Time after time: flowering phenology and biotic interactions

Jelmer A. Elzinga[1,6], Anne Atlan[2], Arjen Biere[3], Luc Gigord[1], Arthur E. Weis[4,5] and Giorgina Bernasconi[1]

[1] Department of Ecology and Evolution, University of Lausanne, CH 1015 Lausanne, Switzerland
[2] University of Rennes, CNRS, ECOBIO, F 35042 Rennes, Cedex, France
[3] Department of Multitrophic Interactions, the Netherlands Institute of Ecology, NL 6666 ZG Heteren, the Netherlands
[4] Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA
[5] Current address: Department of Ecology and Evolutionary Biology, University of Toronto, M5S 3B2 Toronto, Ontario, Canada
[6] Current address: Department of Biological and Environmental Sciences, University of Jyväskylä, FI 40014 Jyväskylä, Finland

The role of biotic interactions in shaping plant flowering phenology has long been controversial; plastic responses to the abiotic environment, limited precision of biological clocks and inconsistency of selection pressures have generally been emphasized to explain phenological variation. However, part of this variation is heritable and selection analyses show that biotic interactions can modulate selection on flowering phenology. Our review of the literature indicates that pollinators tend to favour peak or earlier flowering, whereas pre-dispersal seed predators tend to favour off-peak or later flowering. However, effects strongly vary among study systems. To understand such variation, future studies should address the impact of mutualist and antagonist dispersal ability, ecological specialization, and habitat and plant population characteristics. Here, we outline future directions to study how such interactions shape flowering phenology.

Introduction

For plant reproduction, timing is everything. An individual plant that flowers too early, before it has had time to accumulate sufficient material resources, will have a limited capacity for seed production. One that delays flowering might gain higher capacity, but might also run out of time to use it before the end of the season. Flowering phenology is affected by many environmental factors, among which temperature and photoperiod, which are reliable signals of seasons, are probably the best studied. Accurate detection of such environmental cues and the resulting plastic response of plants enable flowering to occur when climatic conditions are most suitable for reproduction. Thus, resources and conditions impose bottom-up selective forces on phenology.

By contrast, top-down forces act on reproductive timing, particularly those imposed by mutualists (pollinators and seed dispersers) and antagonists (floral pathogens and pre-dispersal seed predators). Here, we review recent progress in understanding some of the top-down selective forces that act on reproductive timing. We highlight what is known,

and what remains to be discovered, about the ways in which the ecological response of these associated species to the population-level abundance of flowers and fruit can shape the evolution of individual plant reproductive schedules (Box 1).

Flowering phenology evolution

Flowering phenology can evolve rapidly, as evidenced by the many examples of phenological divergence between plant populations within the same species, each adapted to their local conditions [1–5]. These microevolutionary shifts imply that plant populations often harbour sufficient genetic variance in phenology for a selection response. This is born out by frequent reports of significant heritabilities for traits such as date of first flowering and duration of the flowering period [4,6–8]. Variation in environmental factors (e.g. light intensity, rainfall, competition and herbivore attack) might also contribute to phenological variation in populations, but these phenotypic plastic responses themselves might also be genetically modulated to some extent [6].

Some researchers have argued that the persistence of phenological variation in plant population implies selective neutrality [9]. Over the past 20 years, however, selection gradient analyses have shown that selection on phenological traits is often measurably strong [10,11]. With the recent strides made in understanding the genetic controls for flowering time [3,12], evolutionary ecologists might soon be able to integrate information from the molecular to community levels to understand the adaptive evolution of phenology. Understanding the role of biotic agents of selection will be an important part of this effort. Such efforts become increasingly relevant, as large-scale disturbances of biotic interactions become more common. First, changes in climatic conditions might act differentially on interacting species and could lead to phenological mismatches that would affect whole communities [13]. Second, biological invasions disrupt coevolved interactions and create opportunities for novel ones.

Plant–plant interactions

Owing to environmental and genotypic variation, plants in a population rarely flower in exact synchrony (Box 2). An important consequence of phenological variation is that it
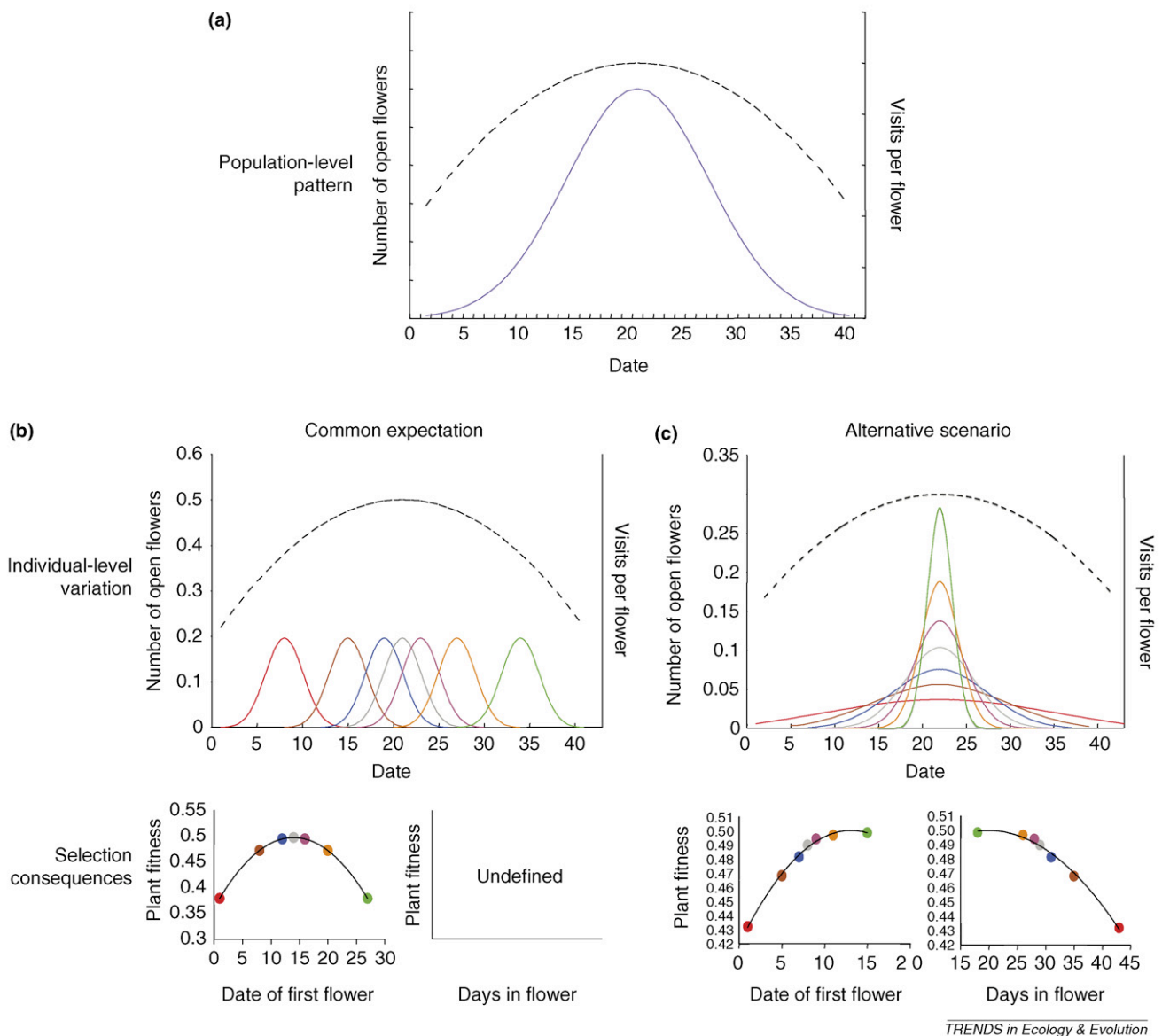
Box 1. Selection on flowering schedules

Many studies indicate that mutualists and antagonists respond to flower density. With pollinators, for instance, the number of visits per flower can rise and fall in synchrony with the population-level flowering curve (i.e. number of open flowers per day) (Figure I). This pattern is commonly expected to impose stabilizing selection on phenology, but sometimes it will not. The form of selection depends on the pattern of variation among the individual flowering schedules, which collectively produce the population flowering curve. Figure I depicts two scenarios with identical flowering curves and pollinator responses, but contrasting selection regimes.

The common expectation of stabilizing selection (Figure Ia) rests on the assumption that, whereas individuals vary in the date of first flowering (red flowers early; green flowers late), they do not vary in the number of days they flower. Plants that start flowering so that their peak flower production coincides with the population-level peak are the most favoured. Because all plants flower for the same number of days, there is no variance in this component of the schedule and

(hence) no selection is possible. If the number of days in flower varies modestly, selection on flowering date will be similar to this scenario, as long as the flowering date and length of the flowering period are uncorrelated.

Figure Ib shows an alternative scenario, in which the date of first flowering of an individual is negatively correlated to its number of days in flower (red flowers early and produces a few flowers each day over many days; green flowers later and produces the same total number of flowers, but over just a few days). Here, correlated directional selection operates on the two flowering schedule components: plants that flower later and flower for fewer days are favoured. Many other scenarios between the two extremes depicted here are possible.

Similar population-level responses of pre-dispersal seed predators to fruit abundance can yield either disruptive or correlated direction selection on fruiting phenology, depending on the pattern of variation in the fruiting schedule components.

Figure I. Relationship between pollinator responses (dashed lines) to population-level flower abundance **(a)** (solid lines), and selection on the components of the flowering schedule. The individual flowering schedules in **(b)** and **(c)** both collectively produce identical population-level flowering curves. (b) All plants flower for the same number of days and so selection on date of first flowering is stabilizing. (c) Date of first flowering is negatively correlated to days in flower, which results in correlated directional selection favouring later flowering and shorter flowering periods. Point colours in the selection graphs correspond to those of the flowering schedules in the individual variation graphs.

Box 2. How to measure flowering synchrony

Flowering phenology can be characterized at the level of population, individual plants within a population, and flowers within an individual. For instance, floral longevity is the number of days that an individual flower can either donate or receive pollen. The flowering period of an individual is the number of days between opening of the first flower and senescence of the last and the flowering season is the number of days between the first and last flower among all individuals in the populations. We can use many additional measure-ments that can be taken on an individual flower, plant or population to characterize flowering schedules in more detail (e.g. flowering start date, peak flowering date, etc.). But how can we quantify overlap in flowering [60]? Simple methods include calculating the number of co-flowering plants or flowers [61], or the differences in mean flowering dates of a focus plant relative to the population mean [62]. In Table I, we give several more complex synchrony indices for within-season synchrony of individual plants.

Table 1. Indices to quantify flowering synchrony

Definition	Parameters	Data required	Suitable for population comparisons?	Refs
$\left(\dfrac{1}{n-1}\right)\left(\dfrac{1}{f_i}\right)\sum_{j=1}^{n} w_j$	N, number of flowering plants; f_i, number of census days plant i flowers; w_j, number of census days plant i and plant j $(j \neq i)$ flower simultaneously	Presence or absence of flowers	Yes	[63,64]
$\dfrac{n\left\{\sum_{t=1}^{k}\left[\left(\dfrac{x_t}{\sum_{t=1}^{k} x_t}\right) p_t\right]\right\} - 1}{n-1}$	x_t, total number of flowers a plant produces on census day t; p_t, proportion of plants flowering on census day t; k, maximum number of census days	Focus plants (number of open flowers); other plants (presence or absence of flowers)	Yes	[24]
$\sum_{t=1}^{p} r_{i,t}$	$r_{i,t}$, rank of plant i based on the ratio of flowers already produced at census day t [until peak flowering (p) in the patch] to the total number of flowers on plant i	Number of open flowers for all plants	No	[23]
$\dfrac{1}{2}\left(2 - \sum_{t=1}^{k}|y_{i,t} - \bar{y}_{j,t}|\right)$	$y_{i,t}$, ratio of flowers produced on census day t to the total number of flowers on plant i	Number of open flowers for all plants	No	[23]
$\sum_{t=1}^{k}\left[\left(\dfrac{x_t}{\sum_{t=1}^{k} x_t}\right) f_t\right]$	f_t, proportion of the total number of flowers in the patch produced on census day t	Number of open flowers for all plants	Yes	[a]

[a]J.A. Elzinga and G. Bernasconi, unpublished data.

affects intraspecific interactions in a way that can influence population genetic structure [14], which can, in turn, affect potential selection responses. If the frequency distribution for the date of first flowering is approximately normal, and flowering times are not spatially aggregated, early and late bloomers should have fewer potential mates in their immediate neighbourhood than should plants blooming near the modal date. To date, studies using paternity analysis indicate that maternal plants receive pollen from more distant donors in the early and late season than during the mid-season [15,16]. In general, asynchronous flowering might blur fine-scale isolation by distance between potential mates, and so reduce bi-parental inbreeding.

Phenological variation can lead to assortative mating: early plants are pollinated by other early plants, and late plants by late plants. Phenotypic assortative mating inflates any underlying genetic variance in the assorting trait (here, flowering schedule [17,18]). Phenological assor-tative mating is not well studied [17], possibly because statistical methods were lacking until recently [8,17,18]. Individual flowering schedules can be used to generate all pairwise mating probabilities [8], from which it is possible to calculate a prospective estimate of the phenotypic cor-relation between pollen donors and recipients. In addition, quantitative genetics can be used to infer retrospectively the actual correlation. Comparing the prospective and retrospective correlation estimates in the field can yield insights into temporal patterns in pollinator visitation [8].

Variation in phenology can also affect interspecific plant–plant interactions through generalist mutualists and antagonists (Box 3). Co-flowering plant species might, for example, either contribute to attract pollinators, or compete for their service. A strategy to avoid the costs of interspecific pollen transfer is to diverge in the daily pattern of pollen release, as observed in an African *Acacia* community [19]. A comparison between plants with abiotic (e.g. wind) and biotic (e.g. by insects) pollination suggests that the latter have more diversified flowering phenologies, which is consistent with the idea that phenological separ-ation reduces competition for generalist pollinators [20]. Thus, the direction and intensity of selection on phenology through mutualists and antagonists is modulated within a community context.

Interactions with mutualists: pollinators

Many plants rely completely or partly on animal pollinators for successful reproduction and there are many correlational studies of the relationships among the temporal pattern of flower production, pollinator visitation and plant repro-ductive success [21–26]. Although these studies generally suggest that pollinators impose selection on flowering phenology, only some demonstrate a causal link between patterns of pollinator visitation rate and patterns of seed set. Demonstration of such a link usually requires exper-imental manipulation. Possible approaches are repeating additional hand pollinations [27] to verify whether decre-ases in seed set coincide with increased pollen limitation and not just increased resource limitation, or working with experimental cohorts of plants. For instance, experimental cohorts of common hound's tongue *Cynoglossum officinale* exposed to pollinators during the normal flowering period had significantly higher seed set than did those exposed before and after, supporting a visitation–selection link;

Box 3. Density effects in space and time: pollination in communities of rewarding and deceptive plants

Flowering phenology is relevant for food-deceptive plants such as many Orchidaceae. Food-deceptive plants do not offer any reward to pollinating insects and, therefore, rely on pollinator inexperience or mistakes for their reproductive success. Both temporal and spatial flowering patterns of food-deceptive species relative to rewarding co-flowering plants appear to have crucial roles for their reproductive success. However, it is difficult to establish whether there is an overall positive or negative effect of co-flowering next to and at the same time as the rewarding species.

At a spatial scale, the remote habitat hypothesis proposes that it would be beneficial for deceptive species to flower at a place where rewarding species are scarce or at low density. A recent field experiment showed that it was detrimental for the food-deceptive orchid *Dactylorhiza sambucina* (Figure I) to flower in patches with a high density of rewarding plants [65]. This result might arise if pollinators learn more quickly to discriminate against deceptive plants if these co-occur next to rewarding plants that pollinators frequently encounter. But the opposite can be true: for some species, deceptive plants receive more visits if they flower where rewarding co-flowering species are at high density, if this increases the overall attractiveness of the patch [65].

At a temporal scale, a fascinating hypothesis is that many of the deceptive orchids, at least European ones, flower early in the season, earlier than most rewarding species. In this way, they might avoid competition for access to pollinators with other (rewarding) species, and/or benefit from higher chances of being visited, because the newly emerged pollinators are relatively inexperienced.

Thus, we cannot yet identify a single, most beneficial strategy of co-flowering in space and time; more experiments are needed to clarify whether a strategy that anticipates flowering phenology of the rewarding species contributes to the maintenance of deceptive plants.

Figure I. Should food-deceptive species flower earlier or later than rewarding species? The food-deceptive orchid *Traunsteinera globosa* (a) flowers relatively late in the season, with numerous co-flowering species, which appears to be beneficial to the reproductive success of the orchids. By contrast, the yellow and purple *Dactylorhiza sambucina* (b) flowers early in the spring, when co-flowering species are scarce, and is pollinated by newly emerged bumblebee queens. Early flowering might also be beneficial to the reproductive success of the orchid, because naïve pollinators did not yet learn to discriminate against deceptive plants. Reproduced with permission from Nicolas Juillet (a) and Antonina Internicola (b).

however, pollen additions showed no effects on seed set, supporting temporal resource limitations [21].

Pollinators are often attracted to a given plant species only after a certain threshold density of individuals or flowers are in bloom [28]. By contrast, a strong flowering peak might satiate pollinator availability [29] and lead to subsequent pollen limitation [30]. Particularly interesting is the case of deceptive plants, which do not offer nectar to pollinators, where density of deceptive flowers reinforces discrimination learning by pollinators [31] (Box 3). For such plants, a high density of flowers might decrease the level of naivety of potential pollinators [31], but low density reduces the attraction [32]. When pollination is density dependent, selection on the timing of flowering will depend on the synchrony between a focus plant and its neighbours (Box 2). Similarly, populations of different densities or sizes might experience different selection on flowering phenology (J.A. Elzinga and G. Bernasconi, unpublished data).

Many studies indicate that pollinator visitation rises and falls in concordance with population-wide flower abundance. The degree to which this imposes stabilizing selection on phenology depends not only on the phenotypic variation in date of first flowering or of peak flowering, but also on the variation in the length of the flowering period, the shape of the flowering schedule (skew and kurtosis, i.e. peakedness of the distribution) and the correlations among them (Box 1). Similarly, post-pollination events can 'censor' the selective impact of pollinator visitation. For instance, supplemental pollinations reveal that pollinators limit fruit set in *Geranium maculatum* early in the season but not later; early-set fruits deplete plant resources so that late flowers are less successful [27]. Despite higher pollinator visitation rate late in the flowering season, seed set was lower owing to increased selfing in *Rhodendron aureum* [33]. In these latter two cases, there might be overall stabilizing selection on phenology, but it would comprise two opposing directional components. Stabilizing selection imposed by pollinators can be nullified if pre-dispersal seed predators impose disruptive selection.

Overall, the temporal correlation between pollinator service and flower abundance over the season is a strong

pattern in natural systems. Pollinators tend to favour peak or earlier flowering (see Online Supplementary Information). The strength and direction of selection on phenology will depend on how strongly other factors affect the relationship between opportunity and outcome.

Interactions with antagonists: pre-dispersal seed predators

Before seeds get a chance of being dispersed, many plants are attacked by herbivores that specifically eat and destroy flowers, seeds or fruits. Most field surveys compare temporal variation in the intensity of seed and flower predation and flowering phenology to infer the direction of selection acting on phenology. Most studies found that flowering off-peak (either early [23,34–37], or late [11,38–41]) is associated with reduced seed predation, probably because fewer herbivores are present at those times. Similar effects are highlighted by studies indicating highest seed predation during peak flowering [42–44], especially when peak flowering attracts more seed predators owing to a higher density of flowers and fruits ([28], J.A. Elzinga and G. Bernasconi, unpublished data). This could impose disruptive selection on the date of first flowering, and/or directional selection for longer flowering periods. However, other studies found constant frugivory over the season [45,46], implying that, in these cases, the herbivore does not exert a selective pressure on flowering date. Alternatively, stabilizing selection towards a peak flowering date might also occur if flowering synchronously during a short period satiates the herbivore [47]. In populations with higher densities of fruits, more fruits can escape predation [28,37] and this can also hold for changes in density over time. In dandelion *Taraxacum officinale*, for example, seeds produced during peak flowering are more likely to escape predation compared with seeds produced by early and late flowers [48]. In an experiment that manipulated tropical shrubs to flower in or out of synchrony with each other to test experimentally the predator satiation hypothesis, synchronous flowering reduced frugivory [49]. However, to date, the evidence for predator satiation is still limited.

Interactions with antagonists: pollinator-transmitted diseases

Pollinator visitation not only ensures reproduction and promotes outcrossing, but might also aid the spread of pollinator-transmitted diseases. Many pathogens, for example fungi use flowers to gain entry into their hosts. In these cases, plant phenology can be a key determinant of disease avoidance, because it can reduce the contact between susceptible host stages and infective pathogen stages (Box 4).

Pollinator-transmitted diseases impose a tradeoff in phenology. A phenological pattern that increases attraction to pollinators might not only increase seed set, but it might also increase the probability of disease transmission (unless spore-producing and pollen-producing reproductive structures have disparate phenologies or pollinators

---

Box 4. Selection on flowering phenology modulated by a pollinator-transmitted fungus

The anther smut *Microbotryum violaceum* systemically infects plants in the carnation family. It sporulates in the anthers, replacing the pollen and sterilizing the hosts (Figure I). Studies in this system have provided insights in the complex relationship between host phenology and avoidance and/or resistance to pollinator-transmitted diseases. Offspring of half-sib families of the host plant white campion *Silene latifolia* planted in healthy and diseased experimental populations show extensive genetic variation in onset of flowering, and presence of the pathogen significantly affects selection on plant phenology (Figure I).

Several mechanisms mediate the effect of phenology on susceptibility. First, pollinator activity declines towards the end of the season. Second, the fungus has a long latent period. Hence, plants that start to flower from mid-season are unlikely to develop symptoms (sterile flowers) within that same season. Third, phenology indirectly affects the probability of infection through genetic correlations. Plants with late onset of flowering also produce fewer flowers, and this reduces the contact rate with pollinators and spores. The other correlation is between late onset of flowering and high values of phenology-independent disease resistance. This reduces the per-contact probability of contracting the disease (Figure Ia, Ib) and illustrates how pathogens can significantly modulate selection on flowering phenology (Figure Ic).
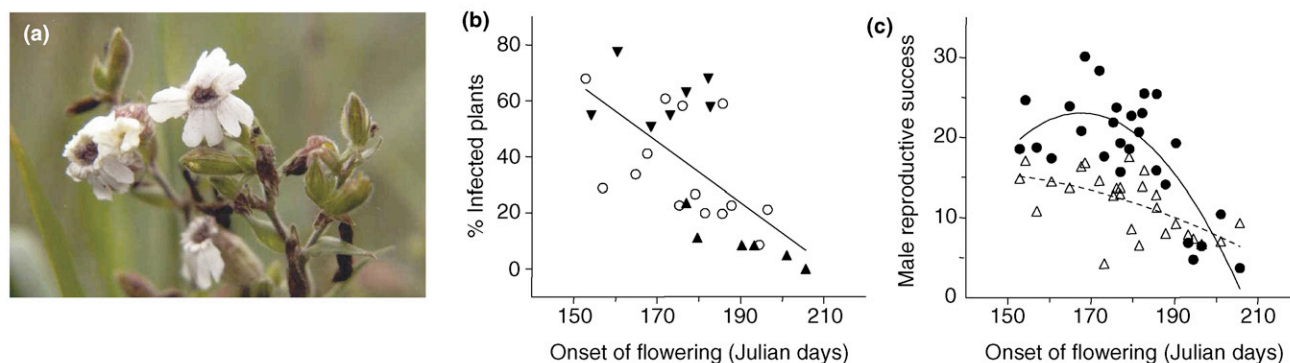

Figure I. A pathogen alters selection on host flowering phenology. **(a)** A *Silene latifolia* plant infected with the anther-smut fungus *Microbotryum violaceum*. **(b)** The proportion of plants per family that become infected by the pathogen decreases for families that start flowering later in the season. Upward- and downward-pointing triangles represent families with high and low values for phenology-independent resistance, respectively; circles represent families with intermediate levels of resistance. **(c)** As a result, the pathogen significantly affects selection on host phenology: in the absence of the pathogen (closed circles, solid line), early-flowering families have the highest male reproductive success (measured as average number of fruits sired per male plant). In the presence of the pathogen (open triangles, dashed line), this advantage diminishes. Each symbol represents a family mean. Reproduced with permission from Arjen Biere (a) and Ref. [52] (b).

discriminate against infected plants). The pollinator-transmitted anther smut fungus *Microbotryum violaceum* significantly alters the pattern of selection on flowering phenology (Box 4). The beneficial effect of late flowering in reducing the risk of acquiring the disease is partly mediated by avoidance of peak pollinator activity.

Effects of phenology on resistance evolution through assortative mating

One recent insight is that plant enemies can alter phenology in ways that either speed up or slow down the evolution of resistance to herbivores. In some cases, herbivore-damaged plants can redistribute their resources and delay reproduction [50] whereas undamaged plants flower 'on time.' In such cases, plants 'mate' (exchange pollen) nonrandomly with others showing a similar level of damage; that is, there can be assortative mating based on damage levels that reflect underlying resistance phenotype. This facilitates fixation of resistance alleles, even if they show a modest net cost in the short term [51].

In other cases, production of a resistance factor by itself delays reproduction (Ref. [52] and references therein; Box 4): susceptible plants flower before resistant plants in the absence of herbivores, but damage-induced delays in susceptibles causes synchrony when herbivores are abundant. In this case, the correlation between selection and genetic variance is reversed, and so fixation of resistance can be delayed.

Multiple interactions and tradeoffs

Plant phenological tradeoffs can also arise when two or more mutualists act discordantly. In the mistletoe *Tristerix corymbosis*, the flowering peak does not coincide with the pollinator peak, but led to a peak in fruiting that corresponded with the peak in seed dispersal [53]. This pattern could be adaptive if variation in plant fitness is more strongly determined by variation in seed dispersal than by variation in pollination [53]. Pollinators and ant guards provide another example of a phenological tradeoff between mutualist interactions. While guarding against herbivores, ants might also deter pollinators [54]. To avoid interference, plants might synchronize flower opening time with the release of extrafloral nectar, thus attracting the ants away from pollinators [54].

Tradeoffs might also occur among multiple antagonistic interactions. In wild indigo *Baptisia australis*, different insect species exert differential selection on phenology, some attacking early flowers, other late flowers, but relative abundances change from year to year, changing the optimal flowering period [55]. In the scarlet gilia *Ipomopsis aggregata*, experimentally varying the presence of two insect herbivore species changed the strength and shape of the selection gradient on flowering start date [50]. In the same plant species, simulating herbivory by mammals delayed flowering and enabled the plants to escape seed predation [39].

More obvious are tradeoffs between mutualists and antagonists, including cases where the same biotic agent is both pollinator and seed predator or disease vector. Indeed, most studies investigating effects of pollinators and antagonists find that they affect phenology in different

ways and, in 40% of these studies, the effects of pollinators counter those of antagonists (see Online Supplementary Information). This might even result in the total seed production being apparently independent of phenology [29,40]. Tradeoffs between pollinators and antagonists might not be restricted to aspects of phenology that are related to the timing of flower production during the season. Increased flower longevity, another component of flowering phenology, might benefit the plant by maximizing the receipt of more, or more-compatible pollen, but this might again increase the risk of acquiring diseases, including those vectored by pollinators.

When plants face such tradeoffs for their reproduction, optimization of reproductive timing might not be possible, because plants cannot simultaneously avoid insufficient pollination, insufficient dispersal, disease acquisition and massive seed predation. Extended flowering duration might serve as a bet-hedging strategy to handle with tradeoffs both between pollinators and seed predators [37,56] and between pollinators and dispersers [53]. Interestingly, biotically pollinated plants flower for longer periods than do abiotically pollinated plants [20], as predicted if extended flowering evolves in response to tradeoffs between interactions with pollinators and other biotic agents.

Future directions

As indicated in the Online Supplementary Material, the intensity of biotic interactions frequently changes over the course of the flowering season. This is a necessary, but not sufficient, cause for these interactions to impose selection on flowering schedules. Constraints can arise owing to correlations between flowering phenology and other fitness-related traits, notably those related to the total number of flowers produced by a plant [57]. Although there is a good understanding of genetic regulation of natural variation in date of first flowering, the genetic architecture of other flowering schedule components, including duration, skew and kurtosis are less well known. Genetic correlations between these phenological characters and other fitness-relevant traits (such as pathogen resistance) also need more attention.

Ecological constraints on selection responses will also arise if the direction favoured by biotic selection is counteracted by other forces such as abiotic selection, resulting in a zero net effect [11]. This calls for future studies that use thorough multivariate selection analyses, experimental manipulation of one of the interacting species, and common garden and transplant experiments to separate environmental and genetic variability in phenological responses.

A little-explored issue is how phenological variation affects the evolution of biotic interactions through assortative mating; in other words, through its effect on genetic variance as opposed to its effect on fitness. Herbivore or pathogen damage often results in delayed onset of flowering of susceptible or non-tolerant hosts. Conversely, costs of resistance can result in delayed onset of flowering of resistant hosts. Both cases will result in assortative mating within resistant and susceptible genotypic classes, yielding varied evolutionary responses [51]. The consequences of

this phenological assortment are just beginning to be explored theoretically and experimentally and open new avenues of research.

An exciting opportunity to study the selection pressures on flowering phenology as they arise through biotic interactions occurs when these interactions have been disturbed. For instance, selection owing to specialist antagonists is often relaxed after biological invasions. Plants of *Silene latifolia* introduced in North America are released from fruit predators and pathogens and flower earlier than are their native counterparts in Europe. The difference is genetically based [5]. Although many factors might be responsible for the shift, one possibility is relaxation from the constraint on earlier flowering imposed by natural enemies in the native habitat [58]. An important future direction would be to establish to what extent traits frequently observed to change in invasions represent an evolutionary response to the new environment [59], and this should include phenological changes, given their demonstrated heritable basis.

Conclusions

The role of biotic interactions in shaping plant phenological patterns has long been a controversial issue. For instance, Ollerton and Lack [9] questioned the need to invoke adaptation to biotic interactions to account for asynchronous flowering within populations, arguing that spatiotemporal variation in effects of phenology on plant fitness prevents fine-scale local adaptation, maintaining much of the variation in phenology that we observe in natural populations. Indeed, approximately one-third of the studies investigating multiple sites or years (Online Supplementary Information) detected spatiotemporal variation in the strength or direction of biotic selection on flowering phenology.

However, although spatiotemporal variation in effects (as well as differential or opposing effects of multiple biotic interactions) might prevent consistent responses to biotic selection, this does not refute the overwhelming support for the conclusion that biotic interactions modulate patterns of selection on flowering phenology. Where such effects have been detected (two-thirds of the studies) pollinators tend to favour shifts towards peak or early flowering, whereas pre-dispersal seed predators tend to favour off-peak or late flowering. There appears to be little support for the hypothesis that synchronous flowering can satiate herbivores; the classic research by Auspurger [49] might be the exception rather than the rule. A caveat is that most studies are correlational. To test for phenological (co-)adaptation between plants and their mutualists and/or antagonists requires experimental manipulation of phenological patterns, a type of study that is still rare and difficult to conduct.

Finally, the physical environment also has strong selective impacts on phenology. Climatic variation might modify selection on flowering phenology as growing seasons expand or contract [2], and this might modify biotic interactions if the phenology of interacting species shifts unevenly with climate. The coming decades may provide unique opportunities to study rapid evolutionary change in phenology as species adapt to new conditions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tree.2007.05.006.

References

1 Antonovics, J. (2006) Evolution in closely adjacent plant populations: long-term persistence of prereproductive isolation at a mine boundary. *Heredity* 97, 33–37

2 Franks, S.J. *et al.* (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1278–1284

3 Mitchell-Olds, T. and Schmitt, J. (2006) Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. *Nature* 441, 947–952

4 Quinn, J.A. and Wetherington, J.D. (2002) Genetic variability and phenotypic plasticity in flowering phenology in populations of two grasses. *J. Torrey Bot. Soc.* 129, 96–106

5 Wolfe, L.M. *et al.* (2004) Increased susceptibility to enemies following introduction in the invasive plant *Silene latifolia*. *Ecol. Lett.* 7, 813–820

6 Mazer, S. and Schick, T.C. (1991) Constancy of population parameters for life-history and floral traits in *Raphanus sativus* L. II. Effects of plant density on phenotype and heritability estimates. *Heredity* 45, 1888–1907

7 O'Neil, P. (1997) Natural selection on genetically correlated phenological characters in *Lythrum salicaria* L (Lythraceae). *Evolution* 51, 267–274

8 Weis, A.E. and Kossler, T.M. (2004) Genetic variation in flowering time induces phenological assortative mating: quantitative genetic methods applied to *Brassica rapa*. *Am. J. Bot.* 91, 825–836

9 Ollerton, J. and Lack, A.J. (1992) Flowering phenology– an example of relaxation of natural selection? *Trends Ecol. Evol.* 7, 274–276

10 O'Neil, P. (1999) Selection on flowering time: an adaptive fitness surface for nonexistent character combinations. *Ecology* 80, 806–820

11 Pilson, D. (2000) Herbivory and natural selection on flowering phenology in wild sunflower, *Helianthus annuus*. *Oecologia* 122, 72–82

12 Putterill, J. *et al.* (2004) It's time to flower: the genetic control of flowering time. *Bioessays* 26, 1–11

13 Visser, M.E. and Holleman, L. (2001) Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proc. R. Soc. B* 268, 289–294

14 Hendry, A.P. and Day, T. (2005) Population structure attributable to reproductive time: isolation by time and adaptation by time. *Mol. Ecol.* 14, 901–916

15 Gérard, P.R. *et al.* (2006) Assortative mating and differential male mating success in an ash hybrid zone population. *BMC Evol. Biol.* 6, 96

16 Kitamoto, N. *et al.* (2006) Effect of flowering phenology on pollen flow distance and the consequences for spatial genetic structure within a population of *Primula sieboldii* (Primulaceae). *Am. J. Bot.* 93, 226–233

17 Fox, G. (2003) Assortative mating and plant phenology: evolutionary and practical consequences. *Evol. Ecol. Res.* 5, 1–18

18 Weis, A.E. *et al.* (2005) Phenological assortative mating in plants: causes and consequences of its inherent frequency-dependence. *Evol. Ecol. Res.* 7, 161–181

19 Stone, G. *et al.* (1998) Partitioning of pollinators during flowering in an african *Acacia* community. *Ecology* 79, 2808–2827

20 Bolmgren, K. *et al.* (2003) Contrasting flowering phenologies and species richness in abiotically and biotically pollinated Angiosperms. *Evolution* 57, 2001–2011

21 De Jong, T.J. and Klinkhamer, P.G.L. (1991) Early flowering in *Cynoglossum officinale* L. Constraint or adaptation? *Funct. Ecol.* 5, 750–756

22 Johnston, M.O. (1991) Natural selection on floral traits in two species of *Lobelia* with different pollinators. *Evolution* 45, 1468–1479

23 Mahoro, S. (2002) Individual flowering schedule, fruit set, and flower and seed predation in *Vaccinium hirtum* Thunb. (Ericaceae). *Can. J. Bot.* 80, 82–92

24 Marquis, R.J. (1988) Phenological variation in the neotropical understory shrub *Piper arieianum*: causes and consequences. *Ecology* 69, 1552–1565

25 Schemske, D.W. (1977) Flowering phenology and seed set in *Claytonia virginica* (Portulacaceae). *Bull. Torrey Bot. Club* 104, 254–263

26 Totland, O. (1993) Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant communities. *Can. J. Bot.* 71, 1072–1079

27 Ågren, J. and Willson, M.F. (1992) Determinants of seed production in *Geranium maculatum*. *Oecologia* 92, 177–182

28 Heithaus, E.R. *et al.* (1982) Cumulative effects of plant-animal interactions on seed production by *Bauhinia ungulata*, a neotropical legume. *Ecology* 63, 1294–1302

29 Gomez, J.M. (1993) Phenotypic selection on flowering synchrony in a high-mountain plant, *Hormathophylla spinosa* (Cruciferae). *J. Ecol.* 81, 605–613

30 Zimmerman, M. (1980) Reproduction in *Polemonium*: competition for pollinators. *Ecology* 61, 497–501

31 Parra-Tabla, V. and Vargas, C.F. (2004) Phenology and phenotypic natural selection on the flowering time of a deceit-pollinated tropical orchid, *Myrmecophila christinae*. *Ann. Bot.* 94, 243–250

32 Sabat, A.M. and Ackerman, J.D. (1996) Fruit set in a deceptive orchid: the effect of flowering phenology, display size, and local floral abundance. *Am. J. Bot.* 83, 1181–1186

33 Hirao, A.S. *et al.* (2006) Seasonal changes in pollinator activity influence pollen dispersal and seed production of the alpine shrub *Rhododendron aureum* (Ericacea). *Mol. Ecol.* 15, 1165–1173

34 Albrectsen, B.R. (2000) Flowering phenology and seed predation by a tephritid fly: escape of seeds in time and space. *Ecoscience* 7, 433–438

35 Lacey, E.P. *et al.* (2003) Multigenerational effects of flowering and fruiting phenology in *Plantago lanceolata*. *Ecology* 84, 2462–2475

36 Pettersson, M.W. (1991) Pollination by a guild of fluctuating moth populations – option for unspecialization in *Silene vulgaris*. *J. Ecol.* 79, 591–604

37 Tarayre, M. *et al.* (2007) Flowering phenology of *Ulex europaeus*: ecological consequences of variation within and among populations. *Evol. Ecol.* 21, 395–409

38 Eriksson, O. (1995) Asynchronous flowering reduces seed predation in the perennial forest herb *Actaea spicata*. *Acta Oecol.* 16, 195–203

39 Freeman, R.S. *et al.* (2003) Flowering phenology and compensation for herbivory in *Ipomopsis aggregata*. *Oecologia* 136, 394–401

40 McIntosh, M.E. (2002) Flowering phenology and reproductive output in two sister species of *Ferocactus* (Cactaceae). *Plant Ecol.* 159, 1–13

41 Pettersson, M.W. (1994) Large plant size counteracts early seed predation during the extended flowering season of a *Silene uniflora* (Caryophyllaceae) population. *Ecography* 17, 264–271

42 English-Loeb, G.M. and Karban, R. (1992) Consequences of variation in flowering phenology for seed head herbivory and reproductive success in *Erigeron glaucus* (Compositae). *Oecologia* 89, 588–595

43 Klips, R.A. *et al.* (2005) Temporal and geographic variation in predispersal seed predation on *Hibiscus moscheutos* L. (Malvaceae) in Ohio and Maryland, USA. *Am. Midl. Nat.* 154, 286–295

44 Molau, U. *et al.* (1989) Predispersal seed predation in *Bartsia alpina*. *Oecologia* 81, 181–185

45 Dieringer, G. (1991) Variation in individual flowering time and reproductive success of *Agalinis strictifolia* (Scrophulariaceae). *Am. J. Bot.* 78, 497–503

46 Ehrlén, J. (1996) Spatiotemporal variation in predispersal seed predation intensity. *Oecologia* 108, 708–713

47 Janzen, D.H. (1971) Seed predation by animals. *Annu. Rev. Ecol. Syst.* 2, 465–492

48 Honek, A. and Martinkova, Z. (2005) Pre-dispersal predation of *Taraxacum officinale* (dandelion) seed. *J. Ecol.* 93, 335–344

49 Augspurger, C.K. (1981) Reproductive synchrony of a tropical shrub - experimental studies on effects of pollinators and seed predators on *Hybanthus prunifolius* (Violaceae). *Ecology* 62, 775–788

50 Juenger, T. and Bergelson, J. (1998) Pairwise versus diffuse natural selection and the multiple herbivores of scarlet gilia, *Ipomopsis aggregata*. *Evolution* 52, 1583–1592

51 Winterer, J. and Weis, A. (2004) Stress-induced assortative mating and the evolution of stress resistance. *Ecol. Lett.* 7, 785–793

52 Biere, A. and Antonovics, J. (1996) Sex-specific costs of resistance to the fungal pathogen *Ustilago violacea* (*Microbotryum violaceum*) in *Silene alba*. *Evolution* 50, 1098–1110

53 Aizen, M.A. (2003) Influences of animal pollination and seed dispersal on winter flowering in a temperate mistletoe. *Ecology* 84, 2613–2627

54 Raine, N. *et al.* (2002) Spatial structuring and floral avoidance behavior prevent ant-pollinator conflict in mexican ant-*Acacia*. *Ecology* 83, 3086–3096

55 Evans, E.W. *et al.* (1989) Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. *Oecologia* 78, 220–230

56 Picó, X. and Retana, J. (2000) Temporal variation in the female components of reproductive success over the extended flowering season of a Mediterranean perennial herb. *Oikos* 89, 485–492

57 Dres, M. and Mallet, J. (2002) Host races in plant-feeding insects and their importance in sympatric speciation. *Phil. Trans. R. Soc. B* 357, 471–492

58 Biere, A. and Honders, S. (1996) Impact of flowering phenology of *Silene alba* and *S. dioica* on susceptibility to fungal infection and seed predation. *Oikos* 77, 467–480

59 Müller-Schärer, H. and Steinger, T. (2006) Predicting evolutionary change in invasive, exotic plants and its consequences for plant-herbivore interactions. In *Genetics, Evolution and Biological Control* (Ehler, L. *et al.*, eds), pp. 137–162, CABI Publishing

60 Bolmgren, K. (1998) The use of synchronization measures in studies of plant reproductive phenology. *Oikos* 82, 411–415

61 Méndez, M. and Díaz, A. (2001) Flowering dynamics in *Arum italicum* (Araceae): Relative role of inflorescence traits, flowering synchrony, and pollination context on fruit initiation. *Am. J. Bot.* 88, 1774–1780

62 Bishop, J.G. and Schemske, D.W. (1998) Variation in flowering phenology and its consequences for lupines colonizing Mount St. Helens. *Ecology* 79, 534–546

63 Augspurger, C.K. (1983) Phenology, flowering synchrony, and fruit-set of 6 neotropical shrubs. *Biotropica* 15, 257–267

64 Primack, R.B. (1980) Variation in the phenology of natural populations of montane shrubs in New Zealand. *J. Ecol.* 68, 849–862

65 Internicola, A.I. *et al.* (2006) Experimental investigation of the effect of spatial aggregation on reproductive success in a rewardless orchid. *Oecologia* 150, 435–441