

Floral thermogenesis: An adaptive strategy of pollination biology in Magnoliaceae

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Floral thermogenesis plays a crucial role in pollination biology, especially in plant–pollinator interactions. We have recently explored how thermogenesis is related to pollinator activity and odour release in *Magnolia sprengeri*. By analyzing flower temperatures, emission of volatiles, and insect visitation, we found that floral blends released during pistillate and staminate stages were similar and coincided with sap beetle visitation. Thus, odour mimicry of staminate-stage flowers may occur during the pistillate stage and may be an adaptive strategy of *Magnolia* species to attract pollinators during both stages, ensuring successful pollination. In addition to the biological significance of floral thermogenesis in *Magnolia* species, we explored the underlying regulatory mechanisms via profiling miRNA expression in *M. denudata* flowers during thermogenic and non-thermogenic stages. We identified 17 miRNAs that may play regulatory roles in floral thermogenesis. Functional annotation of their target genes indicated that these miRNAs regulate floral thermogenesis by influencing cellular respiration and light reactions. These findings increase our understanding of plant–pollinator interactions and the regulatory mechanisms in thermogenic plants.

Thermogenic plants produce an unusually large amount of heat during the floral stages,¹ which has been reported in flowers of several families of seed plants.^{2–8} Thermogenesis is considered a direct energy reward for the insect visitors that usually reside in a floral chamber.⁹ Thermogenesis is also associated with

volatilisation of floral scents, which attract pollinators during the anthesis.^{10–12} Recently, we studied the relationship between thermogenesis and pollinator activity during flowering of *M. sprengeri* by investigating flower temperatures, emission of volatiles, and insect visitation. It was found that release of floral blends and insect visitation were closely associated with heat production. In addition, we found that floral odours during pistillate and staminate stages were fundamentally similar, indicative of odour mimicry of staminate-stage flowers during the pistillate stage. Based on these results, floral thermogenesis plays a role in pollination biology of *M. sprengeri* by promoting emission of floral scents, which act as a signal of food rewards for pollinators.¹³

Flowers of thermogenic plants have the ability to maintain a relatively higher temperature than ambient environments during anthesis.^{14–16} In this study, although the floral temperature of *M. sprengeri* changed constantly throughout anthesis, it was relatively higher than ambient air temperature, and heat production showed 2 noticeable peaks; one at the pistillate stage and the other at the staminate stage. The thermogenic pattern of *M. sprengeri* was identical to that of its close relative, *M. denudata*—both flower during early spring and produce heat in the daytime.¹³ The thermogenic peaks of these species are well-synchronised with the activity of their pollinators. It is interesting to note that pollinators of *M. ovata* (which produces heat at night) are active at night.¹⁶ These studies reveal an association between floral thermogenesis and pollinator visitation.

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Thermogenesis is closely related to the volatilisation of floral odour in *M. sprengeri*. Since floral odour and heat production are dynamic processes, the relative proportions of the odour compounds varied across anthesis. We found that the floral blends released during pistillate and staminate stages were very similar in *M. sprengeri*, indicative of odour mimicry of staminate-stage flowers in pistillate-stage flowers. Previously, it was suspected that staminate-stage flowers mimic pistillate-stage flowers in *M. hypoleuca*, as the former offers no pollen reward but has strong fragrance.¹⁷ Our results confirm odour mimicry between the female and male stage flowers in a *Magnolia* species. In another study, a similarity in the smells of pistillate- and staminate-stage flowers of *M. ovata* was reported.¹⁸ These results reveal that flowers of *Magnolia* species at both pistillate and staminate stages attract pollinators through odour mimicry. Considering that odour emission coincided with the 2 thermogenic episodes, it is possible that thermogenesis promotes odour release, which attracts pollinators at both the pistillate and staminate stages, ensuring reproductive success at low temperatures during the early spring.

Although the ecological significance of thermogenesis is widely recognized,^{19–21} little information on the underlying regulatory mechanisms is available.²⁰ miRNAs

are crucial regulators of various biological processes in eukaryotic cells,^{22,23} but their roles in floral thermogenesis remain unclear. We recently profiled miRNA expression in *M. denudata* flowers during thermogenesis using high-throughput sequencing.²¹ A total of 82 conserved and 32 novel miRNAs were identified in *M. denudata* flowers, among which 17 were differentially expressed between thermogenic and non-thermogenic stages, and thus were thought to play roles in regulating floral thermogenesis. Gene Ontology (GO) enrichment analysis revealed that target genes of these thermogenesis-related miRNAs were enriched in the functional groups of ‘polyprenyl transferase activity’ and ‘photosynthetic electron transport’. Thus, we propose that regulation of floral thermogenesis may be associated with cellular respiration and photosynthesis in *M. denudata*.

Two types of thermogenesis have been identified among thermogenic plants. In some species, floral temperature is maintained within a constant range, independently of ambient temperature, throughout anthesis (Fig. 1A). These are thermoregulatory species, such as *Philodendron selloum*,²⁴ *Nelumbo nucifera*,²⁵ and *Symplocarpus foetidus*.²⁶ In other thermogenic species, heat production usually corresponds to the period when female flower parts are most receptive to

pollination and when floral scents are strongest (Fig. 1B), such as in *Dracunculus vulgaris*, *Helicodiceros muscivorus*,^{24,27} and *Magnolia* species.²⁸ Such thermogenic plants are sometimes referred to as pseudo-thermoregulatory species. Thermogenesis directly rewards pollinators (beetles) with energy, especially at night when no floral scents are released.^{19,29} Compared with thermoregulatory flowers, which produce heat during anthesis, pseudo-thermoregulatory species use less energy, as their heat production is tied to the circadian cycle. They experience intense warming only during peaks of scent emission, promoting odour release to attract visiting insects. The pseudo-thermoregulatory strategy for attracting pollinators may be more efficient than the thermoregulatory strategy in having more benefits and fewer costs. The divergence of the 2 strategies may involve co-evolution with pollinator insects. Further comparative studies of the 2 types of thermogenesis may facilitate our understanding of evolution and of regulatory mechanisms driving floral thermogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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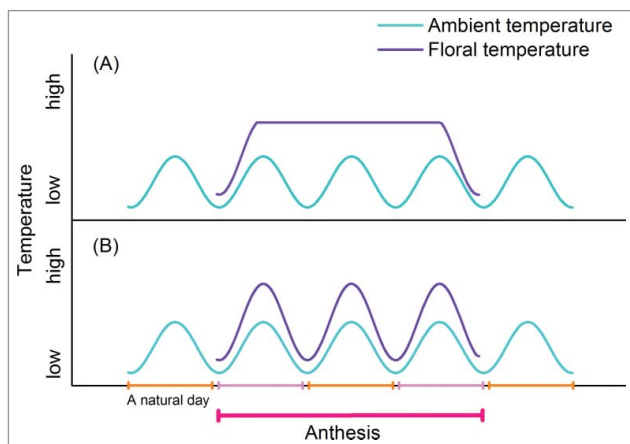


Figure 1. Different patterns of heat production in thermogenic flowers. **(A)** In some thermogenic flowers, heat production is physiologically regulated to maintain a relatively constant floral temperature, independently of ambient temperature, such as in skunk cabbage (*Symplocarpus foetidus*).²⁶ **(B)** Thermogenesis is related to the circadian cycle and greatly increases when scent is released in most thermogenic flowers, including *Magnolia* spp.²⁸

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