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Date March 20, 1986

THE GENETIC CONTROL OF SEX EXPRESSION IN THE FERN *CERATOPTERIS*

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Rodney J. Scott

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ABSTRACT

A study was undertaken to characterize the response of two homozygous strains, H1-n and 176D-4-n, of Ceratopteris richardii (L.) Brongn. to the pheremone antheridiogen, and to determine the genetic basis of this response. Antheridiogen is a readily diffusable substance, produced by the gametophytes of some ferns, which induces undifferentiated gametophytes to produce antheridia (male gametangia). For experimental purposes, antheridiogen was obtained from a crude aqueous filtrate (CAF) of medium which had supported older cultures. This filtrate was used as an external source of antheridiogen in subsequent experiments.

Dose responses to the various concentrations of CAF were measured by determining the percentage of male (exclusively antheridiate) gametophytes in cultures. Other experiments investigated the response of cultures to internally produced antheridiogen and the way in which this effect varied with different gametophyte densities. Results showed that the two parental strains differed greatly in their response to antheridiogen over the entire range of CAF concentrations and densities at which they were studied. The effect of antheridiogen on area and antheridia number for hermaphroditic gametophytes was also determined for different CAF concentrations. A possible trend toward decreasing gametophyte area with increasing CAF concentrations was observed for both strains.

Analysis of spore germination rates for both strains on both plain and CAF supplemented media indicated that germination rates were not affected by the presence of CAF. Germination rates were not significantly different for the two strains. Analysis of the genetic basis of the antheridiogen response was accomplished by studies of reciprocal hybrids of H1-n and 176D-4-n and subsequent F2 generations. Spores from a total of 100 F2 individuals were sown on CAF supplemented medium to determine their response type and to test for segregation from the F1 hybrids. Segregation patterns among the F2's indicated two possible modes of inheritance; one in which response is mediated by two linked genes and another in which two linked genes and a single unlinked gene are responsible. Because either model could account for the responses of the F2's, both are presented and several ways in which they could be distinguished are discussed.

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I. INTRODUCTION

General Characteristics of Antheridiogen

Antheridiogens are pheremones which induce antheridia differentiation on gametophytes of sensitive fern species (Dopp 1950 in Miller 1968, Naf 1979). Dopp first demonstrated the existence of antheridiogen in 1950, when he showed that undifferentiated gametophytes of Pteridium aquilinum could be induced to form antheridia precociously if they were grown on medium from mature cultures (Dopp 1950 in Miller 1968). Subsequently, species in several genera have been shown to have antheridiogen systems (Naf 1979). Of these, the antheridiogens of P. aquilinum (Dopp 1950 in Miller 1968), Anemia phyllitidis (Naf 1959), Lygodium japonicum (Naf 1960) and Ceratopteris thalictroides (Schedlbauer and Klekowski 1972) have been most fully studied. Each antheridiogen (A_{pt}, A_{an}, A_{ly}, and A_{ce}, respectively) has been characterized chromatographically and by its biological activity, and each has been found to be unique (Naf 1975, Schedlbauer 1974, 1976a).

The antheridiogen of Pteridium has been studied extensively and has been shown to be active in many other polypodiaceous ferns. The gametophytes of one such fern, Onoclea sensibilis, generally fail to form antheridia spontaneously in culture, but produce them abundantly in the presence of A_{pt} (Naf 1975). Because of its strong response to A_{pt} under conditions otherwise not conducive to antheridia

formation, O. sensibilis is frequently used as an assay organism for this substance. Naf (1965) showed that O. sensibilis does produce a factor which induces antheridia formation. However, it does not become active until the culture medium which contains it is heated. However, in recent work by Rubin and Paolillo (1983), male gametophytes of this species occurred spontaneously on both agar medium and soil. A_{pt} is completely inactive in the schizaeaceous ferns A. phyllitidis and L. japonicum and in C. thalictroides (Parkeriaceae). There is some cross-reactivity between the two schizaeaceous ferns in that L. japonicum responds to A_{an}. However, A. phyllitidis is unresponsive to A_{1y}. Thus far, A_{ce} is known to be active only within the genus Ceratopteris (Schedlbauer 1974).

In addition to the ability to induce antheridia formation, some antheridiogens are also capable of cancelling the light requirement for germination of fern spores. A_{pt} acts in this manner for some members of the Polypodiaceae (sensu Bowers) and A_{an} and A_{1y} promote dark germination in spores of their own source species and show cross-reactivity similar to that for antheridia induction (Naf 1975). A_{ce} lacks the ability to promote dark germination in the spores of its own source species, or in those of any other tested ferns (Schedlbauer 1976a). However, several authors have reported that dark germination of Ceratopteris spores may occur under the certain conditions, even without applied A_{ce} (see Miller 1968).

Some Gibberellic Acids Have Antheridiogen-Like Activity

It has been shown that some naturally occurring gibberellic acids (GA's) can substitute for antheridiogen, both in antheridia induction and in promoting dark germination, for some schizaeaceous ferns. This effect is lacking in the polypodiaceous ferns and in Ceratopteris (Naf 1975, Schedlbauer 1974). Since GA's and the antheridiogen A_{an} have been shown to share some structural similarities (see below), this relationship is not completely unexpected. However, since no reactivity to GA's occurs for non-schizaeaceous species, and since no information on chemical structures of A_{pt} or A_{ce} exist, no inferences can be made about their relationship to GA's.

Voeller (1964a) tested seven naturally occurring GA's for their ability to induce antheridia. None demonstrated any sensitivity towards O. sensibilis, the species generally used as an assay organism for A_{pt}. He did, however, find a wide range of activities among many of the schizaeaceous ferns. Schraudolf (1964) has ranked these GA's relative to their ability to induce antheridia in gametophytes of the schizaeaceous fern A. phyllitidis: GA7 > GA4 > GA1 > GA3 > GA9 > GA5 > GA8.

In a later study, Takeno and Furuya (1975) tested the same seven GA's by applying them to young protonemal gametophytes of L. japonicum. The authors suggested that this assay was more precise, since only plants of a single developmental stage were used to test for activity, and therefore the response should be more uniform. They observed the following order of responses: GA7 > GA4 > GA9 > GA3 > GA5 > GA1 > GA8.

Weinberg and Voeller (1969) tested 10 members of the family Schizaeaceae and eight species from other families for induction of dark germination by GA3. Other than those species which normally germinate in darkness, only members of the Schizaeaceae responded positively; and of these, only one failed to respond. The authors determined the relative order of effectiveness of the various GA's to be: GA4 = GA7 > GA9 > GA1 > GA3 > GA8 > GA5. In another series of experiments, Weinberg and Voeller (1969) found that normal light-induced germination in A. phyllitidis could be inhibited by AMO-1618, a selective inhibitor of GA biosynthesis.

GA's have also been shown to be capable of inhibiting archegonial formation. Takeno and Furuya (1977) observed this phenomenon in L. japonicum and proposed the following spectrum of activities for authentic GA's, based on the concentration necessary to induce inhibition of archegonial formation in 50% of the gametophytes grown for 8 days in supplemented mediums GA4 = GA9 > GA7 > GA3 > GA1 = GA5 = GA8.

Characterization of Antheridiogen

The observation that GA's have antheridiogen-like activities was significant in and of itself. However, this observation became even more significant when Yamane et al. (1979) actually isolated GA_9 methyl ester from medium supporting L. japonicum gametophytes, and demonstrated its ability to induce antheridia formation and inhibit archegonial development.

In 1971, Nakanishi et al. (1971) carried out the first complete chemical characterization of an antheridiogen. They determined that the chemical formula of A_{an} is $C_{19}H_{22}O_6$, and its structure is similar to that of natural GA's. In fact, they proposed that a compound with this structure could easily be produced from natural GA's by way of a suggested intermediate.

Work done by a number of researchers (Naf 1959, 1960; Nakanishi et al. 1971; Pringle 1961; Pringle et al. 1960; Schedlbauer 1976a) on the four main antheridiogens has indicated that all share a number of basic chemical characteristics. They all behave as weak acids with a pKa around five, all appear to have a carboxyl group which is necessary for their activity, and all have low molecular weights (Schedlbauer 1976a). Although only one antheridiogen has been fully characterized, their similar modes of action and phylogenetic associations suggest that they are chemically similar substances (Schedlbauer 1976a).

Gametophyte Development

When spores of most ferns are moistened and given sufficient light and warmth, germination takes place. Initial cell divisions occur in one plane, and lead to the production of a filamentous protonemata. Subsequent cell divisions cease to be unidirectional and become localized in a meristem region near the apex. Ultimately, this gives rise to a heart shaped, or cordate, gametophyte.

The early stages of growth may be entirely vegetative. When neither male nor female sex organs are present, the gametophyte

is considered to be neuter. When some cells differentiate to form antheridia or archegonia, gametophytes may be classified as males, if they possess only antheridia, females, if they have archegonia alone, or hermaphrodites if both sex organs are present. Most gametophytes are capable of producing antheridia at any growth stage. However, archegonia are produced only following formation of an organized meristem region (Miller 1968).

In multispore cultures, fern gametophytes typically exhibit varying ratios of males:females:hermaphrodite plants. Many studies have demonstrated a correlation between conditions favoring slow vegetative growth and the tendency for unisexual male gametophytes to be produced. Alternately, unisexual, female gametophytes are produced under conditions favoring rapid growth (Miller 1968).

By observing multispore cultures and carrying out assays of media from cultures of various developmental stages, Naf (1975) concluded that gametophytes of P. aquilinum do not begin to produce antheridiogen in effective quantities until they themselves are insensitive to it. According to Naf, this insensitive phase begins shortly after the formation of an organized meristem that leads to the attainment of the heart shape. This proposed sequence would account for the typical range in morphologies which he observed in his cultures. The most rapidly growing individuals reached the archegonial stage without ever producing antheridia. Naf attributes this to a total lack of antheridiogen in the medium. The majority of the remaining gametophytes form a few antheridia, presumably

induced by antheridiogen produced by the rapidly growing gametophytes, before they form archegonia. Those few remaining individuals, whose development is continually being influenced by a high concentration of antheridiogen, become ameristic, exclusively antheridiate gametophytes. Although this pattern of antheridiogen production and gametophyte development has been generally accepted in the literature, recent studies by Hickok and Kiriluk (1984) demonstrated that gametophytes of C. thalictroidies produce small amounts of A_{ce} long before they actually become insensitive to it.

Developmental sequences similar to the one proposed for Pteridium were later demonstrated by Naf (1960, 1967) to occur in A. phyllitidis and L. japonicum. The sequence for A. phyllitidis varies from that of P. aquilinum, however, in that very young gametophytes of A. phyllitidis (0-1 day) initiate a juvenile stage which is insensitive to A_{an} for a period of time. In the development of L. japonicum, Naf observed the same general trends as those which prevail in P. aquilinum. However, instead of older gametophytes becoming completely insensitive to A_{ly}, the region of cells capable of forming antheridia simply becomes more and more basally restricted.

Schedlbauer (1976b) observed multispore cultures of C. thalictroides under many conditions and concluded that a pattern similar to that described for P. aquilinum prevails. Whereas Naf has proposed that the earliest developing gametophytes become cordates in the absence of antheridiogen, Schedlbauer (1976b) has taken this idea a step further for Ceratopteris. He considered three factors,

spore size, germination time and growth rate with regard to how these relate to ultimate gametophyte morphology. He found that spore size provided the best predictor. Individuals destined to develop first and become cordates could be predicted with 74% accuracy based upon spore size. He contended that individuals developing from larger spores had an increased storage capacity for developmental substances (i.e., enzymes, ribosomes, etc.), and therefore developed more quickly.

Mode of Action of Antheridiogen

Based on his own experiments and the observations of other researchers, Naf (1961) has proposed a general theory for the mode of action of antheridiogens. In the ferns for which antheridiogen systems have been studied most completely, it has been shown that gametophytes begin to produce antheridiogen and become insensitive to it at nearly the same stage (Naf 1959, 1960, 1975; Schedlbauer 1976). Naf (1961) has observed that both removal of the meristem tissue of insensitive individuals and temporary plasmolysis of insensitive tissues can restore antheridiogen sensitivity. And perhaps more importantly, he observed that excision of the meristem tissue in young gametophytes can lead to the spontaneous induction of antheridia without the intervention of antheridiogen. These observations prompted Naf (1961) to suggest that antheridiogens exercise their effects simply by overcoming a blcok to antheridia formation which is maintained by some substance produced by the

meristem. It is, he suggests, the accumulation of this substance in maturing gametophytes which ultimately renders them insensitive to antheridiogen. Hickok (1983) recently demonstrated that antheridia induction in C. thalictroides can be blocked by application of abscisic acid (ABA). Along with studies by Cheng and Schraudolf (1974), which show that fern spores contain ABA, this finding may indicate ABA as a possible candidate for the natural antheridia blocking agent; although the role of ABA in spores is not clear.

Natural Significance of Antheridiogen Systems

It is generally agreed that the action of antheridiogen has the effect of reducing the amount of self fertilization which occurs in gametophyte populations (Schraudolf 1985). Because antheridiogen alters the ratios of males to females and hermaphrodites, it changes the likelihood of self versus cross fertilization.

Klekowski (1969a) introduced the terms intra- and inter-gametophytic selfing to refer to fertilization of an egg by a spermatazoid from the same gametophyte and the fertilization of an egg of one gametophyte by a spermatazoid of another gametophyte from the same spore source, respectively. He also used the term intergametophytic mating, which applies to the fertilization of an egg by a spermatazoid originating from a gametophyte produced by a spore from a completely different sporophyte. Clearly, this last situation results in the greatest potential for genetic variability. Intergametophytic selfing provides a more limited

potential and intragametophytic selfing results in no potential for variation, yielding completely homozygous individuals.

Klekowski (1969a) noted that the likelihood that one of these three conditions should prevail in a gametophyte population is dependent on three general types of adaptations: morphological, genetical and populational. Morphological adaptations refer to the orientation of male and female gametangia in relation to each other and the effect this has on promoting self fertilization. Genetical adaptations refer to the presence of self incompatibility systems which prevent, or reduce the likelihood of self fertilization. Populational adaptations are those which affect the relative frequencies of sexual morphologies within a population. This last factor appears to be under the direct control of an antheridiogen system in many species which have been observed under laboratory conditions.

Tryon and Vitale (1977) carried out a series of experiments that appeared to demonstrate the activity of an antheridiogen system in gametophyte populations occurring in nature. They worked with gametophytes which occurred alone as well as with gametophyte populations of Asplenium pimpinellifolium and Lygodium heterodoxum in the field. The gametophytes were collected and catalogued and maps were made for the populations, indicating sizes and the relative proximity to other individuals. Gametophytes were then examined under a light microscope and number of antheridia and archegonia were recorded. These data were compared for individuals in

populations versus those occurring alone. It was found that these comparisons supported predictions which have been made for the activity of antheridiogen systems in nature, based upon laboratory observations. For instance, for gametophytes occurring in populations there was a strong correlation between heavily antheridiate ones and their proximity to large archegoniate ones.

Antheridiogen Bioassays

Since antheridiogen is not commercially available, and must be obtained directly from the experimental organisms, the development of accurate bioassays has been an important aspect of experimentation. Naf (1958) assayed the antheridiogen activity of medium from cultures of various ages of P. aquilinum to determine the time course of the secretion of this substance. He tested medium from cultures of different ages by creating a dilution series of conditioned medium mixed with new medium and determined at what point in the dilution series 50% activity was obtained. For these experiments, he used O. sensibilis as the assay organism. Gametophytes of this species do not form antheridia spontaneously under the prevailing conditions of culture, but do so readily when exposed to Pteridium antheridiogen. Thus, Naf could easily quantify activity by determining what dilution caused 50% of the gametophytes to form antheridia.

Voeller (1964b) carried out similar experiments with P. aquilinum to investigate factors which increase antheridiogen production. He tested various dilutions of conditioned media and

expressed their activities in the following manner: (+3) - nearly all assay gametophytes bore antheridia, (+2) - more than 10% did, (+1) - less than 10% did and (?) - only a few bore antheridia. In the same series of experiments, Voeller showed that Pteridium produced more (or more active) antheridiogen when the pH of the medium was low, when light intensity was between 600 and 800 foot candles and continuous, when temperature was maintained around 20 C, and when coconut milk was added to the basic nutrient media.

Takeno and Furuya (1975) have commented on the assays of Naf and Voeller and concluded that both allow for the existence of too many variable factors to provide clear insight into the amount of antheridiogen actually present. These authors have developed their own assay, in which the activity of the antheridiogen of L. japonicum was assayed against protonemata of this species. In their assay, germinated spores were grown in darkness for three days and then transplanted onto media supplemented with various dilutions of antheridiogen. The cultures were then maintained for a week of additional dark culture. Protonemata were then scored for the presence or absence of a single antheridium. The authors claim that their assay is more accurate than previous ones because the protonemata are more sensitive to antheridia induction and they are all at a uniform growth stage when the antheridiogen is applied. In addition, the entire assay is performed in darkness, thus eliminating photosynthetic and photomorphogenic influences on growth and development.

Genetic Aspects of Antheridiogen Response

Considerable variation appears to exist with regard to the response to antheridiogen within species. Several researchers have observed that different strains of the same species often show characteristic sex ratios when gametophytes are raised in multispore cultures (Schedlbauer 1974, Cousens 1979, Lloyd and Warne 1978, Wane and Lloyd 1981). For instance Schedlbauer (1974) tested the intraspecific activity of A_{ce} from two strains of C. thalictroides and observed different strengths of response, varying with the source of the antheridiogen as well as with the organism being tested (Schedlbauer 1974).

In spite of the apparent intraspecific variability in antheridiogen response, no information is available concerning the genetic basis of this variability. Such information can be of value in determining basic mechanisms of antheridiogen responses. In addition, an understanding of the genetic basis of these responses can provide valuable information for use in studies of the evolution and reproductive biology of ferns.

Statement of Purpose

This research project was undertaken for the purpose of examining the genetic basis of antheridiogen response in Ceratopteris. Ceratopteris richardii, a diploid species (n-39), was chosen as the experimental organism for this study because of its rapid life cycle and ease of handling under laboratory conditions (Hickok 1985).

Ceratopteris completes its life cycle, going from spore to spore producing sporophyte, in about 4 months. Gametophytes can be easily maintained in multispore or isolate cultures, and techniques for inducing selfing or cross fertilization are well established. Two strains of this species, H1-n and 176D-4-n, were chosen for this investigation. These strains were chosen because gametophyte cultures of both respond to the presence of externally supplied antheridiogen, demonstrating that the expression of sex ratio is under the control of an antheridiogen system. However, though both respond to antheridiogen, their sensitivities are markedly different. When grown on supplemented medium with identical concentrations of the filtrate, H1-n exhibits a much higher frequency of male gametophytes than does 176D-4-n. Since the two strains are interfertile and produce a fertile F1 hybrid when crossed, this extreme difference in response has provided an excellent opportunity to study the genetic aspects of the A_{ce} response. The study consisted of two phases: characterization of the responses of the two parental strains and analysis of responses in the F1 and F2 generations in order to determine the genetic basis of the differences.

II. MATERIALS AND METHODS

Spore Source

Spores from two completely homozygous strains, H1-n and 176D-4-n, of C. richardii Brongn. were used for these experiments. Strain H1-n was derived from spores from the herbarium specimen, Killip 44595 (GH) which was collected in Cuba. Strain 176D-4-n was derived from spores collected by R. M. Lloyd in Guyana (coll. #4815). In several experiments, other strains derived from the 176D source and the Hn source were used. In all cases, such strains were derived from individuals genetically identical to strains 176D-4-n and H1-n, respectively.

Medium Preparation

All cultures grown for experimentation were grown in 60 mm X 20 mm petri plates on 10 ml of agar-solidified (1%) Parker/Thompson inorganic nutrient medium. Appendix, Table 3, shows its composition (modified from Klekowski 1969b). The pH of this basal medium was adjusted to 5.4 prior to autoclaving.

Spore Sterilization and Sowing

Spores were sterilized by soaking them in water in a conical 15 ml centrifuge tube overnight and treating with a 1:5 solution of commercial bleach (5.25% Na hypochlorite by weight) and water for 3 minutes. Following this treatment, spores were washed in

ca. 4 ml of sterile distilled water. This water was then removed and the spores were resuspended in sterile distilled water and sown on by depositing drops of suspension on the medium and spreading this with a wire loop. Removal of the water and sterilizing solution in each of the above procedures was accomplished by placing the tip of a Pasteur pipet firmly on the bottom of the conical centrifuge tube and withdrawing the liquid by aspiration, leaving the spores in the tube.

Preparation of Antheridiogen Supplemented Media

Antheridiogen was added to media in the form of a crude aqueous filtrate (CAF) derived from medium which supported mature gametophyte cultures of the H4-n strain. Previous tests have shown that CAF taken from cultures of strain 176D-4-n has a similar effect to that of the H1-n strain. These production cultures were grown axenically on ca. 40 ml of nutrient medium in 100 mm X 15 mm petri plates. A total of 0.01 g of spores from stock H4-n were sown for every 10 plates. These cultures were grown wrapped in unsealed individual plastic bags under cool white fluorescent light (23 Wm^{-2}) at 30 ± 1 C. for 21 or 22 days following soaking (DFS). The cultures were harvested and medium was frozen. To separate the aqueous phase from the agar, the frozen medium was thawed and filtered. Each batch of CAF was coded with a number indicating the culture from which it was derived followed by a "/H" to designate the H4-n spores were grown to produce it. When a batch was divided up into aliquots

to be used for different experiments, each series of aliquots was designated by an a, b, c, d, etc.

Antheridiogen supplemented medium was prepared by adding various quantities of a CAF, volume for volume, in place of distilled water in the basal medium. With the exception of experiments in which some of the CAF concentrations were prohibitively high, the CAF was added to autoclaved medium after its pH had been adjusted to 5.4 and it had been sterile filtered. When CAF concentrations were too high to conveniently add CAF after autoclaving, unsterilized CAF with adjusted pH was added to the medium prior to autoclaving.

Spore Density

For most experiments, a constant spore density was utilized in sowings. This was accomplished by always sowing five drops of a standard density spore suspension onto each culture plate. The standard suspension contained ca. 0.036 mg spores/0.1459 ml water (ca. 0.1459 ml/5 drops), giving a spore density in cultures of ca. 20 spores/cm^2.

For some experiments, sowing density was purposely altered by diluting the standard spore suspension with water. When this was done, only a single drop of diluted suspension was sown on each plate. However, as a control, four drops of sterile distilled water were always added. Gametophytes were sometimes grown as isolates. This was done by filling individual 16 mm wells of 24 well tissue culture clusters (made by Costar) with 2 ml of nutrient medium and inoculating each well with a single spore.

Germination Counts

To determine the percentage of germinated spores, culture plates with spores sown at the standard density (see page 17) were viewed with a dissecting microscope at a magnification of 120X. A 6 mm X 6 mm grid was placed beneath the cultures so that this grid could be seen through the medium. The squares of this grid were used to mark off individual fields of view so that each spore within a square could be scored. Squares were sampled successively until 100 spores had been scored for germination (based on the presence or absence of an observable primary rhizoid).

Sex Ratio Determination

Three characteristic morphologies were present in multispore cultures: 1) exclusively male gametophytes (males) which bore only antheridia and possessed no organized meristem region, 2) gametophytes which did possess an organized meristem region (cordates) and had either produced or were capable of producing both kinds of gametangia, and 3) gametophytes which had neither an organized meristem or gametangia of either kind (neuters).

To score the ratio of males to cordates to neuters (M:C:N) in a culture, a permanent slide was made for each culture. In most instances, this was done at 10 DFS. However, if germination at 10 DFS was < 50%, the cultures were not sampled until 14 DFS. Slides were made by removing all gametophytes from a randomly selected section of the culture and mounting them on a slide with a 2:1 mixture of Hoyer's solution and 0.5% acetocarmine with iron.

Slides used in these experiments normally had at least 80 gametophytes present, although slides made from some of the F2 individuals with low germination had as few as 30 gametophytes. When 200 or fewer gametophytes were present on a slide, all individuals were scored for sexual morphology type. When more than 200 were present, the sex ratio was determined by scoring only 200 individuals at random.

In some instances, sex ratios were determined for samples of isolated gametophytes grown under identical conditions. In such cases, all isolated gametophytes were combined on a single permanent slide for each separate condition. Sex ratios were obtained as described above.

Determination of Gametophyte Size and Antheridia Number

The area and antheridia number of cordate gametophytes were determined by viewing permanent slides at 500X with a dissecting microscope and using a "Bioquant II" software program with a Hipad digitizer in conjunction with an IBM personal computer. By using a drawing tube attached to the dissecting microscope, the image of the slide and the image of the digitizing pad could be viewed in composite. Using a Bausch and Lomb electronic cursor, the image of each cordate was traced and its area was calculated and stored by the computer using the Bioquant program. This program also allowed antheridia number to be tallied using a touch counting device.

Gametophyte Selfing and Hybridization and Sporophyte Maintenance

Completely homozygous sporophytes were obtained by isolating a gametophyte prior to sexual maturity and watering frequently to provide ample opportunity for self fertilization. Gametophytes isolated to induce self fertilization were monitored for the presence of young sporophytes and then transplanted into styrofoam cups. Small holes were then made at the base of each cup and cups were placed in greenhouse trays with standing water in them, which was periodically flushed with fresh water.

Two hybrid sporophytes were produced by reciprocal crosses between the 176D and Hn strains of C. richardii. The first hybrid, F1(176D-3-5 X Hn)8, was produced by L. G. Hickok before this project was begun. The other, F1(H1-n X 176D-4-n)1, was produced in April of 1984. In each combination, the egg parent is listed first and underlined. Hybridizations were achieved by isolating young cordate gametophytes from the intended female strain and inoculating the isolate cultures with a spermatazoid solution derived from a gametophyte culture of the intended male strain. The solution bearing spermatazoids was obtained by watering a culture with abundant antheridia, viewing the culture with a dissecting microscope for the presence of swimming spermatazoids and then transferring the spermatazoid bearing solution to the isolates, by way of a sterile pipet.

Attempted crosses were then examined under a dissecting microscope 3 and 4 days after inoculation and screened for the

presence of an embryo. Gametophytes which failed to produce an embryo were discarded and the rest were retained as putative hybrids. When the sporophytes thus produced reached a sufficient size, they were transplanted into small styrofoam cups filled with soil and maintained as described above. When these plants produced spores, true hybrids were selected from among them on the basis of the expression of an intermediate sex ratio in the subsequent F1 gametophyte generation. The two specific hybrids listed above were selected on this basis.

F2 Production and Analysis

An F2 generation was produced by randomly isolating the spores from the F1 hybrids and culturing them until homozygous F2 sporophytes were produced. These were subsequently potted in soil and maintained in the greenhouse. The first series of F2's was produced by isolating gametophytes from the F1(176D-3-5 X Hn)8 hybrid. All gametophytes were randomly selected simultaneously from among a population of germinated spores. The second series of F2's was produced by isolating spores from the F1(H1-n X 176D-4-n)1 hybrid. This was done at three evenly spaced points in time throughout an 8 day period of active germination. On each of the 3 days, 70 newly germinated spores (those having only the initial rhizoid protruding) were removed and isolated. This was done to prevent a bias toward spores that germinated early and produced cordate gametophytes. F2's from both series were grown as described above and, when fertile

sporophylls were produced, these were removed and placed in large plastic petri plates to facilitate drying and the release of spores. Individuals from the first series were redesignated 176H8-# (the entire series is referred to as AF2-1), and those from the second series were designated H1761-# (the series is referred to as AF2-2).

To analyze F2 individuals, a standard density of spores (see page 17) from each individual was sown on each of three culture plates supplemented with 6% CAF (v/v). Permanent slides were made from each culture and sex ratios were analyzed for each. For the first series of F2's, the analysis was carried out about 2 years after the spores had been collected. For the second series, spores were sown on the 6% CAF supplemented plates 4 weeks (± 3 days) after they had been collected.

Handling of Data

Data on sex ratio and germination were rounded to the nearest whole number when expressed as percentages. When these data were used to produce the figures which accompany this text, the calculations were done by a "Lotus 1-2-3" software program and the values obtained were rounded to the fifth decimal place.

III. RESULTS AND DISCUSSION

Characterization of Parent Stocks

Characterization of the parent strains was accomplished by establishing cultures of both on media supplemented with eight different concentrations of CAF, ranging from 0% to 14% in 2% increments. For each experiment, four cultures were established for each stock at each CAF concentration. The data from four separate experiments are presented in the Appendix (Tables 4, 5, 6, and 7). Experiments 13/Ha, 13/Hb, and 13/Hc were set up using CAF produced from a single batch of medium from older cultures, aliquots of which were frozen and used to make medium after 7, 16, and 33 days of frozen storage, respectively. Experiment 16/Ha was set up using CAF produced from cultures grown under the same conditions, but in this instance, the CAF was not frozen prior to use.

The data from all four experiments, represented as mean percentage of males (± standard deviation) for each CAF concentration, are presented graphically in Figure 1. Although this value represents the percentage of males of all the gametophytes, including neuters, the percentage of neuters was so small (between 0 and 2%) that this was not considered significant in the interpretation of the data.

It is evident from Figure 1 that strains H1-n and 176D-4-n exhibit a marked difference in antheridiogen response. Strain H1-n

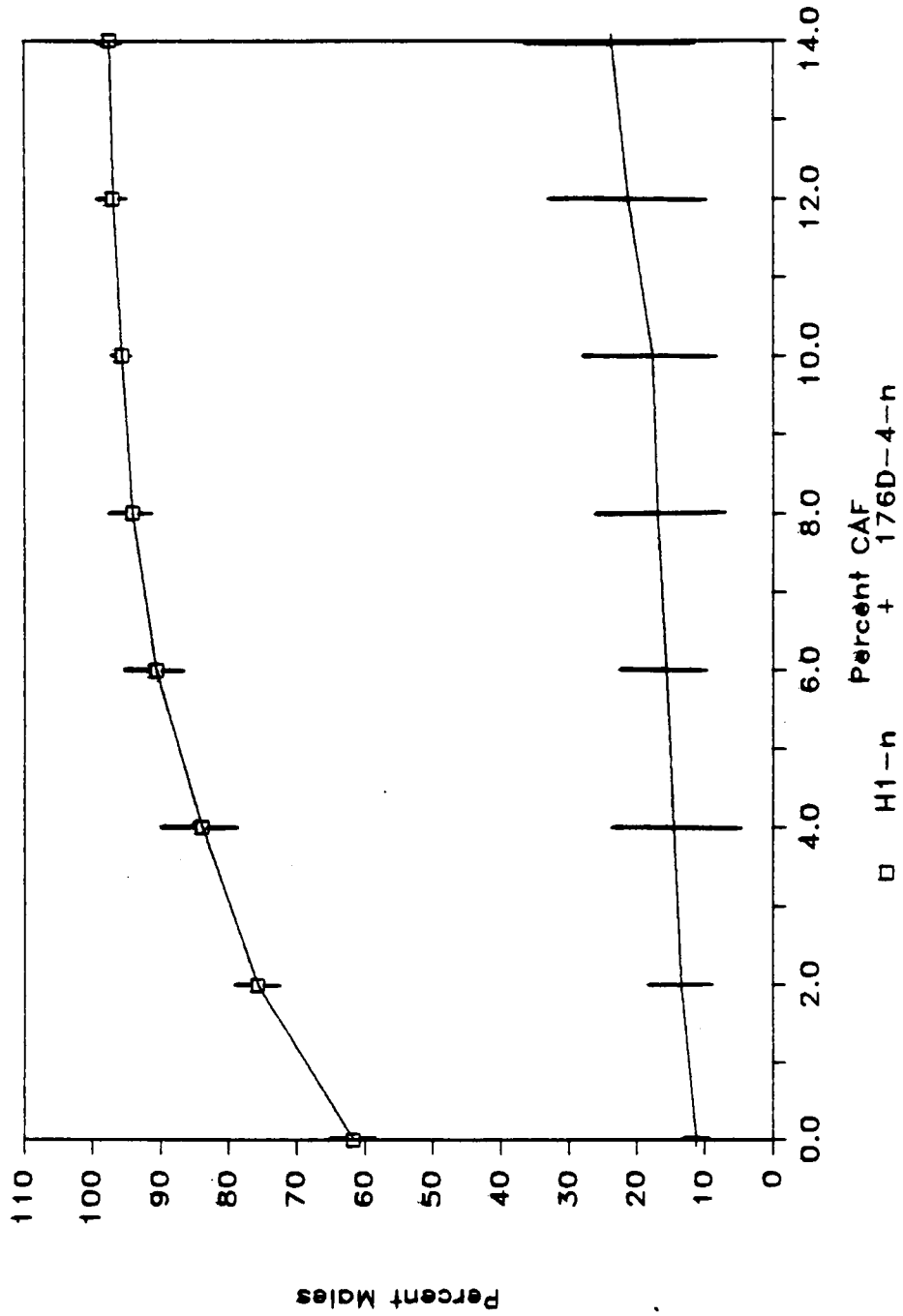


Figure 1. Comparison of strains H1-n and 176D-4-n on various concentrations of CAF. Response measured as change in percentage of male gametophytes. Each point represents the mean ± standard deviation of data from four experiments.

exhibits a high mean percentage of males (> 50%) even at 0% CAF. With the addition of CAF to the medium, this figure rises to > 90% at 14% CAF. Strain 176D-4-n shows much less sensitivity, at 0% CAF, the mean percentage of males is < 15%. With the addition of CAF, there is only a very gradual rise to 23% males on 14% CAF supplemented medium.

Since neither stock reached 100% male gametophytes on CAF concentrations up to 14%, an experiment was designed to test the effects of higher concentrations. Four cultures were grown at each of five different CAF concentrations for each strain. CAF used in this experiment all came from a single batch, 20/Ha. The concentrations used were 14%, 25%, 50%, 75%, and 93.6% (the highest percentage of CAF supplemented medium possible when using the aqueous based nutrient solutions used in these experiments). At high concentrations of CAF it was not clear what effect additional nutrients already present in the CAF might have. Therefore, at 50% and 93.6% CAF, four additional cultures were grown on media with only one-half strength nutrient solutions (sterile distilled water was added to dilute the nutrients) and four more were grown on media with no nutrient solutions (again, water was substituted). This allowed comparison of the results obtained with these three CAF/nutrient conditions (i.e., CAF + 1X nutrients, CAF + 1/2X nutrients, and CAF + 0X nutrients).

Mean percentages of males (± standard deviation) are listed in the Appendix, Table 8, and presented graphically in Figure 2.

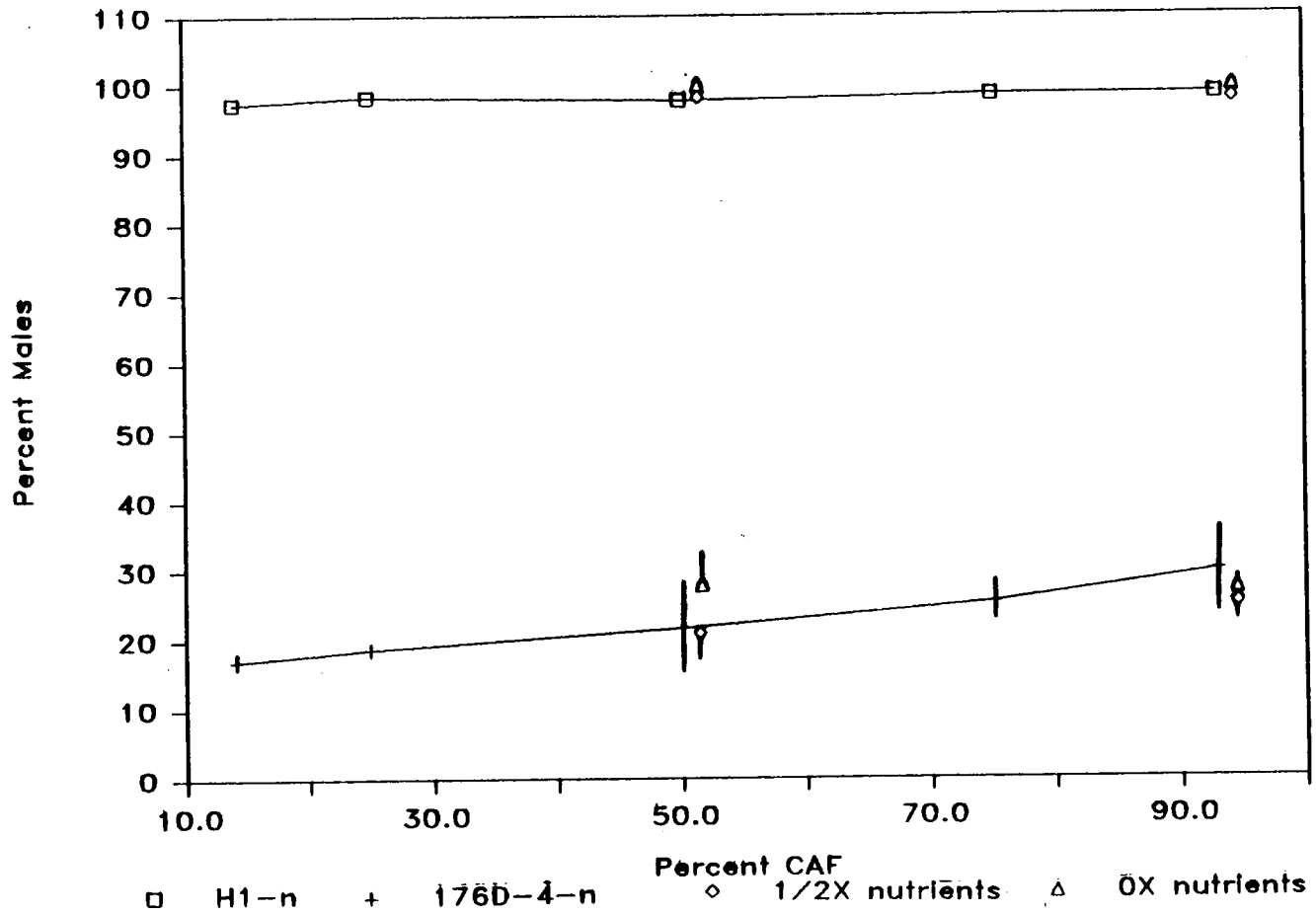


Figure 2. Comparison of strains H1-n and 176D-4-n on high concentrations of CAF. Each point represents the mean ± standard deviation of data from four samples. Results from conditions with nonstandard nutrients are shown offset to the right.

It is evident from Figure 2 that for strain H1-n, the mean percentage of males continued to rise gradually but leveled off at around 98%, never reaching 100% at any concentration. Although neuters were present in most of these cultures, at least a fraction of the gametophytes were fully determined cordates at every CAF concentration. Although pure antheridiogen was not available to test the response at A_{ce} concentrations higher than those attainable with CAF, these experiments indicate that cultures of strain H1-n may have a maximum attainable percentage of males which falls just short of 100. Since H1-n responded at an apparent maximum level at all tested CAF concentrations above 14%, no difference could be discerned for the tests with 1/2X and 0X nutrients.

Strain 176D-4-n again exhibited much less sensitivity to the CAF (Figure 2). The mean percentage of males rose steadily from 17% at 14% CAF to around 27% at 93.6% CAF. Although responses at higher Ace levels could not be determined, the data do indicate that the response levels off at higher CAF concentrations. For instance, from 0% to 14% CAF, the mean increase in percentage of males was 12%, whereas the increase seen from 14% to 93.6% CAF (more than a five-fold increase in CAF concentration) was only about 10%. In both strains, the maximum attainable percentage of males cannot be ascertained until a purified form of antheridiogen is available, since any aqueous filtrate of conditioned media will certainly be composed chiefly of water. In addition, other growth affecting substances may well be present besides antheridiogen.

The response of strain 176D-4-n to the various CAF concentrations was well below 100% at all concentrations (Figure 2), and differences could be discerned between conditions with 1X, 1/2X and 0X nutrient solutions (see Appendix, Table 8). However, at the two CAF concentrations where these comparisons were carried out, no consistency was observed with regard to the order of the strength of response. Therefore, these minor differences can most likely be attributed to chance variations. No evidence exists to indicate that excess nutrients present in the CAF act to modify sex ratios.

All of the experiments described above were carried out at a standard gametophyte density. Because gametophyte cultures exhibit a specific base level percentage of males, resulting from A_{ce} produced within the culture, it was of interest to assess the effect of population density on sexual differentiation. This was done by sowing various dilutions of the standard spore suspension on plain medium and determining the total number of males, cordates and neuters in these cultures after 10 DFS. Six plates were sown for each of 15 dilutions for both strains. However, since the number of spores per plate often varied greatly, even among equivalent dilutions, data for each of the 177 cultures (13 cultures contained no gametophytes) are presented individually, rather than as mean values for each dilution. These data are presented in the Appendix (see Tables 9 and 10) and are graphically illustrated in Figure 3. This figure also contains a point corresponding to 0% males at one

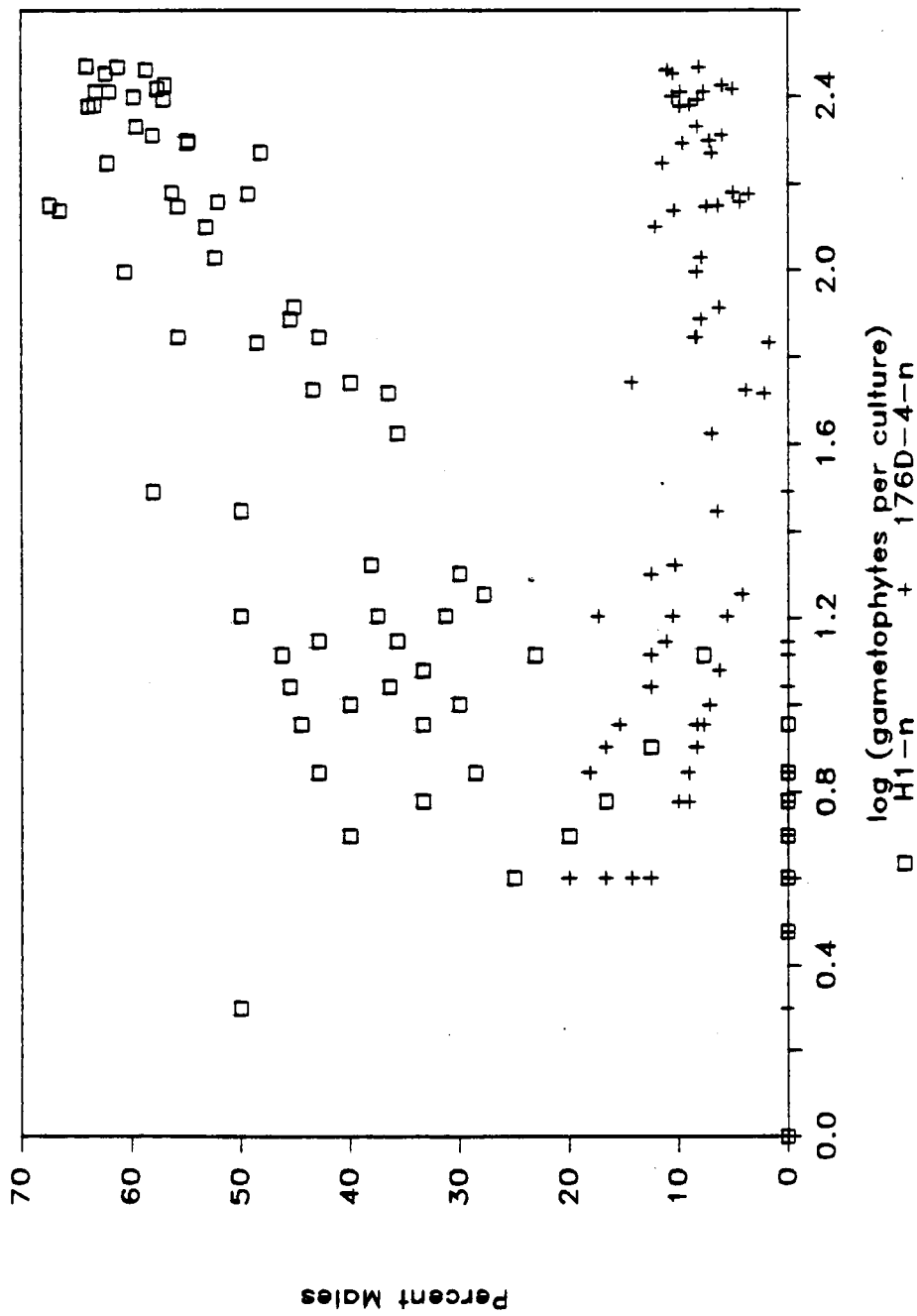


Figure 3. Comparison of the effects of culture density on sex expression for strains H1-n and 176D-4-n. See text for detailed description.

gametophyte per culture. This point was obtained from a separate experiment, not listed in the Appendix, Tables 9 and 10, which is described below.

Since single gametophytes never occurred on any of the plates in the above experiment, an experiment was designed to compare the development of isolated gametophytes with that of gametophytes in cultures of standard density. Four small plates with plain medium and four small plates with 6% CAF supplemented medium were sown for both H1-n and 176D-4-n spores at the standard density. In addition, 204 small isolate culture wells were filled with plain medium and 204 were filled with 6% CAF supplemented medium. Half of the wells for each concentration were inoculated with a single H1-n spore and half were inoculated with a single 176D-4-n spore. These were grown for 10 DFS and sex ratios were obtained as described in Materials and Methods (page 18). These data are presented in Table 1 and the percentage of males in isolate cultures has been incorporated into Figure 3.

The data in Figure 3 are difficult to compare directly to those obtained for standard density cultures on various CAF concentrations because the amount of antheridiogen present cannot be described even in relative terms (such as percent CAF). One might be inclined to assume that the amount of antheridiogen present rises proportionally with the number of gametophytes. However, this cannot be stated unequivocally since it is not known precisely how the amounts of antheridiogens produced by different morphological types

Table 1. Comparison of Sex Expression for Strains H1-n and 176D-4-n in Cultures of Standard Density and in Isolate Cultures.

Strain H1-n				Strain 176D-4-n			
% CAF (v/v)				% CAF (v/v)			
--------0------		-------6-------		--------0------		------6---------	
Cultures of Standard Density							
M:C:N[a]	%M[b]						
119:81:0	60	184:14:2	92	14:184:2	7	14:179:8	7
121:77:2	60	187:11:2	94	11:188:1	6	14:183:3	7
115:82:3	58	186:13:1	93	13:181:6	6	12:188:0	6
138:62:2	69	182:18:0	91	13:187:0	6	24:175:1	12
$\bar{X}$%M ± s[c]	62±5	$\bar{X}$%M ± s	92±1	$\bar{X}$%M ± s	6±0	$\bar{X}$%M ± s	8±3
Isolates							
M : C:N	%M						
0 :90:1	0	66:20:4	73	0:82:3	0	4:81:4	4

[a] M:C:N indicates number of male, cordate and neuter gametophytes.

[b] %M indicates percentage of male gametophytes.

[c] $\bar{X}$%M ± s indicates the mean percentage of males ± the standard deviation of the sample.

compare (i.e., Does A_{ce} production decrease as the percentage of males increases?).

However, some general trends are evident in Figure 3. For instance, there appears to be a range of gametophyte densities for each strain over which sex ratios do not vary greatly or in a predictable fashion. For strain H1-n, this range extends from around 96 to 294 gametophytes per culture (from ca. 1.9 to 2.5 on the log scale shown in Figure 3). For strain 176D-4-n, the extent of this range is subject to much greater interpretation, since the response is so limited. However, it can be seen that the percentage of males remains between 5 and 12% for all densities greater than 140 (ca. 2.1 on the log scale) gametophytes per culture. At or below 140 per culture the frequency of males falls below 5% for 48% of the cultures. For both strains in these experiments, the percentage of males at densities approaching standard sowing densities was similar to the response observed in earlier experiments.

Higher densities have less effect on promotion of males for strain 176D-4-n than for strain H1-n. This is apparent from the fact that cultures of strain H1-n always contained some males at densities greater than 9 gametophytes per plate, whereas gametophytes of strain 176D-4-n grew in cultures devoid of males at densities as high as 32 individuals per plate.

A further observation which can be derived from the data in Figure 3 and Appendix Tables 9 and 10 is that neither strain appears to be capable of producing gametophytes of the male morphology

when grown as isolates on plain medium. This observation concurs with the hypothesis proposed by Naf (1975) which suggests that gametophytes do not produce antheridiogen in effective quantities to induce maleness until they themselves are insensitive to it.

A final observation concerning density effects can be made by comparing percentage of males for cultures containing populations of gametophytes on 6% CAF supplemented medium to that for isolates on the same type of medium (Table 1). These data demonstrate that the antheridiogen produced by gametophytes grown as populations in culture has an effect on sex ratio which is additive to the effect of the antheridiogen supplied as CAF. For strain H1-n, there is a 19% increase in males for cultures grown as populations on 6% CAF as compared to those grown as isolates. A similar, but predictably less dramatic effect is observed for strain 176D-4-n. In this strain, 4% more males were produced in cultures grown as populations on 6% CAF as compared to those grown as isolates.

To assess the effect of antheridiogen on gametophyte morphology, mean area and number of antheridia per gametophyte were determined over a range of CAF concentrations. This was done by measuring the area of 20 cordate gametophytes and tallying numbers of antheridia for each, from each concentration of the 13/Ha, 13/Hb, and 13/Hc dose response experiments described previously. These data are presented in the Appendix, Tables 11 and 12, and Figures 4 and 5 show graphic representations of how these factors relate to each other.

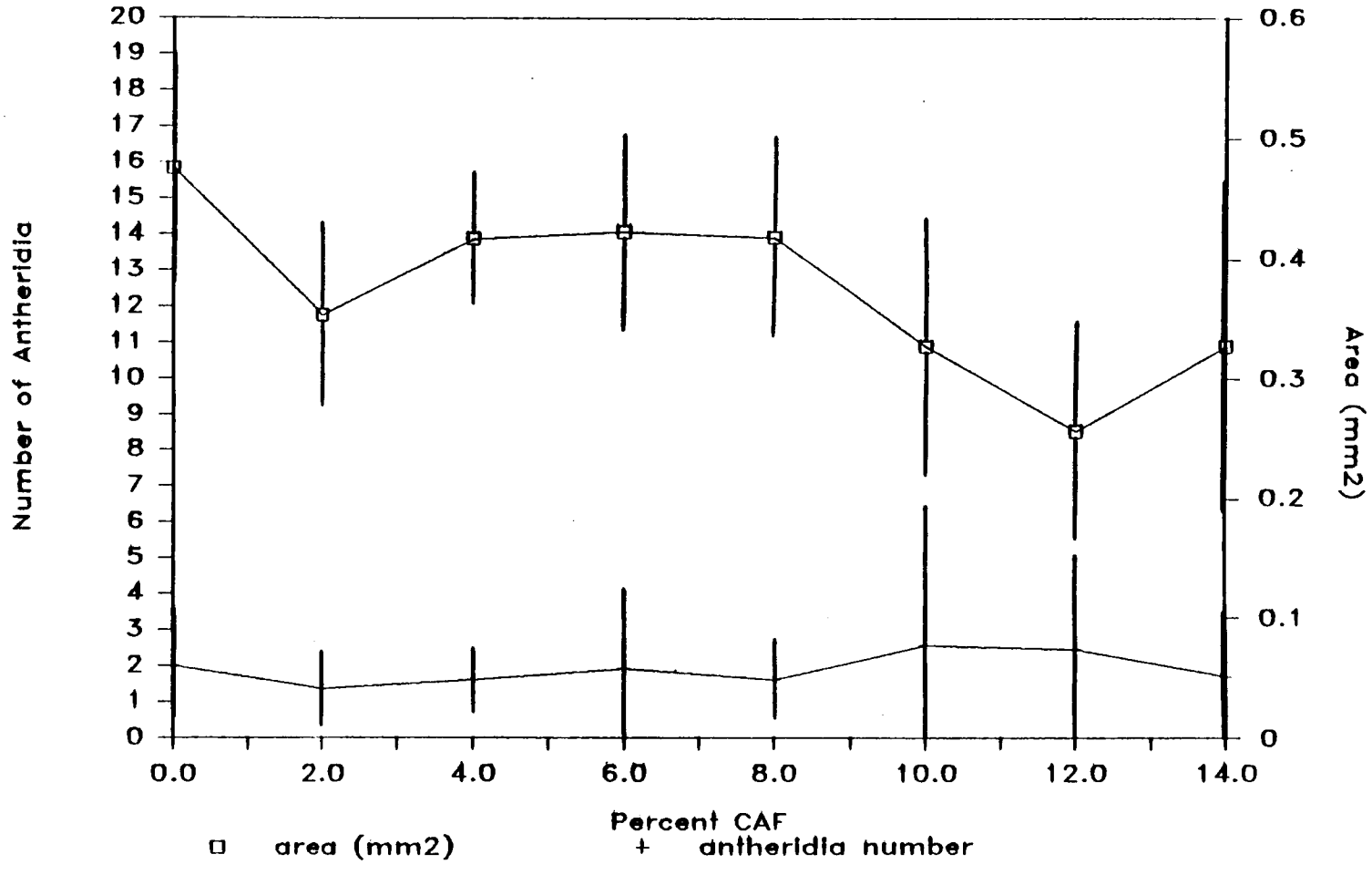


Figure 4. Changes in area and numbers of antheridia for cordate gametophytes of strain H1-n on various concentrations of CAF. Each point represents the mean ± standard deviation for 20 values.

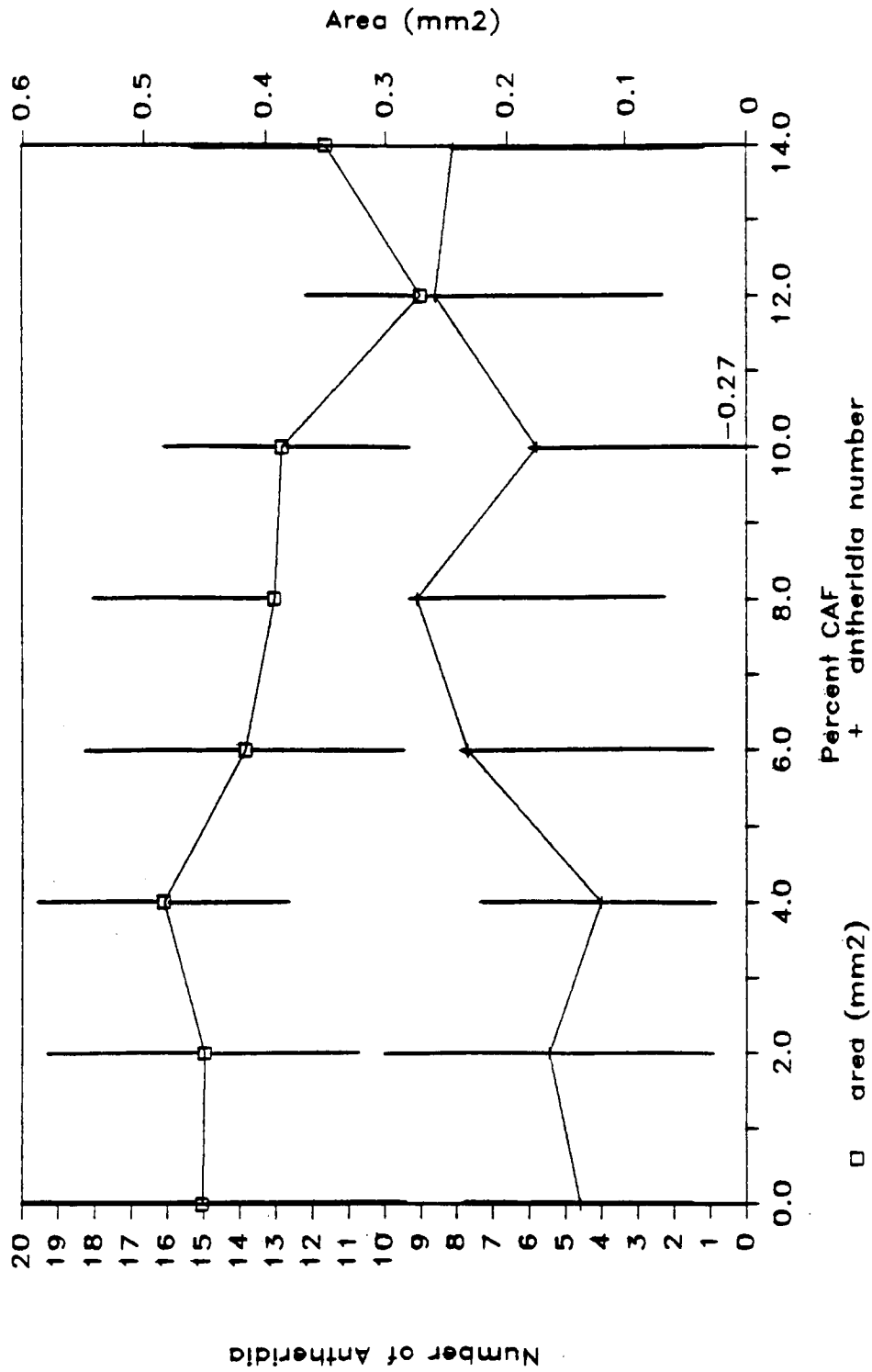


Figure 5. Changes in area and number of antheridia for cordate gametophytes of strain 176D-4-n on various concentrations of CAF. Each point represents the mean ± standard deviation for 20 values.

The data from these experiments showed considerable variability. However, certain trends are evident (Figures 4 and 5). For both strains, there appears to be a general trend toward decreasing mean area with increasing CAF concentrations. The apparent trends for antheridia number at various CAF levels was different for each strain. Mean antheridia number for strain H1-n seems to remain fairly constant over the range of concentrations considered (Figure 4), whereas for strain 176D-4-n, there appears to be a trend toward increasing antheridia with greater CAF concentrations (Figure 5).

There are a number of possible alternatives which could explain the above observations. With regard to the common trend toward decrease in size, it may be that some factor in the CAF simply restricts vegetative growth. It is possible that this factor is the antheridiogen itself, and that this aspect of its activity alone so greatly affects some gametophytes that redirected growth potential alone is responsible for the production of antheridia. This explanation would also account for the increase in antheridia number in strain 176D-4-n. On the other hand, this factor may be a separate entity, different from antheridiogen, which is also present in the CAF. Yet another possibility which would account for the trends seen in strain 176D-4-n, but not in strain H1-n, is that antheridiogen causes an increase in antheridia number and thereby diverts growth potential away from vegetative increase. A final possibility which exists is based on the developmental sequence of gametophytes with regard to sexual morphology. At the higher CAF concentrations,

some cordate gametophytes had the appearance of being individuals which would have been classified as males if cultures had been scored somewhat earlier. These gametophytes possessed a region of meristematic tissue, but were not well developed cordates. It is likely that these individuals developed by way of a different ontogenetic sequence than did the other, more characteristic cordates. The inclusion of data from such individuals in this analysis may have altered the results, if indeed these individuals were different from the more typical cordates. It is apparent that a much more detailed study on the ontogeny of gametophyte populations with regard to sex ratio needs to be carried out.

Strains H1-n and 176D-4-n were also compared on the basis of spore germination rates and the effect, if any, of CAF on germination rate. Five cultures were sown for each strain on plain medium and five on 6% CAF supplemented medium. The percentage of germinated versus nongerminated spores was determined for each culture at frequent intervals throughout a 239 hour period following soaking. A mean percent germination value for each observation has been calculated for the five cultures and these data are presented graphically in Figures 6, 7, and 8.

Figure 6 shows the germination rates for strain H1-n on plain medium and on 6% CAF supplemented medium. The two curves are essentially the same, indicating that CAF has no effect on germination rate for this strain. Figure 7 illustrates germination patterns for strain 176D-4-n under the same conditions. Again, no difference

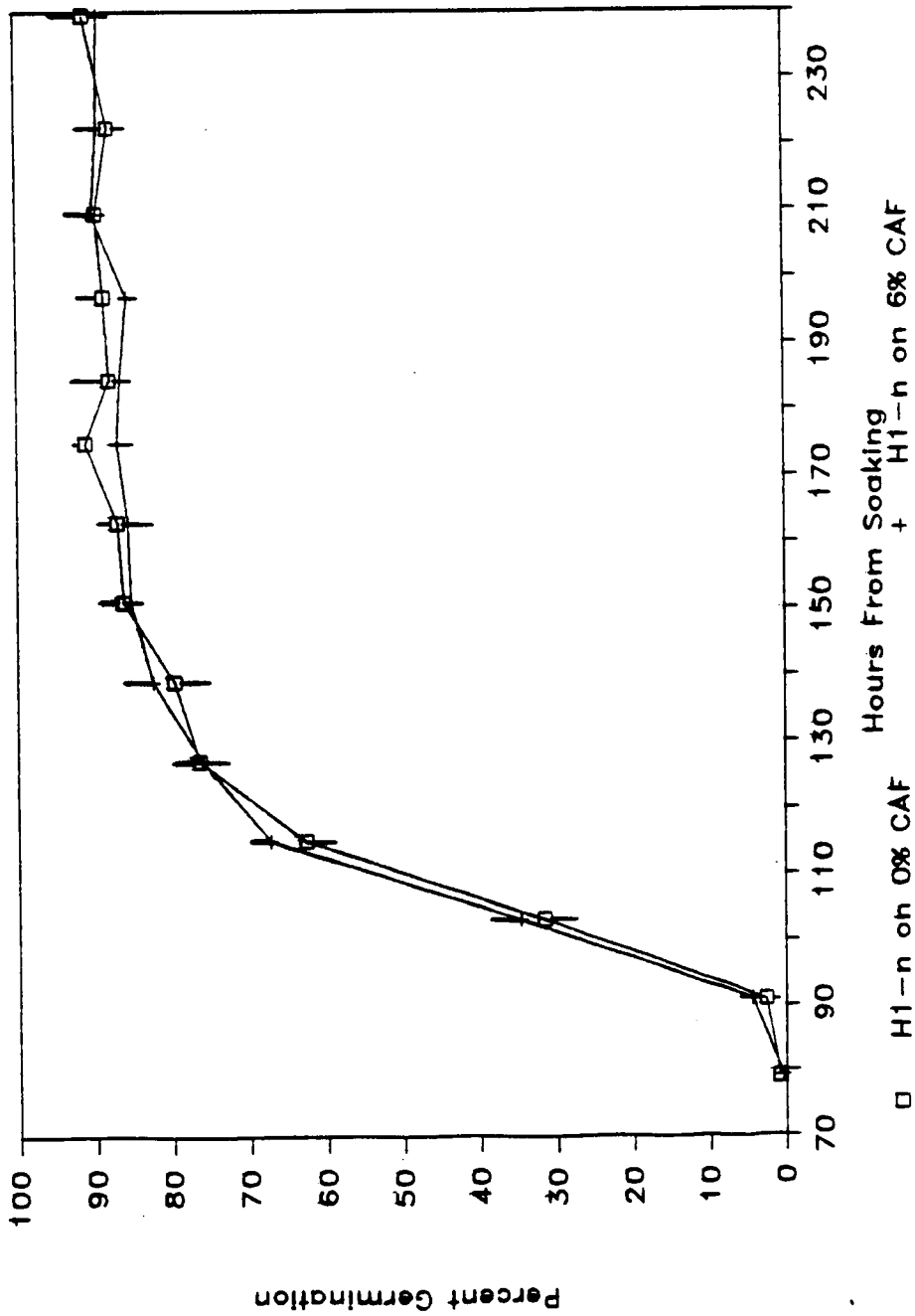


Figure 6. Germination rates for strain H1-n on 0% and 6% CAF supplemented media. Each point represents the mean ± standard deviation of five samples.

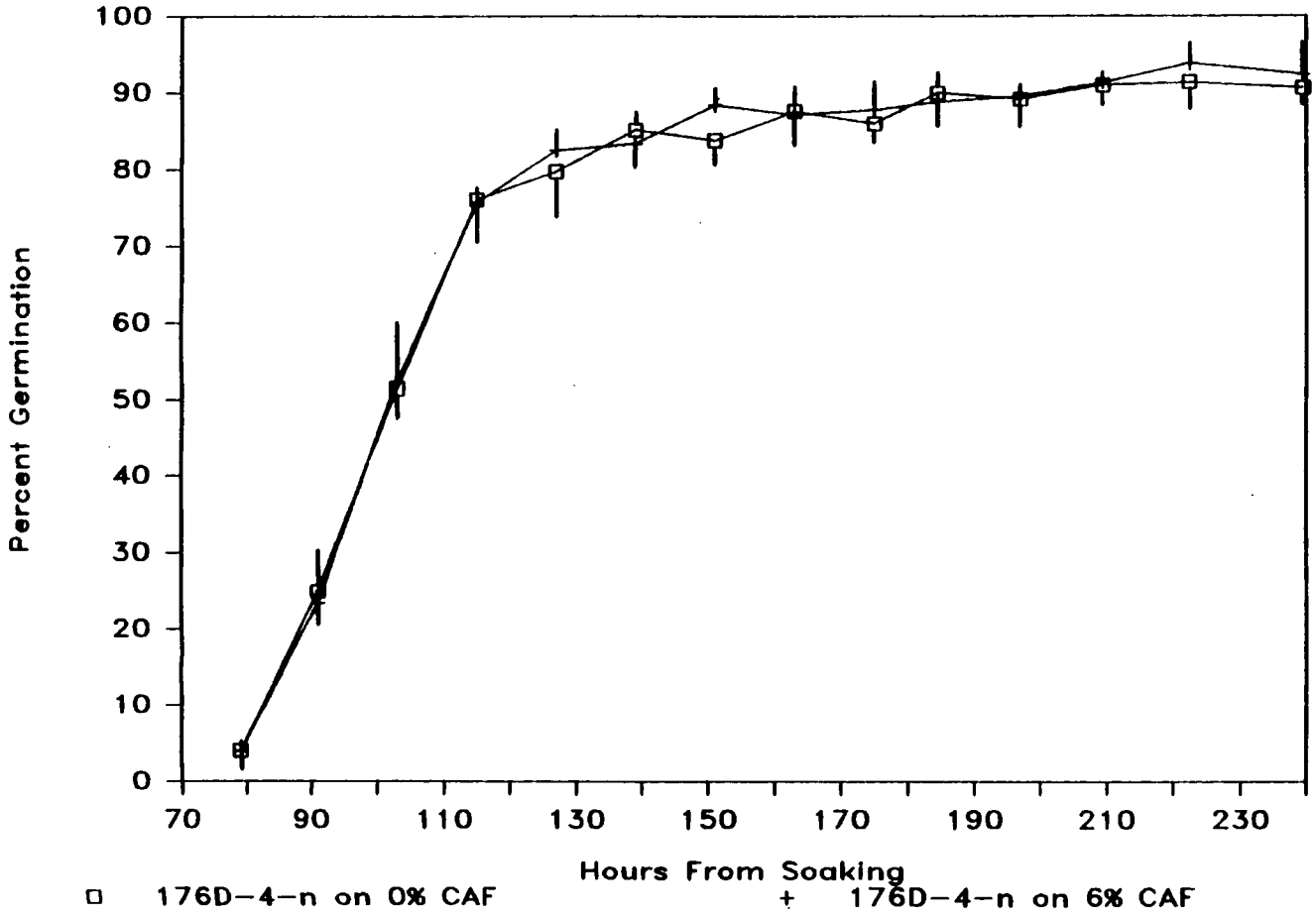


Figure 7. Germination rates for strain 176D-4-n on 0% and 6% CAF supplemented media. Each point represents the mean ± standard deviation of five samples.

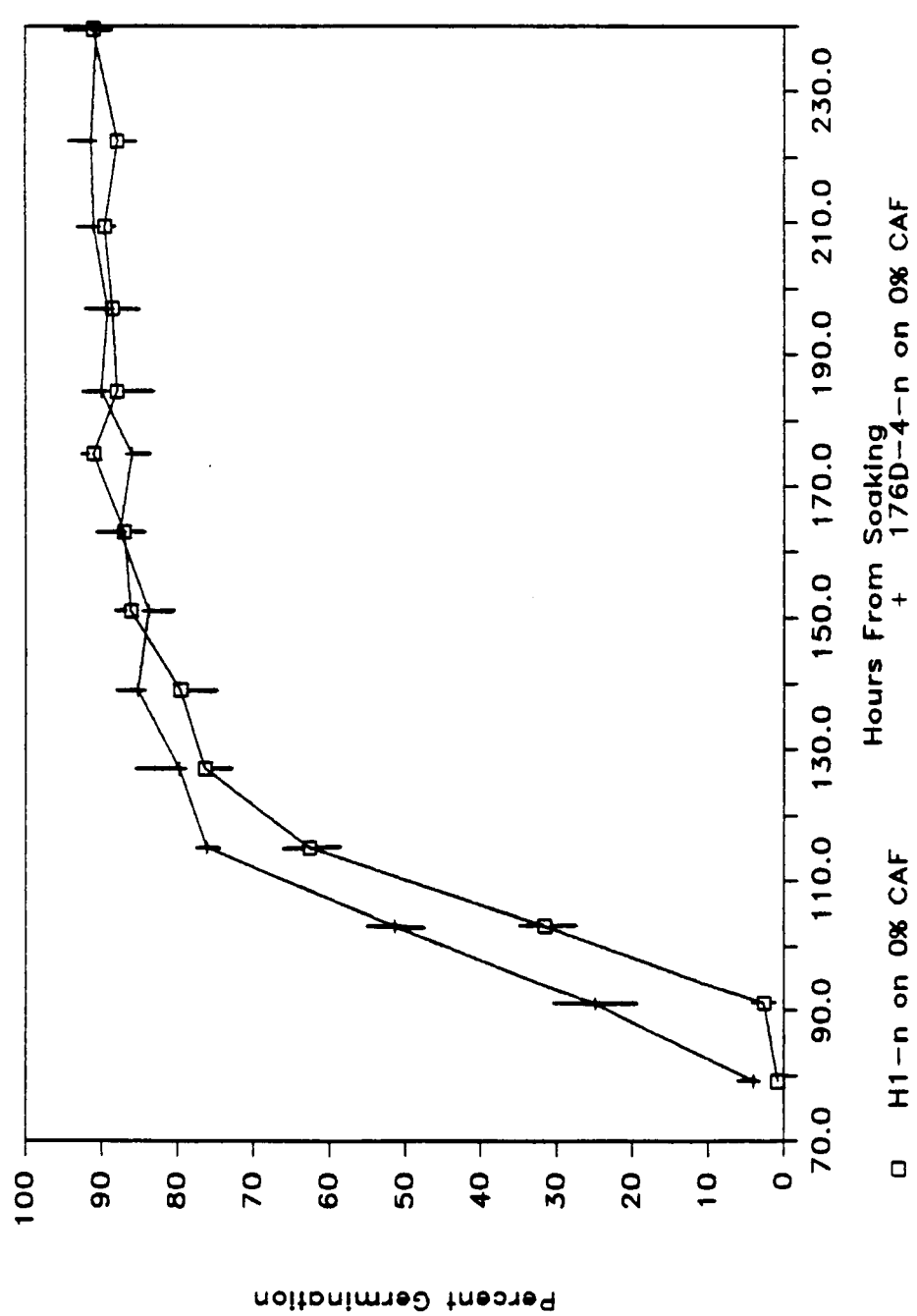


Figure 8. Germination rates for strains H1-n and 176D-4-n on 0% CAF supplemented medium. Each point represents the mean ± standard deviation of five samples.

between the pattern for cultures growing on CAF supplemented medium versus those growing on plain medium is evident. Figure 8 shows a comparison of germination pattern for each strain on plain medium. The curves appear to be essentially the same in shape, with the only major difference being an overall delay in germination for strain H1-n of around 6 to 12 hours as compared to that of strain 176D-4-n. It seems that this delay in germination alone is not sufficient to account for the drastic difference in sex ratios exhibited by the two strains. Thus it seems that germination rates are not directly correlated with sex ratio differences.

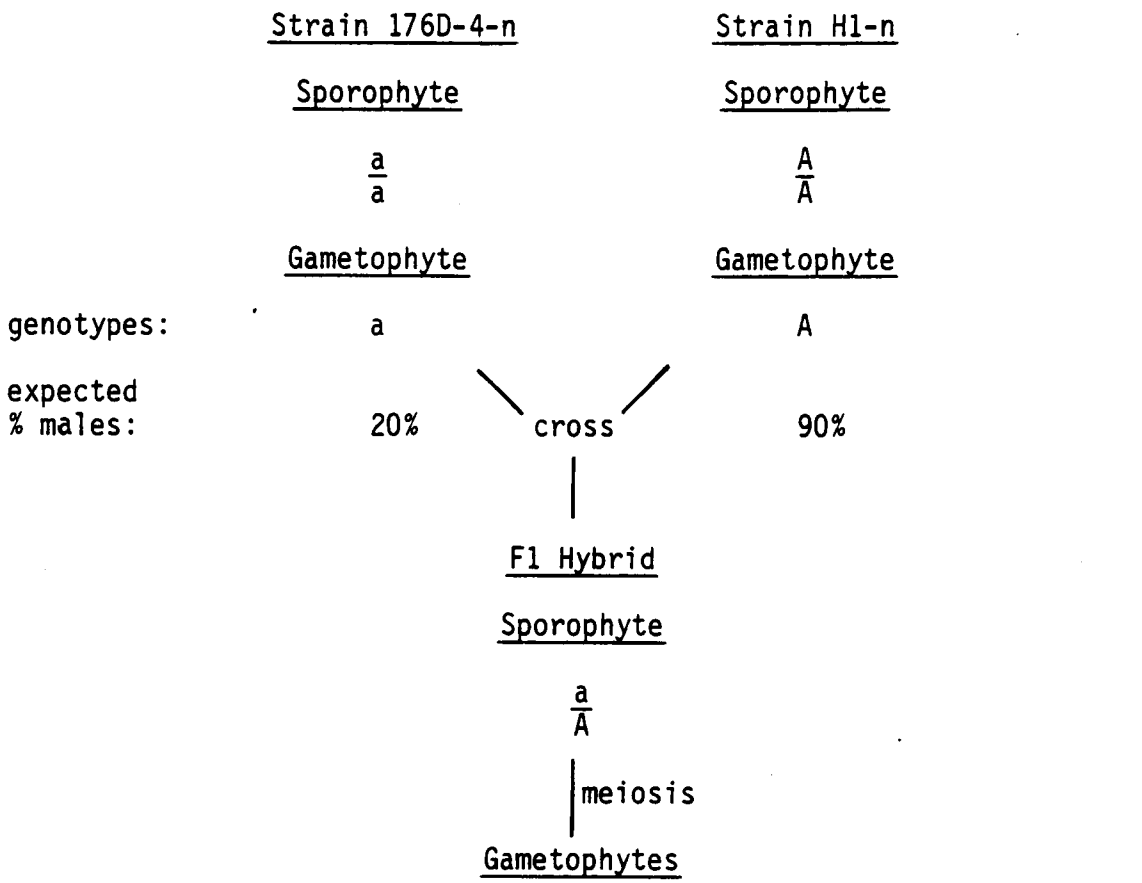
F1 Hybrids

Two F1 hybrids representing reciprocal crosses were utilized in these experiments. F1(176D-3-5 X Hn)8 and F1(H1-n X 176D-4-n)1. Preliminary tests on the first hybrid in 1982, by L. G. Hickok, indicated a sex ratio of 59 males: 41 cordates on plain media. Further details on the experiment (i.e., percent germination, density etc.) are not available. Spores from the second hybrid were sown in April of 1985 using the standard techniques described above. From three cultures, a mean sex ratio of 51 males:77 cordates: 5 neuters was obtained on plain medium. Spores from these hybrids were also sown on 6% CAF supplemented medium in July of 1985, the mean sex ratios from three cultures for each were 108 males:92 cordates:0 neuters for F1(176D-3-5 X Hn)8 and 85 males:93 cordates: 1 neuter for F1(H1-n X 176D-4-n)1, respectively.

All of the above sex ratios are, as expected, intermediate between those of the parental strains under similar conditions. This indicates that both parents contribute to the response of the F1 and therefore it is probably inherited by way of a nuclear gene as opposed to a cytoplasmic one. However, the genetic basis of the inheritance of the antheridiogen response cannot be determined from F1 analysis alone, since such results would be expected whether one, several or many different genes are involved. This situation is illustrated by several examples presented in Figures 9, 10, and 11. In these figures a value is given for the percentage of males which might be expected for each of the parental strains on CAF supplemented medium. In the models where multiple alleles are invoked to account for antheridiogen response, each is assumed to contribute equally and to be additive in its effect. The percentages of males expected for intermediate genotypes were calculated based on these assumptions and such figures are given where appropriate. In all of the given models, as in other likely models, the total percentage of male gametophytes expected in cultures from F1 hybrids is identical. Therefore, since analyses of responses of gametophytes from F1 hybrid spores alone were insufficient for determining a genetic basis for inheritance, studies of the F2 generation and segregation of the response in this generation were necessary.

F2 Segregation

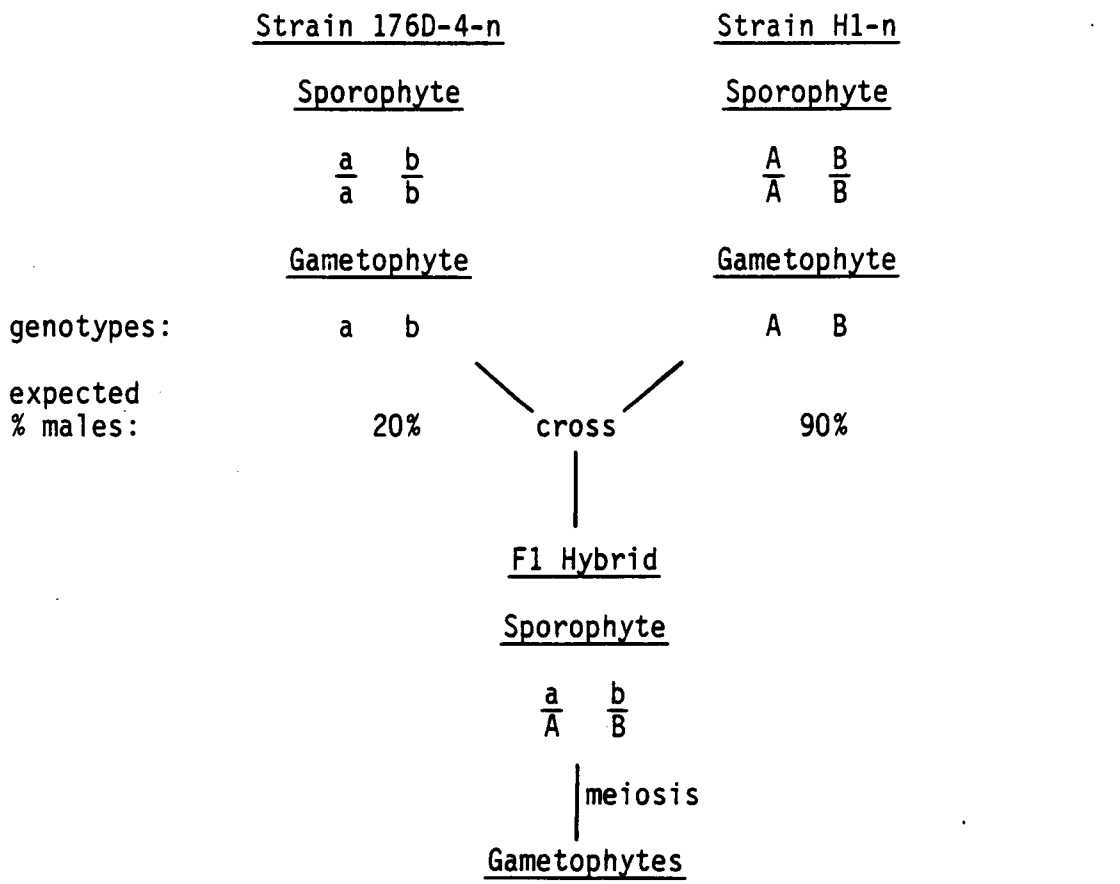
Two series of F2 individuals (AF2-1 and AF2-2), one from each F1 hybrid, were obtained as described in Materials and Methods,



Total expected percentage of males from gametophytes of the F1 hybrid

10% + 45% = 55%

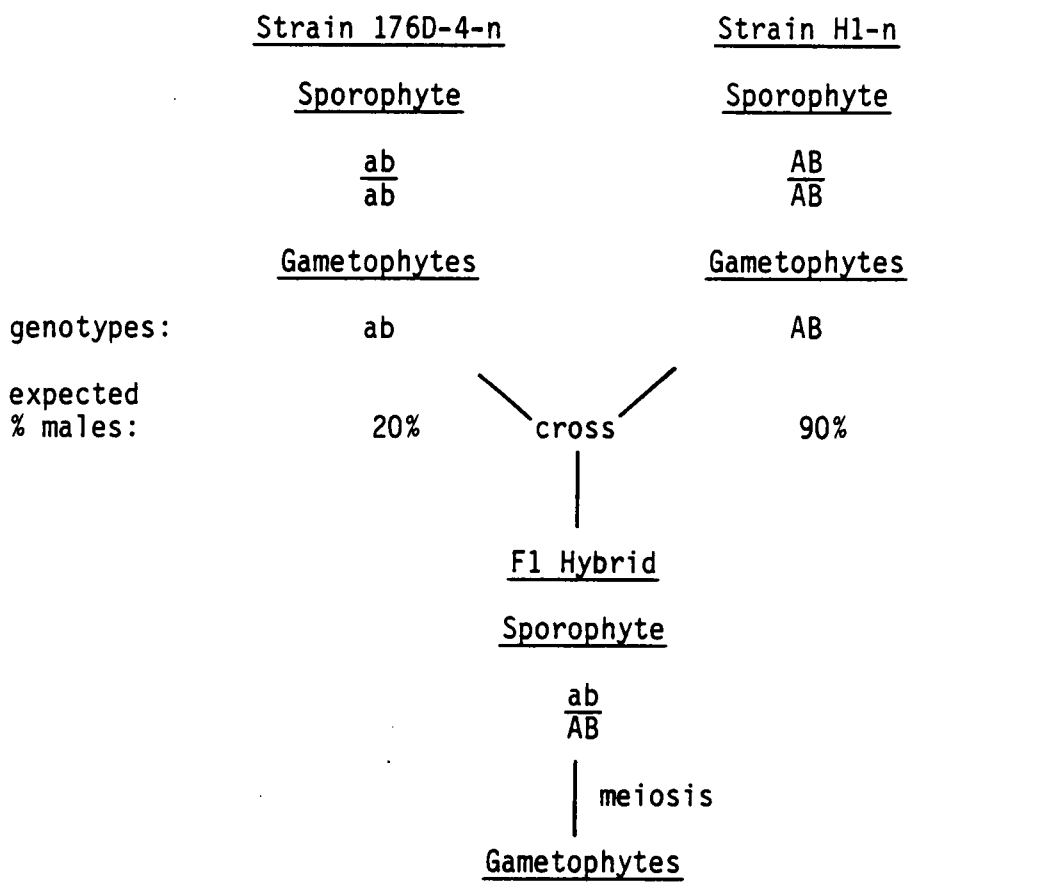
Figure 9. Expected percentage of males from the gametophytes of the F1 hybrid on CAF supplemented medium if response to A_{ce} is controlled by a single gene.



Total expected percentage of males from gametophytes of the F1 hybrid

5% + 13.75% + 13.75% + 22.5% = 55%

Figure 10. Expected percentage of males from gametophytes of the F1 hybrid on CAF supplemented medium if response to A_{ce} is controlled by two unlinked genes.



Total expected percentage of males from gametophytes of the F1 hybrid

$$8\% + 5.5\% + 36\% + 5.5\% = 55\%$$

Figure 11. Expected percentage of males from the gametophytes of the F1 hybrid on CAF supplemented medium if response to A_{ce} is controlled by two linked genes which exhibit 20% recombination.

page 21. Spores from these individuals were grown on 6% CAF supplemented medium, along with spores from the parental strains, to observe segregation of the F2's for antheridiogen response.

Spores from the first series of F2's (AF2-1), those derived from F1(176D-3-5 X Hn)8, were all grown on medium supplemented with CAF from batch 16/Hb, which was frozen for 54 days before being used. All of the plates were made at the same time. The second series of F2 spores (AF2-2) were grown on medium supplemented with CAF from batch 20/H, three separate aliquots of which were frozen and used to make medium at different times. One aliquot, 20/Hb was frozen for 28 days, another aliquot, 20/Hc, was frozen for 35 days and aliquot 20/Hc was frozen for 44 days. Each time medium supplemented with a different aliquot of CAF was used, spores of H1-n and 176D-4-n were grown on some of the culture plates as controls.

The F2 spores were allowed to grow on CAF supplemented medium and, in most cases, permanent slides were made at 10 DFS and sex ratios were determined. Several of the cultures from the AF2-2 series of F2's exhibited very slow spore germination. Therefore, cultures from this series whose percent germination was below 50% at 10 DFS were allowed to grow until 14 DFS. The sex ratios from the AF2-1 series of F2 spores are given as percent males, percent cordates, and percent neuters from three cultures for each individual in Appendix Table 13. Data from the AF2-2 series are presented in the same manner in Appendix Tables 14, 15, and 16.

For each of the series of F2's, a frequency distribution was generated which groups the individual F2 sporophytes together

based on the mean percentage of male gametophytes observed in cultures derived from their spores (Figures 12 and 13). Only a single set of data is available from the AF2-1 series and its distribution is shown in Figure 12. Although the AF2-2's were tested on CAF supplemented media made from three separate CAF aliquots, the responses of the parent strains were similar (Tables 14, 15, and 16) and all are represented in a single distribution (Figure 13).

Figure 12 demonstrates that there is a great deal of variation in the responses within the AF2-1 series when compared to that of the parent strains. In this experiment, 176D-4-n and H1-n exhibited mean percentages of males of 15% and 78%, respectively. The AF2-1's exhibited responses more extreme, similar to and intermediate to either parental strain. Although the sample of AF2-1's tested was small, the distribution appears to be distinctly skewed toward the extreme at which fewer males were present in the cultures. This tendency is probably due to a lack of randomness during the isolation of the gametophytes which produced these individuals. The standard method of producing an F2 generation from a sporophyte is to culture its spores and then isolate large numbers of immature cordate gametophytes shortly after the initial period of germination. This insures that the gametophytes are immature enough that no cross fertilization has been possible, and the resulting sporophytes will all be the products of self fertilization. Generally, this technique does not affect the frequency of specific phenotypes in the F2 generation. However, if antheridiogen response is correlated to the gametophyte

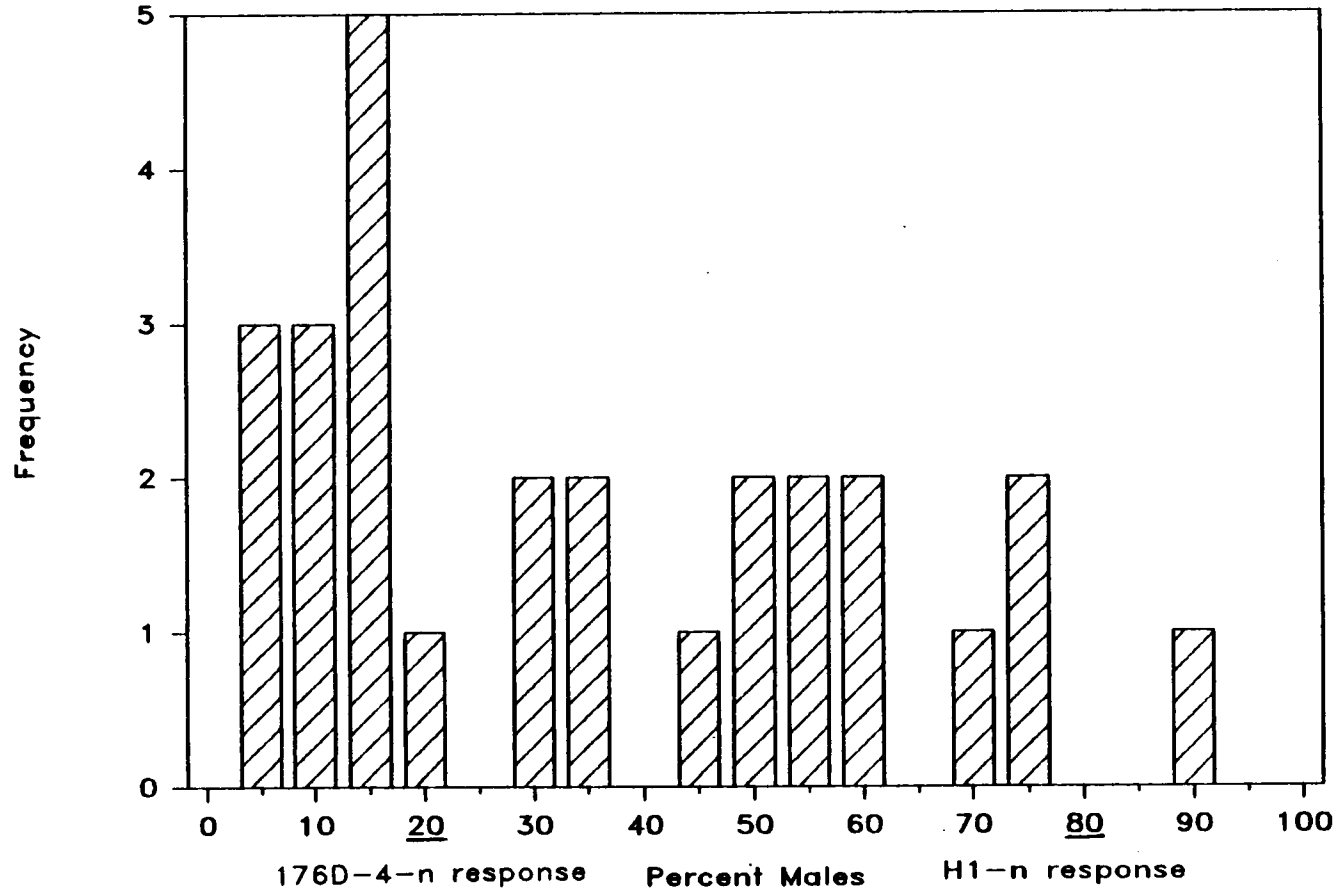


Figure 12. Distribution of response types, based on percentages of male gametophytes, from cultures of the AF2-1 series. Cultures were grown on 6% CAF supplemented medium. The ranges of parental response in this experiment are also shown.

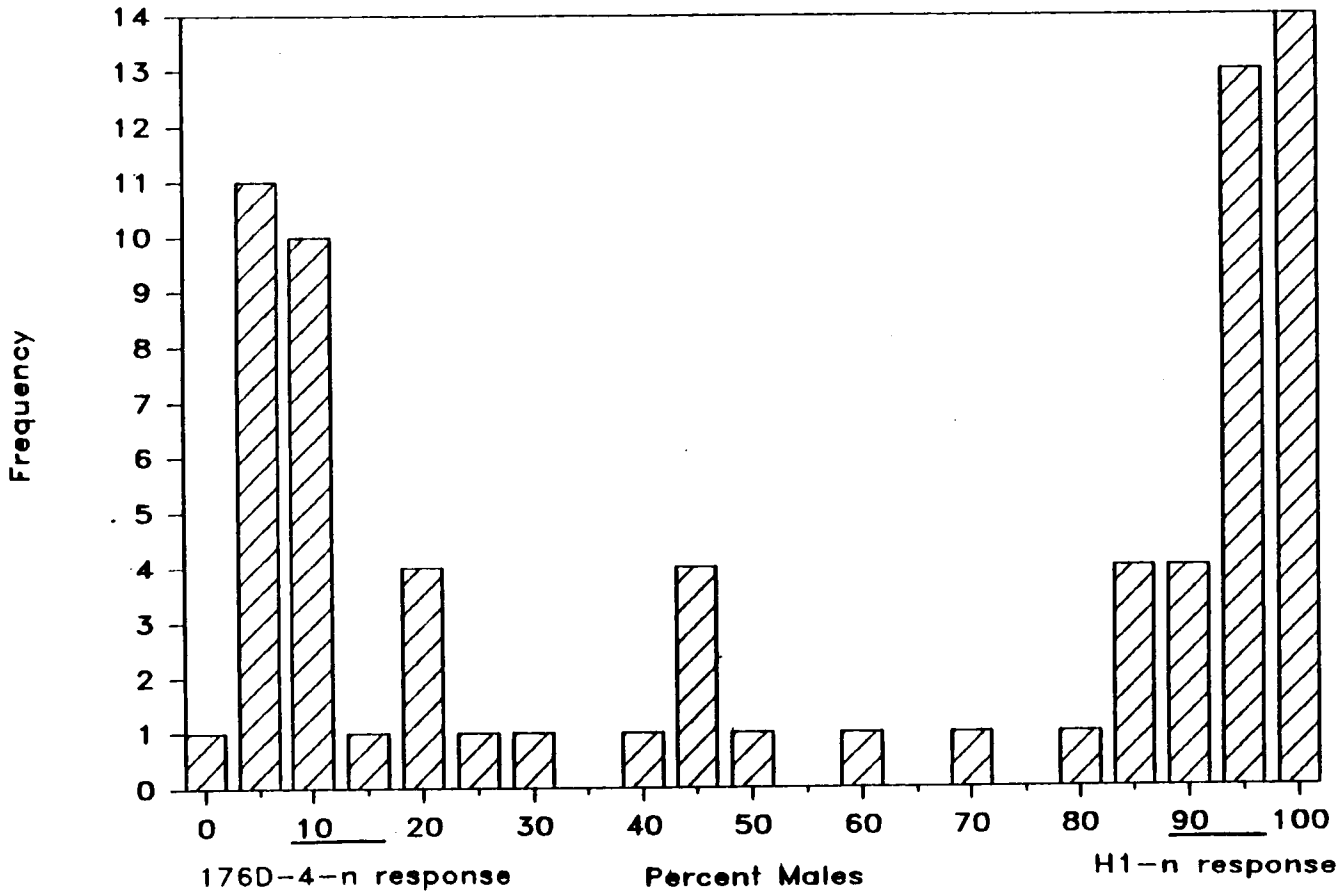


Figure 13. Distribution of response types, based on percentages of male gametophytes, from cultures of the AF2-2 series. Cultures were grown on 6% CAF supplemented medium. The ranges of the parental response in this experiment are also shown.

developmental sequence as was discussed earlier, it would be expected that isolating only cordate gametophytes which germinated early would lead to a bias toward F2's with less sensitivity toward antheridiogen (i.e., 176D-4-n and intermediate types). Therefore H1-n types would be expected to be selected less often and the frequency of occurrence of this type inaccurately represented in an F2 generation produced in this manner.

The distribution of the AF2-2 series, shown in Figure 13, is similar to that of the AF2-1 series except that a larger number of plants were tested and the proportion of individuals at various points along the distribution is somewhat altered as compared to that of AF2-1 (Figure 12). Specifically, the distribution is not skewed as is the one in Figure 12 and shows that there are roughly equal proportions of individuals which approach both parental ranges. In producing the AF2-2 generation, an attempt was made to increase the randomness with which the gametophytes that gave rise to the AF2-2 series were chosen. This involved extending the range of sampling over a longer period of germination (see Materials and Methods, page 21, for more details). Since a larger and less biased sample was used to produce the frequency distribution of Figure 13, it is assumed that this distribution more closely approximates the pattern of segregation of the antheridiogen response in the F2 generation. However, the two patterns (which represent the results from reciprocal crosses) are similar enough that there is no indication of any type of cytoplasmic inheritance. Because of this,

reciprocal crosses can be considered equivalent. Therefore, all subsequent interpretations are based on data from the randomly generated AF2-2 series from the F1(H1-n X 176D-4-n)1 hybrid.

Before considering further the possible mechanisms which may account for the inheritance of the antheridiogen response, one final source of potential variation must be considered. Recent work done by L. G. Hickok and T. R. Warne (personal communication) has shown that spores of Ceratopteris have a maturation requirement which must be met before full germination potential is reached. The consequence of sowing spores before they have fully matured appears to be an increased lag period between the time when spores are soaked and when initial germination occurs, and an increased degree of asynchrony in the cultures. Increased asynchrony may result in altered sex ratios (Hickok and Warne, personal communication). Therefore, an attempt was made in these experiments to monitor possible complications from this phenomenon. Since the spores of the AF2-1 series were about three years old, such precautions were unnecessary. However, for spores of the AF2-2 series this precaution was necessary. AF2-2 spores were seldom kept longer than 4 weeks before they were tested. To monitor the possibility of insufficient spore maturity, germination counts were taken for all AF2-2's on the day that slides were to be made (10 DFS) and if the percentage of germinated spores was below 50%, the cultures were allowed to grow for 4 more days. On the day when slides were actually made, the percentage of germinated spores was recorded. When this

information was plotted against the mean percentage of males for each AF2-2 individual, a graph was produced which appears in Figure 14. This graph provides a great deal of insight concerning the frequency distribution of Figure 13 and how it compares to the actual segregation of F2's. If only those individuals whose spore cultures achieved a level of germination comparable to that of the parental strains are considered, the number of F2's in the intermediate categories declines drastically. This line of reasoning seems well justified, since the F2's with low germination percentages frequently produced cultures of gametophytes with high numbers of neuter individuals (see Appendix, Tables 14, 15, and 16). Obviously, a high proportion of neuters in a culture tends to offset the sex ratio in one direction or the other, depending upon the ultimate sexual type of the majority of those gametophytes.

Further consideration of Figure 14 shows that most of the individuals grouped at either end of the frequency distribution for the AF2-2 series exhibited a fairly high percentage of germinated spores. An examination of sex ratio data for these individuals shows that there was a correspondingly low percentage of neuter gametophytes in the cultures from these plants as well. Thus, there is little doubt that the data for the mean percentage of males for these individuals provides an accurate measure of their response to antheridiogen.

In addition to the large number of individuals which exhibited responses similar to or more extreme than those of the parent strains,

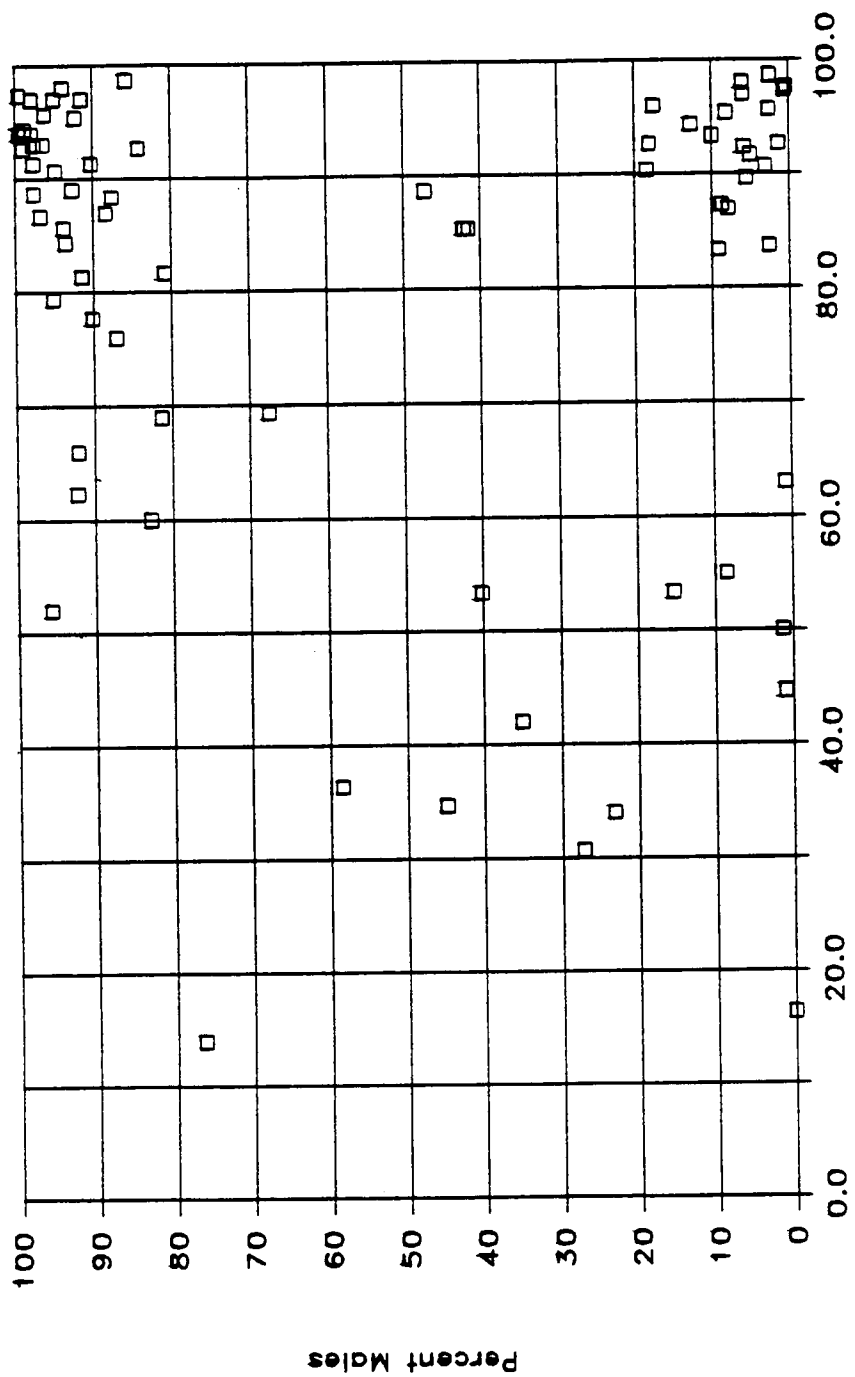


Figure 14. The relationship between percentage of male gametophytes and percentage of germinated spores for the AF2-2 series. Each point represents the response of a single AF2-2 individual to 6% CAF supplemented medium as related to its percentage of germinated spores when sampled.

there were three sporophytes, H1761-12, H1761-58, and H1761-166, which responded at an intermediate level and also had relatively high percentages of germinated spores (89%, 85%, and 85%, respectively). Sex ratios taken from gametophyte cultures of these three sporophytes showed that relatively few neuters occurred in these cultures as well (5%, 4%, and 9%, respectively).

Since the sex ratios for so many of the AF2-2 individuals appear to have been distorted by germination retardation, several were chosen to be retested on 6% CAF supplemented media after storage for about 5 months. The presence of individuals at either end of the response distribution seems well verified. Therefore, three individuals with intermediate percentages of male gametophytes and low germination percentages were chosen to retest. The three individuals chosen, H1761-14, H1761-143 and H1761-151, exhibited percentages of germination of 53%, 36%, and 35% and percentages of male gametophytes of 40%, 58%, and 45%, respectively. Spores from the three sporophytes exhibiting intermediate responses and high germination percentages were also retested on 6% CAF supplemented medium. Spores of both parental strains were grown on the CAF supplemented medium as an internal control. The sex ratios obtained from this second test of these individuals along with the new percentages of germination are shown in Table 2.

For all individuals retested (Table 2), the percentage of germinated spores increased. For those which previously exhibited slow germination, this increase was considerable. In these cases,

Table 2. Retest of Several AF2-2 Individuals After Full Maturation of Spores.

Code	$\bar{X}$%G[a]	M:C:N[b]	%M[c]	M:C:N	%M	M:C:N	%M	X%M±s[d]
H1-n	89	175:24:1	88	174:26:0	87	175:25:0	88	88±1
1760-4-n	88	53:127:0	29	39:98:0	28	18:132:0	12	23±10
H1761-14	95	158:18:2	89	159:23:2	86	172:28:0	86	87±2
H1761-143	96	193:7:0	96	187:13:0	94	188:11:1	94	95±1
H1761-151	98	120:33:0	78	152:34:0	82	131:47:0	74	78±4
H1761-12	98	165:35:0	82	164:36:0	82	177:23:0	88	84±3
H1761-58	88	78:88:10	44	42:73:6	35	54:51:9	47	42±6
H1761-166	95	137:63:0	68	106:92:2	53	102:98:0	51	57±9

[a] $\bar{X}$%G indicates mean percentage of germinated spores from three samples.

[b] M:C:N indicates number of male, cordate and neuter gametophytes. Data from three samples are shown.

[c] %M indicates percentage of male gametophytes.

[d] $\bar{X}$%M±s indicates the mean percentage of male gametophytes ± standard deviation of the sample.

there was also a corresponding change in the mean percentage of male gametophytes. Whereas the mean percentage of males for these three individuals indicated an intermediate response to Ace when first tested, the values obtained in the second test reflect a much higher proportion of males. Two of these individuals, H1761-14 and H1761-143, exhibited mean percentages of males nearly as high or higher than that of H1-n, which was 87% in this test. The third, H1761-151, exhibited a mean percentage of males of 78% in this test. Although this value is not as close to the figure obtained for H1-n as are the others, it reflects a considerable change from the first test in which the mean percentage of males was only 45%.

Of the three AF2-2 individuals which had relatively high percentages of germinated spores and exhibited intermediate responses in the first test, two exhibited intermediate responses in the second test as well (Table 2). H1761-58, which produced cultures yielding 42% male gametophytes in the first test, exhibited a mean percentage of males of 42% in the second test. For H1761-166, this figure was 42% in the first test and 57% in the second test. The third individual, H1761-12, exhibited a mean percentage of males which was much higher in the second test than in the first. In the first test this figure was 47%, whereas in the second test, it was 84%. Since the percentage of germinated spores increased by 9% in this test and the percentage of neuter gametophytes dropped from 5% to 0% for this individual, it is assumed that the interpretation of response for this individual was incorrect and rather than exhibiting

a response which is intermediate to that of the two parents, H1761-12 exhibits a response like that of H1-n.

Genetic Interpretation

The consistent differences in response to Ace exhibited by H1-n and 176D-4-n were demonstrated by obtaining sex ratios from gametophyte cultures of both over a range of CAF concentrations (Figure 1, page 24). Clearly, these two strains exhibited different responses. When the two strains were crossed, gametophyte cultures from reciprocal hybrids each responded at levels which were intermediate between the parental strains on both plain and 6% CAF supplemented medium. Since neither of the parental type responses were exhibited by either hybrid, the possibility that response to A_{ce} is cytoplasmically inherited can be ruled out. This assumption was verified by the appearance of F2's exhibiting responses similar to that of both parental strains in each of the reciprocal F2 generations.

In both series of F2's there appeared to be several fairly distinct classes of response to A_{ce} (Figures 12 and 13, pages 48 and 49, respectively). However, as previously discussed, in the first series the proportion of individuals in each category was altered by a lack of random sampling with regard to the onset of germination in the production of this series. Therefore, the apparent bias in this F2 series toward individuals exhibiting responses similar to the 176D-4-n parent and those with intermediate responses, as

opposed to ones responding like the H1-n parent, was predictable.

The AF2-2 series was produced in a more random fashion and the more proportionate distribution of F2's over the range of responses appears to reflect this. However, while testing the response of this series, it became evident that slow germination among some individuals led to the production of altered sex ratios and misinterpretation of response types. This occurred because such cultures contained an abundance of neuter individuals. Because of the high proportion of neuters, the percentages of males for these cultures were not comparable to those of others. Several AF2-2 individuals were retested after germination rates for these had improved. This retesting indicated that the responses of many if not all of the AF2-2's which exhibited low percentages of germinated spores and an intermediate response in the first test had likely been misinterpreted and that these were actually similar to the H1-n parent strain with regard to A_{ce} response. Retesting of the three AF2-2's which exhibited high germination and intermediate responses indicated two of these actually responded at a level intermediate between the two parental strains.

Since the segregation pattern of Ace response types in the AF2-2 series appeared to have been somewhat obscured by the inclusion of individuals with low percentages of germination, another frequency distribution was generated for the AF2-2 series in which all individuals whose percentage of germination was below 80% were

removed. This distribution is shown in Figure 15. In this distribution, there appear to be three general classes of response, one with individuals which respond similarly to the 176D-4-n parent, one in which response is like that of the H1-n parent and one much smaller category in which responses are intermediate between the two. However, it is also possible that additional classes exist which blend with those of the parental type responses. The presence of AF2-2 individuals whose gametophyte cultures had as small a mean percentage of males as 0% and others with as large a value for this figure as 100% and exhibited high percentages of germinated spores as well, indicates that two classes, one less responsive than the 176D-4-n strain and one more responsive than the H1-n strain, may exist. These responses appear to be significant, since the 176D-4-n strain only exhibited such low mean percentages of males at very low densities and the H1-n strain never exhibited a figure this high, even at 93.6% added CAF (see Appendix Table 8).

Several factors exist which make it difficult to distinguish between the two possibilities described above. Firstly, the response of the parental strains on 6% CAF supplemented medium (see Appendix Tables 14, 15, and 16) was less for the 176D-4-n strain and greater for the H1-n strain than those had been in past experiments (i.e., for testing of the AF2-1 series, see Appendix Table 13). This situation would obscure any separation between individuals expressing subtly different response types in the vicinity of the parental ranges. In addition, although extreme categories may exist, genetic

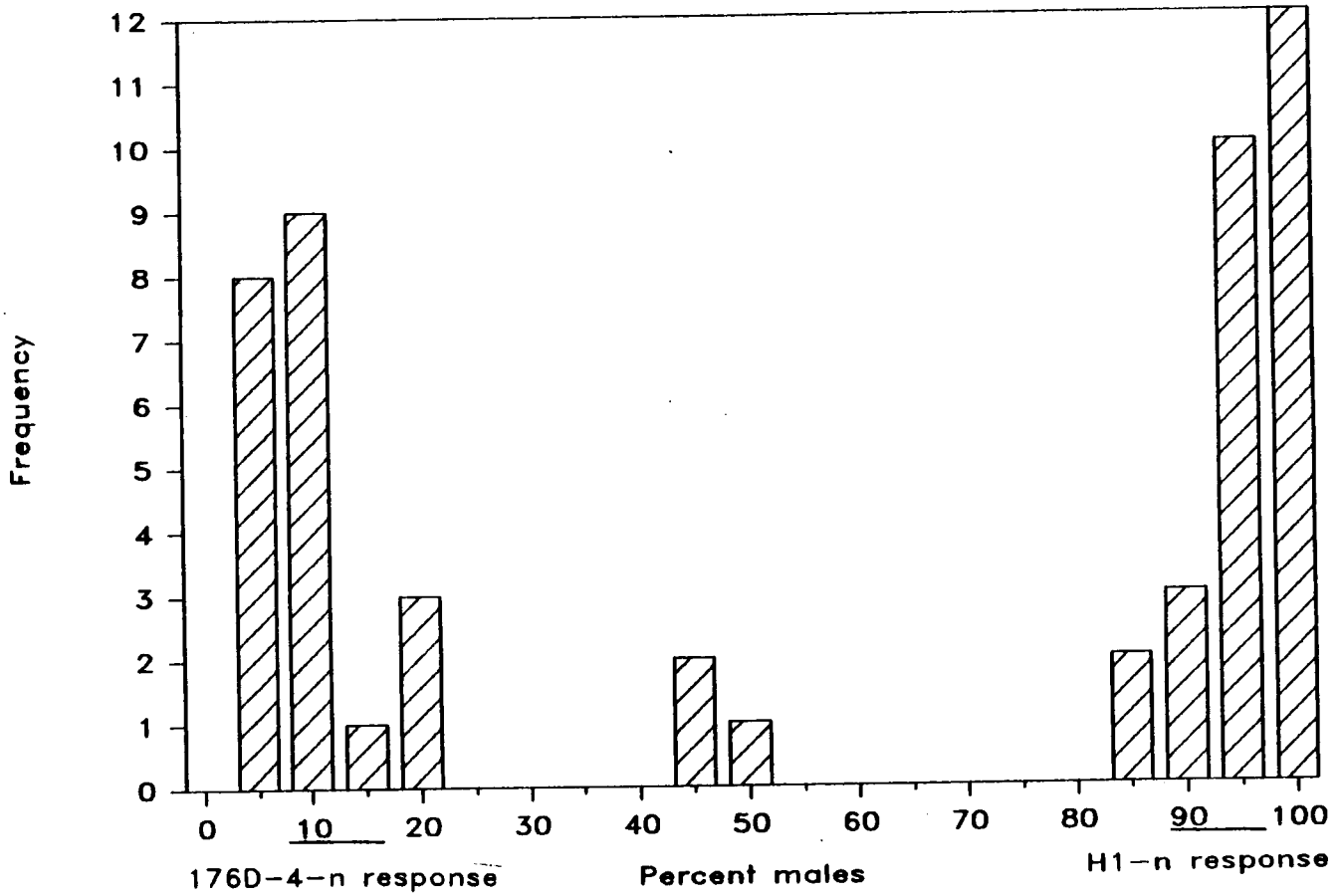
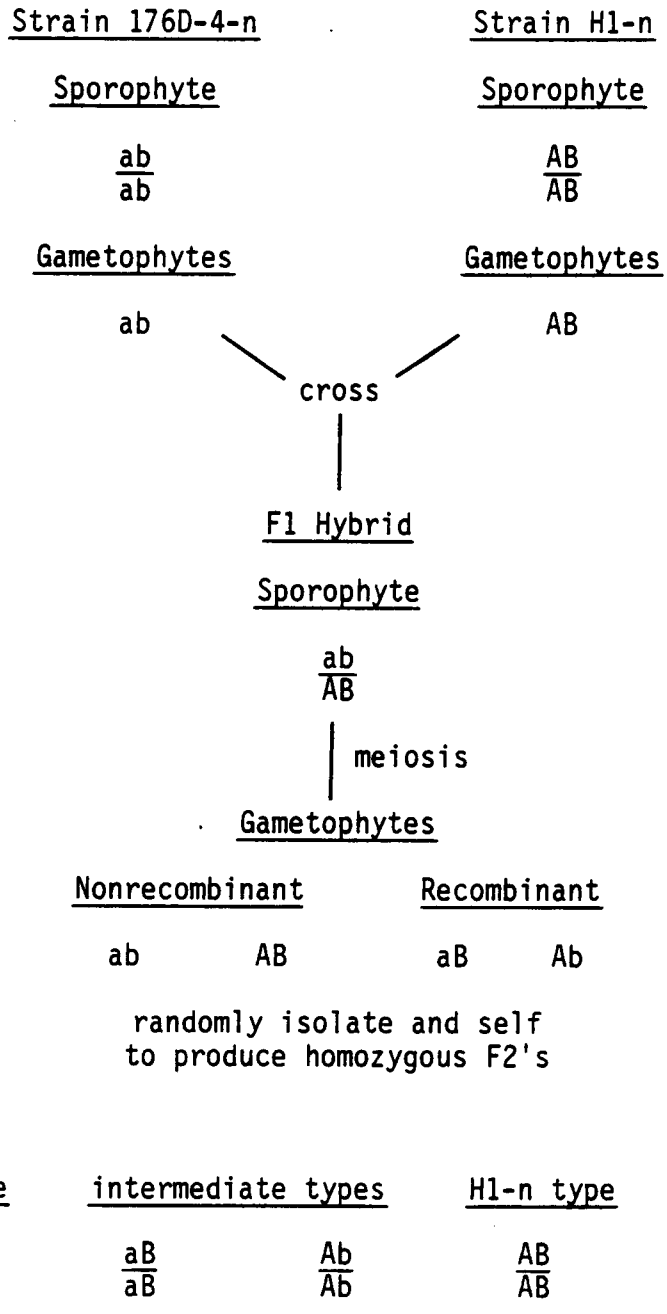


Figure 15. Distribution of response types, based on percentage of male gametophytes, from cultures of the AF2-2 series which exhibited percentages of germinated spores > 80%. Cultures were grown on 6% CAF supplemented medium. The ranges of parental response in this experiment are also shown.

variation among the AF2-2 individuals for traits other than A_{ce} response, creates a range of diverse genetic backgrounds within which this response functions. Since A_{ce} response is almost certainly linked to gametophyte development, there are probably a great many genes besides those that directly control the expression of this trait which greatly influence it. Therefore, individuals that may be genetically identical for major genes controlling the antheridiogen response may not respond in an identical manner because of variation in other loci.

Since it is not possible at this point to distinguish between the two possible patterns of segregation described above, models that account for each are presented in Figures 16 and 17. In the simplest of the two cases (Figure 16), the three categories that occur could be accounted for by a genetic system with two linked genes that exhibit recombination. In this case, a single intermediate category would occur as the result of recombination between the two loci if the genes referred to as A and B contributed equally to Ace response. If A and B were not equal with regard to their contribution to response, two intermediate categories would be expected.

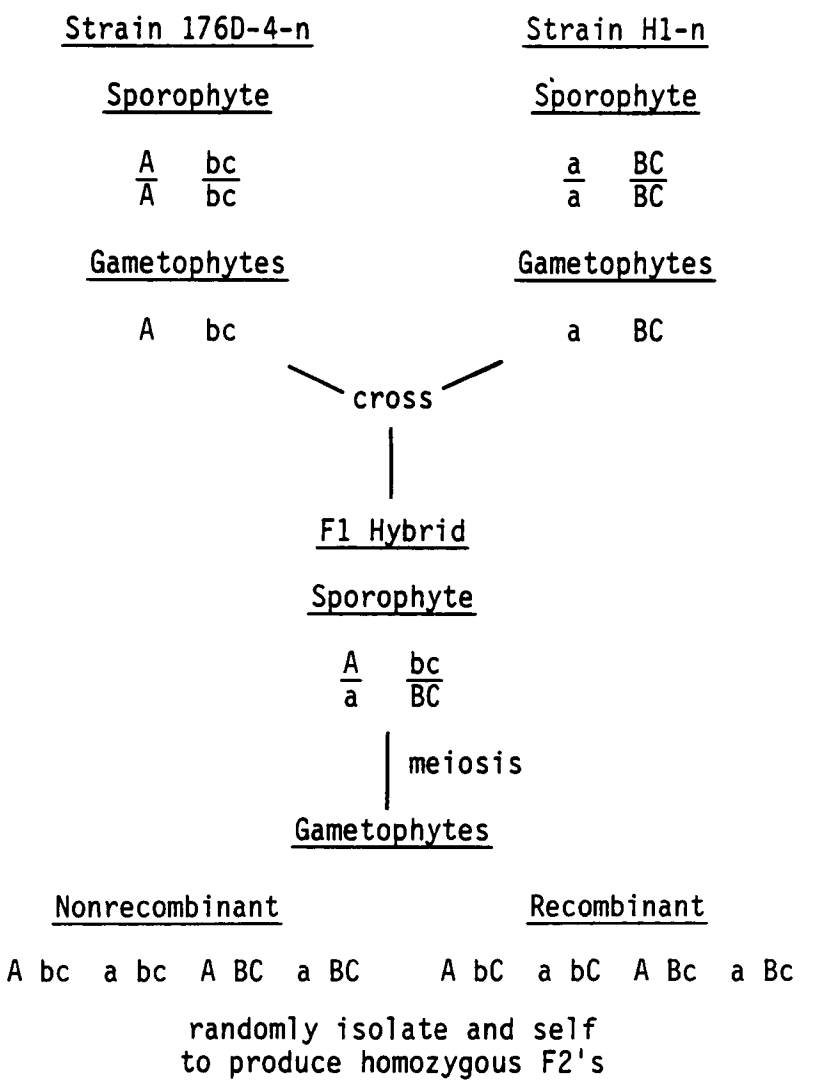
If the more complex pattern of segregation actually occurs and two categories exist that respond more and less extremely than H1-n and 176D-4-n, respectively, the model illustrated in Figure 17 could be invoked to account for it. This model involves two linked genes that exhibit crossing over and one gene that segregates



F2's

176D-4-n type	intermediate types		H1-n type
ab/ab	aB/aB	Ab/Ab	AB/AB

Figure 16. Two gene model for the inheritance of A_{ce} response.



F2's

an extreme type	176D-4-n type	intermediate types	H1-n type	an extreme type
a/a bc/bc	A/A bc/bc	a/a Bc/Bc A/A Bc/Bc a/a bC/bC A/A bC/bC	a/a BC/BC	A/A BC/BC

Figure 17. Three gene model with two linked genes for the inheritance of A_{ce} response.

independently. In this model, the category of response which is more extreme than that of H1-n would be observed when all three effective alleles are brought together. This is possible because the gene labeled A segregates freely from the linked pair B and C. Likewise, the category that is less responsive than 176D-4-n would occur when none of the effective alleles are present. Two or four intermediate categories would be predicted to arise from recombination of the linked genes, depending upon the relative contribution of genes A, B and C to A_{ce} response. If A, B and C contribute equally, two classes, one containing individuals of the genotype ABc and AbC and the other with individuals whose genotype would be aBc or abC would be predicted. If all three genes contribute unequally to A_{ce} response, four separate categories, each containing individuals of a single genotype only, would be predicted.

To determine which of the two models correctly explains the inheritance of the A_{ce} response, further work along several lines will be necessary. One possible course of action is to retest a larger number of the F2's produced in this experiment on CAF from a different batch and perhaps at different concentrations. Such efforts would be aimed at increasing the resolution with which undetected categories that may exist could be discerned. Another option is to select several individuals for which response type is not clear (i.e., those exhibiting higher or lower degrees of response than the parental types) and generating does response curves for these on varying concentrations of CAF. Comparing such curves

to known curves for the parental types could clarify the relationship of these individuals to the parental types. In addition to further tests on the F2 individuals themselves, appropriate backcrosses of F2's to the parental types and crosses among the F2's should be carried out as well. The resulting sporophyte generation derived from these crosses could then be tested for predictable segregation patterns.

IV. SUMMARY

The response to antheridiogen of two homozygous strains was observed over a range of CAF concentrations. The two strains differed greatly over the entire range studied. Strain H1-n always exhibited greater sensitivity than did 176D-4-n. Experiments carried out at high CAF concentrations indicated that each strain had a maximum attainable percentage of males and some cordates would always be present, regardless of antheridiogen concentration. Experiments in which various levels of nutrient solutions were used when media were supplemented with high concentrations of CAF indicated that possible excess nutrients which may have been present in CAF had no effect on modifying sex ratios.

Experiments were performed in which the density of gametophytes was varied on plain medium for both strains. These experiments showed that the two strains varied with regard to their response to antheridiogen produced within cultures in a manner similar to the way they varied in response to added antheridiogen. The response of strain H1-n being greater than that of strain 176D-4-n for all but the lowest densities. Isolated gametophytes of both strains always became cordates.

Measurements of area and counts of antheridia per gametophyte were determined for cordate gametophytes of each strain over a range of CAF concentrations. While these data were inconclusive, a

possible trend toward decreasing area with increasing CAF concentrations was observed for both strains.

Spore germination rates on both plain and CAF supplemented medium for both strains were measured. The rate of spore germination was not affected by the presence of CAF for either strain and this rate was not significantly different for either strain. This indicated that germination rates were not directly correlated with sex ratio differences.

Spores from reciprocal F1 hybrids of the two strains produced gametophyte cultures which exhibited responses to antheridiogen which were intermediate between those of the parental strains. This indicated that nuclear and not cytoplasmic inheritance was probably responsible for the genetic control of antheridiogen response. However, analyses of the responses of cultures from the spores of the F1 hybrids alone was not sufficient to further characterize the inheritance of antheridiogen response.

Reciprocal F2 generations were produced and analyzed for segregation from the F1 hybrids with regard to antheridiogen response on CAF supplemented medium. Segregation patterns among the F2's indicate two possible modes of inheritance; one in which response is mediated by two linked genes and another in which two linked genes and a single unlinked gene are responsible. Because the possibility of slightly overlapping response classes exists, neither model can be ruled out. However, several possible courses of action which may clarify this situation were discussed.

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APPENDIX

Table 3. Composition of Basal Nutrient Medium[c] (Modified from Klekowski 1969b).

Code	Stock Solutions	g/l Distilled Water
A	NH_4NO_3	25
B	KH_2PO_4	20
C	$MgSO_4 \cdot 7H_2O$	10
D	$CaCl_2 \cdot 2H_2O$	13
E	Parker's Micronutrients	- a
F	Iron Solution	- b

[a]Parker's micronutrients solution is composed of 0.025 g of $MnSO_4 \cdot H_2O$, 0.037 g of $CuSO_4 \cdot 5H_2O$, 0.052 g of $ZnSO_4 \cdot 7H_2O$, 0.186 g of H_3BO_3 and 0.0037 g of $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ in 1 l of distilled water.

[b]Iron solution is composed of 2.5 g of $FeSO_4 \cdot 7H_2O$ and 3.7 g sodium EDTA in 1 l of distilled water.

[c]Basal medium is prepared by adding 5 ml of stock A, 25 ml of stock B, 12 ml of stock C, 2 ml of stock D, 10 ml of stock E, 10 ml of stock F and 10 g of agar to 1 l of distilled water, adjusting the pH to 5.4 and then autoclaving.

Table 4. Dose Response of Strains H1-n and 176D-4-n to CAF from Aliquot Series 13/Ha.

	%CAF (v/v)															
	0		2		4		6		8		10		12		14	
Strain	M:C:N[a]	%M[b]	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M
H1-n	90:46:0	66	101:28:1	78	139:19:0	88	103:6:1	94	133:4:1	96	131:2:0	98	100:1:1	98	113:3:0	97
	122:52:2	69	102:40:1	71	180:15:1	92	158:7:0	96	196:4:0	98	121:6:0	95	160:2:0	99	145:4:1	97
	77:35:0	69	91:28:0	76	106:12:0	90	71:10:0	88	130:5:1	96	165:2:1	98	144:2:0	99	143:2:1	98
	82:57:1	59	116:21:0	85	109:9:0	92	96:2:0	98	131:7:0	95	93:2:2	96	96:1:1	98	147:4:2	96
$\bar{X}$%M±s[c]		66±5		78±6		90±2		94±4		96±1		97±2		98±1		97±1
176D-4-n	22:134:1	14	28:110:0	20	35:110:0	24	32:110:2	22	25:138:2	15	31:96:0	24	71:121:1	37	54:113:1	32
	16:151:3	9	19:106:2	15	36:131:0	22	29:100:1	22	36:86:2	30	34:104:2	24	29:84:2	25	46:92:1	33
	13:111:0	11	27:125:1	18	30:138:1	18	22:92:1	19	22:137:0	14	38:109:0	26	51:90:0	36	34:83:3	28
	23:151:0	13	28:97:1	22	46:88:0	34	37:120:1	23	41:111:1	27	30:99:2	23	42:87:1	32	20:101:0	17
$\bar{X}$%M±s		12±2		19±3		24±7		22±2		22±8		24±1		32±5		28±7

[a] M:C:N indicates number of male, to number of cordate, to number of neuter gametophytes.

[b] %M indicates percentage of male gametophytes.

[c] $\bar{X}$%M±s indicates mean percentage of male gametophytes ± standard deviation of the sample.

Table 5. Dose Response of Strains H1-n and 176D-4-n to CAF from Aliquot Series 13/Hb.

	%CAF (v/v)															
	0		2		4		6		8		10		12		14	
Strain	M:C:N[a]	%M[b]	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M
H1-n	132:66:2	66	152:43:5	76	173:24:3	86	180:20:0	90	193:7:0	96	191:6:3	96	198:2:0	99	198:1:1	99
	121:79:0	60	148:51:1	74	173:25:2	86	183:16:1	92	190:9:1	95	192:4:4	96	194:4:2	97	198:1:1	99
	119:79:0	60	146:55:1	73	171:26:3	86	188:11:1	94	188:11:1	94	195:4:1	98	196:3:1	98	199:1:0	100
	120:80:0	60	135:65:0	68	158:40:2	79	182:17:1	91	191:8;1	96	193:4:3	96	197:2:1	98	197:1:2	98
$\bar{X}$%M±s[c]		62±3		73±3		84±4		92±2		95±1		96±1		98±1		99±1
176D-4-n	29:168:3	14	29:171:0	14	45:154:1	22	26:171:3	13	51:147:2	26	44:156:0	22	45:155:0	22	70:129:1	35
	24:176:0	12	40:158:2	20	41:158:1	20	28:171:1	14	48:152:0	24	48:150:2	24	68:129:3	34	76:123:1	38
	25:174:1	12	25:174:1	12	39:161:0	20	54:145:1	27	43:156:1	22	51:145:4	26	37:161:2	18	70:127:3	35
	34:165:1	17	23:173:4	11	26:174:0	13	51:148:1	26	52:146:2	26	56:143:1	28	49:148:3	24	74:124:2	37
$\bar{X}$%M±s		14±2		14±4		19±4		20±8		24±2		25±3		24±7		36±2

[a] M:C:N indicates number of male, to number of cordate, to number of neuter gametophytes.

[b] %M indicates percentage of male gametophytes.

[c] $\bar{X}$%M±s indicates mean percentage of male gametophytes ± standard deviation of the sample.

Table 6. Dose Response of Strains H1-n and 176D-4-n to CAF from Aliquot Series 13/Hc.

	%CAF (v/v)															
	0		2		4		6		8		10		12		14	
Strain	M:C:N[a]	%M[b]	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M
H1-n	109:91:0	54	151:47:2	76	177:21:2	88	175:24:1	88	191:8:1	96	122:4:0	97	101:1:0	99	135:3:0	98
	123:77:0	62	158:42:0	69	162:38:0	81	188:12:0	92	194:6:0	97	190:9:1	95	193:7:0	96	197:3:0	98
	120:78:2	60	167:31:2	84	172:26:2	86	184:15:1	94	190:10:0	95	184:14:2	92	196:4:0	98	195:4:1	98
	138:60:2	69	-	-	172:28:0	86	187:13:0	92	190:10:0	95	197:2:1	98	195:5:0	98	197:2:1	98
$\bar{X}$%M±s[c]		61±6		80±4		85±3		92±3		96±1		96±3		98±1		98±0
176D-4-n	29:168:3	14	19:147:2	11	11:164:2	6	16:181:3	8	16:183:1	8	17:155:0	10	16:183:1	8	19:181:0	10
	17:182:1	8	14:184:2	7	11:149:0	7	15:183:2	8	17:182:1	8	5:193:2	2	19:181:0	10	23:176:1	12
	26:172:2	13	10:188:2	5	6:193:1	3	28:170:2	14	24:172:4	12	23:165:0	12	14:185:1	7	12:187:1	6
	3:150:0	2	11:187:2	6	18:180:2	9	22:178:0	11	12:185:3	6	12:188:0	6	15:184:1	8	11:189:0	6
$\bar{X}$%M±s[c]		9±6		7±3		6±2		10±3		8±3		8±4		8±1		8±3

[a] M:C:N indicates number of male, to number of cordate, to number of neuter gametophytes.

[b] %M indicates percentage of male gametophytes.

[c] $\bar{X}$%M±s indicates mean percentage of male gametophytes ± standard deviation of the sample.

Table 7. Dose Response of Strains H1-n and 176D-4-n to CAF from Aliquot Series 16/Ha.

	%CAF (v/v)															
	0		2		4		6		8		10		12		14	
Strain	M:C:N[a]	%M[b]	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M
H1-n	110:84:6	55	158:42:0	79	147:49:4	74	141:24:0	85	177:23:0	88	167:14:0	92	150:8:1	94	140:2:0	99
	104:59:0	64	133:43:0	76	141:57:2	70	152:18:3	88	181:19:0	90	189:11:0	94	169:8:1	95	107:6:2	93
	124:75:1	62	123:45:1	73	158:38:4	79	165:33:2	82	181:18:1	90	187:13:0	94	192:7:1	96	192:7:1	96
	79:69:0	53	132:65:3	66	107:28:0	79	171:28:1	86	178:20:2	89	132:8:0	94	181:17:2	90	153:5:1	96
$\bar{X}$%M±s[c]		58±5		74±6		76±4		85±2		89±1		94±1		94±3		96±2
176D-4-n	12:88:0	12	14:129:0	10	7:93:0	7	6:114:0	5	16:151:0	10	19:181:0	10	17:124:0	12	25:118:0	17
	23:177:0	12	28:171:1	14	9:141:1	6	16:151:0	10	14:172:1	7	23:155:1	13	32:131:3	19	37:98:0	27
	14:85:1	7	14:133:1	9	10:103:2	9	9:101:0	8	24:142:1	14	16:155:0	9	32:152:0	17	23:132:0	15
	10:141:1	7	14:129:0	10	10:125:1	7	22:151:1	13	-	-	13:103:0	11	17:112:2	13	23:108:2	17
$\bar{X}$%M±s[c]		10±3		11±2		7±1		9±3		10±4		11±2		15±3		19±5

[a] M:C:N indicates number of male, to number of cordate, to number of neuter gametophytes.

[b] %M indicates percentage of male gametophytes.

[c] $\bar{X}$%M±s indicates mean percentage of male gametophytes ± standard deviation of the sample.

Table 8. Dose Response of Strains H1-n and 176D-4-n to High Concentrations of CAF.

	% CFA (v/v)[d]																	
	14		25		50		50[e]		50[f]		75		93.6		93.6[e]		93.6[f]	
Strain	M:C:N[a]	%M[b]	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M	M:C:N	%M
H1-n	195:4:1	98	196:2:2	98	144:1:1	99	198:1:1	99	197:1:2	98	198:1:1	99	200:0:0	100	193:3:1	98	197:2:1	98
	194:5:1	97	197:1:2	98	183:1:0	99	197:1:2	98	194:4:2	97	198:0:2	99	197:1:2	98	195:4:1	98	163:2:1	98
	193:6:1	96	199:1:0	100	197:2:1	98	196:2:2	98	144:1:2	98	196:2:2	98	199:1:0	100	182:1:3	98	197:2:1	98
	197:2:1	98	153:3:1	97	194:4:2	97	195:2:3	98	195:4:1	98	197:2:1	98	170:2:1	98	197:3:0	98	171:1:0	99
$\bar{X}$%M±s[c]		97±1		98±1		98±1		98±0		98±0		98±1		99±1		98±0		98±0
176D-4-n	34:160:6	17	37:154:7	19	54:139:7	27	35:144:3	19	33:164:3	16	41:158:1	20	56:139:5	28	40:124:0	24	49:149:2	24
	32:165:3	16	37:148:4	20	58:109:2	34	48:128:2	27	49:146:5	24	51:147:2	26	44:131:2	25	59:140:1	30	62:111:3	35
	33:162:5	16	37:160:3	18	51:148:1	26	45:152:3	22	59:141:0	30	58:142:0	29	46:126:3	26	47:149:4	24	70:126:4	35
	37:114:0	18	36:162:2	18	48:148:4	24	42:156:2	21	32:164:4	16	46:125:1	27	51:145:4	26	49:149:2	24	42:129:0	25
$\bar{X}$%M±s		17±1		19±1		28±4		22±3		22±7		26±4		26±1		26±3		30±6

[a] M:C:N indicates number of male, cordate and neuter gametophytes.

[b] %M indicates percentage of male gametophytes.

[c] $\bar{X}$%M±s indicates mean percentage of male gametophytes ± standard deviation of the sample.

[d] Unless otherwise noted, all percentages of CAF contain nutrient solutions of standard strength.

[e] Contains one-half strength nutrient solution.

[f] Contains no nutrient solution.

Table 9. The Effects of Culture Density on Sex Expression for Strain H1-n.

Density[a]	M:C:N[b]	%M[c]	Density	M:C:N	%M	Density	M:C:N	%M
2	1:1:0	50	8	1:7:0	12	77	35:42:0	45
3	0:3:0	0	9	4:5:0	44	82	37:45:0	45
	0:3:0	0		0:9:0	0	99	60:38:1	61
	0:3:0	0		3:6:0	33	107	56:50:1	52
4	1:3:0	25	10	4:6:0	40	126	67:59:0	53
	1:3:0	25		3:7:0	30	137	91:46:0	66
	1:3:0	25	11	5:6:0	45	140	78:60:2	56
	1:3:0	25		4:7:0	36	141	95:46:0	67
	0:4:0	0	12	4:8:0	33	144	75:69:0	52
	0:4:0	0	13	6:7:0	46	150	74:76:0	49
	0:4:0	0		3:8:2	23	151	85:63:3	56
	0:4:0	0		1:12:0	8	177	110:65:2	62
5	0:5:0	0	14	5:9:0	36	187	90:97:0	48
	2:3:0	40		6:8:0	43	197	108:88:1	55
	0:5:0	0	16	8:7:1	50	199	109:88:2	55
	0:5:0	0		5:11:0	31	205	119:84:2	58
	1:4:0	20		6:9:1	38	215	128:86:1	60
	0:5:0	0	18	5:12:1	28	238	152:84:2	64
	0:5:0	0		5:12:1	28	240	152:88:0	63
6	1:5:0	17	20	6:13:1	30	247	141:101:5	57
	0:6:0	0	21	8:13:0	38	251	150:99:2	58
	2:4:0	33	28	14:13:1	50	258	160:97:1	62
	1:5:0	17	31	18:13:0	58		163:94:1	63
	1:5:0	17	42	15:26:1	36	262	151:108:3	58
	2:4:0	33	52	19:31:2	37	267	152:112:3	57
7	0:7:0	0	53	23:29:1	43	284	177:106:1	62
	0:7:0	0	55	22:32:1	40	290	170:118:2	59
	2:5:0	29	68	33:35:0	49	294	180:112:2	61
	3:4:0	43	70	30:38:2	43	295	189:106:0	64
8	1:7:0	12		39:31:0	56			

[a]Density indicates the number of gametophytes per culture in a 60 mm x 20 mm petri dish with plain medium.

[b]M:C:N indicates numbers of male, cordate and neuter gametophytes.

[c]%M indicates the percentage of male gametophytes.

Table 10. The Effects of Culture Density on Sex Expression for Strain 176D-4-n.

Density[a]	M:C:N[b]	%M[c]	Density	M:C:N	%M	Density	M:C:N	%M
2	0:2:0	0	12	0:10:2	0	59	5:52:2	8
	0:2:0	0		1:10:1	8	60	5:55:0	8
4	0:4:0	0		2:9:1	17	63	5:56:2	7
	0:4:0	0		1:10:1	8	64	4:59:1	6
5	1:4:0	25	13	2:11:0	15	72	6:66:0	8
	0:5:0	0		1:12:0	8	76	6:70:0	8
	0:5:0	0	14	1:13:0	7	99	12:84:3	12
	0:5:0	0		1:12:1	7	106	11:93:2	10
	0:4:1	0		0:13:1	0	121	9:112:0	7
6	1:5:0	17	16	2:13:1	12	125	108:16:1	6
7	1:6:0	14		1:15:0	6	138	6:131:1	4
8	1:7:0	12		2:14:0	12	140	5:135:0	4
	0:8:0	0	17	0:17:0	0		7:133:0	5
9	0:8:1	0		0:16:1	0	166	19:146:1	11
	0:8:1	0	18	2:15:1	0	172	12:159:1	7
	0:9:0	0		1:17:0	11	177	17:159:1	10
	0:9:0	0	19	2:16:1	6	181	13:168:0	7
10	0:10:0	0	23	4:19:0	11	183	11:172:0	6
	0:10:0	0	24	1:23:0	17	193	16:176:1	8
	1:9:0	10		1:23:0	4	223	22:201:0	10
	1:9:0	10		1:23:0	4	233	21:211:1	9
11	0:11:0	0		3:21:0	12	244	20:224:0	8
	0:11:0	0	29	3:24:2	10	246	26:219:1	11
	0:11:0	0	31	2:29:0	6		19:227:0	8
	1:10:0	9	32	0:31:1	0	254	25:229:0	10
	2:9:0	18	43	3:35:5	7	258	13:243:2	5
	0:10:1	0	46	1:45:0	2	268	16:250:2	6
	1:9:1	9	52	2:48:2	4	286	30:256:0	10
			56	8:45:3	14	318	35:281:2	11
			57	1:56:0	2	320	26:292:2	8

[a]Density indicates the number of gametophytes per culture in a 60 mm x 20 mm petri dish with plain medium.

[b]M:C:N indicates numbers of male, cordate and neuter gametophytes.

[c]%M indicates the percentage of male gametophytes.

Table 11. Effect of CAF on Gametophyte Area and Number of Antheridia for Strain H1-n.

% CAF (v/v)															
0		2		4		6		8		10		12		14	
A[a]	No.[b]	A	No.	A	No.	A	No.	A	No.	A	No.	A	No.	A	No.
.471	1	.329	3	.416	1	.393	0	.483	3	.460	4	.236	3	.433	0
.445	2	.345	2	.465	3	.419	0	.419	4	.177	19	.243	2	.331	1
.620	3	.416	4	.525	3	.350	5	.440	0	.351	5	.393	1	.421	3
.591	2	.514	2	.468	1	.279	9	.416	0	.444	2	.282	3	.572	1
.543	4	.368	2	.479	2	.373	3	.401	2	.336	0	.170	12	.387	1
.529	2	.385	0	.412	1	.325	2	.366	1	.372	3	.261	1	.283	4
.309	1	.298	1	.388	2	.549	1	.194	3	.448	4	.467	0	.299	1
.688	6	.146	1	.307	3	.425	3	.478	2	.327	1	.334	0	.373	2
.470	2	.301	2	.390	2	.422	0	.394	2	.354	2	.193	2	.317	1
.490	3	.371	1	.430	0	.572	0	.528	1	.425	2	.299	1	.452	1
.455	1	.305	2	.427	0	.503	0	.515	4	.385	2	.349	2	.392	1
.338	0	.282	0	.409	2	.344	1	.464	1	.463	1	.239	4	.448	1
.348	1	.345	1	.362	1	.305	1	.489	1	.345	1	.293	7	.094	8
.466	1	.352	0	.359	3	.517	1	.478	1	.109	1	.129	0	.091	2
.472	1	.365	0	.382	2	.478	2	.481	0	.241	1	.254	2	.094	0
.453	4	.361	0	.483	3	.428	1	.333	2	.350	0	.243	2	.111	0
.525	4	.444	2	.346	1	.432	1	.384	1	.169	1	.115	3	.443	2
.361	0	.315	1	.424	2	.389	2	.387	1	.278	0	.200	3	.367	4
.454	1	.373	2	.394	0	.473	5	.299	1	.194	0	.158	1	.303	0
.475	1	.433	1	.437	0	.429	1	.358	2	.261	2	.174	0	.255	1
$\bar{X}$[c]: .475	2	.352	1.35	.415	1.6	.420	1.9	.415	1.6	.324	2.55	.252	2.45	.323	1.7
s[d]: .094	1.556	.074	1.089	.052	1.095	.079	2.245	.081	1.888	.105	4.123	.089	2.800	.137	1.895

[a] A indicates area in mm^2.

[b] Number of antheridia per gametophyte.

[c] $\bar{X}$ indicates mean of all values in the column.

[d] s indicates standard deviation of the sample for the above mean.

Table 12. Effect of CAF on Gametophyte Area and Number of Antheridia for Strain 176D-4-n.

% CAF (v/v)															
0		2		4		6		8		10		12		14	
A[a]	No.[b]	A	No.	A	No.	A	No.	A	No.	A	No.	A	No.	A	No.
.405	8	.328	4	.468	3	.554	1	.498	4	.393	4	.128	12	.275	10
.386	6	.634	1	.705	1	.254	27	.178	11	.507	2	.218	8	.235	24
.540	0	.260	8	.415	4	.268	20	.525	4	.587	4	.184	10	.372	6
.585	3	.475	7	.335	4	.599	3	.224	23	.409	4	.284	4	.421	3
.478	6	.556	2	.616	4	.415	13	.432	1	.371	2	.419	2	.316	7
.827	2	.550	4	.649	3	.541	2	.119	21	.476	1	.485	1	.359	9
.510	3	.317	12	.474	4	.229	13	.523	2	.561	2	.257	13	.227	5
.568	2	.387	12	.550	1	.473	7	.227	19	.256	17	.148	17	.254	13
.369	8	.590	0	.537	3	.653	0	.623	1	.391	11	.326	2	.373	14
.215	7	.337	12	.412	3	.403	8	.328	20	.426	2	.273	4	.337	4
.616	2	.647	2	.557	1	.480	1	.535	8	.376	0	.289	8	.466	0
.406	1	.480	3	.524	2	.331	9	.509	3	.511	0	.150	27	.405	3
.264	11	.338	2	.430	7	.521	2	.444	2	.442	3	.404	6	.436	2
.765	3	.195	11	.471	3	.458	2	.537	4	.275	11	.285	7	.435	7
.220	13	.377	17	.346	6	.384	3	.282	15	.291	2	.292	1	.094	3
.529	2	.453	4	.530	2	.359	12	.364	10	.256	7	.284	9	.441	6
.291	1	.541	6	.324	4	.298	11	.593	4	.277	9	.255	7	.561	2
.316	6	.590	0	.467	3	.315	6	.302	12	.273	2	.179	16	.240	30
.464	5	.479	1	.483	5	.579	0	.347	5	.380	10	.283	2	.462	7
.258	3	.465	1	.380	17	.205	14	.251	13	.263	23	.276	16	.301	7
$\bar{X}$[c]: .451	4.6	.450	5.45	.484	4.0	.416	7.7	.392	9.1	.386	5.8	.271	8.6	.350	8.1
s[d]: .171	3.485	.128	4.968	.103	3.434	.133	7.270	.149	7.276	.105	6.066	.092	6.700	.109	7.447

[a] A indicates area in mm^2.

[b] Number of antheridia per gametophyte.

[c] $\bar{X}$ indicates mean of all values in the column.

[d] s indicates standard deviation of the sample for the above mean.

Table 13. Percentages of Sexual Types for Each AF2-1 Individual and Parental Strains.

Code	%M1[a]	%M2[a]	%M3[a]	Mean %M[b]	sM[c]	%C1[d]	%C2[d]	%C3[d]	Mean %C[e]	sC[f]	%N1[g]	%N2[g]	%N3[g]	Mean %N[h]	sN[i]
H1-n	77	76	79	77	2	24	24	23	23	1	0	0	0	0	0
176D-4-n	17	18	12	15	3	83	82	88	84	3	1	0	1	0	0
176H8-2	12	15	17	15	3	88	85	83	85	3	1	0	1	0	0
176H8-5	15	23	13	17	5	84	77	87	82	5	2	1	1	1	1
176H8-6	57	56	63	58	4	43	44	37	41	4	1	1	1	1	0
176H8-9	84	91	90	88	4	16	10	10	12	4	0	0	1	0	0
176H8-10	19	26	29	28	2	68	73	70	70	2	3	2	1	2	1
176H8-12	22	20	31	28	7	66	80	67	71	8	2	0	3	2	2
176H8-14	14	15	10	13	3	82	81	87	83	3	4	4	3	4	1
176H8-15	56	65	59	60	5	44	35	42	40	5	0	1	0	0	0
176H8-16	79	71	68	72	5	22	30	32	28	5	0	0	0	0	0
176H8-17	41	38	43	41	3	58	63	56	59	3	1	0	1	0	0
176H8-20	27	12	29	31	6	72	62	71	68	6	1	1	1	1	0
176H8-21	12	19	7	10	3	89	88	93	90	3	0	0	0	0	0
176H8-23	13	46	11	14	4	87	82	88	85	4	1	0	1	0	0
176H8-24	38	68	56	47	9	63	54	44	53	9	0	0	0	0	0
176H8-25	75	6	55	66	10	25	32	44	33	10	0	1	1	1	1
176H8-27	5	14	4	5	1	94	95	97	95	2	2	0	0	1	1
176H8-28	14	50	12	13	1	86	86	89	87	2	0	1	0	0	0
176H8-30	52	44	56	53	3	48	51	44	48	3	0	0	0	0	0
176H8-31	51	6	49	48	4	49	56	51	52	3	0	1	1	1	1
176H8-32	9	34	6	7	2	91	94	94	93	2	0	0	0	0	0
176H8-34	28	7	41	34	7	72	66	59	66	7	0	1	0	0	0
176H8-35	11	7	6	8	2	90	93	94	92	2	0	0	0	0	0
176H8-36	3	1	7	6	2	96	91	90	92	3	1	3	3	2	1
176H8-37	3	51	2	2	1	97	99	98	98	1	0	0	0	0	0
176H8-38	61	2	48	53	7	39	49	52	46	7	0	1	1	1	1
176H8-39	2	75	2	2	0	99	99	99	99	0	0	0	0	0	0
176H8-40	65		73	71	5	36	25	28	29	5	0	0	0	0	0

[a] % M1, % M2 and % M3 indicate the percentage of male gametophytes in three samples.

[b] Mean % M indicates the mean percentage of male gametophytes (mean percentages may reflect rounding errors of ±1% which resulted when values were calculated by the Lotus 1-2-3 software program).

[c] sM indicates the standard deviation of the sample for the mean percentage of male gametophytes.

[d] % C1, % C2 and % C3 indicate the percentages of cordate gametophytes for three samples.

[e] Mean % C indicates the mean percentage of cordate gametophytes (mean percentages may reflect rounding errors of ± 1% which resulted when values were calculated by the Lotus 1-2-3 software program).

[f] sC indicates the standard deviation of the sample for the mean percentage of cordate gametophytes.

[g] % N1, % N2 and % N3 indicate the percentages of neuter gametophytes for three samples.

[h] Mean % N indicates the mean percentage of neuter gametophytes (mean percentages may reflect a rounding error of ± 1% which resulted when the values were calculated by the Lotus 1-2-3 software program).

[i] sN indicates the standard deviation of the sample for the mean percentage of neuter gametophytes.

Table 14. Percentages of Sexual Types for AF2-2 Individuals and Parental Strains on Medium Supplemented with 20/Hb CAF.

Code	% Germ[a]	%M1[b]	%M2[b]	%M3[b]	Mean %M[c]	sM[d]	%C1[e]	%C2[e]	%C3[e]	Mean %C[f]	sC[g]	%N1[h]	%N2[h]	%N3[h]	Mean %N[i]	sN[j]
H1-n	89	92	92	88	90	2	6	8	11	8	3	2	1	0	1	1
176D-4-n	92	17	9	8	11	5	91	91	93	91	1	1	2	1	1	1
H1761-12	89	45	50	47	47	3	51	47	47	48	2	5	4	7	5	2
H1761-37	31	36	22	23	27	8	45	41	48	45	3	19	37	29	28	9
H1761-39	84	94	94	93	94	1	0	0	0	0	0	6	6	7	6	1
H1761-41	99	89	81	88	86	4	12	19	13	14	4	0	0	0	0	0
H1761-48	92	4	8	3	5	2	96	93	97	95	2	0	0	0	0	0
H1761-51	45	0	0	3	1	2	48	79	83	70	19	52	21	14	29	20
H1761-55	98	1	1	0	0	0	99	100	100	100	0	0	0	0	0	0
H1761-66	78	88	93	90	90	3	7	4	6	6	1	5	3	5	4	1
H1761-105	87	9	9	6	8	1	85	89	91	89	3	6	2	3	4	2
H1761-137	52	93	97	96	96	2	1	1	1	1	0	6	1	2	3	2
H1761-138	93	98	98	98	98	0	2	1	2	2	1	0	1	0	0	0
H1761-142	91	95	95	96	95	1	5	6	5	5	1	1	0	0	0	0
H1761-146	62	95	94	88	92	4	3	4	4	4	1	3	2	8	4	3
H1761-152	93	12	10	8	10	2	88	91	92	90	2	0	0	0	0	0
H1761-158	94	100	99	100	100	1	0	0	0	0	0	0	2	0	1	1
H1761-165	97	95	89	91	92	3	5	11	9	8	3	0	1	0	0	0

[a]% Germ indicates the percentage of germinated spores on the day the culture was sampled.

[b]% M1, % M2 and % M3 indicate the percentage of male gametophytes in three samples.

[c]Mean % M indicates the mean percentage of male gametophytes (mean percentages may reflect rounding errors of ±1% which resulted when values were calculated by the Lotus 1-2-3 software program).

[d]sM indicates the standard deviation of the sample for the mean percentage of male gametophytes.

[e]% C1, % C2 and % C3 indicates the percentages of cordate gametophytes for three samples.

[f]Mean % C indicates the mean percentage of cordate gametophytes (mean percentages may reflect rounding errors of ±1% which resulted when values were calculated by the Lotus 1-2-3 software program).

[g]sC indicates the standard deviation of the sample for the mean percentage of cordate gametophytes.

[h]% N1, % N2 and % N3 indicate the percentages of neuter gametophytes for three samples.

[i]Mean % N indicates the mean percentage of neuter gametophytes (mean percentages may reflect a rounding error of ±1% which resulted when the values were calculated by the Lotus 1-2-3 software program).

[j]sN indicates the standard deviation of the sample for the mean percentage of neuter gametophytes.

Table 15. Percentages of Sexual Types for AF2-2 Individuals and Parental Strains on Medium Supplemented with 20/Hc CAF.

Code	% Germ[a]	%M1[b]	%M2[b]	%M3[b]	Mean %M[c]	sM[d]	%C1[e]	%C2[e]	%C3[e]	Mean %C[f]	sC[g]	%N1[h]	%N2[h]	%N3[h]	Mean %N[f]	sN[j]
H1-n	82	88	89	88	88	1	12	11	11	11	0	1	0	1	1	1
176D-4-n	88	10	6	8	8	2	90	94	92	92	2	0	0	0	0	0
H1761-16	81	87	94	93	91	4	4	5	3	4	1	8	2	4	5	3
H1761-21	90	9	4	4	6	3	90	91	96	92	3	1	5	0	2	3
H1761-23	97	4	6	8	6	2	96	92	92	94	2	0	2	0	1	1
H1761-35	96	16	20	17	17	3	84	79	84	82	3	1	1	0	1	1
H1761-42	95	93	92	92	92	1	6	9	8	7	1	1	0	0	0	1
H1761-50	93	82	84	86	84	2	16	10	13	13	3	2	6	1	3	3
H1761-52	93	17	19	19	18	1	76	78	75	76	2	6	3	7	6	2
H1761-56	84	3	3	3	3	0	93	95	95	94	1	4	3	2	3	1
H1761-58	85	53	33	40	42	10	43	60	58	54	9	3	7	2	4	3
H1761-67	91	4	2	3	3	1	90	89	92	90	1	6	8	6	7	1
H1761-78	87	97	97	96	97	1	3	3	4	3	1	0	0	0	0	0
H1761-100	69	83	82	80	81	2	12	14	14	13	1	5	5	6	5	1
H1761-111	92	6	8	4	6	2	90	82	87	86	4	4	11	9	8	3
H1761-116	83	9	9	10	9	1	82	85	89	85	3	9	6	2	6	4
H1761-118	95	8	8	9	8	0	91	92	92	92	0	1	0	0	0	1
H1761-119	99	4	1	2	2	2	96	99	98	98	2	0	0	0	0	0
H1761-120	94	15	12	11	13	2	84	87	89	86	2	1	1	1	1	0
H1761-126	34	30	20	20	23	5	61	57	66	61	4	10	23	14	16	7
H1761-136	79	97	94	94	95	2	1	1	1	1	0	1	5	5	4	2
H1761-143	36	60	58	57	58	1	2	5	2	3	2	39	37	41	39	2
H1761-147	87	7	10	9	9	2	92	90	91	91	1	1	0	0	0	0
H1761-151	35	29	56	50	45	14	25	28	11	21	9	46	17	39	34	15
H1761-163	50	1	0	3	1	1	99	100	97	99	1	0	0	0	0	0
H1761-164	14	63	67	100	76	21	0	4	0	1	2	38	29	0	22	20
H1761-166	85	47	37	41	42	5	45	54	49	49	5	9	8	11	9	1
H1761-171	87	82	89	95	88	6	14	11	5	10	4	5	1	0	2	2
H1761-173	91	89	90	92	90	2	7	5	2	5	3	5	4	7	5	1
H1761-173	16	0	0	0	0	0	50	57	59	55	5	50	43	41	45	5
H1761-198	97	100	99	100	100	0	0	0	0	0	0	1	1	0	1	1

[a] % Germ indicates the percentage of germinated spores on the day the culture was sampled.

[b] % M1, % M2 and % M3 indicate the percentage of male gametophytes in three samples.

[c] Mean % M indicates the mean percentage of male gametophytes (mean percentages may reflect rounding errors of ±1% which resulted when values were calculated by the Lotus 1-2-3 software program).

[d] sM indicates the standard deviation of the sample for the mean percentage of male gametophytes.

[e] % C1, % C2 and %C3 indicates the percentages of cordate gametophytes for three samples.

[f] Mean % C indicates the mean percentage of cordate gametophytes (mean percentages may reflect rounding errors of ±1% which resulted when values were calculated by the Lotus 1-2-3 software program).

[g] sC indicates the standard deviation of the sample for the mean percentage of cordate gametophytes.

[h] % N1, % N2 and % N3 indicate the percentages of neuter gametophytes for three samples.

[i] Mean % N indicates the mean percentage of neuter gametophytes (mean percentages may reflect a rounding error of ±1% which resulted when the values were calculated by the Lotus 1-2-3 software program).

[j] sN indicates the standard deviation of the sample for the mean percentage of neuter gametophytes.

Table 16. Percentages of Sexual Types for AF2-2 Individuals and Parental Strains on Medium Supplemented with 20/Hd CAF.

Code	% Germ[a]	%M1[b]	%M2[b]	%M3[b]	Mean %M[c]	sM[d]	%C1[e]	%C2[e]	%C3[e]	Mean %C[f]	sC[g]	%N1[h]	%N2[h]	%N3[h]	Mean %N[i]	sN[j]
H1-n	89	87	94	91	90	4	13	7	10	10	3	1	0	0	0	0
176D-4-n	85	7	9	10	9	2	92	91	90	91	1	2	0	0	1	1
H1761-5	93	98	95	96	96	1	2	5	4	4	1	0	0	0	0	0
H1761-14	53	41	48	32	40	8	16	14	15	15	1	43	38	53	45	8
H1761-18	98	96	92	93	94	2	3	8	5	5	2	1	0	2	1	1
H1761-19	89	99	96	98	98	2	1	4	2	2	2	-	0	0	0	0
H1761-20	94	100	99	99	99	1	0	0	0	0	0	1	2	2	1	1
H1761-22	76	85	92	84	87	4	9	5	10	8	3	6	4	6	5	2
H1761-28	98	5	6	7	6	1	95	94	93	94	1	-	1	0	0	0
H1761-31	96	4	3	1	3	2	95	96	96	96	0	1	1	3	2	1
H1761-38	66	93	93	90	92	2	3	2	4	3	1	4	5	6	5	1
H1761-46	96	95	97	96	96	1	5	3	4	4	1	0	0	0	0	0
H1761-54	97	98	99	98	98	1	3	2	2	2	1	0	0	1	0	0
H1761-62	63	1	1	0	1	1	93	93	94	93	1	5	6	6	6	0
H1761-63	86	95	93	93	94	1	0	0	0	0	0	5	7	7	6	1
H1761-74	90	15	19	21	18	3	83	81	78	81	2	2	0	1	1	1
H1761-81	97	97	92	97	95	3	4	8	4	5	3	0	0	0	0	0
H1761-117	91	98	98	98	98	0	0	0	0	0	0	3	2	3	2	0
H1761-136	93	100	99	99	99	0	1	2	1	1	1	0	0	0	0	0
H1761-144	60	79	86	84	83	4	16	13	16	15	2	5	1	0	2	3
H1761-150	88	92	83	88	88	4	7	13	9	10	3	2	4	3	3	1
H1761-153	93	1	2	2	1	1	99	97	98	98	1	0	1	0	0	0
H1761-161	94	97	98	99	98	1	1	2	1	1	0	2	1	0	1	1
H1761-168	69	68	67	68	67	0	26	18	19	21	4	7	15	16	12	5
H1761-170	55	10	9	6	8	2	87	66	88	80	12	3	3	6	4	2
H1761-177	97	1	1	1	1	0	97	98	98	98	1	2	1	1	2	1
H1761-179	53	17	9	20	15	6	20	37	30	29	9	63	54	50	56	7
H1761-180	82	79	78	86	81	4	11	18	14	14	3	10	4	0	5	5
H1761-196	42	33	40	31	35	5	65	40	59	55	13	2	19	10	10	9
H1761-201	89	93	92	93	93	1	1	4	1	2	2	6	4	6	5	1

[a] % Germ indicates the percentage of germinated spores on the day the culture was sampled.

[b] % M1, % M2 and % M3 indicate the percentage of male gametophytes in three samples.

[c] Mean % M indicates the mean percentage of male gametophytes (mean percentages may reflect rounding errors of ±1% which resulted when values were calculated by the Lotus 1-2-3 software program).

[d] sM indicates the standard deviation of the sample for the mean percentage of male gametophytes.

[e] % C1, % C2 and %C3 indicates the percentages of cordate gametophytes for three samples.

[f] Mean % C indicates the mean percentage of cordate gametophytes (mean percentages may reflect rounding errors of ±1% which resulted when values were calculated by the Lotus 1-2-3 software program).

[g] sC indicates the standard deviation of the sample for the mean percentage of cordate gametophytes.

[h] % N1, % N2 and % N3 indicate the percentages of neuter gametophytes for three samples.

[i] Mean % N indicates the mean percentage of neuter gametophytes (mean percentages may reflect a rounding error of ±1% which resulted when the values were calculated by the Lotus 1-2-3 software program).

[j] sN indicates the standard deviation of the sample for the mean percentage of neuter gametophytes.

VITA

Rodney J. Scott was born in Birmingham, Michigan, on march 24, 1961. In 1979, he graduated from Manatee High School in Bradenton, Florida. In 1981 he received an Associate in Arts degree from Manatee Junior College in Bradenton, Florida. In 1983 he graduated from the University of West Florida in Pensacola, Florida and received a Bachelor of Science degree in Biology. In the fall of 1983 he began his post-graduate studies at The University of Tennessee, Knoxville, in the Department of Botany.