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Effects of ambient temperature and photoperiod on flowering time in faba bean (*Vicia faba* L.)

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Flowering time is a vulnerable stage of plant development and is therefore a significant determinant of adaptation and grain yield in faba bean (*Vicia faba* L.). It is largely controlled by genotype, environmental factors of temperature and photoperiod, and genotype-by-environment interactions. The aim of this study was to evaluate variation in flowering time and the responses of flowering time to ambient temperature and photoperiod in Australian faba bean. Time of sowing experiments were carried out to assess variation among lines for flowering time (measured in days to flowering, thermal time to flowering and node of first flower) and to determine plant sensitivities to ambient temperature and photoperiod by regression analysis in the field, while four controlled environment experiments of differing temperature and photoperiod were undertaken to further analyse the variation in responses. Results showed significant variation in responses to both ambient temperature and photoperiod. Photoperiod was the main factor influencing variation in flowering time, with lines grouped as sensitive, intermediate or insensitive. The responses to ambient temperature were more complex. Most lines fit the traditional linear model, but with possible variation in optimal temperature and/or vernalisation response, while some lines showed temperature insensitivity.

ToC Summary: Flowering time is the most important adaptation trait of plants and is largely controlled by temperature and photoperiod. Evaluation of Australian faba bean genotypes found significant variation in flowering time, and in the plant responses to ambient temperature and photoperiod. This variation could be utilised to breed lines for specific growing environments, increasing yield, yield reliability and possibly expand the production zone into more marginal areas.

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Running head: Environmental control of flowering in faba bean

Additional keywords: floral initiation, legume, pulse, rate of development, reproductive stage.

Introduction

Faba bean (*Vicia faba* L.) is a pulse crop grown on ~2.4 million ha globally (FAO 2016), used primarily for human consumption and animal feed. With a grain protein content of ~30% (Crépon *et al*. 2010), faba bean has been flagged to play a large role in meeting the growing global demand for protein (Multari *et al*. 2015). To help meet the growing demand and ensure food security, the total production needs to increase and the most logical ways of achieving this are to increase the yield in areas already growing faba bean and to increase the production area. In Australia, faba bean was sown

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over a total of 256 000 ha in 2016 (ABARES 2017) across most major winter rainfall-fed agroecological zones, but not to the extent of cereals, which are adapted to a wider range of environments. Enhancing the adaptability of faba bean will be key to increasing yields and production area and one of the most important aspects of plant adaptation is the time of flowering (Patrick and Stoddard 2010). The flowering stage of the plants development is a critical period because the reproductive organs are vulnerable to stresses such as heat, frost and drought (Smith 1982). Therefore flowering and setting of pods needs to occur at a time that avoids these stresses, while also making full use of soil available moisture across the length of the growing season. The flowering time is controlled by the plants genotype, the environment (mainly photoperiod and temperature) and the genotype by environment interactions. Matching genotypes with environments is consequently a vital part of adaptation.

Measuring flowering time is done using several methods, mainly, days to flowering (DF), thermal time to flowering (TTF) and node of first flower (NF) (Evans 1959; McDonald *et al*. 1994). DF is suited to comparing genotypes in a single environment or in different photoperiod environments with the same temperature. Thermal time is calculated by the equation:

$$K = \sum_{i=1}^{n}(T_i - T_b),$$

where K is the thermal time and thermal units are degree-days (°Cd); T_i is the mean temperature of the *i*th day; and T_b is the base temperature, below which no plant development occurs. TTF is suited to comparing genotypes in environments with fluctuating temperatures and across environments with different temperatures. NF is most useful for evaluating the developmental stage that flowering occurs in different environments, where flowering on a higher node shows a delay in the onset of flowering (Collins and Wilson 1974; Murfet 1985). There are also different methods that have been used to measure response (or sensitivity) to photoperiod and temperature. Evaluation of plants grown in a range of environments or times of sowing has been used with the additive model:

$$1/D = a + bT + cP,$$

where *D* is the development period (in days to flowering), *T* is the mean temperature over the period, *P* is the mean photoperiod, and *a*, *b* and *c* are constants that represent values for the intercept, temperature sensitivity and photoperiod sensitivity, respectively (Ellis *et al*. 1988*a*; McDonald *et al*. 1994). Another, simpler method, is to calculate the difference in time to flower of plants grown in controlled environments of either: different photoperiods with the same temperature to measure photoperiod sensitivity, or different temperatures with the same photoperiod to measure temperature sensitivity, as carried out in rice (*Oryza sativa* L.) by Kovi *et al*. (2015).

In faba bean, genotypic variation has been found in flowering time and the responses to photoperiod and temperature (Evans 1959; Ellis *et al*. 1988*a*, 1988*c*; Ellis *et al*. 1990; McDonald *et al*. 1994; Lizarazo *et al*. 2017). Faba bean is generally a long-day plant (requires long days to flower), but day-

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neutral genotypes (that eventually flower regardless of photoperiod) and photoperiod-insensitive genotypes (that flower in the same amount of thermal time regardless of photoperiod) also exist (Evans 1959; Ellis *et al*. 1990; McDonald *et al*. 1994). There are two classifications of temperature that can affect flowering, namely, vernalising (cold) and ambient temperature. Periods of vernalising temperatures decrease the time to flowering in several crops and variation in response to vernalisation has been observed in faba bean (Evans 1959; Ellis *et al*. 1988*a*; McDonald *et al*. 1994), but, the occurrence of a true vernalisation response has been disputed (Ellis *et al*. 1988*b*) and it is not covered in the present study. So, unless stated, further mention of temperature refers to ambient temperature. The variation in response to ambient temperature has not received the same attention as response to photoperiod (or vernalising temperatures). Ellis *et al*. (1990) concluded that all faba bean genotypes require ~1000 degree-days to flower, but, McDonald *et al*. (1994) observed variation in TTF, with different genotypes flowering between 611 degree-days and 972 degree-days in the same environment. Further to this, supra-optimal temperatures have been observed to delay flowering for some species and in a study in wheat (*Triticum aestivum* L.) by Appendino and Slafer (2003), allelic variation at one gene determined whether a plant would have the same TTF in both 16°C and 23°C, or would flower in 342 degree-days *less* under 16°C than 23°C. A study run simultaneously to this one (Catt *et al*. 2017) focussed on detecting quantitative trait loci (QTL) for flowering time and responses to photoperiod and ambient temperature. The study detected eight regions of flowering QTL in an Icarus × Ascot recombinant inbred line population with four of the regions found to be associated with photoperiod response and two of the regions associated with temperature response. The parents of the QTL study flower 14 days apart in a field experiment, but, greater variation in flowering time among breeding lines (51 days between earliest and latest) and released cultivars (30 days between earliest and latest) was observed in the same trial (S. C. Catt, unpubl. data).

Understanding the large variation in flowering time of faba bean and the responses to photoperiod and temperature could be used to assess the suitability of current cultivars to specific environments. More importantly, it will assist in making future breeding decisions and ultimately result in new cultivars with improved yields across the current growing zones and possibly the expansion of the production area into more marginal zones.

The aim of this study was to evaluate the variation in flowering time and responses of flowering time to photoperiod and ambient temperature in Australian faba bean. Time of sowing experiments were carried out to assess the variation among current cultivars and breeding lines for flowering time and response to photoperiod and temperature, and controlled environment experiments were undertaken to further analyse the variation in responses to photoperiod and temperature.

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Materials and methods

2012 and 2013 time of sowing experiments

In 2012, a selection of nine Australian cultivars and breeding lines with varying maturities (Table 1) were sown in pots outside at the Waite Campus, University of Adelaide, Glen Osmond (–34.96°S, 138.63°E). Three replicates were sown of five sowing times (27 April, 11 May, 25 May, 8 June and 22 June), with the sowing times randomised within each replicate and the lines randomised within each time of sowing. Four untreated seeds were sown per pot, which contained a layer of 20-mm drainage bark at the base and were filled with bark mix potting soil. Pots were fertilised with slow release granular fertiliser at the same rate and drip irrigated throughout the duration of the experiment. Each pot was scored for average date of emergence, date at which 50% of the plants had open flowers and average node of first flower (counted from first bifoliolate leaf on whichever stem flowered first). Climate data for daily mean temperature (°C), photoperiod (daylength including civil twilight) (h) and global solar exposure (MJ/m^2) for Adelaide (Kent Town) were obtained from the Bureau of Meteorology (2017). DF was calculated as the number of days between emergence and 50% open flowers. Thermal time to flowering was calculated using the equation:

$$K = \sum_{i=1}^{n}(T_i - T_b),$$

with a base temperature of 0°C assumed, as the error caused by this assumption is minimal (Husain *et al*. 1988). Analysis of mean monthly climate data, DF, TTF and NF was carried out using the one-way and two-way ANOVA functions in GENSTAT 15th Edition (VSN International 2013).

In 2013, four lines that represented the different flowering responses observed in 2012 were selected to repeat the experiment, but sown over 11 dates (10 April, 17 April, 24 April, 1 May, 8 May, 15 May, 22 May, 29 May, 5 June, 12 June and 19 June) and arranged in a nonrandomised fashion to reduce shading interference caused by the large differences in plant size among times of sowing and plant genotype. The experiment was maintained, scored and analysed with the same method as 2012. In addition to 2012, it was noted what stem the NF occurred on (main or secondary) and, if the NF occurred on a secondary stem, the first node to flower of the main stem was also recorded.

Regression analysis of the additive model: $1/D = a + bT + cP$ was done in Microsoft Excel 2013 to determine the coefficients for photoperiod and temperature sensitivity of each line in both years. Instead of using mean photoperiod over the entire period of plant development for *P* like Ellis *et al*. (1988*a*) and McDonald *et al*. (1994), the photoperiod at the time of flowering was used in order to more closely estimate the critical photoperiod for floral initiation.

Evaluation of Australian cultivars and breeding lines in controlled environments

A slightly different selection of Australian cultivars and breeding lines with broader flowering responses were used in the controlled environment experiment (Table 1) over 2014 and 2015.

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The 11 selected lines were grown in a Conviron PGC20 Flex reach-in plant growth chamber (Conviron Ltd, Winnipeg, Canada) in The Plant Accelerator, at the Waite Campus, University of Adelaide, Glen Osmond. Lighting consisted of 54-W high-output fluorescent tubes as well as incandescent lights for infrared output. Plants were grown under four treatments with three photoperiods and two temperatures (Table 2). Each treatment was a randomised complete block design with four replicates per line (run at the same time) and three plants per replicate. Untreated seeds were sown in 0.55-L punnets filled with bark mix potting soil and placed in trays (12 punnets per tray) on the floor of the reach-in chamber. Plants were watered regularly, monitored every 2–3 days and scored for date of emergence, date of first open flower and NF (counted from the first bifoliate leaf on the main stem where possible, or a secondary stem where the main stem failed to flower). DF for each plant was recorded as days from emergence to first open flower. Plants that did not flower by the end of the experiment were given the number of days from emergence to the last day of scoring, plus an additional 14 days as a value for analysis to separate them from the lines that did flower, but were not given a value for NF. The data for DF, TTF and NF was analysed using the two-way ANOVA function in GENSTAT 15th Edition (VSN International 2013).

Results

As the time of sowing experiment was done in the same location, photoperiod was not significantly different between years (Table 3). Global solar exposure was only different between years in September, with a higher value in 2012 (Table 3). Mean monthly temperatures were significantly warmer in 2013 for the months of May, August and September (Table 3). The difference in DF and TTF among lines decreased with a later sowing date and generally the DF and TTF decreased with a later sowing date (Fig. 1). The only significant exception to this was AF03001-1 in 2013, which significantly increased in DF and TTF between the first (10 April) and last (19 June) sowing date. For each sowing date in 2012, the DF and TTF of PBA Samira and PBA Rana were not significantly different from that of Nura, Farah was not significantly different from that of Ascot, and AF08108 was not significantly different from that of AF03001-1 (Supplementary materials fig. 1, as available at journal's website).

In 2013, the NF occurred on a secondary stem for over 50% of Nura and Icarus for the first five sowing dates, and Ascot for the first three. The NF over sowing dates was slightly different in 2012 than 2013 (Fig. 1). In 2012, Ascot and Nura had a significant drop in NF between the first (10 April) and second (11 May) sowing date and then flowered around a consistent node for the remaining sowing dates, whereas, Icarus consistently flowered around the 8th node for all sowing dates and AF03001-1 consistently around the fifth node. In 2013 AF03001-1 remained consistent with flowering on the fifth node, whereas Icarus and Nura generally had a decrease in NF as the sowing date got later, and Ascot had an increase in NF between the first (10 April) and fourth (1 May) sowing date and then decreased from there over the remaining sowing dates. For each sowing date in 2012, the NF of Farah

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was not significantly different from that of Ascot and AF08108 was not significantly different from that of AF03001-1 (Supplementary materials fig. 1). PBA Samira and PBA Rana flowered on a higher node than Nura for the first three sowing dates, the same node for the fourth sowing date and PBA Samira flowered on a significantly higher node than Nura for the last sowing date (Supplementary materials fig. 1).

For Ascot, Nura and Icarus the required thermal time to flowering decreased as the final photoperiod increased, whereas for AF03001-1, the thermal time to flowering remained relatively constant irrespective of the photoperiod at flowering (Fig. 2). Both the minimum TTF and photoperiod at flowering for each line increased in the order: AF03001-1, Ascot, Nura and then Icarus. As expected, AF08108 followed a very similar pattern to AF03001-1, Farah was similar to Ascot and PBA Samira and PBA Rana were similar to Nura (Supplementary materials fig. 2).

In 2012, only AF08108 and Farah had positive, significant temperature sensitivity coefficients (Supplementary materials table 1), whereas the rest of the lines had non-significant values. In 2013, AF03001-1 and Nura had positive values, with AF03001-1 being the most sensitive to temperature (Table 4). For the photoperiod sensitivity coefficients in 2012, all lines were positive and significant, increasing in sensitivity in the order: AF08108, AF03001-1, Farah, Ascot, PBA Rana, Nura, PBA Samira and Icarus (Table 4 and Supplementary materials table 1). The order remained consistent in 2013, but with a significantly higher coefficient than 2012 (other than AF03001-1, which had a non-significant coefficient for photoperiod sensitivity).

Treatments of different photoperiod showed a large amount of variation among lines in response to photoperiod (Fig. 3). Under photoperiods of 10- and 12-h for lines Aquadulce, Ascot, Nura, PBA Rana and Icarus, at least 50% of the individual plants did not flower within the time constraints of the experiment and as such, were deemed to be very sensitive to photoperiod. These lines were delayed by at least 62 to 92 days by the 12-h treatment compared with the 18-h treatment and (within the time constraints of this experiment) were not further delayed by the 10-h treatment. Lines AF03001-1 and AF08108 were not significantly delayed by the 12-h treatment compared with 18 h or by 10 h compared with 12 h, but a decrease from 18 h to 10 h resulted in a delay of 12 and 16 days, respectively (showing relative insensitivity). Doza and PBA Warda were also not significantly delayed by 12 h compared with 18 h, but were delayed by 38 and 46 days by the 10-h compared with the 12-h treatment, respectively. PBA Nasma was not significantly different under the 12-h and 10-h treatments, but these treatments flowered ~40 days later than under the 18-h treatment. Farah was the only line where each decrease in photoperiod resulted in a significant delay in DF, where shortening from 18 h to 12 h caused a 13-day delay and the 10-h treatment caused a further 23-day delay. These lines (Doza, PBA Warda, PBA Nasma and Farah) were considered as having a more intermediate sensitivity to photoperiod as they were not as strongly delayed by shorter photoperiods as the very sensitive lines. NF is not shown to compare photoperiod treatments because in the short photoperiods

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a high number of plants did not flower (36% of plants in 12 h and 41% in 10 h) and 21% of the plants that did flower in the 12-h treatment, flowered on secondary stems rather than the main stem. Secondary stems have fewer nodes than the main stem at the same point of development, skewing the data.

The three methods of measuring time to flower used in the controlled environment experiment told different stories in terms of the temperature sensitivity of each line. Measured in DF, every line took significantly more days to flower under the 11°C treatment than the 22°C treatment, with the 11°C treatment causing delays from 15 (Aquadulce) up to 49 days (Icarus) (Fig. 4). When measured in TTF, only three lines could be said to be delayed by the 11°C treatment (Icarus, AF03001-1 and AF08108), while five of the lines were not significantly different in TTF between the two temperature treatments (Doza, PBA Warda, Nura, PBA Nasma and Ascot), and the remaining three lines (Farah, PBA Rana and Aquadulce) flowered in less thermal time under the 11°C treatment, indicating they were delayed by the 22°C treatment (Fig. 5). Then, when measured in NF, Icarus, AF03001-1 and AF08108 flowered on the same node in both temperature treatments, whereas the other lines flowered on higher nodes under the 22°C treatment than the 11°C treatment, with lines flowering between 2.6 (Doza) and 8.1 nodes (Aquadulce) higher under the 22°C treatment (Fig. 6).

Discussion

The main purpose of this study was to evaluate the variation in flowering time among Australian cultivars and breeding lines of faba bean and to investigate the responses of flowering time to photoperiod and ambient temperature. The results confirmed that variation in flowering time exists within Australian cultivars and breeding lines of faba bean and more importantly provided strong evidence that not only does photoperiod and temperature play a critical role in determining flowering time, but also that the lines tested vary significantly in their response (level of sensitivity) to both photoperiod and temperature.

The flowering times of the tested lines varied significantly across a range of sowing dates and years when grown under natural conditions of temperature and photoperiod at the Waite Campus, South Australia (–34.96°S 138.63°E). Four groups of similarly responding lines were observed, characterised by: AF03001-1 (very early), Ascot (early), Nura (mid) and Icarus (late); confirming previous observations from variety guides and field trials. The effect of sowing date and year on flowering time and the variation among lines can be explained by the variation in responses observed in the controlled environment experiment and by the coefficients determined from regression analysis of the time of sowing experiment.

For photoperiod response, lines tested in the controlled environment experiment can be grouped as: sensitive (Aquadulce, Ascot, Icarus, Nura and PBA Rana), intermediate (Doza, Farah, PBA Nasma and PBA Warda), or insensitive (AF03001-1 and AF08108). This is somewhat backed up by the

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ranking of photoperiod coefficients from the regression analysis, although, whereas lines AF03001-1 and AF08108 had the lowest coefficients, they were still significant in 2012. Photoperiod response has previously been linked to the origin of germplasm in legumes (Roberts and Summerfield 1987), where sensitivity increases as the distance from the equator increases, but no such correlation can be made from this study, with photoperiod sensitive lines coming from the equator (Icarus – Ecuador) and far from the equator (Ascot – Greece, and Aquadulce – Spain). The Australian area of production, however, does seem to have a correlation with photoperiod response. Lines grown in the northern region of Australia (closer to the equator) are less sensitive to photoperiod than those grown in the southern region.

Analysing temperature response was more complex, but can be dissected by looking at the three methods of measurement in the controlled environment experiment. For all lines in this study, the DF decreased with a higher temperature. This was expected because of the increased rate of metabolism and growth rate that comes with higher temperatures (Gillooly *et al*. 2001). The fact that there was variation in the amount the DF decreased among lines, however, showed there was more at play than just increased metabolic rate. The TTF and NF measurements gave more insight to what may have caused this variation. The linear model: $1/D = a + bT + cP$ fit by Ellis *et al*. (1988*a*), assumes that for a given photoperiod, the TTF will be the same for temperatures between the base temperature (T_b) and the optimum temperature (T_o). Most of the lines in this experiment (Ascot, Doza, Nura, PBA Nasma and PBA Warda) fit this model as they had the same TTF for 11°C and 22°C. The fact that some lines (Aquadulce, PBA Rana and Farah) had a higher TTF under 22°C than 11°C could be explained by 22°C being supra-optimal for these lines ($>T_o$) and could therefore still fit the linear model. Ellis *et al*. (1988*c*) found that a selection of lines had optimal temperatures between 19.9°C and 25.4°C and Lizarazo *et al*. (2017) found the ceiling temperature to be 20°C when lines were grown in an 18-h photoperiod, so this is a likely explanation. If there is variation in optimal temperature and temperatures below 22°C are supra-optimal for some lines, this would have consequences for breeding for warmer environments and for flowering times in a warming global climate. An alternate possibility is that these lines had vernalisation requirements that were met by the 11°C treatment and not by the 22°C, which is also feasible (Evans 1959; Ellis *et al*. 1988*a*). Neither supra-optimal temperatures nor vernalisation can explain, however, how some lines (Icarus, AF08108 and AF03001-1) had a lower TTF under 22°C than 11°C and it would be unlikely that 11°C is close to the base temperature for these lines, as the base temperature is commonly assumed to be ~0°C (Ellis *et al*. 1988*a*; McDonald *et al*. 1994; Turpin *et al*. 2003) or up to 2.5°C (Iannucci *et al*. 2008). This means these lines do not fit the linear model. In *Arabidopsis*, mutants that have an altered, non-functioning thermosensory signalling pathway lose their temperature sensitivity and flower at the same time (as measured in leaf number before flowering) across different temperatures (Blázquez *et al*. 2003). If the thermosensory pathway is conserved in faba bean (as suggested by Nelson *et al*. (2010)), an altered

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pathway could explain this response and why they flowered in the same developmental stage (NF) for 22°C and 11°C for a given photoperiod. These 'temperature-insensitive' lines are possibly like the early flowering line 'Kontu', which was described by (Lizarazo *et al*. 2017). All the other lines flowered on higher nodes with the higher temperature, which is more consistent with the results of Evans (1959). From the regression analysis, temperature coefficients did not appear to provide much insight other than the fact that they were much lower and less often significant than the photoperiod coefficients. This may be due to the relatively narrow range of mean temperatures experienced over the sowing dates and the experiment only taking place in one location.

As well as photoperiod and temperature having individual effects on flowering time, plotting TTF against photoperiod for the time of sowing trial suggested an interaction between photoperiod and temperature. Iannucci *et al*. (2008) concluded that faba bean (as well as other legumes) flowers after reaching minimum requirements of photoperiod and thermal time. This study supports their conclusion; however, it is not simply a case of flowering as soon as the minimum requirements are met. Otherwise all points on the graphs would rest on the axes of the relevant minimum requirements and not be sloped in the way they are. This suggests an interaction between photoperiod and temperature, as previously alluded to by Evans (1959), who found that although time to flowering of faba bean was hastened with warmer temperatures under continuous light (to no limit within tested temperatures), under short photoperiods, the time of flowering was delayed by temperatures above a certain limit and the degree of delay increased as the photoperiod decreased. This delaying effect of warm temperatures under short photoperiods has also been described in pea (Berry and Aitken 1979) and chickpea (Daba *et al*. 2016).

With the knowledge of how each line responds to photoperiod and temperature, most of the differences in flowering time over the sowing dates and years can be explained. The difference between 2012 and 2013 is explained by the warmer mean temperatures experienced in 2013 (particularly in May), as photoperiod was consistent over years. Lizarazo *et al*. (2017) found that as well as temperature and photoperiod, solar radiation and water deficit also affects flowering time. For this experiment, however, solar radiation (measured by global solar exposure) was only significantly different in September, after the first three times of sowing had begun flowering, and regular drip watering in both years would reduce the chances of water deficit, although the possibility of small effects of both cannot be ruled out. For photoperiod-sensitive lines Ascot, Icarus and Nura, DF was much the same in both years, whereas TTF was lower in early sowing dates in 2012. These photoperiod-sensitive lines accumulated more degree-days before reaching their photoperiod requirements. AF03001-1 behaved differently because it is relatively insensitive to photoperiod and temperature and is more limited by earliness *per* se and flowers at the same development stage (NF), flowering consistently around the fifth node. Metabolism and growth rate increase exponentially with temperature (Gillooly *et al*. 2001), therefore the earliest sowings that experienced the highest

temperatures likely developed to the fifth node faster and flowered in less DF and TTF than the later sowings. Variation in earliness *per se* is also the most likely cause of differences in flowering times among the lines grown in supposedly optimal conditions (for progression to flowering), such as the long day, high temperature treatment in the controlled environment experiment and the late sowings of the time of sowing experiment.

The NF was found to be inconsistent for making comparisons between years and lines in the natural conditions of the time of sowing experiment. The NF of later flowering lines was erratic and difficult to measure for early sowing dates, as the first flower often appeared on a secondary stem, causing the data to be skewed negatively. A more consistent method of recording NF that allows for situations where the first flower appears on a secondary stem may resolve this issue, possibly by counting the total number of nodes on the main stem at the time when the first flower appears.

Importantly, lines have been detected with greater variation in photoperiod and temperature response than observed in the QTL study run alongside this one (Catt *et al*. 2017). Together with further studies to understand better the mechanisms behind the environmental responses (particularly vernalisation, and optimum temperatures), the loci (and corresponding markers and candidate genes) implicit in conferring the variation in temperature and photoperiod response seen in this study could be identified using lines that represent the greater variation as parents in future QTL mapping populations. Validation of markers and determining additive effects and interactions between markers by a series of multi-locational trials would then provide the opportunity to go down the path of marker-assisted selection for lines with different levels of temperature and photoperiod sensitivity. This would assist in the efficient and more effective breeding for lines adapted to specific growing environments, increasing yield, yield reliability and possibly the expansion of the production zone into more marginal areas.

Conflicts of Interest

The authors declare no conflicts of interest.

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416 **Table 1. Australian cultivars and breeding lines evaluated in the time of sowing experiments**
417 **(TOS) and controlled environment experiment in the Plant Accelerator (TPA)**

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Line	Experiment/s	Australian area of production	Pedigree	Origins of germplasm	Flowering time[B]
AF03001-1	TOS 2012, 2013 and TPA	NA[A]	Acc 46 × Farah	Greece, Spain	Very early
Ascot	TOS 2012, 2013 and TPA	Southern	Fiord selection	Greece	Early/mid
Nura	TOS 2012, 2013 and TPA	Southern	Icarus × Ascot	Ecuador, Greece	Mid
Icarus	TOS 2012, 2013 and TPA	Southern	BPL 710 selection	Ecuador	Late
AF08108	TOS 2012 and TPA	NA	PBA Rana × AF03001-1	Ecuador, Lebanon, Greece, Spain	Very early
Farah	TOS 2012 and TPA	Southern	BPL 1196 selection	Spain	Early/mid
PBA Rana	TOS 2012 and TPA	Southern	974 × (611 × 974)	Ecuador, Lebanon	Mid
PBA Samira	TOS 2012	Southern	(611 × 722) × ((Icarus × Ascot) × Farah))	Lebanon, Ecuador, Greece, Spain	Mid
Aquadulce	TPA	Southern	Local selection	Spain	Mid
Doza	TPA	Northern	Acc383 × STW	Ethiopia, Sudan	Early
PBA Nasma	TPA	Northern	IX38/1 × IX4-16	China, Sudan	Early
PBA Warda	TPA	Northern	SP99046 × SP99081	Ecuador, Greece, Ethiopia	Early

[A]Not applicable.

[B]As per variety guides and observations in field trials at Turretfield, SA.

Table 2. Treatment conditions for the evaluation of Australian cultivars and breeding lines in controlled environments

Treatment	Photoperiod	Temperature
1	18 h	22°C (±2°C)
2	10 h	22°C (±2°C)
3	12 h	22°C (±2°C)
4	18 h	11°C (±2°C)

Table 3. Climate statistics for the growing season in Adelaide (Kent Town) in 2012 and 2013 (Bureau of Meteorology 2017)

n.s., no significant difference between years (l.s.d.; two-way ANOVA, $P \leq 0.05$)

Month	Mean temperature (°C)			Mean daylength (inc. civil twilight) (h)			Mean daily global solar exposure (MJ/m^2)		
	2012	2013		2012	2013		2012	2013	
April	18.4	18.6	n.s.	12.1	12.1	n.s.	14.5	14.0	n.s.
May	13.8	16.5		11.2	11.2	n.s.	9.2	9.7	n.s.
June	11.6	12.5	n.s.	10.8	10.8	n.s.	7.7	7.4	n.s.
July	11.6	12.7	n.s.	11.0	11.0	n.s.	8.5	8.3	n.s.
August	11.8	13.2	–	11.7	11.7	n.s.	10.6	10.9	n.s.
September	14.8	17.5	–	12.7	12.7	n.s.	16.8	14.8	–

Table 4. Coefficients (×10^4) in the Eqn 1/D = $a + bT + cP$ for lines of faba bean determined from regression analysis of sequential sowings in 2012 and 2013 at the Waite Campus

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

	Intercept	Temperature	Photoperiod
	a	*b*	*c*

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Line	Year	*Coeff.*	*s.e.*	*Coeff.*	*s.e.*	*Coeff.*	*s.e.*	R^2
AF03001-1	2012	29.4 n.s.	± 49.8	8.1 n.s.	± 3.9	9.3***	± 1.5	0.77
AF03001-1	2013	–169.9 n.s.	± 113.4	22.1***	± 2.4	7.0 n.s.	± 8.2	0.93
Ascot	2012	–168.5 n.s.	± 83.5	9.9 n.s.	± 6.5	17.8***	± 3.3	0.73
Ascot	2013	–102.4 n.s.	± 83.3	–1.6 n.s.	± 2.8	24.3***	± 4.7	0.88
Nura	2012	–41.6 n.s.	± 101.0	–7.3 n.s.	± 9.4	22.4***	± 5.0	0.64
Nura	2013	–548.8***	± 102.8	9.1*	± 4.0	48.0***	± 5.5	0.91
Icarus	2012	–396.5***	± 73.0	5.6 n.s.	± 7.5	37.7***	± 5.1	0.89
Icarus	2013	–654.0***	± 104.0	5.2 n.s.	± 4.1	58.4***	± 6.2	0.92

Fig. 1. Effect of time of sowing on time to flower of faba bean lines measured in days from emergence to flowering (*a* and *b*), thermal time from emergence to flowering (*c* and *d*) and node of first flower (*e* and *f*) in 2012 (*a*, *c* and *e*) and 2013 (*b*, *d* and *f*) grown in sequential sowings at the Waite Campus. Error bars indicate the least significant difference (l.s.d.; two-way ANOVA, $P \leq 0.05$).

Fig. 2. Thermal time to flower plotted against the photoperiod at the time of flowering of faba bean lines (*a*) AF03001–1, (*b*) Ascot, (*c*) Nura and (*d*) Icarus sown between 27 April and 22 June 2012 (♦) and 10 April and 19 June 2013 (◊). Error bars indicate the least significant difference (l.s.d.; one-way ANOVA, $P \leq 0.05$) for thermal time to flower (vertical) and photoperiod (horizontal) for the 2013 data.

Fig. 3. Average days to flower for lines of faba bean grown in three controlled photoperiod environments (18 h, 12 h and 10 h) at 22°C. Error bars indicate the least significant difference (l.s.d.; two-way ANOVA, $P \leq 0.05$) of 11.82 days.

Fig. 4. Average days to flower from emergence for faba bean lines grown in constant temperatures of either 22°C or 11°C under an 18-h photoperiod in The Plant Accelerator at the Waite Campus. Error bars indicate the least significant difference (l.s.d.; two-way ANOVA, $P \leq 0.05$) of 5.02 days.

Fig. 5. Average thermal time to flower from emergence for faba bean lines grown in constant temperatures of either 22°C or 11°C under an 18-h photoperiod in The Plant Accelerator at the Waite Campus. Error bars indicate the least significant difference (l.s.d.; two-way ANOVA, $P \leq 0.05$) of 83.4 degree-days.

Fig. 6. Average node of first flower for faba bean lines grown in constant temperatures of either 22°C or 11°C under an 18-h photoperiod in The Plant Accelerator at the Waite Campus. Error bars indicate the least significant difference (l.s.d.; two-way ANOVA, $P \leq 0.05$) of 1.84 nodes.