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Blossom colour change after pollination provides carbon for developing seeds

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Summary

1. We tested the hypothesis that greening of the floral (involucral) bracts of *Dalechampia scandens* blossoms after pollination (when bracts are white) increases carbon assimilation and provides photosynthate to developing seeds.

2. We investigated the importance of the involucral bracts for the process of seed development in two ways. First, we removed or shaded bracts of hand-pollinated blossoms to prevent their photosynthesis and tested the effects of these manipulations on seed development. Secondly, we measured the photosynthetic rate of blossoms with white vs. green bracts and compared these rates with those of leaves.

3. After four weeks of development, seeds from blossoms with bracts removed or shaded were lighter than those produced by unmanipulated blossoms. Furthermore, although the area-based photosynthetic rate of green bracts was much lower than that of leaves, it was much greater than that of white bracts. Estimates of the daily carbon budget based on these measurements indicate that photosynthesis in green bracts is sufficient to meet the respiratory demand of the whole blossom, but not so in white bracts.

4. Our results support the hypothesis that colour change in *D. scandens* bracts allows carbon assimilation that contributes to the carbon demand of nearby developing seeds.

Key-words: blossom colour change, carbon assimilation, costs of reproduction, floral photosynthesis, gross photosynthetic rate, plant–pollinator interaction, seed mass

Introduction

Over 450 plant species in 253 genera exhibit ontogenetic floral colour changes. In some, attractive floral parts are retained through the period of seed and fruit maturation (Weiss 1995; Weiss & Lamont 1997). Floral colour change may direct discriminating pollinators to rewarding flowers and enhance fertilization efficiency (Casper & La Pine 1984; Delph & Lively 1989; Gori 1989; Ida & Kudo 2003; Sun *et al.* 2005), while retaining older, unrewarding flowers increases the floral display and facilitates attraction of pollinators (Lamont 1985; Cruzan, Neal & Willson 1988; Gori 1989; Weiss 1991; Suzuki & Ohashi 2014). The retention of senescing flower parts may also enable the translocation or reallocation of nutrients from these structures to the developing fruits and seeds (Weiss & Lamont 1997 and reference therein), provide protection (Armbruster 1997; Sisterson & Gould 1999) and/or enhance carbon supply (Galen, Dawson & Stanton 1993; Herrera 2005; Mokhtar & Houle 2005) during fruiting.

Several studies have reported some significant contribution of floral parts to the assimilation of carbon allocated to developing seeds and fruits (Bazzaz & Carlson 1979; Williams, Koch & Mooney 1985; Reekie & Bazzaz 1987; Galen, Dawson & Stanton 1993; Antlfinger & Wendel 1997; Herrera 2005; see Kozlowski 1992 for review). In *Ambrosia trifida*, an annual colonizer with large seeds, photosynthesis in the whole inflorescence provided up to 57% of the carbohydrate required for seed maturation (Bazzaz & Carlson 1979). Similarly, the removal of sepals of *Floerkea proserpinacoides* (Limnanthaceae) generated a decrease of 53% in fruit production (Mokhtar & Houle 2005). Not all floral structures contribute to carbon assimilation, however. Respiration by petals in *Ranunculus adoneus* generated a deficit in the carbon balance of the

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inflorescence that was compensated by the photosynthetic activity of other floral whorls (Galen, Dawson & Stanton 1993). This suggests that the production and possibly retention of showy structures for pollinator attraction represent a carbon cost that may negatively affect fruit and seed production. In this context, greening of floral parts may allow structures originally devoted to pollinator attraction to gain some photosynthetic activity and help to meet the carbon requirements of the developing fruits or seeds.

In *Dalechampia scandens* (Euphorbiaceae), a Neotropical vine pollinated by bees (Armbruster 1985), blossoms are subtended by two involucral bracts that show ontogenetic change in colour from white, during pollination, to green, during fruit maturation (Fig. 1). The bracts protect the blossoms against florivores and/or seed predators by closing at night during the sexually receptive period and by closing permanently around the developing fruits (Armbruster 1997). The green colour of the bracts during fruit maturation could be interpreted as cryptic coloration to avoid attracting seed predators or pollinators to blossoms that are no longer receptive (Armbruster 1996, 1997). Alternatively (or additionally), this greening could allow bracts to increase their photosynthetic activity and provide a local source of carbon for the developing seeds. We tested this latter hypothesis by first investigating the importance of the involucral bracts for the development of the seeds in *D. scandens*. We compared the development of seeds produced after bracts were removed, shaded or left intact. In order to assess the energy contribution of the bracts in flower, fruit and seed development, we also measured gas exchange and estimated the carbon balance of the bracts and blossoms during the flowering (white bracts) and fruiting phases (green bracts) of the blossom.

Materials and methods

STUDY SYSTEM

Blossoms of *D. scandens* comprise a pair of male and female subinflorescences subtended by two showy bracts (Fig. 1a). Three female flowers, each containing three ovules, form the female subinflorescence, producing a maximum of nine seeds. The male subinflorescence includes ten staminate flowers and a gland producing terpenoid resin collected by bees that use it in nest construction (Armbruster 1984). The blossoms are partially dichogamous. In the female phase, lasting about three days, the bracts are open and female flowers are receptive, with the male flowers remaining closed. The bisexual phase is initiated when the first (terminal) male flower opens, followed by the opening of the other male flowers in succession over a period of approximately 1 week. The plant is self-compatible, although variation in the distance between the anther and the stigma (herkogamy) affects the frequency of self-pollination (Armbruster 1988).

The colour and function of the bracts vary greatly among *Dalechampia* species (Armbruster 1996, 1997, 2002; Armbruster, Antonsen & Pélabon 2005; Bolstad *et al.* 2010). In *D. scandens*, bracts have both signalling and protective functions. By day during the receptive period, the bright white bracts are open and attract pollinators (Pérez-Barrales *et al.* 2013). At night, the bracts close to protect the flowers from florivores. After ca. 10 days, the male subinflorescence abscises, and the bracts turn green and close around the maturing fruits (Fig. 1b,c). After ca. 5 weeks (in the greenhouse), the whole blossom dries out, bracts wither and fall, the sepals of the pistillate flowers spread away from the fruits, and matured seeds are dispersed by explosive dehiscence of the capsules (Armbruster 1982).

EXPERIMENTAL DESIGN

We used the fifth-generation individuals descended from seeds originally collected from 75 maternal plants in Quintana Roo, Mexico (20°13′N, 87°26′W), and maintained as a greenhouse

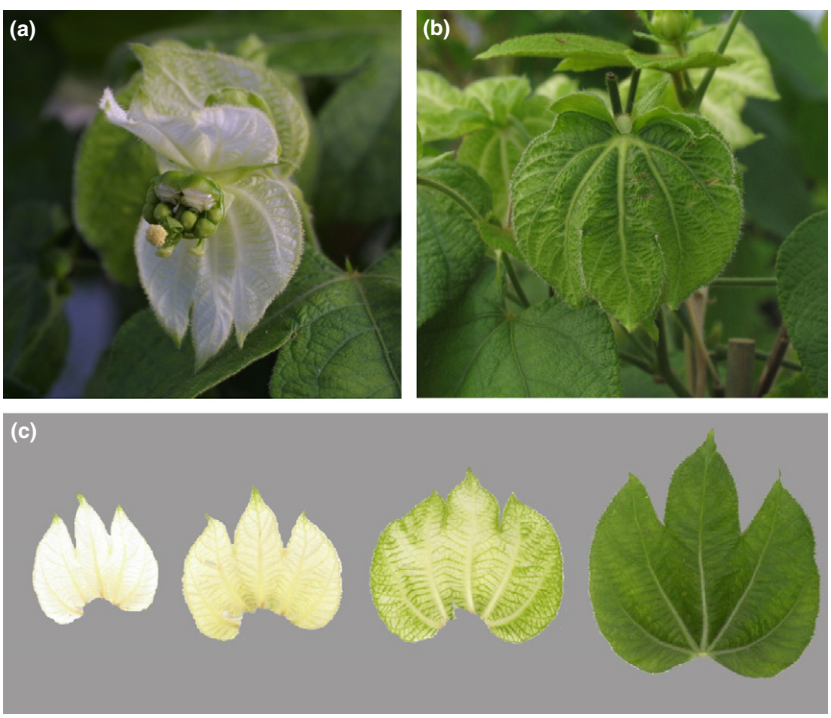

Fig. 1. Different colours and function of the involucral bracts from the *Dalechampia scandens* blossoms. (a) Blossom from the studied population at the first day of the bisexual phase (one male flower open). The upper and lower bracts are white and advertise the blossom to the pollinators. At this stage, bracts open during the day and close at night. (b) After anthesis, the bracts close around the blossom and change colour, the green colour of the bracts making the blossom very cryptic. (c) Change in the colour of the bract. The complete process of colour change from left to right takes approximately 10 days.

population by outcrossing with always more than 200 individuals per generation.

From April to June 2013, we conducted hand pollination on several blossoms per plant and exposed these pollinated blossoms to one of four treatments. We removed the whole male inflorescence from blossoms in female phase and applied pollen from a freshly opened staminate flower from one blossom of a designated 'father plant' on the stigma of each of the three female flowers. We imposed four treatments on randomly allocated blossoms on each of 39 plants: (i) *shaded bracts*: the entire pollinated blossom was enclosed between two sheets of aluminium foil with small holes made for gas exchange; (ii) *removed bracts*: both upper and lower bracts were removed by cutting them off close to their insertion points; (iii) *control for cut bracts*: both upper and lower bracts were cut at ca. 1 mm in along their edge; and (iv) *control*: blossoms were left undisturbed after pollination. For treatments (ii), (iii) and (iv), blossoms were marked with coloured yarn (one colour per treatment). Because blossom size may affect seed mass (Herrera 2009), we measured the diameter of the blossom peduncle as a proxy for blossom size before and after pollination; the average of these two measurements was used as covariate in the analysis of seed mass.

Bract removal or shading may affect either the final seed mass or the timing of seed maturation. Because the exact timing of the explosive dehiscence of the seeds may depend on microclimatic variation in humidity or temperature that are difficult to control in the greenhouse, we decided to standardize the time at which seeds were collected and we harvested all manipulated blossoms four weeks after pollination. We then stored the blossoms in paper envelopes for one week in order to promote the dehiscence of the capsules, and we weighed the seeds individually on a high-precision scale. Consequently, the recorded seed mass is closely linked to seed development, but may not necessarily represent the seed mass at maturation (see Discussion).

PHOTOSYNTHETIC ACTIVITY

To estimate the contribution of the bracts to the primary production of the inflorescence, we measured the rates of respiration (R) and photosynthesis (A) of whole blossoms before and after bract removal on a new set of four blossoms per plant, two with white bracts and two with green bracts, randomly chosen on six different plants. CO_2 exchange was measured using a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE) outfitted with a lighted conifer chamber to accommodate a full blossom. Blossoms still attached to the plant were introduced into the chamber and allowed to equilibrate in the dark for 3 min. An automated LI-6400 program recorded three net gas exchange values and then allowed the blossom to equilibrate for 3 min at 500 μmol m^{-2} s^{-1} before logging 5 more values over approximately 20 min, with chamber temperature maintained at 25 °C and using buffered ambient air throughout the measurement period. Preliminary light response curves showed the selected light level, provided by an internal LED light source, to be saturating but not inhibitory (data not shown). Immediately after this first series of measurements, we removed the bracts and repeated the measurements on the remainder of the blossom. We estimated the respiration rate (R) as the average of the three measures of gas exchange rates taken with the blossom in darkness, with net carbon efflux given as negative value. Gas exchange values in light continued to increase more or less asymptotically throughout the measurement period of 20 min. We used the maximum value obtained during this period as an estimate of the net photosynthetic rate (A_N) and calculated the gross photosynthetic rate (A_G) as the net rate of photosynthesis minus the rate of respiration: $A_G = A_N - R$. We assumed that the differences between the measurements of R and A_G before and after bract removal approximate the contribution of the bracts. We approximated daily total respiration (R_D) and gross primary production (GPP) in mol day^{-1} by assuming constant respiration rate throughout the night and day with 12 h of light at 500 μmol m^{-2} s^{-1}, giving a total daily light dose comparable to that measured on cloudy days in a tropical rainy season environment (Finch *et al.* 2004).

We also measured the respiration rate, the net photosynthetic rate and the gross photosynthetic rate of two leaves per plant on the same six plants, using the same methods. We excised and scanned all measured bracts and leaves, estimated their area using IMAGEJ (Rasband 1997) and used the results to calculate photosynthetic rates on a per area basis.

STATISTICS

We tested the effects of the treatments on seed set and seed development using mixed-effects models where identity of the maternal plants was entered as a random factor. Analyses of the effects of treatments on seed mass were performed using both peduncle diameter and seed set as covariates. Some of the seeds produced were aborted (see Results). We tested whether the number of aborted seeds was affected by the treatments or the identity of the maternal plant by comparing generalized linear models where the proportion of aborted seed was the response variable and treatment and maternal identity were the predictor variables. We used a quasi-binomial link to account for the overdispersion of the data and compared models with likelihood ratio tests. We also tested whether bract manipulation affected the within-blossom variance in seed mass by comparing a model where the variance due to the random effect was common for all treatments, with a model allowing for different variance of the random effect in the different treatments, using the *varIdent* function in the NLME package in R (Zuur *et al.* 2009).

To estimate the effect of bract colour on gross photosynthetic rates, we compared the difference in gross photosynthetic rate before and after bract removal between blossoms with green and white bracts using mixed-effects models, where bract coloration, treatment (intact or removed) and their interaction were fixed factors, and plant identity was a random factor. Finally, we compared the photosynthetic rate per area between white bracts, green bracts and leaves with mixed-effects models where photosynthetic rate per area was the response variable, the type of structure (white bract, green bract or leaf) the predictor variable and plant identity a random factor. Model selection was based on Akaike's information criterion (AIC) obtained from models fitted with maximum likelihood, and parameter estimates were obtained from the best model(s) fitted with restricted maximum likelihood. All analyses were done in R v. 2.15.2 (R Core Team 2013).

Results

EFFECTS OF BRACT REMOVAL AND SHADING

The experiment included 39 plants and the four treatments were completed on 33 of them. In two plants, all four treatments failed (no seed produced), and in four plants, the treatment with bract removal failed.

On average, blossoms produced 8·59 (±0·12) seeds, and we found no difference in blossom seed set between treatments (model with treatment as factor, AIC = 449·25; model without treatment, AIC = 445·50; see Table 1 for descriptive statistics).

Of the 1220 seeds produced, 117 (9·6%) weighted <20 mg and had either a normal seed coat without any

Table 1. Descriptive statistics for the different variables in each treatment. Coefficients of variation (CV) were estimated as the square root of the total phenotypic variance in seed mass divided by the mean seed mass

	Shaded bract	Bract removed	cut-control	Control
Total number of seeds (count)	317	295	317	291
Number of aborted seeds (count)	33	25	39	20
Mean (±SE) seed set*(count)	8·78 (±0·19)	8·44 (±0·19)	8·80 (±0·19)	8·32 (±0·19)
Mean (±SE) seed mass†(mg)	32·64 (±0·79)	33·33 (±0·79)	34·96 (±0·77)	34·67 (±0·79)
CV seed mass	12·15%	10·16%	10·10%	9·14%

*Parameter estimates from the mixed-effect model with treatment as fixed factor and plant identity as a random factor.
†Parameter estimates from the mixed-effect model with treatment and peduncle diameter as fixed factors and plant identity as a random factor.

embryo or endosperm inside, or were very small, with a whitish and empty seed coat. These were classified as aborted and removed from subsequent analyses of seed mass. We found no evidence that the proportion of aborted seeds differed among treatments (comparison of the models including vs. not including treatment: $F_{102,\ 105} = 0{\cdot}43$; $P = 0{\cdot}73$), but aborted seeds tended to be clustered in some plants (comparison of the models including vs. not including maternal plant identity: $F_{105,\ 141} = 4{\cdot}77$; $P < 0{\cdot}001$).

Seeds harvested at four weeks weighted an average of 34·48 (±0·78) mg, nearly 5 mg (14%) lighter than the seed mass recorded at full maturation in the same plants (C. Pélabon, E. Albertsen, M. Falahati-Anbaran, W. S. Armbruster, unpublished data). The diameter of the peduncle positively affected seed mass ($\beta = 13{\cdot}77 \pm 0{\cdot}99$ mg mm^{-1}), while seed set had only a weak positive effect on seed mass ($\beta = 0{\cdot}42 \pm 0{\cdot}36$ mg seed^{-1}) not supported statistically (Table 2). Blossoms with removed or shaded bracts produced seeds that were 1·34 (±0·32) mg (ca. 4%) and 2·03 (±0·32) mg (ca. 6%) lighter, than those produced in the control treatment, respectively (see Table 1 for descriptive statistics and Table 2 for model selection). Seeds from the cut-control treatment were on average 0·29 (±0·32) mg heavier than those from the control treatment, but the difference was not statistically significant. Within-blossom variance in seed mass was highest in the shaded-bract treatment (Table 1) as indicated by the better fit of the mixed-effect model, where treatment-specific variances for the random term were estimated (AIC = 5987·3), as compared to the model including only one term for the random variance (AIC = 6005·6).

Table 2. Model selection for testing the effect of the treatment, peduncle diameter and blossom seed set on the seed mass four weeks after pollination. Treatment represents the manipulation of the blossom (shaded, removed, cut-control and control). Plant identity (plant ID) is included as a random factor in all models

Models	AIC	AIC weights
Peduncle + seed set + treatment + (plant ID)	6006·35	0·42
Peduncle + treatment + (plant ID)	6005·72	0·58
Peduncle + seed set + (plant ID)	6070·67	0·00
Seed set + (plant ID)	6221·11	0·00
Peduncle + (plant ID)	6070·15	0·00
Constant + (plant ID)	6222·85	0·00

PHOTOSYNTHETIC ACTIVITY OF THE BRACTS

Removing white bracts had only a limited effect on the gross photosynthesis of the whole blossom, while the same manipulation of blossoms with green bracts dramatically decreased the gross photosynthesis of the blossom (Fig. 2). This was confirmed by the better fit of the model including an interaction term between bract manipulation and bract colour (Table 3). Furthermore, the photosynthetic rate per area strongly differed between white bracts, green bracts and leaves (models including the type of structure, AIC = 92·0; model without the type of structure, AIC = 167·2). Although green bracts showed a photosynthetic rate higher than that of the white bracts (mean $A_G \pm$ SE of green bracts: $1{\cdot}47 \pm 0{\cdot}23$; white bracts: $0{\cdot}42 \pm 0{\cdot}24$ μmol m^{-2} s^{-1}), these rates were much lower than those measured on leaves (A_G leaves: $5{\cdot}80 \pm 0{\cdot}25$ μmol m^{-2} s^{-1}).

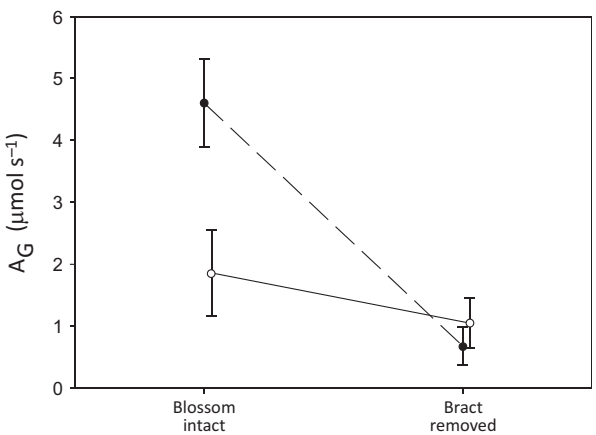

Fig. 2. Effects of bract removal on the gross photosynthetic rate (A_G) of *Dalechampia scandens* blossoms with white (open dots) and green bracts (black dots). The mean (± SE) gross photosynthetic rate (in μmol of C s^{-1}) is presented for intact blossoms and blossoms with bracts removed.

Table 3. Model selection for testing the effect of the bract coloration and bract removal on the gross photosynthetic rate. Colour represents the colour of the bract (white or green) and treatment the manipulation (intact or removed). Plant identity is always included as a random factor in the models (plant ID). Interaction effects are denoted with '×'

Models	AIC	AIC weights
Colour + treatment + colour × treatment + (plant ID)	320·92	1
Colour + treatment + (plant ID)	359·85	0
Colour + (plant ID)	391·84	0
Treatment + (plant ID)	372·55	0
Constant + (plant ID)	397·47	0

While blossoms with white bracts had detectable but low mean gross photosynthetic rate (A_G) in both bracts and flowers, mean respiration rate (R) in both parts of the blossom was large enough to offset photosynthesis, resulting in a net negative carbon balance for the white-bracted blossom as a whole (Fig. 3a). In green bracts, A_G was nearly five times that of white bracts, while A_G in flowers and R in both parts decreased after greening. In both cases, the respiratory demand of the inflorescence was about twice that of the bracts. Consequently, A_G in whole green-bracted blossoms more than compensated for R, resulting in a surplus of fixed carbon available for storage or export. Because the green floral parts are shaded by the bracts in closed blossoms, it is likely that floral A_G in intact blossoms is less than that shown in Fig. 3, with proportionately more of the total A_G occurring in the green bracts. Our preliminary estimates of the daily carbon budget based on these measurements indicate that photosynthesis in the bracts in moderate light should be more than adequate to meet the 24-h respiratory demand of the whole blossom, while white blossoms may require substantial energy influx from other parts of the plant (Fig. 3b).

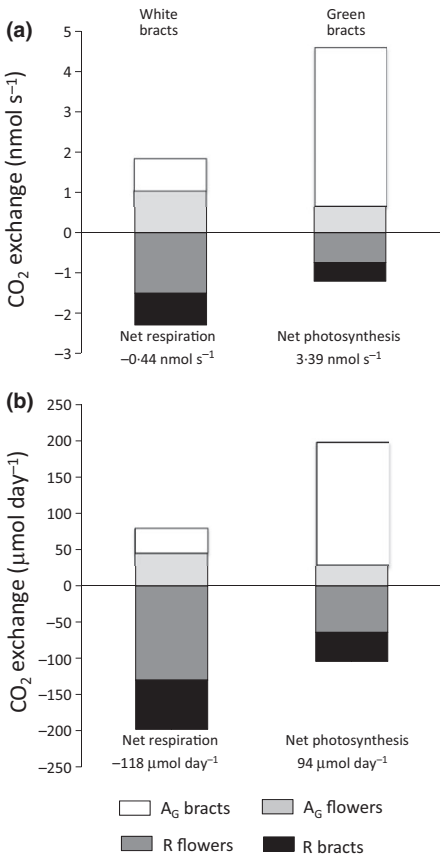

Fig. 3. (a) CO_2 exchange in blossoms of *Dalechampia scandens* with white (left) and green (right) bracts. The respiration and photosynthetic rates are given for the bracts and the rest of the blossom (flowers) separately. In (b), we translated the rate into µmols of C fixed per day by summing-up the effects of the respiration over 24 h and of the photosynthetic rate over 12 h daylight on carbon exchange. The net respiration and net photosynthesis presented below each diagram represent the carbon balance, negative for blossoms with white bracts and positive for blossoms with green bracts.

Discussion

Photosynthetic activity of reproductive structures could provide local sources of energy to offset the costs of reproduction associated with flower development and fruit maturation (Williams, Koch & Mooney 1985; Reekie & Bazzaz 1987; Whiley, Schaffer & Lara 1992; Galen, Dawson & Stanton 1993; Herrera 2005; Sunmonu, Ida & Kudo 2012; see Obeso 2002 and Aschan & Pfanz 2003 for review). In this context, floral greening may increase the photosynthetic rate of floral parts and increase the local production of photosynthate to decrease the costs of reproduction (Salopek-Sondi *et al.* 2000; Salopek-Sondi 2002). Accordingly, our manipulation of involucral bracts preventing their photosynthetic activity during the period of seed maturation negatively affected seed development. Additionally, bract greening after anthesis was correlated with an increase in photosynthetic capacity, although the photosynthetic rate remained much lower than that of leaves on a per area basis. Our rough estimates of daily respiration and gross photosynthesis indicated that green bracts substantially contributed to the energy needs of developing seeds and fruits in the blossom, while blossoms during the sexual phase required energy inputs from the rest of the plant. These results support the hypothesis that bract greening during the period of fruit maturation offsets the carbon costs imposed by the developing seeds.

Although photosynthesis in reproductive structures could meet between 2·3% and 64·5% of their respiratory demands, positive net photosynthetic rate is rarely observed (Obeso 2002). Accordingly, we found that during the period of anthesis, when bracts were white, the net photosynthetic rate of the blossom as a whole was negative. After greening, the photosynthetic rate of the bracts strongly increased, while at the same time, the respiration in other parts of the flower decreased. This generated a positive net photosynthetic rate for the post-pollination blossom (Fig. 3) which contrasted with the decrease in carbon assimilation rate generally observed at the level of the

flower during fruiting (Williams, Koch & Mooney 1985; Antlfinger & Wendel 1997; Sunmonu, Ida & Kudo 2012). We measured the photosynthetic rate in blossoms with green bracts that were in the early phase of seed maturation (within two weeks after anthesis) without further controlling for the time after anthesis or the number of developing seeds. It is therefore possible that the estimated rates of gas exchange are not representative of the whole period of seed maturation and that later during fruit development, the energetic costs of developing seeds exceed the input from the bracts. However, bracts continue expanding during the period of seed maturation and their absolute photosynthetic rate most likely increases during this period. Furthermore, it is possible that the carbon demand of the blossom during anthesis is particularly high in *D. scandens* due to production of resin, a blend of oxygenated triterpenes (C_{30} molecules, hence expensive in carbon terms) (Armbruster 1997; Pélabon *et al.* 2012). Regular measurements during the whole blossom life would be particularly useful for better understanding the dynamics of the carbon balance of the blossom as a whole and the exact contribution of the bracts to carbon assimilation.

The photosynthetic rate in green bracts was much lower than in leaves. Despite being serially homologous organs (Hansen, Pélabon & Armbruster 2007), bracts and leaves in *D. scandens* still differ in several aspects. Bracts show strong canalization against environmental variation in nutrient availability (Pélabon, Armbruster & Hansen 2011) and do not undergo 'adaptive wilting' at midday during pollination peak or under water stress, as do leaves (Pélabon & Armbruster pers. obs.). These characteristics, possibly mediated by lower stomatal density (for wilting), may have evolved at the expense of gas exchange ability and photosynthetic capacity (see also Aschan *et al.* 2005).

The decrease in seed mass when bracts were shaded or removed (4% and 6%, respectively) suggests that the overall contribution of bract photosynthesis for the seed development remains limited or that the plant is able to compensate for the negative effects of the treatments by reallocating photosynthate to the deficient blossoms. Because blossoms were harvested ca. one to two weeks before complete seed maturation (see Methods), the exact effects of the treatments on seed mass at maturation remain unknown. One possibility is that the impact of bract manipulation would be similar or stronger at full maturation since ca. 14% of the mass gain was still to be achieved. Alternatively, it is possible that blossoms compensate the loss of energy intake by longer maturation time and that seed mass at maturation remains unaffected by the treatment, but maturation takes longer when bracts are removed or shaded. Seed mass in *D. scandens* positively affects seedling survival and early growth (Pélabon *et al.* 2005). Therefore, the bracts' contribution to seed maturation may positively affect the fitness of the plant via either an effect on seed mass at maturation or an effect on the duration of maturation.

In species where photosynthesis in fruits strongly contributes to seed development, variation in seed size should decrease due to the decrease in within-fruit competition for resources (Bazzaz, Carlson & Harper 1979; but see Michaels *et al.* 1988). Within-blossom variation in seed mass increased in the treatment where blossoms were shaded, but not in the treatment where bracts were removed. This suggests that the carbon fixation by the bracts may effectively allow blossoms to produce seeds of more constant size, but also that photosynthesis in the fruit (when bracts were removed) may have a similar effect (Michaels *et al.* 1988). However, it is likely that fruit photosynthesis remains limited because the seed mass reduction in the bract removal treatment was nearly equivalent to the reduction observed with a complete shading of the blossom. Alternatively, the increase in within-blossom variation in seed mass may result either from the extra energy demand generated by the respiration of the shaded bracts, or from the microclimate generated by the bag that surrounded the blossom.

Bract persistence into the fruiting stage appears to be adaptive, at least in part because it contributes to the carbon budget of the developing fruits. Macroevolutionary patterns are also consistent with this interpretation. Phylogenetic analyses indicated that bract persistence in *Dalechampia* has evolved at least twice and probably more times (Armbruster 1997; Armbruster, Lee & Baldwin 2009). There is evidence that persistent bracts, once gained, have been lost only once (in the lineage leading to *D. schippii*), with retention of this trait in dozens of lineages. This is arguably consistent with the hypothesis that persistent bracts are adaptive. Also, in all these lineages, once bracts evolved persistence, they quickly evolved the ability to turn green after pollination, and there have been no reversals in this trait. These evolutionary trends together suggest that persistent green bracts are advantageous.

The greening of the involucral bracts after anthesis in *D. scandens* increases their photosynthetic capacity and helps blossoms meet the carbon demands of the developing seeds. This supports the hypothesis that post-pollination colour change in reproductive structures can be an adaptation to decrease the cost of reproduction (Salopek-Sondi *et al.* 2000; Salopek-Sondi 2002). This adds to the already complex functions of the involucral bracts in *D. scandens* and implies that these structures experience multiple sources of selection. It also illustrates how the interplay between development and physiology can allow leaf-like structures to evolve new functions in flowering plants.

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Data accessibility

Data are deposited in the Dryad Digital Repository: doi: 10·5061/dryad.nb675 (Pelabon *et al.* 2015).

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