PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Physiological temperature regulation by flowers of the sacred lotus

Roger S. Seymour and Paul Schultze Motel

Phil. Trans. R. Soc. Lond. B 1998 **353**, 935-943
doi: 10.1098/rstb.1998.0258

The Royal Society

Physiological temperature regulation by flowers of the sacred lotus

Roger S. Seymour* **and Paul Schultze-Motel**

Department of Zoology, University of Adelaide, Adelaide 5005, Australia

Flowers of the sacred lotus, *Nelumbo nucifera* Gaertn. (Nelumbonaceae) are thermogenic and physiologically thermoregulatory. The 42 g flowers remain between 30–36 °C during a 2–4-day period despite fluctuations in environmental temperatures between about 10–45 °C. As the ambient temperature drops, the flowers increase heat production in proportion. Temperature regulation apparently occurs at a cellular level, by a steep, reversible thermal inhibition of respiration at flower temperatures above 30 °C. There was a marked time lag between change in flower temperature and compensatory response, suggesting regulation through a biochemical feedback mechanism rather than structural changes in enzymes or membranes. By oxidizing carbohydrate, the flowers produce up to 1 W, with about half of the heat coming from the 8.5 g carpellary receptacle. The period of temperature regulation begins before petal opening and continues through the period of stigma receptivity. Temperature regulation may reward insect pollinators with a warm, equable environment, or it possibly enhances and coordinates flower development.

Keywords: thermoregulation; flower; *Nelumbo nucifera*; pollination; temperature

1. INTRODUCTION

Members of the lotus genus, *Nelumbo*, are aquatic dicotyledons that are widely distributed in temperate, sub-tropical, and tropical regions of the New and Old Worlds (Borsch & Barthlott 1996). The group is usually considered to contain two species, the sacred lotus *N. nucifera* Gaertner that flourishes throughout the Australasian region, and the American lotus *N. lutea* (Willdenow) Persoon that inhabits southeastern North America, Central America, the Caribbean and the northern coast of South America. *N. lutea*, that appears synonymous with *N. pentapetala*, and *N. nucifera* are so similar in morphology (cf. Moseley & Uhl (1985) and Ito (1986)) that they have been recently combined as subspecies of *N. nucifera* by Borsch & Barthlott (1996).

Nelumbo flowers are large (*ca.* 20 cm in diameter when fully open), and consist of pink to cream petals that surround a conical receptacle (which comprise the carpophore) containing numerous carpels. They are supported on a stiff petiole up to about 1 m above the water. Before the petals open, they form a chamber above the flat surface of the receptacle and press the stamens tightly around its sides. The flowering sequence of the lotus is protogynous and favours outcrossing via insect pollinators (Robertson 1889; Schneider & Buchanan 1980; J. B. Bullin *et al.*, unpublished data). On the 'first day' the petals open slightly at the tip, forming a passage to the floral chamber containing receptive stigmas. Because the petals press the staminal appendages close to the circumference of the receptacle, access to the stamens is prevented. Petals of 'second day' flowers open more widely, revealing pollen-bearing anthers. Thus the flower is primarily allogamous, but can be autogamous via insect intermediaries, because of some overlap between carpel receptivity and pollen availability (Schneider & Buchanan 1980).

Heat production in the lotus flower has been known for a century, and opportunistic measurements showed temperature elevations up to 10 °C above that of the air (Miyake 1898; Schneider & Buchanan 1980). Thermogenesis also occurs in several other species in the aroid, or arum lily, family (Araceae) (Meeuse & Raskin 1988), and in a few water lilies (Nymphaeaceae) (Prance & Arias 1975), palms (Arecaceae and Cyclanthaceae) (Schroeder 1978; Gottsberger 1990; Listabarth 1996), custard apples (Annonaceae) (Gottsberger 1990), and cycads (Cycadaceae) (Skubatz *et al.* 1993). Whereas thermogenesis is an uncommon phenomenon, physiological regulation of flower temperature, in which the rate of heat production increases at lower environmental temperatures, is even rarer, being known in only two species, the aroids *Philodendron selloum* (Nagy *et al.* 1972) and *Symplocarpus foetidus* (Knutson 1974). We recently discovered temperature regulation in the sacred lotus, but were able to report our findings only briefly and superficially (Seymour & Schultze-Motel 1996). Here, we present a full account of the pattern of heat production and thermoregulation, including observations on the time course of responses and the effects of ambient temperature on heat production.

2. MATERIAL AND METHODS

(a) *Intact flowers outdoors*

Nelumbo nucifera was studied in a large outdoor pond in the Adelaide Botanic Gardens between December 1995 and February 1996. The plants had been growing in the garden for over 50 years, and their geographic origin is unknown. Temperatures inside the receptacles and

*Author for correspondence (rseymour@zoology.adelaide.edu.au).

Received 17 March 1997 *Accepted* 2 June 1997

ambient air were measured with Onset 'Hobo' temperature loggers equipped with 60 cm long thermistor leads.

Rates of oxygen consumption were measured with a four-channel, flow-through system that operated on batteries. Each flower was covered with a 2-l clear plastic hood sealed around the stem with plastic cling-wrap and tape, leaving a small opening for entry of air. An umbrella prevented excessive heating of the hood by the sun. Temperatures in the centre of the receptacle and the air inside the hood were measured with thin, PVC-insulated thermocouples. Air from the hood was pumped continuously through PVC tubing (5 mm inside diameter (ID), 6 mm outside diameter (OD)) with a Gil-Air constant flow air-sampling pump at about 400 ml min^{-1}. A vial trapped condensed water from the air before it entered the pump. A flow buffer, consisting of a helium-tight balloon pierced by a disposable pipette tip stabilized the flow through a mass flowmeter (Sierra model 822, calibrated against a Brooks model 1057A Vol-U-Meter calibrator), and then the gas was vented into the atmosphere. To avoid affecting the flow through the hoods, a bank of solenoid valves selected a subsample from three channels in sequence, followed by an empty 'reference' channel of atmospheric air, dwelling on each for 6 min. A separate pump introduced each subsample into a Taylor Servomex model 570A paramagnetic oxygen analyser that was calibrated between runs with high-purity nitrogen and atmospheric air. At 2-min intervals, a Grant model 1203 Squirrel data logger recorded outputs from the instruments. A Davis Instruments Perception II weather station measured barometric pressure and temperature of the instrument case.

The rate of oxygen consumption was calculated from the equation

$$\dot{V}_{O_2} = \dot{V}_E(0.2095(1 - (P_{H_2O}/P_b)))((R - S)/R).$$

$\dot{V}_{O_2}$ is the rate of oxygen consumption (ml stp min^{-1}). $\dot{V}_E$ is the rate of air flow from the hood (ml stp min^{-1}). P_b is the barometric pressure (kPa). P_{H_2O} is the water vapour pressure of saturated air (kPa) which was calculated from the temperature of the instrument case with a polynomial regression of tabled values (Weast & Astle 1979). R and S are the voltage outputs of the oxygen analyser for the reference and sample channels, respectively. It was not practical to absorb CO_2 or water vapour from the gas passing through the analyser. The calculations therefore assume that the measured gas was saturated with water vapour at the temperature of the instrument case and that the respiratory gas exchange ratio $\dot{V}_{CO_2}/\dot{V}_{O_2}$ was 1.0. The first assumption was valid because the flowers were generally warmer than the case, there was usually condensation in the tubing and trap, and the gas in the reference channel was saturated with water vapour in a bottle lined with wet filter paper. The second assumption was validated by direct measurement (see § 2b). The system had a resolution of 0.01% oxygen content difference, and the estimated combined errors of the instruments were less than 10%.

The respirometry system also assumed that all of the gas entering the hood came from ambient air. Because the aerenchyma system of the lotus was known to be pressurized (Dacey 1987), the possibility of gas entering the hood through the flower was tested by measuring the pressure–flow relation in six flowers. The pressure in the emergent petiole was measured by cutting the flower and bending the petiole coming from the rhizome, immersing the cut end into the pond and observing the bubbles from the cut. The end was carefully lowered in the water column until the bubbling stopped and the depth measured to 0.2 cm. Pressure was measured between 10.00 and 14.00 on clear, warm days, and it averaged 0.3 kPa (95% confidence interval, CI = 0.16). Each cut flower was placed in a plastic bag with its petiole in water and taken to the laboratory. Within 1 h, its petiole was sealed under water to a plastic tube and attached to a variable flow-rate pump and a Gilmont F1100 ball flowmeter. Pressure in the air vascular system was measured with a water manometer at selected flow rates. The overall relation between pressure (P, kPa) and flow ($\dot{V}$, ml min^{-1}) was described by a power regression equation: $\dot{V} = 14\,P^{1.38}$ ($r^2 = 0.79$). Therefore, the mean petiole pressure in the field resulted in a flow of gas into the flower at 2.6 ml min^{-1}. The highest calculated flow rate was 4.1 ml min^{-1}. Because these daytime values were expected to be maximal (Dacey 1987) and they were 1% or less of the flow rate through the hood, it was assumed that no oxygen was consumed by the rhizome or other parts of the plant.

(b) *Respiration of cut flower parts*

The contribution to respiration from the parts of the flower was determined by measuring their oxygen consumption rates separately. The petioles of thermoregulating buds were cut underwater and the flower taken to the laboratory for immediate measurement. The flower was divided into three parts: petals, stamens, and receptacle. Petals and stamens were removed intact, and the receptacle was cut from the stem just above the attachment of the stamens and divided vertically into eight wedge-shaped parts to bring the tissue to chamber temperature.

All of the flower parts were weighed and placed into 350-ml plastic chambers lined with wet filter paper to provide high humidity. Stamens and receptacle parts were suspended in the chambers on a plastic mesh to ensure free circulation of air. The three chambers were connected to a flow-through respirometry system and were measured simultaneously. Atmospheric air passed through a desiccant (Drierite), a CO_2 absorbent (soda lime) and more desiccant before being divided into four streams, each metered at about 500 ml min^{-1} with Fischer–Porter ball flowmeters. A total of three air streams then flowed through the chambers inside a constant temperature cabinet and the fourth was an empty reference. Air from the chambers was vented into the atmosphere and sampled in rotation by solenoid valves. Part of each sample was pumped into another column of desiccant, then into an Anarad model AR50 infrared CO_2 analyser and finally into one channel of a Taylor Servomex model OA184 oxygen analyser. The CO_2 analyser was calibrated with CO_2-free gas and a precision mixture of 0.586% (±0.012%) CO_2 in nitrogen. The oxygen analyser was calibrated with high-purity nitrogen and dry, CO_2-free atmospheric air. The outputs of both analysers were recorded at a resolution of 0.001%. Although the flowmeters were corrected for

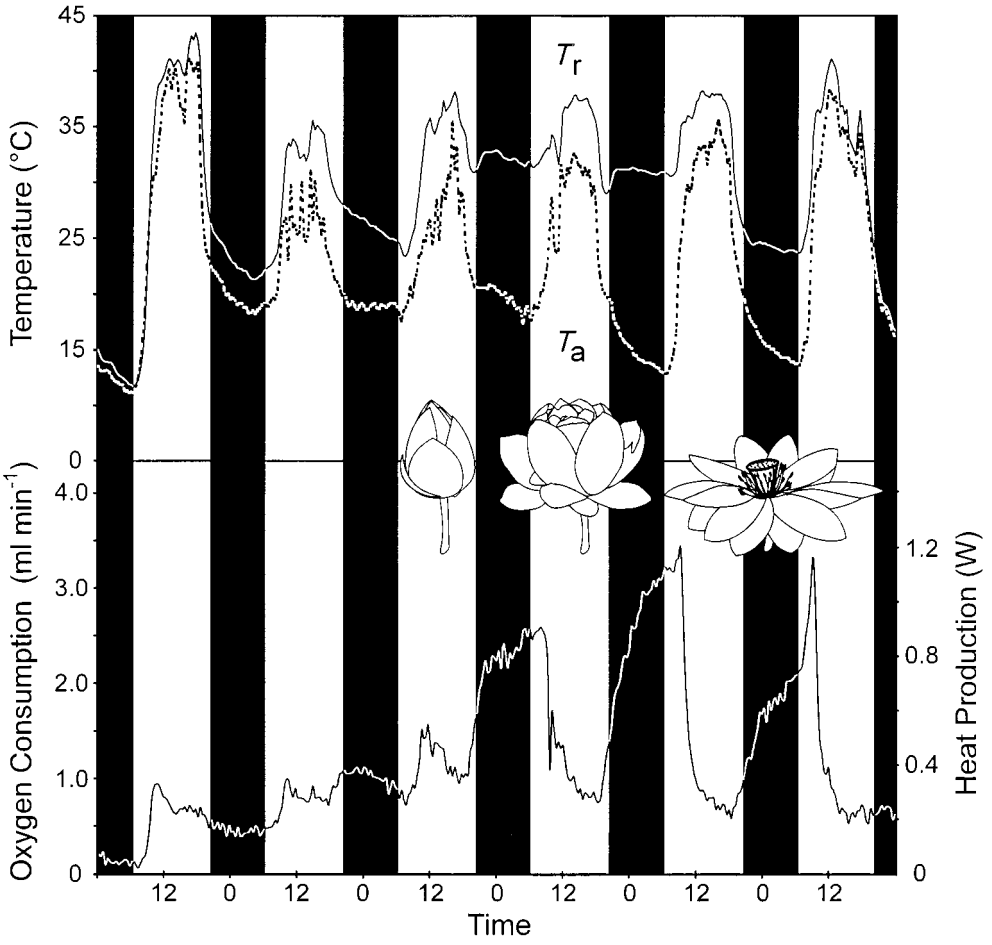

Figure 1. Temperatures of the receptacle (T_r) and ambient air (T_a), and rates of oxygen consumption and heat production, throughout a complete sequence of flowering. Day and night are shown as vertical bars, and stages of petal opening are indicated at the approximate times. Note the constancy of T_r and the reciprocal changes in T_a and oxygen consumption rate, between days 3 and 5.

laboratory temperature and barometric pressure, their accuracy limited our confidence in the data to within ±5%.

Rates of oxygen consumption ($\dot{V}_{O_2}$), and CO_2 production ($\dot{V}_{CO_2}$), and respiratory quotient (RQ) were calculated according to the following equations:

$$\dot{V}_{O_2} = \frac{\dot{V}_I(F_{I_{O_2}} - F_{E_{O_2}})}{1 - (1 - RQ)F_{E_{O_2}}},$$

$$\dot{V}_{CO_2} = \frac{\dot{V}_I F_{E_{CO_2}}}{1 + \left(\frac{1}{RQ} - 1\right)F_{E_{CO_2}}},$$

$$\frac{RQ = \dot{V}_{CO_2}}{\dot{V}_{O_2}}.$$

The new variables are: $\dot{V}_I$ is the rate of air flow into the chambers (ml stpd min^{-1}, where stpd is the standard temperature and pressure of the dry gas (no water vapour)), and $F_{I_{O_2}}$, $F_{E_{O_2}}$, and $F_{E_{CO_2}}$ are the fractional oxygen and CO_2 contents of incurrent (I) and excurrent (E) air. The equations were solved iteratively, first with an assumed RQ of 1, and then with the calculated RQ until it stabilized.

3. RESULTS

(a) *Flowering sequence*

Flowers were categorized in five stages: petals closed and pointed (stage 1), petals opening slightly at their tips only (2a), petals open about 2–5 cm (2b), petals open 5–10 cm and bowl-shaped (2c), and petals horizontal, revealing the stamens (3). Our stage 2 and 3 respectively correspond to 'first day' and 'second day' flowers of Schneider & Buchanan (1980). Stage 1 buds gradually grew during several days before opening, which was anticipated by enlargement and darkening of the pink petal tips. The flowers usually entered stage 2a before sunrise and progressed through 2b and 2c during the subsequent day and night. Stage 3 appeared on the morning of the next day and the flowers remained wide open for most of the day, closing in the evening. On the following day, the petals opened again and eventually fell off, followed by the stamens. Thereafter, the receptacle turned from yellow to green and began to enlarge with developing seeds. In general, the stigmas started to become moist early in stage 2, and they began to dry out by stage 3 when the pollen was shed.

(b) *Pattern of thermoregulation*

Temperatures of the receptacle (T_r) of early buds were similar to ambient temperatures inside the hood (T_a),

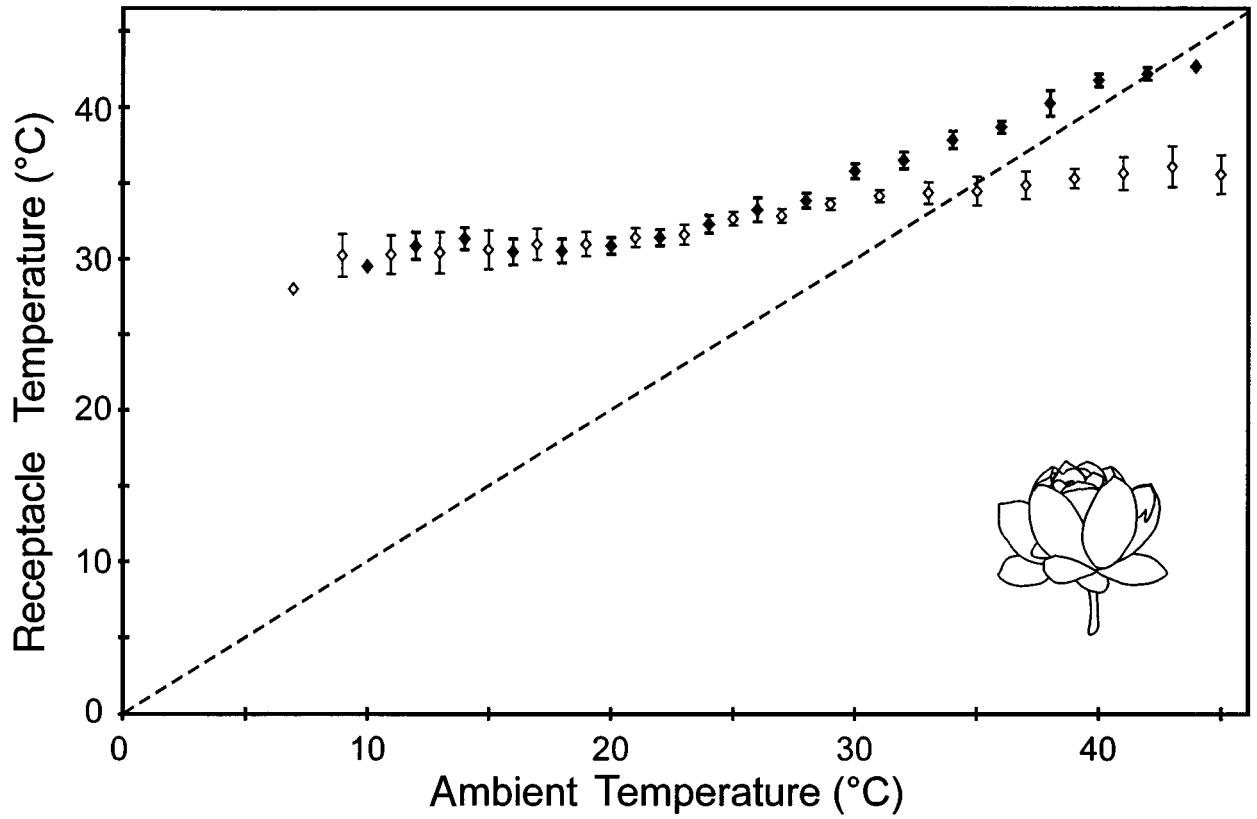

Figure 2. Temperatures of the receptacle and ambient temperature during a 24-h period when the flowers were thermoregulating. Filled symbols are derived from 19 flowers in respirometry hoods; open symbols are from 13 flowers in the free air. For each condition, the data from each flower were grouped in intervals of 2 °C (T_a) and a mean calculated. The figure presents grand means and 95% confidence intervals for all flowers within each 2 °C interval. Inset shows a flower near the end of the homeothermic phase.

but the flower began to warm above ambient about 2 days before the main episode of heating (figure 1). This gradual 'warm-up' transition was characterized by rising $\dot{V}_{O_2}$ and consequently rising temperature difference ($T_r - T_a$). During this period, however, T_r was quite variable and strongly influenced by T_a.

Eventually the 'warm-up' period was replaced by the 'homeothermic' period that was characterized by more-or-less stable temperatures, particularly at night. This period was variable, lasting 1–2 days in 3 flowers, 2–3 days in 16 flowers, and 3–4 days in 5 flowers. The homeothermic period corresponded to flowering stages 1 and 2. At night, T_r remained above about 30 °C, despite T_a dropping as low as 8 °C. When daytime T_a exceeded about 30 °C, T_r of hooded flowers rose a few degrees above it. The pattern of $\dot{V}_{O_2}$ during the homeothermic period was a mirror-image of the pattern of T_a, indicating that more heat was produced as T_a decreased. A spike in $\dot{V}_{O_2}$ often occurred about 2–3 h after sunrise, when T_a began to rise steeply. The pattern of $\dot{V}_{O_2}$ appeared to be related to T_a and not the light cycle; on some cool days when T_a remained below 25 °C, $\dot{V}_{O_2}$ remained high throughout the day (for an example, see Seymour & Schultze-Motel 1996).

After the homeothermic period, the flower entered a 'cool-down' period of about 1 day during which both temperature difference ($T_r - T_a$) and $\dot{V}_{O_2}$ decreased. This period corresponded to flowering-stage 3, when the petals opened widely. Eventually T_r and T_a were identical.

To characterize the relations between T_r and T_a during the homeothermic episode, data from 24-h periods near the middle of the episode were selected (figure 2). There was a difference between flowers in the free air and hooded flowers used for respirometry. In unhooded, exposed flowers, mean T_r was about 30 °C at a T_a of about 10 °C, and rose almost linearly to a T_r of about 36 °C at a T_a of 45 °C. In hooded flowers under umbrellas, the relations were the same below a T_a of 30 °C, but they diverged above it, T_r rising more steeply until T_r and T_a were about equal at 42 °C. The T_a below which the flowers are unable to remain warm is unknown, but we have a record of $T_r = 28$ °C when $T_a = 8$ °C.

At T_a above 30 °C, $\dot{V}_{O_2}$ was nearly constant at about 0.8 ml min^{-1} (figure 3). Below 30 °C, $\dot{V}_{O_2}$ increased practically linearly with decreasing T_a, rising to about 3 ml min^{-1}. This maximum is equivalent to a rate of heat production of approximately 1 W, assuming that 21.1 J of heat are produced per ml of oxygen consumed (Wieser 1986). A least-squares linear regression calculated for the means below 30 °C is: $\dot{V}_{O_2} = -0.105\,T_a + 3.856$ ($r^2 = 0.53$). The slope of the line indicates the thermal conductance of the flowers: 0.105 ml min^{-1} °C^{-1}, or in terms of heat production, 37 mW °C^{-1}.

Replotting the data in figures 2 and 3 revealed the relation between $\dot{V}_{O_2}$ and T_r for stabilized flowers within the homeothermic period (figure 4). There was a steep decrease in $\dot{V}_{O_2}$ at T_r between 30 and 32 °C, followed by a levelling out at about 0.8 ml min^{-1}.

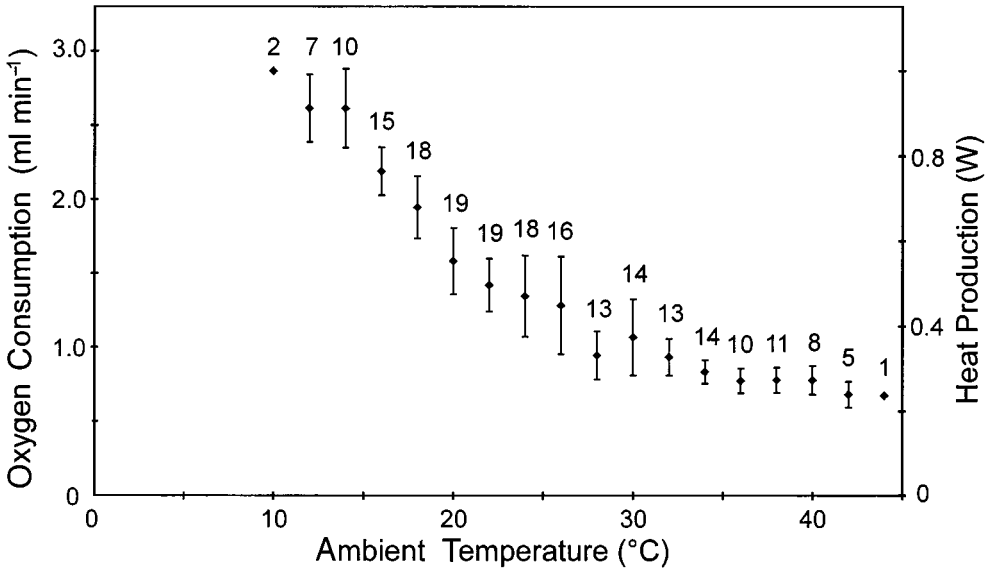

Figure 3. Rates of oxygen consumption in relation to ambient temperature during a 24-h period when the hooded flowers were thermoregulating. The data are derived as in figure 2, and the number of flowers in each 2 °C temperature interval is indicated.

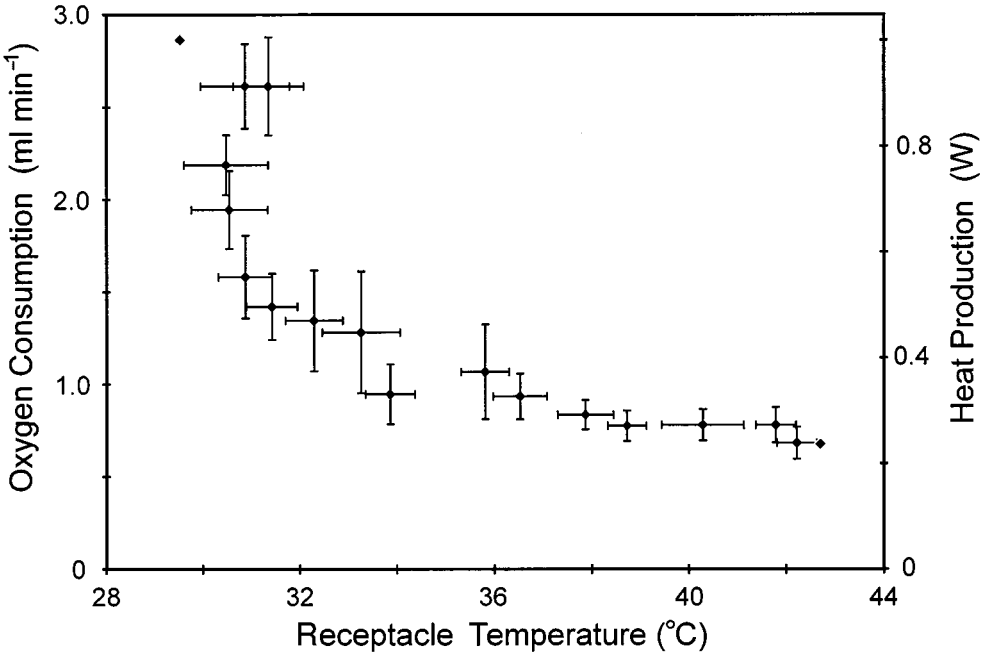

Figure 4. Rates of oxygen consumption and heat production in relation to receptacle temperature during a 24-h period of thermoregulation. The data and statistics are replotted from figures 2 and 3.

Part of the variability apparent in figures 1 and 3 is owing to a marked hysteresis in $\dot{V}_{O_2}$ that depended on the time of day and whether T_a was falling or rising (see figure 5). Between 15.00 and 19.00, T_a decreased quickly, causing a dramatic decrease in T_r, but there was practically no change in $\dot{V}_{O_2}$ until 20.00, after which $\dot{V}_{O_2}$ increased as T_a continued to fall through the night. In the morning, rising T_a was coupled with rising T_r, and $\dot{V}_{O_2}$ peaked at about 07.00 to 08.00. This peak is often more apparent in records from individual plants (figure 1), and is underestimated in figure 5 because of interpolation and averaging.

(c) *Respiration of cut flower parts*

The receptacle of the lotus accounts for only 20% of the entire flower mass, but 54% of the total respiratory rate in cut flowers during the homeothermic period (table 1). Thus, the mass-specific $\dot{V}_{O_2}$ was highest in the receptacle. The petals and stamens accounted for small amounts of heat, but the stamens had considerable mass-specific $\dot{V}_{O_2}$. Rates of CO_2 production ($\dot{V}_{CO_2}$) were essentially identical to $\dot{V}_{O_2}$, and the respiratory quotient was not significantly different from 1.0 in all flower parts.

4. DISCUSSION

(a) *Pattern of thermoregulation*

This study demonstrates that the flowers of *Nelumbo nucifera* are able to maintain a receptacle temperature (T_r) within a relatively narrow range despite ambient temperatures (T_a) that fluctuate widely. During the

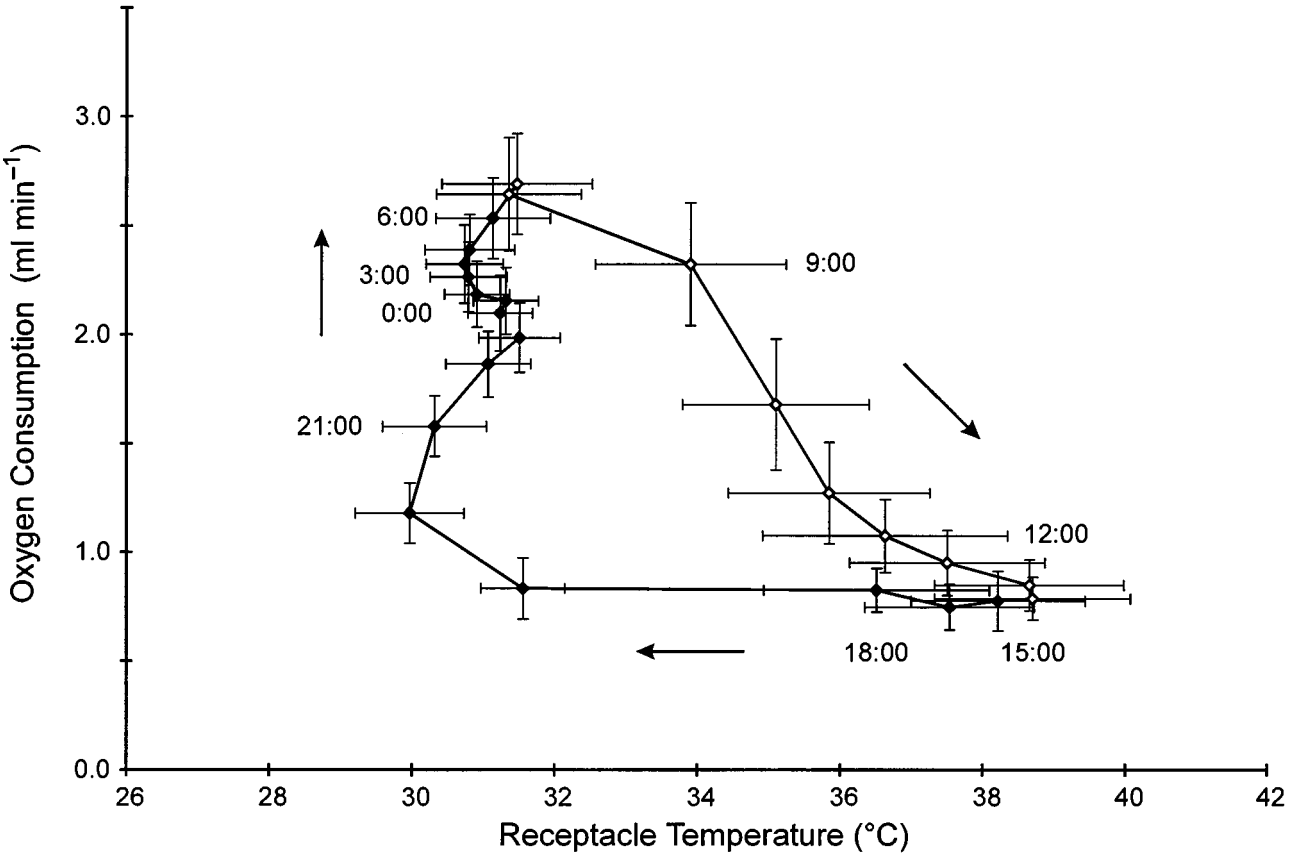

Figure 5. Relation between rate of oxygen consumption, receptacle temperature and time of day during a 24-h interval in the homeothermic period. Raw data that had been collected at 24-min intervals were interpolated to arrive at simultaneous estimates for every hour of the day (as indicated adjacent to selected points), and then the means and the 95% confidence intervals for 18 flowers were calculated. Open symbols indicate rising T_a, filled symbols indicate falling T_a.

Table 1. *Respiratory parameters of* Nelumbo nucifera *flower parts at* 30 °C

(Gas volumes are given at stpd. Six flowers were measured at the late bud stage (2a). CI = 95% confidence interval.)

		whole flower		receptacle		petals		stamens	
variable	units	mean	CI	mean	CI	mean	CI	mean	CI
mass	g	42.22	5.36	8.52	1.33	28.38	3.60	5.32	0.55
O_2 consumption	ml g^{-1} h^{-1}	1.54	0.21	4.21	0.99	0.60	0.08	2.60	0.22
	ml min^{-1}	1.09	0.22	0.61	0.16	0.28	0.04	0.23	0.02
	% total	100		54		25		21	
CO_2 production	ml g^{-1} h^{-1}	1.55	0.21	4.08	1.30	0.63	0.10	2.51	0.27
	ml min^{-1}	1.10	0.24	0.59	0.22	0.30	0.06	0.22	0.03
respiratory quotient		1.01	0.04	1.03	0.04	1.01	0.10	0.95	0.07

'homeothermic' period, T_r remains between about 30 and 36 °C when T_a varies between 10 and 45 °C (figure 2). At low T_a, the flower remains warm by increasing its rate of heat production (figure 3), and at high T_a, it lowers its heat production while evaporative cooling becomes important. The role of evaporation is clear when unhooded flowers are compared with hooded flowers used for respirometry (figure 2). High humidity in the respirometry hood, resulting in much condensation on its walls, inhibited evaporative heat loss from the flower and caused a rise in T_r. However, evaporation from unhooded flowers was less restricted, resulting in T_r below T_a.

The pattern of increasing rates of heat production as T_a decreases constitutes temperature regulation that is remarkably similar to the responses of homeothermic animals. The characteristics of true temperature regulation in animals include: (i) dependence on small changes in body temperature which are the stimuli that elicit thermogenic responses; and (ii) reversibility of the responses. In *N. nucifera*, both criteria are satisfied. When T_a declines, there is a small decrease in T_r (figure 2) that is associated with an increase in heat production (figure 3). Because the homeothermic period lasts during 2–4 days of fluctuating T_a (figure 1), it is clear that the responses are completely reversible.

(b) *Mechanism of temperature regulation*

Temperature regulation in *Nelumbo* apparently occurs at the cellular level, and it may be functionally linked to the cyanide-insensitive respiratory pathway, known to be present in the North American *Nelumbo* (Skubatz *et al*. 1990) and in several aroids (Meeuse & Raskin 1988). This pathway is thermally labile. For example, respiration of isolated aroid mitochondria decreases steeply at temperatures above 40 °C (Chauveau *et al*. 1978), which may account for the reversible thermal inhibition of respiration that is responsible for temperature regulation in the aroid, *Philodendron selloum* (Seymour *et al*. 1983). Thermal inhibition is not exclusively a high-temperature phenomenon, however. Inhibition apparently occurs at temperatures near 15 °C in eastern skunk cabbage, *Symplocarpus foetidus* (Knutson 1974). Inhibition of the cyanide-insensitive pathway in wheat mitochondria begins at temperatures above 17.5 °C, and changes in membrane lipids are thought to be involved (McCaig & Hill 1977). Thermal inactivation of maize mitochondria occurs at about 27 °C (Pobezhimova *et al*. 1996).

The pronounced hysteresis in the relation between $\dot{V}_{O_2}$ and T_r in field flowers (figure 5) may provide a clue to the mechanism of temperature regulation. Thermal inhibition of individual enzymes, or changes in enzyme activity owing to alterations in membrane structure and fluidity are likely to occur immediately with temperature change (Steponkus 1981). However, the response latency of 2–3 h may indicate the involvement of intermediate metabolic-regulators that act on rate-limiting enzymes involved in heat production. A similar hysteresis appeared in experiments on thermal inhibition of heat production in the aroid, *Philodendron selloum*, in which exposure to 45 °C for 5 min resulted in a 40% decrease in $\dot{V}_{O_2}$, that returned to normal only after 20 min at 39 °C (Seymour *et al*. 1983). Such response latency may be associated with slow changes in the concentrations of regulatory substances. It is known, for example, that organic acids such as pyruvic acid activate the cyanide-insensitive pathway in mitochondria of several plant species, including thermogenic aroids (Day *et al*. 1995). Cold exposure for 8 h is also known to activate respiration in a variety of chill-sensitive plant species (Moynihan *et al*. 1995). Alternatively, it is possible that regulation occurs by protein synthesis. Vanlerberghe & McIntosh (1992) reported an increase of alternative oxidase activity in tobacco cells after transfer from 30 to 18 °C owing to *de novo* synthesis of the oxidase protein. Further biochemical investigations on the responses in *Nelumbo* are clearly needed.

(c) *Respiration of cut flower parts*

At 30 °C, over half of the $\dot{V}_{O_2}$ by the flower occurred in the spongy receptacle, with the remainder divided equally between the petals and the stamens (table 1). However, the total $\dot{V}_{O_2}$ of the flower parts (1.09 ml min^{-1}) was lower than the overall $\dot{V}_{O_2}$ (1.75 ml min^{-1}) of field flowers with receptacles at 30 °C (figure 4). This difference may have resulted from either taking measurements in the afternoon and evening when respiratory rates are normally low (figure 5), or from a loss of activity in the flower because of cutting. The fractional distribution of $\dot{V}_{O_2}$ between the floral parts may therefore be different in the field, especially in the early morning when total $\dot{V}_{O_2}$ peaks at about 2.5 ml min^{-1} (figure 5). Previous workers have indicated that the main heat-producing parts of the flower are the staminal appendages (Comi 1939; Skubatz *et al*. 1990), but Vogel (1993) reported a temperature increase in the receptacle. Although the stamens have a mass-specific metabolic rate higher than the petals, it is considerably less than the mass-specific rate of the receptacle and the amount of staminal tissue is small (table 1). Thus, the receptacle is the principal thermogenic tissue of the flower, at least in stage 1 homeothermic flowers. The division of heat production in subsequent stages may change, with the stamens becoming more intense as the pollen is shed, but this idea needs to be confirmed.

We do not know which parts of the flower are responsible for thermoregulation because the effect of temperature on $\dot{V}_{O_2}$ of dissected parts was not investigated. We speculate that high temperature inhibition may reside in the receptacle, the most active part, and respiration of non-regulated parts of the flower may continue at high temperatures. This could explain the levelling of $\dot{V}_{O_2}$ at about 0.8 ml min^{-1} at T_a above 35 °C (figure 2).

The three floral tissues apparently oxidized carbohydrates because their respiratory quotient was 1.0 (table 1). Starch is the most likely substrate and it is present in the staminal appendages and receptacle of *Nelumbo* (Comi 1939; Schneider & Buchanan 1980; Skubatz *et al*. 1990; Vogel 1993). Most thermogenic aroids, including the thermoregulating skunk cabbage, *Symplocarpus foetidus* (Knutson 1974), and the European arum lily, *Arum maculatum* (ap Rees *et al*. 1977), also metabolize starch, but *Philodendron selloum* relies mainly on lipids, as shown by a respiratory quotient of 0.82 (Walker *et al*. 1983; Seymour *et al*. 1984).

(d) *Ecological role of thermoregulation*

Thermogenesis in flowers has long been thought to enhance vaporization of volatile floral scents which make the flower more attractive to insects (Fægri & van der Pijl 1979; Schneider & Buchanan 1980; Meeuse & Raskin 1988). In the case of skunk cabbage that blooms at ambient temperatures well-below freezing, heating is also thought to protect the flowers from cold damage (Knutson 1974). Although Fægri & van der Pijl (1979) dismissed the notion that a warm flower could act as a reward for insect pollinators, the occurrence of temperature regulation in *Philodendron selloum* was evidence that this inflorescence was catering to the thermal requirements of the pollinators, endothermic flying insects (Seymour *et al*. 1983). We hypothesize that, aside from the role of heating in scent production, the adaptive value of temperature stability in *Nelumbo* is related to some biological process that is temperature dependent, possibly linked to the thermal requirements of the pollinators.

Schneider & Buchanan (1980) concluded that the structure of *Nelumbo* is typical of beetle-pollinated flowers, prevalent among primitive angiosperms (Gottsberger 1988). Characteristics include a large flower with petals that form an internal chamber when they close, a large number of carpels with exposed nectar sources, and a multitude of stamens with staminal appendages and masses of pollen. Aside from providing food, beetle flowers may offer a rendezvous point for sexual activity (Fægri & van der Pijl 1979).

The best information on pollination in *Nelumbo* has come from studies by Sohmer & Sefton (1978), Schneider & Buchanan (1980) and J. B. Bullin *et al*. (unpublished data) in the North American lotus. These investigators list a large number of insects visiting the flowers, the species varying daily or seasonally or between different localities. The prominent groups, however, are beetles, bees and flies. Because of the diversity of insect visitors, it is difficult to ascribe pollination to any particular group, but J. B. Bullin *et al*. (unpublished data) suggest that beetles and large bees may be important vectors for first-day flowers when self-pollination is not possible. Second-day flowers can be self-pollinated, but insect vectors are probably required (Schneider & Buchanan 1980). Soldier beetles (*Chauliognathus marginatus*, Cantharidae) may be held overnight inside the floral chamber of second-day (stage 3) flowers where they copulate and consume pollen (Schneider & Buchanan 1980). When the flower opens the next morning, the insects carry pollen away.

The correspondence between flower temperature and activity temperatures of beetles is compelling. Many beetles cannot fly unless their thoracic temperatures rise to about 30 °C. For example, minimum thoracic flight temperature is 27 °C in 1.3 g green fig beetles (*Cotinus texana*, Scarabaeidae; Chappell 1984), 34 °C in 1.2 g grain beetles (*Plecoma* sp., Scarabaeidae; Morgan 1987), and 30 °C in 0.9 g flower beetles (*Pachnoda* sp., Scarabaeidae; Heinrich & McClain 1986). High thoracic temperatures for flight are not restricted to large scarabaeid beetles. Oertli (1989) studied flight thoracic temperatures in 24 species (ten families) of small (3–206 mg) beetles capable of flight. There was large variation, but the study showed thoracic temperatures generally between 25–35 °C. Inexplicably, beetles with larger body mass tended to be thermoconformers and those with small mass appeared to be better regulators, being able to raise their temperatures significantly. For example, thoracic temperatures of 93 mg *Popillia japonica* (Scarabaeidae) ranged from 31 to 38 °C at ambient temperatures between 24 and 36 °C, and tiny 16 mg *Cantharis bilineatus* (Cantharidae) maintained a thoracic temperature of 24–32 °C at ambient temperatures between 16 and 28 °C. However, it is confounding that soldier beetles (*Chauliognathus marginatus*), the 21 mg species found in second-night American lotus flowers (Schneider & Buchanan 1980), are thermoconformers and fly within a body-temperature range of at least 21–30 °C (Oertli 1989).

Flight is only one activity of beetles that demands a high body temperature. Competition for mates and food sometimes depends on the maintenance of a high body temperature (Bartholomew & Casey 1977; Bartholomew & Heinrich 1978; Morgan 1987). Furthermore, many beetles are not well-insulated, lose heat quickly and consequently expend prodigious amounts of energy to stay warm (Heinrich 1993). Assuming that beetles use the lotus flower to forage and find mates, the high, stable temperature inside the floral chamber would provide a direct energetic reward in allowing them to remain active during cool nights without the need to generate their own heat. This hypothesis should be tested by measuring the thermal requirements of the insects in the context of their natural behaviours in the flower. Unfortunately we cannot do this in Australia where the lotus is probably an introduced species (Borsch & Barthlott 1996). Even outside Australia, the widespread distribution of the lotus by humans, because of its dietary and religious significance, may not have included the insect pollinators with which the plant evolved, and the original plant–animal relations may have been obscured.

Although flying honeybees (*Apis mellifera*) maintain thoracic temperatures above 30 °C at ambient temperatures between 7 and 23 °C (Heinrich 1979), it seems unlikely that *Nelumbo* offers any direct energetic reward to them. Significantly, honeybees appear to be more attracted to post-thermoregulatory flowers than to earlier stages, and they usually do not remain in the flower overnight (Schneider & Buchanan 1980). In fact, Schneider & Buchanan (1980) noted that some bees lingering inside the flowers were killed, possibly by the scent, as suggested by Robertson (1889), but also possibly by the CO_2 liberated by the intense metabolism of the flower.

The fact that the lotus enters the homeothermic period about a day before the petals permit access to the interior confounds our proposition that thermoregulation benefits the pollinators alone. Another adaptive explanation for homeothermy is that it may accelerate and coordinate the development of the flower itself. Temperature is known to affect the timing and morphological results of flower development, but adverse effects usually occur at unusually high or low temperatures (Berry & Raison 1981; Kinet *et al*. 1985). It seems unlikely that *Nelumbo* flowers would develop abnormally if maintained at temperatures below 30 °C, but the answer lies in future research.

This project was supported by the Australian Research Council and the Deutsche Forschungsgemeinschaft. We thank Helen Vanderwoude, David Christophel and the staff at the Adelaide Botanic Garden for assistance, and Peter Bernhardt for as yet unpublished information on the pollination ecology of *Nelumbo* (J. B. Bullin *et al*.).

REFERENCES

ap Rees, T., Wright, B. W. & Fuller, W. A. 1977 Measurements of starch breakdown as estimates of glycolysis during thermogenesis by the spadix of *Arum maculatum* L. *Planta* **134**, 53–56.

Bartholomew, G. A. & Casey, T. M. 1977 Body temperature and oxygen consumption during rest and activity in relation to body size in some large tropical beetles. *J. Therm. Biol*. **2**, 173–176.

Bartholomew, G. A. & Heinrich, B. 1978 Endothermy in African dung beetles during flight, ball making, and ball rolling. *J. Exp. Biol*. **73**, 65–83.

Berry, J. A. & Raison, J. K. 1981 Responses of macrophytes to temperature. In *Physiological plant ecology*, vol. 1 (ed. O. L. Lange, P. S. Nobel, C. B. Osmond & H. Ziegler), pp. 277–338. Berlin: Springer.

Borsch, T. & Barthlott, W. 1996 Classification and distribution of the genus *Nelumbo* Adans (Nelumbonaceae). *Beitr. Biol. Pflanzen* **68**, 421–450.

Chappell, M. A. 1984 Thermoregulation and energetics of the green fig beetle (*Cotinus texana*) during flight and foraging behavior. *Physiol. Zool*. **57**, 581–589.

Chauveau, M., Dizengremel, P. & Lance, C. 1978 Thermolability of the alternative electron transport pathway in higher plant mitochondria. *Physiol. Plantarum* **42**, 214–220.

Comi, C. 1939 Ricerche sull' appendice clavata nell' antera del *Nelumbo nucifera*. *Nuovo Giorn. Bot. Ital*. **46**, 600–610.

Dacey, J. W. H. 1987 Knudsen-transitional flow and gas pressurization in leaves of *Nelumbo*. *Pl. Physiol*. **85**, 199–203.
Day, D. A., Whelan, J., Millar, A. H., Siedow, J. N. & Wiskich, J. T. 1995 Regulation of the alternative oxidase in plants and fungi. *Aust. J. Pl. Physiol*. **22**, 497–509.
Fægri, K. & van der Pijl, L. 1979 *The principles of pollination ecology*, 3rd edn. Oxford: Pergamon.
Gottsberger, G. 1988 The reproductive biology of primitive angiosperms. *Taxon* **37**, 630–643.
Gottsberger, G. 1990 Flowers and beetles in the South American tropics. *Acta Bot*. **103**, 360–365.
Heinrich, B. 1979 Thermoregulation of African and European honeybees during foraging, attack, and hive exits and returns. *J. Exp. Biol*. **80**, 217–229.
Heinrich, B. 1993 *The hot-blooded insects. Strategies and mechanisms of thermoregulation*. Cambridge, MA: Harvard University Press.
Heinrich, B. & McClain, E. 1986 'Laziness' and hypothermia as a foraging strategy in flower scarabs (Coleoptera: Scarabaeidae). *Physiol. Zool*. **59**, 273–282.
Ito, M. 1986 Studies in the floral morphology and anatomy of Nymphaeales. IV. Floral anatomy of *Nelumbo nucifera*. *Acta Phytotax. Geobot*. **37**, 82–96.
Kinet, J.-M., Sachs, R. M. & Bernier, G. 1985 *The physiology of flowering. III. The development of flowers*. Boca Raton: CRC.
Knutson, R. M. 1974 Heat production and temperature regulation in eastern skunk cabbage. *Science* **186**, 746–747.
Listabarth, C. 1996 Pollination of *Bactris* by *Phyllotrox* and *Epurea*. Implications of the palm breeding beetles on pollination at the community level. *Biotropica* **28**, 69–81.
McCaig, T. N. & Hill, R. D. 1977 Cyanide-insensitive respiration in wheat: cultivar differences and effects of temperature, carbon dioxide, and oxygen. *Can. J. Bot*. **55**, 549–555.
Meeuse, B. J. D. & Raskin, I. 1988 Sexual reproduction in the arum lily family, with emphasis on thermogenicity. *Sex. Pl. Reprod*. **1**, 3–15.
Miyake, K. 1898 Some physiological observations on *Nelumbo nucifera*, Gaertn. *Bot. Mag. Tokyo* **12**, 112–117.
Morgan, K. R. 1987 Temperature regulation, energy metabolism and mate-searching in rain beetles (*Pleocoma* spp.), winter-active, endothermic scarabs (Coleoptera). *J. Exp. Biol*. **128**, 107–122.
Moseley, M. F. & Uhl, N. W. 1985 Morphological studies of the Nymphaeaceae. XV. The anatomy of the flower of *Nelumbo*. *Bot. Jahrb. Syst*. **106**, 61–98.
Moynihan, M. R., Ordentlich, A. & Raskin, I. 1995 Chilling-induced heat evolution in plants. *Pl. Physiol*. **108**, 995–999.
Nagy, K. A., Odell, D. K. & Seymour, R. S. 1972 Temperature regulation by the inflorescence of *Philodendron*. *Science* **178**, 1195–1197.
Oertli, J. J. 1989 Relationship of wing beat frequency and temperature during take-off flight in temperate-zone beetles. *J. Exp. Biol*. **145**, 321–338.
Pobezhimova, T., Voinikov, V. & Varakina, N. 1996 Inactivation of complex I of the respiratory chain of maize mitochondria *in vitro* by elevated temperature. *J. Therm. Biol*. **21**, 283–288.
Prance, G. T. & Arias, J. R. 1975 A study of the floral biology of *Victoria amazonica* (Poepp.) Sowerby (Nymphaeaceae). *Acta Amazonica* **5**, 109–139.
Robertson, C. 1889 Flowers and insects. III. *Bot. Gaz*. **14**, 297–304.
Schneider, E. L. & Buchanan, J. D. 1980 Morphological studies of the Nymphaeaceae. XI. The floral biology of *Nelumbo pentapetala*. *Am. J. Bot*. **67**, 182–193.
Schroeder, C. A. 1978 Temperature elevation in palm inflorescences. *Principes* **22**, 26–29.
Seymour, R. S., Bartholomew, G. A. & Barnhart, M. C. 1983 Respiration and heat production by the inflorescence of *Philodendron selloum* Koch. *Planta* **157**, 336–343.
Seymour, R. S., Barnhart, M. C. & Bartholomew, G. A. 1984 Respiratory gas exchange during thermogenesis in *Philodendron selloum* Koch. *Planta* **161**, 229–232.
Seymour, R. S. & Schultze-Motel, P. 1996 Thermoregulating lotus flowers. *Nature* **383**, 305.
Skubatz, H., Williamson, P. S., Schneider, E. L. & Meeuse, B. J. D. 1990 Cyanide-insensitive respiration in thermogenic flowers of *Victoria* and *Nelumbo*. *J. Exp. Bot*. **41**, 1335–1339.
Skubatz, H., Tang, W. & Meeuse, B. J. D. 1993 Oscillatory heat-production in the male cones of cycads. *J. Exp. Bot*. **44**, 489–492.
Sohmer, S. H. & Sefton, D. F. 1978 The reproductive biology of *Nelumbo pentapetala* (Nelumbonaceae) on the Upper Mississippi River. II. The insects associated with the transfer of pollen. *Brittonia* **30**, 355–364.
Steponkus, P. L. 1981 Responses to extreme temperatures. Cellular and sub-cellular bases. In *Physiological plant ecology*, vol. 1 (ed. O. L. Lange, P. S. Nobel, C. B. Osmond & H. Ziegler), pp. 371–402. Berlin: Springer.
Vanlerberghe, G. C. & McIntosh, L. 1992 Lower growth temperature increases alternative pathway capacity and alternative oxidase protein in tobacco. *Pl. Physiol*. **100**, 115–119.
Vogel, S. 1993 The receptacle of lotus (*Nelumbo nucifera*): its heat generation, osmophore, and crystal cells. In *XVth International Botanical Congress Abstracts*, p. 337. Yokohama: Pacifico Yokohama.
Walker, D. B., Gysi, J., Sternberg, L. & DeNiro, M. J. 1983 Direct respiration of lipids during heat production in the inflorescence of *Philodendron selloum*. *Science* **220**, 419–421.
Weast, R. C. & Astle, M. J. (eds) 1979 *CRC handbook of chemistry and physics*, 60th edn. Boca Raton: CRC.
Wieser, W. 1986 *Bioenergetik. Energietransformationen bei Organismen*. Stuttgart: Georg Thieme.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES