Function of the heater: the dead horse arum revisited

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The dead horse arum, *Helicodiceros muscivorus*, is a conspicuous, foul smelling and thermogenic plant of the Araceae family. This Mediterranean arum lily copies several aspects of a carcass in order to attract carrion blowflies, which are subsequently exploited as unrewarded pollinators. We have previously shown that this plant exhibits a highly accurate olfactory carrion mimicry, which serves to attract the blowflies. In this study, we have investigated the role of thermogeny in the arum. We show that the thermogeny has a direct effect on the pollinators, altering their behaviour. By manipulating heat and odour release of the plant, we can show that the heat, produced along the appendix, is important to lure the flies to this structure, which is vital as the flies from the appendix are more prone to enter the trap chamber that houses the female and male florets. This study provides rare evidence for a direct functional role of thermogeny.

Keywords: thermogeny; Araceae; mimicry; Diptera

1. INTRODUCTION

The dead horse arum, *Helicodiceros muscivorus* (Araceae: Aroideae) is a most notable example of resource mimicry (figure 1*a*). This plant mimics a carcass in order to attract carrion blowflies that are subsequently exploited as unrewarded pollinators. A prominent feature of the elaborate carrion mimicry is the strong putrid smell (Kite 2000; Stensmyr *et al.* 2002). We have previously shown that the volatile composition of the arum is strikingly similar to a real carcass, so similar in fact that flies are not able to separate the arum from a carcass (Stensmyr *et al.* 2002). Blowflies find the horrific smell, and possibly also the fleshy coloured hairy inflorescence, irresistible and are attracted in large numbers to the plants. Pollination in the arum requires that the attracted flies are deceived in order to enter a trap chamber (figure 1*b–d*) that houses the female florets. Once inside, the flies become trapped in the chamber by spines blocking the exit path, and flies carrying pollen from another plant fertilize the receptive female florets. The flies remain trapped overnight and the spines remain erect until the male florets, at the entrance to the chamber, start producing pollen and the female florets are no longer receptive, upon which the spines wilt and the trapped flies are able to escape. When leaving the chamber, the flies have to pass the male florets and are again coated with pollen.

An interesting aspect of the arum is its capacity to produce heat, or thermogeny, a capacity it shares with many other species in the Araceae family (e.g. Seymour & Schultze-Motel 1999; Gibernau & Barabé 2000; Barabé *et al.* 2002). Although thermogeny is a relatively well-studied phenomenon, few, if any studies have provided answers, supported by strong experimental evidence, as to the function of heat production in plants. Thermogeny has been proposed to have originally evolved as a form of energy reward for beetle pollinators (Seymour & Schultze-Motel 1997). However, at the present time not all thermogenic plants are beetle pollinated and hence a sole role of heat as an energy reward seems unlikely. Accordingly, other ecological roles for thermogeny have been proposed, for example to increase the volatization of specific chemicals directed towards pollinators (e.g. Seymour & Schultze-Motel 1999) and as a carrion mimicry-reinforcing stimulus (e.g. Uemura *et al.* 1993).

We have investigated the functional role of thermogeny in the dead horse arum, and we can show that the presence of heat modifies the behaviour of the pollinators. The produced heat is significant for the success of the plant reproductive system and plays an important role in guiding flies into the trap chamber.

2. METHODS

Dead horse arum plants, on the islands of Cavoli, off the coast of Sardinia, were investigated during the mid-March to late April flowering seasons in 2002–2003. Plant temperature was measured on shadowed plants, which were otherwise not shielded from the environment. Temperature measurements were obtained through a digital thermometer (accurate to 0.1 °C) and were taken every hour from 06.00 to 18.00. Measurements were taken from the spathe and along the appendix. Heat development in carrion was measured on a dead gull (*Larus michahellis*) from the rectal opening; measurements were taken three times per day over the decay process. For the thermogeny manipulation experiments, we heated the appendix through a 0.20 mm thick, 40 cm long nickel–chrome resistance wire (126.7 Ω m^{-1}) coiled around the appendix. The thin wire did not visibly obstruct the flies and was also present, but with the heating current turned off, on plants during day 1 of flowering and also on those plants that were not heated during day 2. Current, converted to heat during the passage through the resistance wire, was supplied by a 6 V, 12 A battery. The appendix was heated to *ca.* 11–12 °C above ambient, level with the output capacity of the natural heater. To replace the natural odour we added synthetic odour to the plants during day 2; 150 μl of a 1 : 1 : 1 mixture of synthetic dimethylsulphide, dimethyldisulphide and dimethyltrisulphide were added to dental cotton rolls and subsequently placed in the trap chamber of the plants (Stensmyr *et al.* 2002). The odorants were obtained from Sigma-Aldrich and were of greater than 98% purity. The cotton rolls were not visible from the spathe and did not obstruct the flies from attempting to enter the trap chamber. Number and behaviour of attracted flies were monitored at 1 h intervals, starting at 09.00, 11.00, 15.00 and 17.00. The number of flies landing, proceeding to the appendix and entering the trap chamber was noted.

3. RESULTS AND DISCUSSION

Initially, we aimed to establish the thermogenic capacity of our study population of dead horse arum plants. As we were interested in the behaviour-modifying effects of thermogeny on the pollinators, we only measured the temperature development during daylight hours, when blowflies are active, and we only recorded the temperature of external parts of the inflorescence. Increased temperatures of the inflorescence were only noted on day 1 of flowering, and we found that heat production was restricted to the appendix. Plotting the average appendix temperature development from 15 plants during the flowering cycle, we found that the highest temperature increase occurred around noon on day 1, and on average the plants held an
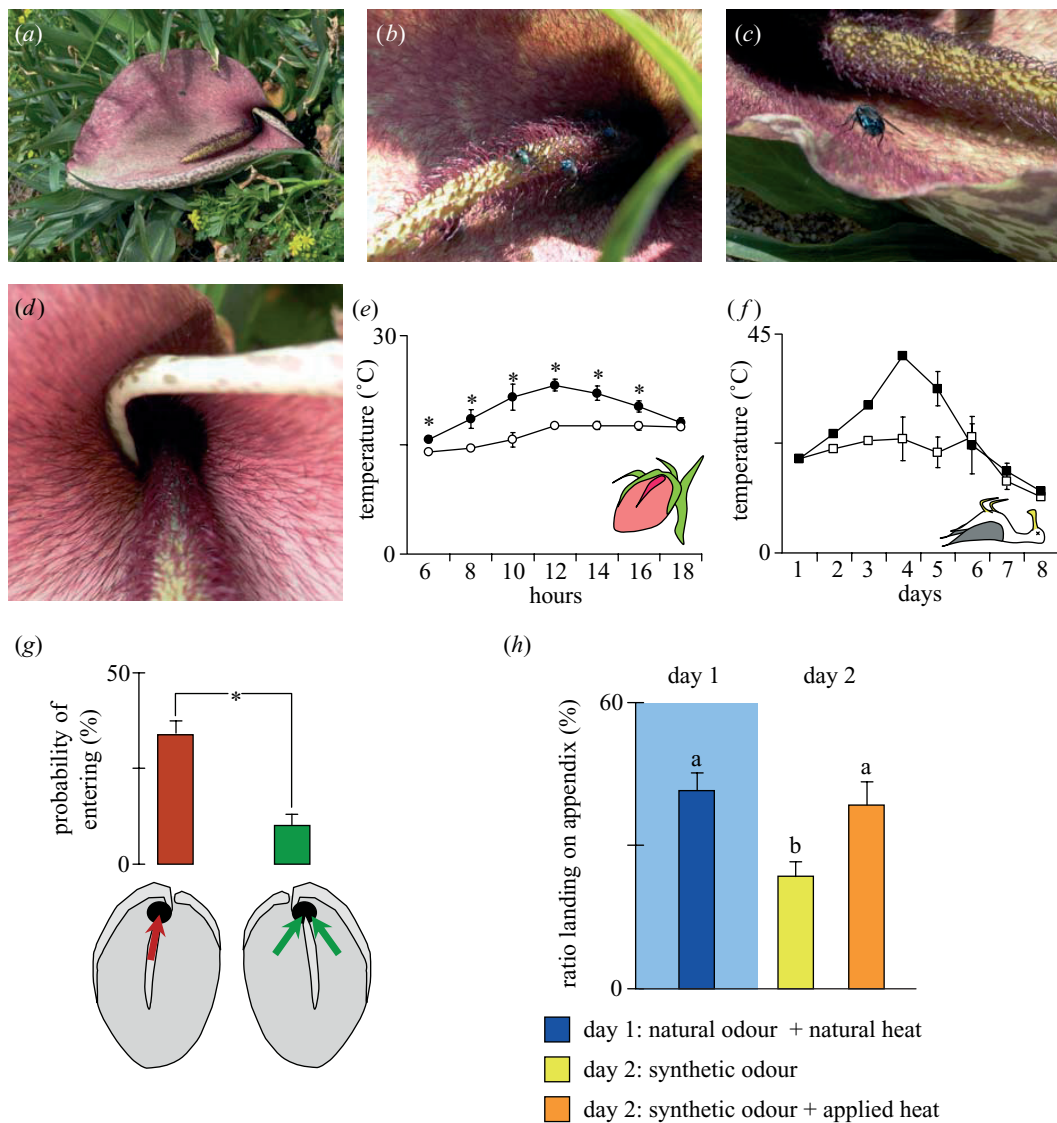
Figure 1. (*a*) The inflorescence of a dead horse arum. (*b*) Several blowflies approaching the trap chamber from the appendix. (*c*) A blowfly (*C. vicina*) on the spathe heading towards the appendix and the trap chamber entrance. (*d*) A close-up view of the trap chamber entrance. The dark opening creates the illusion of a rectal opening, including a hairy tail. (*e*) Heat production of the dead horse arum. Mean ($\pm$ s.e.) appendix temperature measured from 15 arum plants (filled circles) and mean ($\pm$ s.e.) ambient temperature (open circles). Thermogeny was only observed during the first day of flowering (there was no significant temperature increase of the appendix at any measurement points during the second day). The asterisks indicate that the appendix temperatures are significantly different from ambient temperatures (*t*-test, n.s.: $p > 0.05$; $^* p < 0.005$). (*f*) Temperature development of a carcass (dead gull (*Larus michahellis*); $n = 1$) during the decay process. Each data point represents the average of three measurements. Temperature development was measured through the rectal opening. Gull temperature was represented by filled squares and ambient temperature by open squares; (*g*) The probability of a blowfly entering the trap chamber, if coming from the appendix (red) or from the spathe (green). The asterisk indicates a significant difference (*t*-test, $p < 0.01$). Error bars represent standard error. Thus, if a fly locates the appendix it is three times as likely to enter the trap chamber. (*h*) The ratio of blowflies landing on the appendix. On day 1, with natural scent and heat, $42.4 \pm 4.5\%$ of the attracted blowflies visit the appendix. Adding odour, but not heat during day 2 significantly decreases the ratio of flies that visit the appendix to $24.0 \pm 3.4\%$. However, if both odour and heat is applied during day 2, the ratio of flies visiting the appendix is restored to the same level as on day 1 ($39.6 \pm 5.3\%$) ($F = 7.1$, d.f. = 2, $p < 0.05$, one-way ANOVA, *post hoc* Tukey–Kramer). Error bars indicate standard error.

appendix temperature 12.4 °C higher than ambient temperature (figure 1*e*). Individual plants were recorded having a temperature increase of almost 20 °C above ambient temperature. We also measured the heat development in a dead gull, a primary carrion medium on the islands, to establish whether carrion on the island habitat had a temperature development during decay similar to the plants. The temperature of the gull rapidly increased during the decay process, and reached temperature levels comparable with arum plants (figure 1*f*). Having confirmed thermogeny in our sample population, we next proceeded to investigate its ecological significance.

Initial observations suggested to us that most of the attracted flies that continued to enter the trap chamber seemed to do so from the appendix (figure 1*b*) and in fewer cases directly from the spathe (figure 1*c*). To verify this observation, we recorded the behaviour of attracted flies during day 1 of flowering from eight plants and

scored the behaviour of a total of 881 fly visits. Confirming our first observations, we found that the majority—69.4% of the flies that entered—did so from the appendix. Furthermore, we found that the probability of a fly entering was 33.0% if it found the appendix, whereas the probability of entering from the spathe was significantly lower, only 10.2% (*t*-test, $p < 0.01$; figure 1*g*). Thus, by locating the appendix the flies are three times as likely to enter the trap chamber compared with those that do not. As the pollination system of the arum requires that the attracted flies are also deceived to enter the trap chamber, finding the appendix at the outset should be of major importance. Could thermogeny play an important role in this process?

To address this question, we designed an experiment that would show whether or not the presence of thermogeny increases the attractiveness of the appendix. The dead horse arum offers excellent opportunities for manipulation experiments, as the plant stays open for 2 days, and only produces heat and odour during day 1 (Stensmyr *et al.* 2002). We approached the question by manipulating the heat production during day 2 of flowering. We monitored fly behaviour on day 1 and noted the ratio of flies that visited the appendix. On day 2 we added odour to one group of plants ($n = 6$) and in another group ($n = 6$) we added odour plus heat. Heat was supplied through a thin resistance wire that was wound around the appendix. The foul odour, i.e. the oligosulphides, was applied in the trap chamber, as described in § 2. We did not expect heat to enhance the overall long-range attractiveness of the plants, as addition of the oligosulphides alone fully restores the long-range attractiveness during day 2 when compared with day 1 (Stensmyr *et al.* 2002). However, we postulated a difference in behaviour, dependent on the presence of heat, once the flies had landed. Pooling observations from day 1 flowering plants from the two groups, we found that $42.4 \pm 4.5\%$ of the flies that were initially attracted to the plant also found the appendix (1719 fly visits in total monitored; figure 1*h*). In the group where we only added odour during day 2, the proportion of attracted flies that visited the appendix decreased significantly to $24.0 \pm 3.4\%$ (650 fly visits). In the group where we added both odour and heat during day 2, the attractiveness of the appendix was restored to

the same level as on day 1, as $39.6 \pm 5.3\%$ of the attracted flies also visited the appendix (656 fly visits), not significantly different from day 1, but significantly different from day 2 plants with only odour added ($F = 7.1$, d.f. $= 2$, $p < 0.05$, one-way ANOVA, *post hoc* Tukey–Kramer). Thus, adding both heat and odour rendered the appendix as attractive on day 2 as on day 1.

We provide evidence for a direct functional role of thermogeny in modifying pollinator behaviour. The results show that heat production in the dead horse arum is an important part of a complex multisensory targeting mimicry. The heat works in conjunction with the odour cues and fine-tunes the deceit, modifying the behaviour of attracted blowflies. That heat is attractive to blowflies is understandable as true carcasses also have an elevated temperature caused by the microbial processes of decomposition. Heat has also previously been shown to be a crucial oviposition cue for blowflies (Cragg 1956; Erzinclioglu 1996). Apart from odour and thermal cues, visual and tactile cues are probably also important for the overall carrion mimicry and these two sensory stimuli will be the topics of future studies.

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