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The benefits of bathing buds: water calyces protect flowers from a microlepidopteran herbivore

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Protective floral structures may evolve in response to the negative effects of floral herbivores. For example, water calyces—liquid-filled, cup-like structures resulting from the fusion of sepals—may reduce floral herbivory by submerging buds during their development. Our observations of a water-calyx plant, *Chrysothemis friedrichsthaliana* (Gesneriaceae), revealed that buds were frequently attacked by ovipositing moths (Alucitidae), whose larvae consumed anthers and stigmas before corollas opened. Almost 25% of per-plant flower production was destroyed by alucitid larvae over two seasons, far exceeding the losses to all other floral herbivores combined. Experimental manipulation of water levels in calyces showed that a liquid barrier over buds halved per-flower alucitid egg deposition and subsequent herbivory, relative to buds in calyces without water. Thus, *C. friedrichsthaliana*'s water calyx helps protect buds from a highly detrimental floral herbivore. Our findings support claims that sepal morphology is largely influenced by selection to reduce floral herbivory, and that these pressures can result in novel morphological adaptations.

Keywords: floral herbivory; selection; floral evolution; Alucitidae; *Chrysothemis friedrichsthaliana*

1. INTRODUCTION

Herbivory on plant reproductive tissues can have severe consequences on plant fitness, thereby promoting the evolution of defensive floral traits. Floral traits that deter herbivores include physical defences such as resins or sharp trichomes (Armbruster 1997; Abdala-Roberts & Parra-Tabla 2005), extrafloral nectaries that attract enemies of herbivores (e.g. Horvitz & Schemske 1984; Sugiura *et al.* 2006), or enlarged sepals or bracts (e.g. Armbruster & Mziray 1987; Delph *et al.* 1996). Flowers may also be defended by constitutive or induced chemicals such as glucosinolates or nicotine (McCall & Irwin 2006). Although recent work demonstrates experimentally the role of chemicals in floral defence (e.g. Strauss *et al.* 2004; McCall & Karban 2006), physical defences have received considerably less attention.

The outermost floral whorl, composed of sepals (or a calyx when the sepals are fused), is generally thought to function to protect reproductive tissues (Delph *et al.* 1996; Endress 1996; Armbruster 1997). Although floral herbivores are presumed to be repelled by various sepal modifications (e.g. McCall & Irwin 2006; Sugiura *et al.* 2006), such claims are rarely supported experimentally (but see Armbruster & Mziray 1987). We tested the adaptive function of an unusual sepal phenotype, the water calyx.

Water calyces hold and often secrete liquid, causing buds to develop under an aqueous or mucilaginous mixture (Burt & Woods 1975; Endress 1996; figure 1a). Water calyces were first described in *Spathodea campanulata* (Bignoniaceae) over 100 years ago (Treub 1889) and have since been documented in over 10 species of at least six plant families (Endress 1996; J. Carlson & K. Harms 2006, unpublished data), suggesting multiple evolutionary origins of the trait. Water calyx morphology is also variable among families: sepals may be partially or completely fused throughout bud development, and liquid-secreting trichomes may be present or absent on adaxial surfaces.

Skutch (1992) hypothesized that water calyces dissuade herbivores. A comparable function is provided by liquid-filled bracts of some *Heliconia* species, which reduce herbivore damage to floral parts (Wootton & Sun 1990). A non-exclusive alternative is that calyx liquid protects buds from desiccation during drought (Burt & Woods 1975) or may otherwise be important to water-related physiology of flowers. We tested these functional hypotheses in the neotropical plant *Chrysothemis friedrichsthaliana* (Hanst.) H. E. Moore (Gesneriaceae; figure 1a,b) by comparing rates of herbivory and bud abortion in calyces that had been drained of liquid, relative to unmanipulated water calyces.

2. MATERIAL AND METHODS

We monitored over 350 *C. friedrichsthaliana* plants from a large population on the Osa Peninsula of Costa Rica, from August to October within the flowering seasons of 2005 and 2006 (see Carlson (2007) for description of study species and site). Plants were routinely surveyed to identify the most important floral herbivores and their associated damage. We confirmed preliminary observations by dissecting hundreds of flowers, rearing larvae, and viewing over 2500 daylight hours of video on unmanipulated *C. friedrichsthaliana* flowers (collected for a pollination study; Carlson 2007).

The cup-like calyces of *C. friedrichsthaliana* (figure 1a) open 2–3 weeks before the corollas and are lined with liquid-secreting trichomes. After the 2-day protandrous flowers open and abscise, calyces persist for an additional 4–5 weeks (J. Carlson 2005–2006, unpublished data). We chose buds in an early developmental stage (i.e. in unopened calyces) and randomly assigned at least 80 buds to each of three treatments: calyx unmanipulated, calyx drained and calyx drained then refilled. Refilled calyces received liquid from the same or neighbouring calyces to maintain the same chemical composition as unmanipulated controls.

Treatments were applied each afternoon for 21 days or until the flower was consumed or reached anthesis (i.e. flower opened). Number of days until anthesis did not vary among treatments (J. Carlson, unpublished data). After one season, we found that neither floral herbivory nor bud abortion differed between calyces unmanipulated and calyces drained then refilled (linear contrasts for both variables: $t < 0.13$ and $p > 0.90$), suggesting that handling caused no additional bud loss. Thus, we collapsed these two treatments into a single 'calyx undrained' treatment and pooled both years' data.

Each day the treatments were applied, we recorded the condition of individual buds. All buds experienced one of three fates by the end of the experiment: lost to herbivores, lost to premature abortion (remaining small and undeveloped) or open and intact. We subdivided herbivore losses into two categories: flowers were consumed completely prior to opening or flowers were

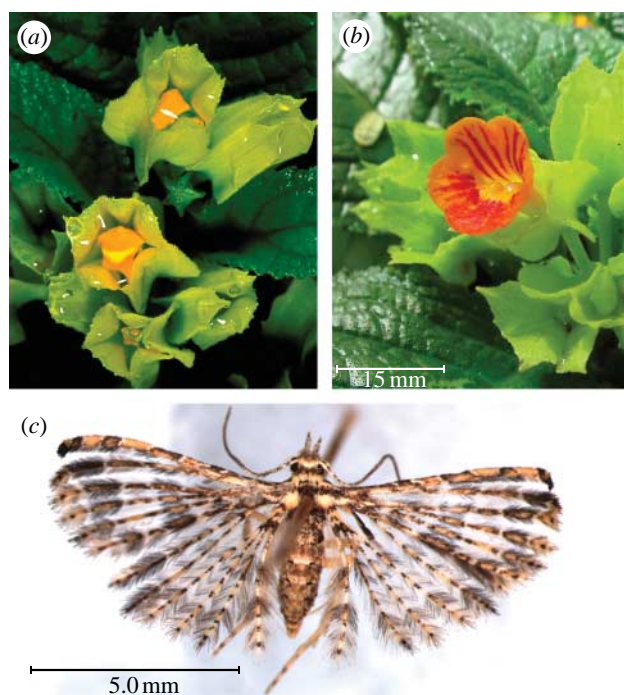


Figure 1. (a) Liquid-filled water calyces and (b) intact flower of *C. friedrichsthaliana*. (c) Adult moth (Alucitidae) that oviposits into *C. friedrichsthaliana* buds. Photo (a) courtesy of Greg Dimijian.

sterilized prior to opening, i.e. anthers and stigmas were consumed entirely, but corollas opened at anthesis.

To determine if buds in water-filled calyces were less susceptible to sterilization, complete consumption or premature abortion (possibly due to desiccation), we performed three binary logistic regressions comparing drained and undrained calyces. First, we compared the probability of flower sterilization between treatments, using only flowers that reached anthesis. Second, we compared the probability of complete flower consumption between treatments, using all flowers. Third, we compared the probability of bud abortion, again using the total number of flowers. We coded all response variables as binary on a flower and tested each using the GLIMMIX procedure in SAS 9.1, including year as a random effect.

3. RESULTS

A moth in the family Alucitidae (*Alucita* sp. cf. *flavicincta* Walsingham; figure 1c) was the most detrimental floral herbivore of *C. friedrichsthaliana*, destroying 38% of unmanipulated flowers in 2005 and 19% in 2006. Adult moths oviposit in immature *C. friedrichsthaliana* buds, and larvae remain inside the buds until anthesis, completely consuming the developing anthers and stigmas, but not the corolla. All flower sterilization could be attributed to the alucitid larvae, and oviposition scars on buds reliably indicated larval infestation, even at early stages in bud development.

Calyx draining more than doubled the odds that individual flowers would be larvae-infested and lack reproductive structures upon opening, relative to flowers in water-filled calyces ($F_{1,241} = 6.87$, $p = 0.009$; odds ratio: 2.18; 95% CI: 1.21–3.92; figure 2). Survival of alucitid larvae was high and invariable across treatments; more than 98% of buds with oviposition scars in both drained and undrained calyces were sterile and contained larvae upon opening. Therefore, differences in flower loss were most probably caused by reduced oviposition in immersed buds, rather than reduced larval survival.

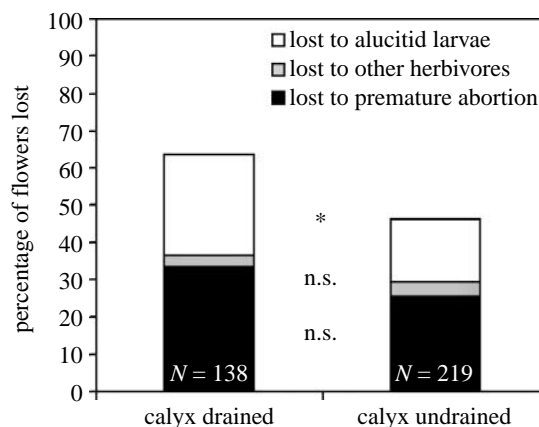


Figure 2. Flower loss in *C. friedrichsthaliana* with drained and undrained water calyces. Flowers that reached anthesis in drained calyces were significantly more likely to contain alucitid larvae; see §3 for details. N , total number of buds in each treatment; * $p < 0.01$; n.s., not significant.

Generalist herbivores, including grasshoppers and katydids, were responsible for some additional herbivory, consuming 4% of unmanipulated flowers. In contrast to alucitid moths, these herbivores consumed flowers completely and did not respond to calyx-draining treatments ($F_{1,355} = 0.02$, $p = 0.88$; figure 2). Finally, a quarter of buds were aborted at an early developmental stage, but abortion was not significantly more probable in drained calyces ($F_{1,355} = 1.21$, $p = 0.27$; figure 2). Almost all aborted buds lacked alucitid oviposition scars, suggesting that abortion generally occurred prior to alucitid attack, and that moths avoided buds that would be subsequently aborted. Thus, we inferred that bud abortion was unlikely to be caused by larval infestation.

4. DISCUSSION

Our experiments indicate that water calyces serve as a defence against floral herbivores. This result supports the widespread but inadequately tested view that sepals are predominately protective in function (e.g. Delph *et al.* 1996; Endress 1996). Although floral herbivores and other enemies are often recognized as important contributors to the evolution of floral phenotype (e.g. Linhart 1991; Irwin *et al.* 2004; McCall & Irwin 2006), our study is among the few that experimentally test evolutionary hypotheses for a physical floral defence.

In *C. friedrichsthaliana*, a liquid-filled calyx protects buds from ovipositing moths, but does not otherwise alter bud survival and development. Bud abortion was fairly high, but was invariant between filled (calyx undrained) and empty (calyx drained) conditions. This suggests that the water calyx is unlikely to serve an anti-desiccation or water balance-related function. The causes of bud abortion were not determined in this study, although other studies have shown that low availability of plant resources can cause high bud abortion rates (e.g. Stöcklin 1997).

Our results support Skutch's (1992) original prediction that *C. friedrichsthaliana*'s water calyces function for herbivore defence. However, the defence is only partial as generalist herbivores were unaffected by filled calyces. Even so, generalists consumed relatively few

flowers overall. Flower loss was much greater to alucitid moths, which were the only herbivores deterred by bud submersion. The moths have a wingspan of approximately 11 mm and have short ovipositors. Thus, it is unlikely that they can reach buds through 5–20 mm of calyx liquid—the range of depths typically observed (J. Carlson, unpublished data).

Oviposition was significantly reduced in water-filled calyces, yet alucitid larvae still destroyed a quarter of buds in the undrained treatment. We explain this observation as follows: calyces occasionally dry out on sunny days, providing opportunities for ovipositing moths to reach buds. Undrained calyces were encountered empty on an average of 17% of days preceding floral anthesis (s.d.=0.14, $n=24$). Most empty calyces were full again within 24 h, although filling rates were variable among plants (Skutch 1992; J. Carlson, unpublished data).

Calyx phenotype may influence the behaviours of floral visitors other than herbivores; for example, pollinators are attracted to brightly coloured sepals (or calyces) on some plants (e.g. Schemske *et al.* 1996). We doubt, however, that the water calyx of *C. friedrichsthaliana* is used by its hummingbird pollinator (*Phaethornis striigularis*) as an indicator of floral rewards, because the yellow-green calyces do not reliably indicate nectar availability. Nectar robbers or thieves may also selectively favour floral modifications (Galen 1999; Irwin *et al.* 2004). Nevertheless, the non-pollinating, nectar-thieving ants that visit *C. friedrichsthaliana* are not deterred by water-filled calyces; even small ants can bypass the water calyx and enter the mouth of the corolla. Furthermore, flowers with ants do not receive fewer pollinator visits, and visiting ants generally do not damage reproductive parts (J. Carlson, unpublished data). Thus, alucitid moths appear to exert the single most important selection pressure currently acting on *C. friedrichsthaliana*'s water calyces.

Cup-like water calyces may function predominately for herbivore defence, yet individual floral traits do not evolve in isolation. For example, pollinators and nectar robbers may influence calyx phenotype through genetic correlations with other floral traits (e.g. Armbruster 1997). Furthermore, selection for one trait, e.g. upright, water-filled calyces, may constrain selection for optimal performance of another, e.g. corolla orientation for most-effective pollination. However, such complex interactions cannot be detected until the effects of all visitors and trait pleiotropisms are known. Detailed studies may reveal how selection by floral herbivores interacts with selection by other visitors to ultimately shape floral evolution.

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