Research Article

Flower closure in the field bindweed (*Convolvulus arvensis*): a field test of the pollination hypothesis

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Abstract: Although the behavioral plasticity of flower traits has received considerable attention, its adaptive value is not thoroughly understood. We experimentally examined flower opening/closure in field bindweed (*Convolvulus arvensis*), a species with short flower persistence (1 day) in which adaptive responses to pollination were not previously expected. In line with the pollination hypothesis we suggested that flower exposure in this species should respond to pollination. More specifically, we predicted that flower closure in the pollinated flowers would be quicker than that in unpollinated flowers. As predicted, the unpollinated flowers were open for a longer time than the pollinated flowers. There was no difference, however, between the self- and cross-pollinated flowers in terms of flower longevity. There was an inverse relationship between flower longevity and fertility, which also suggests that pollination leads to reduced flower longevity. Collectively, our results suggest that field bindweed flowers respond to pollination adaptively, because prolonged flower longevity may increase the likelihood of successful pollination and flower closure reduces energy spent on flower maintenance and/or intraspecific competition with genetically familiar flowers. The behavioral plasticity of flower closure does not seem to be restricted only to plants with high flower longevity.

Key words: Behavioral plasticity, *Convolvulus arvensis*, flower closure, pollination

1. Introduction
Behavioral plasticity is the ability of an individual to produce more than one alternative form of behavior in response to environmental conditions (West-Eberhard, 1989; Betini and Norris, 2012; Berjano et al., 2014), and the degree of plasticity may have important consequences for an individual's fitness (Relyea, 2002; Sih et al., 2004; Miner et al., 2005; Dingemanse and Wolf, 2013). Due to traditional problems with the definition of behavior in plants (Metlen et al., 2009), the research traditionally focused on behavioral plasticity in animals rather than in plants (Sultan, 2003). Most recent research, however, highlights the plastic properties of individual plants (Gagliano, 2013; Frederickson et al., 2013; Fürstenberg Hägg et al., 2013) as well as the individual leaves or branches (reviewed by De Kroon et al., 2005) as adaptive responses that enhance survival.

Plant flowers have attracted human attention since ancient times (Solecki, 1971; Nadel et al., 2013); therefore it is not surprising that investigation of the behavioral plasticity of flowers has a long history (see van Doorn and van Meeteren, 2003 for a review). Flower opening and closure is an extremely conspicuous behavioral adaptation that varies greatly between species. While certain species such as *Hedera helix* or *Oenothera biennis* have open flowers for only a few minutes (Sigmond, 1929, 1930), others have flowers that open for more than 10 days (Schemske, 1980; Willson and Schemske, 1980; Primack, 1985). A number of endogenous (e.g., metabolism, hormonal regulation) and exogenous (e.g., light, humidity) explanations have been provided at the proximate level (i.e. how the mechanism works) regarding flower opening/closure; however, the ultimate reasons why evolution favored this behavioral trait are still unclear (Ashman and Schoen, 1994; van Doorn and van Meeteren, 2003).

Several flowers exhibit flower closure as a behavioral response to pollination that can maximize pollen dispersal or receipt and minimize energy costs associated with flower maintenance (Primack, 1985; van Doorn, 1997). It has been proposed that flower traits correspond with the availability of, and adaptation to, diurnal and nocturnal pollinators (Miyake and Yahara, 1999; Giménez et al., 2007; Yokota and Yahara, 2012), although individual differences in flower opening/closure are less apparent.

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Van Doorn (1997) has suggested that plants that are not ethylene sensitive, such as Asteraceae, do not cease with floral attraction in response to pollination. Fründ et al. (2011) has demonstrated, however, that several individual flowers of the family Asteraceae respond to the presence of pollination with quick flower closure, suggesting that the behavioral plasticity of flowers might differ when compared with earlier views due to an absence of research efforts (van Doorn, 1997). Van Doorn (1997) has also suggested that flowers that are open for up to a day should not exhibit flexible flower closure as a response to pollination; however, Fründ et al. (2011) has questioned whether flowers with roughly circadian patterns (e.g., *Convolvulus*) respond to pollination. Here we test the pollination hypothesis with field bindweed (*Convolvulus arvensis*) flowers, which last only 1 day (Waddington, 1976; Weaver and Riley, 1982) and are insect pollinated (Mulligan, 1972, 1973; Waddington, 1976) and self-incompatible (Mulligan and Findlay, 1970; Gianoli, 2004). Specifically, we predicted that (1) unpollinated flowers flower longer than pollinated flowers in order to attract pollinators, (2) cross-pollinated flowers are open for a shorter time than self-pollinated flowers in order to increase the chance that a pollinator with foreign pollen will visit the flower, and (3) natural experimentally untreated flowers that did not produce seeds are those that have flowers open for a longer time to increase the chances that their flowers will be pollinated as compared with fertile flowers.

2. Methods

2.1. Study site
The experiment was performed in an uncut ruderal habitat near Trnava, Slovakia (N 48°23′, E17°35′), from 31 July to 19 August 2013. We randomly selected 2 plots with bindweeds flowering on the ground rather than coiling on tall plants in order to standardize the experimental conditions. Typical surrounding plants were *Artemisia vulgaris*, *Arrhenatherum elatius*, *Dipsacus sylvestris*, *Cirsium arvense*, *Reseda lutea*, *Sonchus asper*, *Cichorium inthybus*, *Achillea vulgaris*, *Tripleurospermum inodorum*, and others.

2.2 Experimental procedure
On the day of the experiment individual flowers (each belonging to a different plant) with still-closed capitulum were randomly assigned to 1 of 4 treatments: cross-pollinated, self-pollinated, nonpollinated, and control. A total of 120 flowers were individually marked with a ribbon before opening between 0500 and 0700 hours on 31 July and 17 August (60 flowers per experimental day). All flowers except the control group were treated with white tissue with a fine mesh, which prevents any contact between the flowers and the pollinators. The tissue was gently attached to the flower stem with a plastic clip. At this time of day, it was not clear which of the selected flowers would open over the experimental day. Our experiences suggested that pollinators often enter the flower head before it is fully opened (predominantly by Diptera: Syrphidae, P. Prokop, pers. obs.). Treating the flower heads before opening was, consequently, the only way to prevent pollination.

2.3. Hand pollination of the flower heads
Flowers from cross-pollinated and self-pollinated treatments were hand pollinated with a fine brush between 0900 and 1030 hours when the flowers were open. For cross-pollination we used freshly collected flower heads flowering at least 50 m apart from the experimental plots. Each flower in the cross-pollination treatment was pollinated with pollen from a different flower head (n = 21) collected approximately 50 m away from the study plot. In the case of the control flowers, we marked them with plastic clips, similar to the flowers from other treatments, between 0900 and 1030 hours. The flowers were checked every 30 min until closure. Flower closure was defined as full closure that prevents pollinators from entering the flower. We used a flashlight to check the flowers during the night. The plants were later checked every 7 days to ensure the seeds were developing normally, and the seeds were finally collected and counted 30 days after the experiment when they were mature.

2.4. Statistical analyses
The flowers that did not open their heads (44 of 120, 37%) on the day of the experiment were removed from the analyses. The flower opening was calculated from the individual marking until closure. We acknowledge that this time does not precisely follow the opening time; however, as noted earlier, the flower heads are visited by pollinators before they are fully open. Alternatively, we used a time beginning from when the flowers were pollinated (or marked with the plastic clip in the case of the control group) until flower closure; however, the results are identical to the time from when the initial markings were used. As there were no apparent differences between the study sites, the data were combined. Since the flowering exposure time deviated from normal distribution (Shapiro–Wilk test), we used Box-Cox (x + 0.1)–transformed data in statistical analyses. The differences in exposure time between treatments were tested with one-way ANOVA. The question of whether the production of at least one seed (binomial dependent variable) was related to the flowering exposure time (continuous predictor) was calculated with the generalized linear model (GLM). The correlation between the number of seeds and the flowering exposure time was determined with Spearman rank correlation. Statistical tests were performed with Statistica (v8, StatSoft 2007, Tulsa, OK, USA, http://www.statsoft.com).

3. Results

3.1 Predictions 1 and 2

There were no differences in the proportion of flowers that did and did not open on the day of the experiment with respect to treatment (76 out of 120 flowers were open; Pearson chi-square test, $\chi^2 = 2.58$, df = 3, P = 0.46). The mean flowering exposure time (measured from the individual marking of the flowers in the morning until flower closure) was 14.6 h (range: 7.07–42.4 h, SE = 0.83, N = 76). One-way ANOVA with treatment as a factor and the flowering exposure time as a dependent variable yielded significant differences between groups [F(3,72) = 6.7, P = 0.0005]. Unpollinated flowers were open for a significantly longer time than flowers from other groups (Figure 1). This supports prediction 1. However, there were no significant differences in the mean flowering exposure time between the self-pollinated and cross-pollinated flowers, providing no support for prediction 2.

3.2 Prediction 3

The mean number of seeds produced per flower open during the observation time was 0.46 (range: 0–4, SE = 0.13, N = 76). Only untreated, control flowers produced seeds. When considering the control flowers only, the flowering exposure time of flowers that did not produce seeds (mean = 16.9 h, SE = 2.02, n = 6) was longer than the flowering time of those that produced at least one seed (mean = 9.98 h, SE = 1.43, n = 12) (GLM with seed production as the dependent variable; Wald's $\chi^2 = 3.85$, df = 1, P = 0.049). There was a strong negative correlation between the flowering exposure time and the number of produced seeds (Figure 2), suggesting that flowers that were open for a longer time produced fewer seeds than those that were open for a shorter time. Collectively, these data support prediction 3 and suggest that unpollinated
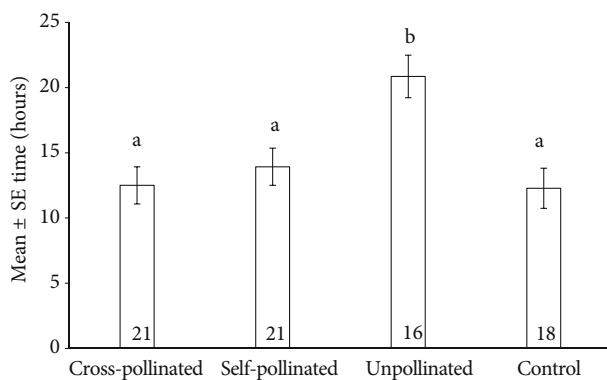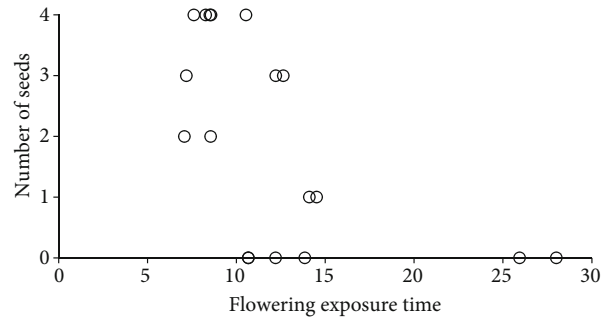

Figure 2. The relationship between the flowering exposure time and the number of seeds produced in the control group (Spearman rank correlation, r = −0.63, P = 0.005, n = 18).

flowers (i.e. those that did not produce seeds) are open for a longer time than pollinated flowers (i.e. those that produced seeds).

4. Discussion

Flower opening/closure has been studied, particularly at the proximate level, although ultimately it is not clear which species of plants demonstrate behavioral responses to external factors, such as pollination, or why (Primack, 1985; van Doorn, 1997; van Doorn and van Meeteren, 2003). Although it has been suggested that plants with flowers that open for up to a day should not exhibit flexible flower closure as a response to pollination (van Doorn, 1997), our experiment with field bindweed, a species with a roughly circadian pattern, provided the first evidence that pollination influences flower closure in ephemeral flowers. In line with the pollination hypothesis (Primack, 1985; van Doorn, 1997; van Doorn and van Meeteren, 2003) we discovered that field bindweed flowers respond flexibly to environmental conditions (West-Eberhard, 1989; Betini and Norris, 2012) and are not exclusively genetically programmed, as suggested previously (Waddington, 1976; Weaver and Riley, 1982). It seems in contrast that the organism's phenotype is determined by a combination of genotype and environment (Ferval et al., 2013; Berjano et al., 2014). Caution must be used when interpreting these data, however, since none of the experimentally treated flowers produced seeds.

Our first prediction, derived from the pollination hypothesis, suggests that pollination should yield a quicker flower closure (van Doorn, 1997; Abdala-Roberts et al., 2007; Fründ et al., 2011). In line with this prediction, unpollinated flowers were open for a significantly longer time than flowers from other treatments. A prolonged flower opening may increase the likelihood of successful pollination and, consequently, the reproductive success of individual flowers (Abdala-Roberts et al., 2007; Fründ et al., 2011). Although short-flowering species such as *Convolvulus* were not expected to respond to pollination

Figure 1. Differences in mean flowering exposure time (untransformed data) from the individual marking until flower closure. The numbers inside the bars are sample sizes. The different letters denote significant differences based on Tukey's post-hoc tests (a vs. b, P < 0.05 and less).

by flower closure (van Doorn, 1997), we suggest that this behavioral response could evolve due to the unpredictable patterns of pollinator availability. Field bindweed often occupies disturbed habitats (Larson et al., 2001) where pollinators may be scarce (Steffan-Dewenter et al., 2002; Kremen et al., 2004; Tylianakis et al., 2005), and the flowers are self-incompatible (Mulligan and Findlay, 1970; Gianoli, 2004). As a result, individual plants responding to pollination availability were favored by natural selection.

The second prediction suggests that self-pollinated flowers are open for a longer time than cross-pollinated flowers, which may increase the likelihood for unfamiliar pollen to be deposited on the stigma. Clark et al. (2007) tested a similar prediction in fireweed (*Chamerion angustifolium*) but found only weak differences in flower closure between self- and cross-pollinated flowers. It seems unlikely that field bindweed is unable to discriminate between self and foreign pollen, although no firm conclusions can be made without additional data. The capacity for self-pollination may vary within species (Brys and Jacquemyn, 2011; Scalone and Albach, 2014), but no data on the European populations of field bindweed are available. It is possible that field bindweed flowers are self-compatible in the study population or that all hand-pollinated flowers only revealed a preliminary response to the presence of pollen. Further research should test the sensitivity of field bindweed flowers on an exactly defined amount of pollen obtained from genetically outbred and inbred lines.

Our final prediction suggested that there would be an inverse relationship between flower opening and fertility. More specifically, we suggested that pollinated flowers need not prolong flowering, for example, due to potential energy costs (Primack, 1985; van Doorn, 1997); thus, quicker flower closure should be expected in previously pollinated flowers. We found full support for this prediction since flower exposure and fertility were negatively correlated. This finding is in line with theories of behavioral plasticity (West-Eberhard, 1989; Metlen et al., 2009; Betini and Norris, 2012; Dingemanse and Wolf, 2013), as the prolonged flower opening may function to increase the likelihood of successful pollination. In addition to possible energy costs associated with flower maintenance, we hypothesize that flower closure may reduce intraspecific competition. Flower closure in response to pollination may positively influence the chance of successful pollination in other, newly opened

flowers of the same plant due to reduced competition. Further experimental research on flowers that are denied closing and pollination visitation will be necessary in order to test this idea.

4.1. Limitations
None of the experimentally treated flowers in this study produced seeds. One explanation may be that we did not use an adequate amount of pollen, which could influence fertility. Alternatively, the low viability of pollen used for hand-pollination may be responsible for the negative results (Dafni and Firmage, 2000). This investigation was carried out by 2 researchers and it is hard to believe that none of the experimental flowers received the required amount of pollen (i.e. that our hand-pollination was incorrectly carried out) or that none of the pollen used for cross-pollination was viable. We have, however, no evidence available to refute these possibilities. Another explanation is that covering the flowers with tissue lowered the overall temperature in the gynaeceum, which could lead to infertility (Patiño et al., 2002; Gianoli, 2004). These explanations cannot be ruled out without additional experiments using an appropriate control group. However, flower closure in the present study revealed adaptive behavioral plasticity, and this corresponds with the pollination hypothesis, which was the primary test in this study.

In conclusion, the field bindweed has flowers that last 1 day, and it has been suggested that flower closure is in all probability purely genetically programmed. These results demonstrate that field bindweed flowers are sensitive to external stimuli, namely to the presence of pollinators. Pollination results in quicker flower closure, which may be an adaptive behavioral strategy that reduces possible energy costs from flower maintenance and/or intraspecific competition with as-yet-unpollinated, genetically familiar flowers. The fertility of untreated flowers provides further support for the pollination hypothesis, as the least fertile flowers were open for a longer time than the fertile flowers, which in all probability increased the likelihood of successful pollination. Flower closure may be used as an estimator of pollinator availability in further studies.

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