Chapter 9
Seasonal Flowering and Evolution: Will Plant Species Be Under Stress from Global Warming?

Rod W. King

Abstract In regulating their seasonal flowering, plants have adapted to many environmental inputs including daylength, temperature, and light intensity. Adaptation to temperature alone explains flowering of *Pimelea ferruginea* (King et al. 1992), a Western Australian perennial shrub. In the laboratory, plants from the 650 km of its north–south coastal distribution show clinal adaptation for thermoregulated flowering: those from higher latitudes require cooler temperatures (15°C) than the more equatorial ones (21°C). This adaptation confers evolutionary advantage because, in reciprocal field transplant nurseries, all lines flowered after a cool field winter (13–15°C), but those originating from cooler sites failed to flower at the warmest (17–19°C) extreme of its latitudinal distribution. Thus, thermoregulated flowering of *P. ferruginea* provides an adaptive advantage. A less extreme response is evident in *Crowea exalata*, another Australian shrub. Increased temperature linearly reduces its flower numbers (16% loss per 3°C) and causes earlier flowering. Clearly, a 3–4°C global warming will restrict flowering and sometimes cause species extinction.

9.1 Introduction

For plants, the seasonal timing and extent of their flowering is critical for survival. They time their flowering and seed set to avoid extreme seasonal environments including frost, heat, and drought. Essentially, they use the seasonal changes in environment to predict and avoid imminent stress.

Studies in the early part of the last century established the importance for flowering time of winter cold (vernalization at temperatures below 10°C; see Gassner 1918; Lang 1965) and daylength (photoperiod; see Garner and Allard

R.W. King
CSIRO, Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia
e-mail: rod.king@csiro.au

P. Pontarotti (ed.), *Evolutionary Biology – Concepts, Biodiversity, Macroevolution and Genome Evolution*, DOI 10.1007/978-3-642-20763-1_9,

1920). In addition, in some species, rainfall, sunlight intensity, and mild temperatures can participate in this seasonal signaling (reviewed in King and Heide 2009). Thus, a species may not only adapt its flowering response to match latitudinal differences in daylength and vernalizing winter temperatures but also to a complex of other environmental inputs.

Recent molecular and genetic approaches have confirmed the heritability of flowering time response (see review in Koornneef et al. 2003), but it has been more difficult to establish evolutionary advantage and species fitness. For example, in a population of plants developed by intermating 19 accessions of the annual European herb, *Arabidopsis thaliana*, Scarcelli and Kover (2009) found that selection pressure in the laboratory for early flowering in the absence of vernalization (cold) led to a decrease in the frequency of alleles of *FRI*, a vernalization-requiring gene. However, *FRI* only accounted for 12% of the variation in flowering time. Furthermore, for another cold sensing gene, *FLC*, in field plantings, its action on flowering time could be overridden in ways yet to be explained (Wilczek et al. 2009).

In domesticated crop plants, the genetics of environmental regulation of flowering time is becoming increasingly well understood (see review in Trevaskis et al. 2007). As a consequence, cultivars can be deliberately adapted to particular growing zones. However, these studies do not directly test evolutionary fitness in natural populations: a limitation also evident in studies with derivative populations (see above for Arabidopsis).

With wild species, although adaptation can be seen from studies in a common field nursery or in controlled environments, a test for selective advantage may need reciprocal field nursery transplantation experiments (Mitchell-Olds et al. 2007). Even so, such field studies must meet a number of specific requirements:

1. To avoid site-to-site shifts in the mix of critical environmental determinants of flowering, its regulation should be as simple as possible and, preferably, involve a response to a single environmental input.
2. To avoid indirect effects including responses involving germination and vegetative growth, transplantation should involve plants, which are still vegetative but fully competent to flower.
3. Arbitrary site selection needs to be avoided. Sites selected for "convenience" may introduce complex and somewhat uncharacterized environmental differences. Even if selected for convenience, the sites should fall within the natural distribution of the species.

This review focuses on adaptation of flowering time to mild seasonal temperatures (10–20°C) coupled with possible effects of global warming on species survival. This scenario relates to a number of recent studies showing links between global temperatures and phenology (seasonal development including flowering time; see review in Forrest and Miller-Rushing 2010).

For one Australian perennial plant, *Pimelea ferruginea*, its past evolution and future survival of global warming is linked directly to its ability to thermoregulate its initiation of flowering. Both controlled environment and a reciprocal field

transplant study show temperature regulation of its floral initiation (King et al. 1996). Difficulties with field transplantation studies (above) have been avoided, so the evidence points to evolutionary selection pressure/adaptive advantage. For a second Australian perennial species, *Crowea exalata*, similar increases in temperature (3°C) cause faster flowering but fewer flowers form (King et al. 2008).

9.2 Thermoregulation of Floral Initiation by Mild Temperatures

Plants of *P. ferruginea* grow vegetatively for at least 2 years if held in a controlled environment glasshouse at a high daily average temperature of 21°C. However, flowers initiate when 4- or 5-month-old plants are shifted to slightly cooler temperatures (12–18°C). The response shown in Fig. 9.1 is for one such line,

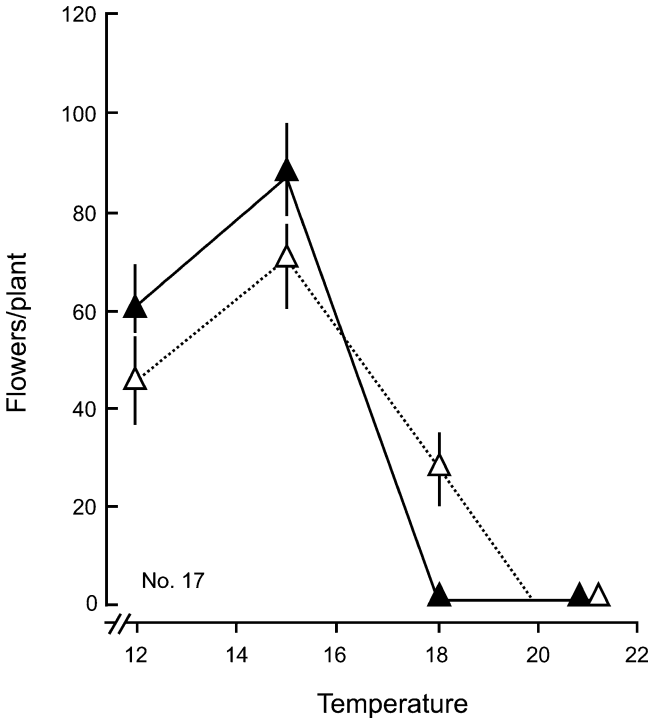

Fig. 9.1 Effect of temperature on flowering (number of open flowers plus buds) of *Pimelea ferruginea*. A batch of ca. 100 clonal plants was vegetatively propagated using cuttings taken from a single field plant (# 17) located at latitude 33° 34′S in Western Australia. There was no flowering over 2 years when the cuttings were growing at 21°C but they flowered rapidly when moved to temperatures of 12°C, 15°C, or 18°C in either short (8 h) or long days (16 h photoperiod). *Vertical bars* are 2× s.e. (Adapted from King et al. 1996)

17, which had been vegetatively propagated by taking cuttings from a single plant in the field.

When examined with a microscope, flower primordia have developed fully by 7 weeks (King et al. 1992). Clearly, mild temperatures with an optima of *ca* 15°C, directly and rapidly induce flowering of *P. ferruginea*. This temperature far exceeds the 4–10°C range accepted for promotion of flower by winter cold (vernalization: see Lang 1965).

Further lines of *P. ferruginea* covering the full latitudinal range of its natural distribution were vegetatively propagated at the same time in batches of approximately 100 plants. For all lines, mild temperatures regulated flowering and there was no photoperiodic effect when tested in daylengths, which span the natural seasonal differences across their latitudes of origin (cf. #17 Fig. 9.1). Thus, a single environmental factor, mild temperature, is the dominant and probably the only determinant of flowering of *P. ferruginea* (see requirement 1 above), There is none of the potential complexity found with Arabidopsis and other species (see King and Heide 2009), where multiple environmental inputs may regulate flowering either interchangeably or in concert.

Such thermoregulation of flower initiation by mild temperatures is not unique to *P. ferruginea* but has been observed for a number of other plant species across a range of families including the grasses (Heide 1994) and dicotyledonous species (see summaries in King et al. 1992 and King and Heide 2009).

All batches of cloned adult plants used in these studies were 4–5 months-old and vegetative when, at the onset of winter, they were either transferred to florally inductive temperatures in controlled environment conditions (above) or returned to field nurseries (see later). Thus, indirect environmental effects on plant establishment and juvenile development were avoided (requirement 2 above). The rapidity of flower initiation (7 weeks) also enhances the specificity of these studies.

9.3 Adaptive Differences in Controlled Environments

P. ferruginea is found over a north–south latitudinal distance of *ca* 650 km along the coast of Western Australia but in an extremely narrow strip of sand plain covering *ca* 200 m adjacent to the ocean (Rye 1988). Aside from occasional residential intrusions, the population is also undisturbed by human activity.

Lines vegetatively propagated from single plants sampled along the full range of their distribution, differed in their thermoregulation of flowering when tested in a controlled environment study (Fig. 9.2). There is clearly a latitudinal adaptation in the temperature permissive for flowering of *P. ferruginea*.

This latitudinal, permissive temperature gradient for flowering matches site temperatures (Fig. 9.2) and both show a 4°C range. Depending on the assumptions made, these two lines could be virtually identical as the upper permissive temperature limit for flowering might equally well be replaced by the 3°C lower optimal temperature for flowering (see Fig. 9.1). Further, site temperatures might

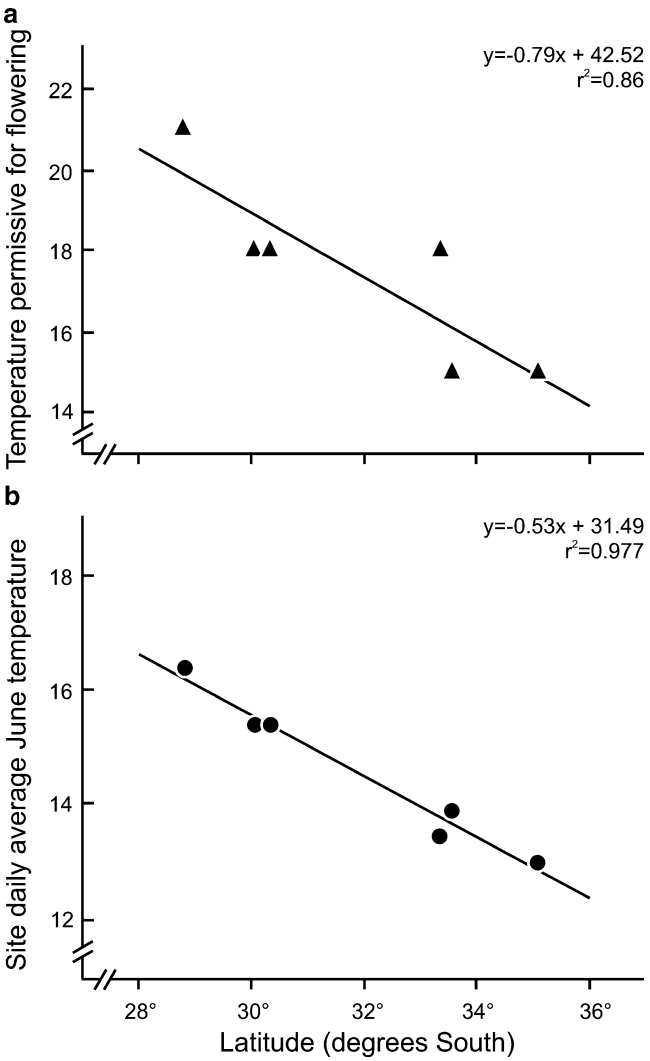

Fig. 9.2 Relationship between latitude of origin of selections of *P. ferruginea* and (**a**) the highest temperature which permitted flowering when tested in controlled environment conditions. (Values are derived from findings reported in full in King et al. 1996 and as shown in Fig. 9.1 for one location.) (**b**) Latitudinal differences in the average daily winter temperature for the month of June based on 100-year averages available from the Australian Bureau of Meteorology

be increased by a small amount as they are only for the first 4 weeks of June, the first month of winter. The estimate of average effective temperature could also have included metrological data on some of the warmer weeks prior to winter as flowering of *P. ferruginea* requires at least a 5-week exposure to mild temperatures (King et al. 1992).

Overall, the close match between site temperatures and adaptation of *P. ferruginea* for thermoregulated flowering implies that temperature has been a dominant evolutionary force in regulating its seasonal flowering and survival.

9.4 Mild Field Temperatures Regulate Flowering of *P. ferruginea*

A more definitive test of evolution and selective advantage was possible with reciprocal field nursery transfers performed across the latitudinal extremes of its distribution. Nurseries were at Denmark in Western Australia (Lat 34° 96′S), a cooler site, and Geraldton in Western Australia (Lat 28° 96′S), the warmest extreme of its natural distribution. The plants of *P. ferruginea* were growing vigorously and vegetatively when transferred at the start of winter from the controlled environment to the field. Thus, all three experimental requirements were satisfied (see Introduction)

At the cooler Denmark nursery, irrespective of their latitude of origin, all lines flowered after about 2 months (Fig. 9.3). Because winter temperatures at this site were always below the upper permissive limit for all lines, they all flowered rapidly. In other words, at this site there was no evidence of selective pressure on the known temperature adaptation between lines from cool or warm sites of origin. Further data (not shown) confirmed this finding for nurseries at this site and another cool site (King et al. 1996). In contrast, at the warmer nursery site (Geraldton), a selection from a warm site (Lat 31°01′) flowered as rapidly as it did at the cooler site but, now, flowering of lines from cooler sites (35° 07′S and 33° 34′S) was considerably delayed or the plants never flowered (33° 55′S). The 4–5°C higher temperature at Geraldton exceeds the temperature adaptation of these latter lines.

There are many reasons why earlier studies of flowering have shown adaptation without proving any evolutionary selective advantage. Here, an important and fortunate feature is the undisturbed and compact distribution *P. ferruginea* in a very narrow zone (*ca* 200 m) up and down the coast of Western Australia. In addition, there was a natural latitudinal temperature gradient of sufficient magnitude for the detection of differences between lines in their "thermostat" set point.

Overall, the action of temperature on flowering of *P. ferruginea* has been a significant selective force in its evolutionary adaptation, a conclusion based on three findings, namely:

1. *P. ferruginea* flowers in response to a single environmental input; mild winter temperatures. Flowering is blocked if the conditions are too warm (>18 to >21°C depending on the line).
2. The natural latitudinal temperatures at the time of its floral initiation match the temperatures tolerated for their thermoregulated flowering in controlled conditions.

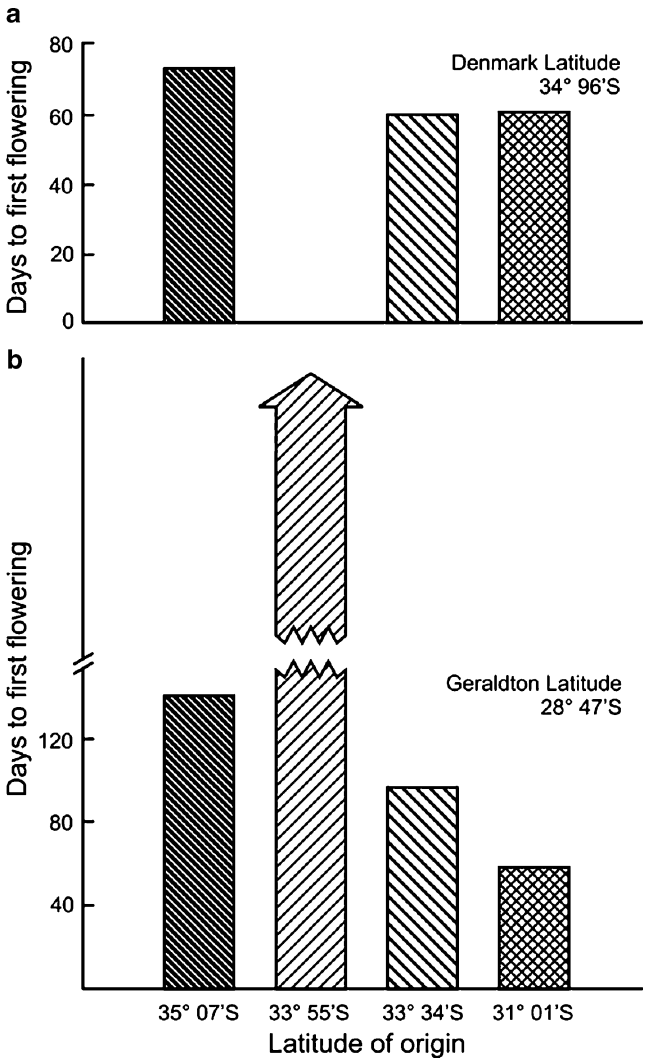

Fig. 9.3 Effect of latitude of origin of selections of *P. ferruginea* on their flowering in a nursery at Denmark (a cooler high latitude site with an average temperature of 13°C at transfer in June) or at Geraldton (a warmer lower latitude site with a high 17°C average temperature at transfer in June). The hatching of the bars is distinctive for the latitude of origin of each selection. (Adapted from King et al. 1996)

3. In reciprocal field nursery transplantation studies across the extremes of its distribution, all lines from warm or cool sites flowered at the cool site. In contrast, temperature at the 4°C warmer site exceeded the tolerance of lines from cooler sites.

9.5 Temperature Effects on Flower Number and Time to Flower

The role of temperature in flower development is well illustrated by findings with *Crowea exalata*, "Bindelong Compact." It initiates flowers when transferred from shade to a higher light intensity (full sunlight). Low temperature was not essential for flowering of *Crowea exalata*, and warmer conditions (up to 30°C) sped up the time to first flower but decreased total flower number at the peak of the display (Fig. 9.4).

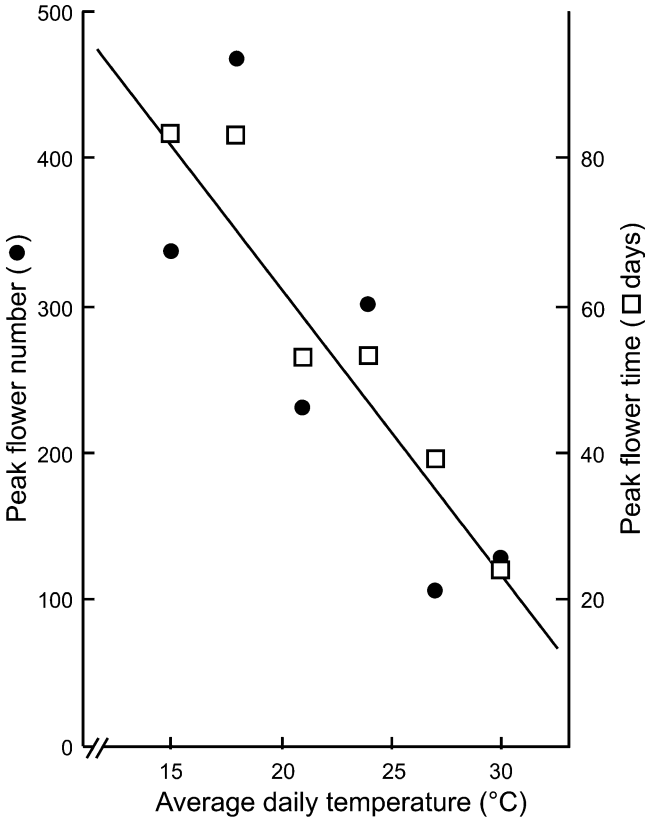

Fig. 9.4 Effect of temperature on flowering of *Crowea exalata*, "Bindelong Compact." The relationship between temperature and either days to peak flowering (*open square*) or, number of open flowers at peak flowering (*closed circle*). Initially, the plants were held vegetative in low irradiances in a green house operating at 21°C. Then the various temperatures were imposed at the time of transferring plants to full sunlight conditions for inducing flowering. The regression line between temperature and flower number and time showed a highly significant fit ($p < 0.001$) to the data. (Adapted from King et al. 2008)

There is no paradox between higher temperatures (>21°C) causing early flowering of *Crowea exalata* but blocking flowering of *P. ferruginea* (see above). These differences reflect independent effects of temperature on sequential developmental processes. Flower initiation may require mild temperatures but flowers develop faster the higher the temperature. These contrasting responses to temperature are evident with *P. ferruginea* where flower initiation is satisfied by a 7-week exposure to mild temperatures. Then, subsequently, higher temperatures are tolerated and speed up floral development (King et al. 1992). On the other hand, *Crowea exalata* only shows the effect of temperature to speed up floral development, its floral initiation is achieved by an increase in photosynthesis (King et al. 2008).

9.6 Thermoregulation and Seasonal Flowering of Other Plant Species

Unlike the findings with *P. ferruginea,* for many other species a combination of daylength and mild temperature is required either simultaneously (e.g., in the Australian perennial legume, *Hardenbergia*; King 1998) or over different seasons and stages of floral development as for some grasses (Heide 1994). Furthermore, the adaptations may be quite complex as with strawberry (*Fragaria* spp), which may tolerate short and long days at low growth temperatures but may only respond to long daylengths at higher temperatures (Heide and Sønsteby 2007).

To simply characterize the daylength and temperature environment, metrological data can be used to calculate seasonal photothermal envelopes for any particular latitude (Heide 1994). Such envelopes change from a pin-point size at the equator to a massive fan at very high latitudes. In theory, by superimposing a species temperature and daylength profile over its site photothermal envelope, inferences can be drawn about species survival. However, unlike the possible outcome of field transplantations, this latter approach provides only a limited test of selective advantage.

The analysis of seasonal regulation becomes even more complex when daylength and both positive and negative temperature responses are important. For example, with *Hardenbergia violaceae,* its flowers initiate in daylengths shorter than 12 h and for temperatures of 18°C and above (King 1998). Then, even as late as visible petal formation, warm conditions (21°C and above) cause all flowers to abort. The net result is that the plants only set seed in short days at 18°C. Thus, in nature, while the onset of flowering before winter allows rapid spring floral development, further rises in temperatures cause rapid loss of formed flowers as well as abortion of immature seed pods (King 1998).

Little is known of how flowering time "thermoregulators" might work in plants. For Arabidopsis, Blázquez et al. (2003) reported extensively on promotion of flowering at a higher temperature (23°C vs. 16°C). Their findings relate to promotion of flowering by warmer conditions imposed over the whole life cycle and were

inconclusive in showing a role for known flowering time genes involved in floral initiation. Very likely, as with *Crowea exalata*, earlier flowering of Arabidopsis at higher temperature reflects a major effect of temperature on floral development not on floral initiation.

9.7 Global Warming, Phenology, and Species Survival

Increasingly, there are reports of effects of global warming on phenology (the timing of seasonal developmental events such as flowering of plants, and bird migration (see Forrest and Miller-Rushing 2010). Here, documentation of past selection pressure on thermoregulated flowering of *P. ferruginea* in its natural environment has provided a unique view of its future survival. Temperature alone controls the phenology of *P. ferruginea* and, based on the reciprocal transfer experiments, a 3–4°C global warming, at cooler sites will block or delay flowering. Furthermore, depending on upper "thermostat" set limits, permissible temperatures for flowering might also be exceeded for lines from warmer sites.

Heritability of temperature-regulated flowering of *P. ferruginea* is indicated by the match across latitudinal sites between a plants "thermostat" setting for flowering and site temperature (Fig. 9.2), a claim further supported by evidence that plants sampled from within one site, showed little or no distinction in their "thermostat" setting (line #17, Fig. 9.1; line # 18 and # 19: King et al. 1996). Nevertheless, the present site sampling is not sufficiently intensive to adequately address the question of natural genetic variation. Even one plant with a 3–4°C greater temperature tolerance could ensure species survival. It seems unlikely this species would survive by migration of warm-tolerant lines to cooler sites. The seed is not wind dispersed and the plant would need to migrate over 650 km in *ca* 50 years.

Compared with *P. ferruginea*, few plant species in their natural environment will provide such ideal material for studying responses to global warming. They may variously show responses to more complex sets of environmental inputs and if spread over wide geographical ranges, there is potential for divergent evolution. Nevertheless, as shown in a recent compilation (see Forrest and Miller-Rushing (2010) and associated reviews), there is growing evidence that global warming is affecting flowering. As one example, from 47 years of records of spring flowering times of 385 species, Fitter and Fitter (2002) reported that 16% had advanced their flowering date by 4.5 days on average but only in association with global temperature increases over the decade from 1990.

What is not yet clear from such phenological information is the impact of earlier flowering on species survival. As discussed above for *P. ferruginea* and *Crowea exalata*, misleading mechanistic assumptions can be introduced where warming will speed up, delay or block floral initiation but speed up floral development. Of even greater concern is the evidence for *Crowea exalata* that earlier flowering at higher temperatures results in fewer flowers forming (Fig. 9.4). Similar responses are also known for a number of species. Wheat, for example flowers earlier at

higher temperatures but then forms fewer florets, grains, tillers, and leaves (see review in Evans 1993). Thus, heat sum models, while adequately predicting maturity and effects of global warming in speeding up plant development (Crauford and Wheeler 2009), could be of limited value in predicting seed production or yield of a crop or of species in the wild.

References

Blázquez M, Ahn JH, Weigel D (2003) A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. Nat Genet 33:168–171

Crauford PQ, Wheeler TR (2009) Climate change and the flowering time of crops. J Exp Bot 60:2529–2539

Evans LT (1993) Crop evolution, adaptation and yield. Cambridge University Press, Cambridge

Fitter AH, Fitter RS (2002) Rapid changes in flowering time in British plants. Science 296:1689–1691

Forrest J, Miller-Rushing AJ (2010) Toward a synthetic understanding of phenology in ecology and evolution. Phil Trans R Soc B 365:3101–3112

Garner WW, Allard HA (1920) Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. J Agric Res 18:553–603

Gassner G (1918) Beiträge zur physiologischen characteristic sommerund winterannueller Gewächse, insbesondere der Getreidepflanzen. Z Botanik 10:417–480

Heide OM (1994) Control of flowering and reproduction in temperate grasses. New Phytol 128:347–362

Heide OM, Sønsteby A (2007) Interactions of temperature and photoperiod in the control of flowering of latitudinal and altitudinal populations of wild strawberry (*Fragaria vesca*). Physiol Plant 130:280–289

King RW (1998) Dual control of flower initiation and development by temperature and photoperiod in *Hardenbergia violacea*. Aust J Bot 46:65–74

King RW, Heide OM (2009) Seasonal flowering and evolution: the heritage from Charles Darwin. Funct Plant Biol 36:1027–1036

King RW, Dawson IA, Speer SS (1992) Control of growth and flowering in two Western Australian species of *Pimelea*. Aust J Bot 40:377–388

King RW, Pate JS, Johnston J (1996) Ecotypic differences in the flowering of *Pimelea ferruginea* (Thymelaeaceae) in response to cool temperatures. Aust J Bot 44:47–55

King RW, Worall R, Dawson IA (2008) Diversity in environmental controls of flowering in Australian plants. Sci Hort 118:161–167

Koornneef M, Alonso–Blanco C, Vreugdenhil D (2003) Naturally occurring genetic variation in Arabidopsis. Annu Rev Plant Biol 56:141–172

Lang A (1965) Physiology of flower initiation. In: Ruhland W (ed) Encyclopaedia of plant physiology, vol 15. Springer, Berlin, pp 1380–1536

Mitchell-Olds T, Willis JH, Goldstein DB (2007) Which evolutionary processes influence natural genetic variation for phenotypic traits? Nat Rev Genet 8:845–856

Rye BL (1988) Revision of Westen Australian Thymeleaceae. Nuytsia 6:129–278

Scarcelli N, Kover PX (2009) Standing genetic variation in FRIGIDA mediates experimental evolution of flowering time in Arabidopsis. Mol Ecol 18:2039–2049

Trevaskis B, Hemming MN, Dennis ES, Peacock WJ (2007) The molecular basis of vernalization-induced flowering in cereals. Trends Plant Sci 12:352–357

Wilczek AM, Roe JL, Knapp MC, Cooper MD, Lopez-Gallego C, Martin LJ, Muir CD, Sim S, Walker A, Anderson J, Egan JF, Moyers B, Petipas R, Giakountis A, Charbit E, Coupland G, Welch SM, Schmitt J (2009) Effects of genetic perturbation on seasonal life history plasticity. Science 323:930–934