

Thermogenesis of three species of *Arum* from Crete

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ABSTRACT

Inflorescences of arum lilies have a three-part spadix with a scent-producing, sterile appendix above two bands of fertile male and female florets. The appendix and male florets are thermogenic, but with different temporal patterns. Heat-production was measured in *Arum concinatum*, *A. creticum* and *A. idaeum*. The male florets of *A. concinatum* showed a 3 d continuous episode of thermogenesis with three waves, and the appendix warmed in a single, 6 h episode. Maximum fresh-mass-specific CO₂ production rate was 0.17 μmol s⁻¹ g⁻¹ to achieve a 10.9 °C temperature elevation by the appendix, and 0.92 μmol s⁻¹ g⁻¹ to achieve a 4.8 °C elevation by male florets. Reversible, physiological temperature regulation was not evident in either tissue. Respiration increased with tissue temperatures with Q₁₀ values of 1.8–3.9, rather than less than 1.0 as occurs in thermoregulatory flowers. Experimental step changes in temperature of appendix and male floret tissues also failed to show thermoregulatory responses. The patterns of thermogenesis therefore appear to be fixed by the temporal sequence of blooming. Thermogenesis in the alpine species, *A. creticum* and *A. idaeum*, was significantly lower than in the lowland *A. concinatum*, possibly related to difficulty in raising floral temperature in their cold and windy habitat.

Key-words: Araceae; arum lily; inflorescence; pollination; temperature regulation.

Abbreviations: $\dot{M}CO_2$, rate of CO₂ production; T_a , ambient temperature; T_{app} , appendix temperature; T_{max} , maximum temperature; T_{mf} , male floret temperature.

INTRODUCTION

Thermogenesis in the flowers and inflorescences of plants has received interest in the literature at least from the time of de Lamarck (1803–1815). It occurs in at least eleven extant families of basal angiosperms and in cycads (Thien *et al.* 2009). Most species occur in the family Araceae, the arum lilies, and thermogenesis in the genus *Arum* has been studied recently in terms of pollination biology (Albre, Quilichini & Gibernau 2003), biochemical regulation of heat production by the cyanide-insensitive pathway

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(Wagner *et al.* 2008) and molecular biology of the alternative oxidase (AOX; Kakizaki *et al.*, unpublished results). The patterns of thermogenesis in selected species have been reviewed (Gibernau, Macquart & Przetak 2004). Measurements of inflorescence temperature with thermocouples, thermistors and thermal imaging cameras reveal multiple thermogenic episodes involving the appendix and male florets on the spathes of *A. maculatum* and *A. italicum* (Skubatz *et al.* 1990; Bermadinger-Stabentheiner & Stabentheiner 1995; Albre *et al.* 2003). The patterns centre around a 1 d protogynous sequence in which heating is closely tied to light cycle and movements of insects from staminate-stage inflorescences to pistillate-stage ones in the afternoon in *A. maculatum* (Bermadinger-Stabentheiner & Stabentheiner 1995) and in the evening in *A. italicum* (Albre *et al.* 2003), when heating of the scent-producing appendix peaks. Thermogenesis of the male florets appears less than that of the appendix from temperature records, and peaks in temperature elevation have been interpreted as episodes of thermogenesis. In both species, there is a peak in male temperatures that occurs during the day of the appendix heating and another on the following day. Mass-specific respirometry carried out on tissues extracted from *A. maculatum* at different developmental stages during the flowering sequence showed intense oxygen uptake during the episode of thermogenesis by the appendix, and a lower peak in male floret respiration preceding it (Lance 1972). However, there are no continuous respiration measurements over the course of blooming in intact inflorescences in the field. Therefore this study aimed to characterise the pattern of blooming and the relationships between respiration rate, stage of blooming, floral temperature and ambient temperature in *A. concinatum* Schott. At the same time, we were able to examine thermogenesis in two other species of *Arum* on Crete, *A. creticum* Boiss. & Heldr. and *A. idaeum* Coust. & Gandoger, with spot measurements of respiration and temperature. These alpine species were chosen because they bloom at lower ambient temperatures than *A. concinatum*, and have different temporal patterns of anthesis.

Some thermogenic flowers are also thermoregulatory, that is, they can increase heat production when ambient temperature decreases and maintain a degree of independence of floral temperature from environmental temperature. Examples of excellent thermoregulatory flowers are *Philodendron selloum* (Nagy, Odell & Seymour 1972;

Seymour 1999), *Symplocarpus foetidus* (Knutson 1974; Seymour 2004) and *Nelumbo nucifera* (Seymour & Schultze-Motel 1998). Temperature regulation has been implied to exist in intact *A. maculatum* by some independence of appendix temperature from ambient temperature, and a model for thermoregulation has been derived from the effects of temperature on respiration of isolated mitochondria (Wagner *et al.* 2008). It is proposed that temperature regulation by *A. maculatum* occurs by an interaction between the AOX protein concentration, and inherent temperature profiles for the activities of AOX and dehydrogenases earlier in the respiratory pathway. The model potentially explains the respiratory behaviour in all thermoregulatory species and represents a breakthrough hypothesis that needs to be tested. Our study, which was carried out before the appearance of the Wagner *et al.* model, nevertheless bears on the question of temperature regulation in *Arum*. We carried out experimental tests to determine whether *A. concinatum* tissues can increase respiration with decreasing tissue temperature, which is a requirement for reversible temperature regulation. The stimulus for these tests was that earlier studies showed that the appendices of the dragon lily, *Dracunculus vulgaris*, and the dead horse arum, *Helicodiceros muscivorus*, are not thermoregulatory (Seymour & Schultze-Motel 1999; Seymour, Gibernau & Ito 2003a). Although male florets of the dead horse arum exhibit some independence from ambient temperature, the pattern is 'pseudo-thermoregulatory', because heat production depends on time of day, rather than ambient temperature. There are no experimental tests of thermoregulatory ability in any *Arum* species.

MATERIALS AND METHODS

Field measurements on *A. concinatum*

Arum concinatum was studied during April and May, 2007 and 2008 in Crete, about 2 km South of Panormos (35°23'53"N, 24°41'56"E). The plants grew in an olive orchard, around the bases of the trees. The site was chosen because of the large number of plants, which, according to the owner, resulted from occasional flooding of the land by an overflowing waterway during the winter. It was also noted that commercial fertilizer had been used in the orchard. Observations were made of the opening of the spathe, the condition of the inflorescence and the insect occupants.

Temperatures of the inflorescences were made with individual loggers (Hobo Temp S08-563-9000 equipped with 40 cm leads and H08-001-02 with short leads, <http://www.onsetcomp.com>). Thermistors were placed into the male florets, appendix and air adjacent to the inflorescence. All loggers were suspended above the ground and covered with plastic wrap to protect from rain, and everything was shaded with a white plastic, open-ended tent. The state of the inflorescence was noted at least once daily. Temperatures of other inflorescences were taken opportunistically

with infrared (IR) thermometric imaging (TVS-500 thermal camera, Nippon Avionics, Tokyo, Japan).

Respiration of inflorescences was measured in the field with an open-flow system described earlier (Seymour & Schultze-Motel 1998; Seymour & Gibernau 2008). Briefly, each inflorescence was instrumented with thermocouples in locations described earlier, and then covered with a hood consisting of a 500 mL plastic bottle with the bottom cut off. The bottom was incompletely sealed to the plant with plastic cling wrap, leaving a small opening to admit air. Air was pumped through the hood at about 250 mL min⁻¹ into the respirometry chain, consisting of a water trap, pump (model Gilair 3, <http://www.sensidyne.com>; Sensidyne, Clearwater, FL, USA), pressure buffer, mass flow meter (Toptrak; <http://www.sierrainstruments.com>; Sierra Instruments, Monterey, CA, USA), selection manifold and O₂-CO₂ analyser (model 280; David Bishop, Warwickshire, UK). Because of the greater sensitivity and stability, only the IR CO₂ analyser was used. It was calibrated with CO₂-free air and a precision 0.49% CO₂ in N₂ mixture. The system had five independent channels, four for samples and the one for humidified reference air, and the selection manifold cycled through these at 6 min intervals, yielding data for each channel every 30 min. The outputs of the analyser, flowmeters and thermocouples were recorded every 2 min with a logger (Squirrel 1203, <http://www.grant.co.uk>). $\dot{M}CO_2$ was calculated the equation: $\dot{M}CO_2 = \dot{M}E(FCO_{2OUT} - FCO_{2IN})$ where $\dot{M}E$ is the air flow rate and FCO_{2OUT} and FCO_{2IN} are the fractional CO₂ contents of the air flowing through the hood. Measurements were taken from whole inflorescences that were completely covered by the hood or separately from the appendix and the male florets. In the latter case, it was necessary to remove the spathe and cover the naked appendix with the hood, whereas a second respirometry channel sampled gas directly from the floral chamber through a tight-fitting hole in the side. Plastic wrap at the top of the floral chamber prevented mixing of the two air-streams. All inflorescences were protected from the sun with tents as described earlier.

After thermal and respiratory measurements, each inflorescence was cut and masses of the floral parts measured to 2 mg with a micro balance (model 1210, Tanita Corporation, Japan). The appendix and male florets were weighed both with and without the spongy stalk. In the latter case, the spadix was split lengthwise and the spongy tissue scraped out with a knife, leaving the thermogenic tissue.

Field respiration of *A. idaeum* and *A. creticum*

Spot measurements were made in the field during the flowering season in May 2008. The site was on the southern slope of Mt. Psiloritis at an altitude of about 1800 m, near the top of the unsealed road from Lochria (35°11'39"N, 24°47'38"E).

Flowers in the female phase were identified as freshly opened and without pollen. The male phase was identified as a turgid, but reflexed, spathe and pollen grains in the floral chamber. Temperatures of the air, the male florets and

centre appendix were measured *in situ* with a needle thermocouple and thermometer (model 52, <http://www.fluke.com>; Fluke, Everett, WA, USA). Each inflorescence was then cut and respiration measured within 12 min. *A. idaeum* was measured at an ambient temperature between 12 and 14 °C, and *A. creticum* was measured at 8–10 °C. The spadix, consisting of the appendix, male florets and female florets, was removed intact and placed in a tight plastic bag, which was the beginning of a similar respiratory train as described earlier, except that a more sensitive CO₂ analyser was used (LI-820, <http://www.licor.com>; Li-Cor, Lincoln, NE, USA). After stabilization in about 4 min, the female florets were cut off and the appendix plus males measured. Finally, the males were removed and the appendix measured alone. Individual values for males and females were obtained by subtraction. This technique was used to reduce cutting and water loss.

Effects of experimental temperatures

It was discovered that tissues excised from the spadix of *A. concinatum* would continue to heat for hours if they were cut with a wet blade and immediately set in a thin layer of water. Subsequently a series of respiration measurements were made from cut male florets and the tip of the appendix. The respirometry chamber consisted of an inverted 20 mL glass vial resting on a cloth over a polystyrene foam base. A polyvinylchloride (PVC) tube was passed through the foam and cloth into sample chamber to sample its air. A section of the spadix consisting of the male florets or the appendix tip was cut and impaled on a needle thermocouple pushed upward through the foam and the bottom of a 14 × 14 × 5-mm-deep polyethylene box containing a little water. This thermocouple measured tissue temperature. A second thermocouple to measure ambient temperature was fixed to the vertical PVC tube. The result was a respirometry chamber with air entering under the lip of the vial and

exiting into the respirometry setup as described earlier. However, the Li-Cor CO₂ analyser was used and sampling of each chamber lasted 2 min. Four such chambers were enclosed in a custom-made constant-temperature cabinet based on heating or cooling with a Peltier element as described earlier (Seymour 2004). The cabinet had an internal volume of ca. 600 mL, and the internal air was stirred with a 40 × 40 mm CPU fan. The strength of the cabinet overwhelmed thermogenesis by the small samples of tissues, so cabinet temperature was largely imposed on the tissues. Once the CT cabinet was in place, air from outside of the building was pumped into it to provide a constant CO₂ level to be sucked into each respirometry chamber. Thus it was possible to measure both appendix and male tissues from two inflorescences simultaneously. The experiment consisted of recording floral responses to 5 °C or 10 °C step changes in cabinet temperature at approximately 3 h intervals over about 15 h. The step changes were either up or down, usually between multiples of 5 °C within the range of 10–30 °C (e.g., one run was 15, 25, 15, 20, 15 °C; another is illustrated in Fig. 8).

RESULTS

Field measurements of *A. concinatum*

The sequence of blooming in *A. concinatum* was characterized by loosening of the spathe on the day before opening (D – 1). The spathe opened on 'D-day' at about 1500 h, when the appendix began to heat (Fig. 1). Thereafter the spathe remained open. Pollen was released in the afternoon of the following day (D + 1).

There was one peak of thermogenesis in the appendix on D-day and three peaks in the male florets, all during the day, with two before, and one after, D-day (Fig. 2a). With each peak of temperature, there was a significant elevation in MCO₂ (Fig. 2b). This pattern of thermogenesis was observed in 13 inflorescences equipped with temperature

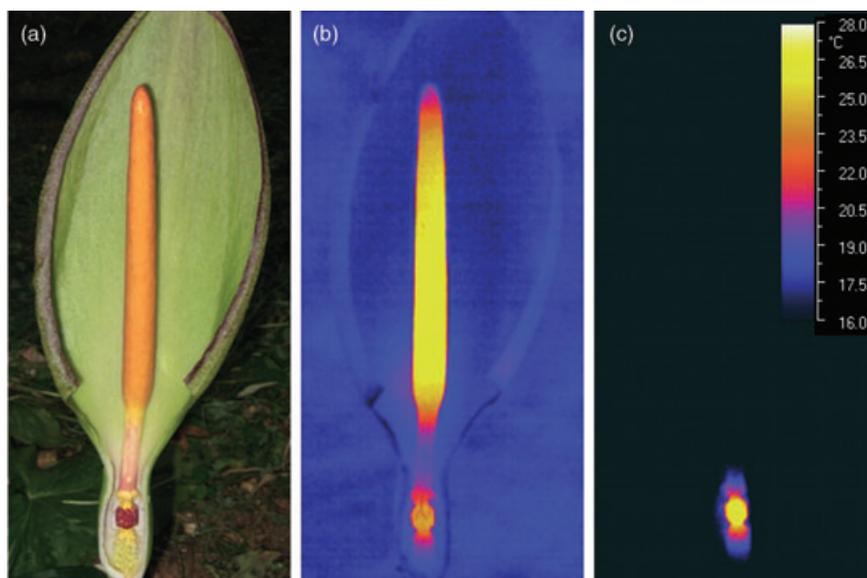


Figure 1. Inflorescence of *Arum concinatum* photographed in the field on 16–17 April 2007. (a) Appearance at 1900 h on D-day, with the orange appendix in front of the open spathe and the wall of the floral chamber cut away to reveal the brown male florets above the yellow female florets. (b) A thermal image taken at the same time, showing the thermogenic appendix and mildly thermogenic male florets. (c) Thermal image of the same inflorescence on the following day at 1020 h, showing intense thermogenicity in the male florets only. The temperature scale for both (b) and (c) is inset.

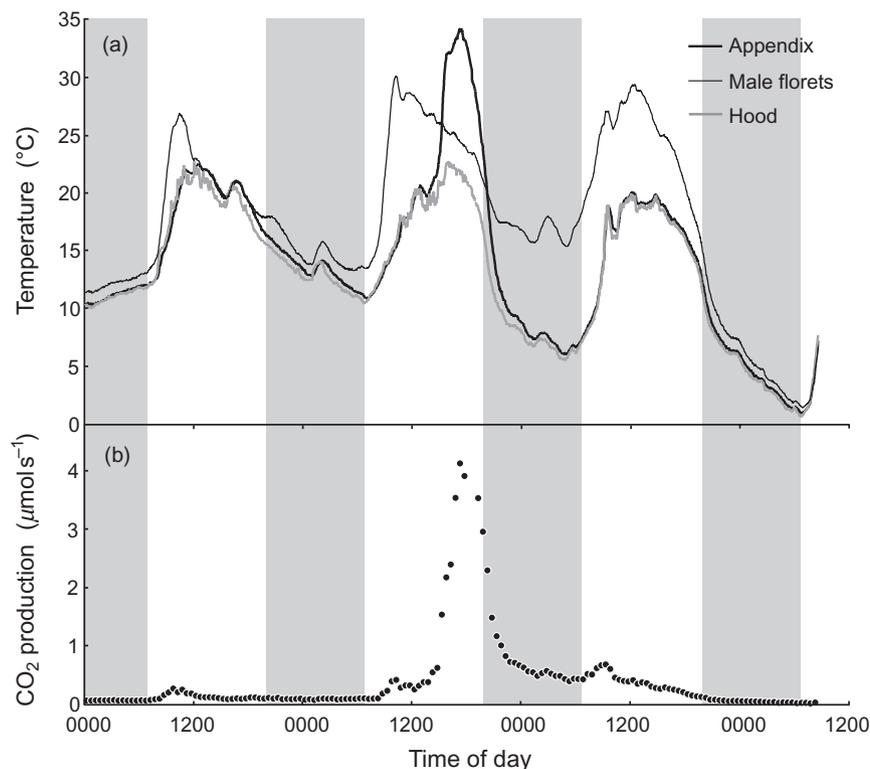


Figure 2. Example of the pattern of thermogenesis in an intact, shaded inflorescence of *Arum concinatum* in the field. (a) Temperatures of central appendix, male florets and air in the respirometry hood, outside of the spathe. (b) Rate of CO₂ production by entire inflorescence. Appendix mass = 11.28 g, male florets = 0.43 g. Shading indicates night-time.

loggers in the open air and two involved with whole inflorescence respirometry. To separate respiration of the appendix from the male florets, the spathe was removed in seven inflorescences, but the pattern of thermogenesis was not affected (Fig. 3). However in all of these cases, the plant was instrumented on D-day, so the early male thermogenesis was not recorded.

Because of the clear, monophasic response of the appendix, it was possible to measure its characteristics precisely (Table 1). There were no significant differences in the timing and maximum temperature reached by the appendix for the methods with and without respirometry, so the data were combined. Peak appendix heating occurred at 1740 h, well before sunset (about 2000 h), but continued into the night (Table 1, Figs 2 & 3). Respiration by the male florets was more continuous, but there were three waves on consecutive days (D - 1, D, D + 1). On D - 1, the male florets warmed a little in the morning (Fig. 2), whereas on D and D + 1, there were larger episodes that lasted throughout the day (Figs 2 and 3). The first episode occurred when the spathe was closed, the second when the spathe was opening and insects were attracted, and the third when pollen was being shed and the insects were leaving the inflorescence. It is also noteworthy that the male florets remained approximately 5–10 °C warmer than the air during the night of insect captivity in the floral chamber.

Maximum appendix respiration was $2.29 \mu\text{mol s}^{-1}$, equivalent to 1.07 W, whereas the maximum male floret heat production was 0.16 W (Table 1). On a mass-specific basis, however, the maximum respiration of the male florets was over five times higher than that of the appendix.

The appendix temperature rose to between 28 and 37 °C in 20 open and hooded inflorescences at ambient temperatures between 17 and 33 °C (Fig. 4). There was a weak, but significant correlation, according to the linear regression equation, $T_{\text{app}} = 0.335 T_{\text{a}} + 25.8$ ($R^2 = 0.28$; $P = 0.017$). However, there were no apparent correlations between $\dot{M}\text{CO}_2$ and either T_{a} or $T_{\text{max}} - T_{\text{a}}$, mainly because of limited T_{a} range in the seven inflorescences in which appendix respiration was measured. There was also no significant effect of appendix mass on $\dot{M}\text{CO}_2$, but a slight tendency for $T_{\text{max}} - T_{\text{a}}$ to increase with mass (regression $R^2 = 0.192$; $n = 20$; $P > 0.05$).

Field data for the appendix and male florets were extracted to correlate tissue temperature (T_{app} and T_{mf}) and $\dot{M}\text{CO}_2$. Because the appendix was strongly thermogenic over a limited time, data were taken over a 6 h period of significant heating (from 1400 h on D-day); data for male florets were taken over a 24 h period (from 1800 h on D-day). Appendix $\dot{M}\text{CO}_2$ ($\mu\text{mol s}^{-1}$) increased exponentially according to the equation: $\dot{M}\text{CO}_2 = 0.022e^{0.136T_{\text{app}}}$ ($R^2 = 0.74$) (Fig. 5). The Q_{10} of this relationship was 3.9. Respiration by the male florets also increased with tissue temperature according to the equation: $\dot{M}\text{CO}_2 = 0.062e^{0.056T_{\text{mf}}}$ ($R^2 = 0.61$) (Fig. 6). The Q_{10} was 1.8.

Thermogenesis in *A. creticum* and *A. idaeum*

Seven attempts were made to log appendix and either male floret or floral chamber temperatures in shaded inflorescences in the field, but high winds and goats dislodged the

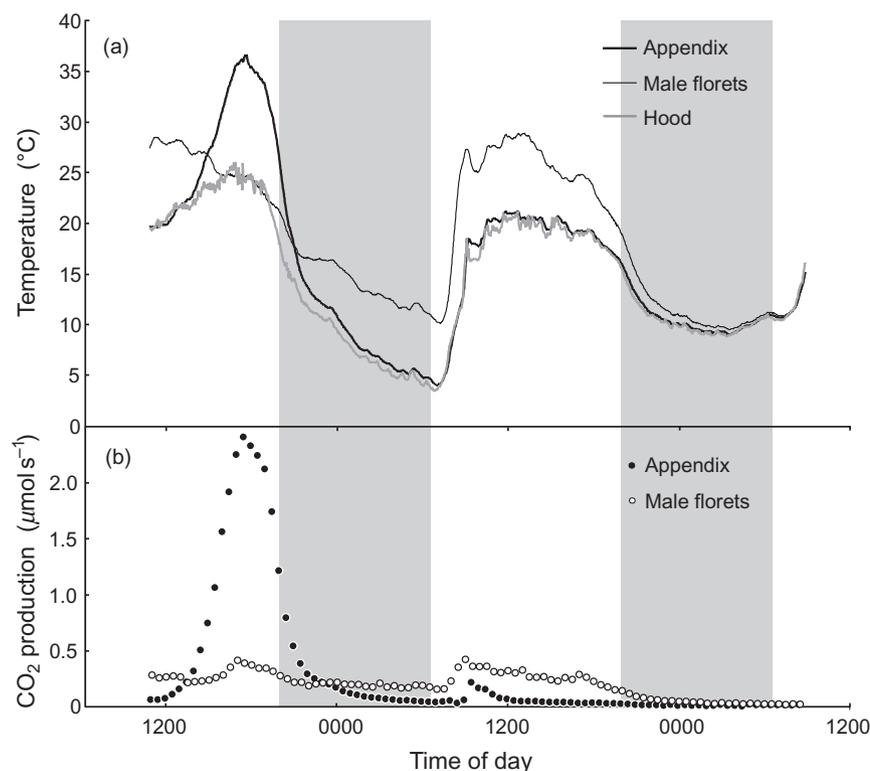


Figure 3. Example of the pattern of thermogenesis in a shaded inflorescence of *Arum concinatum* without a spathe. (a) Temperatures of central appendix, male florets and air in the respirometry hood. (b) Rate of CO₂ production by the appendix and male florets in the floral chamber. Appendix = 19.11 g; male florets = 0.43 g. Shading indicates night-time.

thermistors and sun shades. However, one record of four from *A. creticum* showed the appendix warming between approximately 1430 and 1630 h on the day before, and the day of, spathe opening, but not afterward (Fig. 7). The maximum temperature elevation was 7.7 °C at 1550 h on the second day. None of the three records from *A. ideum* showed temperature elevations that could be definitely associated with thermogenesis rather than solar radiation. Nevertheless, spot measurements of temperatures with needle thermocouples demonstrated thermogenesis in both species, and these were confirmed by spot respirometry

(Table 2). Temperature elevations were small, but the male florets were warmer than the appendix, and this was reflected in a higher respiration rate by the male florets. The appendix had lower thermogenesis, and the female florets had a negligible effect. There was no relationship between respiration rate and temperature elevation measured just before cutting in either male florets or appendix in either species. The temperature elevation may have been affected by solar radiation, wind and, in the case of most *A. creticum*, misting rain. However, the respiration rates are felt to represent the condition in the field at the time, as lab

Variable	Units	Appendix			Male florets		
		Mean	CI	<i>n</i>	Mean	CI	<i>n</i>
Time of onset	Hours	1435 h	0:15	21			
Time at T_{max}	Hours	1740 h	0:17	22			
Time of finish	Hours	2129 h	0:26	21			
Temp. maximum (T_{max})	°C	33.2	1.0	21	26.2	0.9	21
Ambient temp. (T_a)	°C	22.1	1.6	21	21.4	0.8	21
$T_{max} - T_a$	°C	10.9	1.4	20	4.8	0.7	21
Whole mass	g	14.48	1.58	23	0.40	0.03	22
Thermal tissue	g	13.75	1.51	16	0.36	0.03	23
\dot{M}_{CO_2}	$\mu\text{mol s}^{-1}$	2.294	0.602	7	0.334	0.067	7
$\dot{M}_{CO_2}/\text{whole mass}$	$\mu\text{mol s}^{-1} \text{g}^{-1}$	0.169	0.058	7	0.846	0.153	7
$\dot{M}_{CO_2}/\text{thermal tissue}$	$\mu\text{mol s}^{-1} \text{g}^{-1}$	0.175	0.059	7	0.918	0.172	7

Table 1. Data summary for appendix and male floret tissue in *Arum concinatum* in the field

Time of peak appendix temperature is accurate to 2 min; those of the onset and finish are estimated from the bottoms of the steep portions of the respiration curves. Whole mass includes the central pith of the spadix; mass of thermal tissue does not. Respiration attributed to the male florets includes a negligible contribution from the female florets and floral chamber walls. Statistics are means and 95% confidence intervals (CIs).

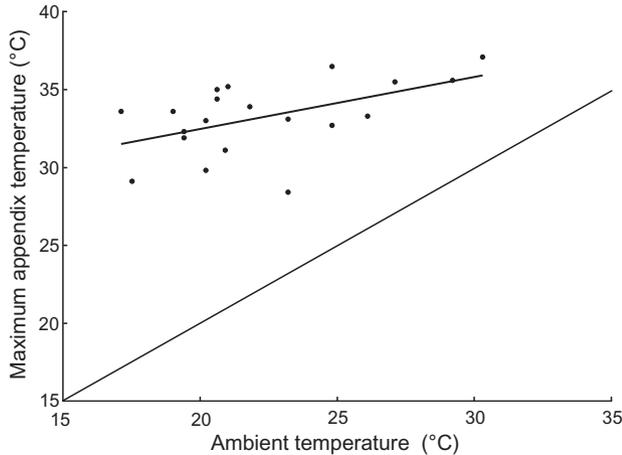


Figure 4. Relationship between peak temperature of the appendix and ambient air temperature in *Arum concinatum* in the field. The equation for the regression line is $T_{app} = 0.33 T_a + 25.8$ ($R^2 = 0.28$). The diagonal line is isothermal.

measurements from other species indicate that there is little change in a few minutes after cutting.

Effects of experimental temperatures

Experiments on cut tissues subjected to step changes in temperature were run on inflorescences cut either in the morning (10 runs started between 0820 and 0930 h and lasting 13–15 h) or evening (four runs started between 2045 and 2230 h and lasting 11–15 h) of D-day, in order to encompass the most thermogenic periods of the appendix or male florets, respectively. The mass of 14 samples was 0.35 g (± 0.07 confidence interval) for male florets and 1.25 g (± 0.20) for appendix tissue. These small sizes caused tissue temperature to follow chamber temperature, and independent temperature regulation was not possible.

If the thermoregulatory mechanism is present in the tissue, the response to a step change is a quick deviation in

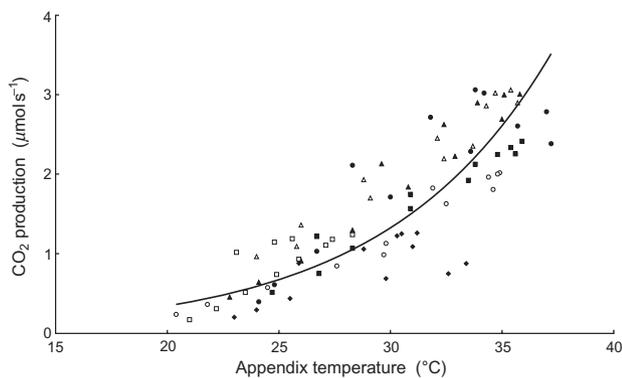


Figure 5. Relationship between respiration rate and tissue temperature of the appendix of *Arum concinatum* during 6 h of the main thermogenic period (1400–2000 h). Symbols indicate seven individual inflorescences.

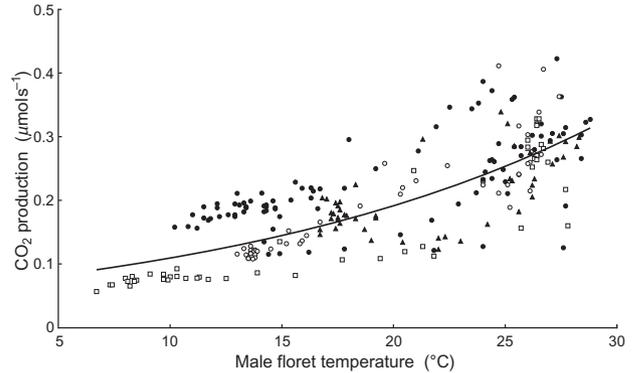


Figure 6. Relationship between respiration rate and tissue temperature of male florets in the floral chamber of *Arum concinatum* during 24 h of the main thermogenic period. Symbols indicate five individual inflorescences.

respiration in the direction of the change (i.e. a drop in temperature results in drop in respiration, and vice versa), followed by a slower reversal (i.e. after the quick change in one direction, respiration begins to move in the opposite direction, eventually to a greater extent). These phases represent the immediate van 't Hoff (Q_{10}) effect on reaction rates, followed by a biochemically regulated activation or inhibition of respiration – the thermoregulatory response. However, the present results showed no consistent effects of temperature on respiration (e.g. Fig. 8). The tissues were responsive to step changes in tissue temperature, often with an immediate change in respiration rate, but not necessarily in a predictable direction. The initial response to an increase in temperature could be the expected increase, but it could also decrease, and vice versa. After the initial adjustment in respiration rate, subsequent reversals occurred in some cases, but not all. For appendix tissue, the pattern of respiration in field inflorescences was apparent under all experimental conditions, peaking at approximately 1600–1800 h regardless of the imposed temperature (Fig. 8). There were also no replicable effects measured with male florets. Of the 46 cases of step temperature changes in male floret and appendix tissue of 14 inflorescences, 24 (52%) were consistent with temperature regulation in male florets and 21 (46%) were consistent in appendix tissue. It is concluded that the mechanism for temperature regulation was not apparent in these experiments.

DISCUSSION

Timing and intensity of thermogenesis

The pattern of thermogenesis in *A. concinatum* was consistent in every specimen studied in the field. There was a major heating of the appendix, coinciding with spathe opening in the afternoon of D-day and peaking about 2.5 h before sunset, coupled with three daytime elevations in respiration by the male florets on successive days (Figs 1–3). We are much less certain about the temporal pattern in *A. creticum* and *A. idaeum*, because the data were limited to

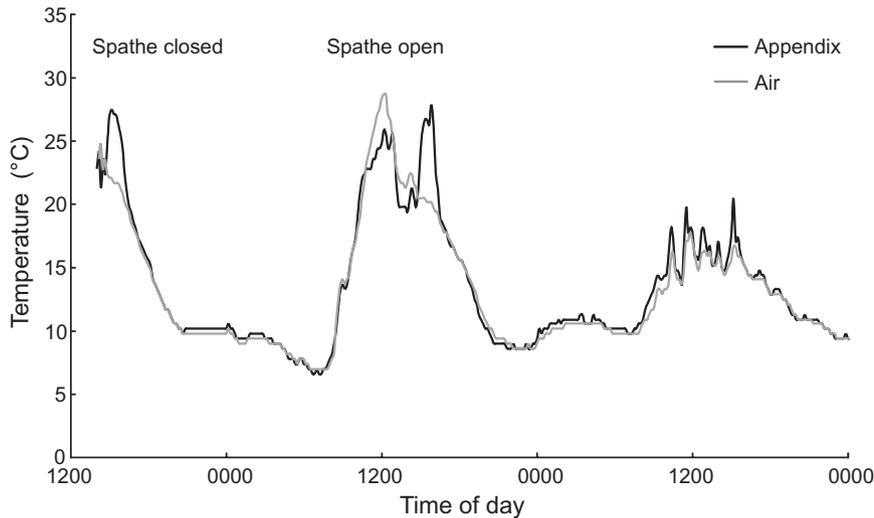


Figure 7. Record of appendix and ambient air temperatures of a shaded inflorescence of *A. creticum* in the field on Mt. Psiloritis.

spot measurements, but one continuous record for *A. creticum* (Fig. 7) and six spot measurements showed that the appendix heated in the afternoon during the pistillate stage. These patterns are generally consistent with data from *A. maculatum* (Bermadinger-Stabentheiner & Stabentheiner 1995) and *A. italicum* (Albre *et al.* 2003). Diaz & Kite (2006) did not mention thermogenicity in *A. idaeum* and *A. creticum*.

The metabolic intensity of *A. concinnatum* was considerably higher than either *A. idaeum* or *A. creticum*, not only because of its larger dimensions, but also on a mass-specific basis (Table 2). Even when corrected to a common

temperature with an assumed $Q_{10} = 2$, the rate of *A. concinnatum* male florets was 2.2 times that of *A. idaeum* and 4.5 times that of *A. creticum*. These data were taken from spot measurements and do not represent maximum thermogenesis, however. Maximum rates taken from continuous records of *A. concinnatum* are even higher.

Respiration of *A. concinnatum* appendix peaked at $0.17 \mu\text{mol s}^{-1} \text{g}^{-1}$ (Table 1), which is considerably lower than the mean value of $0.49 \mu\text{mol s}^{-1} \text{g}^{-1}$ for *in vitro* appendix tissue in *A. maculatum* (Lance 1972). The difference is not caused by the different tissue temperatures of measurement, because Lance used 20°C , and the present

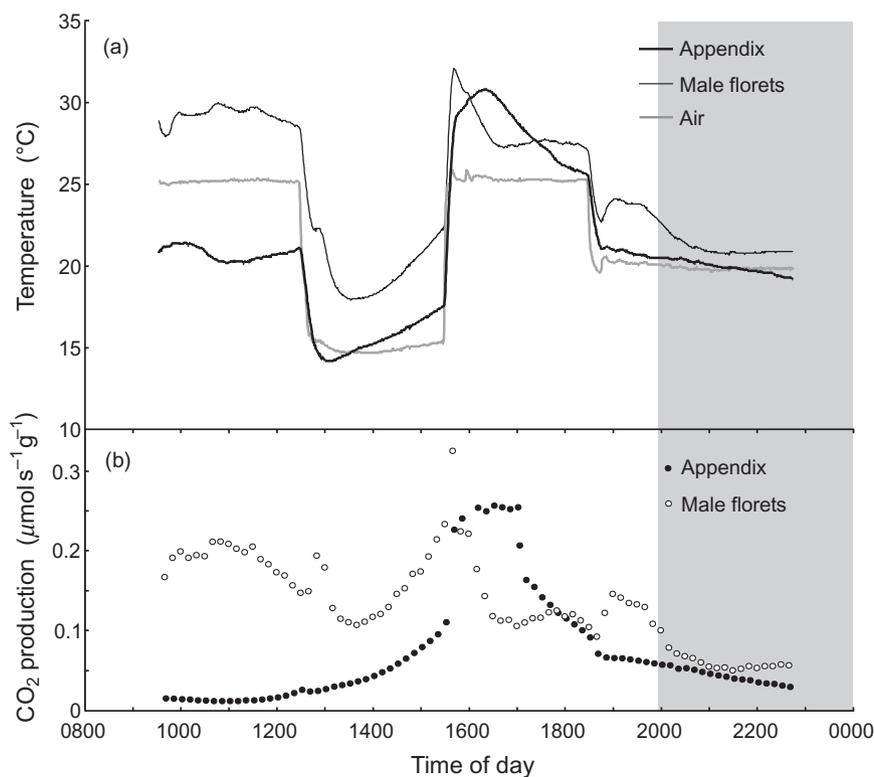


Figure 8. Example results of experimental manipulation of tissue temperature in *Arum concinnatum*. (a) Temperatures of appendix, male spadix and air. (b) Mass-specific rates of CO_2 production for the appendix and male florets. Shading indicates night-time.

Table 2. Data from spot measurements of temperatures and respiration rates of three species of *Arum* on Crete

			<i>A. idaeum</i>				<i>A. creticum</i>				<i>A. concinatum</i>		
			Female phase <i>n</i> = 7		Male phase <i>n</i> = 6		Female phase <i>n</i> = 6		Male phase <i>n</i> = 6		Female phase		
			Mean	CI	Mean	CI	Mean	CI	Mean	CI	Mean	CI	<i>n</i>
Temp.	T_{ambient}	°C	10.6	0.6	13.9	0.9	9.1	1.6	6.4	0.4	22.1	1.6	21
	T_{male}	°C	15.4	1.3	16.9	1.4	12.5	1.9	7.9	0.8	26.2	0.9	20
	T_{appendix}	°C	13.1	0.6	15.8	0.6	9.8	1.3	7.4	0.7	21.9	0.7	9
	$T_{\text{male}} - T_{\text{ambient}}$	°C	4.7	1.3	3.1	1.7	3.4	0.8	1.5	0.6	4.9	0.7	20
	$T_{\text{appendix}} - T_{\text{ambient}}$	°C	2.5	0.7	2.0	0.9	0.7	0.6	1.0	0.3	0.5	0.6	9
Mass	Whole spadix	g	2.80	0.87	2.07	0.41	3.05	1.09	3.09	0.87	16.07	1.69	23
	Appendix	g	1.22	0.42	0.81	0.16	1.88	0.77	1.77	0.46	14.48	1.58	23
	Female spadix	g	1.21	0.41	0.99	0.29	0.43	0.14	0.67	0.25	1.17	0.11	23
	Male spadix	g	0.38	0.10	0.27	0.08	0.82	0.19	0.66	0.24	0.41	0.03	23
	Male florets	g	0.20	0.05	0.14	0.05	0.57	0.12	0.36	0.12	0.36	0.03	23
$\dot{M}\text{CO}_2$	Whole spadix	nmol s ⁻¹	33.7	11.4	20.2	6.5	36.8	9.0	9.5	2.1	368	117	9
	Appendix	nmol s ⁻¹	9.0	3.5			15.7	6.0			215	123	7
	Female spadix	nmol s ⁻¹	4.7	2.9			1.6	2.2					
	Male spadix	nmol s ⁻¹	20.0	7.0			19.5	4.7			164*	64	7
$\dot{M}\text{CO}_2$	Appendix	nmol s ⁻¹ g ⁻¹	7.3	1.0			8.8	1.3			17	10	7
	Male florets	nmol s ⁻¹ g ⁻¹	95.0	18.4			37.3	16.0			435*	180	7

Data for *A. idaeum* and *A. creticum* are spot measurements between 1200 and 1430 h, and between 1500 and 1700 h, respectively. Data for *A. concinatum* were extracted from continuous recordings at 1300 h for comparison with other two species. Numbers with asterisk (*) are measurements that include small contributions from the female spadix and floral chamber. Statistics are means and 95% confidence intervals (CIs).

mean temperature was 33.2 °C (Table 1). Perhaps the mass-specific respiration in *A. maculatum* is higher, because the appendix is smaller, yet it achieves a similar maximum temperature elevation (Bermadinger-Stabentheiner & Stabentheiner 1995). The male florets of intact *A. concinatum* peaked at 0.92 $\mu\text{mol s}^{-1} \text{g}^{-1}$, one of the highest rates of respiration known among plants (Table 1). For some reason, this is considerably higher than 0.04 $\mu\text{mol s}^{-1} \text{g}^{-1}$ measured *in vitro* in *A. maculatum* (Lance 1972), but similar to the 0.82 $\mu\text{mol s}^{-1} \text{g}^{-1}$ for male florets of *Helicodiceros muscivorus* (Seymour *et al.* 2003a).

Lack of temperature regulation

Temperature regulation in thermogenic plants is the characteristic that floral temperature is somewhat independent of ambient temperature. As ambient temperature decreases, floral temperature does not decrease as much, because the rate of heat production increases, partially offsetting higher rates of heat loss. Temperature regulation has three unique features that can be measured to assess its presence and effectiveness. Firstly, the difference between floral and ambient temperature increases at lower ambient temperatures. Secondly, rates of heat production (measured by respiration rate) increase at lower ambient temperatures. Thirdly, because floral tissue temperatures decrease to some extent at lower ambient temperature, respiration is inversely related to floral temperature. The latter characteristic is unusual in physiological systems, because normally the van 't Hoff effect causes respiration to change with

tissue temperature with a Q_{10} of about 2. However, the Q_{10} is less than 1 in thermoregulating flowers that increase respiration at lower temperatures.

The data from field *A. concinatum* satisfy only one of the criteria, and that only partially. Although there is some independence of peak appendix temperature from air temperature (Fig. 4), there was no stability of appendix temperature or independence from ambient temperature during the entire thermogenic episode (Figs 2 & 3). Moreover, the independence of peak temperature apparent in Fig. 4 is weak; the slope of the relationship ($b = 0.33$) is rather poor in the range of 1.0 (no regulation) to 0 (perfect independence). By comparison, the slope is 0.09 in skunk cabbage, *S. foetidus* (Seymour 2004), 0.17 in the sacred lotus, *N. nucifera* (Seymour & Schultze-Motel 1998) and 0.18 in the arum lily, *P. selloum* (Nagy *et al.* 1972). There is also no conclusive evidence that respiration increases at lower tissue temperatures in either the appendix (Fig. 5) or male florets (Fig. 6). The Q_{10} values are 3.9 and 1.8, respectively, not less than 1. Although there was often a progressive increase in the temperature difference between male florets and the air during the night (Figs 2 & 3), indicating that heat production was increasing at lower temperatures, this pattern is apparently 'hard-wired' into the flowering sequence and not true regulation. A similar pattern is evident in inflorescences of the 'pseudo-thermoregulatory' dead horse arum, *Helicodiceros muscivorus* (Seymour *et al.* 2003a).

True thermal regulation can be demonstrated by experimental manipulation of environmental and tissue temperatures, for example in *P. selloum* (Nagy *et al.* 1972),

S. foetidus (Seymour 2004), *N. nucifera* (Seymour, Schultze-Motel & Lamprecht 1998) and *Victoria amazonica* (Seymour & Matthews 2006). Recently, the mechanism has been shown to be present, although somewhat sluggish, in the Neotropical arum lily, *Philodendron melinonii*, by exposure of tissue samples to step changes in temperature (Seymour & Gibernau 2008). It is strongly present in Asian skunk cabbage, *Symplocarpus renifolius*, studied with the same technique (Seymour & Ito, unpublished results). However, despite 46 attempts to demonstrate this response in *A. concinatum* with the same technique, there were no consistent results (e.g. Fig. 6). We do not know what caused the seemingly random changes in respiration and must conclude that there is no thermoregulatory ability or it is simply overwhelmed by some factor other than temperature.

Failure to find physiological thermoregulation in *A. concinatum* does not undermine the proposed biochemically based regulatory mechanism developed from experiments with *A. maculatum* mitochondria (Wagner *et al.* 2008). The mechanism may be inherent in all Araceae, if not all thermogenic flowers, but only fully functional in some species. It would be interesting to look for evidence of reversible temperature regulation in *A. maculatum* inflorescences in the field and under experimental manipulations.

Ecological implications

The differences in metabolic intensity between the three *Cretan* species may be related to the interplay between: (1) the flowering environments; (2) the physics of heat flow; (3) the size of the inflorescences; and (4) the behaviour of the insect pollinators. *A. idaeum* and *A. creticum* have small inflorescences and bloom at high altitudes where the ambient temperatures are low and winds are high, whereas *A. concinatum* has one of the largest inflorescences of the genus and blooms at low altitudes, at higher ambient temperatures and less windy conditions (Table 2). The temperatures maintained by thermogenic flowers depend on the balance between rates of heat production and heat loss (Seymour 2001; Gibernau *et al.* 2005). Heat loss is enhanced if the inflorescences are small, exposed to low ambient temperature or in wind. Thus, in the alpine environments of *A. idaeum* and *A. creticum*, it would require large amounts of heat to increase inflorescence temperature appreciably. Therefore, it is reasonable to hypothesize that prohibitively high energy requirements may have selected against thermogenesis in these species and in turn may have reduced inflorescence size. Conversely, the lowland environments of *A. concinatum* make it easier for the inflorescence to raise its temperature significantly so may have favoured thermogenesis.

There are two roles of thermogenesis that bear on this hypothesis. Thermogenesis is certainly involved in the volatilization of floral scents (Fægri & van der Pijl 1979; Meeuse & Raskin 1988). Not only does warming increase the vapour pressure of scent compounds, but the heated air is less dense and carries the scent away by free convection (Barthlott *et al.* 2009). Thermogenesis is also associated

with an energy reward to insect visitors, by increasing their activities during their residence (Búrquez, Sarukhán & Pedroza 1987; Seymour, White & Gibernau 2003b). Both of these roles may be important in interpreting the responses of the plants. Firstly, in open pastures and treeless slopes at altitude, convective dispersion of scent would not seem to be a problem for *A. idaeum* and *A. creticum*, but it might be valuable to *A. concinatum* that grows preferentially in protected locations, among trees, shrubs and rocky outcrops. Secondly, the pollination systems differ in terms of timing and insect interactions. Inflorescences of *A. creticum* remain open and look fresh for several days, and attract mainly bees and beetles that are not trapped by them, because the spadix lacks the 'occlusion hairs' that prevent escape in other *Arum* species (Drummond & Hammond 1993; Diaz & Kite 2006). Diaz & Kite (2006) propose that *A. creticum* is the only food-reward plant among the 28 species of *Arum*. In contrast, *A. concinatum* is a typical saprophilous trap inflorescence, which has occlusion hairs that retain insects for approximately 24 h and offers no food energy reward (Gibernau *et al.* 2004). Insects trapped in the chamber would benefit from the intense thermogenesis by the male florets, which continues throughout the period of entrapment (Figs 2 & 3) and warms the floral chamber a few degrees (Albre *et al.* 2003).

It is becoming more apparent that thermogenesis by the male florets is not a by-product of pollen production in thermogenic flowers, because heating occurs in more than one episode over 2 d prior to pollen release in some *Arum* species (Gibernau *et al.* 2004). Also, the anthers of the parasitic *Hydnora* sp. produce copious pollen without significant respiration, whereas the scent-producing osmophore tissue is thermogenic (Seymour, Maass & Bolin 2009). Therefore the intense thermogenesis by the male florets has some other significance, and warming the insects is a reasonable hypothesis.

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