

Facultative CAM photosynthesis (crassulacean acid metabolism) in four species of *Calandrinia*, ephemeral succulents of arid Australia

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Received: 12 October 2016 / Accepted: 14 February 2017 / Published online: 4 September 2017
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Abstract Crassulacean acid metabolism (CAM) was demonstrated in four small endemic Australian terrestrial succulents from the genus *Calandrinia* (Montiaceae) viz. *C. creethiae*, *C. pentavalvis*, *C. quadrivalvis* and *C. reticulata*. CAM was substantiated by measurements of CO₂ gas-exchange and nocturnal acidification. In all species, the expression of CAM was overwhelmingly facultative in that nocturnal H⁺ accumulation was greatest in droughted plants and zero, or close to zero, in plants that were well-watered, including plants that had been droughted and were subsequently rewatered, i.e. the inducible component was proven to be reversible. Gas-exchange measurements complemented the determinations of acidity. In all species, net CO₂ uptake was restricted to the light in well-watered plants, and cessation of watering was followed by a progressive reduction of CO₂ uptake in the light and a reduction in nocturnal CO₂ efflux. In *C. creethiae*, *C. pentavalvis* and *C. reticulata* net CO₂ assimilation was eventually observed in the dark, whereas in *C. quadrivalvis* nocturnal CO₂ exchange approached the compensation point but did not transition to net CO₂ gain. Following rewatering, all species returned to their original well-watered CO₂ exchange pattern of net CO₂ uptake restricted solely to the light. In addition to facultative CAM, *C. quadrivalvis* and *C. reticulata* exhibited an extremely small constitutive CAM component as

demonstrated by the nocturnal accumulation in well-watered plants of small amounts of acidity and by the curved pattern of the nocturnal course of CO₂ efflux. It is suggested that low-level CAM and facultative CAM are more common within the Australian succulent flora, and perhaps the world succulent flora, than has been previously assumed.

Keywords Arid · Australian flora · *Calandrinia* · Crassulacean acid metabolism (CAM) · Montiaceae · Succulents

Introduction

Large stem-succulents, such as the cacti of the Americas and the euphorbias of Africa, invariably use crassulacean acid metabolism (CAM), a water-use efficient pathway of photosynthesis, as the predominant contributor to carbon gain (Smith and Winter 1996; Winter and Smith 1996; Ogburn and Edwards 2010). In the large stem-succulents, CAM expression is typically constitutive. Constitutive and obligate are descriptors that are often used synonymously for CAM expression that is part of the pre-set processes of development and growth, i.e. the terms refer to photosynthetic tissues in which CAM always eventually develops.

Although much of Australia is arid or seasonally water-limited, it is a continent without large stem-succulents (Grisebach 1872; Ellenberg 1981) and with few identified endemic terrestrial species with CAM (Holtum et al. 2016). Nevertheless, Australia does support a spatially widespread terrestrial flora of small leaf-succulents.

Calandrinia (family Montiaceae) is the most speciose genus of small succulents within the Australian flora. Following the initial collection of *C. polyandra* in 1699 by the English buccaneer, William Dampier (Dampier 1703;

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George 1999), in excess of 60 species and phrase-name species of *Calandrinia* are now documented from locations of, in the main, ephemeral water supply across the Australian continent, from the Torres Strait in the north to Tasmania in the south, and from coastal Western Australia to coastal New South Wales in the east (Atlas of Living Australia 2016; Australian Plant Census 2016). New species continue to be described (Obbens 2006, 2011, 2012, 2014a, 2014b; Tahir and Carolin 2011; West and Chinnock 2013).

The report of CAM in *C. polyandra* and in two tuberous species designated as *Calandrinia* species A and B (Winter et al. 1981), and the subsequent more detailed study of CAM in *C. polyandra* (Winter and Holtum 2011), led us to explore whether CAM might be more widely present among *Calandrinia*. In *C. polyandra*, the expression of CAM is facultative, a hitherto relatively uncommon form of CAM in which CAM-type dark CO₂ fixation and acid accumulation is induced or upregulated in response to an environmental stress such as drought or high soil salinity (Winter et al. 2008, 2015). Proof that the increase in CAM is truly facultative, i.e. optional, requires demonstration that the effect is fully or largely reversible when stress is removed (Winter and Holtum 2014).

Adding to the recent documentation of facultative CAM in the small Australian native succulents *Crassula sieberiana* and *Anacampseros australiana*, which exhibit characteristics of C₃ photosynthesis when well-watered, and *Portulaca australis* and *P. pilosa*, which exhibit C₄ photosynthesis when well-watered (Winter and Holtum 2017), we report here the expression of facultative CAM in four species of *Calandrinia*: *Calandrinia creethiae* Tratman ex Morrison (= *Parakeelya creethae* [Tratman ex Morrison] Hershkovitz), *C. pentavalvis* Obbens, *C. quadrivalvis* F.Muell. (= *Parakeelya quadrivalvis* [F.Muell.] Hershkovitz) and *C. reticulata* Syeda (= *Parakeelya reticulata* [Syeda] Hershkovitz).

Materials and methods

Species

We retain here the name *Calandrinia* although segregation of the Australian *Calandrinia* into the genus *Parakeelya* has been published (Hershkovitz 1998). There is sufficient molecular and phenotypic support for splitting the Australian and American clades of *Calandrinia* (Carolin 1987; Hershkovitz 1993, 1998) but we follow the names used in the Australian Plant Census (2016). Acceptance of *Parakeelya* awaits determination as to whether another available name, *Rumicastrum*, is congeneric with the Australian *Calandrinia* (Carolin 1987; Obbens 2006; Hernández-Ledesma et al. 2015).

The Australian *Calandrinia* have succulent sausage-shaped or otherwise fleshy leaves, clustered rosette-like at the base of the plant from which stems grow and radiate (Fig. 1; Obbens 2011). Most species rarely rise more than 10 cm above the ground although some may reach 30 cm. Plant size and habit, as well as leaf shape and colour, vary considerably in response to growth conditions (Kapitany 2007, 2010). In general, *Calandrinia* tend to colonise sites of ephemeral water supply such as clay-pans and their fringes, small depressions in sandy or gravelly soils, intermittent watercourses and run-off areas, saline soils, rock seepage lines, skeletal soils on rocky hillsides and dunes (coastal or inland).

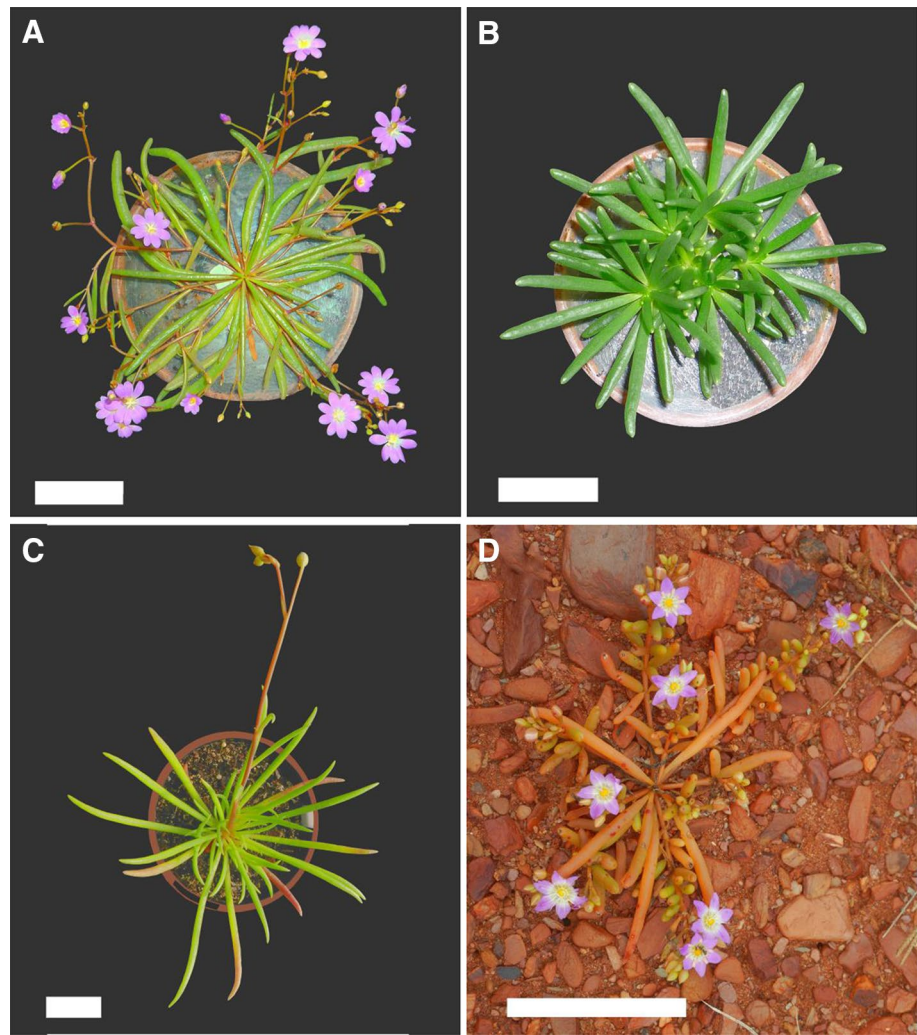
Calandrinia creethiae is a mostly prostrate annual of sandy soils and dry stony ephemeral watercourses in the Gascoyne, Gibson Desert, Little Sandy Desert, Murchison and Yalgoo bioregions of Western Australia (Atlas of Living Australia 2016). It inhabits winter and summer rainfall regions where the median rainfall of 150–250 mm year⁻¹ is generally unpredictable (Figs. 1, 2; Bureau of Meteorology 2016).

Calandrinia pentavalvis is restricted to the Pilbara bioregion of Western Australia where median rainfall of about 300 mm year⁻¹ principally falls during the late-summer and autumn between January and March (Bureau of Meteorology 2016). A semi-erect to erect, sometimes scrambler, *C. pentavalvis* is often associated with *Triodia* grasslands or the grassland understorey of *Acacia* shrublands (Obbens 2011) (Figs. 1, 2).

Calandrinia quadrivalvis is a generally prostrate to ascending succulent annual herb with flowering stems to about 30 cm high (Fig. 1; Western Australian Herbarium 2017). It occurs in moister soils near creeks and swamps along the coastal margins and hinterlands of summer rainfall-dominated tropical northern Australia (Fig. 2). A specimen of *C. quadrivalvis* was collected by Banks and Solander, and sketched by Sydney Parkinson, during the voyage of the Endeavour led by James Cook in 1770 (Stearn 1969).

Calandrinia reticulata is a predominantly inland annual or perennial succulent herb of the central and western Australian arid regions, inhabiting the Burt Plain, Central Ranges, Finke, Great Sandy Desert, MacDonnell Ranges, Pilbara, Pine Creek, Simpson Strzelecki Dunefields, Stony Plains and Tanami bioregions (Figs. 1, 2). Inhabiting regions where the median rainfall of 150–300 mm year⁻¹ is generally unpredictable (Bureau of Meteorology 2016), *C. reticulata* occurs mostly on sandplains and low sandy rises, dunefields, sandy red earth plains and gravelly ranges, often in association with *Acacia aneura* (mulga) (Northern Territory Herbarium 2013).

Fig. 1 Potted plants of *Calandrinia quadrivalvis* (a), *Calandrinia reticulata* (b), *Calandrinia pentavalvis* (c) and *Calandrinia creethiae* growing in situ near Mount Augustus National Park, Western Australia (24.0°25.8'S; 117.35°26.8'E) (d). Plants in a and b were used for the CO₂ exchange studies shown in Figs. 5 and 6. Scale bar 4 cm



Plant material and net CO₂ exchange

Seeds of *C. creethiae* (JAH16; collected near Willuna, WA [latitude 26.591°S, longitude 120.152°E]), *C. pentavalvis* (JAH34; ~5 km east of Port Hedland, WA) and *C. reticulata* (JAH12; north Meekatharra, WA, [latitude 26.025°S, longitude 118.698°E]) were purchased from A. Kapitany (<http://www.australiansucculents.com>). Seeds of *C. quadrivalvis* were obtained from herbarium sheet QH 726990 in the Queensland Herbarium (JAH2; the specimen is labelled as *C. stagnensis* but molecular analysis demonstrates that it is *C. quadrivalvis* [L.P. Hancock, unpublished]). Seeds were germinated in terracotta pots (10 cm upper diameter, 0.4 L) containing potting mix. Plant shoots were enclosed inside a Perspex cuvette (internal dimensions of 11 × 11 × 10 or 20 × 20 × 15 cm) and grown with daily watering. The roots plus pot remained outside the cuvette. Drought treatments were imposed by withholding irrigation until net CO₂ uptake in the light was markedly reduced, after which the plants were rewatered daily.

The gas-exchange cuvette was located inside a controlled environment chamber operating under 12-h light (25 °C):12-h dark (17 °C) cycles. Photon flux density was 800 μmol m⁻² s⁻¹ at the top of the cuvette. The cuvette was supplied with air containing 400 ppm CO₂. The flow rate of air through the cuvette was 1.26 L min⁻¹. Net CO₂ exchange of the plants was measured in a flow-through gas-exchange system consisting of Walz components (Walz GmbH, FRG) and a LI-6252 CO₂ analyzer (Li-Cor, NE, USA) (Holtum and Winter 2003).

Titrateable acidity

In an experiment separate to the gas-exchange experiment, plants were grown outdoors in pots as described above. Mature leaves excised at the end of 12-h day and night were frozen in liquid nitrogen prior to sequential extraction in boiling 50% ethanol and in water. The extracts were titrated with 5 mM KOH to pH 6.5.

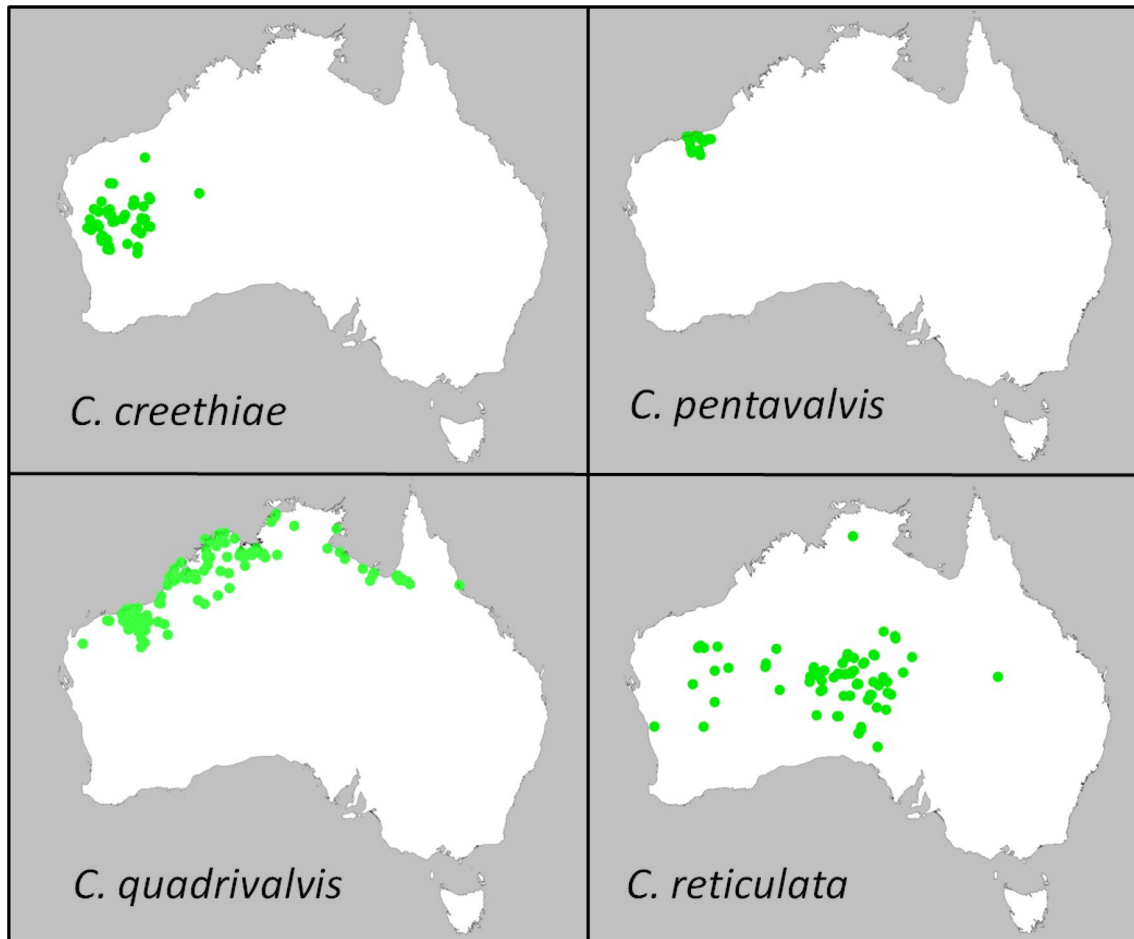


Fig. 2 Collection locations of specimens in Australian herbaria of *Calandrinia creethiae* (45 records), *Calandrinia pentavalvis* (24 records), *Calandrinia quadrivalvis* (149 records) and *Calandrinia reticulata* (136 records) (Atlas of Living Australia 2016)

Results

In well-watered plants, net CO₂ uptake by attached shoots of *C. creethiae*, *C. pentavalvis*, *C. quadrivalvis* and *C. reticulata* was restricted to the light (Figs. 3, 4, 5, 6). CO₂ efflux was relatively constant throughout the night in *C. creethiae* and *C. pentavalvis* (Figs. 3, 4), but exhibited a distinct curvilinear shape in *C. quadrivalvis* and *C. reticulata* (Figs. 5, 6).

Following the imposition of drought, all species exhibited progressive reductions of CO₂ uptake in the light, and a reduction in nocturnal CO₂ efflux that was associated with a more pronounced curvilinear shape (Figs. 3, 4, 5, 6). In *C. creethiae*, *C. pentavalvis* and *C. reticulata* net CO₂ assimilation was eventually observed in the dark, whereas in *C. quadrivalvis* nocturnal CO₂ exchange approached the compensation point but did not transition to net CO₂ gain.

Following rewatering, all species returned to their original well-watered CO₂ exchange pattern of net CO₂ uptake restricted solely to the light coupled with CO₂ loss at night. The CO₂ uptake rates following rewatering exceeded those

at the onset of the experiment because the plants continued to grow inside the gas-exchange cuvette throughout the experiment, although at a reduced rate during the period of drought.

Consistent with the C₃ pattern of gas-exchange, nocturnal acidification was extremely low (*C. quadrivalvis* and *C. reticulata*), or absent (*C. creethiae* and *C. pentavalvis*) in leaves of well-watered plants (Table 1). Following droughting, nocturnal acidification increased in the four species. The increases ranged from 5 μmol (g fwt)⁻¹ for *C. reticulata* to 29.3 μmol (g fwt)⁻¹ for *C. creethiae*. Four days after rewatering, nocturnal acidification had decreased markedly to close to zero but nevertheless remained significantly above zero for *C. creethiae*, *C. pentavalvis* and *C. quadrivalvis*.

Discussion

Calandrinia creethiae, *C. pentavalvis*, *C. quadrivalvis* and *C. reticulata* have the capacity to exhibit CAM, principally

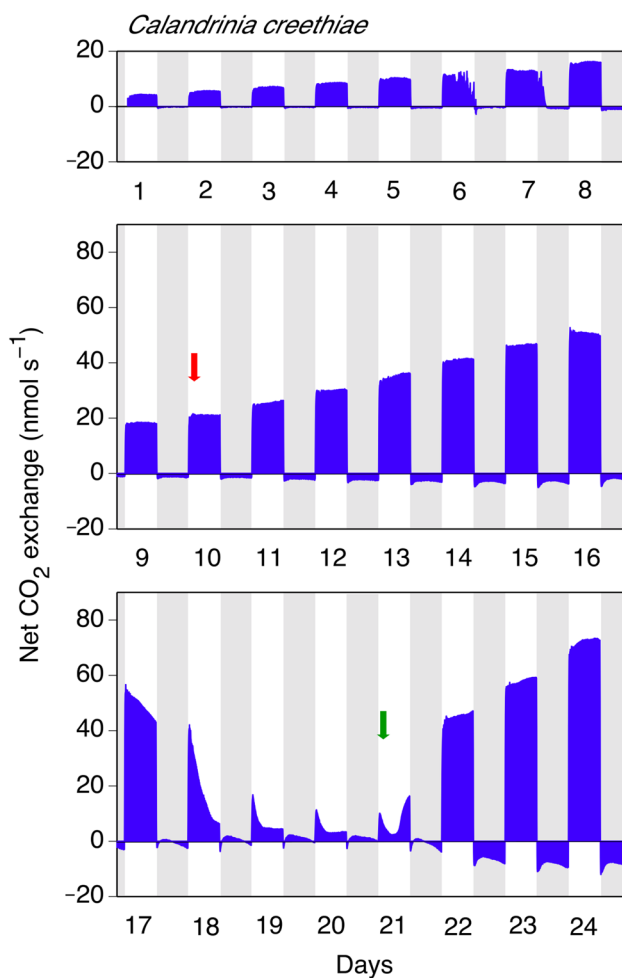


Fig. 3 Twenty-four days of net CO₂ exchange by the above-ground parts of a potted *Calandrinia creethiae*. Watering was withheld on day 10 (red arrow) and recommenced on day 21 (green arrow). Shaded areas represent the 12-h dark periods. The fresh mass inside the cuvette on the last day of the experiment was 10.3 g

facultative CAM. CAM expression is largely facultative because CAM patterns of gas-exchange and nocturnal acidification were present in droughted plants but either absent or close to absent in well-watered plants and in droughted plants that had been rewatered.

The low but nonetheless detectable nocturnal acidification, before droughting, in well-watered plants of *C. quadrivalvis* and *C. reticulata* indicates a small component of constitutive CAM that is independent of drought stress. The acidification was corroborated by gas-exchange in that in these well-watered plants CO₂ efflux decreased during the first half of the dark period and increased in the latter half of the dark period. This curvature in the course of overnight CO₂ exchange when low-level CAM is present, or when facultative CAM is in the process of developing (Figs. 4, 5, 6), is typically not seen in C₃ tissues maintained under constant temperature in the dark (Holtum and Winter 1999; Winter

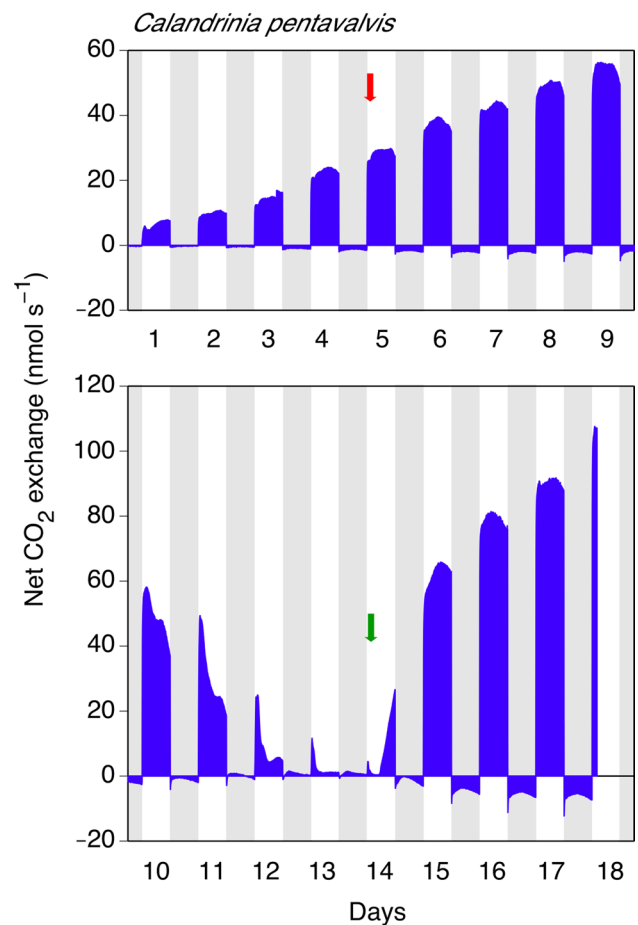


Fig. 4 Eighteen days of net CO₂ exchange by the above-ground parts of a potted *Calandrinia pentavalvis*. Watering was withheld on day 5 (red arrow) and recommenced on day 14 (green arrow). Shaded areas represent the 12-h dark periods. The fresh mass inside the cuvette on the last day of the experiment was 11.4 g

and Holtum 2015). The gradual reduction and subsequent increase of CO₂ efflux is consistent with variations in PEPC activity that reflect changes in PEP supply, PEPC activation and/or inhibition of PEPC by malate (Winter 1982). The brief overshoot of CO₂ loss at the very beginning of each dark period can be explained by the fact that the lower nighttime temperature is not attained instantaneously, as it takes some time for the growth chamber, gas-exchange cuvette and tissue to cool.

In contrast to well-watered *C. quadrivalvis* and *C. reticulata*, well-watered *C. creethiae* and *C. pentavalvis* exhibited no evidence of CAM. Their leaves did not accumulate acidity at night, and there was net CO₂ loss at a more-or-less constant rate for most of the night.

The *Calandrinia* studied here are Australian endemics that inhabit hot, nutrient-poor, seasonally-dry environments where rainfall is unpredictable and water availability tends to be brief. Although studies on the life-cycles of

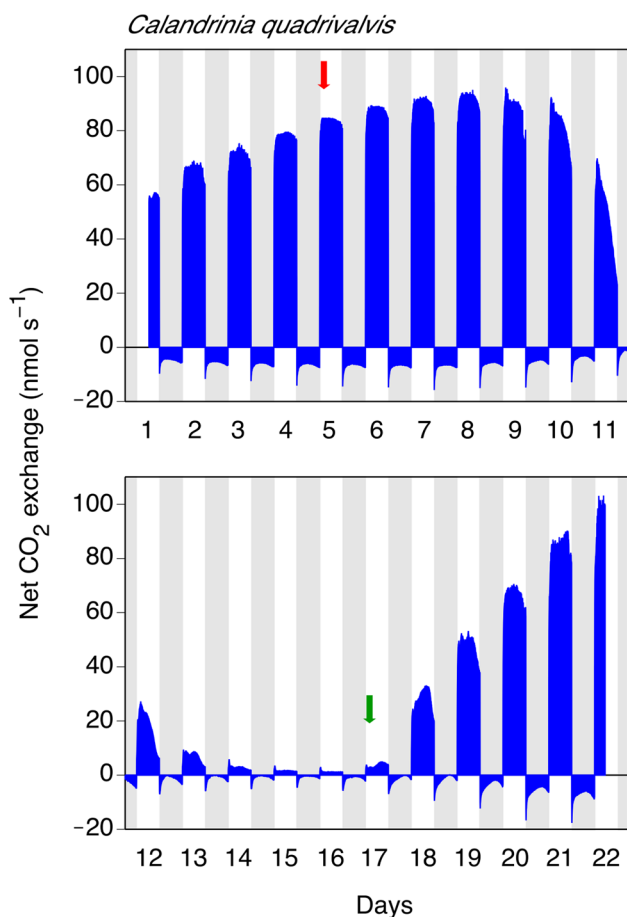


Fig. 5 Twenty-two days of net CO_2 exchange by the above-ground parts of a potted *Calandrinia quadrivalvis*. Watering was withheld on day 5 (red arrow) and recommenced on day 17 (green arrow). Shaded areas represent the 12 h dark periods. The fresh mass inside the cuvette on the last day of the experiment was 15.8 g

Calandrinia growing in their native environments have yet to be conducted, analogy with the role of facultative CAM in *Mesembryanthemum crystallinum* growing in the field (Winter et al. 1978) infers that, for *Calandrinia*, engagement in water-use inefficient C_3 photosynthesis after rainfall enables rapid growth when soil-water is abundant. The transition in *Calandrinia* to water-use efficient CAM when water is scarce would be expected to extend growth and seed- or tuber-filling into the periods when water is even more limiting, as has also been demonstrated for *M. crystallinum* (Winter and Ziegler 1992). Facultative CAM, essentially C_3 (or C_4) with optional CAM, can be considered a trait that prolongs the otherwise short life-cycle. Other characters exhibited by *Calandrinia* that may assist survival in arid and semi-arid habitats with short growing seasons and unpredictable water limitation include succulent leaves that increase the water pool available for growth; the low surface area to volume ratios of cylindrical leaves reduce the evapo-transpirational surface area; small

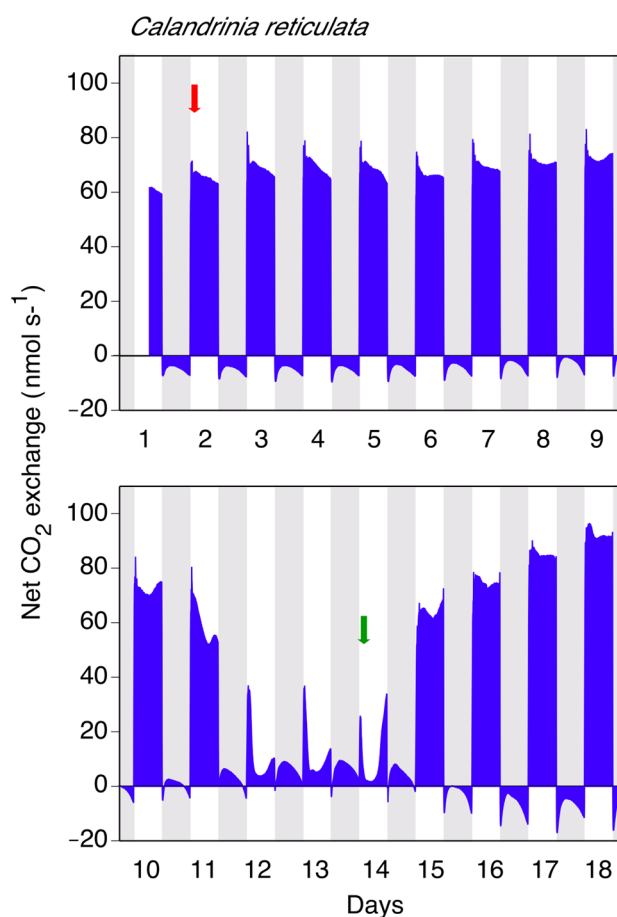


Fig. 6 Eighteen days of net CO_2 exchange by the above-ground parts of a potted *Calandrinia reticulata*. Watering was withheld on day 2 (red arrow) and recommenced on day 14 (green arrow). Shaded areas represent the 12-h dark periods. The fresh mass inside the cuvette on the last day of the experiment was 22.6 g

herbaceous habits enable a short life-cycle; and an ability of seeds to remain dormant permits plants to avoid growth in resource-poor years when survival is less likely (West and Chinnock 2013). Rapid reproduction is supported by abundant colourful nectar-containing flowers that attract a variety of insect pollinators (Kapitany 2010; Holtum and Hancock unpublished). Protandry initially promotes out-crossing but, at least in some species, enables self-crossing if out-crossing is not successful (Obbens 2011). As a consequence, after widespread rain, populations of large *Calandrinia* plants may be abundant over extensive areas of their range, even surviving into a second year if water availability persists, especially in *C. reticulata*. In dry years plants may be absent at a site or, if rain has been local, plants may be small and restricted to small scattered populations (West and Chinnock 2013). Again, what is now needed for the Australian *Calandrinia*, are simultaneous life-cycle studies of CO_2 and acid metabolism, growth and reproduction in the field.

Table 1 Nocturnal variation in leaf titratable acidity in *C. creethiae*, *C. pentavalvis*, *C. quadrivalvis* and *C. reticulata* that were well-watered, droughted for 13 days, and rewatered for 4 days

Species and treatment	Titratable acidity ($\mu\text{mol H}^+ \text{g}^{-1}$ fresh mass \pm s.e.)			
	End of day	End of night	Difference	Significance
<i>Calandrinia creethiae</i>				
Well-watered	0.4 \pm 0.2	0.0 \pm 0.0	-0.3 \pm 0.2	ns
Droughted	2.8 \pm 0.4	31.8 \pm 4.5	29.0 \pm 4.2	\leq 0.05
Rewatered	3.8 \pm 0.5	7.2 \pm 0.9	3.4 \pm 0.7	\leq 0.05
<i>Calandrinia pentavalvis</i>				
Well-watered	1.0 \pm 0.2	1.3 \pm 0.3	0.3 \pm 0.4	ns
Droughted	3.6 \pm 0.4	25.7 \pm 2.0	22.1 \pm 2.0	\leq 0.05
Rewatered	6.9 \pm 1.1	10.0 \pm 0.8	3.1 \pm 1.4	\leq 0.05
<i>Calandrinia quadrivalvis</i>				
Well-watered	0.9 \pm 0.2	1.8 \pm 0.2	0.9 \pm 0.3	\leq 0.05
Droughted	1.8 \pm 0.3	18 \pm 1.7	16.2 \pm 1.9	\leq 0.05
Rewatered	4.1 \pm 0.5	6.3 \pm 1.1	2.2 \pm 1.0	\leq 0.05
<i>Calandrinia reticulata</i>				
Well-watered	1.2 \pm 0.4	4.3 \pm 0.8	3.2 \pm 1.2	\leq 0.05
Droughted	7.8 \pm 0.7	16.0 \pm 3.3	8.2 \pm 3.2	\leq 0.05
Rewatered	7.2 \pm 1.8	10.3 \pm 1.4	3.0 \pm 2.3	ns

Values are means and standard errors for six plants of each species

Differences between end of day and end of night values for samples were compared using paired 1-tailed *t* tests

ns not significantly different

*Significantly different at $P \leq 0.05$

In all of the five Australian *Calandrinia* species tested so far, facultative CAM is part of their photosynthetic repertoire. *Calandrinia quadrivalvis*, a species of northern Australian monsoonal landscapes is the only *Calandrinia* yet examined that inhabits areas where rainfall, be it summer or winter, is reasonably predictable each year. In contrast, *C. creethiae*, *C. pentavalvis*, *C. polyandra* and *C. reticulata* are medium-to-large *Calandrinia* from arid or semi-arid landscapes in Central Australia or north-western Western Australia where rainfall variability is the most extreme for the Australian continent (Bureau of Meteorology 2016). In this context, it is of relevance that the observation of low levels of constitutive CAM in *C. quadrivalvis* and *C. reticulata* is the first evidence that Australian *Calandrinia* have any capacity to express CAM constitutively, albeit expression is low. It will be of interest to observe whether, and if so in what combination, facultative CAM, constitutive CAM and C_3 photosynthesis are exhibited in other as-yet unstudied *Calandrinia*, particularly species such as *C. uniflora*, *C. tumida*, *C. arenicola* and *C. gracilis* that inhabit the northern tropical monsoonal areas, in *C. brevipedata* and other coastal sand-dune species, in prominently tuberous species such as *C. schistorhiza*, in diminutive species such as *C. granulifera*, and in cool-temperate populations of *C. calytrata* and *C.*

granulifera from Tasmania. Such knowledge, in conjunction with phylogenetic studies, will contribute greatly to a better understanding of the evolutionary trajectories and functional significance of CAM in *Calandrinia*.

The maximum CAM signal, as evidenced by CO_2 exchange and nocturnal acid accumulation, is small in the four *Calandrinia* studied here compared to archetypal stem-succulent CAM species, but the presence of the CAM signal clearly differentiates these species from pure C_3 plants. In this context, it is important to note that because CAM in *Calandrinia* is elicited by stress, stress itself restricts the magnitude of CAM, which nonetheless conserves carbon and water.

The detection of low-level CAM in *Calandrinia* is thus of relevance, particularly when interrogating phylogenies about the evolutionary trajectories of the CAM pathway. The detection of low levels of CAM is not trivial experimentally. Isotope surveys do not have the sensitivity to differentiate between standard C_3 species and species that occasionally or permanently exhibit small amounts of CAM relative to C_3 photosynthetic CO_2 uptake in the light (Winter and Holtum 2002). Assessment of low-level CAM in watered, stressed and rewatered plants by gas-exchange requires sensitive equipment that exhibits long-term stability and minimal drift. Determination of nocturnal acidification is probably the most sensitive method for detecting low-level CAM, but tissues harvested for day and night acid measurements must be comparable developmentally and in terms of light exposure. The combination of acidity tissue and gas-exchange measurements, as performed in the study presented here, is clearly the most powerful tool for the detection of low-level CAM (Winter and Holtum 2014).

While past research on CAM tended to concentrate on species with pronounced constitutive CAM (Nobel 2003), it is becoming evident that species with permanently low-level CAM and facultative CAM may be more common than previously thought (Silvera et al. 2005). In the Australian terrestrial flora, a flora that contains no large succulents, in addition to the *Calandrinia* species reported here and in *C. polyandra* (Winter and Holtum 2011), facultative CAM has been described in the arid and semi-arid region leaf succulents *A. australiana*, *C. sieberiana*, *P. australis*, *P. cyclophylla*, *P. digyna*, *P. oleracea* and *P. pilosa* (Brulfert et al. 1991; Guralnick and Jackson 2001; Winter and Holtum 2017; Holtum et al. 2017). In the latter five species, the background photosynthetic pathway is C_4 . This propensity for low-level CAM and facultative CAM may be the product of interactions between plants and an Australian climate during Miocene and post-Miocene aridification but it is probably not just an Australian phenomenon. These Australian taxa, all in the suborder Portulacineae (Carophyllales; Nyfeler and Eggli 2010), have many close relatives in the New World and in Africa, e.g. facultative CAM is long known in

New World portulacinoid *Talinum* (Herrera et al. 1991; Brilhaus et al. 2016) and in *Portulaca oleracea* (Koch and Kennedy 1980). The combined ranges of the Australian species with low-level CAM are extensive, essentially covering the continent, suggesting that the trait is not only suited to the unpredictable rainfall conditions characteristic of the arid regions that constitute around 76% of the Australian land mass. Surveying this landscape for CAM in any of its forms in other structurally similar ostensibly C₃ or C₄ succulent herbs such as *Gunnioopsis* spp. (Aizoaceae), *Sedopsis* spp. (Portulacaceae), *Trianthema* spp. (Aizoaceae) and *Zygophyllum* spp. (Zygophyllaceae) is warranted.

Acknowledgements Gordon Guymer, Peter Bostock and Paul Forster assisted JAMH at the Queensland Herbarium. Frank Obbens (Western Australian Herbarium) tutored LPH and JAMH about *Calandrinia* and guided LPH in the field. Attila Kapitany (australiansucculents.com) supplied seeds and information about collection sites. Aurelio Virgo drew figures. This research was supported by Australian Research Council Discovery Project DP160100098 (JAMH), the Smithsonian Tropical Research Institute and by U.S. National Science Foundation grant DEB-1252901 (EJE).

References

- Atlas of Living Australia (2016) <http://www.ala.org.au/>. Accessed 7 Oct 2016
- Australian Plant Census (2016) Australian plant census (Council of Heads of Australasian Herbaria) IBIS database, Centre for Australian National Biodiversity Research, Canberra. <http://www.chah.gov.au/apc/index.html>. Accessed 19 Jan 2016
- Brilhaus D, Bräutigam A, Mettler-Altman T, Winter K, Weber APM (2016) Reversible burst of transcriptional changes during induction of crassulacean acid metabolism in *Talinum triangulare*. *Plant Physiol* 170:102–122
- Brulfert J, Güclü S, Kluge M (1991) Effects of abrupt or progressive drought on the photosynthetic mode of *Crassula sieberiana* cultivated under different daylengths. *J Plant Physiol* 138:685–690
- Bureau of Meteorology (2016) Australian Government Bureau of Meteorology. <http://www.bom.gov.au/jsp/ncc/climate/averages/rainfall-variability/index.jsp>. Accessed 7 October 2016
- Carolin R (1987) A review of the family Portulacaceae. *Aust J Bot* 35:383–412
- Dampier WA (1703) A voyage to New Holland etc in the year 1699. James Knapton, London
- Ellenberg H (1981) Ursachen des Vorkommens und Fehlens von Sukkulente in den Trockengebieten der Erde. *Flora* 171:114–169
- George AS (1999) William Dampier in New Holland: Australia's first natural historian. Bloomings Books, Victoria
- Grisebach A (1872) Die Vegetation der Erde nach ihrer klimatischen Anordnung: Ein Abriss der vergleichenden Geographie der Pflanzen. W. Engelmann
- Guralnick LJ, Jackson MD (2001) The occurrence and phylogenetics of crassulacean acid metabolism in the Portulacaceae. *Int J Plant Sci* 162:257–262
- Hernández-Ledesma P, Berendsohn WG, Borsch T, Von Mering S, Akhani H, Arias S, Castañeda-Noa I, Eggli U, Eriksson R, Flores-Olvera H, Fuentes-Bazán S, Kadereit G, Klak C, Korotkova N, Nyffeler R, Ocampo G, Ochoterena H, Oxelman B, Rabeler RK, Sanchez A, Schlumpberger BO, Uotila P (2015) A taxonomic backbone for the global synthesis of species diversity in the angiosperm order Caryophyllales. *Willdenowia* 45:281–383
- Herrera A, Delgado J, Paraguatey I (1991) Occurrence of inducible crassulacean acid metabolism in leaves of *Talinum triangulare* (Portulacaceae). *J Exp Bot* 42:493–499
- Hershkovitz MA (1993) Revised circumscriptions and subgeneric taxonomies of *Calandrinia* and *Montiopsis* (Portulacaceae) with notes on phylogeny of the portulacaceous alliance. *Ann Mo Bot Gard* 80:333–365
- Hershkovitz MA (1998) *Parakeelya*: a new genus segregated from *Calandrinia* (Portulacaceae). *Phytologia* 84:98–106
- Holtum JAM, Winter K (1999) Degrees of crassulacean acid metabolism in tropical epiphytic and lithophytic ferns. *Aust J Plant Physiol* 26:749–757
- Holtum JAM, Winter K (2003) Photosynthetic CO₂ uptake in seedlings of two tropical tree species exposed to oscillating elevated concentrations of CO₂. *Planta* 218:152–158
- Holtum JAM, Hancock LP, Edwards EJ, Crisp MD, Crayn DM, Sage R, Winter K (2016) Australia lacks stem succulents but is it depauperate in plants with crassulacean acid metabolism? *Curr Opin Plant Biol* 31:109–117
- Holtum JAM, Hancock LP, Edwards EJ, Winter K (2017) Optional use of CAM photosynthesis in two C₄ species, *Portulaca cyclophylla* and *Portulaca digyna*. *J Plant Physiol* (in review)
- Kapitany A (2007) Australian succulent plants. Kapitany Concepts, Boronia
- Kapitany A (2010) The Australian *Calandrinia*. *Spinette* 2010:1–20
- Koch K, Kennedy RA (1980) Characteristics of crassulacean acid metabolism in the succulent C₄ dicot, *Portulaca oleracea* L. *Plant Physiol* 65:193–197
- Nobel PS (2003) Environmental biology of Agaves and Cacti. Cambridge University Press, Cambridge
- Northern Territory Herbarium (2013) FloraNT—Northern Territory flora online. Department of Land Resource Management. <http://eflora.nt.gov.au>. Accessed 12 Jan 2017
- Nyffeler R, Eggli U (2010) Disintegrating Portulacaceae: a new familial classification of the suborder Portulacinae (Caryophyllales) based on molecular and morphological data. *Taxon* 59:227–240
- Obbens FJ (2006) A review of the tuberous *Calandrinia* species (section Tuberosae), including three new species for Western Australia. *Nuytsia* 16:95–115
- Obbens FJ (2011) Five new species of *Calandrinia* (Portulacaceae) from Western Australia with additional information on morphological observations. *Nuytsia* 21:1–23
- Obbens FJ (2012) Three new species of *Calandrinia* (Portulacaceae) from the Eremaean and South West Botanical Provinces of Western Australia. *Nuytsia* 22:351–362
- Obbens FJ (2014a) Two new species of *Calandrinia* (Portulacaceae) from southern Western Australia. *Nuytsia* 24:27–43
- Obbens FJ (2014b) *Calandrinia butcherensis* and *C. rubrisabulosa* (Portulacaceae), new species from the Midwest of Western Australia. *Nuytsia* 24:207–214
- Ogburn RM, Edwards EJ (2010) The ecological water-use strategies of succulent plants. *Adv Bot Res* 55:179–225
- Silvera K, Santiago LS, Winter K (2005) Distribution of crassulacean acid metabolism in orchids of Panama: evidence of selection for weak and strong modes. *Funct Plant Biol* 32:397–407
- Smith JAC, Winter K (1996) Taxonomic distribution of crassulacean acid metabolism. In: Winter K, Smith JAC (eds) Crassulacean acid metabolism. Springer, Berlin, pp 427–436
- Stearn T (1969) A Royal Society appointment with Venus in 1769: the voyage of Cook and Banks in the 'Endeavour' in 1768–1771 and its botanical results. *Notes Rec R Soc Lond* 24:64–90
- Tahir SS, Carolin RC (2011) A new species of *Calandrinia* (Portulacaceae) from Northern Territory, Australia. *Proc Linn Soc NSW* 133:11–14

- West JG, Chinnock RJ (2013) *Calandrinia mirabilis* (Portulacaceae), a spectacular new species from Western Australia with notes on its ecology, seed germination and horticultural potential. *J Adel Bot Gard* 26:97–102
- Western Australian Herbarium (2017) FloraBase—the Western Australian flora. Department of Parks and Wildlife. <https://florabase.dpaw.wa.gov.au>. Accessed 12 Jan 2017
- Winter K (1982) Properties of phosphoenolpyruvate carboxylase in rapidly prepared, desalted leaf extracts of the crassulacean acid metabolism plant *Mesembryanthemum crystallinum*. *Planta* 154:298–308
- Winter K, Holtum JAM (2002) How closely do the $\delta^{13}\text{C}$ values of CAM plants reflect the proportion of CO_2 fixed during day and night? *Plant Physiol* 129:1843–1851
- Winter K, Holtum JAM (2011) Induction and reversal of CAM photosynthesis in *Calandrinia polyandra* Benth: effects of soil moisture and nutrients. *Funct Plant Biol* 38:576–582
- Winter K, Holtum JAM (2014) Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. *J Exp Bot* 65:3425–3441
- Winter K, Holtum JAM (2015) Cryptic crassulacean acid metabolism (CAM) in *Jatropha curcas* L. *Funct Plant Biol* 42:711–717
- Winter K, Holtum JAM (2017) CO_2 -exchange patterns demonstrate facultative CAM photosynthesis (crassulacean acid metabolism) in four small C_3 and C_4 leaf-succulents. *Aust J Bot* (in press)
- Winter K, Smith JAC (1996) An introduction to crassulacean acid metabolism. In: Winter K, Smith JAC (eds) *Crassulacean acid metabolism*. Springer, Berlin, pp 1–13
- Winter K, Ziegler H (1992) Induction of crassulacean acid metabolism in *Mesembryanthemum crystallinum* increases reproductive success under conditions of drought and salinity stress. *Oecologia* 92:475–479
- Winter K, Lüttge U, Winter E, Troughton JH (1978) Seasonal shift from C_3 photosynthesis to Crassulacean acid metabolism in *Mesembryanthemum crystallinum* growing in its natural environment. *Oecologia* 34:225–237
- Winter K, Osmond CB, Pate JS (1981) Coping with salinity. In: Pate JS, McComb AJ (eds) *The biology of Australian plants*. University of Western Australia Press, Perth, pp 88–113
- Winter K, Garcia M, Holtum JAM (2008) On the nature of facultative and constitutive CAM: environmental and developmental control of CAM expression during early growth of *Clusia*, *Kalanchoë* and *Opuntia*. *J Exp Bot* 59:1829–1840
- Winter K, Holtum JAM, Smith JAC (2015) Crassulacean acid metabolism: a continuous or discrete trait? *New Phytol* 208:73–78