Functional Ecology 2003
17, 886–894

Thermogenesis and respiration of inflorescences of the dead horse arum *Helicodiceros muscivorus*, a pseudo-thermoregulatory aroid associated with fly pollination

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Summary

1. In central Corsica, *Helicodiceros muscivorus* (Schott ex. K. Koch) produces a protogynous inflorescence that resembles the anal area of a dead mammal and produces a foetid scent during the few hours after sunrise. Flies enter the floral chamber, pollinate the female florets and become trapped until the next morning, when pollen is shed from the male florets and the flies are released.

2. The exposed appendix exhibits a strong, unimodal episode of thermogenesis associated with scent production, reaching a maximum of 30 °C at 15 °C ambient temperature. The male florets in the floral chamber are highly thermogenic throughout the second night and generally maintain stable floret temperatures of about 24 °C at ambient temperatures down to 13 °C.

3. Maximum respiration rates of the appendix (0·45 μmol CO_2 s^{-1} g^{-1}) and the male florets (0·82 μmol s^{-1} g^{-1}) may be the highest recorded for plant tissue.

4. Thermogenesis of the appendix does not depend on ambient temperature, but that of the male florets increases with decreasing ambient temperature in most cases. However, the pattern of heat production by the males appears related more to time than to ambient temperature, hence the term 'pseudo-thermoregulation'.

5. The behaviour and thoracic temperatures of flies emerging from captivity suggests that male floral warming does not enhance their activity.

Key-words: Araceae, flower, heat production, pollination, protogyny

Functional Ecology (2003) **17,** 886–894

Introduction

The dead horse lily *Helicodiceros muscivorus* (Schott ex. K. Koch) is a member of the arum lily family (Araceae). Its distribution is limited to Corsica, Sardinia and the Balearic Islands in the western Mediterranean Ocean (Mayo, Bogner & Boyce 1997; Fridlender 2000). In Corsica, 30 known populations are located on limestone and granite rock crevices on islets or along the coast, and on inland rocky cliffs up to 700 m on the main island (Fridlender 2000). The protogynous inflorescence resembles the anal area of a dead mammal, complete with a hairy spathe and a 'tail' consisting of the hairy appendix of the spadix, and it emits a putrid scent that attracts necrophilic insects (Knuth 1909; Bown 2000; Stensmyr *et al*. 2002). It is apparently pollinated mainly by flies (Knuth 1909). Arcangeli (1883) recorded 371 flies from a single inflorescence [primarily *Lucilia* (*Somomyia*) and *Calliphora* species] and only seven beetles; in another inflorescence he collected 385 flies, 107 of which were *Lucilia caesar*. These insects are enticed to enter a floral chamber where they deliver pollen to receptive female florets, mainly between sunrise and noon. They are trapped in the chamber and remain there overnight, presumably while the receptivity of the female florets wanes. Early the next morning the male florets begin to produce pollen, which coats the insects before their escape and attraction to another inflorescence. *Helicodiceros muscivorus* has been thought to be carnivorous, because dead insects are commonly found at the bottom of the floral chamber (Fridlender 2000). The smaller carrion flies attracted by the strong putrid odour were thought to be unable to escape from the floral chamber because they were held firmly by sticky hairs and digested by their secretion (Knuth 1909). However, there is no evidence that the plant gains any nutrients from the flies.

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Many species in the arum lily family are thermogenic (Meeuse & Raskin 1988), but the extent and pattern of thermogenesis in *H. muscivorus* have not been measured. The pattern in its close relative, the beetle-pollinated *Dracunculus vulgaris*, is triphasic, with a single, intense thermogenesis in the appendix during the day, bracketed by two episodes of warming of the male florets in the floral chamber at night (Seymour & Schultze-Motel 1999). Thermogenesis of the appendix of *D. vulgaris* is an unregulated burst associated with scent production, but the male florets regulate their temperature on the second night by changing respiratory rate inversely with ambient temperature changes. Thus the inflorescence of *D. vulgaris* regulates floral temperature physiologically, as do other monocot arum lilies such as *Philodendron selloum* (Nagy *et al*. 1972; Seymour 1999) and *Symplocarpus foetidus* (Seymour & Blaylock 1999) and the dicot *Nelumbo nucifera* (Seymour & Schultze-Motel 1998). The physics and physiology of the phenomenon are known, but the molecular basis for temperature control is not (Seymour 2001). Temperature regulation seems to be primarily associated with pollination by beetles that may benefit from the warm environment in the floral chamber during their incarceration overnight (Seymour & Schultze-Motel 1997). Beetles are often endothermic, raising their body temperatures by endogenous heat production during activity, but carrion-loving flies seldom are (Heinrich 1993). Temperature regulation in the floral chamber of *H. muscivorus* might not be expected, therefore, because flies are the main pollinators. Alternatively, temperature regulation by the male flowers suggests that it is associated with some other function, such as regulation of the rate of floral development, including receptivity of the female florets and pollen shedding by the males. This is the first study to investigate the relationship between thermogenesis and pollination biology in an essentially fly-pollinated arum lily under natural field conditions.

Materials and methods

PLANTS AND BREEDING SYSTEM

A local population of *H. muscivorus* was located at Caporalino, 10 km south of Ponte Leccia, central Corsica. The plants were located on a steep, rocky slope above the railway tracks, as described by Fridlender (2000), and were studied during the latter part of the flowering season between 22 April and 6 May 2002.

The sequence of flowering was observed daily, usually in the early morning, between about 06:00 and 11:00. Notes were taken on the appearance of each inflorescence. The presence of pollen was determined by cutting a small window in the floral chamber and touching the male florets with a cotton-tipped stick covered with a piece of cellophane tape with its sticky side out. The tape was then examined for pollen.

INFLORESCENCE TEMPERATURES AND RESPIRATION

Small thermocouples were inserted into the appendix and male florets in lateral holes made by a 15-gauge hypodermic needle. This required that a $\approx 5 \times 10$ mm window be cut in the wall of the floral chamber. (The window also released any trapped flies in a few cases where the inflorescence was instrumented after some flies had entered.) Respiration rates of the appendix and floral chamber were measured separately with methods similar to those described earlier (Seymour & Schultze-Motel 1999). After thermocouples were inserted, a close-fitting PVC hood, connected to 5 mm PVC tubing, was placed over the appendix and sealed loosely with plastic cling-wrap near the entrance to the floral chamber. Another PVC tube was sealed into the window of the floral chamber with plastic duct tape. Gas samples from the appendix hood and floral chamber were pumped into a portable respirometry setup capable of measuring rates of oxygen consumption and carbon dioxide production in up to four channels simultaneously. The apparatus, and the methods and assumptions, are described in an earlier paper (Seymour & Schultze-Motel 1998), but the components were upgraded. Briefly, sampling pumps (Gilian Instruments Corp., Gilian Sensidyne, Clearwater, FL, USA, model Gil-air R) pumped gas from the plant, through mass flow meters (Sierra Instruments Inc., Monterey, CA, USA, model 822) and into a combination oxygen and CO_2 analyser (David Bishop Instruments, Bacharach Europe, Warwick, UK, model COMBO 280). A gas flow selection manifold, incorporating 12 V solenoid valves (Lee Company, Westbrook, CT, USA, model LFAA1200210H) sampled gas at 6 min intervals from five channels: four from the appendix and floral chamber of one or two inflorescences, and the fifth from atmospheric air via a humidifier chamber. A data logger (Grant Instruments, Cambridge, UK, model 1200) recorded the outputs from the analyser, flow meters, thermocouples and a barometer at 2 min intervals to assure a stable reading, uninfluenced by changes in channel. A 12 V, 43 A-h automotive battery, which was replaced with a recharged one daily, powered the apparatus. All inflorescences were protected from direct sunlight under cardboard shades. Ambient temperature was recorded in the shaded air, next to the inflorescence. After the flowering sequence, the inflorescences were cut and sealed in plastic bags until the floral parts were measured with callipers and a balance.

The oxygen analyser was calibrated with pure nitrogen before the study and with humidified atmospheric air automatically at 30 min intervals throughout recording. The CO_2 analyser also periodically compared the sample gas with atmospheric air, and its output was scaled against the oxygen data to achieve an overall respiratory quotient of 1·0, assuming that carbohydrate was the energy source (James & Beevers 1950; ap Rees,

Fuller & Wright 1976; ap Rees, Wright & Fuller 1977; Bulpin & ap Rees 1978; Seymour & Schultze-Motel 1998; Seymour & Blaylock 1999). The CO_2 analyser was about 10 times more sensitive than the oxygen analyser, so its data provided a clearer pattern of respiration. Therefore CO_2 data were selected for presentation and statistical analysis.

Thermal images of the appendix and floral chamber were made with an infrared colour LSD camera (TVS-600, Nippon Avionics Ltd, Tokyo, Japan) on one inflorescence raised in a botanic garden in Adelaide, Australia. Images were saved as IRI files on the PCMCIA memory card of the camera and were subsequently analysed for temperature determination with the image analysis program provided by the manufacturer (Thermal Video Systems, Nippon Avionics).

BEHAVIOUR AND TEMPERATURES OF POLLINATORS

Flies trapped in the floral chamber of non-instrumented inflorescences were captured in gauze bags as they emerged in the early morning of the second day. Thoracic temperatures were measured with a 22 ga needle thermocouple and thermometer (Fluke, Corp., Everett, Washington, USA, model 55), by immobilizing the fly with the bag and puncturing it through the fabric, being careful to avoid contact with fingers. The types and level of activity of flies before puncture were noted, and flies were taken to base, chilled in a freezer and weighed to 0·02 mg.

STATISTICS

All reported errors are 95% confidence intervals for the mean. Regressions are least squares linear (model 1).

Results

PLANTS AND BREEDING SYSTEM

On the day before anthesis, the spathe loosened slightly. It opened sometime during the first night and was always wide open when the plants were inspected at about 06:30 on the first day. The spathe was usually a deep pink, and its hairs were erect. The appendix lay flat against the spathe. The spathe remained open on the second day, but it fell slightly away from the appendix and lost some colour intensity. On subsequent days its edges turned up, forming a trough around the appendix, and eventually the edges touched. Then both spathe and appendix gradually withered.

A putrid odour was apparent early on the first day, and it increased in intensity after about 07:30 when the sun rose from behind the mountains and shone on the flowers. It became less apparent in the afternoon and on subsequent days.

The breeding system appeared consistent with protogyny, as in other Araceae. There was never any pollen evident on the first day of flowering when flies were attracted and trapped. Pollen release occurred late in the following night or early in the morning of the second day. In several second-day inflorescences, pollen was apparent at first examination between 06:15 and 06:30. Observations with cellophane tape showed the first pollen at 06:45 for one inflorescence, the other having released its pollen before 06:15. Thus pollen appears to be released shortly before sunrise.

INFLORESCENCE TEMPERATURES AND RESPIRATION

Thermal images taken on the morning of the first day indicated that there were two main sites of thermogenesis, the appendix and the male florets in the floral chamber (Fig. 1). Maximum surface temperatures at 08:10 were 21·8 °C for the appendix and 21·6 °C for the male florets. The ambient temperature was 12·6 °C.

Detailed quantitative observations were made on 10 inflorescences at Caporalino. Not all records were complete, so statistics from seven to nine inflorescences are given in Table 1. The appendix began to heat during the first night, beginning at 01:10 in the single specimen that was measured from the day before anthesis (Fig. 2). Measurements from eight others began on the first day, between 07:22 and 09:45 (e.g. Fig. 3). Maximum respiration rates of the appendix occurred between 07:50 and 11:17 on the first day (Table 1), but maximum appendix temperature (T_{ap}) of 32·1 ± 1·7 °C occurred somewhat later, at 11:25 ± 1:01, when ambient air temperature (T_a) was 22·3 ± 2·8 °C. The respirometry hood and shading of the flower may have

Table 1. Morphology and respiratory maximum CO_2 production rate ($M\mathrm{CO}_{2\mathrm{max}}$) of *Helicodiceros muscivorus* inflorescences

	Mean	95% CI*	*n*
Spathe length (mm)	197	27	9
Appendix			
Length (mm)	124	23	9
Mass (g)	3·2	1·7	8
$M\mathrm{CO}_{2\mathrm{max}}$ (μmol s^{-1})	1·40	0·61	8
$M\mathrm{CO}_{2\mathrm{max}}$ (μmol s^{-1} g^{-1})	0·45	0·10	8
T_{ap} at $M\mathrm{CO}_{2\mathrm{max}}$ (°C)	29·8	3·2	7
T_a at $M\mathrm{CO}_{2\mathrm{max}}$ (°C)	15·2	1·5	7
Time at $M\mathrm{CO}_{2\mathrm{max}}$	8:56	0:52	7
Floral chamber			
Total height (mm)	81	8	9
Female floret height (mm)	13·2	1·3	9
Female floret mass with stalk (g)	1·04	0·27	9
Male floret height (mm)	12·0	1·7	9
Male floret mass with stalk (g)	0·65	0·21	9
$M\mathrm{CO}_{2\mathrm{max}}$ (μmol s^{-1})	0·55	0·12	8
$M\mathrm{CO}_{2\mathrm{max}}$ (μmol s^{-1} g^{-1})†	0·82	0·13	8
T_m at $M\mathrm{CO}_{2\mathrm{max}}$ (°C)	24·3	0·8	7
T_a at $M\mathrm{CO}_{2\mathrm{max}}$ (°C)	12·8	1·7	7
Time at $M\mathrm{CO}_{2\mathrm{max}}$	03:37	1:16	7

*95% confidence interval.
†Assumes male florets responsible for all respiration.

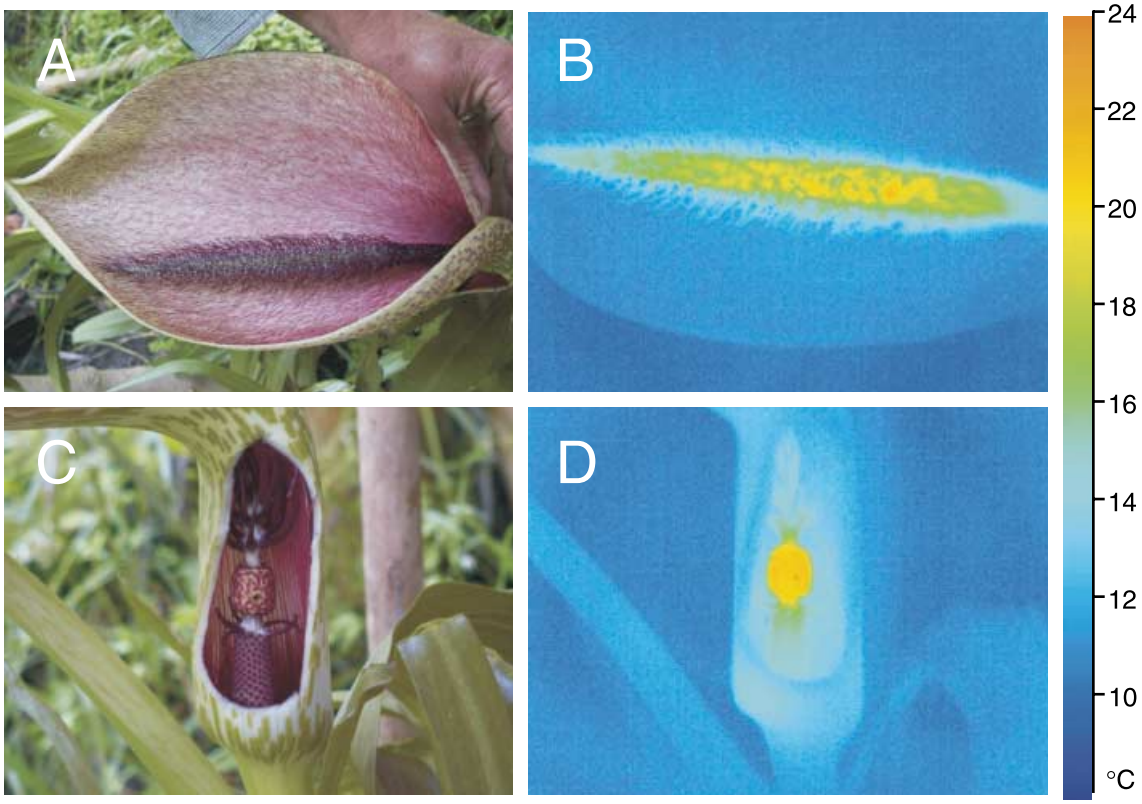

Fig. 1. Photographs of *Helicodiceros muscivorus* on the morning of the first day of anthesis, showing the appendix on the hairy spathe (a) and the male florets above the female florets in the opened floral chamber (c). Thermal images show intense heating of the appendix (b) and male florets (d). Surface temperature of the appendix reached 21·8 °C, while that of the male florets reached 21·6 °C. At the time the air temperature was 12·6 °C.

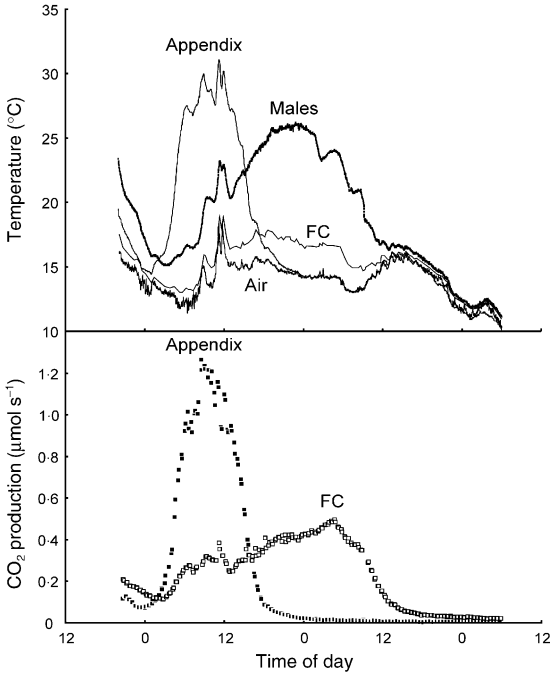

Fig. 2. Time course of temperatures and respiration in a shaded *Helicodiceros muscivorus* (#9) in the field. Temperatures of the appendix and male florets were obtained with thermocouples inserted into the tissue. Temperatures were also obtained in the lower floral chamber (FC) and ambient air adjacent to the inflorescence. Total respiratory rates were measured as CO_2 production independently in a hood around the appendix and in the floral chamber.

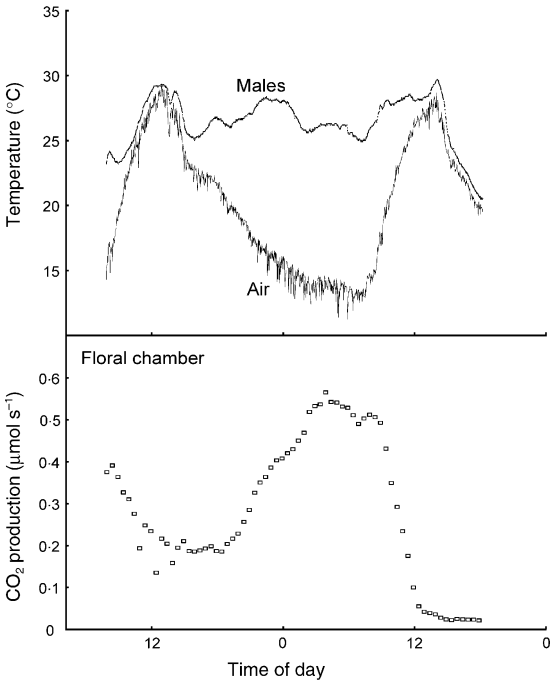

Fig. 3. Temperatures of the male florets and ambient air (top) and respiration rate of the floral chamber in *Helicodiceros muscivorus*. Note the constancy of floret temperature and an inverse relationship between respiration rate and ambient temperature.

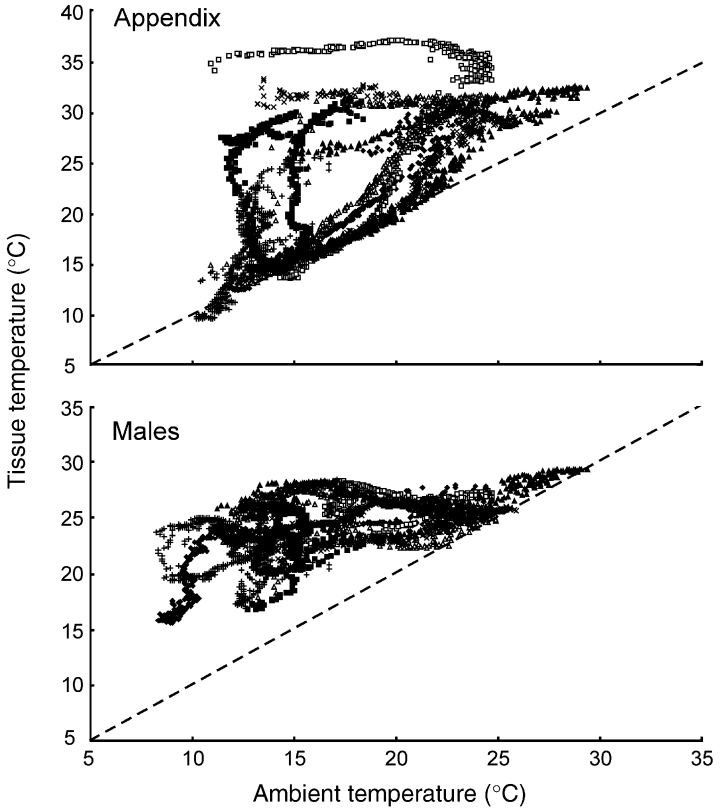

Fig. 4. Relationships between temperatures of the appendix and male florets to ambient air temperature in *Helicodiceros muscivorus*. Data were selected for 24 h periods, beginning at midnight on the first night for the appendix and 08:00 on the first day for the floral chamber, to encompass the main peaks of thermogenesis. Symbols represent different inflorescences and are consistent between figures.

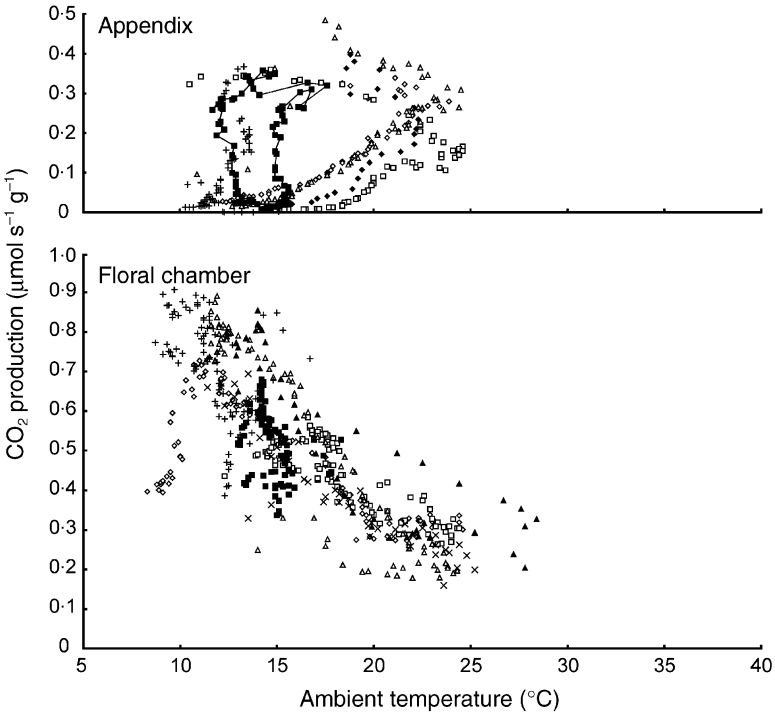

Fig. 5. Relationships between mass-specific respiration of the appendix (top) and the male florets in the floral chamber (bottom) to ambient temperature in *Helicodiceros muscivorus*. Data set and symbols as in Fig. 4. Filled squares connected by lines are for the appendix in inflorescence #9, illustrated in Fig. 2.

influenced T_{ap}, but the effects of the former were slight, because there were no distinct discontinuities in the profile of T_{ap} when the hood was purposefully removed between 08:15 and 09:15 during peak thermogenesis in one inflorescence with a 1·87 g appendix. The hood may have had a significant effect in larger appendices, but it was not measured.

There were two main episodes of heating of the male florets on the first and second nights, respectively. Male floret temperatures (T_m) were elevated at 20:00 in the evening of the first night and remained about 2–4 °C higher than T_a throughout the night (Fig. 2). After sunrise T_m increased, reaching a mean peak in the second night at 23:40 ± 1:35, when T_m averaged 26·2 ± 1·1 °C and mean T_a was 14·8 ± 1·9 °C in eight inflorescences. After this, T_m decreased to approximately ambient at 12:00–14:00 of the second day. The air in the floral chamber was considerably cooler than T_m (Fig. 2), averaging 16·6–18·9 °C, but this was 1·6–6·5 °C warmer than T_a in three individuals measured over the 24 h period. Peak respiration rate in the floral chamber occurred on the second night, about 4 h after peak T_m, when T_a was low (Table 1).

The pattern shown in Fig. 2 occurred only in two inflorescences (#9 and #10) when overcast weather kept T_a relatively stable. In seven others, T_m remained relatively stable as T_a decreased during the night; respiration and T_a were inversely related (Fig. 3). Occasionally, if T_a fell below about 12 °C (range 8·2–15·1), T_m began to decrease at about 03:00, but recovered at dawn, when T_a began to increase.

T_{ap} and T_m were often above T_a, but the patterns were different (Fig. 4). T_{ap} was not consistently related to T_a, because the unimodal T_{ap} curve was superimposed on a sigmoid curve of T_a (Fig. 2). However, T_m appeared to be somewhat independent of T_a, and the difference between T_m and T_a increased as T_a decreased (Fig. 4). At the lowest T_a, there was a sharp decrease in T_m in some individuals.

For the appendix, there was a linear relationship between mass (M_{ap}) and maximum temperature difference $(T_{ap} - T_a)_{max}$ according to the equation: $(T_{ap} - T_a)_{max} = 1{\cdot}85M_{ap} + 8{\cdot}18$ ($R^2 = 0{\cdot}94$). The maximum difference ranged from 9·2 °C in a 1·33 g appendix to 23·9 °C in an 8·88 g appendix. Absolute maximum T_{ap} was also related to M_{ap}: $T_{ap(max)} = 1{\cdot}11M_{ap} + 27{\cdot}28$ ($R^2 = 0{\cdot}73$). For the male florets, maximum T_m ranged from 25·0 to 29·3 °C, and maximum temperature difference between T_m and T_a ranged from 11·9 to 15·5 °C; both were unrelated to floret mass ($R^2 = 0{\cdot}12$ and 0·04, respectively), which ranged from 0·356 to 1·298 g.

Respiration (rate of CO_2 production, M_{CO_2}) of the appendix was not related to T_a (Fig. 5, top). It increased as T_a both decreased during the late night and increased in the morning. After about 11:00, M_{CO_2} continued to decrease as T_a increased in the afternoon and decreased at night. On the other hand, M_{CO_2} of the male florets showed a strong tendency to increase at lower T_a (Fig. 5,

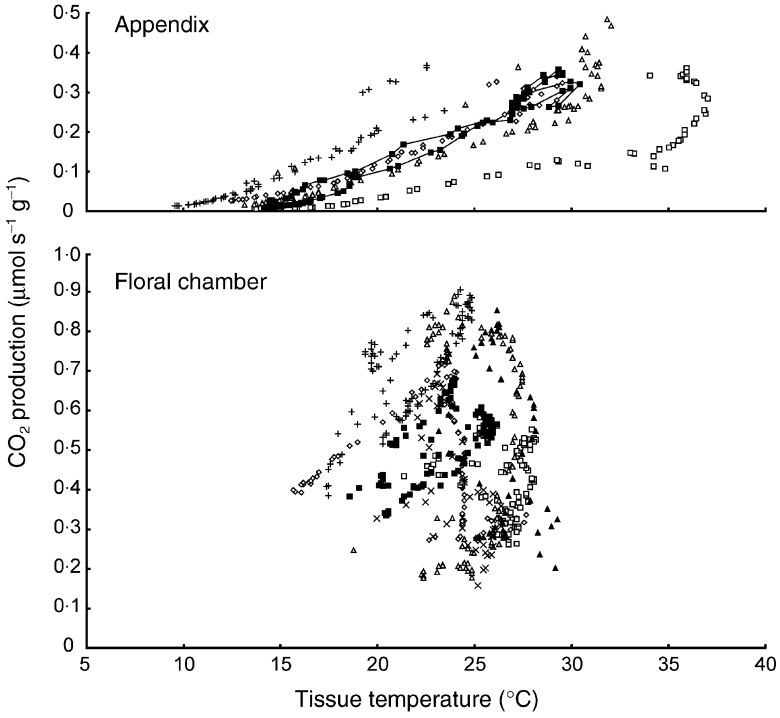

Fig. 6. Relationships between mass-specific respiration of the appendix (top) and male florets in the floral chamber (bottom) to respective tissue temperatures in *Helicodiceros muscivorus*. Data set and symbols as in Fig. 4. Filled squares connected by lines are for the appendix in inflorescence #9, illustrated in Fig. 2.

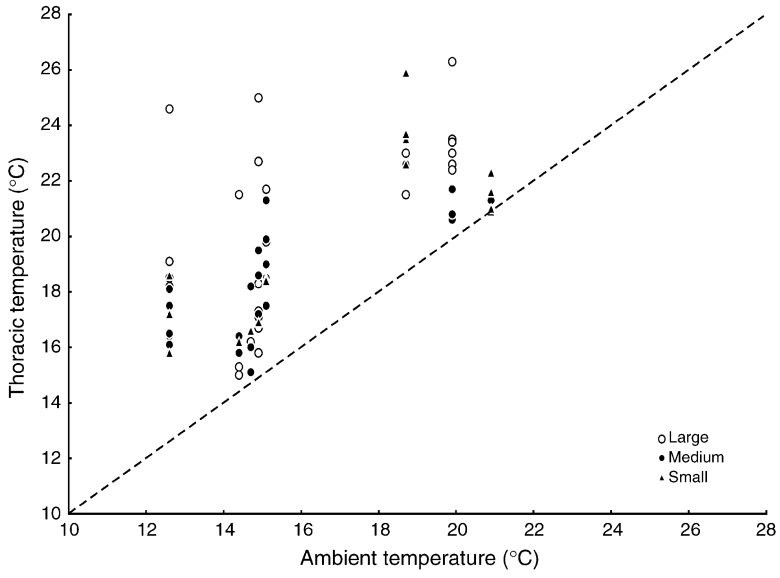

Fig. 7. Thoracic temperatures of flies emerging from *Helicodiceros muscivorus* inflorescences on the morning of the first day. Flies were captured in gauze bags and pierced with a needle thermocouple. Each point represents one individual within three size groups.

bottom). Regressions of M_{CO_2} on T_a for six inflorescences showed R^2 between 0·67 and 0·92, but data from #9 and #10 were 0·13 and 0·27, respectively. The latter corresponded with cloudy conditions, rain showers and much smaller diurnal changes in T_a (Fig. 2).

Respiration of the appendix increased with higher appendix temperatures (Fig. 6, top). This occurred mainly because the peak in respiration occurred during the day when T_a was elevated and high respiration heated the tissue further. Conversely, respiration of the male florets was not significantly related to their temperature (Fig. 6, bottom).

BEHAVIOUR AND TEMPERATURES OF POLLINATORS

Although the inflorescences were emitting a putrid odour when initially observed on the first day, flies were attracted only after the sun shone directly on them during the morning. No flies were ever found in the floral chamber before sunrise. Flies would usually land on the spathe and walk over it and the appendix. Some would enter the neck of the floral chamber and emerge again, but others would enter and not emerge.

Fifteen second-day inflorescences were bagged and the insects collected and identified. In all, 301 Diptera were collected, of which 242 (80·4%) were Calliphoridae and 59 (19·6%) were Anthomyiidae. Of the Calliphoridae, 75% belonged to two species of *Calliphora*: 146 *C. vomitoria* (16% of which were males) and 35 *C. vicina* (14% males); whereas the other 25% were only females of *Lucilia* species: 50 *L. regalis*, five *L. bufonivora* and six *Lucilia* sp. The anthomyiid flies were also only females of the genus *Fannia* (49 *Fannia* sp1, eight *Fannia* sp2) and two undetermined specimens. No other insects were found. In the field, and before identification, we categorized the flies roughly as large, medium and small. The large flies were mainly *Calliphora*; medium ones were mainly *Lucilia* and *Calliphora*; and small ones were mainly *Fannia*, although there was considerable overlap.

Five inflorescences were covered with gauze bags on the first day, after many flies had entered. Before the sun shone on the inflorescences on the second day, a few flies had emerged into the bags (they may have emerged during the previous afternoon), but most remained in the floral chamber. Trapped flies were covered with pollen, especially on the hairy thorax, and excess pollen accumulated on the floor of the floral chamber. Flies emerging from windows cut in the floral chamber were very sluggish before sunrise and could not fly well; they fell to the ground when thrown into the air. Several flies showed 'buzzing' behaviour upon release and some flew one or two metres. The thoracic temperatures of flies emerging into the bags were variable when measured between about 07:30 and 11:30 (Fig. 7). On average the flies were 3–5 °C above ambient, but the difference ranged between 0 and 12·0 °C. There was no significant difference apparent between the fly species. The average body mass of 135 flies collected from these inflorescences was 48 ± 7 mg. Within size groups, 52 large flies (*Calliphora*) weighed 77 ± 6 mg; 51 medium flies (mainly *Calliphora* and *Lucilia*) weighed 42 ± 5 mg; and 32 small flies (mainly *Fannia*) weighed 9 ± 2 mg.

Discussion

PATTERN OF THERMOGENESIS

Heat was produced at two locations in the inflorescence, the appendix and the male florets (Fig. 1), and the patterns of temperature elevation were different in each organ (Fig. 2). The changes in respiration rate roughly paralleled changes in temperature elevation. The appendix showed a unimodal peak at the beginning of the first day, but the male florets had a bimodal response on two nights, with greater thermogenesis on the second. This pattern is similar to that in *Dracunculus vulgaris* (Seymour & Schultze-Motel 1999).

There are three aspects of the data that are consistent with temperature regulation by the male florets. First, male floret temperature is somewhat independent of ambient temperature when all inflorescences are viewed together (Fig. 4). This indicates that the respiration rate must increase with decreasing ambient temperature, the pattern observed (Fig. 5). Second, there was no effect of floret mass on either maximum temperature or maximum temperature difference. If respiration in the tissue were unregulated, one would expect larger masses to attain higher temperatures, because larger masses have relatively lower surface areas for heat loss, and retained heat would increase tissue temperature and hence respiration rate. Such size effects were clearly seen in the appendix: maximum temperatures were strongly related to appendix size. Third, male floret temperature was fairly constant throughout the night in most cases, except when ambient temperature dropped below about 12 °C late at night and respiration rates suddenly began to decrease, only to recover when the air began to warm the next day. Such failure to thermoregulate has also been seen in *Philodendron selloum* (Nagy, Odell & Seymour 1972) and studied in detail in *Symplocarpus foetidus* (Seymour & Blaylock 1999). Because of surface–volume relationships, some small inflorescences of these thermoregulating species cannot produce enough heat to maintain floret temperature within the regulated range during exposure to low ambient temperature. Thus regulation is 'switched off' at ambient temperatures below a certain threshold, but it can 'switch on' if the ambient temperature rises above it (Seymour 2001).

On the other hand, some aspects of the current study are inconsistent with thermoregulation. In thermoregulating species, one always sees a steep inverse relationship between respiration rate and temperature of the respiring tissues, for example in *P. selloum* (Nagy *et al*. 1972; Seymour 1999), *S. foetidus* (Seymour & Blaylock 1999) and *Nelumbo nucifera* (Seymour & Schultze-Motel 1998). Some unknown control mechanism causes respiration to increase at lower tissue temperatures within the regulated range. For *Helicodiceros*, decreased ambient temperature was correlated with decreasing tissue temperature (Fig. 4) and increasing respiration rates (Fig. 5), but there was no overall relationship between respiration rate and tissue temperature (Fig. 6). Individually, two inflorescences showed significant negative relationships as expected, but two others showed strongly positive relationships and three showed no relationship. The inflorescences #9 and #10 were particularly inconsistent with thermoregulation (Fig. 2). Not only did they show a positive relationship between male floret respiration and tissue temperature, but also there was little stability in floret temperature during the recording period. These plants were unusual because they bloomed during a cloudy and rainy period when ambient temperatures were relatively stable. Under these conditions one would expect that a thermoregulating inflorescence would be more constant than those exposed to more variable conditions, but the opposite was true. However, the pattern of male floret respiration in these inflorescences was the same as in all the others, peaking between 02:00 and 05:00, suggesting that the pattern of heat production is determined by the developmental sequence of the flowers rather than by ambient temperatures. Therefore a rising and falling respiration rate causes a peaked pattern of male floret temperature when ambient temperature is constant (Fig. 2), but stability when ambient temperature dips during the night (Fig. 3).

Because of the above inconsistencies in our results, we call *H. muscivorus* a 'pseudo-thermoregulatory' species. Male floret temperatures may appear independent of ambient temperature as it decreases during the night, but the control mechanism is not related to ambient or tissue temperature, but rather to a circadian pattern. Pseudo-regulation applies only to the male florets. There is absolutely no evidence of thermoregulation in the appendix, which shows a pattern of warming related to time of day and associated with scent production. Lack of temperature regulation in the osmophore is also characteristic of the appendix of *D. vulgaris*, but its male florets demonstrate true regulation (Seymour & Schultze-Motel 1999).

Two sites of thermogenesis in *H. muscivorus* suggest at least two roles in the biology of the plant. Unregulated heat production of the appendix probably enhances the vaporization of the volatile elements of the scent, as often suggested (Meeuse & Raskin 1988; Kite 1995). High temperature elevations of up to 23·9 °C above air temperature in this study (Fig. 4) contrast with a mean elevation of only 2·5 °C in *D. vulgaris* (Seymour & Schultze-Motel 1999), but the difference is related to appendix size. The mean appendix mass was 3·2 g in *H. muscivorus*, but 47·2 g in *D. vulgaris*. The former compensates for a smaller appendix by having a maximum respiration rate of 0·45 μmol s^{-1} g^{-1}, which is 6·2 times higher than the 0·072 μmol s^{-1} g^{-1} in *D. vulgaris* (Seymour & Schultze-Motel 1999). Total respiration of the appendix is 1·4 μmol s^{-1} in *H. muscivorus* and 3·6 μmol s^{-1} in *D. vulgaris*, so the total rates of thermogenesis are not greatly different.

Maximum respiration rate by the male florets appears to be the highest ever recorded in plants. Assuming a respiratory quotient of 1, it is possible to compare mass-specific M_{CO_2} data analysed in this study to M_{O_2} data from earlier studies on arum lilies. Thus the mean values are: *H. muscivorus* male florets 0·82 μmol s^{-1} g^{-1} (Table 1); *D. vulgaris* male florets 0·48 μmol s^{-1} g^{-1} (Seymour & Schultze-Motel 1999); *Arum maculatum* appendix tissue 0·45 μmol s^{-1} g^{-1} (Lance 1974); *Philodendron selloum* sterile male florets 0·36 μmol s^{-1} g^{-1} (Nagy *et al*. 1972; Seymour, Bartholomew & Barnhart 1983); and *Symplocarpus foetidus* bisexual florets 0·36 μmol s^{-1} g^{-1} (Seymour & Blaylock 1999). Two of the *H. muscivorus* inflorescences exceeded 1 μmol s^{-1} g^{-1}, which surpass the highest individual value (0·89 μmol s^{-1} g^{-1}) for *Arum maculatum* (Lance 1972). The function of this intense, pseudo-regulated heat production of the male florets is not clear. Its effect is to maintain a relatively stable temperature throughout nights of clear weather. This might be associated with the timing of pollen maturation and synchronizing pollen release in second-day inflorescences with appendix warming in other first-day inflorescences. There remains the possibility that warming in the floral chamber is a form of direct energy reward to the insects trapped there (Seymour & Schultze-Motel 1997), but our observations on trapped flies are equivocal.

BEHAVIOUR AND TEMPERATURES OF POLLINATORS

Helicodiceros muscivorus in Corsica appears to attract and trap flies exclusively. There were no beetles in any of the 15 inflorescences we collected or in the several others we examined. The flies were mainly *Calliphora* and *Lucilia*, genera of blowflies that are typically among the first attracted to carrion (Mégnin 1894). The anthomyiid flies (*Fannia* spp.) attracted to the inflorescences are also known to be carrion flies (Mégnin 1894). This is in contrast to the situation with *D. vulgaris*, also distributed in the north Mediterranean regions between south-west Turkey and Sardinia, and a close relative to *H. muscivorus* (Boyce 1994; Mayo *et al*. 1997). *Dracunculus vulgaris* attracts both flies and beetles, but the flies appear to be excluded from the floral chamber, while the beetles fall in and are trapped overnight (Seymour & Schultze-Motel 1999). Carrion beetles (*Saprinus*, *Dermestes*) have been found to be its pollinator (Arcangeli 1883; Arcangeli 1890). The upright spathe of *D. vulgaris* acts as a slippery chute that funnels falling beetles into the chamber. On the other hand, the hairy spathe and appendix of *H. muscivorus* are usually sloped upward toward the chamber opening, and the flies must climb into it. Perhaps spathe design is one mechanism that isolates the two species, especially where they potentially contact in Sardinia. Differences in scents are obviously another possibility that awaits analysis.

Flies remain in the floral chamber overnight and are presumably trapped there – they readily escape if a window is cut through the chamber wall in the early morning of the second day. The sluggishness of the emergent flies indicates that their activity is scarcely enhanced by any heat generated by the male florets in the chamber. At the time of natural emergence, the air temperature in the floral chamber was about 16–19 °C, although the male florets were close to 25 °C. One reason for their initial inactivity is that many flies, and *C. vicina* in particular, possess circadian rhythms naturally entrained by light cycle and characterized by locomotor activity only in the light phase (Saunders 1982; Saunders & Hong 2000). Another reason is that they might be affected by elevated CO_2 in the floral chamber. After emergence from the inflorescence in the morning, the flies often buzzed in the collection bags, thereby generating their own heat to raise the temperature of their flight muscles. Flies in the mass range of those recorded in this study are large enough to elevate their thoracic temperatures significantly. Syrphid (≈ 30 mg), asilid (≈ 100 mg) and tachinid (≈ 135 mg) flies are endothermic, and raise their thoracic temperatures to about 25–38 °C at ambient temperatures ranging from about 10–25 °C (Heinrich 1993). Our data are somewhat variable, but indicate thoracic temperatures as high as 12 °C above the air in flies with a mean mass of 48 mg (Fig. 7). This is in contrast to data from another calliphorid blowfly, *Phaenicia sericata*, which was able to reach only 1·2 °C above air (Yurkiewicz & Smyth 1966). Because of their inactivity in the floral chamber just before sunrise, and their subsequent self-warming, we conclude that the flies visiting *H. muscivorus* do not appear to benefit from the warming of the male florets just prior to their departure in the morning. However, until we know more about the activities of flies in the floral chamber throughout the night, we cannot completely dismiss the possibility that they are affected by plant thermogenesis.

Acknowledgements

This study was supported by the Australian Research Council and the Program for Promotion of Basic Activities for Innovative Biosciences (Japan). Permits to study *Helicodiceros muscivorus* were obtained by M.G. from the Conservatoire Botanique National Méditerranéen de Porquerolles, thanks to M. Boillot. Robin Seymour helped in the field.

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Received 5 May 2003; revised 12 August 2003; accepted 8 September 2003