Respiration, temperature regulation and energetics of thermogenic inflorescences of the dragon lily *Dracunculus vulgaris* (Araceae)

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Dracunculus vulgaris is a protogynous arum lily with thermogenic inflorescences consisting of male and female florets on a spadix within a floral chamber. Above the chamber, an odour-producing appendix and a carrion-coloured spathe attract flying insects. The inflorescence shows a triphasic warming pattern. The floral chamber warms weakly on the first night as the spathe opens. Then the appendix produces a large amount of heat and a powerful scent during the first day. As the appendix cools on the second night, scent production ceases and the floral chamber rewarms. Warming ceases when the pollen is shed on the second day. The heating pattern is associated with attraction of pollinating insects by the appendix on the first day, entrapment in the warm chamber at night and release after pollen shedding. The temperature in the floral chamber is regulated at around 18 °C during the second night. The oxygen consumption rate of the florets is inversely related to the ambient temperature as in other thermoregulatory flowers. Conversely, the oxygen consumption rate of the appendix is directly related to the ambient temperature, indicating that it does not thermoregulate. Thus, temperature regulation is not associated with scent production, but with some activity inside the floral chamber.

Keywords: arum lily; inflorescence; thermoregulation; oxygen consumption; temperature; pollination

1. INTRODUCTION

Several members of the arum lily family (Araceae) produce inflorescences that warm themselves during the sequence of blooming (Meeuse & Raskin 1988). Some are able to raise their inflorescence temperature as much as 35 °C above ambient temperature and regulate it with surprising precision (Seymour & Schultze-Motel 1997). Heating in many species is associated with the production of pungent aromas that attract carrion-loving insects. The inflorescences are protogynous and male and female florets often reside in a floral chamber that acts as an insect trap. A striking example is the dragon lily, *Dracunculus vulgaris* Schott, which belongs to the subfamily Aroideae (Bown 1988). It occurs naturally in the eastern and central Mediterranean, from Greece to Corsica (Prime & Webb 1980), but ornamental cultivars thrive in regions as far ranging as Ireland (Blackith & Blackith 1993) and southern Australia (present study). Reaching nearly 1 m in length (Schmucker 1930), the single inflorescence of *D. vulgaris* presents an extraordinary appearance, with an erect, dark purple spike (properly termed the appendix of the spadix) suspended over a liver-coloured, velvety spathe. The spathe resembles decaying meat and the appendix gives off a powerful aroma which has been likened to a cadaver (Delpino 1874), 'long-dead, half-dried fish' (Meeuse & Hatch 1960) or 'fermenting human faeces' (Knoll 1926). The appearance and scent are highly

attractive to flies and beetles in its native geographical distribution (Piccioli, cited by Delpino 1874; Schmucker 1930) and elsewhere (Meeuse & Hatch 1960; Blackith & Blackith 1993).

As part of continuing investigations of thermal and energetic relationships between thermogenic plants and their pollinators, we measured floral temperatures and rates of oxygen consumption and evaporation continuously throughout blooming in *D. vulgaris*. This report documents the pattern of heat production in relation to the protogynous flowering sequence and relates it to the morphological changes in the inflorescence and the activities of insect visitors.

On the basis of temperature measurements, thermogenesis in *D. vulgaris* has been considered to be weak. For example, Schmucker (1930) measured only a 1–2 °C temperature rise in the appendix and the floral chamber and concluded that heating did not have any ecological significance. Skubatz *et al.* (1990) used a thermal imaging technique which indicated a short period of warming of the male florets and almost no warming of the appendix. However, a temperature rise may not be a good indication of heat production, because much of the heat can be lost by evaporation. Therefore, this study includes measurements of oxygen consumption and evaporation for a more direct analysis of metabolic heat production and evaporative heat loss.

A thermal study of *D. vulgaris* is also valuable in addressing the question of the ecological role of heat production and temperature regulation in thermogenic flowers. Heating may be adaptive because it increases the

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production and dispersal of attractant scents (Meeuse & Raskin 1988; Kite 1995). This compelling explanation occurs widely in the literature, but it may not be the only one. In the case of *Symplocarpus foetidus*, heating may be beneficial in protecting the inflorescence from damage by frost or in speeding its development in cold weather (Knutson 1979). We have suggested that heating in general and temperature regulation in particular may be a direct energy reward to insect visitors by providing them with a warm, stable environment in which they may remain active (Seymour & Schultze-Motel 1997). In all detailed studies of thermoregulating flowers (*Philodendron selloum* (Nagy *et al.* 1972; Seymour *et al.* 1983), *S. foetidus* (Knutson 1974, 1979; Seymour & Blaylock 1999) and *Nelumbo nucifera* (Seymour & Schultze-Motel 1996, 1998)), the sites of heat production and scent production lie within the floral chamber, so it has been impossible to separate the effects of heating on scent production and thermal conditioning of the insect enclosure. In *D. vulgaris*, on the other hand, scent production occurs largely in the appendix which lies completely outside the floral chamber. Our study uses this separation to demonstrate for the first time that temperature regulation is not closely associated with scent production.

2. MATERIAL AND METHODS

(a) *Plants and breeding system*

D. vulgaris Schott was studied outdoors between October and November during four flowering seasons (1995–1998) in gardens near Adelaide, South Australia. Observations of the timing of blooming and the behaviour of insect visitors were made visually and photographically with a time-lapse camera. Flowers were staged each morning using the following classifications: Y (young stage with spathe closed tightly), S1 (stigma stage with spathe loosening at the top of the floral chamber but still wrapped around the spadix), S2 (spathe open, upright and revealing appendix), S3 (spathe folded back from the appendix) and P (pollen visible). An Olympus 8 mm × 250 mm borescope was used to view pollen emergence in relation to time and ambient temperature in 26 flowers.

The breeding system was determined by exclusion experiments. Treatment inflorescences were chosen randomly and completely covered with thin muslin bags (circumference 60 cm and length 95 cm) from the Y stage until they began to wither. The bags covered the entire flower and were suspended from overhead to prevent the bag from interfering with anthesis. Six inflorescences were used for each treatment, 21 for controls and several extra flowers were temporarily bagged as pollen donors. The treatments were (i) self-pollination (bagged with no other manipulations), (ii) insect-facilitated self-pollination (bagged and two *Creophilus erythrocephalus* (Staphylinidae) beetles introduced into the floral chamber on the S2 day where they remained until several days after the P stage), (iii) cross-pollination 1 (bagged and pollen collected from pollen donors (P stage) with a long thin paintbrush and brushed onto the female florets on the S2 day), (iv) cross-pollination 1A (bagged and as for cross-pollination 1, but to prevent self-pollination after cross-pollination, a thin plastic-wrap collar was placed over the male florets and secured tightly with thread at the male–female floret junction), (v) cross-pollination 2 (bagged and as for cross-pollination 1, but flowers were manually cross-pollinated on the S3–P1 day) and (vi) control (not bagged or
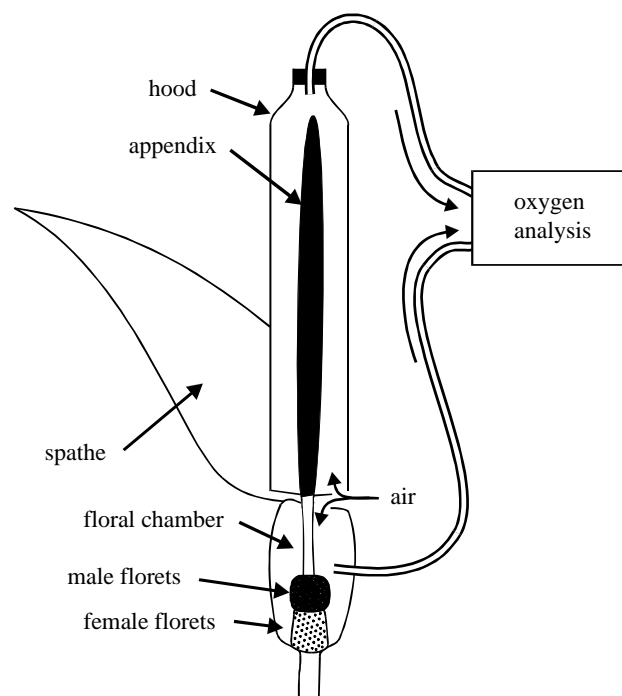

Figure 1. Diagram of a *D. vulgaris* inflorescence equipped for independent respirometry of the appendix and floral chamber.

manipulated). All flowers were assessed for seed set three weeks after the final flowers had reached the S2 stage.

(b) *Respiration and evaporation*

Oxygen consumption rates were measured with a portable, flow-through system that permitted continuous logging of respiration for several days. Oxygen was measured with a Taylor Servomex oxygen analyser and the output recorded with a Grant data logger. Continuous air flow was maintained in each of three sample channels and one empty reference channel and flow rates were measured with mass flow meters. A subsample of each channel in turn was diverted to the oxygen analyser for 6 min, giving a total cycling time of 24 min. Details of the respirometry equipment, calculations and assumptions are provided in Seymour & Schultze-Motel (1998).

Some inflorescences, including spathes, were fully covered with transparent plastic hoods, sealing the open end around the stem with plastic cling-wrap and tape, but leaving a narrow opening for entry of outside air. Gas from the hoods was pumped at *ca.* 400 ml min^{-1} into the analysis equipment. Respiration of the appendix and lower spadix were measured separately in other inflorescences (figure 1). In these cases, the spathe was carefully unwrapped and the appendix enclosed in a hood, as described. The floral chamber was used as its own respirometry chamber by partially sealing it with plastic wrap stuffed into the narrow opening between the spathe and the spadix and inserting the air sampling tube through the side of the spathe directly into the chamber. Temperatures in the middle of the appendix, in the male florets inside the floral chamber, in air inside the hood and in the ambient air were measured with fine PVC-coated copper-constantan thermocouples and logged at 2 min intervals. The inflorescences were shaded with umbrellas and 70% shade cloth, where necessary, to reduce direct heating by the sun.

In a separate set of experiments, temperature regulation in the floral chamber was confirmed by measuring the oxygen

uptake in the chambers of four inflorescences equipped with a water jacket to control the ambient temperature artificially. The water jacket was a 2 litre urine-collecting bag which was loosely wrapped around the floral chamber and insulated on the outside with layers of foam and plastic wrap. Tubing attached to ports on the top and bottom of the bag connected to a Julabo refrigerated water bath and the weight of the entire jacket was supported by a post above the inflorescence. The equipment was installed on S2 inflorescences in the late afternoon with the water bath at 10 °C. At approximately midnight the temperature was raised to 20 °C and reduced to 10 °C again in the morning.

Evaporation rates were measured in two inflorescences with a similar flow-through system which involved a plastic hood on the naked appendix. Air was pumped from the hood at *ca.* 7 l min^{-1}, through a flow meter and into a Vaisala Humitter® 50Y humidity and temperature sensor. Another sensor monitored the air entering the hood. The sensors were calibrated above saturated salt solutions and the flow meter was calibrated with a 20 l volumetric respirometer. The water vapour density ρ_{H_2O} (mg l^{-1}) was calculated from the relative humidity (RH) (%) and a polynomial regression we derived from tabled data for saturated water vapour density and air temperature (T_a) (°C): $\rho_{H_2O} = 0.01 RH (0.0004 T_a^3 + 0.0006 T_a^2 + 0.4439 T_a + 4.6078)$. The evaporation rate (mg min^{-1}) was calculated as the product of the flow rate and the difference in the water vapour densities of the inlet and outlet air.

(c) *Bomb calorimetry*

Tissue samples cut from inflorescences before and after the thermogenic period were weighed and frozen in glass vials at -18 °C. The samples were then freeze-dried, weighed again, powdered, oven-dried at 50 °C for 1 h and stored over silica gel. The energy content was measured with a 1107 semimicro bomb of a Parr 1261 bomb calorimeter after calibration with dry benzoic acid.

3. RESULTS

(a) *Morphology and anthesis of the inflorescence*

Each *D. vulgaris* plant produces a single inflorescence which consists of a central spadix ending in a long sterile appendix which is encircled by a large spathe (figure 1). The base of the spathe forms a floral chamber within which male and female florets form separate zones around the spadix stalk. The dimensions and masses of the inflorescence parts are given in table 1.

Casual observations and time-lapse photography of the flowering sequence revealed a relationship between spathe opening, scent production, pollen release and insect behaviour. The timing of flowering was arbitrarily divided into (i) the first night when heating occurred in the floral chamber, (ii) through the first day when the appendix produced heat and scent, (iii) the second night when the floral chamber heated again and, finally, (iv) the second day when the sequence ended. The spathe began to open in the evening of the first night and the appendix became visible at approximately midnight (stage S2). Scent production increased greatly in the morning and continued throughout the first day. The male florets were all dark purple and smooth during the first day. Throughout the first day, the spathe usually stood nearly upright, parallel to the spadix, but bending slightly back at the tip (stage S2). It never closed, but

Table 1. *Morphometrics of* D. vulgaris *inflorescences*

	mean	95% CI[a]	*n*
length of lower spadix with florets (mm)	34.4	2.2	17
length of appendix (mm)	614	32	18
mass of male florets (g)	1.89	0.29	17
mass of female florets (g)	2.75	0.45	17
mass of lower spadix with florets (g)	8.2	1.2	17
mass of appendix (g)	47.2	8.1	19

[a] 95% confidence intervals.

gradually relaxed, so by the morning of the second day, it arched well back with the tip pointing downwards (stage S3). On this morning there was no pollen at 09.00 in 26 plants observed with the borescope, but it appeared (stage P) between 09.00 and 11.00 (mean 10.02 ± 00.42 CI).

All of the inflorescences that were cross-pollinated on the first day set seed, including those with plastic-wrapped male florets. None of the self-pollination treatments, either with or without beetles or those cross-pollinated on the second day when pollen was released set any seed. Of the 21 control inflorescences only two set seed.

(b) *Insect behaviour*

Changes in the characteristics of the floral chamber were indicated by the behaviour and abilities of the insects that visited the flowers. On the first day, the stinking appendices attracted a large numbers of flies. The species that we observed on or near thermogenic inflorescences included *Hydrotaea rostrata* (Muscidae), three unidentified species (Muscidae), *Sarcophaga crassipalpis* (Sarcophagidae), *Lucilia sericata*, *Onesia tibialis*, *Calliphora vicina* and *Calliphora dubia* (Calliphoridae) and two unidentified species (Piophilidae and Faniidae). They flew around the inflorescence and landed on the appendix and spathe, but they rarely entered the floral chamber. In addition, several species of beetle, including *C. erythrocephalus*, *Aleochara brachialis* and *Aleochara speculifera* (Staphylinidae), *Saprinus* sp. (Histeridae), *Dermestes frischii* (Dermestidae), *Ptomaphila* sp. (Sylphidae) and *Carphurus* sp. (Melyridae), visited the inflorescences. In contrast to the flies, the beetles typically flew towards the inflorescence, landed clumsily on the spathe, lost their grip and fell into the floral chamber. Beetles that entered the chamber voluntarily and ones that were placed inside experimentally were unable to crawl out of the vertical inflorescences on the first day, either up the walls of the chamber or up the base of the appendix. However, they could emerge from horizontal inflorescences on the first day by crawling along the base of the spadix or along the walls. Beetles inside the floral chamber appeared much less active on the first day compared to the second day when they continually tried to escape. They were able to emerge from second-day flowers if they crawled up the base of the appendix which now provided traction. However, they often had to make several attempts, climbing up part way and then falling to the bottom and

Table 2. *Summary of respiration and energetics of* D. vulgaris

(Data are means 95% confidence intervals (number of inflorescences).)

| parameter (units) | floral chamber | | appendix | entire inflorescence |
	first night	second night		
basal $\dot{M}O_2$ ($\mu mol\,s^{-1}$)	0.025 ± 0.007 (4)	0.025 ± 0.007 (4)	0.103 ± 0.062 (4)	0.136 ± 0.036 (9)
duration (h)	11.2 ± 1.3 (5)	28.7 ± 3.1 (4)	26.3 ± 3.3 (5)	47.8 ± 4.0 (9)
$\dot{M}O_{2max}$ ($\mu mol\,s^{-1}$)	0.335 ± 0.074 (11)	0.880 ± 0.099 (11)	3.62 ± 1.15	—
$\dot{M}O_{2max}$ ($\mu mol\,s^{-1}\,g^{-1}$)	0.043 ± 0.011 (11)	0.113 ± 0.017 (11)	0.072 ± 0.013 (11)	—
net $\dot{M}O_2$ (mmol)	5.2 ± 2.7 (4)	37 ± 7 (3)	125 ± 87 (4)	127 ± 22 (7)
T_{flower} at $\dot{M}O_{2max}$ (°C)	17.9 ± 2.1 (11)	18.6 ± 2.5 (11)	26.6 ± 3.1 (11)	—
T_a at $\dot{M}O_{2max}$ (°C)	14.2 ± 1.9 (11)	10.3 ± 1.7 (11)	24.1 ± 2.9 (11)	—
ΔT at $\dot{M}O_{2max}$ (°C)	3.7 ± 1.2 (11)	8.4 ± 2.1 (11)	2.5 ± 0.7 (11)	—

picking up pollen from the male florets on the way down. Upon emerging from the floral chamber, they lost traction on the appendix, fell onto the spathe and usually flew away.

(c) *Temperatures, respiration and evaporation*

Warm weather appeared to trigger flowering; simultaneous opening of many plants often occurred on warm nights. This effect is apparent in the significantly higher mean ambient temperature (T_a) on the first night (14.2 °C) than on the second night (10.3 °C) (table 2).

A consistent pattern of heating and the oxygen consumption rate ($\dot{M}O_2$) appeared in 11 inflorescences (figure 2). The temperatures of the appendix (T_{ap}) and male florets in the floral chamber (T_{fc}) were similar to T_a until the evening before spathe opening when a minor episode of warming by the florets caused the mean T_{fc} to rise 3.7 °C above T_a and $\dot{M}O_2$ to increase to a maximum of *ca.* 0.33 $\mu mol\,s^{-1}$ (figure 2 and table 2). The floral chamber's $\dot{M}O_2$ returned to basal values during the second half of the night. The spathe opened in the early morning, exposing the appendix, which began warming rapidly, reaching a peak of *ca.* 3.6 $\mu mol\,s^{-1}$ and a maximum temperature excess of 2.5 °C over the internal hood temperature (T_h) at about noon (table 2). Otherwise T_h was practically superimposible on T_{ap} (and therefore not shown in figure 2). Thermogenesis in the appendix decreased steeply during the afternoon, reaching basal levels by the evening. As heat production in the appendix decreased, the florets in the floral chamber began to warm to a maximum of 8.4 °C above T_a when $\dot{M}O_2$ reached 0.88 $\mu mol\,s^{-1}$. Aside from being more intense than the first heating of the floral chamber, the second heating persisted more than twice as long (table 2). Heat production faded to baseline during the subsequent day.

Floral temperatures were related to ambient temperatures. To summarize the patterns, temperature data for the appendix and floral chamber were collected within 2 °C intervals of the 'ambient temperature' (figure 3). Hood temperatures were used for the appendix and for floral chambers that were covered with a hood during combined respirometry of the whole inflorescence. Ambient air temperatures were used when the floral chamber was unhooded. For this analysis, 24 h data sets were taken from noon to noon (described as first or second night of the floral chamber) or from midnight to

midnight (described as first day for the appendix). There were linear relationships between the floral and ambient temperatures and the regression equations were as follows: (i) appendix, $T_{ap} = 1.07\,T_h - 0.10$ ($r^2 = 0.99$); (ii) floral chamber first night, $T_{fc} = 0.77\,T_a + 6.0$ ($r^2 = 0.99$); and (iii) floral chamber second night, $T_{fc} = 0.58\,T_a + 11.8$ ($r^2 = 0.98$).

The effect of T_a on $\dot{M}O_2$ was analysed in the same way (figure 4). The ambient temperature affected the $\dot{M}O_2$ of the appendix and floral chamber in opposite directions. The appendix $\dot{M}O_2$ increased with increasing T_h (figure 4a). Because T_{ap} was nearly equal to T_h, $\dot{M}O_2$ increased with T_{ap} as predicted from the Van't Hoff principle, with a Q_{10} of 3.8. T_{ap} usually increased and decreased unimodally with T_a during the day but, on one occasion, it dipped *ca.* 10 °C during 4 h around noon (figure 2a, inset). This caused $\dot{M}O_2$ to decrease and then rise, showing that $\dot{M}O_2$ depended on T_{ap}, not time of day. In contrast to the appendix, the $\dot{M}O_2$ of the floral chamber was low and nearly independent of T_a on the first night, but increased greatly at lower T_a on the second night (figure 4b). A hysteretic relationship between $\dot{M}O_2$ and T_a appeared in the floral chamber during the 24 h period including the second night; at a given T_a, $\dot{M}O_2$ was lower when T_a was decreasing than when it was increasing. Due to averaging, this relationship is obscured in figure 4.

Four water-jacketed inflorescences in which the oxygen consumption of the floral chamber was measured showed similar responses to an artificial ambient temperature change. The water bath temperature was abruptly changed in the middle of the second night (between 00.00 and 02.00). $\dot{M}O_2$ was averaged for 2 h before the change and for another 2 h period that began 1 h after the change. Before the change, the mean T_a was 10.2 °C (± 0.2 CI), T_{fc} was 14.7 °C (± 2.3) and $\dot{M}O_2$ was 0.54 $\mu mol\,s^{-1}$ (± 0.17). After the change, T_a rose to 20.1 °C (± 0.9), T_{fc} to 22.5 °C and $\dot{M}O_2$ dropped to 0.37 $\mu mol\,s^{-1}$ (± 0.08). A paired *t*-test was significant ($p < 0.05$).

The characteristics of the energy expenditures during the three episodes of heating were quantified from records of $\dot{M}O_2$ (table 2). Complete records were available from four inflorescences involving separation of the appendix and floral chamber and from five whole inflorescences. Nearly complete records were used from two others. To calculate the maximal $\dot{M}O_2$ ($\mu mol\,s^{-1}$), the
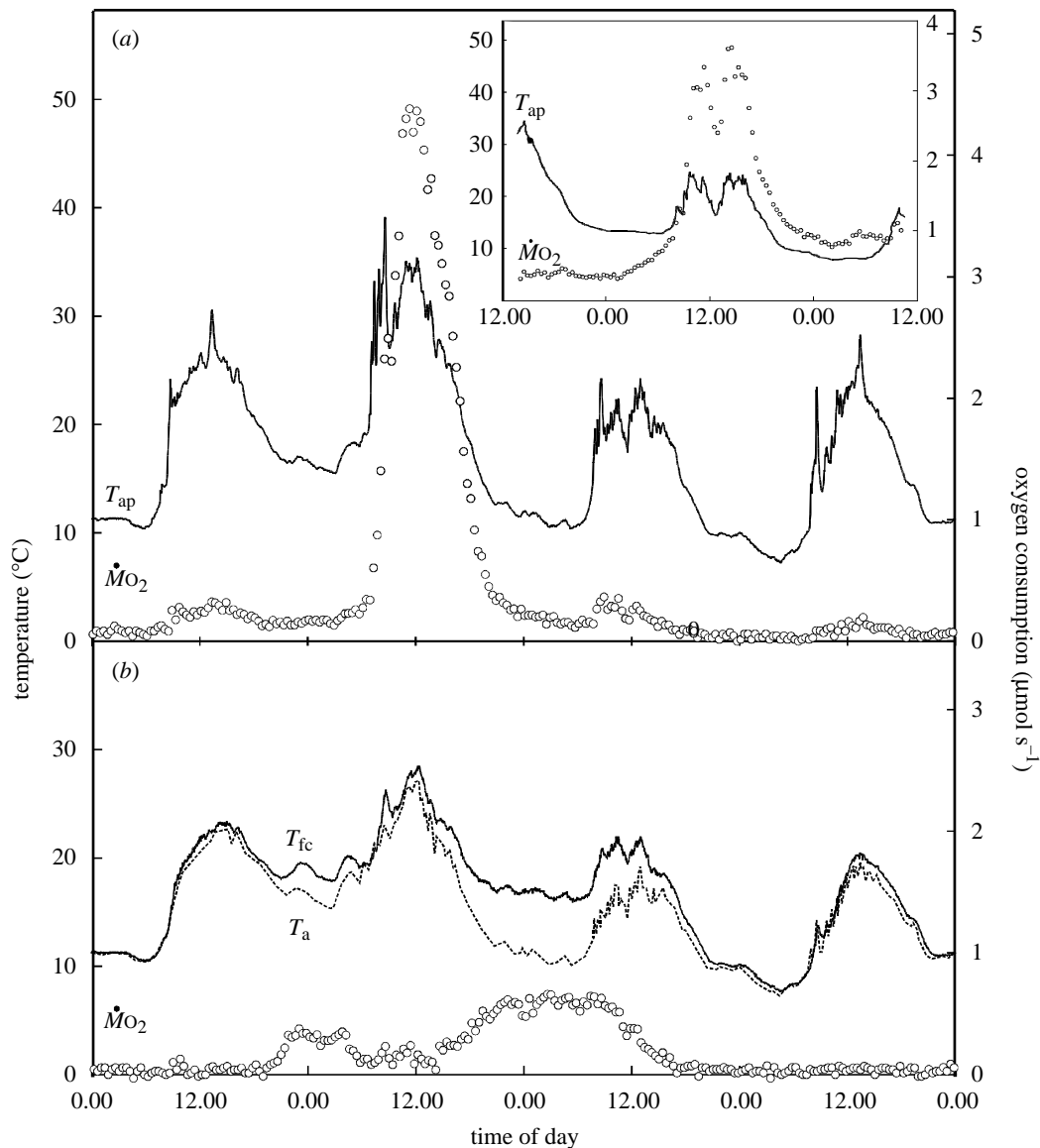
Figure 2. Time-course of temperatures of (*a*) the appendix (T_{ap}) and (*b*) the floral chamber (T_{fc}) and ambient air (T_a) along with oxygen consumption rates ($\dot{M}O_2$, circles) during thermogenesis in a *D. vulgaris* inflorescence throughout a complete sequence of blooming. The inset in *a* shows data from the appendix of another inflorescence when T_a dipped during the middle of the day.

absolute maximum was averaged with the two preceding and the two following values ($n = 5$) from the original data file, comprising an interval of 96 min. The maximal mass-specific $\dot{M}O_2$ ($\mu\text{mol s}^{-1}\text{g}^{-1}$) was calculated by dividing this average value by the mass of the appropriate tissue, either the whole appendix or the lower spadix with florets and adjusting the time unit. The total oxygen consumed during each heating episode was calculated by adding all individual measurements for $\dot{M}O_2$ ($\mu\text{mol s}^{-1}$) and multiplying by 1440 s, i.e. the duration of a sampling interval. The beginning and end of a warm-up period were marked by a 30% elevation of $\dot{M}O_2$ above the basal level. The basal $\dot{M}O_2$ was calculated as a 24 h average of $\dot{M}O_2$ before or after the heating episodes. The net amounts of oxygen consumed during an episode were obtained by subtracting an amount calculated from the basal rate from the total oxygen consumed. When $\dot{M}O_2$ had been measured from whole inflorescences rather than separately for the appendix and the floral chamber, it was

assumed that the peaks of $\dot{M}O_2$ during the first and second night were entirely due to metabolic activity of the floral chamber and the large peak on the first day was due to the appendix only. It was not possible to separate the total energy expenditure of the appendix and floral chamber in whole flowers, because the episodes overlapped.

The appendix produced the most heat. Not only was the maximum $\dot{M}O_2$ of the appendix four times higher than that of the floral chamber, the net oxygen consumed was 3.3 times higher (table 2). However, the mass-specific $\dot{M}O_2$ of the appendix was 0.072 $\mu\text{mol s}^{-1}\text{g}^{-1}$, which was lower than the 0.113 $\mu\text{mol s}^{-1}\text{g}^{-1}$ of the lower spadix. The $\dot{M}O_2$ contributions by the two floral types is unknown, but their mass-specific rates were apparently high. Assuming that the male florets were totally responsible (Skubatz *et al.* 1990), the mean rate was 0.48 $\mu\text{mol s}^{-1}\text{g}^{-1}$; if both males and females were involved, the rate was 0.20 $\mu\text{mol s}^{-1}\text{g}^{-1}$. Overall, there was a linear relationship
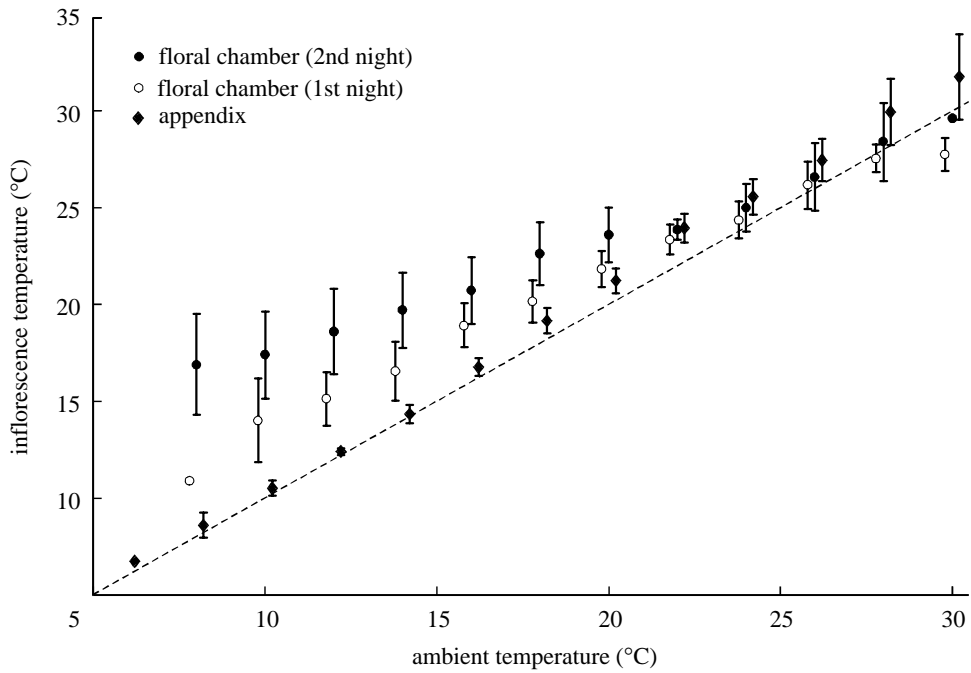
Figure 3. Effects of ambient temperature on temperatures of the appendix and floral chamber in *D. vulgaris*. Ambient temperature was taken as the hood temperature when the appendix and floral chamber were surrounded by a respirometry hood; otherwise the ambient temperature is the adjacent air temperature. The dashed line is isothermal. Means and 95% confidence intervals are given for data obtained within each $2\,^{\circ}C$ interval of ambient temperature. Sample sizes are up to five inflorescences; single points represent one or two. Points slightly shifted for clarity.
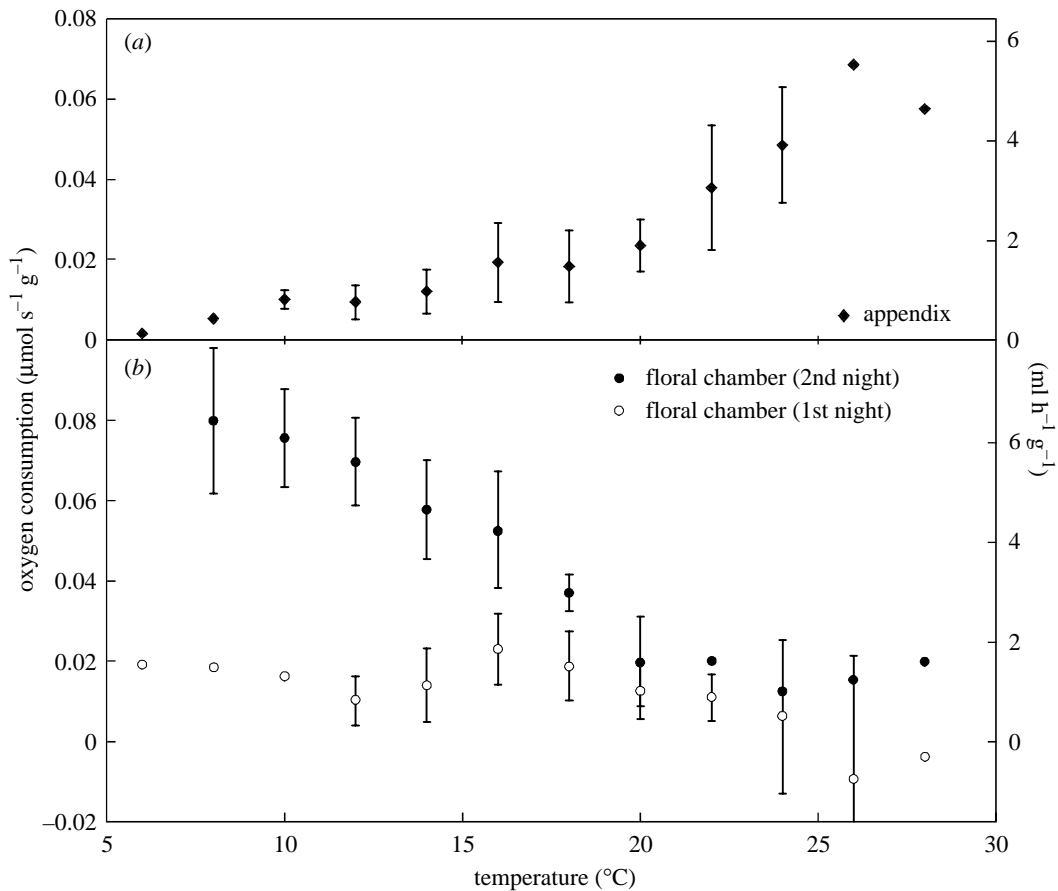

Figure 4. Effects of ambient temperature on the $\dot{M}O_2$ of (*a*) the appendix and (*b*) the floral chamber in *D. vulgaris*. Ambient temperatures are hood temperatures for the appendix and adjacent air temperatures for the floral chamber. Open circles represent the first night of heating of the floral chamber and closed circles represent the second night. Statistics are as in figure 3.

between the maximum MO_2 and mass of the appendix, but regression analysis showed that the mass-specific MO_2 was independent of appendix mass ($r^2 = 0.002$). The total oxygen consumed by the thermogenic tissues ($MO_{2_{tot}}$) (mmol) was directly related to their mass (M) (g): $MO_{2_{tot}} = 2.77M - 4.46$ ($r^2 = 0.87$).

The evaporative water loss from the naked appendix in two inflorescences was less than $0.44\,\mathrm{mg\,s^{-1}}$ on the day before warming and 0.03–$0.1\,\mathrm{mg\,s^{-1}}$ on the first and second nights. On the first day, coinciding with the metabolic warm-up, it increased to a maximum of $1.03\,\mathrm{mg\,s^{-1}}$.

(d) *Energy and water contents*

The tissues examined were the appendix, female florets and male florets cut from the spadix stalk. There was no significant difference in the energy density ($\mathrm{J\,mg^{-1}}$ of dry mass of floral tissues) before and after thermogenesis. The means s.d. (n) before and after flowering, respectively, were as follows: (i) appendix, 15.5 (2) versus 15.7 ± 2.6 (5); (ii) female florets, 16.1 ± 0.9 (3) versus 15.7 ± 1.2 (5); and (iii) male florets 16.3 ± 0.5 (3) versus 17.8 ± 1.2 (5). The mean water contents of these tissues were 79.3, 83.4 and 70.3% wet mass, respectively.

4. DISCUSSION

(a) *Pattern of thermogenesis*

There are three phases of heat production in *D. vulgaris*: (i) a minor warming of the male florets in the floral chamber on the first night when the spathe opens, (ii) a major heat production by the appendix during the next day when a powerful scent is produced and (iii) a moderate warming of the floral chamber on the second night, before the pollen is released on the second day (figure 2). This triphasic pattern has also been documented in the arum lily *Arum maculatum* by Bermadinger-Stabentheiner & Stabentheiner (1995) who used infrared thermography. The biphasic warming of the male florets in the floral chamber is divided by a peak in the appendix warming associated with intense production of a putrid odour. However, while the entire sequence occurs within one day in *A. maculatum*, it requires two days in *D. vulgaris*, so there are major differences in the timing of the phases. The sequence of warming in *Arum italicum* and *Arum dioscorides* is also short, as brief as 1 h, but the pattern does show discrete warming events in the male florets and appendices (Skubatz *et al.* 1990).

The relationship between heat production and inflorescence temperature differed greatly between the appendix and the floral chamber. While the MO_2 of the appendix was directly related to T_a, they were inversely related in the floral chamber (figure 4). The inverse relationship was also evident in the water-jacketed inflorescences which showed a decreased MO_2 with artificially increased T_a. This relationship is a characteristic of other thermoregulating plants (Nagy *et al.* 1972; Knutson 1974, 1979; Seymour *et al.* 1983; Seymour & Schultze-Motel 1996, 1998; Seymour & Blaylock 1999).

(b) *Physiological implications of temperature regulation*

The opposite responses of the appendix and male florets suggest that the biochemical control of thermogenesis is fundamentally different in the two tissues. Heat generation in some arum lilies is triggered by salicylic acid (Raskin *et al.* 1989) and is associated with activity of both the normal cytochrome electron transport pathway and the alternative, cyanide-insensitive pathway (Meeuse & Raskin 1988; Skubatz *et al.* 1990). However, the mechanism by which rising temperature reversibly inhibits respiration in thermoregulatory tissues is not yet known. It is known, however, that the appendix of the voodoo lily *Sauromatum guttatum* is sensitive to salicylic acid while the male and female florets are not (Skubatz *et al.* 1991), which indicates that there may be inherent differences in the responses of different tissues in the same inflorescence.

The temperature regulation in the floral chamber of *D. vulgaris* is less precise than in other thermoregulating plants. The slope of the regression line for T_{fc} on T_a quantifies thermoregulatory precision; nearly perfect regulation produces slopes approaching zero (T_{fc} nearly independent of T_a) and no regulation produces slopes near 1.0. The slope is 0.59 in *D. vulgaris* (figure 4), compared to 0.18 in *P. selloum* (Nagy *et al.* 1972) and 0.17 in *N. nucifera* (Seymour & Schultze-Motel 1998). In *S. foetidus* the slope for large inflorescences is 0.29 (Knutson 1974), but in small ones it is 0.51 (Seymour & Blaylock 1999). The slope of this relationship is an essential component in temperature regulation at the cellular level and it is determined by the shape of the relationship between heat production and floret temperature in the region where temperature becomes inhibitory to the thermogenic biochemical pathways (Seymour *et al.* 1983). If the tissue is very sensitive to thermal inhibition, then temperature regulation is more precise. The present data suggest that the male floret tissue is less sensitive to thermal inhibition than in other species. It may be significant that inhibition occurs at relatively low temperatures in *D. vulgaris* (*ca.* 18 °C) and *S. foetidus* (*ca.* 20 °C), both of which have less thermoregulatory precision compared to species that regulate at higher temperatures, for example *P. selloum* (*ca.* 39 °C) and *N. nucifera* (*ca.* 32 °C).

(c) *Energetics of heat production*

The maximum rates of mass-specific MO_2 by the appendix (0.072 $\mathrm{\mu mol\,s^{-1}\,g^{-1}}$) and the male florets (0.113 $\mathrm{\mu mol\,s^{-1}\,g^{-1}}$) were modest among thermogenic flowers. Appendix tissue of *A. maculatum* can briefly reach 0.5 $\mathrm{\mu mol\,s^{-1}\,g^{-1}}$ (Lance 1974), intact sterile male florets of *P. selloum* climb to 0.37 $\mathrm{\mu mol\,s^{-1}\,g^{-1}}$ (Seymour *et al.* 1983), whole spadices of *S. foetidus* reach *ca.* 0.27 $\mathrm{\mu mol\,s^{-1}\,g^{-1}}$ (Knutson 1974; Seymour & Blaylock 1999) and the entire receptacle of *N. nucifera* peaks at *ca.* 0.14 $\mathrm{\mu mol\,s^{-1}\,g^{-1}}$ (Seymour & Schultze-Motel 1998). It would be interesting to know whether these differences in aerobic metabolic capacity are matched by differences in mitochondrial density of the thermogenic tissue.

At maximum heat production, the appendix produced *ca.* 1.7 W (table 2) (assumed caloric equivalent of oxygen = 473 $\mathrm{J\,mmol^{-1}}$). Lower rates of heat production by *N. nucifera* are sufficient to raise the flower temperature more than 20 °C above the environmental temperature (Seymour & Schultze-Motel 1998), but the temperature of the *D. vulgaris* appendix did not rise appreciably (table 2). Two explanations account for this difference. First, the

appendix loses heat quickly to the air because it has a large surface area and it is unprotected by a spathe. Second, it loses considerable heat by evaporation. Based on an evaporation rate of $0.92 \, \mathrm{mg \, s^{-1}}$ and a latent heat of evaporation of $2.46 \, \mathrm{J \, mg^{-1}}$, the rate of evaporative heat loss is *ca.* $2.2 \, \mathrm{W}$. Therefore the appendix can evaporate more heat than it generates, accounting for the similarity of T_{ap} and T_a.

Although we have no measure of the change in the total energy content of individual inflorescences, we can conclude that energy was imported during heating. The total heat produced by the floral chamber during the first and second nights ($20 \, \mathrm{kJ}$) (table 2) was more than that contained in pre-warm-up male and female florets combined ($16.5 \, \mathrm{kJ}$ and dry mass $= 1.02 \, \mathrm{g}$) and the heat production by the appendix ($59 \, \mathrm{kJ}$) (table 2) was 39% of its total initial energy content ($152 \, \mathrm{kJ}$ and dry mass $= 9.8 \, \mathrm{g}$). It appears, therefore, that substrates were imported, at least by the florets. The situation is the same in *S. foetidus* which imports carbohydrates into the spadix during its lengthy thermogenic period (Knutson 1974; Seymour & Blaylock 1999).

(d) *Role of thermogenesis in pollination*

Our exclusion experiments show that cross-pollination is obligatory in *D. vulgaris* and the fact that seed set in a few of the control plants suggests that insects are pollen vectors. We feel that this inflorescence caters primarily for carrion beetles. Most authors record that both flies and beetles are attracted and our collections included species of five families of coleopterans and five families of dipterans, most of which are typically attracted to carrion (J. F. Wallman, personal communication). Schmucker (1930) found comparatively few pollinators in *D. vulgaris* which he studied within the native distribution in Crete, but these consisted mainly of beetles (staphylinids, dermestids and others) and flies. Delpino (1874) observed the blooming of *D. vulgaris* in Italy and noted that the inflorescences were visited by flies and beetles. He considered that flies were the most important pollen vectors because they moved faster, although he and an entomologist (Piccioli) found as many as 200 beetles in a single inflorescence. Numerous flies were netted around a single ornamental plant in Ireland by Blackith & Blackith (1993), who recorded that staphylinid beetles were absent on the plant. Our observations agree with those of Meeuse & Hatch (1960) who observed the plants in Washington State, USA. They noted that, although a large number of flies were attracted to the inflorescences, they usually did not enter the floral chamber. On the other hand, they remarked that many beetles did enter and, on one occasion, they obtained 162 beetles (mostly histerids) from a single first-day inflorescence.

The structure of *D. vulgaris* has some characteristics typical of so-called 'beetle flowers', namely large size, broad landing area, large internal floral chamber, many carpels and prodigious production of pollen (Gottsberger 1988). However, unlike many flowers that beetles remain in voluntarily, *D. vulgaris* is a sliding trap that does not appear to offer a food reward. The spathe and floral chamber are well designed to direct beetles into the plant during the stage of female receptivity and to release them after the pollen is shed. The upright spathe forms a chute that promotes entry into the floral chamber during the first day and the slippery walls of the chamber and the base of the spadix prevent escape until the next day. Beetles are trapped overnight and are coated with pollen during their struggles to escape. Eventually, the lower spadix provides traction and the beetles emerge and drop onto the relaxed spathe which creates a platform for departure.

We hypothesize that thermogenesis in the appendix is related solely to scent production which attracts the insects and that warming of the floral chamber is directed at their well being while resident during the second night. Several characteristics of the flower are consistent with this idea. Although the appendix produces considerable heat, it does not warm up very much (figure 3). It is unprotected by a spathe and its heat is lost immediately by evaporation and convection to the air. Beetles attracted to it do not remain near it, but fall immediately into the floral chamber. On the other hand, the floral chamber is surrounded by the spathe which tends to retain the heat and its temperature rises considerably (figure 3). Despite warming of the floral chamber, the odour of the inflorescence decreased dramatically, suggesting that heating of the floral chamber is not closely associated with scent production. Therefore, it is significant that the only part of the plant that warms is designed to trap insects during the second night of blooming. It is also consistent in that heating of the floral chamber ceases just after the insects are released to carry pollen away to attractive first-day inflorescences.

It might be argued that warming of the floral chamber is associated with the production of other scents that are less intense than that from the appendix. Kite (1995) collected no less than 56 volatile odour compounds from inflorescences of the related arum lily *A. maculatum*, most being associated with the appendix, but one in particular emanating from the floral chamber. For the same species, Bermadinger-Stabentheiner & Stabentheiner (1995) also reported a fruit-like scent when the male florets began heating. However, it is not clear what advantage would be accrued by exposing an already trapped insect to an odour peculiar to the floral chamber. Heating of the chamber may also be associated with maturation of the pollen or some other synthetic activity of the florets. It is true that the heat comes mainly from the male florets (Skubatz *et al.* 1990), but the actual shedding of pollen occurs during the cooling phase of the floral chamber. Moreover, direct and indirect calorimetry of thermogenic *P. selloum* (Seymour *et al.* 1983) and *N. nucifera* (Lamprecht *et al.* 1998) showed that heat production is not associated with any measurable synthesis of floral tissue.

In *D. vulgaris*, the floral chamber is maintained at *ca.* $18 \, ^{\circ}\mathrm{C}$ (table 2). Although a temperature similar to that maintained by the skunk cabbage *S. foetidus* (Knutson 1974; Seymour & Blaylock 1999), it is considerably lower than the range of 28–$44 \, ^{\circ}\mathrm{C}$ maintained by other thermoregulating flowers that are associated with beetle pollination (Seymour & Schultze-Motel 1997). Many beetles require body temperatures above *ca.* $30 \, ^{\circ}\mathrm{C}$ to initiate flight and they may require similar temperatures to engage in other activities such as mating or digesting. However, until we have information about the thermal requirements of the insects that have evolved with specific thermoregulating plants, we will be unable to test this hypothesis.

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