

LETTER

Linné's floral clock is slow without pollinators – flower closure and plant-pollinator interaction webs

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Abstract

Temporal patterns of flower opening and closure within a day are known as Linné's floral clock. Time of flower closure has been explained mainly by light in the traditional botanical literature. We show with a set of experiments that Asteraceae flower heads can close within three hours after pollination, whereas un-pollinated flower heads stay open until the late afternoon. This suggests that closing time strongly depends on pollinators. Using plant-pollinator interaction webs we further demonstrate that the daily pattern of flower opening and the rapid response to pollination can impose strong temporal dynamics on interspecific interactions within a single day. We observed pollinator species turnover and changes in facilitation vs. competition among plants. Our results show for the first time that pollination induces rapid flower closure on the community level. This causes imprecision in Linné's floral clock with far-reaching consequences for plant-pollinator interactions.

Keywords

Capitulum closure, Cichorioideae, circadian, *Crepis capillaris*, flower opening, interaction networks, mutualism, pollination response, temporal specialisation, temporal turnover.

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INTRODUCTION

It has long been recognised that many flowers are open for a restricted time of the day and this inspired Linné (1783) to propose the concept of a flower clock based on observed flower opening and closing times of different plant species (Fig. 1). Later, detailed observations and experiments showed that flower opening and closure depend on light and temperature, with some evidence for influence of endogenous rhythms and humidity (Oltmanns 1895; Burgerstein 1901; Todt 1962; Ewusie & Quayle 1977; Tanaka *et al.* 1988; van Doorn & van Meeteren 2003; von Hase *et al.* 2006) including some knowledge about the underlying modes of inheritance (Nitta *et al.* 2010).

However, the daily (circadian) pattern of flower opening and closure has not been connected to pollination. It is known for some plants, e.g. many orchids, that unpollinated flowers remain attractive longer than pollinated flowers (Fitting 1909; van Doorn 1997; Abdala-Roberts *et al.* 2007; Clark & Husband 2007). Most floral responses to pollination are too slow to affect the daily pattern of flower opening and closure: in *Epilobium angustifolium* flowers began to close about 10 h after pollination (Clark & Husband 2007), and in *Gentiana straminea* pollination-induced closure occurred after 2 days (He *et al.* 2005). The few plants known to react within a few hours (e.g. petal abscission in Geraniaceae: Fitting 1911) do not close in a daily pattern. Here, we focus on liguliflorous Asteraceae (subfamily Cichorioideae), which are textbook examples of floral movements (e.g. Sitte *et al.* 2002, p. 476). This group includes 27 of the 44 species in Linné's floral clock, it is an important part of European vegetation (Mémott 1999; Lázaro *et al.* 2009) and invasive elsewhere (Alexander *et al.* 2009; Hao *et al.* 2010).

We provide evidence that flower heads (capitula) of a number of species in this group close rapidly after pollination, but stay open much longer without pollination. The questions we address in the first part of the article are:

- (1) Do pollinated flower heads close earlier than non-pollinated flower heads (i.e. does pollination induce advanced capitulum closure)?
- (2) Is this effect consistent for a number of common species and for closure time on the level of single flower heads as well as populations?

Despite the importance of plant-pollinator interactions and the high interest in them, within-day patterns have received only limited attention in the last few years. While it is known that for example nectar production varies throughout the day (Willmer & Corbet 1981; Pleasants & Chaplin 1983), time of day is rarely considered in community approaches (but see Stone *et al.* 1996, 1998; Hoehn *et al.* 2008). The network approach for plant-pollinator interactions has gained popularity (Bascompte & Jordano 2007; Vázquez *et al.* 2009), but within-day patterns are not considered in such studies, while temporal dynamics on larger time scales have recently been highlighted (Alarcón *et al.* 2008; Olesen *et al.* 2008; Petanidou *et al.* 2008). However, changes in flower opening of dominant plant species within a single day may affect the whole network e.g. by dynamics of competition for pollinators and temporal specialisation, which may be further complicated by pollination-dependence of flower closure.

In the second part of this article, we analyse whether or not an influence of early flower closure of Cichorioideae can be found in plant-pollinator interaction webs, addressing the following questions:

- (1) Do interactions differ between morning and afternoon? Are the pollinators of early-closing Cichorioideae only foraging during the morning or do they switch to other plants in the afternoon?
- (2) Do co-flowering plants experience increased competition for pollinators during the morning when Cichorioideae are open?
- (3) Are field data consistent with the experimental evidence for pollination as a determinant of flower closure time (i.e. are Cichorioideae flower heads open for longer in webs with low visitation rate)?



Figure 1 A floral clock drawn after the *Horologium florum* by Carl von Linné, where he listed opening and closing times for a number of plant species and proposed that these could be used to accurately predict the time of the day. Drawing by Ursula Schleicher-Benz. From: Lindauer Bilderbogen (Series 1, No. 5) ed by Friedrich Böer © Jan Thorbecke Verlag, Lindau/Reutlingen, 1948.

We show that pollination can induce rapid flower closure and this in turn can effect the community-level pattern of interactions, causing temporal shifts of plant-pollinator interactions within a single day.

MATERIAL AND METHODS

Experimental set-up

In 2008 a large field experiment was set up in Göttingen, Germany, to study the relationship between wild bee diversity and pollination of a plant community (Fründ *et al.*, unpublished). In April 59 beds of 4 m × 2 m were seeded in a fixed pattern (separate patches) with 16 species of wild herbs and covered by gauze cages to exclude insects. At the beginning of July wild bees were released in 40 of the cages until mid-August. Different combinations of five wild bee species (*Bombus terrestris*, *Osmia bicornis*, *Megachile centuncularis/versicolor*, *Heriades truncorum*, *Hylaeus communis*) were used with the same total abundance per cage (Mean = 20.5 bee individuals). For this article, they will be referred to as ‘bee cages’ regardless of the species. Twelve cages served as controls (without bees), and seven beds were left uncaged or with open cages allowing access to pollinators from the surrounding area (‘open’). Among the 14 herb species that flowered during the experiment one was a member of the subfamily Cichorioideae within the Asteraceae: *Crepis capillaris* (L.) Wallr., which flowered primarily in July and August in each of two patches per cage.

Circadian pattern of flower opening in different pollinator treatments

On 2 August 2008, all cages were monitored for openness of *C. capillaris* flower heads. Starting at 10:50 h, when almost all flower heads had opened in all cages, the number of open flower heads

(opening angle > ~ 90°) were counted (to the nearest 10) from the same relative position for each cage. Counting was repeated three times, at 13:30 h, 17:00 h and 19:00 h. From these estimated numbers of open flowers, the proportion of open flowers at a time relative to the first count was calculated.

Hand pollination of flower heads

In a cage identical to the control cages, the relationship between pollination and capitulum closure was tested by hand pollinating single flower heads on 14 August 2008. All flying insects were removed from the cage. Twenty-four flower heads of *C. capillaris* on several individuals were marked with thread and numbered. Twelve flower heads were assigned to the pollination treatment and 12 neighbouring flower heads were left as controls (controls were always near to a treatment flower head and on the same plant, Fig. 2a). At 11:00 h, when all flower heads had opened, treatment flowers were hand-pollinated with three flower heads from other *C. capillaris* individuals. After hand pollination, the opening angle (Fig. 2b) of each flower head was noted every 30–60 min until 19:10 h, when all flower heads were closed. Flower heads were also assessed three times the next day.

Similar experiments were conducted for other sets of plants and different species in 2008 and 2009: *Crepis biennis* L. (five naturally growing plants), *C. capillaris* (a second set of 12 plants, sown into pots in spring 2009), *Leontodon autumnalis* L. (six plants from a ruderal area in Göttingen, planted into two 5 L pots) and *Taraxacum officinale* [(L.) Weber] (naturally growing plants). Experiments in 2009 were performed in a cage of the same type as in 2008. Further details on all hand pollination experiments can be found in Table S1 of

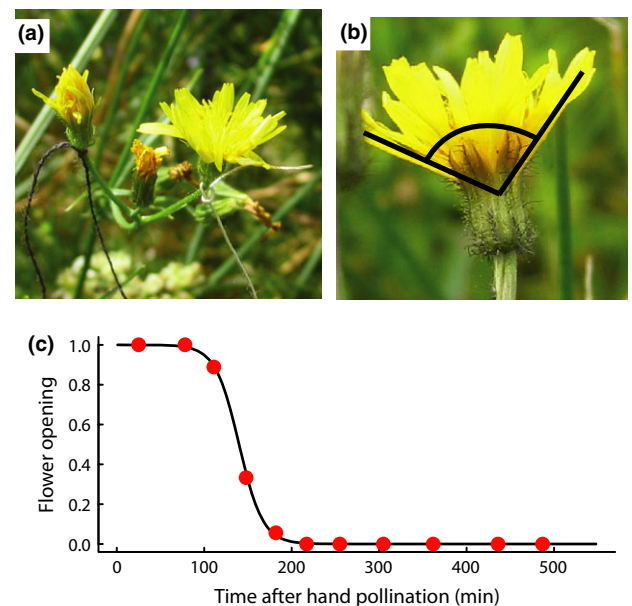


Figure 2 Closure of flower heads in hand pollination experiments. (a) A pair of flower heads of *C. capillaris*, outer left = cross pollinated, outer right = control. (b) Opening angle was used to quantify opening and closure of flower heads, the photo shows a flower head with 98° opening. Photo credit Kristian Peters, GNU licence. (c) Logistic curve fitted to temporal flower opening data of one example flower head of *C. capillaris*. This curve has the parameters $b = 0.0736$ and $t_0 = 138.9$ (see Material and methods for explanations, see Table S2 for fitted parameters for all flower heads). Note that the y-axis indicates proportional opening in relation to maximum opening degree.

Supporting Information. To check whether the measured response to pollination was just a response to mechanical stimulation, additional controls were used in two of the experiments: for *C. biennis*, three flower heads were touched with flower heads of another species (*Picris hieracioides* L.) in the same manner as in the pollination treatments, and for *C. capillaris* in 2009, six flower heads were self-pollinated with flower heads from the same plant individual.

Data analysis Part 1 – flower closure

All analyses were performed using R 2.11.1 (R Development Core Team 2010). To analyse the effects of hand pollination on capitulum closure, we first fitted a logistic function (Fig. 2c) with non-linear least squares regression:

$$y = 1 - \frac{1}{1 + e^{-b \cdot (t - t_0)}}$$

where y is standardised opening degree (opening angle divided by the initial estimate of opening angle, which sometimes slightly deviated from 180°), t is time after pollination treatment and b and t_0 are the fitted parameters. Parameter b measures the ‘slope’ (i.e. speed of closure after initiation), while parameter t_0 represents the inflection point of the curve (i.e. the time until the flower is half closed). This function described the temporal process of flower closure generally very well (Fig. 2c). In a few cases in which flower heads were still fully open at the end of the observations (opening angle > 150°, five controls in *C. biennis*), curves could not be fitted and we used a conservative estimate of 600 min for t_0 (which would represent immediate closure after the last measurement). The effect of hand pollination was tested by comparing the parameter t_0 (relative time of closure) between flower heads of the different treatment groups. All hand pollination experiments were analysed using ANOVA with treatment, experimental set and their interaction as explanatory variables.

Part 2: Effects of early flower closure in interaction network data – data and analysis

We explored the evidence for effects of Cichorioideae flower closure on community-wide plant-pollinator interactions with a dataset of quantitative plant flower-visitor networks from 27 meadow sites in southern Germany. Details of the study can be found in Fründ *et al.* (2010). Flower visitation data were collected in transects and observation plots on single day, between 8:30 h and 19:00 h (all webs have data before 12:00 h and after 13:00 h). The number of flowers or flower heads was counted for each of ten 2 m² observation plots per network. All flower visitors on all plants were noted to family and morphotype, and bees and hoverflies were captured and determined to species level. In contrast to the original article, here we used both captured and non-captured flower visitors and we included two different single-day networks for five of the sites (resulting in a total of 32 webs). For these five sites, resamples were at least three weeks apart, when floral composition had already changed. On average, these networks comprised 219 observed flower visitors, of which 61 bees and hoverflies were determined to species level. We used these data to characterise circadian patterns of flower visitation.

Time of the day was noted for each observation plot (15 min) and sub-transect (*c.* 30 min). For the analyses, we assigned the mean time of each observational unit (i.e. observation plot or sub-transect) to all

interactions observed in the respective unit. Because we did not collect temporally resolved data for actual flower opening in the field study, we focussed on patterns of flower visitation in this part of the article as an indirect measure of flower closure.

To analyse the temporal dynamics of plant – pollinator interactions within a single day, we separated the networks into morning- (AM) and afternoon- (PM) subwebs. For this purpose, ‘noon’ was defined as the time of solar zenith (13:00 h). Thus, AM-subwebs include times until 12:59 h. Web plots were created with the R-package bipartite 1.12 (Dormann *et al.* 2009). We compared the identity of pollinators and the identity of links between the two subwebs for each network using Bray – Curtis dissimilarity of relative frequencies including only specimens determined to species level. To account for the fact that dissimilarity values are sensitive to sample size (see Appendix S1), we focussed on the difference to a null model. This null model re-assigned observations among the two subwebs (times of day), while fixing the total frequency per species (or link) and subweb (function `r2dtable` in R, mean of $N = 1000$ replicates; see Appendix S1 for further details). Consequently, species richness (or connectance) was constant for each web, but variable for each subweb. The proportion of visits to Cichorioideae among all visits was calculated to quantify the dominance of these flowers in the network and their importance for the temporal turnover of pollinators and interactions.

To check for the influence of Cichorioideae flower closure on visitation to other plants, we calculated separately for morning and afternoon (i.e. before and after 13:00 h) visitation rate to the two plant species without closing mechanism observed in plots of more than 10 networks: *Achillea millefolium* L. (Asteraceae), a species with a visitor spectrum overlapping with Cichorioideae, and *Trifolium pratense* L. (Fabaceae), which attracts a very different pollinator spectrum. Visitation rate was calculated as the number of observed flower visitors in observation plots divided by the number of inflorescences in observation plots for each of the two time periods. To quantify the importance of afternoon visitation, the proportion of visits received during the afternoon was calculated as the visitation rate during the afternoon divided by the sum of morning and afternoon visitation rates.

To check for evidence of pollinator-dependent flower closure in the field, we compared the morning visitation rate by bees and the temporal pattern of visitation to Cichorioideae, i.e. estimated time of flower closure. The latter was expressed as the time from sunrise until at least 90% of visits were observed, thereby adjusting for differences in day length. In this case, the only web where *Cichorium intybus* L. was the dominant Cichorioideae was excluded because this species was an unusual Cichorioideae in the dataset (however, its inclusion would only strengthen the observed pattern). Webs with < 2 observed visits to Cichorioideae were also excluded. Using visitation rate by all flower visitors instead of bees showed a similar pattern but with less explanatory power ($\Delta_{AICc} > 10$, comparison of the two models, see Burnham & Anderson 2001).

RESULTS

Circadian pattern of flower opening in different pollinator treatments

On average, flower heads of *Crepis capillaris* closed first in the open cages, second in the bee cages and last in the control cages. All flower

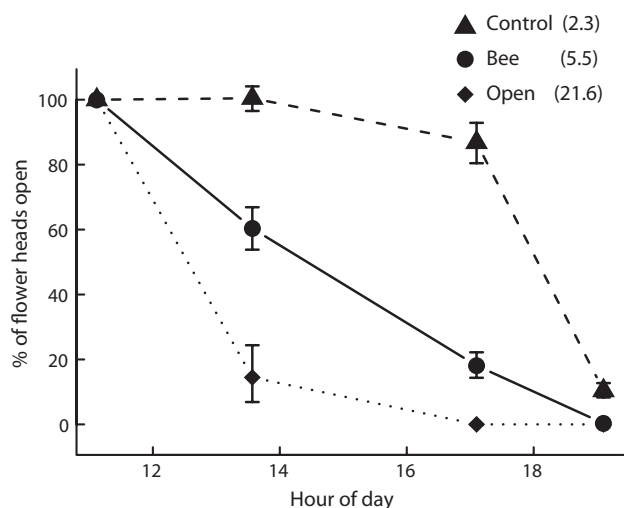


Figure 3 Pattern of flower opening in relation to time of the day for *C. capillaris* under three different pollinator treatments (cage types). Number of open flower heads is shown in relation to the first estimate. Mean \pm SEM [calculated from arcsine (square-root)-transformed data] are shown, and the mean number of visits to *C. capillaris* per cage is indicated for each treatment in the legend.

heads were closed at night. Even in the control cages most had closed by 19:00 h. In open cages and some of the bee cages, however, most flower heads closed already 5 h earlier (Fig. 3). The proportion of closed flower heads differed significantly among cage types (Kruskal – Wallis tests: $P < 0.001$ for all three times after the reference count). Differences in the number of observed bee visits to *C. capillaris* flowers due to different bee community composition corresponded well to the proportion of flower heads closed before 13:30 h (correlation across 39 ‘bee cages’: $P = 0.009$, Pearson’s $r = 0.41$). Among the control cages there were two different sub-treatments: in eight of the cages, hoverflies were present and regularly visited *C. capillaris*, while in four of the control cages, all flying insects were regularly excluded. This difference in the presence of hoverflies corresponded only to a minor difference in early closing flower heads (significant difference only at 19:00 h, $t = 2.9$, $P = 0.021$, Mean \pm 1 SD = $9.0 \pm 5.0\%$ and $16.9 \pm 4.1\%$ with and without hoverflies, respectively).

Hand pollination of flower heads

Cross pollination by hand strongly effected closing time in all species except *Taraxacum officinale* (Fig. 4; interaction of treatment by experimental set, $F_{4,62} = 3.2$, $P = 0.019$; excluding *T. officinale*: interaction $F_{3,48} = 1.1$, $P = 0.33$, treatment effect $F_{1,48} = 50.9$, $P < 0.001$). Capitulum closure was advanced by 3 h in *Crepis capillaris* in 2008. In this case, the reaction started ~ 137 min after pollination (mean time to 90% openness according to fitted curves) and flowers were closed after ~ 209 min (10% openness).

Neither heterospecific pollination in *C. biennis* nor self pollination in *C. capillaris* reproduced the closing reaction to intraspecific cross-pollination: in both cases, the additional control closed significantly later than cross-pollinated flower heads, but did not differ from un-pollinated flower heads (see Table S3). For flower heads, closure was not necessarily permanent: younger flower heads opened again the next day exposing fresh, previously immature florets. In *C. biennis*

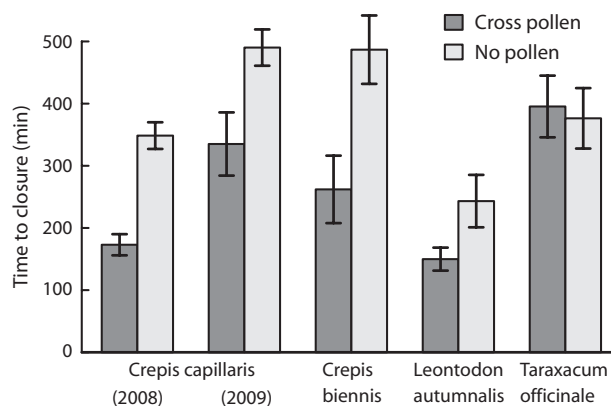


Figure 4 Effect of hand pollination on closure of flower heads in Asteraceae: Cichorioideae. Time to closure (Mean \pm SEM) refers to the time from treatment until half closure estimated from a logistic curve fitted to flower closure dynamics per flower head (see Fig. 2c).

we counted the number of seeds, which corresponded well with pollination treatments and time of closure (see Table S4).

Effects of early flower closure in interaction network data

Plants of the subfamily Cichorioideae were important in the networks dataset, comprised 17% of all observed interactions and $> 10\%$ of interactions in 18 of 32 networks. Interactions with these plants happened early within the day, mostly between 10 to 11:00 h, and $> 91\%$ of visits were observed before 13:00 h (see Figure S1). This predominance of interactions in the morning was not apparent for all other plant species combined and could not be explained by sampling effort which was relatively homogeneously distributed across the main period of bee activity (9:00 h to 17:00 h). Cichorioideae in this dataset were *Picris hieracioides*, *Leontodon autumnalis*, *Hypochaeris radicata* L., *Crepis biennis* (all > 100 observed visits) and seven less frequently observed species.

Strong differences between morning and afternoon subsets of plant-pollinator interaction webs were found (Figs 5 and 6). These differences were particularly dramatic for networks with a high proportion of Cichorioideae. The dissimilarity between interactions of the two subwebs increased with the proportion of Cichorioideae in the network ($F_{1,28} = 11.7$, $P = 0.002$, response = difference to null model, Fig. 6a; see Figure S3 for raw dissimilarity values). Likewise, pollinator communities differed more strongly between morning and afternoon in networks with higher proportions of Cichorioideae ($F_{1,28} = 14.4$, $P < 0.001$, Fig. 6b, Figure S3). The intercept was significant for interaction dissimilarity ($P = 0.003$), but not for the difference in pollinator communities ($P = 0.19$).

Flower visitation to *Achillea millefolium* was reduced during the morning in networks with a high proportion of Cichorioideae, and for this plant species the relative contribution of visitation during the afternoon increased with dominance of Cichorioideae (linear regression, $F_{1,10} = 13.4$, $P = 0.004$, Fig. 6c; see also Figure S3). This relationship was also significant when the dominance of Cichorioideae was defined by % of flower area rather than % of flower visits. The same relationship was not significant for *Trifolium pratense* L.

Estimated time of Cichorioideae flower closure in the network study was earlier in networks with high bee visitation rate to Cichorioideae, and later in webs with low bee visitation rate: the time

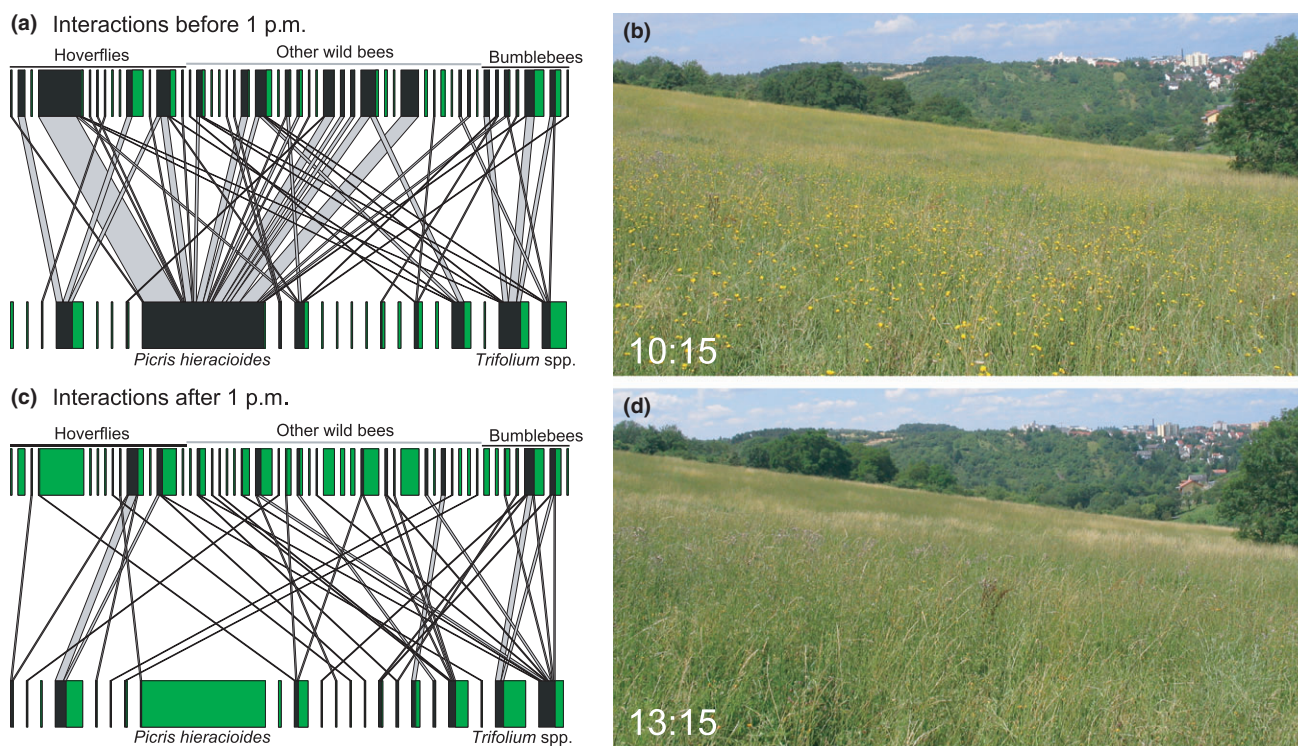


Figure 5 Flower-visitor interaction networks change between morning and afternoon. The graphs on the left show a quantitative plant-pollinator network (close to Gerbrunn, Southern Germany; pooled across five sampling days on four sites). (a) Morning subweb (i.e. all interactions observed before 13:00 h), (c) afternoon subweb (after 13:00 h). (b and d) show part of the meadow complex where the web was compiled, both pictures were taken on the same day, but with a time-lag of 3 h, during which almost all of the yellow flowers of *Picris hieracioides* had closed. In (a and c) width of the marginal rectangles is relative to a species' interaction frequency in the total network and interactions not occurring during the focal time period are marked in green. In the network graphs, plants are sorted by family, and flower visitors by functional group. Full species names are available in the Supporting Information (Figure S2, Tables S5, S6).

from sunrise until at least 90% of visits to Cichorioideae had been observed decreased significantly with visitation rate by bees, while weather had an additional influence with estimated flower closure on sunny days being about half an hour earlier than on partly cloudy days (multiple regression; weather: $F_{1,16} = 8.02$, $P = 0.01$; \log [bee visitation rate + 0.05]: $F_{1,16} = 51.8$, $P < 0.001$, Fig. 6d; excluding webs from overcast days to avoid intercorrelated independent variables). This decrease was also significant when hour of day was used as the response variable (ranging between 11:00 h and 15:00 h; see Figure S3).

DISCUSSION

Our study clearly shows that plants within the subfamily Cichorioideae of the Asteraceae close their flower heads shortly after pollination and that this effect is fast enough to affect the circadian pattern of flower opening and closure, the so called 'floral clock'. We further show that this early flower closure can be an important component of plant-pollinator interaction webs influencing the dynamics of interaction patterns within the course of a day. Both parts of the article suggest that the 'floral clock' is delayed when only few pollinators are visiting.

Experimental approaches

Differences in flower closure among the cages reflect differences in pollination. In the open cages and some of the bee cages, *C. capillaris*

was pollinated well and most flower heads had closed by noon, suggesting that flowers were pollinated during the first two hours after opening. Microclimatic conditions were identical at least in the closed cages, such that only pollinator visitation differed. Early flower closure in open cages can also be explained by pollination. The proportion of open flower heads at intermediate times could thus be used to estimate pollination success.

The hand pollination experiments strikingly confirmed that pollination caused advanced flower closure. Three of the four tested species showed this effect, suggesting that it is widespread among Cichorioideae. The only species that did not respond to pollination was *Taraxacum officinale*, which is often apomictic (Collier & Rogstad 2004) meaning that pollen application does not lead to pollination (presumably also in the studied population, as seeds were produced also in the non-pollinated flower heads). Observed differences in the closure time of unpollinated flower heads may be explained by differences between species and experimental conditions (e.g. weather conditions or the time from the first measurement until sunset). The time from pollination to closure is likely also influenced by pollen quality and quantity. Correspondingly, visits by hoverflies had a much smaller effect on opening patterns in the cages than visits by bees, probably reflecting their lower rate of (conspecific) pollen deposition (Alarcón 2010).

Our findings suggest that the capitulum closure is controlled by a combination of pollination and other factors. The time of flower opening seems to be determined by light (Stoppel 1910), and light is probably the reason that pollinated and unpollinated flowers will eventually be closed at night. However, pollination is probably more

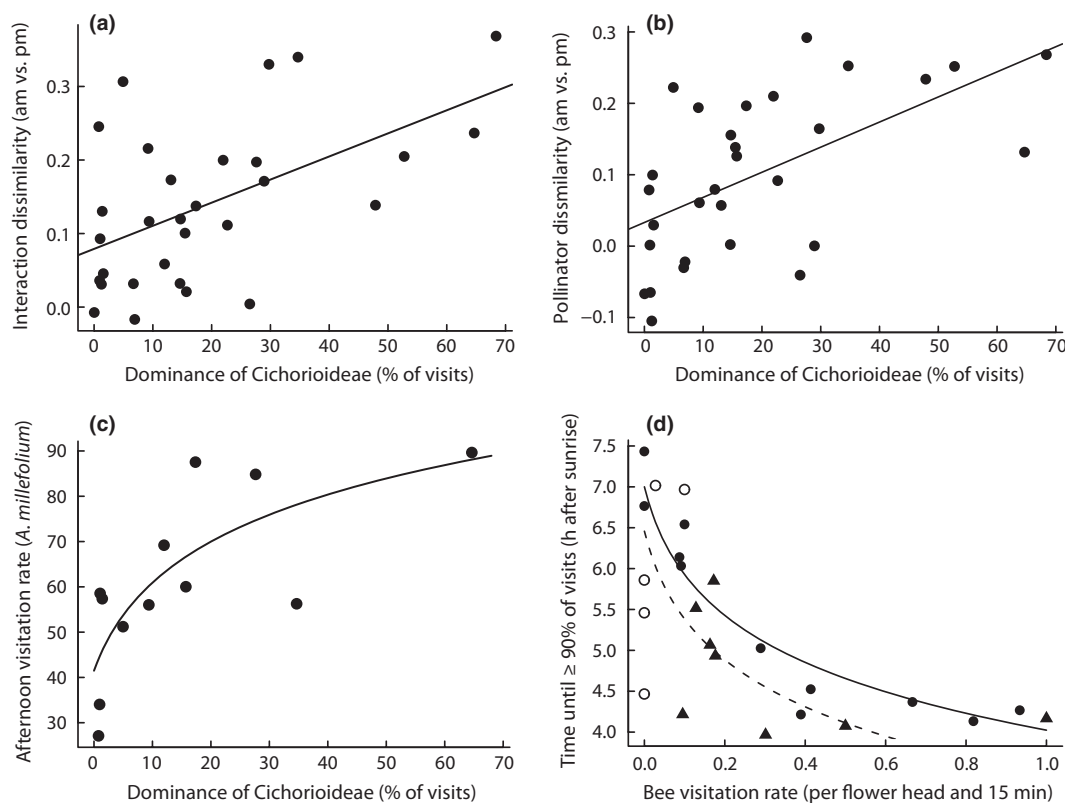


Figure 6 Patterns in a set of flower-visitor networks, highlighting the consequences of early flower closure in the field. (a – c) show the influence of the proportion of Cichorioideae (closing early during the day) in the web on community interactions, and (d) shows the influence of visitation rate on temporal patterns of Cichorioideae. (a,b) Difference between morning and afternoon subsets of plant-pollinator interaction webs. (a) Differences in interactions, (b) differences in pollinator communities. Dissimilarity is measured as Bray – Curtis distance subtracting the expected value for sampling from a homogeneous community (i.e. the null model mean). (c) ‘Importance’ of afternoon visitation to *Achillea millefolium* (in % of AM + PM visitation) increases with the proportion of Cichorioideae. (d) Negative feedback – relative time to flower closure of Cichorioideae (estimated by visitation) in relation to bee visitation rate. The y-axis reflects estimated closure time relative to sunrise, i.e. controlling for seasonal differences in day length. Dashed line and triangles = sunny days, solid line and filled circles = partly cloudy, open circles = overcast days (not included in regression).

important in determining the time of closure in typical field situations. As many pollinator species are also influenced by light and weather conditions (Kevan & Baker 1983; Fründ *et al.* 2010), it is difficult to tease apart the different factors influencing flower closure in field scenarios. Our controlled cage experiments showed that plants of the Cichorioideae have the potential to visibly respond to pollination within 1–2 h. This is by far the fastest known flower closure response to pollination (van Doorn 1997) and combined with high pollinator visitation it makes early flower closure on the population level possible.

Our data suggest that many species within the Cichorioideae might show such a response, given that capitula of probably all Cichorieae perform closure movements. However the effect of pollinator-mediated flower closure will not be found in all Cichorioideae. Several species of Cichorioideae can produce seeds asexually or following self-pollination (Fryxell 1957; Ferrer & Good-Avila 2007). The strength of the closure response may be related to a species’ breeding system, with apomictic species not responding to deposition of cross pollen. Furthermore, the lack of response after self pollination in *C. capillaris* suggests that there is a very fast mechanism for detecting effective pollination and for transferring this signal from the stigma to the involucre bracts that perform the closure movement. The underlying physiological mechanisms should be identified by future laboratory experiments.

Compared to published examples of flower closure in response to pollination, the example shown here differs because it refers to flower heads that may re-open after closure (if immature flowers are still present), and thus represents a plant movement rather than a simple sign of senescence. It is particularly surprising because van Doorn (1997, 2002) proposed that Asteraceae do not cease floral attraction in response to pollination because they are not sensitive to ethylene. Ethylene is considered to be the main signal in floral pollination responses (O’Neill 1997; van Doorn 2002). Whether plants from other taxa that also show flower opening and closure movements in a roughly circadian pattern (e.g. *Convolvulus*, *Legousia*, *Calendula*, *Cucurbita*) also respond to pollination remains an important question for future studies.

Early flower closure in the field and plant-pollinator interactions

Interactions between Cichorioideae and their flower visitors almost exclusively happened during the morning, probably due to the circadian pattern of flower opening and closure. As these plants were also highly-visited and abundant components of plant communities, early flower closure had a strong impact on plant-pollinator interaction networks.

There was a strong difference between morning and afternoon subwebs. This adds a new dimension to temporal dynamics of plant-

pollinator networks. The temporal turnover within single day was mainly driven by the presence of Cichorioideae closing their flowers around noon. In networks with a relevant proportion of Cichorioideae, morning subwebs included a number of pollinators specialised on this subfamily of Asteraceae, e.g. *Lasioglossum villosulum* and *Panurgus calcaratus* (Westrich 1989). These and other pollinators were absent from the afternoon subwebs. It is difficult to separate how much of the declining foraging activity of this subset of pollinators can be explained by a preference for Cichorioideae or by a circadian activity pattern (i.e. peak activity during the morning). Circadian patterns of pollinator activity due to abiotic or biotic reasons have already been recognised (Willmer & Corbet 1981; Stone *et al.* 1999; Lienhard *et al.* 2010). The non-significant intercept of community difference suggests that in our system there was only a limited turnover of pollinator species within a day other than the decrease in 'Cichorioideae specialists'. However, as the intercept for interaction dissimilarity was significant, a certain amount of circadian dynamics independent of Cichorioideae flower closure also seem to be present.

Despite the absence of some pollinators from the afternoon networks, there was also evidence that some generalist pollinators switched from Cichorioideae to other plant species offering floral resources also in the afternoon. Correspondingly, *Achillea millefolium* was mainly visited during the afternoon in networks with high proportion of Cichorioideae, while visitation rate was higher during the morning in networks with a low proportion of Cichorioideae. This result suggests that visitation rate to *A. millefolium* is reduced during the morning due to competition for pollinators from Cichorioideae, but it receives more visits in the afternoon by pollinators that remain active. It is unknown whether this adds up to a net facilitation or competition effect, as pollinator abundance might also vary between networks and the outcome for the plant (i.e. seed set) also depends on the receptivity of the flowers throughout the day. Similar dynamics of competition can be expected for other plant species if they share a sufficient part of their pollinator community (see also Lázaro *et al.* 2009; Mitchell *et al.* 2009), which was not the case for *Trifolium pratense*. These observations show temporal dynamics in mutualistic and competitive interactions similar to patterns suggested for larger temporal scales (phenology: Mosquin 1971): competition for pollinators (Mitchell *et al.* 2009) seems to be more important in the morning, while pollination facilitation (Ghazoul 2006) and competition between pollinators are probably more pronounced during the afternoon. While optimal flower opening times can be predicted by models (Miyake & Yahara 1999), models on the consequences of pollination driven flower closure for community and network dynamics are lacking.

Current theory on mutualistic networks suggests that generalist core species make the network stable and tolerant to extinctions (Memmott *et al.* 2004; Bascompte & Jordano 2007). Our findings show that different links are not equivalent, questioning the assumption of functional redundancy. For example, pollinators with a long activity period may need different plant species providing resources at different times of day. However, pollination-induced flower closure also suggests a flexible link structure, enabling plants to establish new links when pollinator communities change. Furthermore, our findings caution against homogeneous assumptions when considering the relationship between pollinator specialisation and pollination success. Intraday turnover of interactions should particularly be considered in the interpretation of networks

without whole-day sampling: important interactions may be missed in studies with sampling restricted to either morning or afternoon, and interday turnover may be overestimated in studies with variable sampling times. This is of general importance as many plant-pollinator communities include species of Cichorioideae (see Table S7: 22 of 37 studies), and similar circadian patterns of interactions may be expected also for systems without Cichorioideae: many plant species show intraday patterns of flower opening, nectar and pollen availability (Ewusie & Quaye 1977; Pleasants & Chaplin 1983; Stone *et al.* 1998; Hoehn *et al.* 2008). However, it is yet unknown whether those patterns are also driven by pollination responses.

The network data suggest that pollination advances flower closure in the field similar to the experimental results. The negative relationship between bee visitation rate and estimated time of flower closure suggests a delay in flower closing time by several hours on sites or days with low pollination, with limited additional influence of day length and weather conditions. However, this pattern must be interpreted with caution, because it is only indirect evidence of advanced closure and also plant species composition differed between networks. The correlation between closing time and visitation rate suggests that the observed closure response to pollination is not restricted to the species tested in our experiments, but rather widespread among Cichorioideae.

Temporal dynamics within plant-pollinator networks might enhance biodiversity maintenance through temporal niche partitioning (Mosquin 1971; Abdala-Roberts *et al.* 2007), which is also possible on a daily basis (Stone *et al.* 1996, 1998). Diverse plant communities in particular should benefit from this pattern due to reduced hetero-specific pollen delivery. The finding that flowers are quickly closed in response to pollination introduces also a flexible component to circadian niche partitioning. This probably contributes to the selective advantages that might have led to this 'plant behaviour': on the one hand, it might lead to improved pollinator behaviour by guiding pollinators to flowers that still require pollination and on the other hand, rapid flower closure might help to reduce costs of open flowers such as increased water loss and susceptibility to antagonists (Ashman & Schoen 1994). Plasticity in the timing of flower closure combines the benefits of short flower opening and temporal specialisation while avoiding the cost of pollination failure.

CONCLUSIONS: CONSEQUENCES FOR FLOWER AND POLLINATOR SURVEYS

The circadian dynamics of flower opening and plant-pollinator interactions that we demonstrated here need to be considered when designing field surveys on pollinators, flower availability and plant-pollinator interactions. If sampling is only performed in the afternoon, it will systematically miss parts of the pollinator community and interactions, and this bias will increase with the dominance of plants with early flower closure. Flower availability and pollinator diversity will probably be most strongly underestimated on sites or dates with highest pollination service (to plants responding with rapid closure). Apart from helping to avoid bias in field studies, our findings are also promising for multiple applications: flower closure may be used as a fast and effective proxy for estimating pollination success in field and laboratory experiments and assessments. Literally speaking, we show that Linné's floral clock needs to be adjusted in times of pollinator declines – flowers

originally closing at 15:00 h. (such as *Leontodon autumnalis*) may remain open until 18:00 h without pollination.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

- Table S1** Experimental details of hand pollination experiments.
- Table S2** Fitted parameters for closure curves per flower head.
- Table S3** Summary statistics for additional hand pollination controls.
- Table S4** Summary statistics for number of seeds in experimental *Crepis biennis*.
- Table S5** Plant species codes for Figure S2.
- Table S6** Pollinator codes for Figure S2.

Table S7 Prevalence of Cichorioideae in plant-pollinator network studies.

Figure S1 Circadian pattern of flower visitation in the networks dataset.

Figure S2 Network graphs with all species codes.

Figure S3 Alternative Figures to Fig. 6a–d, presenting uncorrected response variables including summary statistics.

Appendix S1 Explanation of randomization procedures for dissimilarity analyses.

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