The physiology of some sapromyophilous flowers

B. J. D. MEEUSE

Botany Department, University of Washington, Seattle, Washington, U.S.A.

An effort will be made to provide some physiological and biochemical background to St. Vogel's beautiful descriptions of the pollination-syndrome in certain arum lilies, and to L. van der Pijl's pioneer experiments on the role of light and darkness in the opening of some flower-species. The highly thermogenic, cyanide-insensitive respiration which characterises the so-called appendix of *Sauromatum* on the first day of flowering is triggered by a hormone ('calorigen') which originates in the primordia of the staminate flowers and begins to leave these about one day before opening-time. The formation (or the release?) of the hormone is, in its turn, controlled by the particular light/dark regime to which the developing inflorescence has been exposed. A single dark-period of at least 6 hours' duration, given at the right moment to inflorescences first allowed to develop in constant light, leads to the characteristic 'respiratory explosion' about 45 hours later. It will be shown that the opening of certain flowers, often thought of as a very simple act, may likewise depend on a series of 'programmed' events set in motion two days before actual opening-time.

KEY WORDS:—aroid—*Sauromatum*—calorigen—respiratory explosion—dark period—sapromyophilous.

CONTENTS

INTRODUCTION

It is probably fair to say that aroids began to attract the attention of scientists 2000 years ago. The drawing of *Arum maculatum* or *Arum italicum* found in the A.D. 412 version of the famous herbal of Dioscorides (*De Materia Medica*) may well be based on sketches by Crateuas, going back to 120 B.C. (Prime, 1960). Studies on the thermogenicity of aroids are about 200 years old, for it was in 1778 that the great Jean Baptiste de Monet, Chevalier de Lamarck, recorded the spectacular temperature-rise displayed by the inflorescences of *Arum maculatum*. Floral biology, which started a little later (Sprengel, 1793) has given us an understanding of the survival-value of the heat-development (Knoll, 1926; Vogel, 1963; see below).

It is both intriguing and depressing that an area of study with such impressive historical credentials—potentially a happy meeting-ground for floral ecologists

on the one hand and plant physiologists and biochemists on the other—remains rife with controversy. Undeniably, linguistic barriers have played a role in creating this stage of affairs. A sad testimonial is provided by Knoll's splendid contributions on *Arum*, published in 1926, which remained virtually unknown in English-speaking countries, until, in 1960, Dormer drew attention to them in "The truth about pollination in *Arum*". Although linguistic barriers are now less substantial, new ones have sprung up as a result of the fact that biochemically oriented investigators no longer speak the same scientific language as colleagues of a "natural history" bent. It is the purpose of this article to demonstrate that the two groups can provide *each other* with valuable clues for further research and understanding. The approach taken here will be comparative as well as analytical and predictive; a springboard will be provided by the situation in *Arum*, which is not only of historical importance, but happens to highlight several problems as well.

POLLINATION EVENTS IN THE GENUS *ARUM*

Knoll (1926) has correctly interpreted the inflorescences of *Arum nigrum* and *Arum maculatum* as 'trap-flowers' which 'collect' their pollinators (small beetles and midges, respectively) in a floral chamber into which the visitors fall when they are denied a foothold on the slippery surface of the spathe, and of the appendix: the heat and smell producing osmophore, *sensu* Vogel, which represents the naked, sterile upper end of the fleshy central spadix. The stiff hairs or barrier-organs at the entrance of the floral chamber serve as a sieve or collander, keeping out larger animals such as bluebottle and greenbottle flies which might also be attracted by the smell of the appendix. The inflorescences display a very pronounced protogyny; the pistillate or 'female' flowers surrounding the base of the fleshy spadix in the floral chamber are receptive when the appendix is active in producing its heat and smell. The visitors, some of which may be carrying pollen from another *Arum*-inflorescence which was in the pollen-shedding or 'male' stage, feed on a stigmatic secretion produced by the pistillate flowers and pollinate them in the process. It is only several hours later that the staminate or 'male' flowers, bunched together in a ring higher up on the spadix, dehisce and shower the captive insects with a rain of pollen, so that the next day these animals are covered with pollen. At that time, the barrier-organs have wilted so that they are no longer functional, while the stigmatic secretion as well as the heat and the smell have disappeared; the visitors leave, with the pollen which they have received and may visit another inflorescence which is still in the smelly, attractive, 'female' stage. It would be difficult to find a better-designed, more foolproof mechanism to insure cross-pollination than the one just described. For the plant physiologist, the interest lies in the importance of timing-mechanisms.

FUNCTION AND TIMING OF THE HEAT-DEVELOPMENT IN *ARUM* LILIES,
AND THE RELATIONSHIP BETWEEN HEAT AND SMELL

Knoll convincingly demonstrated that the heat *per se* does not act as an attractant: models of *Arum*-inflorescences with small, heat-providing light-bulbs in the floral chamber failed to attract pollinators, whereas models provided with a mixture of rotting blood and glycerol (an antidesiccant) did. The biological

function of the heat is to aid the evaporation of odoriferous compounds (a mixture of various amines, ammonia, and indole or skatole). The heat-production, which in *Arum maculatum* can lead to a temperature-difference between the appendix surface and the environment of about 15°C (in other aroids it may be even higher: 22°C in *Alocasia pubera* and *Schizocasia portei*), is based on a starch-consuming respiration process unparalleled in the plant kingdom, so that in the course of a single day the dry weight of an appendix may fall from about 32% to about 6%. When this phenomenon reaches its peak, the intensity of the metabolic process is comparable to that of a flying hummingbird; oxygen-consumptions as high as 72,000 mm³ of oxygen per gramme wet weight per hour have been recorded (Lance, 1972). As explained below, respiration in this case is of a special 'uncoupled' and cyanide-insensitive type so that very little ATP or other high-energy compounds are generated; practically all the energy locked up in the respiratory substrate (starch) is expended in the form of heat, an unusual situation which in this case, however, has a high survival-value. I have demonstrated the heat-production visually by putting on the appendix a thin film of a vaseline-like mixture of liquid crystals; since the manner in which such crystals 'handle' light is temperature-dependent, a striking sequence of colours ranging from copper-red to peacock-blue manifested itself as the appendix, in the process of heating-up, passed through the 25°–28·5°C range. Smith & Meeuse (1966) as well as Chen & Meeuse (1971) have analysed (to some extent) the mixture of odoriferous compounds emanated by arum lily inflorescences of various species. A high degree of specificity is evident; thus, *Sauromatum guttatum* produces, among other things, large amounts of indole, while *Arum dioscoridis* yields skatole instead. The differences are usually quite obvious to the human nose; e.g. inflorescences of *Arum orientale* have the characteristic smell of spent fire-crackers (due to the presence of sulphur compounds?) while those of *Arisarum vulgare* are reminiscent of dead freshwater fish. In consonance with the different chemical composition of the emanations, the visitor-spectra too are different; thus, *Helicodiceros muscivorus* is pollinated almost exclusively by large flies; *Arisarum proboscideum* (which displays beautiful mushroom-mimicry: Vogel, 1973) by fungus-gnats; *Dracunculus vulgaris* by beetles. It is plausible that there is also a good correlation between the life-style of the pollinators and the hour of the day at which the production of heat and smell reaches its peak; in *Arum maculatum*, that peak occurs in late-afternoon or early-evening, in *Sauromatum* around noon. Obviously, sympatric species of aroids avoid competition for pollinators.

Leick (1915) was probably the first investigator to compare various species of Araceae with regard to the number of heating-periods through which each inflorescence goes during the flowering-sequence. It is here that the enormous advantage, in terms of energy, of the 'invention' of the floral trap becomes obvious. Certain primitive Araceae lack a true, functional floral chamber and possess a spadix which is covered with small hermaphroditic flowers from top to bottom. Protogyny is still very much in evidence, and the production of heat and smell must therefore occur at least twice: the first time to attract pollen recipients. In contrast, an inflorescence with an effective trap, such as that of *Arum maculatum*, essentially secures for itself a 'captive audience' and can therefore restrict itself to only one pronounced heating-up period at the time the pistillate flowers are

receptive, although a small second heating-peak will still manifest itself when the pollen reaches maturity. A myth which has persisted for some time among certain floral ecologists is that the expenditure of much heat is necessary for the production of amines and other chemical attractants. It is based on a complete misunderstanding of thermodynamics. In plant cells, 'heat' is essentially a useless form of energy, a waste-product, and there are mechanisms in both plant and animal cells for trapping the energy of the respiratory substrate as ATP rather than permitting early heat-production. The appendices of arum lilies are truly exceptional. It can be demonstrated that the bulk of their heat-production occurs in the decomposition (into water and oxygen) of hydrogen peroxide which arises as the final product of their peculiar respiration-process. It is difficult to see this as something connected with a synthetic act. It is true that a large production of heat is indicative of a large breakdown of starch, and it is a matter of simple observation that this breakdown is accompanied by the production of certain odoriferous compounds. In a quantitative sense, however, there is a large discrepancy between the two processes. The breakdown of starch can be expressed in grams while the concomitant production of amines etc. is expressed in milligrams. If the only biological 'purpose' of the starch breakdown were the generation of energy for amine production, the process would be very inefficient indeed, and it is unlikely that such a process would have been maintained in the highly selective process of evolution.

In the aroid inflorescences heat should be seen as a volatiliser. However, there are exceptional cases, such as that of *Symplocarpus foetidus* (eastern skunk cabbage) where the developing inflorescences will push themselves up through the snow even when the ambient temperature is below freezing. Knutson (1972, 1974) has demonstrated the existence of a regulatory mechanism which maintains the temperature-constancy of the floral parts, regardless of the prevailing environmental temperature: the lower the latter, the higher the intensity of respiration. Self-pollination seems to be normal in *Symplocarpus* in Iowa, although later in the season simulids and even honeybees are visitors.

The question as to whether the production of odoriferous compounds and of heat are just two aspects of one and the same process, respiration, has also received attention. An affirmative answer would imply that heat and smell *must* manifest themselves at the same time (if both are present). This has occasionally been challenged. It has also been emphasised, in some quarters, that certain Araceae produce smell, without giving off appreciable heat. The power of the latter argument is somewhat less than impressive as long as it is not accompanied by the presentation of very accurate and sophisticated temperature-measurements. Certainly, it should not be used as evidence against the idea that heat acts as a volatiliser; the tacit assumption is that every pollination mechanism has to be perfect (or 'complete'), and this is incorrect. Much stronger evidence for the functional independence of heat-production and smell would be the observation that heat can occur in aroids in the absence of smell. To the best of our knowledge, however, such an observation has never been made. If it had been, it would of course have provided very strong evidence indeed that the heat *per se* can act as an insect-attractant (see above). The production of amines by aroid appendices can indeed be seen as an aspect of respiration in the broad sense, whether it be due to decarboxylation of amino acids as suggested by Simon (1962) and Richardson

(1966), or to transamination of aldehydes, as championed by Hartmann, Dönges & Steiner (1972a, b). Free ammonia can potentially be formed in the oxidation of glutamic acid by glutamic acid dehydrogenase, an enzyme which in the appendix of *Sauromatum* reaches its highest activity when there is maximal heat-production (Meeuse, unpubl.). Further evidence for the *very* close metabolic connection between heat-production and smell (or at least between heat-production and one smell-compound, indole) has been provided by Chen & Meeuse (1972) and by McIntosh (unpubl.).

THE BIOCHEMICAL BASIS FOR THERMOGENICITY IN *ARUM* LILIES, AND THE TRIGGERING OF THE METABOLIC EXPLOSION IN THE APPENDIX

The cyanide-insensitivity of the appendix-respiration, reported above, resides in the mitochondria. When isolated from the tissue, these contain a dual pathway for respiratory electron transfer: the classical, cyanide-sensitive electron transport system which is coupled to the generation of high-energy phosphate (ATP), and a cyanide-insensitive pathway which branches off from the classical one at the ubiquinone link and is phosphorylative to a much lesser extent. When cyanide is added to respiring mitochondria in a laboratory experiment, the electrons coming from the respiratory substrate are *forced* to go through the alternate pathway only; very little ATP is generated, and the energy of the respiratory substrate quickly appears as heat. Hess & Meeuse (1967) have provided evidence that on the first day of flowering in *Sauromatum* the alternate pathway is followed even in the absence of cyanide, i.e., spontaneously. Presumably, the same situation obtains in related species such as *Arum*. It is as if a switch has been thrown, releasing heat. How is this achieved? On the basis of the pioneer work done by van Herk (1937a, b, c) with *Sauromatum*, it is now possible to postulate that the agent responsible is a plant hormone, calorigen, which is produced in the primordia (buds) of the staminate flowers and begins to leave these to move into the appendix about 22 hours before the metabolic peak is reached. Meeuse and coworkers have succeeded in concentrating and purifying two compounds with calorigen-activity. They are low-molecular, aromatic compounds of considerable stability; complete elucidation of their chemical structure and properties seems only a matter of time (Buggeln & Meeuse, 1971; Chen & Meeuse, 1975). In the purification, a bio-assay based on the formation of indole by pieces of immature appendix treated with calorigen-containing extracts has been very helpful. The long lag-time between the exposure to calorigen and the appearance of the metabolic peak argues in favour of the idea that calorigen-triggered synthesis of new enzymatic protein (as the result of an unblocking of certain genes?) is essential. This hormone would mediate the switch to the cyanide-insensitive heat-producing pathway at the ubiquinone link. This idea is now being tested by treating immature pieces of *Sauromatum*-appendix with calorigen and then adding inhibitors such as cycloheximide and puromycin which interfere with protein synthesis; these inhibitors can be added simultaneously with the calorigen or a specified number of hours afterwards (McIntosh & Meeuse, unpubl.).

Earlier, Simon & Chapman (1961) had already obtained evidence relevant to the question of protein synthesis. With the aid of electron micrographs, these authors compared mitochondria from *Arum maculatum* appendices collected on

the day of heat production ('D' day), with mitochondria from earlier stages. They found the number of cristae per mitochondrion to be significantly higher for D-day mitochondria, as compared with younger ones; and, since cristae represent 'enzymatically active surface area', the results clearly favour the idea of protein synthesis during development. However, a considerable proportion of this takes place before there is any triggering by calorigen (which is also found in *Arum*; see Buggeln & Meeuse, 1971). It is also true that in *Sauromatum* there is a positive response to the addition of calorigen even in pieces of appendix so young (D minus 3 days, for instance) that the cristae are still highly underdeveloped and the amount of enzymatically active protein is low. Obviously, the metabolic explosion can be brought about even with relatively incomplete metabolic machinery, and the question of control remains open; perhaps only certain key cofactors have to be synthesised, or perhaps crucial cofactors have to be juxtaposed to other metabolites in a different manner than before, a process in which membrane-permeability changes may be very important. It is also important to keep in mind that an almost explosive mitochondrial respiration has to be 'supported' by a spectacular boost in glycolysis. Which factors play a key role in that process is a problem that has been addressed by Hess & Meeuse (1968) and by Johnson & Meeuse (1972). There is evidence that phosphofructokinase plays a major role in this boost.

THE ROLE OF THE LIGHT/DARK REGIME IN THE FLOWERING OF *SAUROMATUM* AND *ARUM*

Although van Herk's publications create the impression that the flowering-sequence in *Sauromatum* proceeds normally even in constant light, B. J. D. Meeuse and coworkers have clearly shown that this is not so. Confirming earlier observations by Schmucker (1925), they found that a regime of constant darkness prevents the development of heat and smell. Constant light permits 'half-hearted' anthesis, but the fact that this is not synchronised in a group of individuals, in contrast to the natural situation in which *Sauromatum* goes through a peak at noon as described in this article, shows clearly that an alternation of light and dark periods is required. Buggeln, Meeuse & Klima (1971) raised *Sauromatum* inflorescences in constant light until they were sure that D-day would normally have passed. Imposition of a 6-hour 'dark shot' at that time led to anthesis, with the metabolic peak occurring 42–45 hours after the beginning of the dark period. Surprised by the length of the lag-period, Meeuse then initiated a literature-search which showed that the *Sauromatum*-situation can by no means be considered unique. It is tempting to see the actual opening of flowers in general as a very simple act, controlled by such things as turgor changes in certain cells of the petals or a temperature-induced difference in growth-rate between the upper and the lower layers of a petal or tepal, as is the case in crocuses. In a number of cases, however, it is obvious that about two days before flower-opening, the bud is already 'committed' to that act. Once the 'triggering' has taken place, it is of minor importance what happens during the long lag-period. Among the best pieces of evidence is the elegant investigation carried out by Arnold (1959) on species of *Oenothera*. Normally, the flower buds open early in the evening, e.g. at 18.00 hours. When day and night are reversed, the buds continue to open at their accustomed time

for two days; it is only on the third day that the new time-schedule is adopted, so that flower-buds open at 06.00 hours. In cases like this, it is again tempting to postulate the synthesis of new enzymatic protein. One could formulate the hypothesis that for the ultimate act of flower-opening, certain osmotic phenomena (turgor changes) are essential, but these may require free sugars, which first have to be produced by the hydrolysis of starch present in the buds, a process which in turn may require the presence of certain enzymes, made available by the activation of certain genes. A whole new field of investigation thus awaits investigation, and it is gratifying that studies on aroids have contributed towards that development.

REFERENCES

ARNOLD, C.-G., 1959. Die Blütenöffnung bei *Oenothera* im Abhängigkeit vom Licht-Dunkel-Rhythmus. *Planta, 53:* 198–211.

BUGGELN, R. G. & MEEUSE, B. J. D., 1971. Hormonal control of the respiratory climacteric in *Sauromatum guttatum* (Araceae). *Canadian Journal of Botany, 49:* 1373–1377.

BUGGELN, R. G., MEEUSE, B. J. D. & KLIMA, J. R., 1971. Control of blooming in *Sauromatum guttatum* Schott by darkness. *Canadian Journal of Botany, 49:* 1025–1031.

CHEN, J. & MEEUSE, B. J. D., 1971. Production of free indole by some arum lilies. *Acta botanica neerlandica, 20:* 627–635.

CHEN, J. & MEEUSE, B. J. D., 1975. Purification and partial characterization of two biologically active compounds from the inflorescence of *Sauromatum guttatum* Schott (Araceae). *Plant Cell Physiology, 16:* 1–11.

DORMER, K. J., 1960. The truth about pollination in *Arum. New Phytologist, 59:* 298–301.

HARTMANN, T., DÖNGES, D. & STEINER, M., 1972a. Biosynthese aliphatischer Mono-amine in *Mercurialis perennis* durch Aminosäure-Aldehyd-Transaminierung. *Zeitschrift für Pflanzenphysiologie, 67:* 404–417.

HARTMANN, T., ILERT, H.-I. & STEINER, M., 1972b. Aldehydaminierung, der bevorzugte Biosyntheseweg für primäre, aliphatische Mono-amine in Blütenpflanzen. *Zeitschrift für Pflanzenphysiologie, 68:* 11–18.

HERK, A. W. H. Van, 1937a. Die chemischen Vorgänge im *Sauromatum*-Kolben. *Recueil des travaux botaniques néerlandais, 34:* 69–156.

HERK, A. W. H. Van, 1937b. Die chemischen Vorgänge im *Sauromatum*-Kolben. II. *Proceedings K. Nederlandse akademie van wetenschappen, 40:* 607–614.

HERK, A. W. H. Van., 1937c. Die chemischen Vorgänge im *Sauromatum*-Kolben. III. *Proceedings K. Nederlandse akademie van weterschappen, 40:* 709–719.

HESS, C. M. & MEEUSE, B. J. D., 1967. The effect of various uncouplers on the respiration of appendix tissue slices of *Sauromatum guttatum* Schott (Araceae) at various stages of anthesis. *Acta botanica neerlandica, 16:* 188–196.

HESS, C. M. & MEEUSE, B. J. D., 1968. Factors contributing to the respiratory flare-up in the appendix of *Sauromatum* (Araceae). I. *Proceedings K. Nederlandse akademie van wettenschappen (Ser. C), 71:* 443–455.

JOHNSON, T. F. & MEEUSE, B. J. D., 1972. The phosphofructokinase of the *Sauromatum*-appendix (Araceae). Purification, and activity-regulation *in vitro. Proceedings K. Nederlandse akademie van wetenschappen (Ser. C), 74:* 1–19.

KNOLL, F., 1926. Insekten und Blumen. Experimentelle Arbeiten zur Vertiefung unserer Kenntnisse über die Wechselbeziehungen zwischen Pflanzen und Tieren. IV. Die *Arum*-Blütenstände und ihre Besucher. *Abhandlungen der Zoologisch-botanischen Gesellschaft in Wien, 12:* 379–482.

KNUTSON, R. M., 1972. Temperature measurements of the spadix of *Symplocarpus foetidus* (L.) Nutt. *American Midland Naturalist, 88:* 251–254.

KNUTSON, R. M., 1974. Heat production and temperature regulation in Eastern Skunk Cabbage. *Science, 186:* 746–747.

LAMARCK, J. B. de., 1778. *Flore française, 3:* 1150. Paris.

LANCE, C., 1972. La respiration de l'*Arum maculatum* aucours du développement de l'inflorescence. *Annales des Sciences naturelles, Botanique (12e ser.), 13:* 477–495.

LEICK, E., 1915. Die Erwärmungstypen der Araceen und ihre blütenbiologische Deutung. *Bericht der Deutschen botanischen Gesellschaft, 33:* 518–536.

PRIME, C. T., 1960. *Lords and Ladies,* London: Collins.

RICHARDSON, I, 1966. Studies on the biogenesis of some simple amines and quaternary ammonium compounds in higher plants. Isoamylamine and isobutylamine. *Phytochemistry, 5:* 23–30.

SCHMUCKER, Th., 1925. Beiträge zur Biologie und Physiologie von *Arum maculatum. Flora, 118:* 460–475.

SIMON, E. W., 1962. Valine decarboxylation in *Arum* spadix. *Journal of Experimental Botany, 13:* 1–4.

SIMON, E. W. & CHAPMAN, T. A., 1961. The development of mitochondria in *Arum* spadix. *Journal of Experimental Botany, 12:* 414–420.

SMITH, B. N. & MEEUSE, B. J. D., 1966. Production of volatile amines and skatole at anthesis in some arum lily species. *Plant Physiology, 41:* 343–347.

SPRENGEL, C. C., 1793. *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen.* Berlin.

VOGEL, S., 1963. Duftdrüsen im Dienste der Bestäubung. *Abhandlungen Mathematisch-naturwissenschaftliche Klasse, Akademie der Wissenschaften und der Literatur, Mainz, 10:* 599–763.

VOGEL, S., 1973. Fungus mimesis of fungusgnat flowers. In N. B. M. Brantjes & H. F. Linstens (Eds), *Pollination and Dispersal:* 13–18. University of Nijmegen: Publ. Dept. Botany.
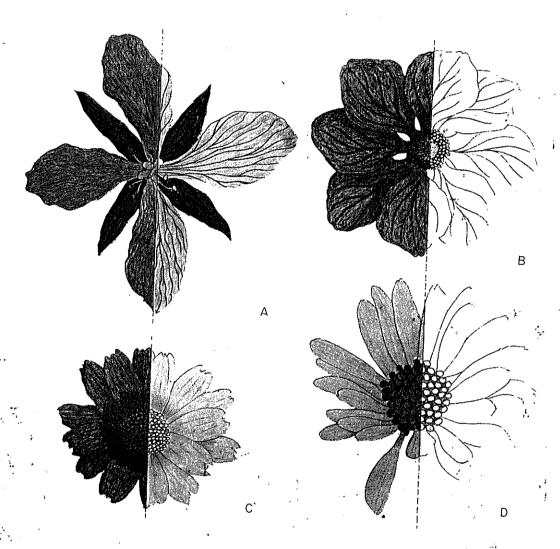
118 879

Marc, Cet article a l'air pas mal sur les odeurs de l'Arum mais surtout sur l'explication bioch de l'ouverture de la spathe et product odeur !!

The Pollination of Flowers by Insects

A

B

C

D

edited by

A. J. Richards