for amino acid analyses on a Beckman-Spinco model 120C analyzer.

On the basis of the minimum molecular weight from the amino acid analysis and the molecular weight of the purified enzyme determined by ultracentrifugation in acidic sucrose gradients, we calculated the amino acid composition of acrosomal proteinase (Table 1). The results demonstrated great similarity in amino acid composition between rabbit acrosomal proteinase and the pancreatic trypsins, but especially with human trypsin. The glycine content was identical in both enzymes, while the structurally similar amino acid pairs lysine and arginine, threonine and serine, and tyrosine and phenylalanine were also present in identical numbers of residues when taken in pairs. That is, acrosomal proteinase has one less lysine, but one more arginine; it has two less serines, but two more threonines; and it has two less tyrosines, but two more phenylalanines. The other amino acids all differ by only one residue, with the exception of glutamic acid, valine, and isoleucine. Acrosomal proteinase has six less valine and four less isoleucine residues, but six more glutamic acid or glutamine residues when compared to human trypsin. Because of the small quantities of enzyme available to work with, we have not yet obtained an accurate determination of half-cystine and tryptophan.

Unfortunately, the amino acid composition of rabbit trypsin has never, to our knowledge, been reported; and the pancreatic trypsins analyzed for their amino acid content are from widely divergent species. However, we did compare the antigenic properties of these enzymes by preparing, with the use of Freund's adjuvant, an antiserum to crystalline bovine pancreatic trypsin since it is apparent from Table 1 that acrosomal proteinase is similar to bovine pancreatic trypsin as well as to human trypsin. To accomplish immunodiffusion with acrosomal proteinase the gel must be buffered at *p*H 5.0. At higher *p*H acrosomal proteinase forms dimers and larger aggregates within the well of the immunodiffusion plate, and the enzyme never diffuses into the gel in sufficient quantities to form precipitation bands. At *p*H 5.0, distinct cross reactions were obtained between rabbit antiserum to bovine pancreatic trypsin with human, bull, rhesus monkey, and rabbit acrosomal proteinases, confirming the structural similarities between acrosomal proteinase and trypsin (Fig. 2).

The similarity between acrosomal proteinase and trypsin is remarkable considering their widely different physiological roles, and it will be interesting to determine how similar the structures of these two enzymes are in the same species. It seems that this loss of some hydrophobic amino acid residues might allow a partial unfolding of the polypeptide chain, giving rise to the tendency of acrosomal proteinase to reversibly form dimers and larger aggregates at low *p*H, a phenomenon that we have observed many times in our laboratory when sucrose density gradient centrifugation was used for molecular weight determinations both at high and low *p*H. Whether any aggregation occurs between acrosomal proteinase molecules and other enzymes in vivo remains to be determined; but the enzyme does appear to be bound to the acrosomal membrane (*6*, *9*).

Table 1. Amino acid composition (number of residues) of rabbit acrosomal proteinase, and of human (*10*) and bovine (*11*) trypsin; N.D., not determined.

Amino acid	Acrosomal proteinase	Human trypsin	Bovine trypsin
Glycine	20	20	25
Lysine	10	11	14
Arginine	7	6	2
Threonine	12	10	10
Serine	22	24	33
Tyrosine	5	7	10
Phenylalanine	6	4	3
Histidine	4	3	3
Asx*	22	21	22
Proline	8	9	9
Alanine	14	13	14
Methionine	2	1	2
Leucine	13	12	14
Glx†	27	21	14
Valine	10	16	17
Isoleucine	8	12	15
Total	190	190	207
Half-cystine	ND	8	12
Tryptophan	ND	3	4

* Aspartic acid or asparagine. † Glutamic acid or glutamine.

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Heat Production and Temperature Regulation in Eastern Skunk Cabbage

Abstract. *The spadix of* Symplocarpus foetidus *L. maintains an internal temperature 15° to 35°C above ambient air temperatures of −15° to +15°C. For at least 14 days it consumes oxygen at a rate comparable to that of homeothermic animals of equivalent size. Temperature regulation is accomplished by variations in respiratory rate.*

The inflorescence of eastern skunk cabbage (*Symplocarpus foetidus* L.) weighing 2 to 9 g maintains a temperature 15° to 35°C above air temperature for a period of at least 2 weeks during February and March when air temperatures are from −15° to +15°C. The tissues of the inflorescence (spadix) are not frost-resistant and escape freezing by maintaining a high respiratory rate. An elevated respiratory rate [which Buggeln *et al.* (*1*) refer to as respiratory climacteric] in the inflorescences of members of the Arum family (Araceae) is common, but maintenance of that elevated respi-

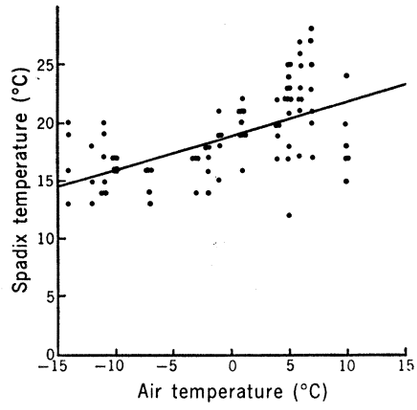

Fig. 1. Temperatures of eastern skunk cabbage inflorescences (spadices) at various air temperatures. All measurements were made in the field on intact plants.

ration for more than a few hours is uncommon and may be unique to eastern skunk cabbage. The measured rate of oxygen consumption in inflorescences attached to intact plants varies inversely with air temperature, providing evidence that eastern skunk cabbage floral structures regulate their own temperature in a way comparable to that reported for *Philodendron* (*2*).

Eighty-seven paired measurements of spadix temperatures and air temperatures over a 3-year period (Fig. 1) indicate some dependence of spadix temperature on air temperature (*3*). The calculated regression line has a slope of 0.288, the correlation coefficient is .58, and the slope is significantly different from zero. The time of day when temperature was measured had no apparent influence on spadix temperature, although no continuous recordings of temperature were made. Two selected spadices measured daily or twice daily at various hours of the morning, afternoon, and night averaged 19.2° and 21.9°C over air temperature for 12 and 14 days, respectively. At nearly all air temperatures some spadices were at or above 20°C.

The spadix has no starch storage in its tissues and begins to cool immediately on being severed from the parent plant (*4*). The massive root of eastern skunk cabbage (Fig. 2) stores large quantities of starch and is the obvious source of the respiratory substrate needed to maintain elevated temperatures for 2 weeks or more.

In an effort to estimate the respiratory rate of intact plants calculations of the rate of heat loss were made; these were based on a measured specific heat (0.85 cal g^{-1} $°C^{-1}$), a caloric equivalent of 4.6 kcal per liter of oxygen, and the rate of temperature change in severed spadices heated to 30°C and cooled to 10°C in 0°C air. The total heat loss in calories per minute varies directly with spadix weight (the slope equals 0.73) for spadices weighing 2.5 to 9.5 g. The calculated rate of oxygen consumption for a 4.5-g spadix maintaining a temperature of 20°C at an air temperature of 0°C is 12.8 ml g^{-1} $hour^{-1}$ (Fig. 3), which is roughly comparable to the rate for equivalent-sized small mammals (*5*). Pearson (*5*) suggests that the lowest possible weight for thermoregulating organisms is 2.5 g, which coincides very well with the weight of the smallest spadices observed. A limited number of measurements of oxygen consumption in 2.8-g spadices indicate a respiratory rate nearly double that of 4.5-g spadices at an air temperature of 12° to 13°C (6.68 ml g^{-1} $hour^{-1}$ for a 4.5-g spadix and 11.58 ml g^{-1} $hour^{-1}$ for a 2.8-g spadix).

The actively respiring tissue of eastern skunk cabbage is a layer of fleshy-petaled flowers tightly packed on the surface of the spadix. These constitute about 79 percent of the total fresh weight of spadix and are approximately 12 percent protein, based on Kjeldahl nitrogen determinations. Respiratory rates based on the weight of flowers range up to 15.2 ml g^{-1} $hour^{-1}$ for small spadices at 12°C, and could be as high as 30 ml g^{-1} $hour^{-1}$ at the lowest temperatures observed.

Direct measurements of oxygen consumption were made in the field with a modification of a constant pressure respirometer sealed over the spadix. Figure 3 indicates a near doubling of oxygen consumption for a 10°C drop in air temperature. Respiratory rates of severed flowers of eastern skunk cabbage have been measured in the laboratory (*6*), but are generally lower than those measured in intact plants.

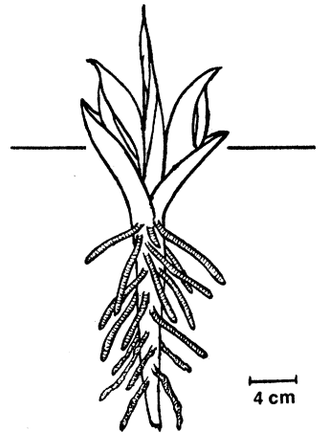

Fig. 2. Slightly stylized drawing of complete eastern skunk cabbage plant during the blooming period. The plant shown has two inflorescences, each consisting of an enclosing leaflike spathe which surrounds the spadix. The horizontal line indicates ground level.

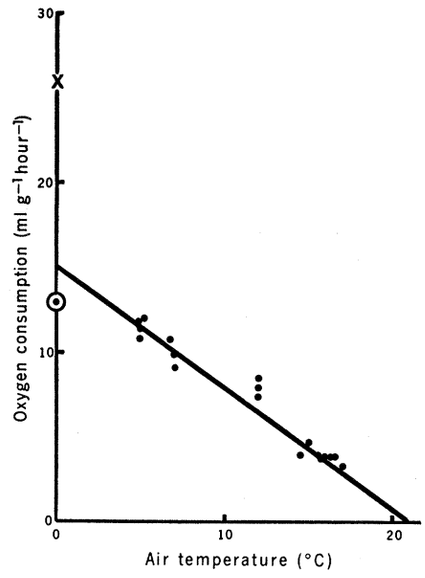

Fig. 3. Oxygen consumption of eastern skunk cabbage spadices measured in the field at available air temperatures. All values are for spadices weighing about 4.5 g. (⊙) Calculated oxygen consumption based on heat loss from a 4.5-g spadix. (×) Rate of oxygen consumption projected at −15°C, the lowest temperature at which spadices were observed.

Maintenance of elevated temperatures in the relatively small skunk cabbage spadix is apparently possible because of the large percentage of actively respiring tissue in the spadix and the nearly inexhaustible supply of respiratory substrate in the root.

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