Why do flowers close at night? Experiments with the Lesser celandine *Ficaria verna* Huds (Ranunculaceae)

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Although the mechanisms of flower closure have been widely studied, the evolutionary processes underlying this phenomenon are largely unknown. Open flowers may face a trade-off between successful pollination/pollen transfer on the one hand and destruction of reproductive organs caused by exogenous factors (e.g. rain) on the other. The cost of flower opening was investigated by treating flowers of the Lesser celandine both in the field and under laboratory conditions. No differences were found in anthesis and reproductive success between treated and untreated flowers in any of the experiments. However, treated flowers in the field experiment suffered from significantly higher predation rates, in all probability caused by slugs and roe deer. It is suggested that flower closure may decrease the rate of being eaten by some herbivores, particularly in early-spring plants that flower at times of insufficient food availability. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **118**, 698–702.

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INTRODUCTION

Individual plants are able to receive or transfer pollen from another flower and produce seeds when opening flowers. Certain species only open flowers once (Sigmond, 1929, 1930; Prokop & Neupauerová, 2014), whereas others have flowers with nyctinasty (intermittent opening; Kerner von Marilaun, 1895; Stoppel, 1910; Pfeffer, 1904). A number of experiments have confirmed that exogenous (e.g. light, humidity, temperature) and endogenous factors (e.g. hormones, nutrition) influence flower opening/closure (van Doorn & van Meeteren, 2003; van Doorn & Kamdee, 2014), thereby maintaining pollen viability (Franchi, Nepi & Pacini, 2014).

Although proximate mechanisms causing flower opening/closure have received serious attention (van Doorn & Kamdee, 2014), the ultimate evolutionary reasons for this phenomenon are much less clear (van Doorn & van Meeteren, 2003; De Kroon *et al.*, 2005; Pacini, 2008). It has been hypothesized that flower closure is induced by pollination (van Doorn, 1997; Abdala Robers, Parra Tabla & Navarro, 2007; Fründ *et al.*, 2011), thereby inhibiting intraspecific competition with nonpollinated flowers (Prokop & Neupauerová, 2014) and/or reducing additional energy costs associated with flower maintenance (Ashman & Schoen, 1997). Flower closure may be considered a strategy for preventing pollination with pollen of low viability (Franchi *et al.*, 2014) or it may favour nectar re-absorption, protecting the remaining nectar from the bacteria and mould spores carried by flower visitors (Herrera *et al.*, 2009). van Doorn & van Meeteren (2003) have suggested that early-spring flowering species (e.g. crocus) respond to temperature drop by closing flowers to protect the reproductive organs from being covered by snow, or from being damaged by hail or rain, at a time when the pollinator activity is low.

Natural selection favours strategies that reduce costs and maximize reproductive fitness (Williams, 1966; Hirsfield & Tinkle, 1975). Flower closure appears to have a protective function for reproductive organs, which means that there is a trade-off

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between costs associated with damage of reproductive organs (van Doorn & van Meeteren, 2003; Herrera *et al.*, 2009) and benefits resulting from successful pollination (Primack, 1985; Ashman & Schoen, 1996; Rathcke, 2003; Castro, Silveira & Navarro, 2008). As far as we are aware, no study has experimentally investigated the costs of flower opening/closure both under natural and laboratory conditions. We used Lesser celandine (*Ranunculus ficaria* L.), a native, ephemeral perennial species often available in the leafless canopy of forested habitats in the early spring (Swearingen *et al.*, 2002), aiming to investigate the reproductive costs of flower closure. This species has flowers with nyctinasty (Andrews, 1929). The flowers not only open regularly in the morning and close in the evening, but also when the the weather is wet or during rain (P. Prokop, pers. observ.), suggesting that protection against rain can be a plausible explanation for flower closure. Open flowers were experimentally treated with a wire to prevent flower closure both in the field and under laboratory conditions. It is hypothesized that flowers that remain open throughout anthesis will have lower reproductive success as a result of rain damage compared with those that are allowed to close the corolla.

MATERIAL AND METHODS

FIELD EXPERIMENT

Study area
The field experiment was performed from 10 to 25 April 2015 on a fragment of original floodplain forest in western Slovakia near the city of Trnava (48°23′N 17°34′E). The daily and night temperatures during the field study varied between 12 and 20 °C and 6 and 9 °C, respectively. Rain occurred twice (up to 2 mm of rainfall per day). Flower buds ($N = 99$) were individually marked and numbered on a ribbon attached to a peduncle to ensure that the flowers did not open previously. Selection of flowers and their assignment to treatments was spatially and temporally random.

Procedure
Flowers were randomly assigned into one of three groups on the first day that the flowers opened. The flowers were treated with a wire that prevents flower closure (Wire) (Fig. 1A). A control group with a wire placed outside of the flower was to ensure that the wire does not influence pollinator visitation (Wire-Control) (Fig. 1B) because the Lesser celandine is usually pollinated by dipterans (Marsden-Jones, 1937). The third group consisted of untreated flowers (Control). The flowers were controlled daily between
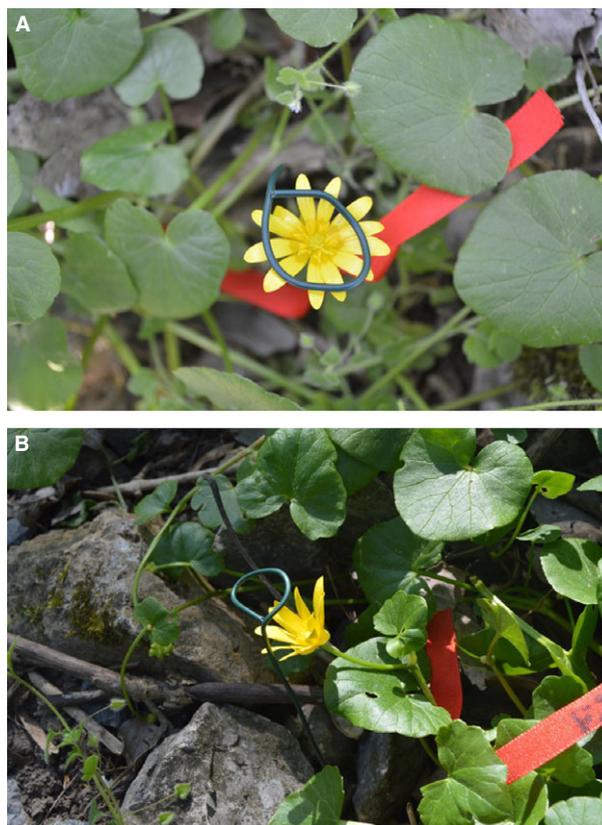

Figure 1. Experimental flowers from (A) Wire and (B) Wire-Control treatments.

09.00 h and 16.00 h until the end of anthesis, defined as the loss of at least 80% petals. At this stage, the petals are unable to close and wind or rain could easily drop them out of the corolla (P. Prokop, unpubl. data). The total length of anthesis was measured from the first to the final day (in days) and flower fertility was defined as the number of diaspores on the flower immediately after anthesis ended.

Predation
A number of flowers were predated over the course of the experiment. Some flowers were browsed during the experiment. One of the predators was *Arion distinctus* (P. Prokop, pers. observ.). If the flowers were 'decapitated', with the remainder of the stem intact, these events were attributed to roe deer (*Capreolus capreolus*) (cf. Wallach, Inbar & Shanas, 2009).

LABORATORY EXPERIMENT

Laboratory conditions
The laboratory experiment took place between 13 and 24 April 2015. Flowering individuals of the Lesser celandine ($N = 70$) were removed in various localities, planted into flower pots, and cultivated

outdoors in individual pots as of April 2014. In total, 62 plants were brought to the laboratory with a constant temperature (approximately 20 °C), relative humidity (approximately 50%), and natural photoperiod in April 2015. The natural photoperiod stimulated flower closure in a manner similar to the natural environment (no flowers remained open in the evening).

Procedure

The flowers were marked with a ribbon prior to anthesis, identical to the field experiment. The open flowers were randomly assigned into one of three groups on the subsequent day: (1) flowers treated with a wire, preventing flower closure, and which were sprayed daily with water (2 mL) (Wire-Water) each evening at 20.00 h when the untreated flowers were closed; (2) flowers treated with a wire but not sprayed with water (Wire-Control); and (3) untreated flowers (Control). We used an atomizer from which the water was sprayed from a close distance (approximately 2 cm from the flower) to mimic downpour. On the first day of flowering, all flowers were hand pollinated between 09.00 h and 11.00 h with pollen from flowers brought from a different locality to mimic a situation where closure should protect embryo/seeds. The entire length of anthesis and flower fertility was measured and defined identically as in the field experiment.

STATISTICAL ANALYSIS

Continuous data were checked for normality (the Shapiro–Wilk test) and, because normality was achieved, parametric analysis of variance (ANOVA) was used to test differences between treatments in terms of flowering time and seed production. The effects of treatment on the predation rate were examined with logistic regression based on the likelihood ratio test where treatment was defined as categorical predictor, and flowering time and seed production were continuous predictors. If the flower was browsed, it was dummy coded as 1 and, if the flower was intact, it was dummy coded as 0 (binomial dependent variable). We also calculated interaction terms between independent variables.

RESULTS

FIELD EXPERIMENT

Flowering time and fertility were similar ($F_{2,96} = 2.24$ and 0.5, $P = 0.11$ and 0.61, respectively) between the three treatments (Table 1). Three flowers were partially eaten by slugs, *Arion distinctus*, and five flowers were decapitated, which means that

Table 1. Descriptive statistics for flowering time (days) and fertility (number of diaspores) in Lesser celandine in the field

Treatment	Flowering time Mean ± SE	Fertility Mean ± SE	N
Wire	5.16 ± 0.24	14.11 ± 0.82	45
Wire-Control	5.38 ± 0.31	13.27 ± 1.07	26
Control	5.96 ± 0.30	14.75 ± 1.03	28

they were probably eaten by roe deer. All the browsed flowers belonged to the Wire treatment. A logistic regression was performed to determine the effects of treatment, fertility, and flowering time on the likelihood that flowers would be browsed. The model was significant (likelihood ratio: $\chi^2 = 14.9$, d.f. = 4, $P = 0.005$) and explained 33% (r^2) of the variance in the predation rate. Only treatment (likelihood ratio: $\chi^2 = 13.7$, d.f. = 2, $P = 0.001$), and not flowering time or fertility (likelihood ratio: $\chi^2 = 0.002$ and 1.41, d.f. = 1, $P = 0.96$ and 0.23, respectively), significantly influenced the risk of being eaten. The interaction terms were not statistically significant (all $P > 0.86$). The reduced logistic regression model, in which nonsignificant variables were omitted, showed once again that the predation rate was influenced by treatment (likelihood ratio: $\chi^2 = 13.5$, d.f. = 2, $P = 0.001$) and that this effect explained 30% (r^2) of the variance.

LABORATORY EXPERIMENT

One-way ANOVA indicated that there was no difference in the flowering time and fertility ($F_{2,59} = 0.19$ and 1.57, $P = 0.83$ and 0.22, respectively) between the three treatments (Table 2).

DISCUSSION

The present study used experimentally treated flowers to determine the benefits resulting from flower closure in the Lesser celandine. By contrast to hypotheses suggesting that closed corolla protect

Table 2. Descriptive statistics for flowering time (days) and fertility (number of diaspores) in Lesser celandine in the laboratory

Treatment	Flowering time Mean ± SE	Fertility Mean ± SE	N
Wire-Water	4.35 ± 0.20	15.15 ± 0.95	26
Wire-Control	4.16 ± 0.23	16.79 ± 1.11	19
Control	4.29 ± 0.25	13.94 ± 1.18	17

reproductive organs from rain (van Doorn & van Meeteren, 2003), the field and laboratory experiments failed to show any difference in the reproductive success of treated flowers compared to untreated flowers. Rain during the experiment was scarce and neither hail, nor snow occurred. Consequently, these additional factors need to be investigated further because they would also destroy petals and reduce flower attractiveness for pollinators.

The present study introduces predation as a new possibility for regarding selective pressures on flower closure, a possible defensive strategy against herbivores (Burns, 2014; Lev-Yadun, 2014; Vermeij, 2015). Apparent closing of flowers in the evening correlates with the foraging activity of roe deer, which are particularly active at dawn and dusk (Wallach, Shanas & Inbar, 2010). Similarly, the activity of slugs such as *A. distinctus* is apparently highest at night and before dusk (Hommay *et al.*, 2003). Wet weather causing flower closure in Lesser celandine (P. Prokop, pers. observ.) may also correlate with slug activity because slugs are more mobile in humid environments relative to dryer conditions (Crawford-Siebotham, 1972).

The present study determined that 11.3% of treated flowers (Wire and Wire-Control treatments) in the field were eaten. This rate need not necessarily be representative because a detailed study on roe deer found that, on average, 30% (locally 75–80%) of the wood anemone (*Anemone nemorosa*) flowers, a familiar species from the same family Ranunculaceae, were predated by this large mammal (Mårell, Archaux & Korboulewsky, 2009). The study area was a fragment of the original floodplain forest, where roe deer individuals were repeatedly observed (P. Prokop, pers. observ), although their activity could have been suppressed by human visitors (Hewison *et al.*, 2001).

Self-pollination could be a confounding factor in the present study because flower emasculation was not performed. Although the Lesser celandine may be self-compatible in the field (Sell, 1994), it was observed that nonpollinated flowers (not included in the present study) in the laboratory never produced seeds (P. Prokop, pers. observ). This suggests that pollinator activity is required for fertilizing flowers (Marsden-Jones, 1937) and the presence of anthers on flowers would not influence the results of the present study.

In conclusion, flowers opening during night had higher rates of being browsed in the Lesser celandine. Predators, such as slugs and herbivore mammals, could supress the reproductive success of these flowering plants, particularly in early spring when the food of herbivores is scarce. Furthermore, plants occupying habitats (e.g. meadows) where grazing is intense (e.g. *Bellis perennis*) may also benefit from

nyctinasty with respect to decreasing rates of being browsed by herbivores.

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