Floral longevity in *Campanula americana* (Campanulaceae): a comparison of morphological and functional gender phases[1]

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Plastic responses to pollination and/or pollen removal may shift a flower's realized longevity closer to an optimal longevity that maximizes reproductive output per unit resource input. In particular, conditional responses to pollen removal and pollen deposition are expected in flowers of protandrous species in which the lengths of the male and female phases may be adjusted independently. We investigated plasticity in floral longevity in *Campanula americana*, a protandrous, insect-pollinated herb. In greenhouse studies, we found that the longevity of the morphological male phase was shortened by pollen removal and that the longevity of the morphological female phase was shortened by pollen deposition. In a natural population, male and female sexual functions saturated within a few hours of morphological gender phase onset. In contrast to theory, morphological gender phases did not terminate immediately upon saturation of sexual function. These findings are discussed in the context of current floral longevity theory.

Key words: *Campanula americana*; Campanulaceae; floral gender; floral longevity; pollen deposition; pollen removal; pollinator activity; protandry.

The factors that control floral longevity, the period of time between anthesis and floral senescence, have been a focus of recent theoretical and empirical attention (Ashman and Schoen, 1994, 1996; Schoen and Ashman, 1995; Charnov, 1996; Ishii and Sakai, 2000, 2001; Sargent and Roitberg, 2000). While flowers are necessary for sexual reproduction in angiosperms, they are expensive structures for plants to build and maintain. Costs include floral construction (Primack, 1985; Schoen and Ashman, 1995), nectar production (Schemske, 1978; Harder and Barrett, 1992; Ashman and Schoen, 1997), floral respiration (Ashman and Schoen, 1994), and floral transpiration (Nobel, 1977). A recent model balances these costs of floral construction and upkeep against rates of male and female fitness accrual to predict optimal floral longevities (Ashman and Schoen, 1994, 1996; Schoen and Ashman, 1995).

One assumption of this model is that natural selection has optimized floral longevity by acting upon heritable variation in that trait (Schoen and Ashman, 1995; Ashman and Schoen, 1996). However, the longevity of individual flowers may also be influenced by conditional, plastic responses to pollen dissemination and/or pollen deposition, which are correlates of male and female fitness accrual, respectively (Ashman and Schoen, 1996; Ishii and Sakai, 2000). Specifically, floral longevity may be shortened in response to pollen removal or pollen receipt. Such flexibility in floral longevity may optimize reproductive output per unit resource input. A number of studies have found a decrease in floral longevity with pollination

(Harrison and Arditti, 1976; Motten, 1986; Ackerman, 1989; Primack and Hall, 1990; Proctor and Harder, 1995; Clayton and Aizen, 1996; reviewed in van Doorn, 1997) or with pollen removal (Bell and Cresswell, 1998). These studies lend support to the idea that conditional responses to fitness accrual may be common. However, responses in floral longevity to pollen removal or pollen receipt are not directly included in Schoen and Ashman's (1995) model. In addition, few studies have examined the effect of pollen removal and deposition on the longevities of the individual male and female gender phases (Devlin and Stephenson, 1984, 1985; Richardson and Stephenson, 1989; Preston, 1991).

Flowers of protandrous plant species are morphologically and functionally male prior to becoming morphologically and functionally female. This temporal separation of gender phases makes protandrous species ideal for use in studies that analyze the male and female functions independently. A flower's morphological gender can be characterized by the presence of either pollen-presenting structures or stigmatic surfaces, and its functional gender can be determined by whether it is disseminating pollen or accumulating pollen (see Preston, 1991, for a similar concept). In protandrous flowers, conditional responses to fitness accrual may shorten the lengths of the morphological male and female phases to optimize reproductive success and resource use. In this case, the morphological male phase should terminate shortly after complete pollen dissemination and the morphological female phase should end shortly after pollination (i.e., pollen deposition and ovule fertilization). A conditional response to pollination is expected to be most common in species in which floral senescence upon pollen receipt would not interfere with further pollen dissemination from a flower (e.g., in protandrous species, in which male function precedes female function; Ashman and Schoen, 1996).

Using both experimental and observational studies, we examined the longevity of floral gender phases in *Campanula americana* L. (Campanulaceae), a protandrous, insect-pollinated, woodland herb (Johnson, Delph, and Elderkin, 1995; Galloway, 2001; Galloway, Cirigliano, and Gremski, 2002).

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First, we asked whether the longevities of the morphological male and female phases in *Campanula americana* flowers vary with degree of pollen removal or pollen deposition under greenhouse conditions. Next, we surveyed flowers in a natural population, to address the following questions: what are the average longevities of the morphological male and female phases? What are the average longevities of the functional male and female phases? How do the morphological gender-phase longevities compare to the functional gender-phase longevities? Finally, what are the daily patterns of pollinator activity in this population? In total, we explored conditional responses to pollen dissemination and pollen receipt as well as qualitatively tested Schoen and Ashman's (1995) floral longevity theory in a protandrous species.

MATERIALS AND METHODS

Study system—In members of the genus *Campanula*, pollen is presented to pollinators on pollen-collecting hairs (PCH) located on the surface of the style of each flower (Shetler, 1962; Nyman, 1993). In a *C. americana* flower, the five anthers surround the style, dehisce, and release pollen onto the style's PCH shortly before anthesis (Shetler, 1962). During anthesis, the anthers wither and become nonfunctional. After anthesis, the PCH slowly retract from the tip to the base of the style, releasing pollen (Nyman, 1993). The stigmatic lobes open following the retraction of all PCH. In several species of *Campanula*, retraction of the PCH is hastened by physical stimulation roughly equivalent to that experienced during pollinator visitation (Nyman, 1993).

We conducted three studies during peak flowering season (10 July to 2 August 2000) in greenhouse and natural populations of *C. americana* on Salt Pond Mountain in Giles County, Virginia, USA. In these studies, we defined the following stages of floral development. Floral anthesis occurred when a flower's petals had separated enough to expose the style to pollinators. We defined morphological male phase as the time between anthesis and the opening of the stigmatic lobes at the tip of the style and morphological female phase as the time between stigmatic lobe opening and floral senescence. Floral senescence progressed in two stages: wilt, at which time the petals had wilted but style remained turgid, and complete senescence, at which time both the petals and style were wilted and may have abscised.

In the natural population study, the floral gender phases were also defined functionally. A flower was considered functionally male from anthesis until pollen was completely removed. Functional female phase was defined as the period between the opening of the stigmatic lobes and maximum pollen accumulation. This definition is likely to result in overestimation of the functional female phase because the amount of pollen necessary to fertilize all ovules may be smaller than the amount present on a stigma at maximum pollen accumulation. However, this definition is useful in providing an outer limit to the functional female phase.

Pollen removal and morphological male-phase length—A greenhouse experiment was performed to determine whether the longevity of the morphological male phase is plastic in response to pollen removal. Greenhouse plants were raised from seed collected from the population used in the Natural Population Survey (see below). Twenty-six *C. americana* were haphazardly chosen for study. Three buds of similar size were selected on each plant. One of the following treatments was randomly assigned to each of the three buds, such that all of treatments were represented on each plant: 0, 50, or 100% pollen removal. Shortly after floral anthesis, the assigned amount of pollen was removed from each flower's style by gentle strokes with a paintbrush moistened with water. The gender phase of each flower was recorded every 2 h between 0900 and 1900 until onset of morphological female phase. Because little developmental change occurs in flowers overnight and in the early morning (L. Evanhoe, personal observation), all days were scaled to 13 daylight hours. A fixed-effect, one-way analysis of variance (ANOVA) was performed to determine if morphological male-phase duration (square-root transformed) varied among the three removal treatment groups (PROC GLM; SAS Institute, 2000).
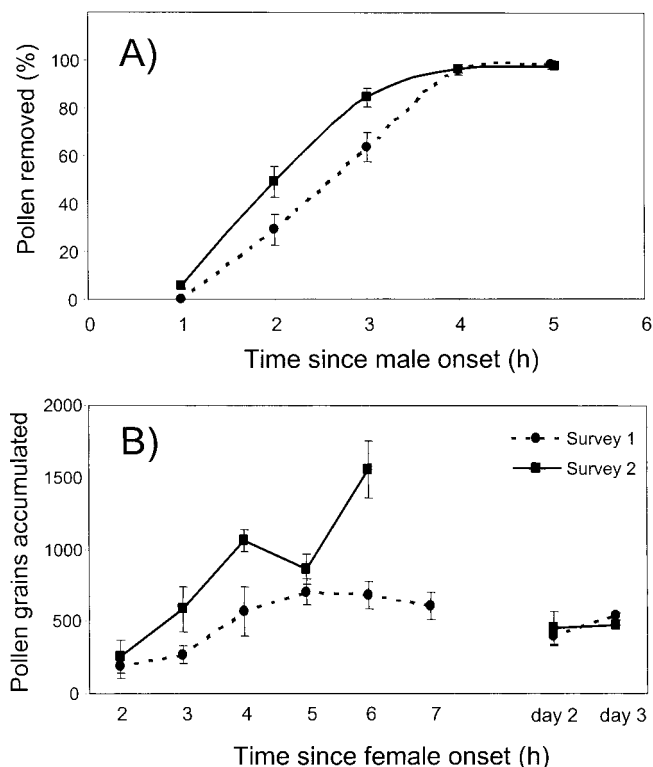

Fig. 1. Durations of the functional gender phases in a natural population. Mean (±SE) percentage pollen removed from male phase flowers (A) and number of pollen grains accumulated on the stigmatic lobes of female phase flowers (B) were plotted against time since gender phase onset. $N = 25$ for male phase flowers; $N = 6–14$ for female phase flowers for times up to 6 h and on day 2; and $N = 2–3$ for female phase flowers at 7 h and on day 3. In some cases, error bars are obstructed by symbols.

Pollen deposition and morphological female-phase length—A second greenhouse experiment was performed to evaluate whether the longevity of the morphological female phase is plastic in response to pollen accumulation. Ninety-eight *C. americana* were haphazardly chosen for study. Individuals were exposed to pollinator activity for several hours to facilitate pollen removal from male phase flowers, then returned to the greenhouse. On each plant, one male phase flower was selected, and any pollen remaining on the style was removed as described above. Each flower was randomly assigned one of the following outcross pollen deposition treatments ($N = 24–25$ flowers/treatment): no pollen deposited; about 30 grains of pollen deposited; one stigmatic lobe coated with pollen (about 300 grains); or three stigmatic lobes coated with pollen (about 1000 grains). The treatments were based on levels of pollen deposition observed in natural populations (cf. Fig. 1). Because flowers may have up to 100 seeds, the low pollen deposition treatment is unlikely to have saturated female function (L. Galloway, personal observation).

Pollen deposition treatments were applied within approximately 2 h of the opening of a flower's stigmatic lobes. The about-30-grain deposition treatment was applied by drawing a single, pollen-speckled hair across a flower's stigmatic lobes. The one-lobe and three-lobe deposition treatments were applied by swiping a pollen-loaded paintbrush across the appropriate number of lobes until the lobes were thoroughly coated. After pollen deposition, the developmental phase of the flowers was evaluated at 2-h intervals between 0900 and 1900; both time of petal wilting and time of complete senescence were noted. A one-way ANOVA with deposition treatment as a fixed effect was performed for both variables (ln transformed) to determine whether morphological female-phase length varied among the four treatment groups (PROC GLM; SAS Institute, 2000).

Natural population survey—Two 5-d surveys were conducted to examine the longevities of the morphological and functional gender phases in a natural *C. americana* population. Each survey tracked the development of one flower on each of 25 plants from the time of anthesis to the time of complete floral senescence. At every hour between 1000 and 1700 and again at 1900, each flower's morphological gender was recorded and its degree of pollen dispersal or accumulation was determined. When each flower was in male phase, the amount of pollen removed was estimated by measuring the number of millimeters of pollen present on the flower's style using a calibrated wire bent to the same curvature as the style.

Quantifying a female phase flower's level of pollen accumulation requires harvesting the stigma. Therefore, on each plant, two additional flowers that were developmentally synchronous with the survey flowers were selected and tagged prior to onset of female phase. Every hour following the onset of female phase, four flowers from this additional group were randomly selected and their stigmas harvested and mounted in basic fuchsin gel on microscope slides (Beattie, 1971). The number of pollen grains on each stigma was counted using a dissecting microscope.

Pollinator activity was monitored during each 5-d survey. Every hour, one plant with approximately 20 flowers (20.53 ± 0.28 flowers, mean $\pm$ 1 SE) was observed for a 10-min period. In total, between four and six replicates of each hourly observation period were performed. During the observation, the number of flowers on the plant, the number of pollinator visits to these flowers, and pollinator identities were noted. In this population, *C. americana* are pollinated primarily by *Bombus* foraging for nectar and pollen (Galloway, Cirigliano, and Gremski, 2002). *Bombus* exhibit no preference between male- and female-phase flowers (Galloway, Cirigliano, and Gremski, 2002).

Diurnal patterns of pollinator activity were evaluated using analysis of covariance with number of visits per flower in an observation period (ln[number of visits/number of flowers]) as the dependent variable. Linear and quadratic forms of time of day were covariates, and day of observation was included as a blocking effect (fixed) (PROC GLM; SAS Institute, 2000).

RESULTS

In our study populations, anthesis occurred fairly synchronously among flowers at midday (1238 ± 8 min, mean $\pm$ 1 SE; $N = 122$). On the day following anthesis, the stigmatic lobes opened at approximately the same time (1322 ± 10 min; $N = 143$).

Pollen removal and morphological male-phase length—The longevity of morphological male phase in flowers from which pollen had been experimentally removed was significantly shorter than the male phase length of those that had no pollen removed (Fig. 2A). However, male phase lengths of flowers that had 50 and 100% of their pollen removed were similar.

Pollen deposition and morphological female-phase length—The longevity of the morphological female phase decreased with increasing pollen deposition (Fig. 2B). The length of time from female onset to petal wilting was twice as long for flowers that had no pollen deposited as for flowers that received pollen (Fig. 2B). The time to petal wilting was also significantly longer for flowers that had approximately 30 grains of pollen deposited than for flowers that had either one or three stigmatic lobes coated with pollen. However, there was no difference in the length of time to petal wilting for flowers that had one or three stigmatic lobes coated with pollen. The length of time from female onset to complete senescence showed similar patterns (Fig. 2B).

Natural population survey—Flowers exposed to pollinators exhibited morphological male-phase longevities comparable to
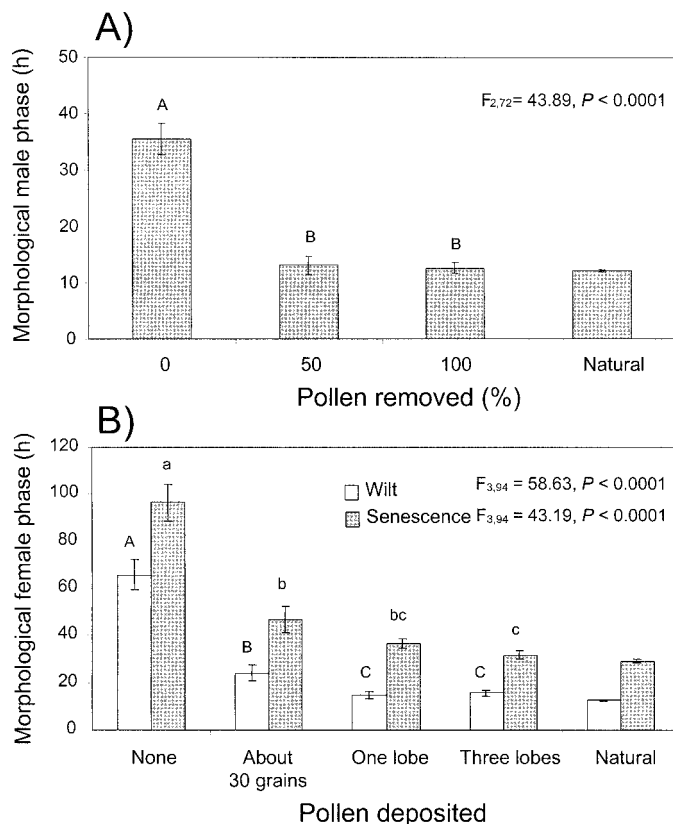

Fig. 2. Mean ($\pm$SE) durations of the morphological gender phases for male phase (A) and female phase (B) flowers receiving experimental pollen removal or deposition. Bars marked with the same letter do not differ significantly at $\alpha = 0.05$ with a Tukey multiple comparison test. Note that natural population plants (''Natural'') were not included in the ANOVA and that 1 d is equivalent to 13 h.

those of greenhouse flowers with half or all pollen removed (Fig. 2A) and female phase longevities similar to those of greenhouse flowers with at least one stigmatic lobe fully coated with pollen (Fig. 2B). For both surveys, the percentage of pollen removed from male phase flowers increased linearly during the first 4 h of morphological male phase (Fig. 1A). Within these 4 h, flowers had achieved at least 96% male function saturation—nearly complete pollen removal. Maximum levels of pollen accumulation on stigmas were reached within 5–6 h of onset of female phase, indicating a maximum length of functional female phase (Fig. 1B). Less pollen was observed on the stigmatic lobes collected on day 2 and 3, at which time corollas had wilted (although stigmas were turgid) and rain had fallen.

On average, 18 ± 1.62 bees visited during the 10-min observation periods, resulting in 2.4 ± 0.2 visits per flower. The level of pollinator activity was low in the morning, peaked in late afternoon, and dropped as evening approached (Fig. 3). There was a significant negative quadratic relationship between visits per flower and time of day ($\beta_{time} = 1.20$, $\beta_{time}^2 = -0.037$, both significant at $P < 0.0001$). Pollinator activity also varied significantly among days of observation ($F_{5,41} = 7.70$, $P < 0.0001$). Daily variation in pollinator activity explains the difference in pollen removal rates between the two surveys (Fig. 1A) but only partially accounts for differences between pollen deposition rates (Fig. 1B).
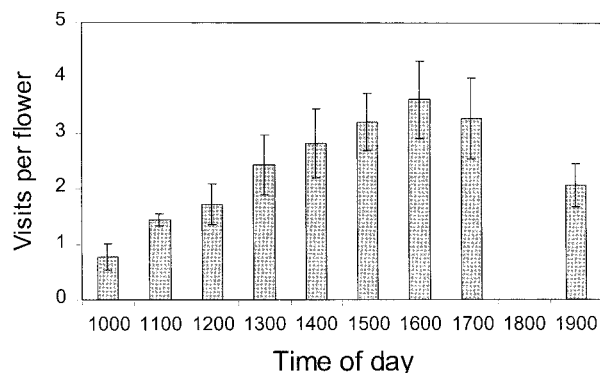
Fig. 3. Mean (±SE) number of pollinator visits per flower between 1000 and 1700 over 6 d. No data were taken at 1800.

DISCUSSION

Longevities of both the male and female morphological phases in *C. americana* changed in response to degree of fitness accrual. Morphological male-phase longevity was shortened by pollen removal and morphological female-phase longevity was shortened by pollen deposition. This decrease in morphological female-phase longevity with increasing female fitness accrual is consistent with findings of previous studies on protandrous species (Devlin and Stephenson, 1985; Richardson and Stephenson, 1989; Preston, 1991; Sargent and Roitberg, 2000). Morphological male-phase length has been found to decrease with increasing pollen removal in other protandrous species (Devlin and Stephenson, 1984, 1985; Richardson and Stephenson, 1989; Sargent and Roitberg, 2000). These results follow Schoen and Ashman's (1995) expectation that if the costs of floral maintenance are constant through time, floral longevity should be shortest when fitness accrual rates are high.

The floral longevity model by Schoen and Ashman (1995) predicts optimal floral longevities of individual flowers based on properties of those flowers (i.e., per-flower fitness accrual rates and resource demands). In a qualitative test of the model's predictions, Ashman and Schoen (1996) examined the mean floral longevities and pollinator visitation rates associated with many individual plant species as reported in the literature. They found that *taxa* that had historically experienced high levels of pollinator activity exhibited shorter floral longevities, on average, than those that experienced low levels of pollinator visitation (Ashman and Schoen, 1996). In this study, we investigated proximate factors that might influence floral longevity within individual flowers. Our results at the level of an individual flower parallel Ashman and Schoen's (1996) findings at the taxon level. This suggests that their model could be expanded to allow floral longevity to change plastically with degree of pollen removal or pollen receipt.

Ashman and Schoen (1996) report that in each of the species they studied, floral longevity measured under field conditions was very close to the maximum for the species. In contrast, the longevity of *C. americana* flowers in the field (3–5 d) was much shorter than the species' maximum floral longevity in the absence of pollinators (7–10 d; L. Evanhoe, personal observation). Additionally, the longevities of the morphological gender phases in the field were nearly identical to those of greenhouse flowers receiving high levels of pollen removal and deposition. From these similarities in gender

phase length and the knowledge that experimental treatments saturated sexual function, we can infer that floral development in the field proceeded as quickly as possible. Frequent pollinator visitation and rapid rates of male and female fitness accrual underlie the swift development of flowers in the natural population. The realized floral longevity we witnessed in the field likely represents a genetically determined minimum for *C. americana*.

In the natural population, male and female sexual functions saturated within a few hours of morphological gender-phase onset. However, the morphological gender phases did not end as soon as their sexual functions were complete. Male phase flowers in the natural population reached maximum levels of pollen removal (i.e., approximately 100%) within 4–6 h of becoming male. Despite the fact that they had achieved their male sexual function, they did not become morphologically female until a day later. Thus, the observed functional male phase of 4–6 h was considerably shorter than the morphological male phase of 1 d. Within 2–3 h of becoming female, flowers in the field reached pollen deposition levels equivalent to the experimental midlevel pollen deposition treatment. This amount of pollen deposition, approximately 300 pollen grains, is six times the typical seed production (L. Galloway, unpublished manuscript). Within 5–6 h, female phase flowers had accumulated pollen loads equivalent to maximum experimental pollen deposition treatments. This yielded a functional female-phase length of 5–6 h—less than half of the total time that flowers were morphologically female in the natural population.

Several possible explanations exist for the differences between the longevities of the morphological and functional gender phases. First, pollinator activity patterns may explain why morphologically male flowers do not become morphologically female until the day after complete pollen removal. By the time of day that all pollen has been removed from male phase flowers, pollinator activity is dwindling; when the flowers become female the next day, pollinator activity is reaching its peak (Fig. 3). Perhaps the transition to morphological female-phase is postponed so that fresh stigmatic surfaces can be exposed to pollinators at the most effective time possible for pollination.

Second, a flower's morphological female phase may be extended past the time of maximum pollen deposition if the flower's cue to senesce (e.g., pollen grain germination or ovule fertilization) does not occur until hours after pollen deposition. Consider ovule fertilization as a possible cue for floral senescence. Although maximum numbers of pollen grains had been deposited on morphologically female flowers in the natural population within 5–6 h of female onset, the grains might not have all germinated, grown full pollen tubes, and fertilized the ovules until a day after maximum pollen deposition. If so, the petal wilting that we observed on the day following maximum pollen deposition would be appropriately timed. Such an explanation seems particularly plausible in light of studies on other species in which ovule fertilization has been found not to occur until 24 or more hours after pollination (e.g., 10–48 h in *Raphanus raphanistrum*, Mazer, Snow, and Stanton, 1986; 24–72 h in *Erythronium grandiflorum*, Cruzan, 1989).

Third, a flower may still be useful to a plant after it has completed its sexual functions if, by remaining turgid and contributing to a large floral display size, it increases the plant's attractiveness to pollinators (reviewed in Snow et al., 1996; Ishii and Sakai, 2001; Galloway, Cirigliano, and Gremski,

2002). As the number of open flowers on a plant increases, so may the number of pollinator visits the plant experiences. Heightened visitation rates may increase a plant's fitness through pollen removal and receipt. The postponement of floral senescence until a day or more after maximum pollen deposition may serve this function in *C. americana*. However, sustaining a large floral display may be costly for a plant. Large displays can be a significant resource drain and can result in increased geitonogamy (i.e., transfer of pollen between flowers on an individual plant) (Barrett and Harder, 1996; Snow et al., 1996). Geitonogamy may lead to inbreeding depression in selfed progeny and fewer opportunities for outcrossing.

In summary, results presented here confirm the potential for a plastic response in floral longevity to fitness accrual and support the idea that this factor should be incorporated in current models of floral longevity. The observed disparity between the lengths of the morphological and functional gender phases in the field suggests that additional factors (e.g., timing of pollinator activity and schedules of pollen germination and pollen tube growth) may shape floral longevity. Finally, our finding that *C. americana* flowers show decreased longevity at higher degrees of fitness accrual lends support to the idea that resource-use efficiency may be a primary determinant of floral longevity.

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