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Regulation of thermogenesis in flowering Araceae: The role of the alternative oxidase

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ABSTRACT

The inflorescences of several members of the Arum lily family warm up during flowering and are able to maintain their temperature at a constant level, relatively independent of the ambient temperature. The heat is generated via a mitochondrial respiratory pathway that is distinct from the cytochrome chain and involves a cyanide-resistant alternative oxidase (AOX). In this paper we have used flux control analysis to investigate the influence of temperature on the rate of respiration through both cytochrome and alternative oxidases in mitochondria isolated from the appendices of intact thermogenic *Arum maculatum* inflorescences. Results are presented which indicate that at low temperatures, the dehydrogenases are almost in full control of respiration but as the temperature increases flux control shifts to the AOX. On the basis of these results a simple model of thermoregulation is presented that is applicable to all species of thermogenic plants. The model takes into account the temperature characteristics of the separate components of the plant mitochondrial respiratory chain and the control of each process. We propose that 1) in all aroid flowers AOX assumes almost complete control over respiration, 2) the temperature profile of AOX explains the reversed relationship between ambient temperature and respiration in thermoregulating *Arum* flowers, 3) the thermoregulation process is the same in all species and 4) variations in inflorescence temperatures can easily be explained by variations in AOX protein concentrations.

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1. Introduction

It is well documented that the inflorescences of a large number of species such as those from the Arum lily family are able to increase their temperature considerably above ambient temperature during thermogenesis. Inflorescences generate heat via a mitochondrial respiratory pathway that is distinct from the cytochrome chain and involves a cyanide (CN)-resistant alternative oxidase (AOX) [1]. This alternative pathway branches from the cytochrome pathway at the level of ubiquinone (UQ) and consists of a single protein, a ubiquinol: oxygen oxidoreductase [2–4]. Although no high-resolution AOX structure has been determined to date the current structural model predicts that AOX is an integral (~32 kDa) interfacial membrane protein that interacts with a single leaflet of the lipid bilayer, and contains a non-haem diiron carboxylate active site [5,6]. This model is supported by extensive site-directed mutagenesis studies [7–9] and EPR spectroscopic experiments have confirmed the presence of a binuclear iron centre [10,11]. The redox energy released during AOX activity is not conserved for the production of ATP, but is liberated as heat [12]. In addition to the alternative oxidase, cDNAs encoding uncoupling proteins (UCPs) have been isolated from several plants [13–15] and although some tissues have been shown to co-express UCPs with AOX [16,17] their role in promoting thermogenesis still remains unclear [18,19]. In thermogenic inflorescences, respiration via AOX can achieve rates comparable to that observed in 'a hummingbird in flight' [20]. As a result of this activity, the temperature of the inflorescence may rise up to 35 °C above ambient temperature [21] (see Fig. 1). This heat release may be used to volatilise malodorous compounds and/or maintain a warm environment in the flower [1,22], thereby attracting insects for pollination.

It has been reported that members of the Araceae, such as *Philodendron selloum* [23], *Symplocarpus foetidus* [21,24–28] and *Dracunculus vulgaris* [29], in addition to non-aroids such as the sacred lotus, *Nelumbo nucifera* [18,30,31], regulate their heat production in such a way that a constant temperature is maintained which is independent of the ambient temperature [32]. The question therefore arises as to what is the mechanism of thermoregulation in these plants. If, for instance, the ambient temperature increases, the process generating the heat (respiration) slows down in order to maintain a constant flower temperature [23,32,33]. Since the process has been shown to be reversible, however, it is highly unlikely that denaturation of respiratory enzymes is the mechanism responsible for thermoregulation [33]. A more popular explanation for thermoregulation is the temperature sensitivity of AOX itself [33,34]. However, scrutiny of published data reveals that the answer to the question is not that straightforward. Firstly, the flower temperature is species-dependent and although the temperature optimum of respiration via AOX has also been reported to be species-dependent, there appears to be no direct correlation between the two. For example it has been observed

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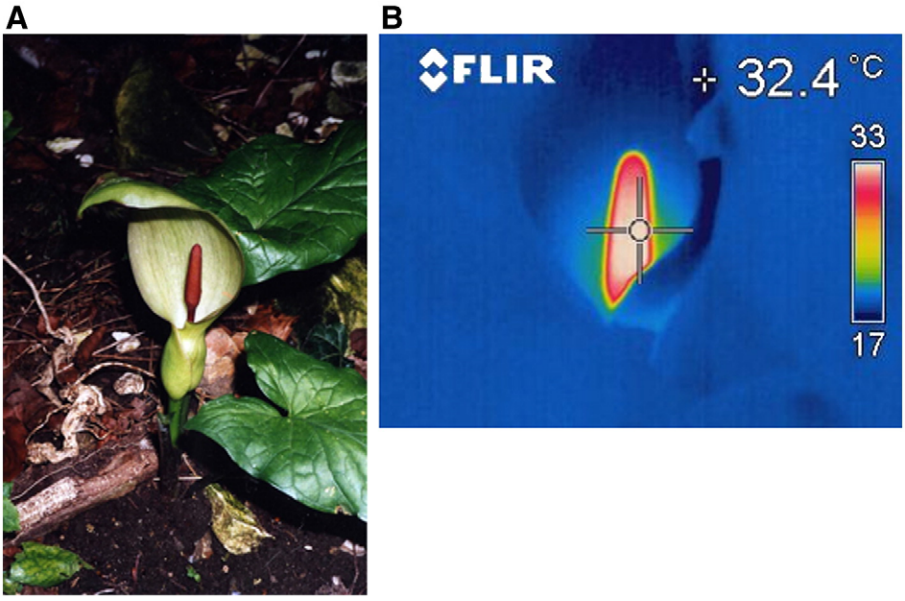

Fig. 1. Thermographic analysis of *Arum maculatum* spadix. (A) Structural features of an *A. maculatum* flower used for thermographic analysis. The unfolded spathe reveals the appendix (the spadix) which is the thermogenic organ. (B) Thermal imaging of the plant shown in A using a high-resolution infrared thermal imaging camera. The temperature scale of the thermographic analysis is shown on the right and the temperature of the spadix is indicated on the top right of the figure. The temperature measurement was taken at 18.00 h and within 2 h the temperature of the spadix had returned to the ambient temperature of 17 °C.

that although the flower temperature in the cuckoo pint (*Arum maculatum*) varies from 25 °C–35 °C [35], requiring respiration to slow down above an ambient temperature of 25 °C, the optimum temperature of respiration via AOX in *A. maculatum* mitochondria is greater than 30 °C [36]. Secondly, it has been reported that in *A. maculatum* and potato tuber callus mitochondria the temperature characteristics of AOX are dependent upon the oxidizable substrate [36,37] with temperature optima for AOX of 15 °C–17 °C as well as >25 °C [36–39] whilst more recently AOX activity in potato leaf mitochondria was found to be temperature insensitive between 15 and 25 °C [40]. Such data argue against a simple relationship between the optimum temperature of AOX and the flower temperature.

In an attempt to clarify this relationship we have used flux control analysis to investigate the influence of temperature on the rate of respiration through both cytochrome and alternative oxidases in mitochondria isolated from the appendices of intact thermogenic *A. maculatum* inflorescences. Results are presented which indicate that at low temperatures, the dehydrogenases are almost in full control of respiration but as the temperature increases flux control shifts to the AOX such that above 35 °C, AOX has almost full control of respiratory flux. We suggest that it is the temperature profile of AOX that explains the reversed relationship between ambient temperature and respiration in aroids and propose a simple model for the regulation of the flower temperature which not only accommodates the temperature dependence of AOX but can also account for the variations observed in inflorescence temperatures.

2. Materials and methods

2.1. Plant material

Appendices of *A. maculatum* were collected from plants growing at various sites on the campus of the University of Sussex (Brighton, UK) and in the garden of one of the authors (AMW) in Zoetermeer, The Netherlands.

2.2. Temperature measurements

Temperature of the appendices of thermogenic plants was measured *in situ* with a thermocouple (Electronic Thermometer model 1604, Comarck Electronics Ltd., Hertfordshire, UK) at about 1 cm below the appendix surface. Temperatures from non-thermogenic plants were measured after transferring the plants to pots and placing them in incubators set at various temperatures. For continuous temperature measurements, the upper half of the spathe of an intact plant *in situ* was removed to reveal the appendix, and a thermocouple connected to a recorder was inserted.

Heat loss was measured in three appendices of about the same size (±7 g) freshly cut from non-thermogenic inflorescences. Appendices were placed in incubators set at various temperatures for 30 min. Rates of heat loss were measured upon transfer of the appendices to an incubator set at 4 °C.

Thermal images were obtained using a FLIR E45 thermal imaging camera (ThermaCAM - FLIR Systems, USA). The specified temperature resolution was below 0.01 °C at room temperature and images were analyzed directly on the camera for temperature determination using the image analysis software provided by the manufacturer (QuickView, FLIR Systems USA).

2.3. Isolation of mitochondria and measurements of mitochondrial respiration

Mitochondria from *A. maculatum* appendices were isolated and purified on Percoll gradients as described by Moore et al. [41]. O_2 consumption was measured at 20 °C in 2 mL of reaction medium containing 0.3 M mannitol, 1 mM $MgCl_2$, 5 mM KH_2PO_4, 10 mM KCl, and 20 mM Mops, pH 7.2, in a glass vessel housing a Rank O_2 electrode. A mixture of succinate (20 mM), malate (10 mM), NADH (2 mM) and NADPH (5 mM) was given as the respiratory substrate. ADP (0.15 mM), pyruvate (5 mM) and DTT (10 mM) were present in all determinations. KCN (0.1 mM) was added to inhibit respiration via the Cyt pathway, and 2.5 mM salicylhydroxamic acid (SHAM) was added to inhibit AOX.

Mitochondrial protein was determined by the method of Bradford [42], using BSA for calibration.

2.4. Measurements of UQ reduction

UQ reduction in intact tissue and mitochondria was measured as described in [35,43] respectively. In this technique pieces of appendix tissue of 0.5 to 1 g were cut and immediately dropped in liquid N_2, ground to a fine powder with a mortar and pestle, and 10 mL of 0.2 M $HClO_4$ in methanol (0 °C) was added. The mixture was transferred to a tube with 10 mL of petroleum ether (boiling point 40 °C–60 °C) and vortexed for 1 min. After the mixture was centrifuged at 1500 *g* for 2 min, the upper petroleum ether phase was removed, transferred to a test tube, and evaporated to dryness under a flow of N_2. A further 10 mL of petroleum ether was added to the lower phase, and the vortex and centrifugation steps repeated. The upper phase was added to the one previously obtained. After evaporation, extracts could be stored for at least 1 day under N_2 at −20 °C. Immediately before use, the extracted UQ was resuspended with a glass rod in 100 µL of N_2-purged ethanol, and analyzed by HPLC at the VU Universiteit in Amsterdam with a pump system (Gilson, Villiers le Bel, France) and detector (model 811, Perkin-Elmer), and at the University of Sussex with an absorbance system (model 160, Beckman), a solvent-delivery module (model 110B, Beckman), and a reverse-phase column (model 10-RP 18 Lichrosorb, Chrompack, Bergen op Zoom, The Netherlands; 4.6×250 mm in size). The column was equilibrated with N_2-purged ethanol–methanol (3:2, v/v) and this mixture was used as the mobile phase. Detection of UQ was performed at 290 nm.

2.5. Estimation of flux control distribution in the respiratory chain

The flux control distribution in respiring *A. maculatum* mitochondria was estimated with the following approximations (see Fig. 2):

1. The kinetics for both UQ-reducer (dehydrogenases, DEH) and UQH_2-oxidizer (AOX) is approximately linear at all temperatures (cf. the results depicted in Fig. 6A and B for 15 °C and 25 °C).
2. The rate v is 0 at both the minimal reduction level of the UQ pool q_{min} (no substrates) and the maximal reduction level of the UQ pool q_{max} (complete inhibition of UQ oxidation).

With these approximations, the procedure follows three steps:

1. Estimation of the slopes of the DEH and AOX kinetics from the steady-state values of rate v and UQ reduction q. This procedure is illustrated in Fig. 2 and yields Fig. 6C.
2. Calculation of the elasticity coefficients ε_q^{deh} and ε_q^{aox} by normalizing the slopes of the DEH and AOX kinetics. Note that the values of these coefficients only depend on the three UQ reduction levels:

$$\varepsilon_q^{deh} = \frac{q}{q - q_{max}} \qquad \varepsilon_q^{aox} = \frac{q}{q - q_{min}}$$

3. Calculation of the flux control coefficients from the elasticity coefficients using the summation and connectivity theorems of metabolic control analysis [44], to yield:

$$C_{deh}^{J} = \frac{q_{max} - q}{q_{max} - q_{min}} \qquad C_{aox}^{J} = \frac{q - q_{min}}{q_{max} - q_{min}}$$

The values of the flux control coefficients are plotted as a function of temperature.

2.6. Modelling

Warming up (increasing T_{app} by heat production by respiration) and cooling down (decreasing T_{app} by heat loss to the environment at T_{amb}) was modeled as a temperature change described by a third order polynomial (see Fig. 7):Heating up:

$$\frac{dT_{app}}{dt} = [protein] \cdot \left(4.24 + 4.7123 \cdot T_{app} - 0.21339 \cdot T_{app}^2 + 0.0023841 \cdot T_{app}^3\right)$$

Cooling down:

$$\frac{dT_{app}}{dt} = -\left(0.13422 \cdot (T_{app} - T_{amb}) + 0.0001669 \cdot (T_{app} - T_{amb})^3\right)$$

For the modelling Stella II software (High Performance Systems Inc. Hanover NH, USA) was used.

3. Results

During previous work from our laboratories with *A. maculatum* spadices [35], it was noted that the temperature which the spadix achieved during thermogenesis was relatively independent of the ambient temperature. Such observations are summarised in Fig. 4 which indicates that in non-thermogenic plants there is a linear relationship between ambient and appendix temperature whereas in thermogenic inflorescences such a relationship exists to a much lesser extent. Such results indicate that *A. maculatum* regulates its flower temperature in a similar manner to that reported for a number of other aroid species [21,23,30,31]. Hence it is logical to surmise from the above that respiration of the thermogenic appendix decreases with increasing ambient temperature.

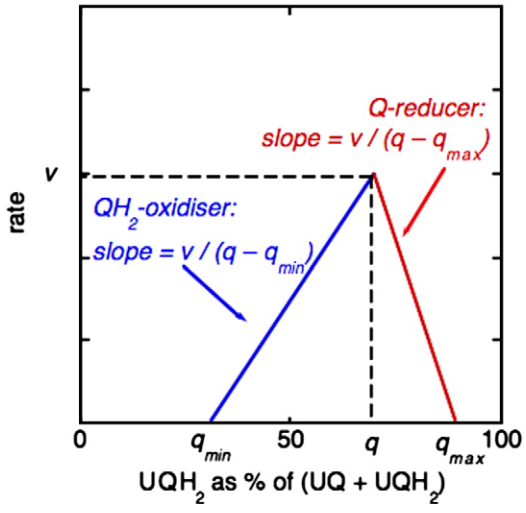

Fig. 2. Modelling of theromoregulation in thermogenic plants. Estimation of flux control distribution in the respiratory chain of *Arum maculatum* mitochondria. For further details see Materials and methods.

Fig. 5 shows respiratory activity and ubiquinone (UQ)-pool reduction in mitochondria isolated from the appendices of thermogenic inflorescences either in the presence or absence of respiratory inhibitors. It is apparent from Fig. 5A that although respiration is linearly dependent upon the assay temperature it is inhibited at assay temperatures above 32 °C. Since flower temperatures range between 25 °C and 35 °C, a direct relationship between flower temperature and mitochondrial respiratory activity apparently cannot simply account for thermoregulation. Furthermore Fig. 5A clearly shows that the activity of the cytochrome pathway (respiration in the presence of SHAM), is very low in mitochondria isolated from thermogenic appendices of *A. maculatum* [45,46] and hence electron transport via the AOX can be regarded as the main respiratory pathway in thermogenic inflorescences [46]. Previously we reported [35] that in intact, thermogenic inflorescences of *A. maculatum*, the UQ pool is almost fully reduced during thermogenesis. Fig. 5B shows that this high level of *in vivo* UQ reduction occurs at appendix temperatures from 24 °C–30 °C. In isolated mitochondria, however, it is apparent that even in the presence of a mixture of substrates (thereby engaging all dehydrogenases and hence ensuring that the reduction of UQ is maximal), the UQ pool was no higher than 50% reduced at 24 °C, and only at assay temperatures of 35 °C–40 °C, did it become as reduced as that observed during *in vivo* thermogenesis. Such a result suggests that in isolated mitochondria, the dehydrogenases (DEH) apparently cannot achieve activities, relative to AOX, as those observed *in vivo*. In other words, the redox level of the UQ pool is determined by the balance between the activities of DEH and AOX. When the UQ pool is maximally reduced it is AOX that controls the respiratory flux and when UQ is fully oxidized it is DEH that exerts the most control.

Since both AOX and DEH use UQ as a substrate, a plot of rate against UQ reduction can be used to characterize both processes kinetically [47] and the results are depicted in Fig. 6. In Fig. 6A the UQ-oxidizing pathway (AOX) is plotted, whereas in Fig. 6B the UQ-reducing pathways (all dehydrogenases engaged) are shown. It is apparent from Fig. 6A that when the rate of succinate oxidation is varied through titration with malonate a linear relationship between UQ reduction and the respiratory rate is obtained which is comparable to that previously observed in thermogenic *Arum* mitochondria [45,46]. Interestingly Fig. 6A also reveals that, although the uninhibited respiratory rates increase when the temperature increases from 15 °C to 25 °C, the slope of the line representing the relationship between AOX and substrate concentration (reduced UQ), is lower at 25 °C than at 15 °C. Since the slope is a measure for the potential activity of AOX, this means that at 15 °C AOX activity is higher at a certain level of reduced UQ than at 25 °C.

In a similar manner, the temperature characteristics of DEH activities have been estimated. DEH uses UQ as the substrate and the rate of DEH will be zero when UQ is fully reduced. We determined the characteristics of DEH by measuring the reduction level of UQ and varied the rates by titrating with 1 mM SHAM. Fig. 6B shows that the total rate of the dehydrogenases is also linearly dependent upon UQ reduction, in accordance with previous results [47]. However, in contrast to the reaction of AOX to temperature, the slope of the line, representing the relationship between DEH rate and substrate concentration (oxidized UQ), is higher at 25 °C than at 15 °C.

In order to further establish the temperature profiles for the UQ-oxidizing and -reducing pathways, we determined slopes at a range of temperatures, using the data presented in Fig. 5. The slopes for total respiration, AOX, the cytochrome pathway and DEH were determined for each temperature using the respiratory rates and UQ reduction

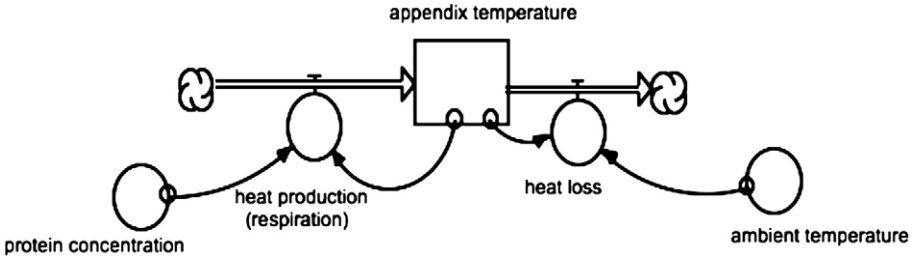

Fig. 3. The Stella model for thermoregulation in aroids. For details see Materials and methods.

levels measured in Fig. 5 along with the UQ reduction levels measured in the absence of substrate and following full inhibition with CN and SHAM. In this manner three points for each assay temperature were obtained, from which the slopes for DEH and AOX can be calculated for each temperature (see Fig. 2; Materials and methods for methodology). A comparison of the temperature profiles of the slopes (Fig. 6C) reveals that in *A. maculatum* the optimum temperature for total respiration and AOX is approximately 16 °C, whereas the optimum temperature for DEH is approximately 35 °C. It is also apparent from Fig. 6C that the slopes for the cytochrome pathway are very low, again indicating that respiration via this pathway does not significantly contribute to total respiration, and furthermore that the slopes of the cytochrome pathway are higher at 25 °C than at 15 °C.

Flux control coefficients of AOX and DEH can be calculated from the slopes plotted in Fig. 6C. Fig. 6D shows that at low temperatures DEH is almost in full control of respiration, but as the temperature increases, flux control shifts from DEH to AOX. It is apparent that at those temperatures where UQ is fully reduced in mitochondria (above 35 °C), AOX has almost full control of the respiratory flux.

4. Discussion

A principal objective of the present study was to determine whether there was a simple relationship between the optimum temperature for AOX activity and the flower temperature as current data provides conflicting results. An answer to this question is of importance since a strong correlation between these two parameters would suggest that it is the alternative oxidase pathway activity that is responsible for the temperature rises observed in such tissues and furthermore would argue against AOX possessing differing temperature sensitivity that is species-dependent. The results expressed in Figs 5 and 6 clearly demonstrate that although both the redox poise of the UQ pool and total respiration are linearly dependent upon the assay temperature this is the case only up to 32 °C. Above this temperature, although the UQ pool becomes further reduced, total respiration is severely inhibited suggesting that since flower temperature varies between 25 and 35 °C a simple relationship between respiration and flower temperature cannot exist. In addition, the finding that cytochrome pathway activity during thermogenesis only contributes less than 15% of total respiration (Fig 5A) confirms recent oxygen isotope results which suggest that in thermogenic tissues the alternative pathway is probably operating close to full capacity [18].

Since the optimal temperature for maximal mitochondrial respiration is 32 °C yet the flower temperature is between 25 °C and 35 °C (thereby necessitating a decrease in AOX activity at temperatures above 25 °C) it is clear that, *in vivo*, the rate of respiration via AOX must be regulated differently in comparison to isolated mitochondria. Two mechanisms have been suggested to explain the regulation of AOX

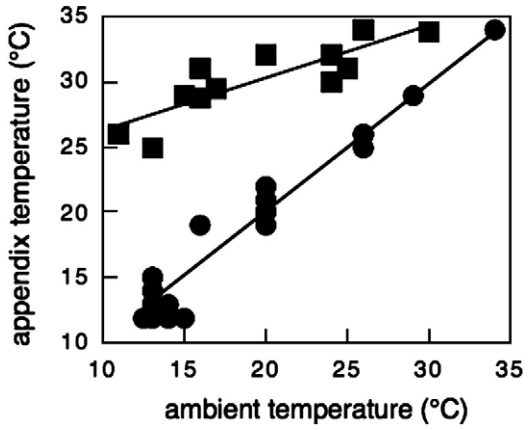

Fig. 4. Temperatures of appendices of intact thermogenic (■) and non-thermogenic (●) *A. maculatum* inflorescences. Lines are linear fits with slopes of 0.98 (non-thermogenic) and 0.39 (thermogenic).

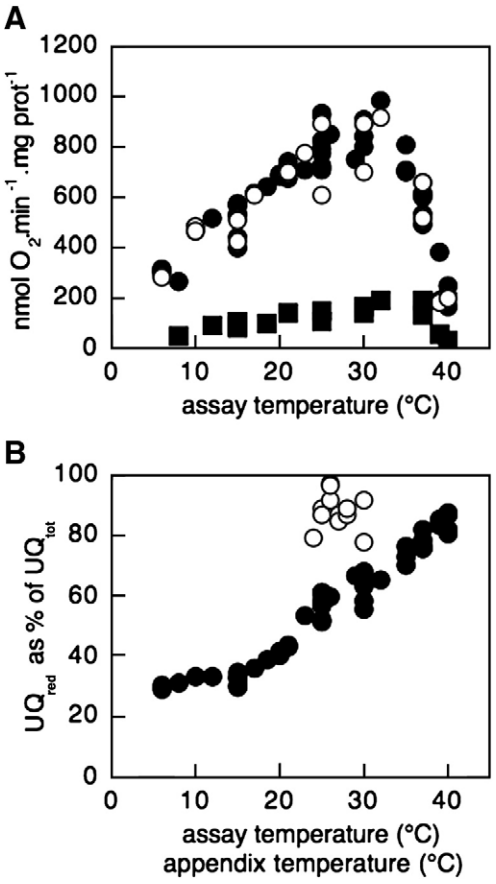

Fig. 5. Respiration and UQ reduction. (A) The influence of assay temperature on total respiration (●) and respiration in the presence of KCN (0.1 mM, O) or SHAM (2.5 mM, ■) in mitochondria isolated from the appendices of intact thermogenic *A. maculatum* inflorescences. Each point represents a separate determination. Results from 4 different mitochondrial preparations are combined. (B) UQ reductions in intact appendices from thermogenic *A. maculatum* (O) and UQ reductions in mitochondria isolated from thermogenic appendices (●) respiring at various temperatures. Mitochondrial samples were taken after respiration was measured (data in panel A).

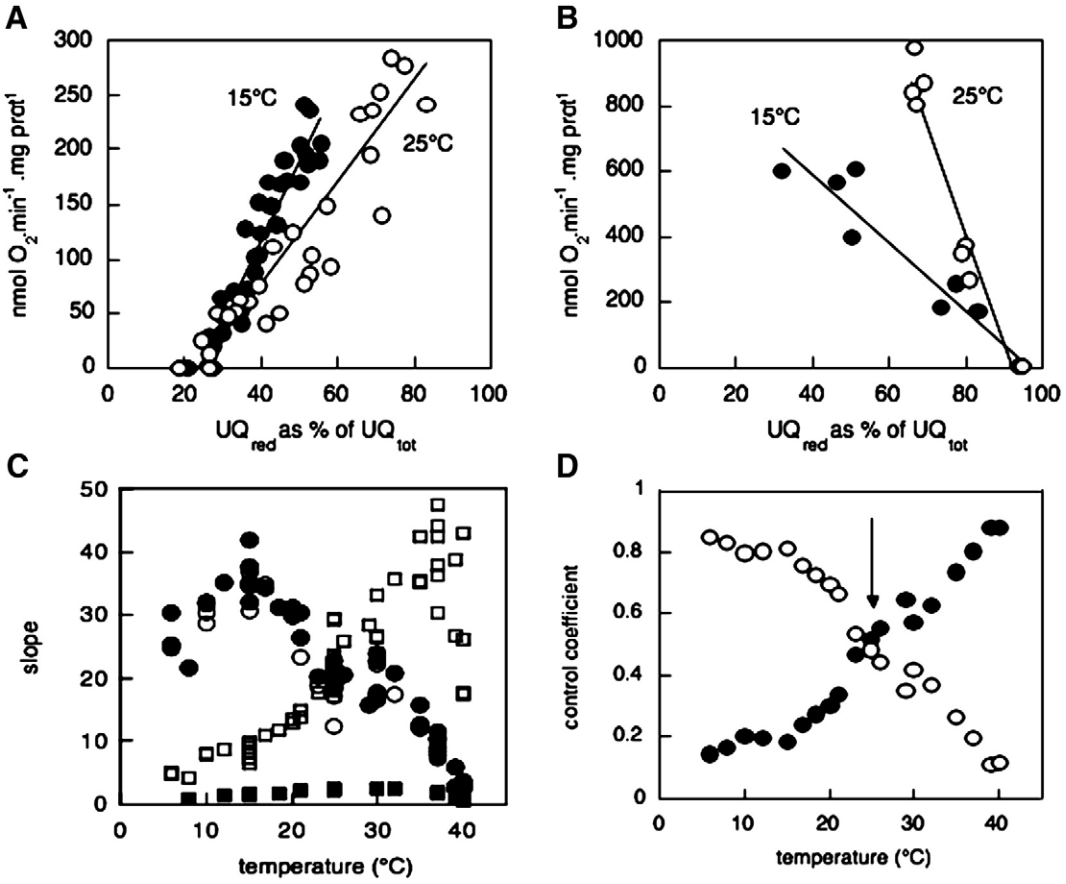

Fig. 6. Temperature profiles and control of respiration. (A) and (B) Relationships between rates and substrate concentrations for the UQ-oxidizing pathway (AOX, A) and for the UQ-reducing pathways (DEH, B) in mitochondria isolated from thermogenic *A. maculatum* appendices at assay temperatures 15 °C (●) or 25 °C (○). (C) Slopes of the lines representing the relationship between substrate and total respiratory rates (●), DEH (□), AOX (○) and the cytochrome pathway (■), obtained by using the data in Fig. 5A and B. (D) Flux control coefficients for AOX (●) and DEH (□) at various assay temperatures. Arrow represents the shift in control from DEH to AOX. Data from Fig. 5A and B were used for the calculations.

activity [for reviews see Refs. 3,4,48]. One involves the reduction of the disulphide bridge that connects the two halves of the AOX dimer [49], whereas the other involves the interaction of alpha keto-acids, principally pyruvate, both of which stimulate AOX activity [50–53]. However, whether either of these two mechanisms plays important roles in the regulation of AOX activity *in vivo*, is debatable [3,48]. Importantly, we were unable to detect any change in the reduction of the disulphide bridge of AOX within whole thermogenic tissues upon incubation at various temperatures, nor were any significant differences in pyruvate concentrations observed when whole plants are incubated at a range of temperatures from 8 °C–25 °C (A. M. Wagner, unpublished results). There are, however, two other factors that may determine enzyme activity *in vivo*, namely substrate and protein concentration. The substrate for AOX is ubiquinol (UQH_2), which is the product of the dehydrogenases [2]. This means that the activity of AOX is not only determined by its protein concentration, but also by the rate by which the dehydrogenases can reduce UQ. Here, in our opinion, lies the explanation for the apparent discrepancy between flower temperature and AOX temperature dependence. For instance, respiration is a sequence of metabolic processes which involve not only AOX but many other enzymes (i.e. glycolysis, citric acid cycle, dehydrogenases) all acting in concert. Each of these processes will have its unique temperature profile. The temperature profile of the respiratory flux will thus depend on the profiles of these processes and on the control of each process exerted by respiratory fluxes [39].

In order to explain the discrepancy in temperature optimum of *in vivo* respiration compared to that observed in isolated mitochondria, we suggest that the activity of AOX has a lower temperature optimum than that of the DEHs. During thermogenesis *in vivo* UQ is always highly reduced (Fig. 5B) and AOX exerts a high control on respiration. As a consequence of the above the temperature dependence of respiration *in vivo* will almost be completely determined by the temperature dependency of AOX. In isolated mitochondria, however, UQ reduction increases with increasing temperatures but is always much lower than in intact thermogenic tissue (Fig. 5B) and therefore in isolated mitochondria the temperature profile of the DEH will co-determine the net temperature profile of mitochondrial respiration thereby, resulting in a higher temperature optimum for mitochondrial respiration compared to *in vivo* respiration.

We propose that in intact thermogenic tissues AOX has a similar temperature profile to that observed in isolated mitochondria with an optimum at around 16 °C. The temperature profile of the whole respiratory chain is thus determined by the amount of control exerted by AOX and DEH respectively. In non-thermogenic tissues AOX is present in a fairly low concentration, and when the cytochrome pathway is inhibited by cyanide, UQ is generally fully reduced and AOX exerts a high flux control. This is consistent with the findings that, for respiration in potato tuber callus and wheat mitochondria [37,38] utilizing AOX, the temperature optima are in the range of 16 °C. Interestingly, when the activity of AOX in potato tuber callus was stimulated by keto-acids (thus decreasing the level of control by AOX) the temperature dependency changed to a higher optimum [37] as predicted by this hypothesis.

It should be noted that Atkin et al. [39] determined the temperature profile of AOX in mitochondria isolated from soybean cotyledons in a similar manner to that outlined in this paper by determining the relationship between UQ reduction levels and respiratory rates (thereby excluding an effect of a switch in control from AOX to DEH). These authors found an increase in AOX rate with increasing temperatures up

to 25 °C. An explanation for this apparent discrepancy may lie in the finding that in higher plants there are two gene families of AOX (*Aox1* and *Aox2*). *Aox1* type genes are present in both monocotyledon and dicotyledon plants, whereas *Aox2* type are expressed only in dicotyledons [54,55]. Moreover, in soybean, *Aox1* expression has only been reported in exceptional conditions such as upon respiratory inhibition by antimycin A in cell cultures [56]. It is not inconceivable that *Aox1* and *Aox2* gene products may differ in temperature optima, with an optimum at around 16 °C for AOX1 and a much higher optimum (>25 °C) for AOX2. Our results with potato tuber callus [37] coupled with the recent results of Covey–Crump [40], in both of which a temperature optimum of 16 °C was observed, are completely in line with this proposal since only AOX1 is expressed in potato [54,55].

An implication of the model presented in this paper, in which the temperature profile of AOX in aroids explains the reversed relationship between ambient temperature and respiration, is that thermogenic tissues cannot maintain an inflorescence temperature below 16 °C. Indeed, as far as we are aware, the lowest temperatures reported for such tissues appear to be approximately 15 °C in *D. vulgaris* and *S. foetidus* [21,29].

For species with a higher inflorescence temperature than that observed in *A. maculatum*, it is possible that if the temperature optimum shifts to a higher temperature the switch in control (see Fig. 6D) also rises to a higher temperature. However, experimental data indicates that although *Amorphophallus krausei* maintains a maximum flower temperature of 38 °C, the level of UQ reduction measured in intact appendices is 91% during thermogenesis [35]. This suggests that AOX always retains full control during thermogenesis, a result that is inconsistent with the notion that there is a shift in control from DEH to AOX. A change in the balance between heat production and heat loss could, of course, also account for the maintenance of a higher temperature.

We therefore propose that in all aroid flower appendices at the onset of thermogenesis, when substrate supply increases due to increased glycolytic rates (and potential contribution of uncoupling proteins [14,17]), UQ becomes highly reduced and AOX assumes almost complete control over respiration. Under such conditions the maximum achievable temperature will be dependent upon the balance between heat loss and the respiratory rate.

This can be readily illustrated by a very simple model in which;

1) the dependence of heat production on inflorescence temperature is proportional to the fit of the AOX temperature profile shown in Fig. 6C (see Fig. 7A for the used fit)
2) heat loss is proportional to the difference between ambient temperature and temperature of the inflorescence (Fig. 7B).

The model (see Fig. 3), which has as inputs the ambient temperature and the AOX protein concentration, calculates the inflorescence temperature that the system can achieve. When the AOX protein concentration is set to the arbitrary unit of 0.1, an inflorescence temperature of 26 °C is predicted when the ambient temperature is set at 13 °C (Fig. 7C).

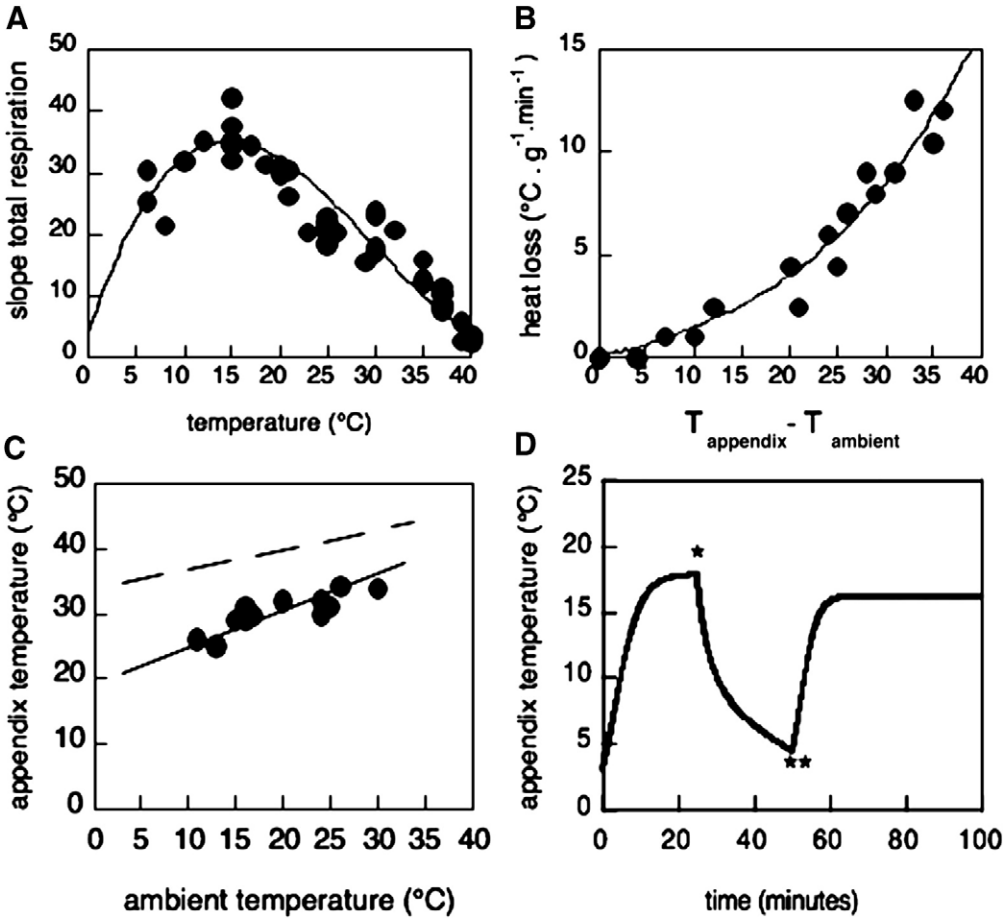

Fig. 7. Modelling for thermoregulation. (A) 3rd order polynomial fit to the AOX temperature profile used for the modelling of heat production. (B) 3rd order polynomial fit to cooling down used for the modelling of heat loss. (C) Simulation of thermoregulation with arbitrary protein concentrations of 0.1 (straight line) and 1.0 (dotted line). The measured appendix temperatures at various ambient temperatures for *A. maculatum* (Fig. 4) are included for comparison (●). (D) Simulation of the effect of a sudden cold shock. With a protein concentration of 0.075 and ambient temperature 3 °C, a change in ambient temperature of −10 °C (*) leads to loss of thermoregulation. An increase in protein concentration to 0.15 (**) restores thermoregulation.

Fig. 7C predicts that at this protein concentration, inflorescence temperatures at ambient temperatures from 10 °C–30 °C are regulated between 26 °C and 35 °C. As can be seen from Fig. 7C such simulations are in very good agreement with the experimental data obtained for *A. maculatum*. When the protein concentration is increased (to an arbitrary unit of 1), inflorescence temperatures become higher, resembling the situation in for instance *P. selloum* [23]. Interestingly, the model also shows that the higher the temperature of the inflorescence, the less is the predicted variation of the flower temperature over the range of ambient temperatures (Fig. 7C). Again, this result is in accordance with experimentally observed data [33].

Fig. 7C furthermore clearly shows that in order to maintain thermoregulation, for a certain species at a most favourable temperature, adjustment of the heat production (i.e. amount of AOX) relative to heat loss is all that is required. Protein concentration, therefore, seems an important factor in the control of thermoregulation. It has been reported that upon sudden drastic temperature changes, plants temporarily lose the capability to regulate the temperature. For instance, when snow is packed around *S. foetidus*, the inflorescence initially cools, but after a short time heat production increases again and the temperature begins to rise. It has been suggested that in such situations protein concentration is simply insufficient to cope with the increased heat demand [33]. Fig. 7D mimics such a situation in which a severe drop in ambient temperature causes the loss of the thermoregulatory power of the appendix. A twofold increase in protein, however, can restore thermoregulation to a level not very different from that before the temperature drop. An alternative possibility for a loss in thermoregulation is that, upon drastic environmental changes, UQ reduction levels temporarily drop (due to a decrease in glycolytic rates) thereby resulting in a shift of control to the DEH.

In conclusion, a very simple model is proposed that can explain thermoregulation at various flower temperatures without the need to postulate varying temperature optima for AOX activity in various aroid species. Furthermore we propose that the thermoregulation process is the same in all species and is due to a metabolic explosion resulting in a very high supply of mitochondrial substrate, and hence a fully reduced UQ pool, which enables AOX to set and maintain the flower temperature through regulating its protein concentration.

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