Mar	4-Mar	3720	3218	3469	355	35	31	33	2.8
166	25-Feb	8-Mar	2329	3567	2948	875	21	34	28	9.2
172	11-Mar	4-Mar	2600	3915	3258	930	37	38	38	0.7
194	1-Mar	10-Mar	4081	5504	4793	1006	40	60	50	14.1
199	4-Mar	9-Mar	4948	5444	5196	351	53	57	55	2.8
202	25-Feb	5-Mar	5819	7156	6487	946	63	80	72	12.0
211	1-Mar	7-Mar	4522	6362	5442	1301	50	65	58	10.6
215	29-Feb	10-Mar	6089	6553	6321	328	67	67	67	0.0
218	25-Feb	5-Mar	4146	5144	4645	706	45	54	50	6.4
226	29-Feb	3-Mar	3976	5253	4614	903	39	54	47	10.6
239	15-Mar	11-Mar	6791	7139	6965	246	72	77	75	3.5
241	10-Mar	3-Mar	4154	3915	4034	169	42	37	40	3.5

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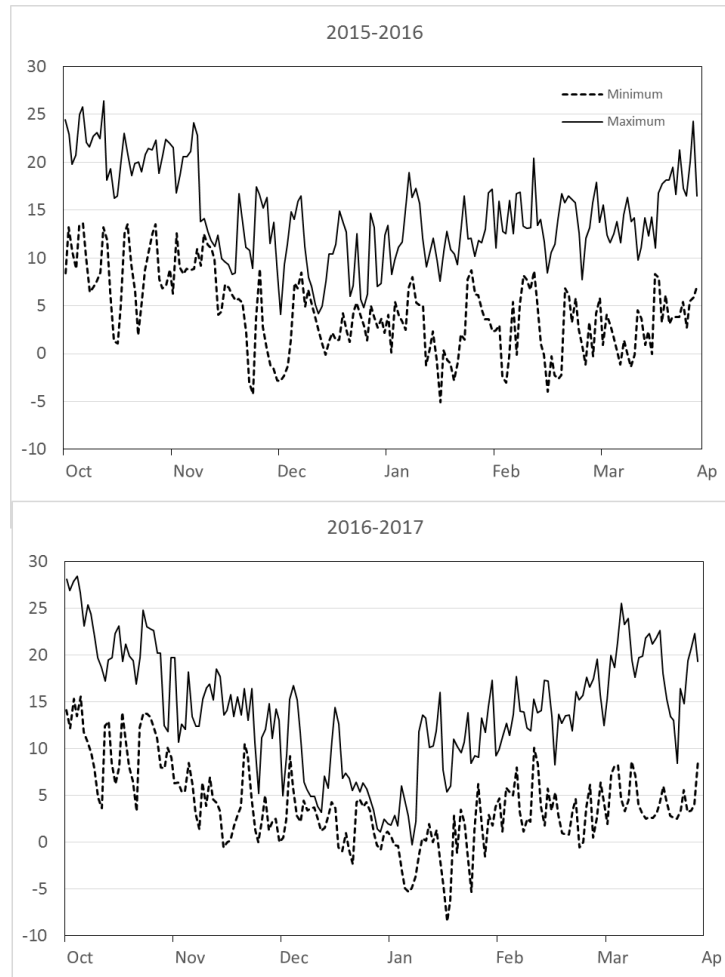
457

458   Fig. S1. Maximum and minimum daily temperatures registered in the period October-April in

459   2016 and 2017 in Gimenells (Lleida).

460
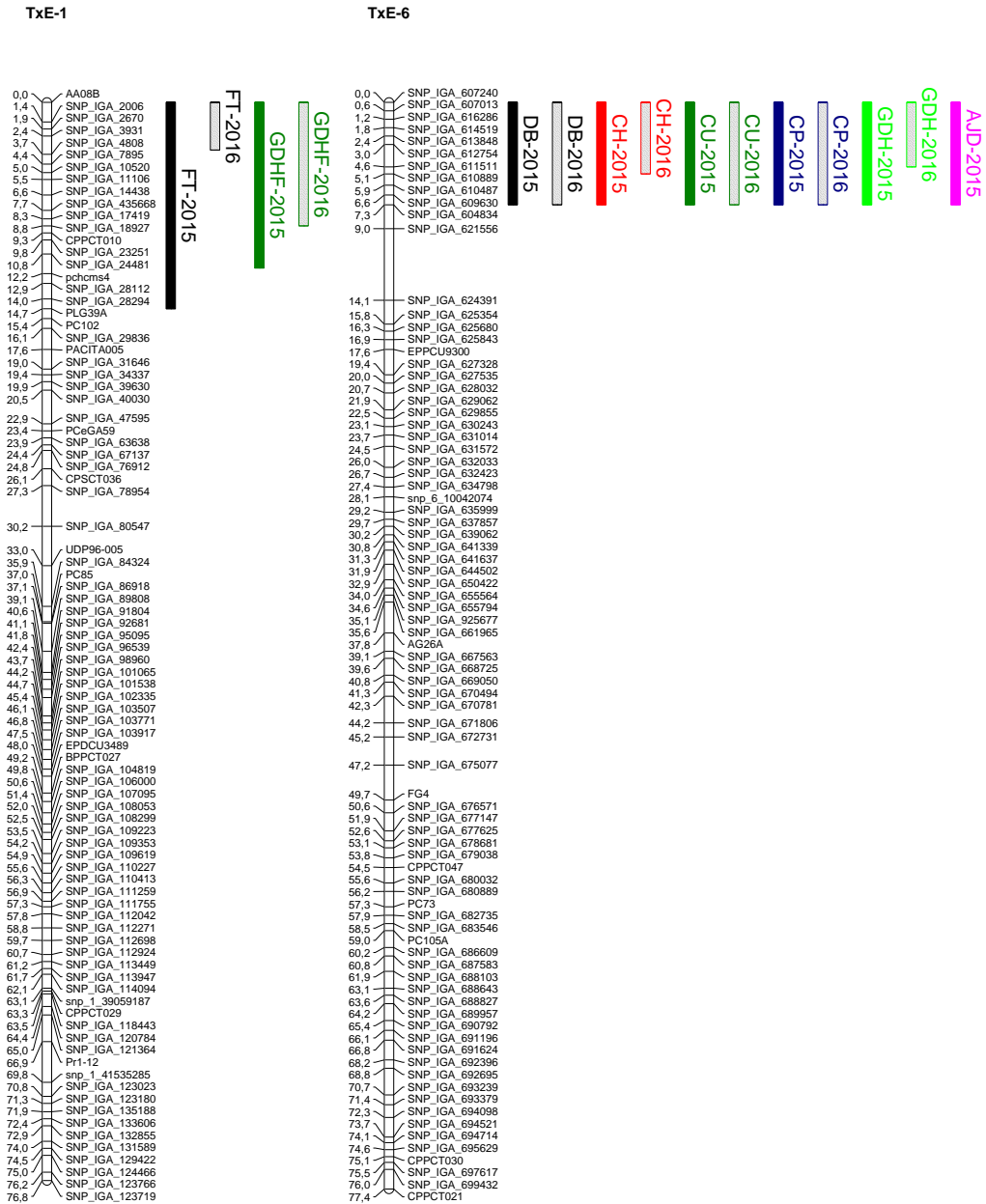
Fig. S2. Map of the G1 and G6 of the TxE population with the positions of the consistent QTLs

mapped in this work. Bars of QTLs indicate the LOD-1 intervals and are named with the trait

and the year of evaluation. Abbreviations: FT, flower time; GDHF, total growing degree hours;

DB, dormancy breaking; CU, chill units; CP, chill portions; GDH, growing degree hours; ΔJD,

days from dormancy breaking to FT.

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