

Pollination biology of *Bergeranthus multiceps* (Aizoaceae) with preliminary observations of repeated flower opening and closure

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Little is known about pollination of the Aizoaceae (Mesembryanthemaceae). There are sparse reports of generalist pollination in the family by a variety of insects (predominantly bees). Furthermore, most species are self-incompatible in cultivation. In this study, observations were made on two populations of *Bergeranthus multiceps* (Salm-Dyck) Schwantes growing in the Eastern Cape province of South Africa. Insects visiting the flowers were collected and examined for pollen. While 79 individual insects (in 24 genera representing 14 families and four orders) were collected visiting the flowers, the majority (43 individuals) were female *Allodapula variegata* bees (Apidae, subfamily Xylocopinae, tribe Allodapini) collecting pollen. All other bee visitors were also female, suggesting pollen collection as the primary activity at the flowers. The protandrous flowers were found to be self-incompatible, pointing to the importance of bee-mediated xenogamy in this species. The flowers of *B. multiceps* are bright yellow in the human visual spectrum. In addition, the petals of this species reflect ultraviolet light. In contrast, the yellow anthers absorb UV. Flower opening and closing is common in the Aizoaceae. Interestingly, in *B. multiceps* flowers open at about 15:30 and remain open for approximately three hours before closing again in the late afternoon. These afternoon flower opening events were found to be closely correlated to ambient temperatures above 23°C, relative humidity lower than 50% and vapour pressure deficit below 1.05 kPa measured from as early as 09:00 on the days when flowers opened.

Introduction

Little is known about the pollination of Aizoaceae (including Mesembryanthemaceae), estimated to be the second largest plant family in South Africa and an important component of the flora of the arid areas of this country.¹ The predominance of bright, showy petals and the presence of functional nectaries do, however, suggest insect pollination as the primary pollen vector.^{2,3} Chesselet *et al.*⁴ discuss the role of floral nectaries in pollination within the family and note the importance of nectar as an attractant to insects. The abundant pollen complements of many species also support the view that insect pollination is common in the family as pollen is an important reward for many insects.⁵ However, Bittrich⁶ suggests that abundant pollen might be an adaptation for wind pollination, although both Chesselet *et al.*⁴ and Ihlenfeldt³ indicate that this requires further investigation. Ihlenfeldt³ also notes that many flowers within the Aizoaceae attract a wide spectrum of floral visitors and that most species are protandrous and self-sterile. Gess^{7,8} reports that the general distribution of the Aizoaceae coincides markedly with that of the Afrotropical masarine wasps. Furthermore, she

reports that the main flowering season of the family in the Western Cape province coincides with the peak flight period of the masarines. Struck (cited in ref. 7), however, recorded a wide range of generalist visitors to Aizoaceae in the Goegab Nature Reserve in Namaqualand. The available data on pollination of Aizoaceae (ref. 7 and references therein; also refs 3, 8–10) is restricted to the arid and semi-arid winter rainfall areas of the western part of southern Africa, which is considered to be the ancestral distribution for the family.¹

Hammer¹¹ reports that flowers of the Aizoaceae last for about a week on average and are maximally receptive on the fourth or fifth day. Flowering time is well synchronized within populations of Aizoaceae, the period of flowering is usually short and the repeated opening of flowers is usually restricted to a certain period of the day.² Groen and Van Der Maesen¹² have observed mixed populations of *Bergeranthus* Schwantes, *Faucaria* Schwantes and *Orthopterum* L. Bolus flowering simultaneously and suggest that this could indicate a combined pollinator attraction by flower synchrony.

As has been reported for other genera in the family Aizoaceae,^{2,11} *Bergeranthus* flowers are protandrous. The stigma, which are at first shorter than the stamens, later elongate, spread and finally recurve, at which point they become receptive on the 4th or 5th day (S. Hammer, pers. comm.) and the flowers enter their female phase. Hartmann² describes four broad pollination syndromes in the Aizoaceae, of which *Bergeranthus* flowers are described as melittophilous (bee pollinated). This group is characterized by open flowers, the presentation of large quantities of pollen, hidden nectaries and diurnal opening of bright shiny petals. The nocturnal, fragrant flower of *B. artus*, however, has characters described for phalaenophilous (moth pollinated) flowers.²

Repeated opening and closing of flowers of the Aizoaceae has been previously documented.^{2,3} However, surprisingly little is known about flower opening and closing in general, and the repetitive opening and closing of a single bloom in particular. Van Doorn and van Meeteren¹³ recently reviewed this limited literature.

The very limited data on the pollination biology of a few species within the Aizoaceae come from the southwestern parts of South Africa. Here we report on: 1) observations of the pollination biology of *Bergeranthus multiceps* (*sensu* Dold¹⁴), a species restricted to the Albany Centre of Endemism;^{15,14} and 2) the abiotic cues that are correlated with flower opening.

Materials and methods

The study species

Bergeranthus multiceps (Salm-Dyck) Schwantes is a low-growing, perennial, clump-forming leaf succulent with a thickened rootstock and triquetrous lanceolate leaves. The cyme of bright yellow diurnal flowers opens in the mid-afternoon and closes at sunset (Fig. 1A). The lifespan of individual flowers is approximately six days, and they open and close each day if conditions are suitable. Flowering of *B. multiceps* peaks between November

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and January but appears linked to rainfall, with flowering as early as August and as late as April (pers. obs.).

The species is widespread in the Eastern Cape from Joubertina in the west to the Keiskamma River in the east and as far north as Michell's Pass near Hogsback, and occurs in a wide range of habitats across seven biomes at altitudes from 20 to 1520 m.¹⁴

Breeding system

As *B. multiceps* has a low, cushion-like growth form, whole plants with unopened buds were bagged with small gauze bags, which were pegged into the ground around the plants using tooth-picks. The buds were then monitored until the flowers opened, whereupon the pollen was removed from the flowers using cotton buds and stored in Eppendorf tubes. The tubes were kept open and refrigerated at ~6°C for three to five days until the stigmas matured. The pollen was then used either to self-pollinate or cross-pollinate flowers on the various plants. Additional flowers were left untreated to test for autogamy (spontaneous self-pollinations) and agamospermy (asexual production of seeds).

Flower colour

Flowers were photographed using an SLR camera with a 100-mm macro lens and a B + W 403 black filter that transmits light primarily between 300 and 400 nm. Konica VX 400 black and white film (sensitive from about 360 to 700 nm) was used and so effectively exposed the film to light reflected from the flowers between 360 and 400 nm. A suitable greyscale was used to judge for correct exposure.¹⁶ Control photographs were taken as above, but without the B + W 403 black filter. For all photographs, the flowers were illuminated constantly during exposures using two 12-V spotlights.

Observations of insect visitors

Two populations of *Bergeranthus multiceps* were visited during November and December 2000 and January 2001, to observe and document insect visitation. One population comprised approximately 20 plants within 8 m² of open rocky grassland on Grahamstown aerodrome flats (represented by *Dold 4186* in GRA), and the second population comprised approximately 30 plants over ~18 m² of open rocky grassland at Thomas Baines

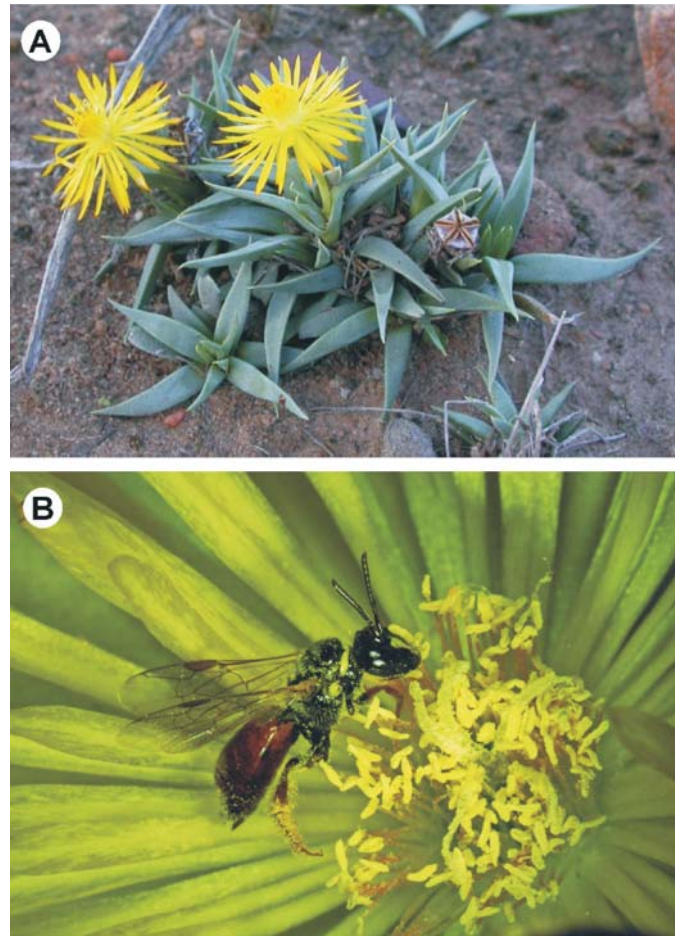


Fig. 1. A, *Bergeranthus multiceps* with open flowers in late afternoon (approximately 16:30); **B,** *Allodapula variegata* visiting *B. multiceps*. Note the considerable pollen loads on the legs, abdomen and thorax of this bee.

Nature Reserve, 10 km southwest of Grahamstown (*Dold 4203* in GRA). Our observations coincided with flower opening times (15:30 to 18:30) on clear, windless, warm days during the peak flowering season and represent approximately 33 hours of observations.

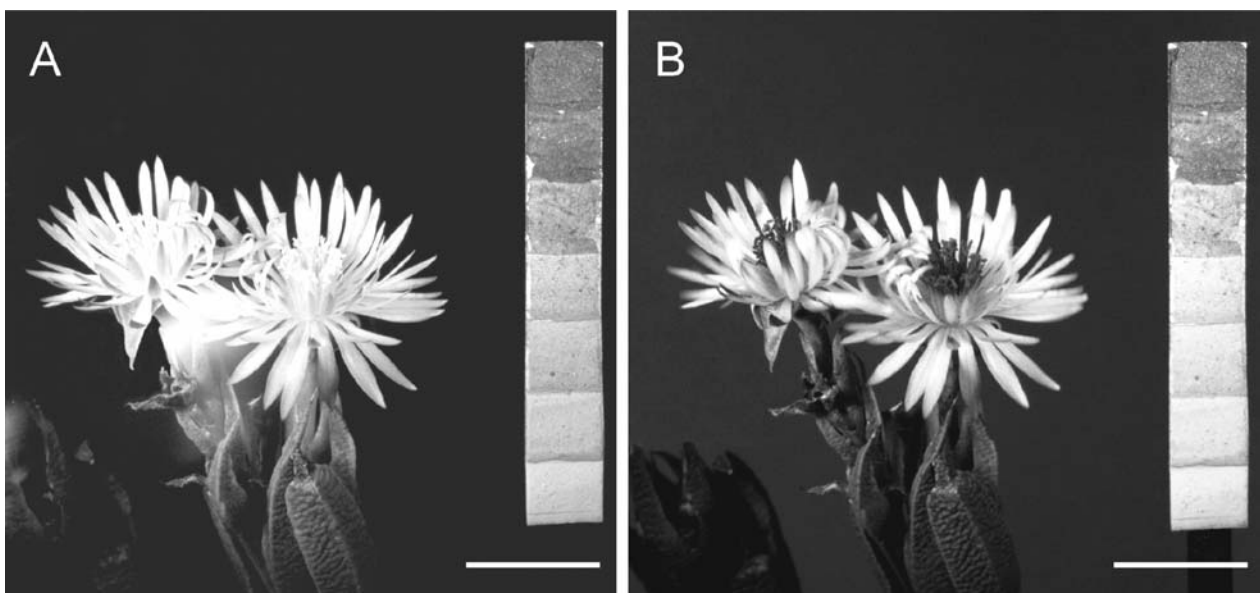


Fig. 2. Appearance of *Bergeranthus multiceps* flowers in **(A)** the human visual spectrum (400–700 nm) and **(B)** in the near-UV region of the spectrum (~360–400 nm). Scale bars = 10 mm.

Visiting insects were collected and subsequently identified by the staff of the Albany Museum Entomology Department. Many more insects were observed visiting flowers than were caught. However, only specimens so collected have been included in this investigation. Insects were inspected for pollen loads using fuschin gel.¹⁷ In addition, scanning electron microscopy was used to examine pollen deposited on the limbs of *Allodapula variegata* bees caught visiting the flowers of *B. multiceps*.

Flower opening

The seasonal flowering period was observed in the field over a period of three years. Forty plants were collected and grown under natural conditions in Grahamstown. Voucher specimens are housed in the Selmar Schonland Herbarium (GRA). The daily flower opening/non-opening events of the potted plants were monitored over a period of 42 days during the peak of the flowering season of these plants. Temperature and relative humidity measurements were made at hourly intervals and the state of the flowers (open or closed) was recorded. In addition, vapour pressure deficit (VPD) was calculated following Peter and Ripley.¹⁸ Environmental data collected on days on which flowers opened were compared to data from days on which the flowers remained closed, using a simple one-way ANOVA.

While this study focused on *B. multiceps*, we also observed an additional 170 potted individuals from eight other day-flowering *Bergeranthus* species at the same time and exposed to the same conditions. The data presented in Fig. 4, however, refers only to the *B. multiceps* plants.

Results

Breeding system

None of the self-pollinated flowers produced capsules ($n = 12$), whereas 50% of the out-crossed flowers produced functional capsules ($n = 10$). Six additional untreated but bagged flowers also failed to produce capsules, pointing to a likely absence of autogamy or agamospermy.

Flower colour

The flowers of *B. multiceps* are bright yellow to the human eye (Fig. 1A). However, photographs of the flowers taken in the spectral range of 360–400 nm indicate that the petals are UV reflective, while the centres, primarily the anthers of the flowers, absorb UV (Fig. 2B).

Flower visitors

In total, 79 insects representing 14 families and four orders were collected (Table 1). It is clear from the range of taxa collected over a relatively short period that *B. multiceps* attracts a broad spectrum of visitors. However, 66% ($n = 52$) of all the visitors collected belong to the order Hymenoptera, of which 83% ($n = 43$) are *Allodapula variegata* (being 54% of the total number of visitors). All *A. variegata* were female. Of the Hymenoptera, the remaining 17% ($n = 9$) belong to the genera *Lasioglossum*, *Amegilla*, *Megachile*, and *Ceratina*, all of which were also female (Table 1).

All the bees examined had considerable pollen loads. *A. variegata* bees, the most frequent visitors to the flowers, had on average 601 grains of *B. multiceps* pollen (range 89 to 2512) and only two pollen grains (range 0–5) from other taxa ($n = 15$). The three *Amegilla* individuals also had substantial pollen loads, as did a single *Megachile* species (Table 2). The three individuals of *Lasioglossum* and the single *Ceratina* bee had smaller pollen loads (Table 2).

Table 1. Insects collected visiting *Bergeranthus multiceps* flowers.

Order COLEOPTERA (beetles)
Family Chrysomelidae
Genus indet.
species indet. A × 4 specimens
Genus indet.
species indet. B × 1 specimen
Family Meloidae (blister beetles)
Genus indet.
species indet. A × 2 specimens
Family Scarabaeidae
Subfamily Melolonthinae
Tribe Hopliini (monkey beetles)
Genus indet. × 1 specimen
Family indet. × 3 specimens
Order DIPTERA (flies)
Family Bombyliidae (bee flies)
Genus indet. × 3 specimens of 3 unidentified taxa
Family Syrphidae (hover flies)
Genus <i>Metasyrphus corollae</i> (Fabricius) × 1 specimen
Genus <i>Ischiodon aegyptius</i> (Wiedermann) × 1 specimen
Genus indet.
species indet. A × 2 female specimens
Genus indet.
species indet. B × 1 specimen
Genus indet.
species indet. C × 1 specimen
Family Tachinidae
Genus indet.
species indet. A × 1 specimen
species indet. B × 1 specimen
Order HYMENOPTERA (bees, wasps, ants)
Family Halictidae
Subfamily Halictinae
Tribe Halictini
Genus <i>Lasioglossum</i> species A × 1 female specimen
Genus <i>Lasioglossum</i> species B × 1 female specimen
Genus <i>Lasioglossum</i> species C × 1 female specimen
Genus <i>Lasioglossum</i> species D × 1 female specimen
Family Megachilidae
Subfamily Megachilinae
Tribe Megachilini
Genus <i>Megachile</i> (<i>Eutricharaea</i>) <i>semiflava</i> (Cockerell) × 1 female specimen
Family Apidae
Subfamily Xylocopinae
Tribe Ceratinini
Genus <i>Ceratina</i> (<i>Ctenoceratina</i>) <i>pencilata</i> Friese × 1 female specimen
Tribe Allodapini
Genus <i>Allodapula variegata</i> (Smith) × 43 female specimens
Subfamily Apinae
Tribe Anthophorini
Genus <i>Amegilla punctifrons</i> (Walker) × 1 female specimen
Genus <i>Amegilla spilostoma</i> (Cameron) × 2 female specimens
Order LEPIDOPTERA (Butterflies, moths)
Family Nymphalidae
Genus <i>Vanessa cardui</i> (Linnaeus) (painted lady) × 1 female specimen
Family Lycaenidae
Genus <i>Freyeria trochylus</i> (Freyer) (grass jewel blue) × 1 specimen
Family Pieridae
Genus <i>Pontia helice</i> (Linnaeus) (meadow white) × 1 specimen
Family Hesperidae
Genus <i>Gegenes niso</i> (Linnaeus) (common Hottentot skipper) × 2 male specimens

Table 2. Pollen loads of both *Bergeranthus multiceps* pollen and pollen from other taxa, counted from a sample of insect visitors collected on *B. multiceps* flowers. Unless otherwise indicated, $n = 1$.

Species	<i>B. multiceps</i> pollen loads	Other taxa pollen loads
HYMENOPTERA		
<i>Allodapula variegata</i> ($n = 15$)	$\bar{x} = 601.1^*$	$\bar{x} = 1.6^*$
<i>Amegilla punctiferous</i>	113	16
<i>Amegilla spilostoma</i> ($n = 2$)	469	62.5
<i>Megachile semiflava</i>	234	0
<i>Lasioglossum</i> sp. 3	109	7
<i>Lasioglossum</i> sp. 2	3	7
<i>Lasioglossum</i> sp. 1	17	3
<i>Ceratina penicullata</i>	9	3
DIPTERA		
Bombyliidae sp. 1	2	5
Bombyliidae sp. 2 ($n = 2$)	$\bar{x} = 0.5$	$\bar{x} = 1$
<i>Metasyrphus corollae</i>	136	4
<i>Ischiodon aegyptius</i>	1	0
Tachinidae sp. 1	3	5
Tachinidae sp. 2	1	1
Syrphidae sp. 1 ($n = 2$)	$\bar{x} = 78$	$\bar{x} = 11.5$
Syrphidae sp. 2	1	2
Syrphidae sp. 3	8	0

*Range 89–2512; *range 0–5.

Fourteen per cent ($n = 11$) of the insects visiting *B. multiceps* belong to the order Diptera, 14% ($n = 11$) to the Coleoptera (primarily flower-feeding beetles) and 6% ($n = 5$) to the Lepidoptera. The majority of the flies had insignificant pollen loads. Only a species of syrphid and an individual *Metasyrphus corollae* had significant loads of *B. multiceps* pollen (Table 2).

Allodapula variegata, being the most frequent flower visitor, is most likely to be the primary pollinator of *Bergeranthus multiceps* (Fig. 1B). Field observations attest to this bee being extremely fast, moving rapidly from one flower to the next and collecting pollen that is stored on the legs. Some of this collected pollen is probably unavailable for pollination (Fig. 3A,B). However, there is abundant pollen scattered over the entire body, and this is potentially available for pollination (Figs 3C, 1B).

Flower opening

In *B. multiceps*, flowers were observed to open between 15:30 and 16:00 and to close by 18:30. Flower opening was correlated with ambient temperature, relative humidity and vapour pressure deficit (Fig. 4). At 09:00, if relative humidity dropped below $59 \pm 16\%$, temperature rose above $20.6 \pm 3.6^\circ\text{C}$ and vapour pressure deficit increased above 1.05 ± 0.66 kPa, then the flowers opened later that afternoon. In all cases, relative humidity stayed below this threshold for the rest of the morning and into the afternoon for the flower-opening period (15:30 to 18:30). Similarly, temperature and VPD measurements stayed above the key thresholds of 20.6°C and 1.05 kPa. Thus it is apparent that although the flowers open only in the late afternoon, the conditions to which flower opening was correlated were significantly different as early as 09:00 on the day of flower opening (Fig. 4).

All other species of *Bergeranthus* except for *B. katbergensis* and *B. artus* showed the same response. In *B. katbergensis*, the timing of flower opening is similar, except that it occurs earlier in the day, from approximately noon, and the flowers close again by mid-afternoon. *B. artus* opens after dark and closes again at approximately midnight.

Discussion

Data presented here add to a meagre body of knowledge about the pollination biology of the Aizoaceae, a large family (approximately 1800 species¹) of crucial importance to the flora of the arid areas of South Africa. *Bergeranthus multiceps* is visited by a suite of different insects but the incidence of female bee visitors suggests that pollen collection is their primary activity at the flowers. While minute quantities of nectar have been observed, the volumes are too small to measure (less than $0.02 \mu\text{l}$). Of the female bees, *Allodapula variegata* females were by far the most frequent visitors and carried *B. multiceps* pollen almost exclusively, indicating that at this site and time of year the bees are specializing on the flower of *B. multiceps*. We found no masarine wasps visiting the flowers of *B. multiceps* in contrast to the results of Gess^{7,8} working on various other species of Aizoaceae and

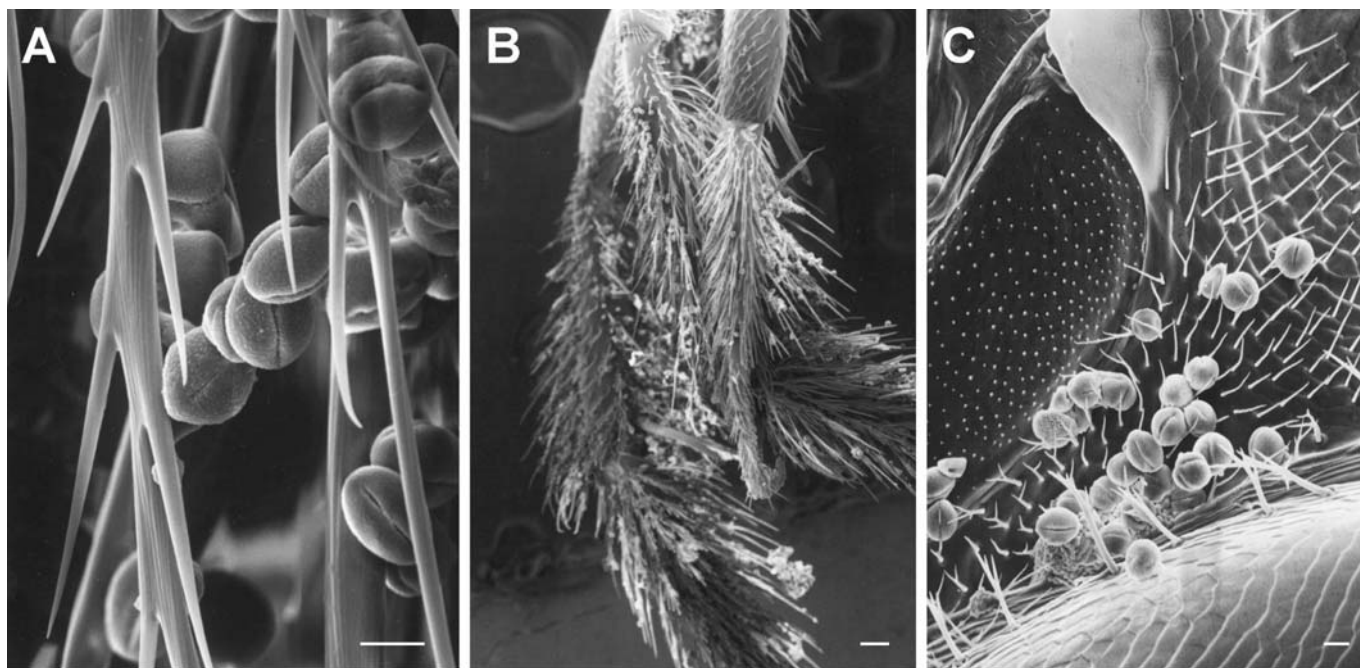


Fig. 3. *Bergeranthus multiceps* pollen on *Allodapula variegata*. **A**, Pollen on upper hind leg (scale bar = 10 μm); **B**, pollen collected on hind legs for transportation to nest (scale bar = 100 μm); **C**, pollen on thorax available for pollination (scale bar = 10 μm).

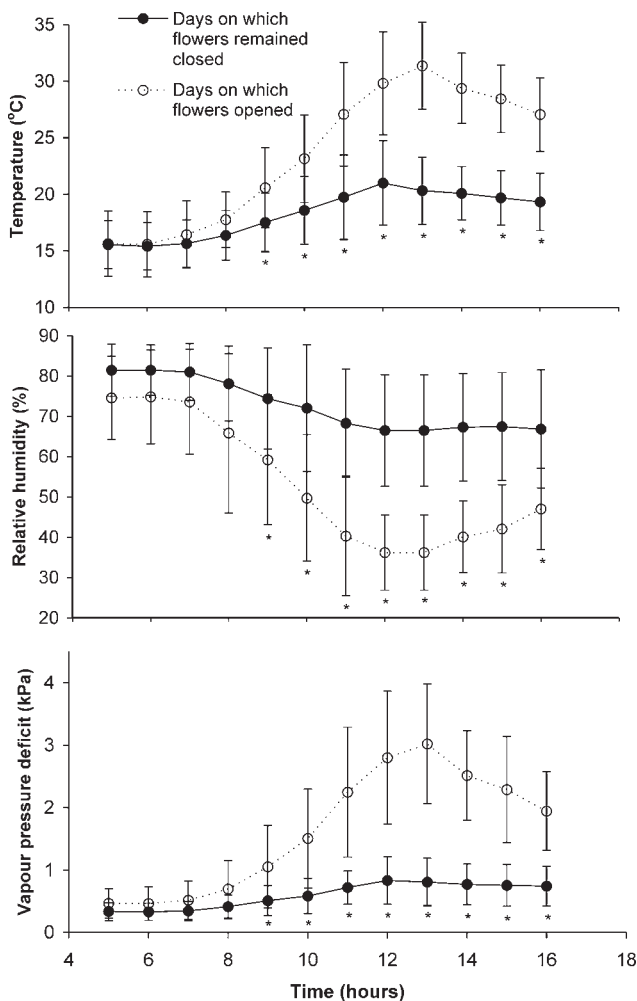


Fig. 4. Mean values of ambient temperature, relative humidity and vapour pressure deficit recorded at set times (over 42 consecutive days) correlated to *Bergeranthus multiceps* flower opening/non-opening events (bars represent standard deviations from the mean). Asterisks indicate those parameters that differ significantly (at the 95% confidence level) between days on which flowers opened and days on which flowers remained closed.

other taxa. Other visitors to *B. multiceps* flowers include beetles (almost exclusively destructive, flower-feeding species), butterflies (nectar-foraging insects) and a few nectar- or nectar-and-pollen-feeding flies (Bombyliidae, Tachinidae and Syrphidae). The majority of these flies had small pollen loads.

While the flowers of *B. multiceps* are bright yellow to humans, in the UV region of the spectrum there is marked contrast between the UV-reflective petals and the UV-absorbent anthers. A number of other species with pollen-rich flowers in the Asteraceae are also frequented by female *A. variegata* bees (C.I. Peter, unpubl. data; R.J. McKenzie, S.K. Gess and F.W. Gess, pers. comm.) and have similar colour patterns with UV-absorbent disk florets (C.I. Peter, unpubl. data), which suggests possible convergence.

Our breeding system experiment conducted on wild-growing plants confirms the experience of horticulturists.¹¹ Only plants receiving xenogamous pollen produced capsules. We also rule out autogamy and agamospermy. This experiment also indirectly precludes wind pollination as the large pore size of our bags would not have excluded wind-blown pollen grains as shown by Neal and Andersson.¹⁹ Other evidence including bright petals, the presence of nectaries and the abundant flower visitors, also indicates that Bittrich's⁶ suggestion of wind pollination is unlikely, at least in *B. multiceps*. Afternoon foraging female

bees, primarily *A. variegata*, are therefore the most important pollinators of *B. multiceps*. This supports Hartmann's² hypothesis, based on observation of flora characters, that *B. multiceps* belongs to the bee-pollinated (melittophilous) syndrome.

The second intriguing question relates to the cues, mechanisms and evolutionary reasons behind the late afternoon opening and subsequent closure of the flowers of *B. multiceps*. Our preliminary data support the idea that temperature and relative humidity (combined as vapour pressure deficit) are probably important cues determining flower opening on a particular afternoon. We do not consider light, which has been shown to be an important cue in other studies.^{13,20}

Van Doorn and van Meeteren¹³ reviewed endogenous and exogenous factors affecting the repeated opening and closing of single flowers. What is of interest to us is that the exogenous factors to which flower opening was correlated were significantly different many hours before the actual opening events on opening and non-opening days. Further experimentation requires more controlled conditions to tease out the relative importance of these different variables and to determine causal relationships. Interestingly, previous studies have not considered vapour pressure as an important exogenous factor driving flower opening and closure. This is despite VPD inducing water loss from plants²¹ and the fact that plants are known to respond to VPD.²²

At this stage we can only speculate on the mechanisms behind the rapid movement of petals during flower opening and closure. A one-minute photographic exposure recorded petal movements of approximately ten degrees. Given this rapid movement, some of the mechanisms reviewed by van Doorn and van Meeteren¹³ can be ruled out. Differential cell growth and cellular death in specific parts of the petals are unlikely, given the rate of petal movement and the fact that these movements occur many times over the lifespan of a particular flower.

Flower opening and closure in the Aizoaceae, primarily the groups included in the subfamily Mesembryanthemoideae, is common³ and may well represent an ancestral condition. This behaviour also accounts for the common name of this subfamily — 'midday flowers' — as well as the original name, 'Mesembryanthemaceae', coined by Breynia in 1689.¹ It is therefore not so much the fact that flowers show repetitive opening that is interesting, but the specific timing of these events in the late afternoon that requires an explanation. It seems likely that this is a mechanism to filter out generalist pollinators most active at midday, rather targeting specific group of insects, primarily *Alloeadapula variegata* bees, still active in the late afternoon.

In addition, we speculate that *Bergeranthus* flowers (with the exception of the nocturnal *B. artus*) remain closed at low ambient temperature and vapour pressure as a mechanism to protect the pollen from moisture. It is well known that pollen fertility in most plants is negatively affected through contact with water⁵ and this has been tested specifically for species of Aizoaceae, including *Bergeranthus* (ref. 11; S. Hammer, pers. comm.). This may be a mechanism to protect pollen from water on cool humid days and from dew at night.

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