

**POST-FLORAL PERIANTH FUNCTIONALITY:
CONTRIBUTION OF PERSISTENT SEPALS TO SEED
DEVELOPMENT IN *HELLEBORUS FOETIDUS*
(RANUNCULACEAE)¹**

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The perianth persists after flowering in many plants, yet its post-floral functionality has been little investigated, and the few studies available provide ambiguous evidence. This paper tests the hypothesis that the green persistent sepals of the perennial herb, *Helleborus foetidus*, contribute to the plant's fitness by enhancing seed number and/or size. Post-floral contribution of the calyx to fruit and seed development was evaluated by manipulating sepal number and measuring the effect on follicle size, seed set, seeds per follicle, and mean seed mass. The allometric relationship between calyx size and follicle mass was examined for flower buds, open flowers, and immature fruits differing in follicle number. Calyx manipulation had no significant effect on follicle size, seed set, or number of seeds per follicle, but it did have a significant influence on the size of individual seeds. Calyx size and mean seed mass were positively, linearly related. The calyx mass/seed number ratio declined with increasing number of follicles per fruit. The persistent sepals of *Helleborus* do contribute resources to the development of seeds, although there is not evidence of post-floral allometric adjustment of the calyx mass/follicle number ratio that could compensate for variations in seed number per fruit.

Key words: allometry; calyx size; *Helleborus foetidus*; perianth persistence; Ranunculaceae; seed size; sepal photosynthesis.

In many species of flowering plants, the calyx, the corolla, and/or some floral ancillary structures (e.g., bracts) persist well beyond completion of anthesis, often until fruit maturation and seed release. The protracted presence of structures whose genuine function is to contribute to the success of flowers (pollen export and import) suggests that, after anthesis, they are co-opted for new, different functions unrelated to the pollination process. The nature of this post-floral function is fairly evident in some cases, as in species where the fleshy "fruits" made up of juicy bracts or hypanthia, or the spiny, plumose, or otherwise modified calices, play an essential role in seed dispersal (Ridley, 1930; van der Pijl, 1982). In fact, the "recycling" of pre-existing floral structures seems one of the main evolutionary avenues leading to the diversification of seed dispersal mechanisms in higher plants (Stebbins, 1974). In other instances, however, the post-floral function of persistent perianth parts is much less clear and can only be tentatively guessed. Consider, for example, the dry persistent corollas found in so many plant families (Cronquist, 1981), the inflated calyx of *Physalis* (Sisterson and Gould, 1999), or the large accrescent calices of species of Olacaceae, *Hypericum*, or *Helleborus* (among many others; Heywood, 1978; Mathew, 1989).

Investigations on the post-floral functions of perianth parts other than those related to seed dispersal are strikingly scarce (Sisterson and Gould, 1999; Salopek-Sondi et al., 2000, 2002), and these few studies do not always confirm an immediate utility or presumable adaptive value of perianth persistence. In *Physalis angulata*, for example, the large inflated calyx pro-

vides a protection from parasitoids to the caterpillars of the *Physalis*-specialist herbivore *Heliothis subflexa* while they feed on the fruit enclosed within the calyx, a herbivore-protective function that can hardly be interpreted as enhancing plant fitness (Sisterson and Gould, 1999). In cultivated persimmon fruits (*Diospyros kaki*), intense photosynthesis by the large, persistent green calyx lobes does not contribute to fruit development (Nakano et al., 1997). Further investigations on a variety of species in diverse ecological scenarios are needed to assess the functional significance and possible adaptive value of perianth persistence, a widespread but poorly understood floral feature. In addition, an improved understanding of the functionality of persistent perianth parts past flowering would allow one to address the more general issue of whether, because of contrasting selective pressures operating at the floral and post-floral stages, multifunctionality of a given floral structure may contribute to the evolution of compromise floral attributes, an aspect that remains essentially unexplored.

This paper evaluates the hypothesis that the large, persistent green sepals of the perennial herb, *Helleborus foetidus* L. (Ranunculaceae), make some measurable contribution to the plant's fitness in terms of enhanced seed number or size. In the genus *Helleborus* the petals have become modified into nectaries, and sepals are enlarged and look like petals (Mathew, 1989; Tamura, 1993). Depending on the species, they may be green or, less often, white, pink, or purple at anthesis, turning green afterwards and persisting through the fruiting stage. This observation has fueled the traditional view that *Helleborus* sepals "assist in photosynthesis" (Mathew, 1989, p. 40), "helping the seed-pods to swell" (Rice and Strangman, 1993, p. 8). Strong indirect support for this idea was recently provided by Salopek-Sondi et al. (2000, 2002) for *Helleborus niger*. In this species, fruit initiation triggers post-floral expansion of the white sepals and regreening through transformation of leucoplasts into chloroplasts, which confers a photosynthetic capacity similar to those of young leaves. This finding

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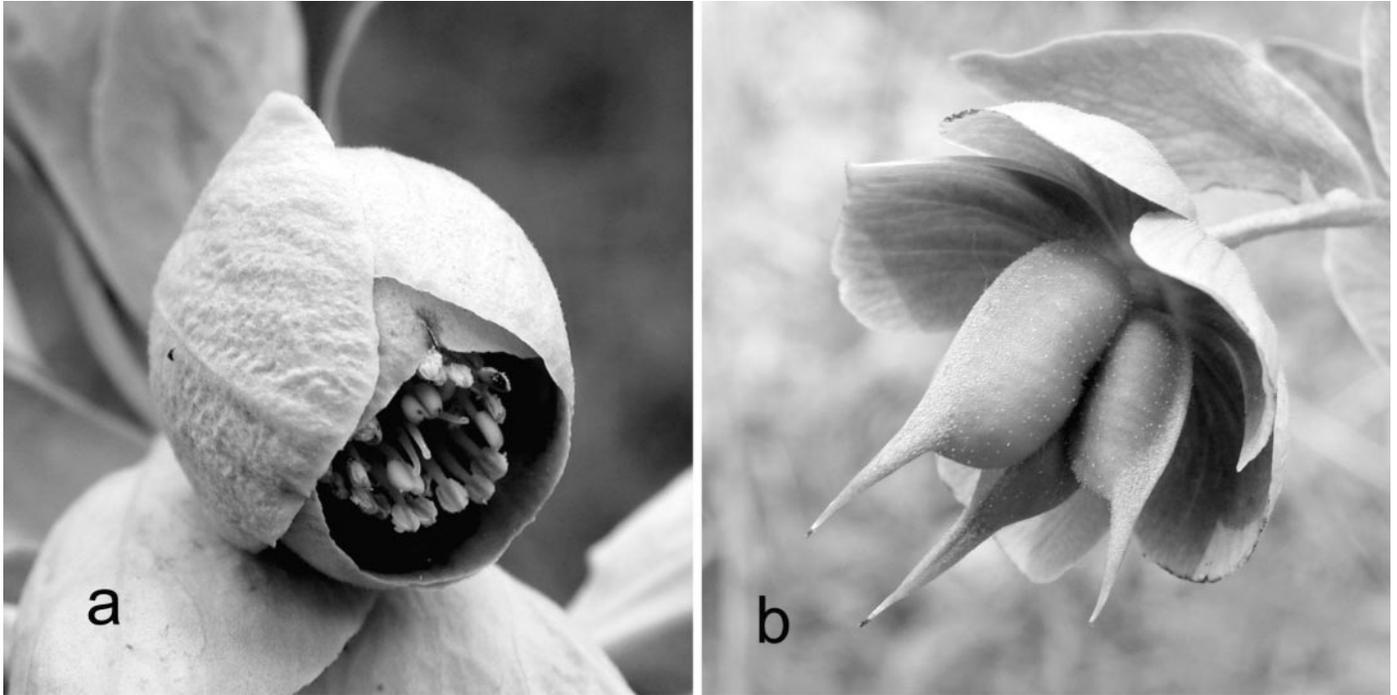


Fig. 1. Flower (a) and developing "fruit" (b) of *Helleborus foetidus*. After flowering, the green sepals spread out and remain, subtending the apocarpous follicles until the follicles complete development, and fruit ripening and seed release take place.

suggests that chlorophyll-containing sepals may be a source of assimilates for the developing seeds in this and other species of *Helleborus*, but this hypothesis has not been tested directly. The main objective of this study was to determine if, under natural field conditions, experimental modification of calyx size after flowering affected the size of the associated fruit and/or the number and size of seeds produced, and if so, whether calyx size and the response variable(s) were linked by a linear relationship. If selection had enhanced the photosynthetic contribution of the calyx to developing seeds, one would expect calyx mass and total fruit mass to be linked by a proportionality relationship independent of variations in fruit size. A second objective of this study was to examine the allometric relationship between calyx and fruit size, specifically whether there is some post-floral adjustment of calyx size to variations in the number of fruit follicles and developing seeds.

MATERIALS AND METHODS

Study plant—*Helleborus foetidus* is a perennial herb widely distributed in western and southwestern Europe (Werner and Ebel, 1994). In the Iberian Peninsula, it typically occurs in clearings, forest edges, and the understory of montane forests, mainly on limestone substrates. Flowering mainly takes place during January–March. Flowers are hermaphroditic and self-compatible, and although some spontaneous autogamy occurs when pollinators are excluded experimentally, successful reproduction requires insect pollination. Bumble bees and anthophorid bees are the main pollinators (Herrera et al., 2001). The green, pendent, bell-shaped flowers (Fig. 1a) are 14–19 mm long and 12–17 mm wide. The outer floral whorl consists of five, large, overlapping sepals, which are green at anthesis. The nectaries are shaped like more or less flattened horns forming a ring between the stamens and the sepals, and produce copious nectar (Herrera and Soriguer, 1983; Vesprini et al., 1999). Flowers are apocarpous, the number of carpels per flower ranging between 1–6 (mostly 1–3). Following flowering, the green sepals expand and spread, forming a conspicuous, dish-shaped, 3–4 cm wide structure subtending the developing

follicle(s) (Fig. 1b). Sepals remain green and persist until the follicles mature in June–early July and seed dispersal takes place. For convenience, the composite structure made up of the developing follicle(s) plus the subtending sepals (Fig. 1b) will be termed here a "fruit," and the set of sepals will be referred to as the "calyx" regardless of whether they are part of a flower or a developing fruit. The term "follicle" will be used to designate either the carpels of flowers or flower buds, or the true follicles of developing fruits. Further details on the reproductive ecology of *H. foetidus* in the Iberian Peninsula may be found in Garrido et al. (2002), Herrera et al. (2002), and Guitián et al. (2003).

Study area and methods—This study was carried out during March–June 2002 at a large, more or less continuous population of *H. foetidus* located at 950–1100 m elevation in wooded slopes around the small village of Vadillo-Castril, in the Parque Natural Sierras de Cazorla-Segura-Las Villas, Jaén Province, southeastern Spain. Plants were growing in the understory of pine (*Pinus pinaster* and *P. nigra*) and evergreen oak (*Quercus ilex*) mixed woodlands.

Flower buds, open flowers, and immature fruits were collected to examine patterns of variation in calyx size from the flower bud through the nearly mature fruit stages, and also to determine the allometric relationships between calyx size and follicle size in flowers and fruits differing in follicle number. Collections were done on five different dates between 7 March and 25 May from a large number of flowering and/or fruiting plants scattered over the study area. Only structures without signs of herbivore damage were collected. The sample of fruits comprised all developmental stages from newly withered flowers up to nearly mature fruits in roughly similar proportions. Efforts were made to sample the whole range of follicle number occurring in the population (range = 1–5), yet flowers and fruits with five follicles were too scarce to obtain adequate samples, and the analyses were limited to structures bearing between 1–4 follicles ($N = 327$). Each collected structure was dissected within a few hours of collection, the number of follicles recorded, and the fresh calyx weighed to the nearest 0.1 mg. Fresh mass was considered to provide a more accurate assessment of calyx size than, e.g., linear measurements, which were likely to be influenced by other sources of variation like shape changes. On the negative side, however, using calyx mass to measure size

TABLE 1. Summary of linear mixed model testing for the effects of developmental stage (flower bud, flower, unripe fruit), number of follicles per flower or fruit (1–4), and their interaction, on natural variation of calyx fresh mass in *Helleborus foetidus*. Based on bud, flower, and fruit samples collected at the study area over the 2002 flowering and fruiting season. Significant *P* values are shown in boldface. See also Fig. 2.

| Effect | df | <i>F</i> | <i>P</i> value |
|--------------------------|--------|----------|-------------------|
| Developmental stage (DS) | 2, 308 | 144.13 | <0.0001 |
| Number of follicles (NF) | 3, 308 | 4.02 | 0.0079 |
| DS × NF | 6, 308 | 0.93 | 0.47 |

had the drawback of requiring destructive sampling, thus precluding repeated measurements of the same structure over time.

The possible post-floral functionality of the calyx was evaluated experimentally by manipulating its size and subsequently measuring the effect of induced size variations on per-flower seed set, seeds per follicle, mean seed mass, and mean follicle size as measured by follicle wall mass. The experiment was set up during 6–26 March, according to a randomized block design, with $N = 17$ individual plants treated as blocks and $N = 175$ individual flowers (after elimination of unusable data, such as herbivore-damaged fruits) used as experimental units. The main factor, calyx size, had three levels: five sepals (control), three sepals, and zero sepals. Sepal number was reduced by excising the unwanted sepals with scissors immediately after flowering (necaries and stamens already shed), so that any eventual effect of calyx size manipulation on the reproductive success of the subtended follicles would exclusively reflect its post-floral functionality. To assess if the effect of calyx size was contingent on the number of follicles in the fruit, the follicle number was incorporated into the experiment as a discrete covariate with three levels (1, 2 or 3 follicles). Experimental fruits were individually marked and collected in early June when they had reached final size, shortly before the mature follicles started to dehisce. Fruits were dissected individually to separate seeds and follicle walls. Follicle walls were weighed to the nearest 0.1 mg, and an average mass per follicle was computed for each fruit. The total number of full-sized, sound seeds (NSEEDS) were counted and weighed collectively to the nearest 0.1 mg, and an average seed fresh mass value was obtained for each fruit. Undeveloped ovules and empty seeds that were aborted before maturation were counted (NABORT), for an estimation of the initial number of ovules as $NOVULES = NSEEDS + NABORT$. Seed set could then be obtained for each fruit as $NSEEDS/NOVULES$.

Statistical analyses—All statistical analyses were conducted using the SAS software package (SAS Institute, Cary, North Carolina, USA). Observational data on natural variation in calyx size and experimental results on the effects of calyx size and follicle number (covariate) on seed mass, number of seeds per follicle, and follicle wall mass, were analyzed by fitting linear mixed models to the data using the MIXED procedure and restricted maximum likelihood estimation. Experimental results on the effects of calyx size and follicle number on seed set were tested by fitting a generalized linear mixed model, as implemented in SAS macro GLIMMIX (Littell et al., 1996). The response variable ($NSEEDS/NOVULES$, i.e., the per-flower probability of an individual ovule producing a sound seed) was modeled as a binomial response, using logits as the link function. In the analyses, calyx size and follicle number were treated as fixed effects, and plants (i.e., blocks) were included as a random effect. All means and associated standard errors reported are model-corrected means obtained with the LSMEANS statement in the MIXED procedure.

When a significant effect of calyx size on a response variable was found, orthogonal polynomials (e.g., Steel and Torrie, 1980) were used to further test for a trend between the response variable and the quantitative levels of the factor and to assess if any trend had significant linear and/or quadratic components (i.e., whether the response variable changed with decreasing sepal number, and if it did, whether the relationship was linear or quadratic). Coefficients of orthogonal polynomials for the three unequally spaced treatment

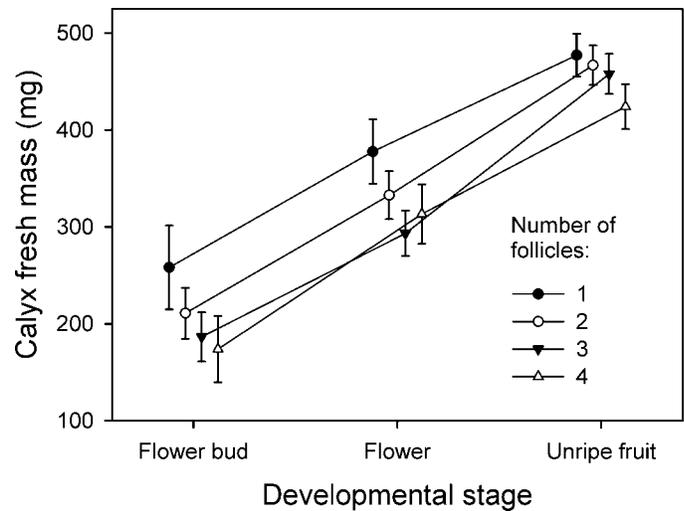


Fig. 2. Variation in mean fresh mass of the persistent calyx of *Helleborus foetidus* flowers over the developmental sequence of bud to flower to unripe fruit, plotted separately for structures bearing different number of follicles. Vertical segments extend over ± 1 SE of the mean. See Table 1 for significance tests of the two main effects (developmental stage and number of follicles) and their interaction.

levels (5, 3, and 0 sepals) were computed using SAS macro ORPOLY (Schabenberger, 1996) and incorporated into the appropriate CONTRAST statements constructed for use with procedure MIXED.

RESULTS

Natural variation in calyx size—There was a strong, statistically significant effect of developmental stage on calyx size as assessed with fresh mass (Table 1). Calyx mass increased steadily from the flower bud (mean ± 1 SE = 207.4 ± 22.6 mg, $N = 47$, all follicle numbers combined; hereafter, all means are reported ± 1 SE) through the flower (329.2 ± 20.7 mg, $N = 69$) to the immature fruit stage (456.5 ± 19.1 mg, $N = 211$) (Fig. 2). On average, fresh mass of the calyx increased by roughly 39% from flowers to immature fruits.

Calyx mass was significantly related to the number of follicles (Table 1). On average, structures bearing single follicles had the heaviest calices (371.1 ± 24.9 mg; buds, flowers and fruit combined), those with four follicles the lightest ones (303.7 ± 23.1 mg), and those with two (336.8 ± 20.4 mg) and three follicles (312.5 ± 19.9 mg) were intermediate. This pattern remained consistent across the bud–flower–fruit developmental sequence (Fig. 2), as revealed by statistical non-significance of the stage \times follicle number interaction effect (Table 1). As a consequence of the inverse relationship between calyx mass and number of follicles, there was a steep decline in mean calyx mass per individual follicle with increasing number of follicles per fruit, from an average of 371 mg/follicle in single-follicle fruits down to 169 mg/follicle, 102 mg/follicle, and 76 mg/follicle in two-, three- and four-follicle fruits, respectively. Because individual follicles had, on average, the same number of seeds (8.2 ± 0.15 seeds, $N = 272$) irrespective of the number of follicles in the fruit ($F_{2,253} = 0.34$, $P = 0.71$), this result reveals a steep decline of the calyx mass/seed number ratio with increasing number of follicles.

TABLE 2. Summary of linear mixed models testing for the effect of experimentally induced variation in calyx size (5, 3, and 0 sepals) on seed set (modeled as per-flower probability of one ovule producing a sound seed), number of seeds per follicle, mean follicle size (mean fresh mass of follicle wall), and mean seed mass, all computed on a per-fruit basis. Follicle number (1–3) was included in all models as a covariate. Significant *P* values are shown in boldface.

| Dependent variable | Effect | df | <i>F</i> | <i>P</i> value |
|--------------------|----------------------|--------|----------|----------------|
| Seed set | Calyx size (CS) | 2, 113 | 0.16 | 0.85 |
| | Follicle number (FN) | 2, 113 | 0.06 | 0.94 |
| | CS × FN | 4, 113 | 0.27 | 0.90 |
| Seeds per follicle | Calyx size (CS) | 2, 113 | 0.13 | 0.88 |
| | Follicle number (FN) | 2, 113 | 0.60 | 0.55 |
| | CS × FN | 4, 113 | 0.55 | 0.70 |
| Mean follicle size | Calyx size (CS) | 2, 113 | 0.26 | 0.77 |
| | Follicle number (FN) | 2, 113 | 4.57 | 0.012 |
| | CS × FN | 4, 113 | 0.77 | 0.55 |
| Mean seed mass | Calyx size (CS) | 2, 112 | 4.14 | 0.018 |
| | Follicle number (FN) | 2, 112 | 4.38 | 0.015 |
| | CS × FN | 4, 112 | 1.17 | 0.33 |

Calyx size manipulation—Seed set, mean number of seeds per follicle, and mean follicle size (as estimated by fresh mass of follicle wall) were not significantly affected by experimentally induced variations in calyx size (Table 2). Follicle number significantly affected the mean size of individual follicles, which was larger in fruits with one follicle (416 ± 30 mg) than in those with either two (345 ± 23 mg) or three follicles (356 ± 27 mg; all calyx sizes combined).

The experimental treatment had a significant effect on mean seed mass (Table 2). For fruits of all follicle numbers combined, mean seed mass declined from 25.9 ± 0.8 mg in fruits with five sepals (control), to 25.1 ± 0.8 mg and 23.7 ± 0.8 mg in those with three and zero sepals, respectively. This effect occurred consistently in fruits with a different number of follicles, as revealed by the nonsignificant calyx size × follicle number interaction effect (Table 2). Mean seed mass was also significantly affected by follicle number (Table 2), declining from 26.5 mg in single-follicle fruits, to 24.5 mg and 23.7 mg in fruits with two and three follicles, respectively (fruits with all sepal numbers combined).

Trend analysis for the relationship between calyx size and seed mass using orthogonal polynomials demonstrated a significant linear relationship between the two variables ($F_{1,112} = 7.77$, $P = 0.006$), while the quadratic component was not significant ($F_{1,112} = 0.64$, $P = 0.43$). After statistically accounting for the effect of follicle number variation, mean seed mass was therefore linked to calyx size (as assessed by sepal number) by a linear relationship. Additional support for a linear relationship between seed mass and calyx size was obtained by taking advantage of the information on mean calyx mass of fruits with one, two, and three follicles reported in Fig. 2. From this data, estimates of mean calyx mass per follicle were obtained for each of the nine experimental fruit groups, i.e., three sepal number levels (5, 3, 0) by three follicle number levels (1, 2, 3). Across fruit categories, mean seed mass was significantly, linearly related to estimated mean calyx mass per follicle (Fig. 3). Because, as noted before, individual follicles had the same number of seeds irrespective of the number of follicles in the fruit, this result reveals that increasing calyx mass per seed predictably translated into a proportional increase in mean seed mass.

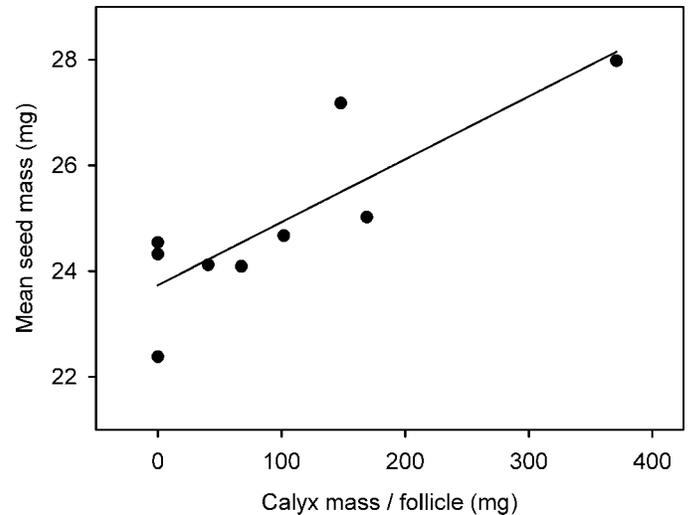


Fig. 3. Relationship between mean seed mass and estimated mean calyx fresh mass per follicle across the nine experimental fruit groups (i.e., three sepal number levels × three follicle number levels). The line shown is the least squares-adjusted linear regression ($y = 23.73 + 0.0119x$; $R^2 = 0.72$, $F_{1,7} = 17.62$, $P = 0.004$).

DISCUSSION

Results of this study provide compelling evidence that the green, persistent calyx of *H. foetidus* contributes resources to the development of seeds, hence supporting the traditional interpretation of its post-floral functionality (Mathew, 1989). Although the manipulation of calyx size had no significant effect on follicle wall mass, seed set, or the number of seeds produced per follicle, it did significantly influence the size of individual seeds. Galen et al. (1993) likewise found that, in *Ranunculus adoneus*, preventing carbon fixation by developing infructescences had no effect on seed number per flower, but did result in significantly smaller seeds. In *H. foetidus*, failure of experimental calyx reduction to induce a decrease in the number of seeds produced is consistent with the results of earlier investigations that, in the Sierra de Cazorla study region, seed production by *H. foetidus* flowers is mainly limited by pollination rather than resource availability. There, the number of pollen tubes is inferior to the number of ovules in about 50% of follicles, the number of seeds produced per follicle is closely correlated with the number of pollen tubes in the style, and supplemental hand-pollination increases seed production (Herrera et al., 2001; Herrera, 2002). Under this prevalently pollen-limited seed production regime, it is not surprising that reducing or suppressing the resources furnished by the calyx were ineffective at inducing a reduction in the number of seeds produced. The possible role of the calyx in enhancing fruit set and/or seed set could only be safely ruled out if experimental manipulations of calyx size were performed on developing fruits from flowers that were not pollen-limited (e.g., receiving supplemental hand-pollination).

In addition to the green leaves which are the primary sources of photosynthate production, higher plants may use almost all vegetative (stem tissues, roots) and reproductive (flowers, fruits) structures to perform photosynthetic CO_2 assimilation (reviews in Blanke and Lenz, 1989; Aschan and Pfanz, 2003). In both cultivated (Vemmos and Goldwin, 1994; Nakano et al., 1997; Smillie et al., 1999) and wild (Williams et al., 1985)

plants, the photosynthetic capacity of green sepals at the flowering or early post-flowering stages may be comparable or even exceed that of leaves. This has been demonstrated for the persistent green sepals of some *Helleborus* species as well. Chlorophyll fluorescence and O₂ gas-exchange measurements indicate that the photosynthetic capacity of sepals is about 50–60% that of mature leaves in *H. niger*, and about 20% in *H. viridis* (Salopek-Sondi, 2000; Aschan and Pfanz, 2003). On this basis, Salopek-Sondi (2000) suggested that the chlorophyll-containing sepals may be a major source of assimilates for the developing seeds in species of *Helleborus*. This hypothesis, however, requires direct validation, because even if the photosynthesis of reproductive structures reaches a positive carbon balance (which is not always the case; e.g., Werk and Ehleringer, 1983; Palliotti and Cartechini, 2001), it may (e.g., Galen et al., 1993; Ogawa and Takano, 1997) or may not (e.g., Marcelis and Hofman-Eijer, 1995; Nakano et al., 1997; Carrara et al., 2001) eventually translate into a significant enhancement of fruit or seed development. In the case of persistent, green sepals, the assimilates produced may remain in the sepals without being translocated to other parts of the fruit, as found by Nakano et al. (1997) for *Diospyros kaki* or, if exported, architectural constraints may drive them to storage organs or metabolic sinks other than nearby seeds (Watson and Casper, 1984). For *H. foetidus*, this study does support that the green, persistent sepals are a significant source of assimilates for developing seeds as proposed by Salopek-Sondi (2000) for *H. niger* for the following reasons: (1) Calyx size reduction resulted in a highly significant reduction in mean seed mass, and this effect took place consistently, irrespective of the number of follicles in the fruit. Removing the calyx reduced seed mass by an average of about 10% (from 25.9 mg in control fruits to 23.7 mg in those with the five sepals removed). (2) The trend that linked calyx size, which may be taken as a rough surrogate for its photosynthetic capacity, and mean seed mass was significantly linear. Linearity of the relationships between seed mass and both number of sepals and calyx mass/follicle, also corroborates that the variation in the size of the calyx, rather than some unsuspected consequence of the artificiality of the experiment (cutting sepals with scissors), was ultimately responsible for observed variation in mean seed mass.

A direct relationship between intraspecific variation in seed size and seedling emergence, survival and/or growth, has been documented for many species (e.g., Herrera et al., 1994; Leishman and Westoby, 1994; Bonfil, 1998; Susko and Lovett-Doust, 2000; Rey et al., 2004; among many others). *Helleborus foetidus* is not an exception to this general pattern. Seed germination rate tends to increase linearly with increasing seed mass in this species, and natural variation of seed mass similar in magnitude to those induced in this study by calyx size variation has been proven consequential for germination rate (Garrido, 2003). It thus seems reasonable to suggest that persistence and size expansion of the calyx after flowering is an adaptive feature favored by natural selection because of its effects on seed size and, ultimately, on seedling emergence and survival. It must be noted, however, that enhancement of seed mass and seedling performance need not be the only or even the main function played by the persistent calyx in *H. foetidus*. In the study region, the developing follicles of this species are eaten by lepidopteran larvae (*Noctua* spp., *Trigonophora flammea*; Noctuidae; Herrera et al., 2002). Sepals might also play a defensive function, acting as a physical hindrance to the foraging larvae, and/or diverting their feeding

activity away from the follicles, because they often feed on the sepals without damaging the follicles. The present investigation was originally designed to test this protection hypothesis of sepal functionality too, but the incidence of lepidopteran herbivores on experimental plants during the study season was too low to produce useful data.

As noted in the Introduction, identifying some post-floral functionality of a persistent floral part prompts questions about the extent to which its features have been shaped by selective pressures linked to this putatively secondary function. In *H. niger* and other *Helleborus* species with colored sepals, selective pressures unrelated to the floral function most likely account for the subsequent enlargement and transformation of the calyx into a green, photosynthetic structure (Salopek-Sondi et al., 2000, 2002). In the green-flowered *H. foetidus*, sepal color does not change after flowering, but the bell-shaped perianth spreads out and, as shown by this study, sepals increase considerably in size from the flower to the developing fruit stage. This most likely reflects selection for post-floral modification of the calyx to enhance its photosynthetic function. An accurate description of the calyx growth trajectory based on repeated resampling of the same fruit was precluded by the destructive sampling used in this study. Nevertheless, because calyx mass estimates were based on random samples taken from the same population at different times of the flowering and fruiting season, results can confidently be interpreted as indicative that the calyx of *H. foetidus* grows significantly past flowering.

Although post-floral growth of the calyx does support an adaptive interpretation of calyx persistence in *H. foetidus* and other congeneric species (Salopek-Sondi et al., 2000, 2002), the allometric relationship between calyx size and follicle number found in this study does not, because post-floral calyx growth did not compensate differentially for variations in the number of follicles. The mean number of seeds per follicle did not vary with the number of follicles in the fruit, thus constancy of the calyx/seed mass ratio across follicle number classes would have required calyx size to be directly proportional to the number of follicles. The observed trend was just the opposite, because calyx mass was inversely related to the number of follicles in buds and flowers, and the trend remained invariant after flowering. There was not, therefore, any compensatory growth of the calyx after flowering that could offset the lower calyx/seed mass ratio found in multi-follicle fruits. This may partially explain the observed decline in mean seed mass with increasing number of follicles. In conclusion, although the green persistent calyx of *H. foetidus* plays a function that is expected to be enhanced by natural selection, no evidence was found of adaptive fine-tuning of the calyx mass/seed number relationship that would account for variations in seed number per fruit due to variations in the number of follicles.

Because of their photosynthetic activity, nonfoliar structures may supply a significant fraction of their own carbon and energy requirements (Bazzaz and Carlson, 1979; Bazzaz et al., 1979; Williams et al., 1985; Galen et al., 1993; Antlfinger and Wendel, 1997; Ogawa and Takano, 1997). The extent to which plant reproductive organs contribute to their own pools of fixed carbon thus has important implications for resource allocation theory, and investigations on the photosynthetic contribution of reproductive structures have traditionally tended to consider it exclusively in the context of reproduction costs (review in Obeso, 2002). From an evolutionary viewpoint,

however, it is also important to consider to what extent photosynthesis by reproductive structures actually promotes plant fitness via enhanced seed production. In natural populations, there are very few experimental demonstrations that the number or size of seeds produced by plants are actually reduced in the absence of carbon assimilation by reproductive structures (e.g., Galen et al., 1993). This study, in addition to documenting the post-floral functionality of the perianth in a species with persistent sepals, also provides one of the few examples to date of enhanced seed production promoted by the photosynthetic contribution of a reproductive structure.

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