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FARRAR, DONALD R., and ROBERT D. GOOCH (Department of Botany and Plant Pathology, Iowa State University, Ames, Iowa 50010). Fern Reproduction at Woodman Hollow, Central Iowa: Preliminary Observations and a Consideration of the Feasibility of Studying Fern Reproductive Biology in Nature. *Proc. Iowa Acad. Sci.* 82(2): 119-122, 1975.
Field observations of spore availability, gametophyte establishment and survival, and sporophyte production were made over a one-year period. Maximum spore release for most species occurred shortly after spore maturation in mid to late summer, but some spores remained on sporophyte fronds through the winter and were available for germination the following spring. Gametophytes of *Cystopteris fragilis, Woodsia obtusa* and *Adiantum pedatum* became established in late summer and fall. Production of sporophytes occurred both in fall and in the following spring. Both gametophytes and juvenile sporophytes survived the winter in relatively unchanged condition. Results indicate that it is feasible and important to correlate field studies with current laboratory studies of fern reproductive biology.
INDEX DESCRIPTORS: Ferns, Fern Gametophytes, Fern Reproduction, Woodman Hollow in Iowa.

The process by which sexual reproduction is accomplished in ferns has considerable bearing upon the interpretation of their past evolution as well as their present distribution and ecology. Our knowledge of sexual reproduction in these plants has increased greatly during the last two decades, but almost entirely from research conducted in the laboratory. Differing conclusions which can be drawn from different aspects of this research leave it unclear as to what methods of sexual reproduction occur in nature. It seems unlikely that these differences will be totally resolved without the aid of field observations. It is the purpose of this investigation to determine the feasibility of conducting field studies on sexual reproduction in the ferns.

The laboratory research of greatest relevance to fern evolution and ecology may be grouped into two categories, the hormonal control of antheridium formation and the genetic response to self-fertilization. Studies in the first area have been conducted primarily by Döpp (1950, 1959), Schraudolf (1966), Näf (1963, 1969), and Voeller (1969, 1971). They have demonstrated conclusively that, in several species of ferns, older or faster growing gametophytes of a population produce a soluble hormone which induces younger or more slowly growing gametophytes to produce antheridia. The end result of this system is the assurance of the simultaneous maturation of male and female gametangia and promotion of cross-fertilization within the population and thus a decrease in the probability of self-fertilization of hermaphroditic gametophytes. An additional effect of the same hormone is the promotion of dark germination of spores which may be buried too deeply in the substratum to germinate alone but which when stimulated by the hormone will produce gametophytes capable of producing additional antheridia.

This hormone system is apparently widespread in the ferns, so much so that it has been possible to set up a phylogeny of the ferns based upon the interaction of various species to the hormones of one another (Voeller, 1971). It is important to note that the effectiveness of this hormone system is dependent upon the close proximity of gametophytes growing in a relatively dense population. Such a system is unlikely to have evolved unless this is a common means of reproduction, i.e., *by relatively dense populations.*

On the other hand, studies by Klekowski (1971, 1973) and co-workers on the genetics of ferns have brought together persuasive evidence suggesting that ferns, throughout their evolutionary history, have reproduced sexually to some extent by self-fertilization of bisexual gametophytes. The principal evidence for this conclusion is the relatively high basal chromosome numbers of ferns, probably the result of ancient polyploidy (Klekowski and Baker, 1966). The selective pressure for this polyploidization is the protection it provides the organisms against the expression of lethals in a totally homozygous state which results from self-fertilization of bisexual gametophytes. If this interpretation is accepted, then one must conclude that the polyploid system in ferns would not have evolved if there were not an appreciable amount of reproduction in nature on the single gametophyte level, i.e., *by self-fertilization of single, bisexual gametophytes.*

These two lines of evidence thus suggest an appreciable degree of reproduction both by cross-fertilization between gametophytes in a population and by self-fertilization of isolated gametophytes. Additional studies in the laboratory may also predict which method predominates for which species. However, verification of these predictions by field observations is lacking, e.g., as stated by Holbrook-Walker and Lloyd (1973) in their study of the reproductive biology of *Sadleria:* "Therefore, further testing of the hypothesis put forward above rests with an ecological study of the gametophyte generation in nature."

Is it possible to study fern gametophytes in nature? The paucity of such studies has been largely ascribed to the difficulties of finding gametophytes in nature and of identification of species in the gametophytic stage. While these difficulties do exist, we feel that the observations presented here indicate that they need not preclude productive field studies if study sites and methods of study are selected carefully.

Growth conditions for gametophytes in the field are unlikely to be similar to those in laboratory studies. Thus it is important to consider as many aspects as possible of the life

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history of gametophytes in nature when making comparisons to laboratory data. One must consider such factors as the growth conditions, the age of reproducing gametophytes, and especially the effectiveness of reproduction. A full understanding of natural reproduction cannot be based solely upon collection of populations of gametophytes at one time of year and analysis of their sexual condition. It is necessary to follow gametophyte and sporophyte growth throughout the year and to determine which gametophytes or populations of gametophytes produce sporophytes that subsequently mature to participate in further reproduction.

## PROCEDURE

The study site we have chosen is the Woodman Hollow State Preserve, a relatively isolated canyon in central Iowa. The canyon, cut into glacial till and Pennsylvanian sandstone, is about one-half mile long and 50 to 150 feet deep, with a small intermittent stream. It supports a mature deciduous forest typical of the midwest (Nieman and Landers, 1974) and contains 13 species in 11 genera of ferns (Table 1). Identification of species in the gametophyte stage is most difficult between species of the same genus; this problem is nearly eliminated in Woodman Hollow because only two genera have more than one species present. In these genera (as well as in the remainder of the species) the species could be differentiated by spore morphology and eventually by the sporophytes produced.

TABLE 1. FERN SPORE PRODUCTION AND RELEASE AT WOODMAN HOLLOW STATE PRESERVE, CENTRAL IOWA

| Species | *First Release (approx. date)* | *Maximum Release (approx. date)* | *Type of Frond Maturation* |
|---|---|---|---|
| *Matteuccia struthiopteris* | Mar. 20 | April 17 | flush |
| *Cystopteris fragilis* | May 31 | June 10 | flush |
| *Dryopteris spinulosa* | June 10 | July 20 | flush |
| *Polypodium virginianum* | June 10 | July 20-? | sequential |
| *Dryopteris goldiana* | June 30 | July 20 | flush |
| *Botrychium virginianum* | June 30 | June 30 | flush |
| *Athyrium filix-femina* | June 30 | July 20 | flush |
| *Cystopteris bulbifera* | July 5 | July 20 | flush |
| *Osmunda claytoniana* | July 5 | July 5 | flush |
| *Cryptogramma stelleri* | July 10 | July 20 | flush |
| *Woodsia obtusa* | July 10 | July 20-? | sequential |
| *Adiantum pedatum* | Aug. 4 | Aug. 20-? | sequential |
| *Asplenium rhizophyllum* | Sept. 19 | Sept. 30-? | sequential |

To outline a procedure of study we posed several questions relative to sexual reproduction of the species in Woodman Hollow. When are spores available for germination? When does reproduction occur and how is it influenced by micro- and macroclimates? When and by what breeding systems are sporophytes produced? Does sexual reproduction occur in nature on a regular basis for all species? To gather data in answer to these questions we began bi-monthly observations in the study area which were continued throughout the year. In addition to recording presence and condition of sporophytes and gametophytes, we began a long-term study of the physical parameters of the habitats in which reproduction occurs. Correlation of reproduction with habitat parameters including temperature, light intensity, relative humidity, and soil characteristics will be reported in a later paper.

## OBSERVATIONS

### *Spore Availability*

Information in the literature on spore availability is limited to relatively few accounts of the time of spore maturation and release by various species (Hill and Wagner, 1974; Lloyd and Klekowski, 1970). Times indicated in such listings are precise only for the given area and year in which the observations are made, but they are valid as general indications of the time of first availability of spores from sporophytes of a given season.

Approximate dates of spore release for the species in Woodman Hollow for 1972 are given in Table 1. The period of maximum release is related to type of sporangium, the type of soral maturation, and type of maturation of fertile fronds. In *Botrychium virginianum* and *Osmunda claytoniana* the sporangia are very large and smooth-walled and mature nearly simultaneously. Thus, release of their spores occurs very rapidly after maturation and the dates of first and of maximum spore release are approximately the same. In the remaining species, differential maturation of the sporangia within the sorus, combined with the small size and density of the sporangia, results in a period of maximum release extending usually one to three weeks. Although the fertile fronds mature in a single flush in most of the species, the period of release in *Polypodium, Woodsia, Asplenium* and *Adiantum* is also extended by the sequential maturation of fertile fronds, which may continue until the time of first frost. *Matteuccia struthiopteris* is a special case because the fertile fronds mature in the fall but spores are presumably not released until the following spring. Although in 1972 first release of spores was observed in March, we have subsequently observed spore release from *Matteuccia* fronds as early as December.

Time of first release and period of maximum release do not give the total picture of spore availability. After release, the spores remain viable in most species for at least a year (Sussman, 1965) (green-spored genera such as *Osmunda* and *Matteuccia* may be exceptions [Lloyd and Klekowski, 1970; Hill and Wagner, 1974]); thus, spores which have landed in a given habitat are available at the time when environmental conditions required for germination are first attained. While most probably occurring soon after release, these conditions may first occur at any time of the year, notably in early spring following a dry fall.

Further complicating the picture of spore availability is the fact that not all spores are shed from the sporophyte fronds during the summer of their maturation. Preliminary examination of the species in Woodman Hollow revealed that in April viable spores remained on the fertile fronds of the previous year (Table 2). In several species 10% or more of the sporangia were unopened, and even in the species where all sporangia were open, spores remained in and on the sporangia and on the frond surface. These were easily dislodged by light tapping of the dried fronds. Figures listed in Table 2 for numbers of spores present are very conservative and can probably be multiplied tenfold in most cases. The old sporo-

TABLE 2. SPORE AVAILABILITY IN APRIL FROM FRONDS OF THE PREVIOUS YEAR

| Species | Spores/frond | Spore Viability Relative to 50% | Sporangial Condition—% Opened |
|---|---|---|---|
| *Athyrium filix-femina* | >1000 | + | 100 |
| *Asplenium rhizophyllum* | >1000 | – | 90-100 |
| *Polypodium virginianum* | >1000 | + | 75-90 |
| *Woodsia obtusa* | >1000 | + | 75-90 |
| *Cryptogramma stelleri* | >1000 | none | 50-75 |
| *Matteuccia struthiopteris* | >1000 | + | actively opening |
| *Cystopteris fragilis* | 100-1000 | + | 100 |
| *Dryopteris goldiana* | 100-1000 | + | 100 |
| *Cystopteris bulbifera* | <100 | + | 100 |
| *Adiantum pedatum* | <100 | – | 90-100 |
| *Dryopteris spinulosa* | no fertile fronds found | | |
| *Osmunda claytoniana* | no fertile fronds found | | |
| *Botrychium virginianum* | no fertile fronds found | | |

phyte fronds in the early spring are generally flattened against the substrate and spore release into the air currents would be greatly reduced. Nevertheless, some spores could certainly germinate in the immediate vicinity of the sporophyte frond if a suitable habitat were available.

*Gametophyte Establishment*

Established populations of gametophytes in Woodman Hollow were first observed in mid-September. They appeared in great numbers in the vicinity of sporophytes of *Cystopteris fragilis, Woodsia obtusa,* and *Adiantum pedatum,* covering exposed as well as protected areas of bare rock or soil. By late September, gametophytes of these species could be found in 23 of 25 one-square-meter plots which were within 10 meters of a fertile sporophyte, and plots within five meters contained 500 to 3000 individuals.

During the month of October, gametophyte numbers were steadily reduced, largely through erosion from exposed sites. By early November, populations of gametophytes were largely restricted to protected pockets of less than 100 $cm^2$ and contained usually 75 to 100 individuals per 100 $cm^2$. Most of the gametophytes were mature by November and many had produced the first leaf of the new sporophyte. At this time, one population of *Cystopteris* gametophytes was harvested to determine the feasibility of studying the sexual constitution of natural populations. The gametophytes were examined for presence and type of gametangia. Of the 75 individuals in the population, 67 were sexual, 46 bearing both antheridia and archegonia, and 21 bearing antheridia only. Thus, this population apparently had potential for both cross- and self-fertilization. Hormonal control of antheridial production is also suggested by the large number of male gametophytes, but these observations must be combined with laboratory studies before definite conclusions are drawn. Nevertheless, this observation indicates that correlations between field and laboratory investigations on breeding behavior can be made.

From November through February, temperatures in Woodman Hollow remained near or below freezing and little growth in the gametophytes of juvenile sporophytes could be detected. Many populations became covered with snow in December and remained so into February. In March, when the populations could again be examined, we found that although some gametophytes and sporophytes had been killed, in most populations, 75% or more of the number present at the end of November had survived the winter in essentially unchanged condition. By April, young sporophytes produced in the fall had resumed growth and new sporophytes appeared on other gametophytes which had survived the winter. Through April and May the young sporophytes in most populations grew vigorously and produced dense stands one to three centimeters tall. By June this growth had largely stopped, presumably due to one or more of the factors of high population density, inadequate substrate, and dense shading by an herbaceous canopy. Relatively few individuals in favorable sites continued growth which could be expected to reach maturity.

These observations were made during the fall of 1972, which was wetter than average for central Iowa. This was also the best year for gametophyte establishment in Woodman Hollow we have observed. Yet establishment of gametophytes was observed for only four of the thirteen species present. In addition to the three species mentioned, gametophyte establishment to a much smaller degree was observed for *Dryopteris goldiana.* Although this species produced spores in great abundance, and habitats of bare, moist rock and soil were available, only a few scattered gametophytes were found. Spores of all species were tested in the laboratory and found to be viable. The reasons for the failure of gametophyte establishment are obscure, especially for *Dryopteris goldiana* and *Athyrium filix-femina,* which are very common in the area. However, these are larger, perennial plants which grow in less disturbed areas than do *Adiantum, Woodsia* and *Cystopteris fragilis,* which are colonizers of the most rapidly eroding parts of the canyon. Factors to be considered in explaining the absence of gametophytes of the remaining species include the reduced period of viability of green-spored species, specific requirements for habitats not abundantly available, and occurrence of some species in habitats not as moist as those of *Adiantum, Woodsia* and *Cystopteris.* Establishment of the subterranean gametophytes of *Botrychium virginianum* is of course not readily observable; however, we found young sporophytes with attached gametophytes, indicating that reproduction of this species is occurring in Woodman Hollow.

Regardless of the reasons for lack of gametophyte establishment for certain species, we must conclude that some species reproduce via gametophytes much more readily than others. The species occurring in Woodman Hollow may be tentatively grouped into categories as indicated in Table 3. Weedy species which reproduce annually either sexually or asexually include the two species of *Cystopteris* and the species of *Adiantum* and *Woodsia.* These species may be perennial but are frequently short-lived due to the unstable character of their habitat. *Athyrium* and *Matteuccia* are also weedy species in central Iowa, but individuals are longer-lived, and in Woodman Hollow they apparently do not reproduce annually. The two species of *Dryopteris* and the species of *Osmunda, Polypodium* and *Asplenium* are found only in the most mature forests of central Iowa and cannot be considered weedy, although *Dryopteris goldiana* is very abundant in Woodman Hollow. These species apparently do not reproduce annually here and are also long-lived perennials.

Two species are not included in Table 4, *Botrychium virginianum* because of the difficulty of studying its reproduc-

TABLE 3. FERN REPRODUCTION AT WOODMAN HOLLOW STATE PRESERVE IN CENTRAL IOWA

I. Weedy, short-lived perennials reproducing annually by asexual reproduction
*Cystopteris fragilis*
*Woodsia obtusa*
*Adiantum pedatum*

II. Weedy, short-lived perennials reproducing annually by sexual reproduction
*Cystopteris bulbifera*

III. Weedy, long-lived perennials not reproducing annually
*Athyrium filix-femina*
*Matteuccia struthiopteris*

IV. Non-weedy, long-lived perennials not reproducing annually
*Dryopteris spinulosa*
*Dryopteris goldiana*
*Osmunda clatoniana*
*Polypodium virginianum*
*Asplenium rhizophyllum*

tion, and *Cryptogramma stelleri* because of its atypical occurrence here on the extreme southern boundary of its range. *Botrychium virginianum* is weedy in central Iowa and probably reproduces annually. *Cryptogramma stelleri* in Woodman Hollow occurs in three small populations on large, relatively stable cliffs. It is at the extreme southern edge of its range here and although we observed no reproduction, this may not be typical of its behavior further north.

## SUMMARY

We have presented here a number of observations which we believe indicate the feasibility of field studies of fern reproduction. Results presented are non-quantitative and will need to be verified by further observation and experimentation not only in Woodman Hollow, but in other areas as well. When a sufficient number of such studies have been completed it should be possible to make a more meaningful integration of present laboratory data on fern reproduction into the study of fern evolution and ecology.

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