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Reproductive biology of isolated fern gametophytes

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REPRODUCTIVE BIOLOGY OF ISOLATED FERN GAMETOPHYTES

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Reproductive biology of isolated fern gametophytes

by

Carol Jacobs Peck

A Dissertation Submitted to the
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DOCTOR OF PHILOSOPHY

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INTRODUCTION

Ferns are pteridophytes, land plants with two distinct plant forms which alternate in the life cycle. The more conspicuous form is the diploid generation, consisting of a vascularized sporophyte plant which meiotically produces large numbers of wind dispersed spores. Derived from those spores is the haploid generation, consisting of a non-vascularized gametophyte plant that produces gametes by mitosis. Male and female gametes fuse to form a zygote which develops into a new sporophyte plant.

Within the ferns, there are two variations of this basic life cycle: heterospory and homospory. The heterosporous ferns, like seed plants, produce two types of spores, smaller microspores which give rise to strictly male gametophytes which bear antheridia with sperm cells, and larger megaspores which give rise to female gametophytes which bear archegonia, each containing an egg cell. For heterosporous ferns, two gametophytes are required for reproduction and establishment of new populations by sexual reproduction.

Homosporous ferns produce a single kind of spore which gives rise to a gametophyte that is viewed as potentially bisexual. Theoretically, a single gametophyte of a homosporous fern could self-fertilize to establish a new population.

Three distinct types of mating are possible in homosporous pteridophytes (Klekowski, 1969a), each representing a different
level of inbreeding. Mating between male and female gametes from the same gametophyte has been termed intragametophytic mating or intragametophytic selfing (Klekowski, 1969a). Because gametes are produced mitotically, this type of mating will result in a totally homozygous sporophyte. This level of inbreeding is unparalleled in heterosporous plants. There are two requirements for the success of this type of reproduction. Male and female sex organs must be produced simultaneously, and the gametophyte genotype must be free of any lethal genetic defects. If these requirements are met, the resultant sporophyte will be entirely free of genetic load.

If mating occurs between male and female gametes derived from two gametophytes descended from the same parent sporophyte, this is referred to as intergametophytic selfing (Klekowski, 1969a). Because gametes are derived from separate meiotic events, this type of mating is equivalent to the highest level of inbreeding possible in heterosporous plants. Although such inbreeding tends to lower the level of heterozygosity, total homozygosity is not very likely to be attained in a single generation.

Finally, mating between gametes derived from gametophytes descended from two different sporophyte plants is referred to as intergametophytic crossing (Klekowski, 1969a). This is equivalent to outbreeding in heterosporous plants.

The more general term intergametophytic mating is used if
gametes are derived from separate gametophytes, but the parentage of the gametophytes is not specified (Klekowski, 1969a). In populations where intergametophytic mating predominates, some level of heterozygosity and genetic load will probably be maintained due to sheltering of recessive genes, including lethals, in the heterozygous condition. If some deleterious or lethal genes are favored in the heterozygous state (heterosis), the level of genetic load maintained in the population will be even higher (Goodenough, 1978).

Because the unit of dispersal is the spore, the establishment of new sporophyte populations is dependent upon spore dispersal and upon the subsequent establishment, growth, and reproductive potential of the gametophyte generation. Two kinds of dispersal may be considered, multispore dispersal close to the spore source and single spore dispersal some greater distance from the spore source. Sites near parent sporophyte plants are likely to be saturated with spores. Conditions favorable for gametophyte growth may result in development of dense gametophyte populations, and sporophytes may result from intergametophytic mating. As the distance from the spore source increases, spore density decreases rapidly, such that for most species, colonization of distant sites is probably by single spores.

Although there are some spectacular instances of long distance dispersal in ferns, most families and genera of ferns
have the same basic geographic patterns as flowering plants (Smith, 1972; Wagner, 1972). Thus, the advantage of producing a large number of wind dispersed spores, each theoretically capable of initiating a new fern population, has not been sufficient to give fern species a significant dispersal advantage over seed plants. This apparent contradiction may be explained in part by ineffectiveness of single spore reproduction.

The consequences of a fern species relying on the success of a single, isolated spore to initiate a new population are far-reaching in terms of genetics, development, sexuality, and ecology. The isolated spore must germinate and develop into a bisexual gametophyte free of genetic load. Since any sporophyte produced from such a gametophyte will be load free, and may have inherited a tendency toward bisexuality, such sporophytes would initially have a high probability of reproductive success by isolated spores. However, genetic load may develop in populations over time.

The sources of genetic load include somatic mutations which may be accumulated in long-lived individuals or clones. These mutations, along with mutations originating during the meiotic process, will be transmitted through the next generation of gametophytes. Normally outcrossing populations will retain or accumulate some of this genetic load of lethals sheltered in heterozygotous individuals. Since even one lethal gene would
prevent effective intragametophytic reproduction, sporophytes with very high genetic load might be far less successful in giving rise to spores and gametophytes that could self-fertilize to produce viable sporophytes. The presence of any genetic load would prevent establishment of viable sporophytes from most isolated gametophytes. This, in turn, could dramatically reduce colonizing potential.

In terms of homosporous fern distribution, the gametophyte and the spore from which it forms, are typically viewed as constants that affect all species equally. For example, Tryon (1970; 1972) noted that most ferns produce a large number of wind blown spores, that these spores should therefore reach all available habitats over time, and that each spore has the potential to initiate a new population. Accordingly, fern distribution should be limited only by availability of suitable habitat and sufficient time to reach it. Although habitat suitability and length of time available for colonization are certainly important factors in species distribution, differences among other aspects of species biology need to be considered.

The biological success of fern may be viewed as the completion of a series of stages. Those species which have the greatest potential to be most successful at all stages of their life cycle in the widest range of habitats and circumstances are naturally the most widespread and abundant species. Stages
influencing success of homosporous fern species include sporophyte vegetative reproduction, spore production, spore dispersal, spore germination, and appropriate development of sexually competent gametophytes.

Total relative dispersability of different fern species was considered by Peck (1980). He assessed various factors influencing reproductive success potential, such as abundance of sporophytes, percentage of fertile fronds, number of spores per frond, dates and duration of spore release, and completeness of spore release. He noted distinct differences among the 14 fern species he studied.

The question which remains largely unexamined is whether, in fact, all spores are inherently equal in reproductive potential. This is not to say there is no available information bearing on this question. An extensive literature has been published on various aspects about fern gametophytes since their role in the fern life cycle was recognized some 120 years ago. Some investigators have conducted research to clarify fundamental biological processes while others have attempted to aid and improve schemes of classification of ferns. For example, Hofmeister (1862) explained the various forms of alternation of generations in plants in a manner that gave a prominent place to the pteridophyte free-living generation. Campbell (1908) examined gametophytes of a wide range of pteridophytes to elucidate
phylogentic relationships. Later workers followed two general paths. One was experimental, often using fern gametophytes as a convenient tool to study chemical influences, developmental processes, and genetic problems (e.g., Dyer, 1979b; Lloyd, 1974a; Klekowski, 1972b; Miller, 1968; Naf et al., 1975). The second avenue was descriptive, using patterns of development and morphology to contribute to fern systematics (e.g., Atkinson, 1973; Atkinson and Stokey, 1964; Nayar and Kaur, 1971; Stokey, 1951). However, very little of this work has been related to the ecology of the fern gametophyte (Page, 1979).

With few exceptions, previous gametophyte studies have been studies of gametophyte populations. Results from these studies are frequently cited as if they were equally applicable to isolated gametophytes or gametophytes in populations. The development and behavior of isolated spores and gametophytes has generally been inferred from studies of populations, even though there are many indications that a number of significant interactions occur among gametophytes in populations. Current information about such interactions suggests that isolated gametophytes may express different 1) germination potential, 2) developmental patterns, 3) sexual sequence and expression, and 4) reproductive capability than do gametophytes in populations. However, because there have been relatively few studies of isolated gametophytes, these possibilities are virtually untested.
Most works utilizing isolated gametophytes have been genetic load studies that focused on the presence or absence of lethal genes. Cousens (1969; 1979; 1981) and Cousens and Horner (1970)) have focused more directly on sexual expression and ecological interpretations. To date, work with isolated gametophytes has examined relatively few species, and the numbers of gametophytes observed has often been low. Furthermore, isolation has usually been achieved by transfer of young gametophytes from populations, allowing early intergametophytic interactions. The effects of transfer are presently not fully known, but they may disrupt polarity and early morphological development, which in turn may influence the sexual status of the mature gametophyte.

One important example of a potentially erroneous interpretation is a statement frequently repeated in the literature that most fern gametophytes or that most gametophytes of any fern species are protandrous (first male then bisexual). These statements are based almost entirely on observations of gametophyte populations. However, it is also known that gametophytes of many fern species respond to a chemical antheridogen. It has been demonstrated that in dense cultures of gametophytes from a species with an antheridogen system, the first gametophytes to form an organized meristem begin to secrete antheridogen. Gametophytes secreting antheridogen are insensitive to its effect and become female, and at the same time they induce
the many less developed gametophytes to become male.

This suggests that in a given species the innate development of the gametophyte may be protogynous, and that male or protandrous gametophytes may occur only as a result of being induced with an antheridogen from neighboring gametophytes. In a population, very few gametophytes may develop as females, but their role is critical in determining the sexual status of the entire population. Thus, sampling of gametophytes in populations where antheridogens are active results in data suggestive of a male or protandrous sexual systems, whereas in reality, a female or protogynous system enhanced by antheridogen was present. Thus, it is difficult to derive data from gametophyte populations that are useful or pertinent to a consideration of the ecology, reproductive biology, and genetic implications of single, isolated fern gametophytes.

The initial impetus for this study was an ecological one. Long distance dispersal must depend on the ability of single spores to produce a self-compatible and reproductively competent isolated gametophytes. Unpublished observations by D. R. Farrar on Adiantum pedatum L. led to the development of the concept of isolate potential, originally defined as the ability of isolated gametophytes to produce viable sporophytes. He postulated that differences among species in isolate potential might partially explain differences in species dispersability.
Thus, two models exist relevant to fern gametophyte reproduction. One is oriented toward inbreeding by isolated, single gametophytes. This type of reproduction both requires and maintains low genetic load or load free populations. The other model is oriented toward outbreeding by densely stocked populations in which sexuality is influenced by an antheridogen system. The relative importance of the first model to interpretations of colonizing species and disjunct populations is the theoretical basis for this dissertation.
LITERATURE REVIEW

Introduction

Survey of the literature suggests that gametophytes growing in isolation may differ from gametophytes growing in populations in a number of ways. Reported effects of gametophyte density and hormonal control of sexual development in populations suggest that isolation may have profound effects on many aspects of gametophyte growth and development which in turn could influence isolate potential.

In addition, numerous interactions with or influences by the physical and biotic environment, including specific culture conditions, could alter gametophyte development in ways that might enhance or decrease isolate potential. Isolate potential is relevant not only to ecological and distributional questions, but to biological questions relating to the intrinsic developmental patterns and sexuality of a gametophyte growing without the influence of neighboring gametophytes. Sexual development of isolated gametophytes may also clarify whether homosporous ferns are uniformly homothallic.

To adequately relate these ideas to the present study, the following section reviews and analyzes the literature pertinent to fern reproduction by isolated gametophytes. The following topics were considered to have a fundamental bearing on the study: dispersal; factors affecting spore germination; factors affecting
gametophyte development, morphology, sexuality, fertilization, and genetic load; and previous reports based upon monospore culture. In order to evaluate the limitations in interpretations of results of the present study, literature dealing with all phases of gametophyte development was evaluated in relationship to culture conditions and possible effects on isolate potential.

Dispersal

In order for new populations to be established, several prerequisite phenomena must be successfully completed, including adequate spore formation, effective spore dispersal, lodgement in a suitable microsite, spore germination, gametophyte development to sexual maturity, simultaneous presence in the gametophyte population of viable, compatible sperm and egg, conditions conducive to fertilization, and suitable conditions for sporeling growth (Peck, 1980).

As the distance from the parent sporophyte spore source increases, the probability that gametophytes will be spatially isolated from one another increases (Hamilton and May, 1977; Harper, 1977), suggesting that for ferns any new plant produced some distance from the parent sporophyte will most probably be initiated by a single spore. The degree to which this is true has a direct bearing on the significance of isolate potential to species dispersability. Consequently, a review of dispersal literature is presented here. The review first considers general
observations on fern geography, then local dispersal, long
distance dispersal, and finally relictual hypotheses.

Fern geography

Wagner (1972) stated that homosporous pteridophytes include
some species with the widest distributional ranges of all vascular
plants, such as Lycopodium clavatum, Osmunda regalis, Cystopteris
fragilis, and Asplenium trichomanes. Yet, the general patterns of
fern distribution are similar to those of seed plants, with the
same metropolitan areas, common examples of narrow endemism, and
long distance disjunctions frequent (Christ, 1910; Tryon, 1969;
Winkler, 1938). Wagner (1972) referred to the pattern of
"metropolis" and "outliers" with the "metropolis" frequently being
ancestral. The transition from metropolis to outlier reflects a
distribution within the range from common in the metropolis to
rare, rare and local, or sporadic as outliers which is typical
across eastern North America.

Smith (1972) noted that the pattern of ranges for genera of
ferns is similar to that of seed plants, further supporting the
contention that no special phenomena exist that regulate fern
distribution or speciation other than those noted for seed plants
(Klikowski, 1972b).

Although "disjunct" has no precise definition in terms of
distance, Erhlich and Raven (1969) assert that distances of only a
few miles or less may suffice to isolate plant populations from
effective gene flow. The distance sufficient to isolate fern sporophyte populations is probably similar. In contrast, Tryon (1972) suggested that fern populations 400 km apart are "essentially continuous" in their distribution, while Wagner (1972) noted that disjunctions of 250 km or greater are noteworthy.

Analysis of outlier or disjunct populations has contributed much to the discussion of the geography of ferns. There have been essentially two views of such populations. One, that they represent examples of recent long distance dispersal. Two, that they are relics of older distributional patterns with intervening populations now eliminated. There apparently has been no satisfactory way to answer whether populations differ in some way which discriminates as to whether their long distance dispersal was recent or old, and as to whether recent populations from long range dispersal differ from relict populations that have persisted through time (Cushing, 1965).

**Local dispersal**

The general character of local dispersal of fern spores is dependent on spore production and release from sporophyte plants and the transport and deposition of spores on wind currents. Although there have been a few studies of local fern dispersal, the interpretation of such research relies heavily on studies of the local dispersion of pollen, pollutants, or pathogenic fungal
spores (Page, 1979).

Data on the production of spores by ferns are limited. Conway (1957) reported that Pteridium aquilinum could produce up to 300 million spores per frond. Page (1979) reported estimates of spore production for single fronds of 12 cultivated ferns, ranging from 750 thousand to 750 million. Peck (1980) reported estimates of spore production per frond and per plant of 14 fern species at Woodman Hollow, Iowa, ranging from 54 thousand to 81 million per frond and 54 thousand to 324 million per plant.

The seasonality, duration, and completeness of spore release also varies among ferns. Hill and Wagner (1974) noted that the seasonality of spore release was related to frond morphology, spore storage products, and spore longevity. Farrar and Gooch (1975) reported that the duration of spore release was related to sporangial type, sorus development, and foliar maturation. Farrar (1976) noted that although some species quickly release all of their spores, some species have a prolonged interval of spore release and some species retain appreciable quantities of spores on dead, overwintering fronds. Peck (1980) noted that the timing of optimum spore release for 12 of 14 fern species at Woodman Hollow, Iowa, occurred at the time of maximum forest canopy development. Peck (1980) also noted that completeness of spore release was highly dependent upon the extent of environmental moisture; wet years favored spore retention, while dry years
favored complete spore release.

As fronds mature, drying of the sporangium activates a natural dehiscence mechanism that is capable of ejecting spores 1-2 cm from the fronds (Ingold, 1939). Once away from the fronds, spores may be treated as particles subject to prevailing physical factors, such as gravity, turbulence, temperature, rainfall, and electrostatic forces (Wright, 1952; Gregory, 1945), much as long palls of smoke disperse from a smoke stack.

Deposition takes place when eddy currents bring a portion of the air mass into contact with the ground (or vegetation) or when rain cleanses the air mass of particulates. The general effect is that the vast majority of spores are deposited relatively near their source. From this maximum, the number of spores deposited per area drops dramatically as distance from the source increases. A graph of deposition with increasing distance would show a leptokurtic distribution with a rather long tail of fairly uniform but very low levels of deposition (Hamilton and May, 1977; Harper, 1977).

Magraw and Musselman (1979), Schneller (1975), and Peck (1930) mapped spore dispersal from source plants. All reported a non-random pattern of dispersion, reflecting the importance of wind currents in removing spores in a directional manner. Although some spores escaped from the vicinity of source plants, all three reports concluded that most spores remained adjacent to
the parental sporophytes.

Dispersal within, into, and escape from the closed environment of sheltered forest vegetation is complex and difficult (Raynor, Ogden and Hayes, 1976). Wind velocity and air turbulence is greatly reduced. Spores which escape the immediate environment of the parent will tend to be dispersed more slowly by diffusing forces while being subject to the same rate of fall due to gravity. In addition, spores will tend to be trapped by surrounding vegetation (Janssen, 1972; Tauber, 1967). Trapping efficiency increases as surface area complexity increases relative to volume. Thus, the more complex the habitat, the more difficult it is for spores to be transported or to escape, and conversely, the more difficult it would be for spores to penetrate from the atmosphere to suitable sites within the forests (Whitehead, 1969).

This suggests that ferns from open habitats, such as _Pteridium_, will be more successful in escaping the local environment and dispersing their spores over long distances. Similarly, open habitats are more likely to be rapidly and effectively colonized.

Both within the local habitat and at distant sites, spore loss is undoubtedly high. Evidence indicates that large numbers of released spores are eaten by microarthropods, such as collembola (Conway, 1953; Schneller, 1975) and are destroyed by fungi (Conway, 1953).
Long distance dispersal

Direct evidence of long distance dispersal of fern spores is provided by spore trap data from mid-ocean regions and high altitudes (Erdtman, 1937; Newman, 1948; Polunin, 1951). Similar evidence over countryside environments was reported by Gregory and Hirst (1957) who indicated that a fairly large quantity of spores may be transported some distance from the nearest spore source. They reported from 4 to 36 spores of Pteridium aquilinum per cubic meter of air during sampling that lasted all summer long. Bracken fern was not present within several miles of their sampler.

A measure of the effectiveness of long distance dispersal of fern spores is to note newly formed fern populations. Within historic times, areas known to be denuded of vegetation have been successfully colonized by ferns. For example, ferns were well-represented among the recolonizers of Krakatoa (Campbell, 1909), an isolated island volcano with a known date of catastrophic eruption.

Similarly, recent dispersal to man-altered habitats also allows observation on fern immigration with respect to a maximum allowable time. Wagner (1972) reported that a pine plantation in Michigan which was planted in the 1920s in an old pasture now supports a 320 km disjunct population of Polystichum braunii, along with other colonizing pteridophytes: Lycopodium digitatum, Dryopteris spinulosa, Onoclea sensibilis, and Asplenium
platyneuron. The appearance of the other species, while not as dramatic, suggests that they have a potential to invade new sites.

Crist and Farrar (1983) reported ferns colonizing Iowa coal spoils one hundred years old and younger. These spoils are a man-made habitat quite different from the glacial till soils covering Iowa. The age of the spoils and general rarity of the colonizers elsewhere in Iowa (Asplenium platyneuron, Botrychium dissectum, Dryopteris cristata, and Dryopteris spinulosa) was notable especially considering the absence of many of the species more common in Iowa.

Based upon an increased number of recent collections where it had not been reported, Wagner and Johnson (1981) believed that Asplenium platyneuron was increasing its range northwestward in the Upper Midwest. However, Peck (1982) suggested that the range was not expanding; he felt that the new records he reported in the Driftless Area probably reflected previous undercollection and lack of attention to pteridophyte distribution in that region, rather than coincidental expansion with intensive collection. The records Peck (1982) reported were not from a special habitat nor from a datable new habitat.

A more convincing case of range expansion for North American ferns can be derived from observation of alien fern species that have spread across North America. Leonard (1972) reported on the spread of Thelypteris torressiana across the Coastal Plain from
Florida northward to North Carolina and westward to Texas since its introduction in the early 1900s. As this species is not native, any population is new and readily noted as such. A summary of the spread of weedy ferns might be very instructive for understanding the potential ferns have for spreading to new locations if suitable habitats have not yet been occupied.

Tryon (1970; 1972) suggested that ferns have virtually unlimited dispersability. From studies of oceanic island floras, he concluded that dispersal was relatively frequent at distances of about 300 km, and increasingly frequent at shorter distances. Dispersal was usually rare at 1600 km and became very rare at greater distances. He noted that immigration to islands at distances of over 3200 km did occur.

Consequently, Tryon (1970; 1972) considered 400 km to be within easy dispersal distance, and ranges with such gaps to be essentially continuous. Tryon (1972) suggested that at such distances gene flow is maintained through repeated immigration or emigration because little or no endemism develops on islands isolated by up to about 300 km.

Tryon (1970) noted that a species with a broad range is more likely to immigrate to an island. He attributed this to broadly adapted phenotypes. However, this would also correlate with the concept of isolate potential. Every population established by an isolated spore is a population with no load and presumably with
high bisexual potential. Reproduction by isolated spores selects for high isolate potential; reproduction within a population does not necessarily select for isolate reproduction.

As a population develops genetic load, those gametophytes which become bisexual and self-fertile will have higher and higher rates of reproductive failure and will thus contribute less to succeeding generations. To the extent that bisexuality is under direct genetic control, it will be selected against. A colonizing species by frequently establishing and abandoning new populations will maintain a high fraction of populations with low load and high isolate potential.

Another way to compare dispersability was noted by Tryon (1972), who compared sexual and apogamous races of the same species (Selaginella rupestris, Pellaea andromedifolia, P. intermedia, and P. ovata). The apogamous races had ranges averaging 4 times larger (1.2 to 9 times) than the sexual races. He considered the two races equivalent in dispersability and sporophyte habitat requirements, but the apogamous race was better adapted for establishment in that it did not require water for fertilization. Apogamous species of higher ploidy level also had larger spores, more rapid gametophyte growth, more rapid sporophyte formation (Tryon, 1968; Whittier 1968, 1970). All apogamous species may be considered to have 100% isolate potential, whereas the isolate potentials for the sexual races are
unknown.

Tryon (1970; 1972) considered that restricted range was due to narrow or specific habitat requirements. He did not consider intrinsic factors such as low isolate potential. A species with a uniformly high genetic load and or a low potential for forming bisexual gametophytes, may seldom or never produce a spore which can in isolation initiate a new population in any habitat.

The actual mode of long distance spore dispersal by ferns is most likely by single spores. Alternatively, spore dispersal by spore tetrads or synaptospory has been hypothesized (Kramer, 1977) which may function for local dispersal as well. In this instance, spores would be dispersed as a unit. Klekowski (1979) observed that during the winter, entire sporangia are shed by plants of Matteuccia struthiopteris. He suggested that these propagules, transported by spring snowmelt, would form clusters of male and female gametophytes upon germination. These types of multispore dispersal would allow for local establishment by a population of gametophytes (4 to 64). With or without an antheridogen system operating, outcrossing among gametophytes could result from a single dispersal event. The probability of both male and female sex organs occurring within the population would certainly be enhanced. Crossing between the unlike meiotic products could prevent expression of recessive lethals and could reestablish the parental sporophyte genotype. Genetically, this
might be equivalent to long distance vegetative reproduction. Selfing would result in 100% homozygosity with resultant expression of any recessive lethals. Genetically, this is equivalent to dispersal by a single spore.

The importance of such multispore dispersal over long distances has not been demonstrated. Catastrophic storms might transport intact sporangia, frond fragments, and entire plants could be moved considerable distances by hurricanes, typhoons, or tornados. Such storms would have the ability to penetrate the closed canopy of the fern habitat which normally reduces movement of spores from fern plants or local habitats. If viable reproductive structures are then redeposited during the same storm event some distance from where they were picked up, there could be increased availability of colonizing sites from storm damage, such as scoured rocky banks, bare soils, and high moisture levels.

Relictual hypotheses

Although evidence supports the occurrence of long distance dispersal in many cases, an alternative explanation, that some outliers or disjunct populations are actually remnants or relicts of an older dispersion, also has merit.

Many botanists in Iowa have suggested that some of the more graphic disjunctions in the Iowa flora reflect glacial and post-glacial vegetation distributions rather than recent anomalous long distance dispersions. Most of these disjunctions involve mesic
species occurring in isolated, sheltered sites in Iowa valleys and canyons (Pammel and King, 1901; Pammel, 1905; Gleason, 1922; Conard, 1952; Thorne, 1964; Eilers, 1965; Niemann, 1975; Plouffe, 1977; Peck, 1980; Peck, 1982). Other authors (Shimek, 1948) considered that the prairie occurred in Iowa immediately after glacial retreat, and that the mesic species were very recent invaders which had arrived since the hypsithermal period of greatest drought stress approximately 5000 years ago.

In a study of ferns at Woodman Hollow, a sandstone canyon in central Iowa, Peck (1980) theorized that after glaciation most of the species could have been in the immediate vicinity in the upland mesic woods soon after glaciation. During the following drying and warming trend which resulted in the prairie upland, the species could have survived within the canyon at Woodman Hollow. Established plants within Woodman Hollow would have had a competitive edge to populate the valley, but they were probably not very effective in getting their spores out of the valley; thus, would have contributed little to the fern populations elsewhere in central Iowa.

Peck (1982) also examined the status of ferns of the Driftless Area. He suggested that the region may have served as a long-term refugium during the last part of the glacial epoch. That species might have been introduced during or after glaciation of the surrounding areas was also likely. The diversity of habitats in
the region is large, leading to a large fern flora, especially when compared to the region to the west of the Driftless Area. One striking species in the Driftless Area flora is *Thelypteris simulata*, which is disjunct from eastern Pennsylvania. It occurs from New Foundland to New Jersey, with one disjunct population in Tennessee at Newfound Gap (Moran 1981). Peck (1982) suggested that since he could locate 34 populations of *T. simulata* in 5 counties of Wisconsin within the Driftless Area, that the occurrence of this disjunct was probably not the product of a recent long range dispersal.

Unfortunately, phytogeographic data are often open to more than one interpretation. Tryon (1972) suggested that fern species are everywhere they can be; they are habitat limited. Smith (1972) did not agree, noting that species have many differences in ecology and reproductive biology which make them less than equal in ability to form new populations. Singh and Roy (1977) related poor reproductive potential to scattered disjunct populations, whereas Crist and Farrar (1983) related the scattered disjunct populations of *Asplenium platyneuron* to high isolate potential.

The same geographic pattern, theoretically, could result from very different dispersal and reproductive capacities expressed over different time spans. Observations on single spore ecology might, provide some useful observations to resolve or clarify these discussions.
Factors Affecting Germination

A variety of factors have been reported to influence the initiation of fern spore germination, the percentage of spores which are able to germinate, and the rate of germination. These factors include external (biotic and abiotic) factors and internal factors. Some of these factors are or may be differentially effective for isolated spores versus populations of spores. All of these factors must be considered in designing and interpreting any laboratory or field study in which differences in germination response could play a significant role. Several general reviews have been published on this topic; they form a basis for the following discussion (Miller, 1968; Page, 1979; Sussman, 1965).

External factors

External factors that influence germination include abiotic factors (e.g., light, temperature, pH, and media composition) and biotic factors (e.g., seasonality of spore collection, environmental condition during spore formation, nutritional status of parent plant, spore maturity at time of collection, spore age, storage conditions, and spore density). Some of these factors are generally within experimental control, while others are generally or effectively outside experimental control.

Abiotic factors A number of workers have examined the influence of abiotic factors, with particular emphasis on the effects that light, temperature, and pH have on spore germination.
Light has been investigated more intensively than the other chemical or physical abiotic factors. Mohr (1963) reported that light is almost universally required for spore germination in ferns. Weinberg and Voeller (1969) found that light was required for 30 of 33 species (8 families) tested. Included were Athyrium felix-femina and Woodsia obtusa of the species included in the present study. The absolute light requirement for germination was demonstrated to be a requirement for red light, apparently a red/far-red phytochrome induction-reversion system. As far as it has been tested, the light requirement for germination may be replaced by hormones having an antheridogen effect on the species tested (Naf, 1979).

Under natural conditions this kind of control could be adaptive in favoring outbreeding. Gametophytes growing in the light could stimulate nearby spores in soil and rock crevices to germinate and contribute gametes. It may also be related to control of germination by day length. Miller (1968) reported that long days resulted in better germination and were necessary for subsequent growth. His report resulted in many British workers starting all spore germination investigations in late Spring (Page, 1979).

In addition to light quality, light quantity and duration also influences germination. For example, Pietrykowska (1962b) found that percent germination of Matteuccia struthiopteris spores
increased with increasing light intensity (to 3000 lux) and increasing day length (to continuous light).

Hill (1971) examined three fern species and found that different species germinated over different ranges of light intensities and that optimum germination occurred at different light intensities. This correlation was considered to be significant and adaptive to their respective habitats. The effect of light intensity may be increased with older spores. Smith and Robinson (1975) found that high light levels increased the delay in germination of older spores, although the final percent germination was similar for fresh and old spores.

Temperature Hill (1971) found that the three species he studied germinated over different temperature ranges and that the optimum germination temperature differed for the three species. Again, these differences were correlated with and adaptive to the habitats of the species studied. Similar observations were made by Hartt (1925) and Hevly (1963), who had studied temperature effects on Onoclea sensibilis and cheilanthoid ferns, respectively. Warne and Lloyd (1930) reported that the temperature range and optima for germination for Ceratopteris thalictroides and C. pteridoides differed from those measured for Matteuccia struthiopteris.

A number of workers have examined germination and growth relative to substrate pH. Hill (1971), as with temperature
and light intensity, found spores of different species to germinate over a range of pH values and to have different pH optima. Mohr (1956) determined pH optima for *Dryopteris filix-mas*.

**Effects of spore surface sterilization**  In an attempt to eliminate influences from contaminant organisms, researchers frequently use a dilute solution of sodium hypochlorite to surface sterilize spores (Miller, 1963; Dyer, 1979a).

According to Hutchinson and Fahim (1958), Huré-Py (1950) reported the percentage of spores germinating is reduced by surface sterilization of the spores.

Kotenko (1983) reviewed previous reports on the effects of surface sterilization of spores. She reported that Iarenço (1956) observed that the sterilant removed the perispore from *Onoclea* spores; Crotty (1967) indicated that the sterilant inhibited cell wall growth; Rowland and Boyd (1974) found *Pteridium aquilinum* spore germination reduced as much as 50% following brief sterilization; Dyer (1979a) noted that brief sterilization reduced synchrony and percentage of germination.

**Effects of media**  Bell (1958) suggested that variations in germination commonly observed among different spore sowings when cultured under uniform conditions could be due to growth substances produced by different samples of agar in response to proteolytic enzymes diffusing from spores and young
gametophytes. He cited Mohr (1956) and Kulescha (1948) to support this possibility.

**Biotic factors** A number of biotic influences emanating from fungi, fern sporophytes, and other fern gametophytes have been reported to influence spore germination. In addition, a variety of plant hormones, exudates, and extracts are reported to influence spore germination.

**Effects of fungi on germination** It has been shown that some fungal contaminants may enhance germination rates and percentages of spores germinating. Bell (1958) reported that germination in cultures inoculated with an unidentified fungus was significantly stimulated compared to sterile spore cultures for three species of ferns: *Thelypteris palustris* Schott, *Polystichum lobatum* (Huds.) Woyner, and *Dryopteris filix-mas* (L.) Schott. The effect was apparently due to a volatile or diffusible substance, as the hyphae of the mycelium had not spread to the agar surface on which the spores were sown. Furthermore, fungi have been shown to produce a variety of potentially biologically active volatile compounds including aldehydes (acetaldehyde and formaldehyde), ethanol, methanol and numerous other unidentified compounds (Robinson and Park, 1966).

**Growth regulators** Various plant hormones (ethylene, auxin, and gibberellic acid) and growth factors which influence spore germination are known to be produced by fungi, and by fern
gametophytes (Smith and Robinson, 1971; Smith et al., 1973) and sporophytes. Hormone effects on fern spore germination were reviewed by Howland and Edwards (1979).

Edwards and Miller (1972a, 1972b) found ethylene to inhibit spore germination. Ethylene effects could be significant in multisporocyte culture or in crowded conditions. Under crowded conditions gametophytes naturally produce ethylene causing a sequencing effect by delaying slower germinating spores. This would reduce competition in the short run and provide an additional reserve of spores for the long run if the first crop of gametophytes were not successful in producing sporeling plants. This or any other factor which favors sequencing of germination could also influence sex ratios if an antheridogen system is present.

As cited in Weinberg & Voeller (1969), Schraudolf (1962) demonstrated that gibberellic acid (GA) could stimulate spore germination in the dark, replacing the obligate red light requirement for germination.

Naf (1966) reported germination stimulation of Lygodium japonicum spores by filtrates of media on which L. japonicum gametophytes had grown. This may be due to antheridogen effects. It has been demonstrated that an antheridogen may replace the light requirement for germination in species which produce precocious antheridia in response to that antheridogen (Naf,
Allelopathy Allelopathic and autotoxic effects have been demonstrated using leachates and extracts between sporophyte-gametophyte and gametophyte-gametophyte for several species. Petersen and Fairbrothers (1973) identified the gametophyte as the weakest link in the fern life cycle, making it the most likely to be sensitive to and easiest to demonstrate the effectiveness of allelopathic or autotoxic compounds. Such chemical effects need to be understood to design meaningful laboratory experiments and to properly study field populations of gametophytes. The importance of chemical influences in nature is not known.

Sporophyte-gametophyte interactions were demonstrated by Froeschel (1953), who reported that water extracts of Polypodium aureum and Lycopodium clavatum sporophytes decreased the growth rates of gametophytes of four fern species. Davidonis and Ruddat (1973, 1974) reported sporophyte-gametophyte interactions based on roots and fronds of Thelypteris normalis, which contain thelypterin A and B, which inhibits cell division in the gametophytes of T. normalis, Pteris longifolia, and Phlebodium aureum. Davidonis (1976) reported thelypterin A and B from T. dentata roots, thelypterin A in T. novaboracensis leaves, and thelypterin B in the roots of two Pteris species. Davidonis (1976) also reported the presence of an unidentified growth inhibitor in the leaves of Osmunda cinnamomea. Star and Weber
(1978) reported that exudate from sporophytes of *Pityrogramma* species inhibits spore germination and gametophyte development in *P. calomelanos*.

Munther and Fairbrothers (1980), testing leaf leachates and extracts of *Dennstaedtia punctilobula*, *Osmunda cinnamomea*, and *Osmunda claytoniana* obtained from New Jersey and Vermont populations, demonstrated geographic differences in allelopathic and autoxic responses among these species in regard to spore germination. Peck (1980) could not demonstrate an allelopathic or autotoxic effect among 14 species of ferns at Woodman Hollow, Iowa, based upon spore germination on moistened stipe residue and debris. Perhaps living material has a stronger effect or these species or populations do not have strong allelopathic or autoxic responses.

Gametophyte-gametophyte interactions were noted by Bell (1958), who reported that an aqueous extract of *Dryopteris filix-mas* gametophytes stimulated spore germination and prothallial growth in *D. borreri*, but that gametophyte debris of *D. filix-mas*, when incorporated into the medium, prevented *D. borreri* spore germination. Petersen and Fairbrothers (1980) demonstrated reciprocal suppression of cell division by gametophytes when subjected to extracts of different genera, specifically *Osmunda cinnamomea* on *Dryopteris intermedia* and *Dryopteris intermedia* on *Osmunda cinnamomea*. This phenomenon would favor the first
gametophyte to occupy a site, preventing or reducing the potential for competition, and the effect would be intensified with sporophyte production.

**Internal Factors**

There are intrinsic differences in rate of germination among species. Hill (1971) showed that among the three species he studied, there were innate differences in the length of time between sowing and germination, and in the interval over which germination occurred. In addition, the species showed optimum germination and growth at different temperatures, pH levels, and light intensities. These differences were correlated with and adaptive to differences in their optimal habitat. One result of intrinsic factors is that all spores would not germinate at once, resulting in a phased array of ages of gametophytes, that would be available to replace those which are not successful (Pickett, 1914) and to provide new antheridogen sensitive gametophytes.

**Effect of time of collection** Some intrinsic differences among spores of the same sample could occur as a result of different developmental ages of the spores at the time of collection.

Weinberg & Voeller (1969) found that a sequence of spore samples of *Anemia phylitisidis* collected from greenhouse plants at different times varied in the time required for initiation of germination after light or GA stimulation. GA concentration or
length of light exposure required for optimum germination also
varied among these spore samples. There was no direct correlation
with season or time progression, but rather, spores produced
during periods of maximum spore formation tended to give higher
germination percentages.

Mohr (1963) also noted variation in germination levels for
spore batches of two *Osmunda* species collected at different times
of the year.

**Effect of spore size** It has been reported that larger
spores of *Ceratopteris thalictroides* (Schedlbauser, 1975) and
*Dryopteris pseudo-mas* (Dyer, 1979a) tended to germinate more
rapidly than smaller spores.

**Effects of spore age** Sussman (1965) reviewed reports of
spore viability after different periods of storage under a variety
of conditions. Variation among species was considerable. Spores
from herbarium specimens of *Asplenium serra* and *Dicksonia
antarctica* were found viable after 48 and 22 years respectively.
Green-spored species of *Onoclea*, *Osmunda* and *Matteuccia*, however,
showed low viability after even brief storage (Hartt, 1925; Lloyd and
Klekowski, 1970; Sussman, 1965). After one month of storage, only
10% of *Osmunda regalis* spores germinated; no germination occurred
after two months or more. Reports for *Matteuccia struthiopteris*
were similar. However, Peck (1980) found spores of this species
to be 94% viable after 15 months of refrigerated storage.
Conditions of storage are important. Dry and cold conditions have been found to improve storability (Sussman, 1965; Dyer, 1979a). Even under these conditions changes occur over time. Changes include reduction in percent germination, in rate of germination, and in rate of development after germination.

Smith and Robinson (1975) studied the effects that storing spores after collection had on germination in *Polypodium vulgare*. They found that viability of refrigerated spores declined significantly after three years. Maximum spore death occurred at year four, followed by a slower decline in viability, increased delay in germination, and a decrease in the rate of germination. Laage (1907) reported that spore age also affected the ability of spores to undergo dark germination.

**Interactions among various factors**

A number of workers have reported interactions among different factors which influence spore germination.

Light requirements interact with or are influenced by other factors. According to Sussman (1965), Heald (1893) reported dark germination after treatment with high temperatures. Gibberellic acid, nutritional factors, and antheridogen may substitute for red light requirements.

Weinberg and Voeller (1969) reported strong pH dependence of gibberellic acid stimulation of dark germination in *Anemia phyllitidis*. Maximum dark germination occurred at pH 4.5 and
lower with virtually no germination at values over pH 6.0, whereas spores in the light germinated well from pH 3.5 to 7.0.

Smith and Robinson (1975) found a higher rate of germination for aged or isolated spores of *Pteridium aquilinum* could be achieved when spores were sown on media which previously supported gametophytes of that species. Final germination, however, was not altered significantly. They also found that low spore density and high light levels increased the delay in germination of older spores, although final percent germination was not affected. In their discussion, they related the similar effects caused by spore age and low spore density to "the declining ability of the germinating spores to produce adequate levels of metabolites essential for development" and to the inability of the isolated spore to accumulate high enough metabolite levels in the media to promote growth.

Hutchinson and Fahim (1958) suggested that some loss of metabolites or synthetic abilities by aging spores could be counteracted by substances provided by the presence of living fungal hyphae.

**Factors Affecting Morphology**

Factors affecting morphological development of fern gametophytes are significant to isolate potential because morphological development may, in part, determine the ultimate sexual expression of isolated gametophytes. Pertinent factors for
this study are those factors which may cause an isolated
gametophyte to have a different reproductive potential than would
a genetically identical individual growing in a population. Also
important are those factors in the environment which might cause
isolate potential to differ in nature from that found in the
laboratory. In other words, how might culture conditions which
influence gametophyte morphology and development influence the
reproductive success of the gametophytes being tested?

**Relationship of morphology to sexuality**

Emigh and Farrar (1977) elucidated a mechanism for generating
a bisexual clone from a single unisexual (female) gametophyte of
*Vittaria lineata*. Female gametophytes produced antheridogen, but
were themselves insensitive to it, and thus, were unable to form
antheridia and attain bisexuality. However, such female
gametophytes also produced asexual gemmae which after dropping
from the parent gametophyte were sensitive to antheridogen. These
gemmae produced abundant antheridia. Thus, a single parent
gametophyte, which itself was hormonally fixed as female, could
nevertheless generate a bisexual clone in which sexual
reproduction became possible.

A similar pattern is possible through simple proliferation of
non-gametiferous gametophytes of other genera. A number of authors
have referred to the presence of antheridia on gametophyte
proliferations or branches originating from the base of an "old"
female gametophyte.

For example, Mottier (1927) found in *Matteuccia nodulosa* that archegonia were largely confined to the undersurface of the midrib while few or no antheridia developed there. Antheridia did form on smaller lateral proliferations. Similarly, in *Osmunda claytoniana* gametophytes with many cordate lobes, he observed it to be very rare for both sexes to occur on the same lobe (Mottier, 1925). In other instances, archegonia were restricted to the midrib and antheridia occurred only on the margins of the wings or on proliferations from the wing margins.

Haufier & Gastony (1973a) reported antheridia "on the lobes of old, large, abundantly branched gametophytes in which lobes were separated from the archegonial portion by necrotic tissue. Gametophytes lacking extensive branching in unispore culture became and remained exclusively archegonial."

Shedlbauer & Klekowski (1972) reported that two distinct morphologies were present in populations of *Ceratopteris thalictroides*: cordate bisexual gametophytes and spatulate, ameristic, male gametophytes. The males developed into bisexual gametophytes only long after initial sexual maturity and only by proliferating a cordate lobe that bore the archegonia.

Cousens (1969) reported that older female gametophytes of *Dryopteris ludoviciana* occasionally produced basal outgrowths which became male.
Saus and Lloyd (1976) found that gametophytes that were isolated during development produced archegonia in 15-17 days and remained unisexual for another 23-120 days. Antheridia were formed only on proliferations from the prothallial wings.

Peck (1980) reported that unisexual gametophytes of Woodsia obtusa regenerated bisexual lobes after winter tissue death.

Naf (1979) reviewed experimental evidence indicating that if portions of antheridogen-insensitive prothalli were isolated from the meristem, they regained sensitivity and formed antheridia. Tissue isolation has been achieved by excision of the meristem, excision of wing tissue, separation from the meristem by a row of dead cells, cuts between the meristem and a portion of the wing, and plasmolysis of gametophytes. In Onoclea, some regenerative cell division always occurred before antheridia production began.

From observations such as these, it can be predicted that isolated gametophytes which produce tenuously connected or physiologically disconnected (Naf, 1979) outgrowths or branches might have a mechanism, analogous to the gemmae system in Vittaria, by which they may produce antheridogen sensitive tissue and thus overcome antheridogen insensitivity. Damage caused by frost, desiccation, insect predation, or other natural causes might also serve as release mechanisms. It would be expected that such gametophytes would be more likely to achieve bisexuality than would those with a simple cordate morphology.
There is some evidence of variation among species in bisexual potential. In *Dryopteris mollis*, Mottier (1925) found large and small proliferative lobes to be bisexual. In some, each secondary prothallus bore a sporophyte.

**Types of deviation from normal morphology**

Most reports, particularly older ones based on sequential sampling from multigametophyte cultures, present morphological development as a single invariant pattern of gametophyte development and sexual sequence. However, a variety of deviations from species "normal" morphology have been observed under typical culture conditions as well as in response to particular experimental manipulations. Morphological aberrations or changes include apolar growth, dwarfing, failure of meristematic development, calluslike growth, prolongation or resumption of two-dimensional growth, twinning or early branching of gametophytes, production of marginal or basal proliferations (especially on older gametophytes). Responses to specific chemical treatments include effects on rate or occurrence of cell division, cell enlargement, and cell elongation.

Many of the morphological variations reported in the literature have been due to proliferations associated with aging of the prothallus. These proliferations have been observed on numerous species. They are highly varied in form and site of origin.
According to Mottier (1927), it was well-known that prothalli of Polypodiaceae "when grown beyond the ordinary time for the production of sporophytes, may branch both dichotomously and by means of lateral proliferations. The proliferations may spring from both the upper and under sides of the midrib and from the margins of the wings of the gametophyte."

Mottier (1927) reviewed earlier reports by Hofmeister (1862), Kny (1872), Goebel (1877) and Campbell (1892, 1908). Lateral proliferations, branching, secondary prothalli, and "adventitious shoots" were reported. These originated from the margins, from the surface and from under the midrib. Goebel (1877) observed varied shoots from young, mature and old tissue of Gymnogramma leptophylla.

Mottier (1927) observed gametophytes of Matteuccia nodulosa and Osmunda claytoniana in soil cultures for a period of four years. He reported the following types of proliferations: 1) cylindrical projections with central vascular patch arose near notch, 2) cordate proliferations, 3) marginal proliferations with many antheridia, 4) small granular proliferations, and 5) rounded wartlike, granular appearing, protrusions covered with antheridia.

Mottier (1925) noted that Pteris longifolia and Dryopteris mollis, in addition to the species noted above, developed branches or proliferations after prothallia reached a certain sex or age.
In addition to branching and formation of two-dimensional prothalli, Steeves et al. (1955) reported five types of calluslike proliferations in Pteridium cultures. These were: 1) filamentous proliferations primarily composed of branched filaments, but which may also formed small archegoniate lobes, 2) pincushion proliferations, hemispherical masses, composed of elongate, mostly unbranched filaments with antheridia, 3) corraloid proliferations which arose from the archegonial cushion near the apical notch and were composed of masses of clublike branches with rhizoids (these proliferations were bisexual, but the sex organs occurred in separate groups), 4) filamentous pseudocallus composed of rhizoids and branched filaments of isodiametric bubblelike cells which grew rapidly, and 5) parenchymatous callus composed of a true tissue callus. The latter two types were generally observed only after prolonged culture. They bore no sex organs. Their inability to regenerate normal prothalli was associated with high (3n-4n or more) and irregular chromosome numbers, but it was concluded that these did not initiate the proliferations, but were secondarily derived. The first three types not only produced apparently normal sex organs, but retained the capacity for regeneration of normal prothallial lobes.

Thus, a variety of morphological aberrations are known to occur. These are often associated with a change in gametophyte sexual status. Although individual gametophytic lobes are often
reported to be uniformly unisexual, some proliferations have been reported to be bisexual. Whether this is strictly species specific or simply environmental is not clear.

**Factors which influence morphology**

Reports of experimentally oriented studies offer a myriad of factors and combinations of factors which influence gametophyte growth and morphological development. For example, Dyer and King (1979) summarized experimental treatments reported to initiate filamentous or proliferative growth. Treatments included surgery, plasmolysis, colchicine, UV radiation, high temperature, centrifugation, and darkness or low light followed by high light. Many of the factors which influence germination also affect development. There are external influences, both abiotic and biotic, as well as, internal or intrinsic factors. Factors included in the following discussion are: effects of spore surface sterilization, gametophyte transfer, light, temperature, pH, media-substrate, gametophyte–gametophyte interactions (including antheridogen and density effects), auxins, fungi–gametophyte interactions, innate differences among species, and interactions among various factors.

**Effects of spore surface sterilization**

In her review of reports on the effects of sterilization upon gametophyte growth and development, Kotenko (1983) reported that Steeves *et al.* (1955) observed more proliferative and irregular gametophytes in
sterilized cultures; Crotty (1967) indicated that the sterilant inhibited cell wall growth. Kotenko found that sterilization for less than two minutes with dilute calcium hypochlorite reduced the rate of cell division in Onoclea gametophytes, but produced no significant effect on morphology and antheridium formation.

**Effects of transfer** Isolate gametophyte cultures used for genetic load studies (Table 1) have been established by transferring gametophytes prior to sex organ development. Although almost no information is available on the effects of transfer on gametophyte growth, there are some indications that development may be profoundly disrupted (Steeves et al., 1955; Kotenko, 1983).

Kotenko (1983) established multispore cultures by transferring either germinating spores (a spore with protruding rhizoid) or young gametophytes (three to six cell stage). She found that growth of gametophytes in transferred cultures was retarded compared to nontransferred gametophytes. Many of the gametophytes in the transferred cultures died after two to three weeks growth; death of gametophytes in nontransferred cultures was negligible.

Prothallial colonies of the type observed by Hurel-Py (1950) were reported to be more likely to develop from small transplanted prothalli than from large gametophytes (Steeves et al., 1955).

Farrar (pers. comm.) found that age of transfer was
significant. In experiments with *Adiantum pedatum*, disruption of morphological development, possibly due to disruption of polarity, was more profound for gametophytes which were younger at the time of transfer than it was for older gametophytes, probably because the likelihood was greater that young plants were not replaced in their original orientation.

**Effects of light** There is both a quantitative and qualitative requirement for light.

Light quantity directly influences growth rate through photosynthesis (Miller and Miller, 1961). Pietrykowska (1962a) confirmed light quantity effect on gametophyte development and morphogenesis as previously noted by Miller and Miller (1961).

Kill (1971) reported that optimal light intensities for growth of *Adiantum pedatum*, *Thelypteris palustris*, and *Woodwardia virginica*, as measured by gametophyte width, distinctly differed among species, and that these differences correlated with differences in light intensities as measured for the typical habitats of these species.


Atkinson and Stokey (1964) found that change in direction of
the light source was very disruptive to morphological development, especially for young gametophytes.

Mohr (1963) reported that blue light was required for transition from filamentous to two- and three-dimensional growth in *Dryopteris filix-mas* and *Alsophila australis*. The intensity of light required to elicit the change differed for the two species.

**Effects of temperature** In reviewing the effects of temperature on gametophyte culture, Dyer (1979a) found few publications concerned with the influence of temperature on development.

Hill (1971) found that optimal temperatures for growth, as measured by gametophyte width, differed for three species studied. *Adiantum pedatum* grew best at 15-25 C with 25 C optimum. *Thelypteris palustris* grew well at 25-30 C with 30 C optimum. For *Woodwardia virginica*, the optimal temperature was 25 C; growth was significantly reduced at all other temperatures tested.

Dyer and King (1979) reported that high temperature (27.5 C in white light and 25 C in red light) induced branching filamentous growth in gametophytes of *Dryopteris pseudo-mas*. Morphology of the filaments varied depending on the temperature and light regime. The authors indicated that this effect had not been previously reported for any species.

**Effects of pH** Hill (1971) found that *Adiantum pedatum* gametophytes grew fairly well at pH 5 to pH 8 with optimum growth
at pH 8. For *Thelypteris palustris*, optimal growth occurred at pH 5; growth was significantly reduced at all other levels measured. *Woodwardia virginica* grew best from pH 5 to pH 7 with optimal growth at pH 6. Optimal pH for all species corresponded very closely to the pH measured for the native habitat of each species.

**Effect of media-substrate**  
In Dyer's (1979a) review of gametophyte culture methods, he noted that although growth generally occurs on any medium containing complete macro-nutrients, "the rate and even the detailed pattern of development can be affected by the concentration and composition of the medium." He lists 34 different macronutrient formulas which have been used for fern gametophyte cultures. He found that although some authors have noted that a particular species performed better on one medium or concentration of nutrients than on some other, few comparative studies have been made.

It has been noted that irregularities of growth are more frequent in aseptic or agar cultures (Bell, 1958), but different morphologies have been noted on a variety of substrates and from nature. In reviewing reports of such effects, Atkinson and Stokey (1964) suggested that media responses differed among species. Some authors (DeMaggio, 1961; Ward and Wetmore, 1954) reported that gametophyte growth of some species (*Todea barbara* and *Phlebodium aureum*) followed very similar developmental patterns whether grown in sterile culture or on other media.
In contrast, Hurel-Py (1950) reported that gametophytes of Gymnogramma calomelanos, which grew normally on soil, proliferated extensively in sterile culture. According to a review of this report by Atkinson and Stokey (1964), the initial lobe forms secondary lobes. "Each lobe proliferates further, either from the margin or surface, until a prothallial colony of upright plates is formed, all attached to, but easily separated from, the original thallus. These colonies expand at the periphery and die off at the center and can be grown indefinitely by transferring fragments of the colony to fresh medium every 2-3 months. The proliferated thalli are fertile and form sporophytes; as these grow the regeneration of thalli continues."

Atkinson and Stokey (1964) stated that these proliferations differ from those on soil-grown gametophytes. In soil-grown gametophytes, they found that, "regeneration usually takes place from a single cell which in time can duplicate in all respects the original thallus and such regeneration usually takes place during old age or after an injury." They noted that the tendency toward regenerative proliferation varied among species.

Whittier (1970) reported that apogamous gametophytes of tetraploid Pellaea glabella exhibited a shorter filamentous stage and grew faster in sterile culture than had been previously reported for soil cultures.

Cousens (1979) noted proliferations in soil cultured
gametophytes of *Blechnum spicant*.

Schedlbauer and Klekowski (1972) noted that the distinct bisexual and male morphologies of *Ceratopteris thalictroides* which they reported from agar cultures, although not yet collected from wild populations, had been reported on sand (Kny, 1875), mineral solution (Javalgekar, 1960; Nayar and Kaur, 1969) and a soil mixture (Pal and Pal, 1963).

Mottier (1927) observed a variety of gametophyte proliferations, including male calluslike forms, from soil cultured gametophytes.

Steeves et al. (1955) found that soil cultures of *Pteridium* differed from agar cultures. Initial filamentous growth was brief in time and extent, and there was no evidence of the prolongation of the juvenile filament on soil. Later populations were composed largely of bisexual and small, irregular, crowded males. If large cordate gametophytes were not fertilized on soil, then some proliferation was observed. In general, however, aberrations occurred on both soil and agar culture, but were more frequent on agar.

Mohr (1956) attributed growth differences in replicate sowings to differences in agar quality. Agar was known to yield growth promoting substances in the presence of certain proteolytic enzymes (Kulescha, 1948). It is possible that substances of this kind liberated in varying amounts from different samples of agar
are another cause of erratic behavior in culture.

Bell (1958) suggested that "The more regular behavior of spores on soil than on agar may also result in part from the physical uneveness of the surface of the former and the smaller opportunity for outward diffusion" on soil than on the even and uniform agar surface and films.

Peck (1980) noted that gametophytes collected from nature were very regular in morphology as compared to agar grown gametophytes.

Although possibly rare, morphological irregularities are not totally unknown in nature. Cousens (1979) reported a single field-collected gametophyte with proliferations on the dorsal surface. These were initiated as filaments, but progressed to spatulate and cordate plates.

Observations discussed above suggest that attainment of bisexuality may be in part dependent upon a proliferative morphology. If gametophytes from agar cultures are more proliferative, they may be more bisexual than would be expected for gametophytes from soil cultures or from nature.

Gametophyte-gametophyte interactions Knowledge of gametophyte development comes primarily from observations of gametophyte populations. There are a very few reports of gametophytes grown in single spore cultures. Comparisons of both types of cultures suggest that gametophyte growth and development
Antheridogens One of the most important influences within gametophyte populations is the operation of antheridogen systems. In 1950, Dopp reported that medium from mature Pteridium aquilinum gametophyte cultures and extracts from those cultures induced precocious antheridia formation in gametophytes of this species and those of Dryopteris filix-mas (Naf, 1979). Substances which produce this effect have been termed antheridogens (Pringle, 1961). Subsequently, observations on antheridogens have been elaborated upon by a number of workers (see reviews by Naf, 1961, 1969, 1979; Naf et al., 1975; Voeller and Weinberg, 1969; Voeller, 1971).

Native antheridogens have been demonstrated in at least 10 fern species (Naf, 1979). It has been determined that several of these chemically differ from each other (Naf, 1960, 1962, 1968; Schedlbauer, 1976).

The only antheridogen which has been fully defined, the antheridogen in the Schizaeaceous genus Anemia, has a gibberellin-like structure (Endo et al., 1972). It has been found that gibberellins can mimic antheridogen action within the Schizaeaceae (Schraudolf, 1962, 1966; Naf, 1968; Voeller, 1964a, 1964b) and in the Vittariaceae (Enigh and Farrar, 1977). Antheridogens from Schizaeaceous species, although showing some cross effectiveness within the family, are not as broadly
effective as are gibberellins. Neither native antheridogens of the Schizaeaceae nor gibberellins show any activity in ferns from the Polypodiaceae (sensu lato), Osmundaceae, or Cyatheaceae (Voeller, 1964a; Xaf, 1959, 1960, 1963).

Pteridium antheridogen has been shown effective for 28 species in the Polypodiaceae (sensu lato), but was ineffective for the genus Polypodium and for the families Osmundaceae, Cyatheaceae, and Schizaeaceae (Xaf, 1979).

Evidence to date indicates that antheridogens influence sexuality according to the following pattern. In populations having an antheridogen system, the larger or faster growing gametophytes secrete an antheridogen into the environment which induces neighboring gametophytes to become males. When very slow-growing gametophytes are induced, they form small irregular asexual male gametophytes. With continued exposure to antheridogen, these gametophytes remain male and never form an organized meristem. It has been suggested that this is because "antheridium formation in the growing region of the juvenile prothallus interferes with the organization of the meristem that leads to the attainment of the archegonial phase" (Xaf, 1979).

If gametophytes which are growing more rapidly are induced with antheridogen, they produce antheridia, but also form an organized meristem. Meristem organization is associated with development of insensitivity to antheridogen. These gametophytes
then cease antheridia formation and begin to form archegonia. In *Pteridium* populations approximately 80% of the gametophytes may show this pattern of bisexuality (Naf, 1958).

The most rapidly growing gametophytes, those which are first to secrete antheridogen, develop into meristic females. It is thought that by the time effective concentrations of antheridogen are achieved, the antheridogen-secreting gametophytes have reached the insensitive stage. In *Pteridium* populations, only about 20% of the gametophytes develop as meristic unisexual females. However, in isolation, virtually all gametophytes are reported to follow this pattern (Naf, 1958).

The antheridogen assures the production of eggs by robust gametophyte plants that are likely to be able to support the young sporeling plant. If other gametophytes were present, a supply of sperms would be made available and synchronized to the production of the egg. This would assure that other gametophytes present would produce only sperms, resulting in reduced intraspecific competition among too many sporophytes (limitation of the number of egg bearing gametophytes) and possibly reduced interspecific competition by gametophytes (exclusion of other species from trying to colonize the site) (Willson, 1981). This control of sexual expression, sequence, and reproduction clearly favors outcrossing, but it could doom the isolated gametophyte which would have no neighboring gametophytes to convert to males.
In isolated gametophytes, the only expected effect of an antheridogen system would be the possible effect of developing female cordate gametophytes which produce antheridogen, but are unable to respond to it, and thus, may be incapable of attaining bisexuality. This could be overcome by production of physiologically isolated gametophytic proliferations as discussed above.

**Density effects on gametophytes** Early reports noted that crowding in cultures promoted maleness and that isolate or single prothallus cultures were much retarded in germination and growth (Kurel-Py, 1950; Bell, 1958; Miller, 1968).

Albaum (1938) cited reports by Kny (1872) and DeBary (1878) indicating that prothalli growing in crowded cultures of Osmunda regalis or Pteris cretica produced proliferations of secondary cordate prothalli.

The effects of high culture density were summarized by Smith and Rogan (1970). Goebel (1905) noted delay of two dimensional growth. Pietrykowska (1962b) found that prolongation of filamentous growth by crowding of Matteuccia struthiopteris gametophytes was irreversible after a few weeks.

Smith and Robinson (1969, 1975) found low density combined with high light levels increased the percentage of apolar gametophytes in Polypodium vulgare. Apolar gametophytes ranged from branched filaments to callus-like clusters of cells. The
authors indicated that these differed from the filamentous gametophytes favored by low light levels or high density. They noted that although no apolar gametophytes were present at 26-710 lux regardless of density, 5% were present at 1600-3600 lux at 10 gametophytes/mm² and that at 1 gametophyte/mm², 25% were apolar. As spores aged, the effect increased; up to 30% were apolar. If isolated (or aged) spores were grown on media supplemented with extracts of old media, the percentage of spores producing a rhizoid only, and the percentage of apolar gametophytes were reduced.

Steeves et al. (1955) noted prothallial colonies of the type reported by Hurel-Py (1950). He observed that isolated gametophytes were frequently sources of such "colonies prothalliennes".

Bell (1958), noting that isolated spores of Thelypteris palustris and Dryopteris filix-mas produced abnormal gametophytes, suggested that a minimum concentration of required metabolites may be necessary for normal gametophyte development (see LITERATURE REVIEW, Monospore Culture for additional review of effects of isolation). This concentration may not be attained with isolated spores or gametophytes, especially if diffusion into a relatively large volume of medium is unrestricted. Consistent with this view, Bell (1958) suggested that the behavior of spores of Thelypteris palustris and Dryopteris filix-mas in agar culture are
conspicuously more regular than that of *Pteridium aquilinum*. The spores of the former possess a perispore, which may retard diffusion of metabolites into the medium.

**Other factors**  
Other promotive and inhibitive effects may occur in gametophyte populations.

Albaum (1938) reported that indole acetic acid (IAA) was present in fern gametophytes and that it had been found to stimulate growth in low concentration and inhibit growth in high concentration.

Bell (1958) noted that a water extract of old prothalli of *Dryopteris filix-mas* promoted the growth of gametophytes of *D. borreri*, but the prothallial debris and the agar on which the spores and gametophytes had been growing strongly inhibited germination and growth.

Bell (1958) noted that varying proportions of aberrant prothalli, obtained when different species are sown on the same medium, may result from differences in metabolism and spore morphology. For example, the presence of a perispore might reduce diffusion.

**Effects of auxins**  
Albaum (1938) studied the relationship of auxin to production of secondary cordate prothalli which arise from individual prothallial cells. He provided an extensive review of reports of this type of proliferation. As indicated by Albaum (1938) and reports cited above, these proliferations have
been associated with a variety of factors, including abortive prothalli, old prothalli, crowding, low light, different wave lengths of light, exposure to x-rays, plasmolysis, and experimental cutting of prothalli.

Albaum's experiments established that auxin originating in the apical meristem is transported polarly and inhibits formation of secondary prothalli in a manner analogous to inhibition of lateral buds by the apical meristem in flowering plants. If gametophyte meristems were excised or separated from basal portions by dead cells, proliferations formed on the parts separated from the meristem. Auxin applied to the apical cells of the separated portion fully replaced the inhibitory effect of the meristem. Albaum suggested that factors previously associated with proliferations were compatible with the hypothesis that they acted through reduction in meristem activity and auxin production, through interruption of auxin transport, or through reduction of auxin concentrations to ineffective levels.

For example, in very large old gametophytes basal cells may be far enough removed from the apex to be released from inhibitory effects. Similarly, loss of auxins to the agar medium may prevent attainment of inhibitory concentrations within prothallial cells.

This association of auxin production with active growth of the meristem offers the possibility that any factor which reduces apical growth may also reduce auxin production and thereby allow
initiation of proliferative growth.

**Effects of fungi** Some fungi have been reported to have a stimulatory effect on gametophytes growing in culture, some have influenced gametophyte morphology, and others have been found to be inhibitory.

Hutchinson and Fahim (1958) reviewed and extended previous work on the effects of fungi on bracken fern gametophytes. One dramatic instance of the stimulatory effect was observed by Wilkie (1954) who cultured spores from a "crested" population of *Pteridium aquilinum* that developed only to a single-cell stage gametophyte in pure culture. However, the presence of any of a variety of fungi or actinomycetes allowed the gametophytes to develop into normal adults. He was not able to duplicate the effect by substituting a variety of nutrients, vitamins, or growth substances.

Hutchinson (1967) examined the effects of volatile metabolites from 16 fungal species on growth of *Pteridium aquilinum*. Fourteen stimulated growth compared to controls. Eleven produced statistically significant increases. The increase in gametophyte surface area ranged from less than 50% to over 500%. Two fungi inhibited growth. Pares (1958) conducted similar studies.

Bell (1958) reported an unknown fungal contaminant that stimulated germination and gametophyte growth of *Thelypteris*
palustris compared to gametophytes grown in sterile cultures.

Smith and Robinson (1969) studied the effects of fungi on the morphogenesis of gametophytes of Polypodium vulgare grown for over 3 months. *Fusarium oxysporum* added to pregermination cultures prolonged one dimensional growth, and if added after two dimensional growth had been attained, it caused growth to revert to one dimensional growth. *Geotrichum candidium* and *Aspergillus niger* had similar, but less pronounced effects. The authors suggested that fungal metabolites, such as ethanol and acetaldehyde may have been involved, since they inhibit two dimensional growth and rhizoid production (Miller et al., 1970). Tests of these chemicals produced similar anomalous growth. In general, gametophytes that showed early branching or apolar growth did not produce normal adult plants. Although not conclusive, the evidence suggests that the initial unequal division, giving rise to rhizoid and protonemal cell, was an essential prerequisite for normal morphological development.

Response to fungi may differ among plants within a species. Hutchinson (1976) noted that different prothallial strains of *Pteridium aquilinum* were differentially susceptible to fungal attack. With special effort to expose gametophytes to well-established fungal colonies under favorable conditions, Hutchinson (1976) found that 18 of 20 tested pathogens could initiate at least local infections. However, in ordinary non-sterile cultures
of thousands of prothalli and with sterile spores sown on a
variety of native soils of bracken sites, he found only two
naturally occurring fungal contaminants which were clearly
pathogenic in green cells. Reports by other workers vary.

Mottier (1927) and Hurel-Py (1950) reviewed the early work on
vegetative proliferation of prothalli which formed colonies of
secondary prothalli. Hurel-Py (1950) was of the opinion that this
only occurred in pure culture, suggesting that fungi may have some
positive regulatory effect on gametophytes. It was then
demonstrated that pure cultures with glucose, sucrose or fungal
contaminants added were stimulated in growth and did not produce
abnormalities.

Campbell (1905) reported that endophytic fungi occur in the
green prothalli of Marattia douglasii Baker, Kaufussia
aesculiflora Bl., Ancopteris evecta Hoffm., Gleichenia
polypodioides Sm., G. dichotoma Willd., G. laevigata Hook., G.
pectinata Presl., and Osmunda cinnamomea L. Growth was normal and
no disruption of morphology was noted as a result of fungal
symbionts. Mottier (1927) found an endophyte in older portions of
a long-cultured gametophyte of Osmunda claytoniana. The fungus
was confined to portions of the midrib, with filaments passing
from cell to cell through pits in the cell wall. The prothallus
was apparently not injured by the presence of the fungal
endophyte.
Hepden (1960) and Harly (1969) reported that mycotrophic fungi were widespread in their association with gametophytes of leptosporangiate ferns. Pirozynski and Mallock (1975) reported that endotrophic vesicular-arbuscular fungi were conspicuous in the gametophytes or sporophytes in all groups of extant ferns. They considered this association as probably obligate.

**Internal factors**  Intrinsic factors affecting growth and development include spore age and spore size. These may influence growth rate and growth form. Different species have shown optimal growth responses to different levels of light, temperature, and pH.

Smith and Robinson (1975) reported that increased spore age was associated with a lower rate of cell division and a higher percentage of abnormal gametophytes. They suggested that older spores declined in ability to synthesize metabolites essential for cell growth. At low spore densities, this would lead to abnormal growth patterns, while high density spore cultures would be able to compensate by the additive effect of neighboring plants.

**Interactions among factors**  Interactions among factors, such as the compensation by increasing spore density for declining competency due to increased spore age (Smith and Robinson, 1975), suggest that profoundly confounding results may be obtained. Effects which might be apparent and possibly significant in the less favorable circumstances met under natural conditions may well
be masked under the generally highly favorable growth conditions in artificial culture (Robinson and Park, 1966).

Interpopulational Variation

The preceding review adequately indicates that substantial variation in many features of growth and development may exist among fern species. The following section presents a few examples to indicate that variation is possible and does occur among populations of the same species.

Pray (1968) reported that sexual populations of Pellaea andromedaefolia differed in gametophyte morphology.

Variation among populations of Ceratopteris thalictroides was noted for gametophyte morphology, sex ratios, and frequency of sporophytic lethals (Klekowski, 1970c).

Two populations of Anemia mexicana were found to differ in the ability of spores to germinate in isolation, gametophyte morphology, timing of initiation of developmental stages, and percentages of different morphological types (Nester, 1979; Nester and Schodlbauer 1981, 1982).

Even these few examples suggest that populations of the same species may vary in isolate potential or features that influence isolate potential. Thus, reproductive potential determined for a single population cannot be assumed valid for the species as a whole.
Gametophyte Sexuality

According to Klekowski and Lloyd (1968), in order for intragametophytic mating to be possible, "obviously, the most important factor is the attainment of a hermaphroditic condition." Unfortunately, except for the very few studies of gametophytes in monospore culture, nothing is known about the sexuality of gametophytes developing from isolated spores.

In their review of fern gametophyte development, Atkinson and Stokey (1964) indicated that one focus of interest for investigators of the late 1800s was "whether the fern thallus, especially that of the Polypodiaceae (e.g.), was monoecious or dioecious, that is, whether sex organs were produced on the same or different thalli." Stokey and Atkinson asserted that, "It has now become obvious that the dioecious thallus, in this, or any other homosporous fern group is rare, if indeed it exists at all."

Relative to the sequence of sexual development, they reported that, "In homosporous ferns, antheridia appear before archegonia and continue to develop while the archegonia are mature." Archegonia were reported to appear "one to several weeks later than the antheridia."

Atkinson and Stokey (1964) had studied the development of many different species of gametophytes. Their generalizations about the bisexuality of fern gametophytes have been frequently
cited. Although Atkinson and Stokey (1964) reviewed work on antheridogens, the full significance of antheridogen influences was not yet appreciated. In reporting that, "Antheridia are often found, especially in the higher ferns, on filamentous or amercistic thalli which frequently grow in tangled rhizoids of mature thalli," they did not suggest antheridogen action as a possible cause. Rather, they presumed that these were "specimens whose growth has been retarded because of unfavorable environment."

Because it is known that antheridogen systems alter sexual development in gametophyte populations, reports of sexual development from studies of gametophyte populations (e.g., Nuyama, 1975a, 1975b) cannot be considered valid for isolated gametophytes.

Atkinson and Stokey (1964) also reported an influence of light upon sexuality. They found that bisexual gametophytes placed under low light for several weeks produced only antheridia, but production of archegonia resumed if gametophytes were returned to higher light conditions.

Warne and Lloyd (1960) reported that temperature had a dramatic effect upon sexuality of Matteuccia struthioteris gametophyte populations. While gametophyte populations grown at 11 C and 16 C included male, female, and bisexual gametophytes after 95 and 65 days respectively; populations grown at 21 C, 24
C, and 27 C were exclusively male after 102 days.

Spore size has been reported to influence sexuality. The homosporous fern *Platyzoma* shows a bimodal distribution of spore size with the smaller spores developing into male gametophyte and the larger spores developing into female gametophytes. It has been proposed that this species shows "incipient heterospory" (Tryon, 1964; Tryon and Vida, 1967). Other reports show an association of spore size with sexuality primarily through differential timing of germination among spores and differential growth rates interacting with antheridogen effects (Schedlbauer, 1976; Wester, 1979).

Factors Affecting Fertilization

The possibility that factors other than genetic load may influence fertilization success has received very little consideration.

Hottier (1925) reported that fertilization was influenced by light levels and time of day. He indicated that "the best time was found to be near midday during sunny weather."

The work of Evans and Bozzone (1977, 1978) indicated that pH of 5.6 or below reduced sperm motility and pH below 4.2 reduced the rate of sporophyte production in *Pteridium aquilinum* by 50%. Also, the presence of chloride, nitrate and sulfate ions in buffered solutions reduced sperm motility and sporophyte production at all pH levels tested. Reduction was significant.
Addition of 86 mM sulfate solution reduced motility and effective fertilization by 50% or more.

Sufficient water must be present to facilitate fertilization. Nester (1979) reported that motile sperm of *Anemia mexicana* were not observed unless the gametophytes were completely covered with water. A thin film of water was not sufficient for antheridia to open.

Peck (1980) found that supplemental watering substantially increased sporophyte formation in natural gametophyte populations at Woodman Hollow, Iowa. Water was a primary limiting factor for sexual reproduction in these populations. In his studies of natural populations of *Woodsia obtusa* gametophytes, he felt that lack of water may have influenced the breeding system by favoring intragametophytic selfing.

Kleckowski and Baker (1966), noted that archegonial necks of most homosporous ferns "are curved inward, away from the apical notch of the gametophyte, thereby pointing their mouths toward the antheridia and rhizoids. This appears to be an adaptation to self-fertilization. If this were a cross-fertilizing system, selection should favor archegonial necks curved in the opposite direction." Although this assertion has been repeated in subsequent publications (Lloyd, 1974a), there appears to be no experimental evidence indicating that the curvature of archegonial necks influences fertilization in any way.
Genetic Load

Diploid organisms may shelter a number of recessive deleterious or lethal genes in the heterozygous state. Dobzhansky and Spassky (1953, 1963) experimentally produced Drosophila with genotypes homozygous for a single chromosome. They found that, "the homozygotes are often lethal, semilethal, subvital, sterile, or show various physiological or structural abnormalities" and that, "almost all chromosomes, at least in the populations studied, are more or less deleterious when homozygous." They concluded that "most or all individuals of sexually reproducing, diploid and outbreeding species carry in heterozygous condition genetic variants which are deleterious or even lethal in homozygotes." This concealed detrimental genetic variation constitutes the "genetic load" of the individual or population.

Studies indicate that significant levels of genetic load may be present in outbreeding organisms (Dobzhansky et al., 1963). In Drosophila, the percentage of chromosomes which were lethal or semilethal in the homozygous condition ranged from 9.5% to 60% in various populations studied (Dobzhansky et al., 1977). Morton et al. (1956) estimated that an average person carries 3-5 genetic lethals which affect late fetal through early adult stages. Since this did not include undetected embryonic deaths or post-maturity lethals (including infertility), he assumed true levels to be at least 2-3 times higher.
Load is presumed to be of two types. In the first type, mutational load, the allele in question is either neutral or somewhat detrimental in the heterozygous condition. This load will tend to be eliminated to the extent it is exposed to selection in the homozygous state. In sexually reproducing populations, an equilibrium will be established between mutation rate and selection.

In the second type, heterotic or balanced load, the detrimental allele is maintained at a relatively high level in the populations because, although it is deleterious or lethal when homozygous, it is favored in the heterozygous condition (i.e., it exhibits heterosis). The classic example of heterotic load is the sickle-cell trait in humans (Goodenough, 1973).

Because homosporous ferns have the potential to form completely homozygous sporophytes in a single generation of intragametophytic selfing, they were perceived to be ideal organisms for the study of genetic load. Since the late 1960s, approximately 30 studies have examined genetic load in 50 species of homosporous ferns (Table 1). Estimates of genetic load reported in these studies ranged from 0% to 100%.

Approximately 25% of the reports indicate no genetic load for the plants or populations tested. A similar proportion of the studies revealed genetic load to be 20% or above. Variation is present both within and among species. The extent of variation
Table 1. Systematic list of fern species studied in genetic load investigations

<table>
<thead>
<tr>
<th>Species</th>
<th>% Genetic Load/# Gametophytes</th>
<th>Reference</th>
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<td></td>
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<td>Adiantium capillus-veneris</td>
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<td>Haufler &amp; Gastony (1978a)</td>
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</tr>
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<tr>
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<tr>
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<tr>
<td></td>
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<td>% Genetic Load/ # Gametophytes</td>
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<td>0-8/959</td>
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<td>Singh &amp; Roy (1977)</td>
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<td>36-67/213</td>
<td>Hasuyama (1979)</td>
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<td>Ganders (1972)</td>
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<td><em>Cibotium glaucum</em></td>
<td>26-84/?</td>
<td>Lloyd (1974b)</td>
</tr>
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</table>
differed among species. Cousens (1979) reported a range of genetic load from 10% to 98% for *Blechnum spicant* and a similarly wide range has been reported for *Osmunda regalis*. However, *Ceratopteris thalictroides* and *Acrostichum danaeifolium* show a much narrower range of variation.

The methodology utilized in these studies, originally outlined by Klekowski and Baker (1966), has been reviewed and updated (Klekowski, 1979). Gametophytes are initially grown in multipore culture. Prior to attainment of sexual status, a random sample (usually 25 gametophytes) of morphologically normal gametophytes are transferred to isolate culture (usually in 60 x 20 mm plastic petri dishes). Upon becoming bisexual, gametophytes are watered weekly for several months. Gametophytes are then scored for the presence of sporophytes. Those bisexual gametophytes which do not form sporophytes are presumed to carry lethal genes affecting gametes or early sporophyte development.

In some instances, the genetic basis has been further supported by cloning isolates and demonstrating the formation of sporophytes when these clones are paired with others bearing nonallelic lethals.

The level of genetic load has been interpreted as being indicative of the population's breeding system. Absence of genetic load was considered evidence that the fern being studied was inbreeding since successful intragametophytic selfing requires
absence of genetic lethals. Lethal and deleterious genes would be totally expressed in the completely homozygous offspring and would be eliminated by selection. Presence of significant levels of genetic load were considered to be evidence of an outbreeding mating system. Load was present because the deleterious alleles were sheltered in the population in the heterozygous condition. Klekowski (1982) indicated that it was not known whether the genetic load observed in ferns was due to heterotic or to mutational load.

If, or when, genetic load is heterotic, the level of genetic load in a population will be closely related to the mating system operating within the population. Outbreeding allows heterotic load to increase; inbreeding reduces or eliminates load.

To the extent that observed genetic load is mutational, the level of genetic load in the population will be dependent upon mutation rates and the age of individuals in the population, as well as, the mating system. New load generated through mutation may or may not be heterotic. Only mutations which are deleterious in the heterozygous condition will tend to be reduced to low equilibrium levels in outbreeding populations.

Because many fern populations exhibit heterozygosity for recessive lethals, some fern biologists have assumed that such levels of genetic load must be due to heterotic selection (Klekowski, 1984 cites Lovis, 1977).
Through analysis of lethal gametophytic mutants in the long-lived clonal species *Onoclea sensibilis* and *Matteuccia struthiopteris*, Klekowski (1984) concluded that somatic mutation rates alone could account for high levels of genetic load. Previous studies presented models showing that plants, such as most fern sporophytes, with permanent single-celled meristems are more likely to retain somatic mutations than are typical higher plant apices (Klekowski and Kazarinova-Fukshansky, 1984a, 1984b). Chromosomal mutants which affect meiosis but not mitosis could also accumulate in such long-lived clones. These would produce defective meiotic products, but, unlike the gametophytic mutants considered above, there would be variation in morphological expression, and ratios of the different types would be non-Mendelian (Klekowski, 1984).

Klekowski (1982) has shown that the potential of fern gametophytes for simple polyembryony (Etter, 1923; Mottier, 1925) reduces expressed genetic load to zero in panmictic populations, thus, reducing selection against recessive lethals. This allows a much higher frequency of heterozygosity for recessive lethals to occur in the population. This also leads to strong inbreeding suppression and selection against recessive lethals under conditions of restricted mating. The most extreme restriction would be the required intragametophytic mating necessary for the establishment of a new population from a single spore.
Homoeologous Chromosome Pairing

It is axiomatic that heritable variation is essential for evolutionary potential. This concept, demonstrated mathematically for a single locus and experimentally at the organismal level, is known as the "The Fundamental Theorem of Natural Selection", which states that "the rate of increase in fitness of a population at any time is equal to its genetic variance in fitness at that time." In other words, "natural selection can only occur if there is hereditary variation. The more genetic variation there is in a population, the greater the opportunity for the operation of natural selection" (Dobzhansky et al., 1977).

In 1966, Klekowski and Baker published a landmark paper for fern genetics. They pointed out the potential for intragametophytic selfing and consequent total homozygosity in homosporous ferns. They recognized that if this type of reproduction was common in the homosporous ferns, genetic variability and thus, evolutionary potential would be minimized in these ferns.

They showed that homospory was associated with high chromosome numbers, presumably due to ancient and modern polyploidy (Wagner and Wagner, 1980). It was suggested that high levels of polyploidy were a mechanism to compensate for reduction in genetic variability and heterozygosity resulting from intergametophytic mating. Duplication of the genome through
polyploidy could allow individuals which were homologous homozygotes to be homoeologous heterozygotes. Homoeologous pairing of chromosomes at meiosis would allow recombination and release of this variability, producing genetically varied spores from totally homologous-homozygote parents (Klekowski, 1973a).

Since the Klekowski and Baker paper, a variety of evidence has been presented in support of the occurrence of homoeologous chromosome pairing (Klekowski, 1973; Hickok and Klekowski, 1973; Klekowski and Hickok, 1974; Bierhorst, 1975; Hickok, 1978; Chapman et al., 1979).

However, Lovis (1977) noted that homoeologues generally fail to show any pairing in wide hybrids. He suggested that "if homoeologues fail to pair when there are no apparent restrictions to prevent them from doing so, it is extremely difficult to see why they should ever do so when faced with competition from true homologues." That other explanations are possible for the genetic phenomena attributed to homoeologous pairing has been suggested (Lovis, 1977; Chapman et al., 1979).

Whether frequent homoeologous pairing is required as a special mechanism to compensate for elevated levels of homozygosity due to regular intragenetophytic selfing depends upon the prevalence of these events. Only recently has evidence of levels of heterozygosity in natural populations been presented. Evidence for widespread homozygosity has not yet materialized.
Electrophoretic evidence suggests that patterns of heterozygosity and segregation of alleles from naturally occurring sexually reproducing populations of some homosporous fern species are compatible with Hardy-Weinberg expectations for segregation of Mendelian alleles.

Gastony and Gottlieb (1982) demonstrated that heterozygosity in two natural populations of *Pellaea andromedaefolia* was due to allelic heterozygosity at single gene loci. In comparisons of sexual and apogamous populations of this fern, electrophoretic data showed heterozygosity for both, but segregation was observed only in sexual populations (Gastony and Gottlieb, 1985). The authors concluded that "the results suggest that genetic variation in sexual homosporous vascular plants is produced by cross fertilization of genetically different gametes and may not result from pairing between homoeologous chromosomes carrying duplicated loci as previously thought."

Haufler and Soltis (1984) found levels of polymorphic loci and individual heterozygosity in the fern *Bommeria hispida* to be similar to those reported for other outcrossing plant species.

Gastony and Darrow (1983) analyzed multiple-banded enzyme phenotypes found in electrophoretic study of *Athyrium felix-femina* (L.) Roth. Such patterns have been considered to be evidence of duplicated loci occurring on homoeologous chromosomes of polyploid taxa (Chapman et al., 1979). However, Gastony and Darrow, found
that *A. felix-femina*, 2n=80, was functionally diploid; the multiple banding patterns could be resolved into single non-duplicated loci for isozymes compartmentalized in either the cytosol or chloroplast fractions of sporophyte leaf tissue.

In addition, many studies of inheritance of mutant characters have shown segregation ratios characteristic of diploid heterozygotes (Andersson, 1923, 1927; Andersson-Kotto, 1931; Haigh and Howard, 1970; Klekowski, 1969b, 1984; Lang, 1923).

To date, evidence for homoeologous chromosome pairing in homosporous ferns is no more compelling than for flowering plant polyploids. It seems best to consider homoeologous pairing, if it occurs in ferns, to be an occasional aberration rather than a regular feature of fern reproduction. Whether intragametophytic selfing and consequent homozygosity are significant reproductive features for some homosporous ferns is yet to be determined.

Gametophytes in Nature

Most of our understanding of sexual reproduction in ferns is derived from laboratory studies of gametophyte cultures. Because of the rarity and unpredictability of their occurrence, the most pertinent observations of gametophytes in nature, the sexual and reproductive potential of isolated gametophytes growing outside parental sporophyte populations, are not amenable to study. The few ecological studies of gametophytes in nature have focused on gametophyte populations growing within parental sporophyte
populations. Peck (1980) summarized the problems inherent in these studies.

Controlled studies and observations of natural populations have shown temperate homosporous fern gametophytes to be physiologically highly capable of withstanding winter extremes of temperature (Sato and Sakai, 1980, 1981). In nature, the majority of individual gametophytes failed to survive more than a calendar year (Happen, 1964; Schneller, 1975; Farrar and Gooch, 1975; Peck 1980; Cousens, 1979, 1981). Mortality was due mainly to desiccation and substrate heaving from frost. These workers also observed that some gametophytes can overwinter to the following growing season. Populations which they observed occurred mainly as small high density gametophyte populations. These were located in the vicinity of parental sporophyte populations.

This contrasts with observations by Farrar (1967, 1974, 1976), who reported on perennial populations of independently reproducing gametophytes of four tropical fern genera surviving in temperate North America. These gametophytes, which occur in highly moderated microhabitats, survive freezing weather; however, they seemingly have lost most of their potential for sexual reproduction. Their dispersal is most probably only vegetative, by gemmae.

Tryon and Vitale (1977) provided evidence for activity of an antheridogen system in natural populations of *Asplenium*
pinpinellifolium Fee and Lygodium heterodoxum Kze. They studied the population with respect to gametophyte size, spacing, and number of antheridia and archegonia. Small gametophytes growing in colonies usually had high numbers of antheridia. Gametophytes of the same size, growing at least 15 cm from the nearest neighbor had few antheridia. Heavily antheridiate gametophytes in colonies grew in close proximity to large archegoniate gametophytes.

This population structure contrasts with that reported by Peck (1980), who found that isolated gametophytes of Woodsia obtusa were predominantly male (74%), while only 5% were females, and 21% were bisexuals. Gametophytes in the laboratory showed evidence of an antheridogen system. Populations in nature were highly antheridiate (80%–50%), but increasing density was not found to correlate with an increase in maleness. Although ameristict males were observed in agar culture, none were found in nature. Females increased from 5% to 30% as population density increased. Bisexual gametophytes accounted for 8–20% of the gametophytes in all populations.

Farrar and Gooch (1975) noted a high percentage of bisexual gametophytes in a natural population of Cystopteris tenuis gametophytes. But whether sex ratios alone are indicative of reproductive patterns is not clear. Peck (1980) found that although the sex ratios indicated an outbreeding mating system, bisexual gametophytes of Woodsia obtusa produced sporophytes in
numbers out of proportion to their relative abundance in the
gametophyte population.

Even if inbreeding is the most common form of sporophyte
production, homozygous sporophytes may not be as likely to survive
and contribute to subsequent generations as would heterozygotes
produced by outcrossing. Combining breeding system analysis with
electrophoretic study of different age-classes in the population
might indicate the relative contribution of each type of
reproduction.

Monospore Culture

Information relating to the biology of isolated gametophytes
is quite sparse. In the literature, references to isolated
gametophytes often refer to gametophytes in a multispore culture,
but with no near neighbors. Substances diffusing from
gametophytes may influence development of other gametophytes over
some distance. For example, Voeller (1971) demonstrated
antheridigen to be effective over 25 cm in agar culture.
Therefore, reports of 'isolated' gametophytes in multispore
cultures, though possibly suggestive, can not be relied upon for
information about the intrinsic developmental potential of the
isolated gametophyte.

There have been about 30 reports based on single gametophyte
culture (see Table 1 and literature cited below). These dealt
with 50 species, only about four-tenths of one percent of the
known fern species.

However, most of these publications report studies of genetic load (Table 1) which were based on gametophytes that were transferred from multispore culture to isolate culture at some stage prior to attainment of bisexuality. While these studies provide valuable information regarding the ability of transferred gametophytes to attain bisexuality and the occurrence of genetic load, they provide limited information regarding the development potential of single spores.

Because of population effects on spore germination, early gametophyte-gametophyte interactions which influence morphology and sexuality, and the disruptive effects of transfer, only monospore culture can provide the fundamental data on the potential for reproduction from isolated spores which is essential for evaluating the potential of various fern species to establish new populations by long distance dispersal.

All of these formed abnormal gametophytes. Bell stated that, "each grew slowly into a nodular mass of cells reaching about 3 mm in diameter, eventually producing a cluster of cordate prothallial lobes over its surface and filamentous outgrowths from its base."

Cousens (1969) sowed 50 Dryopteris ludoviciana spores in monospore culture on soil. He reported the sexual status of eight of these at day 45; one was neuter, six were female, and one was bisexual. Sexual ratios of gametophytes transferred with intact soil mass were similar. No males were observed in any isolate culture, whereas males and bisexual gametophytes were common in multispore cultures. Ameristic males were present in dense cultures, suggesting the activity of an antheridogen system. Sexuality was delayed in isolate cultures as compared to cultures of moderate and high density.

Mester (1979) established monospore cultures from two populations of Anemia mexicana. In one population, she found germination greatly reduced (62%) compared to multispore cultures (94%). Of those germinating, only 12% survived to two weeks of age. The morphology of the surviving isolates was highly abnormal. Each gametophyte had several lobes showing unorganized growth. From the second population, 67% of the spores germinated compared to 82% in multispore cultures. Gametophyte growth was slower in monospore culture than in multispore culture. Isolates were all female first. Only one isolate became bisexual and only
after initiating a secondary prothallial lobe. In multispore cultures, 76% of the gametophytes were male, only 6% were female, and almost none were bisexual.

Although Schneller (1979) does not indicate method of transfer or isolate establishment, or number of spores observed, he states that, "isolated males of *Athyrium felix-femina*, if growing well, develop into bisexuals and then functional females, whereas, isolated neuter gametophytes or isolated spores develop first into females."

Schedlbaur and Klekowski (1972), in their study of antheridogen activity in *Ceratopteris thalictroides*, have given the most complete report to date of development of gametophytes in monospore culture. Information on speed of germination relative to spore size, sex ratios relative to spore size, sex ratios relative to heterozygosity or homozygosity of the spore parent, differences in sex ratios in response to old-media extract (antheridogen) supplemented media versus response to non-supplemented media, morphological and developmental data for male gametophytes on supplemented media and bisexual gametophytes on non-supplemented media were followed and compared. Over 400 gametophytes were studied for one or more of these characters. Male gametophytes were the most common type in multispore cultures, but were not reported from unsupplemented isolate cultures. Isolates from all unsupplemented cultures became
Wilkie (1963) established 133 monospore cultures of *Pteridium aquilinum*. He reported that approximately 20% developed into aneristic males under three conditions (sterilized, non-sterilized, and sterilized on a nutrient supplemented medium). Cultures with 50, 100, and 500 gametophytes produced approximately 30%, 36%, and 62% aneristic males. The presence of aneristic males in isolate culture, though at a lower rate than at higher densities, cannot be explained as antheridogen induced. These observations warrant repeating.

These studies suggest that isolated gametophytes may show poorer germination, slower growth, higher levels of aberrant morphology, and retarded sexual development in comparison to gametophytes from multispore cultures. As predicted from studies of antheridogen action, some species formed only females in isolate culture, but variation among species was indicated.
MATERIALS AND METHODS

A total of 164 sporophyte plants of 14 species were chosen for study, including four plants of *Dryopteris cristata* (L.) Gray from Pilot Knob State Preserve, Hancock Co., Ia.; five plants of *Polypodium virginianum* L. (two from Hardin Co., Ia., and three from Warren Co., Mo.; five plants of *Cystopteris bulbifera* (L.) Bernh. from a ravine in Warren Co., Mo.; five plants of *Thelypteris novaboracensis* (L.) Nieuwl. from Rickett's Glen, Luzern Co., Pa.; five plants of *Thelypteris palustris* (L.) Schott from the New Jersey Pine Barrens; fifty plants of *Thelypteris simulata* (Davenp.) Nieuwl. with five plants from each of ten populations across five counties within the "driftless" area of Wisconsin; five plants of *Dryopteris marginalis* (L.) Gray from Iowa Co., Wi.; and five plants of each of the following species from Woodman Hollow State Preserve, Webster Co., Ia.: *Athyrium angustum* (Willd.) Presl, *Cryptogramma stelleri* (S. G. Gmel.) Prantl, *Cystopteris tenuis* (Michx.) Desv., *Dryopteris goldiana* (Hook.) Gray, *D. spinulosa* (O. F. Muell.) Watt, *Matteuccia struthiopteris* (L.) Tod., and *Woodsia obtusa* (Spreng.) Torr.

Scientific names of these species generally reflect usage in Mickel (1979) and Peck (1976, 1982). These species and plants were chosen for the following reasons: availability of spores, availability of previous studies providing important background
information, and status of species or plants as potentially
disjunct or peripheral populations which other authors have
suggested as being post-Pleistocene relicts.

Spores were collected from study plants by removing fronds or
portions of fronds from individual plant apices. Although the
limits of individual plants could not be reliably determined for
rhizomatous species, distinctly separated apices were chosen
within each sample population. For convenience, these will be
referred to as separate plants, even though their genetic
distinctness could not be ascertained absolutely.

Fronds were rinsed, and fertile pinnae were removed and
allowed to dry inside glassine envelopes for 1-3 days. The
envelopes were tapped to facilitate spore discharge. The pinnae
fragments were discarded, and the envelopes were sealed with
cellophane tape. The sealed spore packets were then labeled with
species name, collection locality and date. All spore packets
were stored under dark refrigeration at 5 C.

Culture medium consisted of a 0.8% agar solidified medium
containing Bold's macronutrients (Bold, 1957) and Nitsch's
micronutrients (Nitsch, 1951), supplemented with cobalt chloride
and ferric chloride. For multispore cultures, medium was poured
into 100 x 20 mm plastic petri dishes, which were placed in clear
plastic boxes (10 x 20 x 26 cm vegetable crispers). For isolate
cultures, 4 ml of medium were poured into each 1.5 x 3 x 4 cm
clear plastic jelly mold (Carolina Biological Supply, Burlington, N. C.). Four or five sheets of jelly molds, each sheet with twenty-five molds in a 5 x 5 array, were stacked alternately with clear cellulose-acetate cover sheets and placed in clear plastic boxes, described above.

Spores were sown from the packets to the culture dishes with aseptic precautions. Multispore sowings were conducted by poking several small holes in the packet with a needle, and then gently tapping the packet over the opened petri plate. Resulting cultures were reasonably free of debris and contamination.

Multispore cultures were sown for each plant of each species. Three plates were sown per plant. Two plates were watered to facilitate sexual reproduction. As a control to check for apogamous sporophyte formation, the third plate was not watered.

Single spore sowings were conducted by poking several small holes in the spore packet with a needle, then tapping the packet over an empty petri dish. From the petri dish, individual spores were selected, picked up with a fine glass needle, and transferred to the surface of the agar solidified media. The needle tip was moistened by first touching the agar surface, then brought into contact with a single spore. Each culture chamber was examined with a dissection microscope to confirm the presence of a single spore. Twenty-five single spore cultures were sown for each plant of each species.
Cultures were maintained under continuous, cool-white fluorescent illumination of approximately 5000 lx (350-500 ft-c). Temperature was ambient, between 20-30 C. These conditions were representative of laboratory culture conditions reported by other workers (Dyer, 1979a).

Gametophyte cultures were examined weekly to assess germination success and subsequent initiation of gametangia. By week four, an initial observation of gross morphology was recorded, such as "typical development", "callus", "mound" or "multilobed".

After week four, when sex organs were observed on most gametophytes, through week 14, cultures were watered weekly with sterile, double glass-distilled water. Multispore cultures were flooded with water, then covered and allowed to stand for a minimum of one-hour before the excess water was poured off. The water could easily be drained off by tipping the covered petri dish.

Single spore cultures were watered by pipetting into each jelly mold enough water to cover the gametophyte, but not with enough water to allow water to be drawn between neighboring jelly molds (allowing sperm transfer to neighbors). Just as for the multispore cultures, water was allowed to remain for at least one hour on single spore cultures. Excess was then removed using short wicks made from sterilized paper towels. When the
gametophytes were larger, water was removed using an aspirator tipped with a glass pipette.

Two weeks after the final watering (week 16) data were collected on gametophyte size, morphology, sexual status and sporophyte production.

To compare morphological development among species and assess the relationship of morphology to other aspects of reproductive potential, gametophytes were classified into four groups: normal, multilobed, bushy mound, and callus.

Normal morphology was broadly interpreted to include gametophytes with one to a few (3-4) large cordate lobes. Multilobed gametophytes were dominated by numerous large cordate lobes. Bushy mound morphology included gametophytes with large numbers of proliferations originating from a central core. These hemispherical mounds were composed of branched filaments, two-dimensional straps of tissue, and/or small cordate lobes. The callus morphology included only very small gametophytes consisting of amorphous cell masses which lacked two-dimensional plates.

These morphological types formed a continuum. Changes from callus to bushy, or from bushy to multilobed occurred as callus types seemed to occasionally overcome some developmental hurdle and began to form two-dimensional lobes, or large dominant lobes formed from previously bushy gametophytes. Although separation
into these groups was somewhat arbitrary, the relative numbers in the four classes gives an approximation of the degree of morphological normality or abnormality for each species.

Gametophytes were censused for sporophyte production. Isolated gametophytes which produced sporophytes were divided into two groups: one-half were stained with basic fuchsin and examined microscopically to determine the sexual status of the gametophytes, while the other one-half were transplanted to soil-containing peat pots to maintain the sporophytes. Survival of these sporophytes was assessed after one year.

Of the isolated gametophytes which did not produce sporophytes during 16 weeks of culture, one-fourth were sacrificed and examined microscopically to determine sexual status. One-fourth of the gametophytes were retained as isolates. These were transferred to fresh medium, watered weekly for four additional weeks, and examined at six weeks (22 weeks after sowing) for sporophyte production and sexual status.

Percentages of spores or gametophytes attaining various reproductive states were calculated as indicated in Table 2.

Germination-development potential was based on the formation of viable gametophytes (as opposed to rhizoid emergence or production of the first prothallial cell). This combination of germination and establishment was considered more relevant to reproductive success than was an estimate based on a narrower
Table 2. List of defined terms used to contrast reproductive potentials of the study species

<table>
<thead>
<tr>
<th>Term</th>
<th>Formula</th>
</tr>
</thead>
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<tr>
<td>1. Germination-development Potential</td>
<td>( \frac{# \text{Gametophytes}}{# \text{Spores}} \times 100 )</td>
</tr>
<tr>
<td>2. Bisexual Potential</td>
<td>( \frac{# \text{Bisexual Gametophytes}}{\text{Total } # \text{Gametophytes}} \times 100 )</td>
</tr>
<tr>
<td>3. Selfing Potential</td>
<td>( \frac{# \text{Sporophytes}}{# \text{Bisexual Gametophytes}} \times 100 )</td>
</tr>
<tr>
<td>4. Genetic Load</td>
<td>( \frac{# \text{Non-sporophyte Producing Gametophytes}}{# \text{Bisexual Mature Gametophytes}} \times 100 )</td>
</tr>
<tr>
<td>5. Gametophyte Isolate Potential</td>
<td>( \frac{# \text{Sporophytes}}{# \text{Mature Gametophytes}} \times 100 )</td>
</tr>
<tr>
<td>6. Spore Isolate Potential</td>
<td>( \frac{# \text{Sporophytes}}{# \text{Spores}} \times 100 )</td>
</tr>
</tbody>
</table>
interpretation of germination.

Because there are a large number of factors that may affect germination, bisexual potential and gametophyte isolate potential were based on the number of mature gametophytes, rather than the number of spores sown. The number of non-sporophyte producing gametophytes per number of bisexual gametophytes was used as the measure of genetic load because it is most comparable to measures of genetic load used in fern studies cited in Table 1.

Spore isolate potential was included for comparative purposes, even though questions remained about the validity of the germination component.

The number of Iowa counties in which each species occurs was tabulated from Peck (1982). This number was used as an index of commonness for the study species.

The percentages of each morphological type, the percentages of each sexual type, the reproductive potentials, and the relative commonness for each species, as determined above, provided 16 variables for comparison. These 16 variables were examined for the extent of association between them through calculation of correlation coefficients (r) on an Epson QX-10 microcomputer with a statistical analysis program, STATPAK, prepared by Northwest Analytic, Inc., Eugene, Oregon. The r values were tested for statistical significance using the critical values provided in Table 25 of Rohlf and Sokal (1981).
RESULTS AND DISCUSSION I: SPECIES ACCOUNTS

Introduction

In the following section, results are presented separately for each species. Each species account includes background information, results of the present study, and a discussion of those results.

Multispore cultures were generally not analyzed. Although contamination was initially low, watering of these cultures tended to spread contamination and apparently contributed to an overall population decline before the 16 week analysis date.

Athyrium angustum

Introduction

Athyrium angustum is a large fern with upright erect fronds. It is typical of the mesic woods in northeastern North America. The species is abundant throughout its range (Mickel, 1979) and common in Iowa (Peck, 1980). The population occurring at Woodman Hollow, Webster Co., Iowa, that was used in this study is a peripheral outlier on the western edge of the range.

This species is part of a wide ranging, circumboreal complex of Athyrium felix-femina (Lady Ferns) which have been variously treated by different authors. This complex is particularly interesting because it has had several contemporary treatments of sporophytes (Liew, 1972) and gametophytes (Masuyama, 1975a, 1975b; Schneller, 1979; Peck, 1980). It seems to be generally accepted
that there are two distinct taxa in the eastern United States and Canada (A. \textit{angustum} (Willd.) Presl. primarily in the northeast and A. \textit{asplenioides} (Michx.) Desv. primarily in the southeast), neither of which is conspecific with the European A. \textit{felix-femina} (L.) Roth. (Butters, 1917; Wherry, 1948; Liew, 1972). The taxa are reported to be diploid (n=40) (Wagner and Wagner, 1966; Schneller, 1979).

Although typically occurring in mesic situations, Lady Fern apparently has a wide tolerance of habitat factors. Butters (1917) reports collections of "sun forms" occurring in "very dry open woods" and sunny roadsides that seem to indicate a tolerance for drier, less sheltered habitats.

There have been two important studies of the natural history of Lady Fern (Schneller, 1979; Peck 1980) that include sporophyte phenology, gametophyte development in laboratory and natural populations, and genetic load.

According to Schneller (1979), in nearly all instances, sporophyte populations are composed of many plants (14-57/100 sq m) and only rarely occur as isolated plants. He noted morphological variation among sporophytes, particularly as to stipe color (red or green), with both forms present in all populations, and suggested that the plants were established for a long time and gave rise to the two forms. He considered it less likely that populations were initially established from multiple
colonizations because inbreeding depression was noted within populations.

Schneller (1979) estimated an average spore production of 75 million spores per plant (15 million per frond x 5 fertile fronds per plant) and an average population density of 3 plants per 20 sq m. Peck (1980) estimated that plants of this species at Woodman Hollow produced an average of 53.1 million spores/plant. With approximately 500 fertile plants at Woodman Hollow, this made a total annual spore production of 26 billion spores.

Schneller (1979) reported an average spore germination of 84% (50-95%). He also noted that overwintered spores were still viable and that spores on soil (overwintered) germinated in spring. Peck (1980) measured even higher spore germination (average 94-95% with a range of 84-97%) at the time of maximum release, and a 2% reduction in spore germination after 15 months of storage at 5 C.

Peck (1980) estimated that he observed the best year in 20 years for gametophyte and sporophyte production at Woodman Hollow. Gametophytes of other species were present and often abundant in Woodman Hollow for the eight years of his study. *Athyrium angustum* occurred within 10 m of 35 gametophyte populations studied by Peck (1980), but in spite of proximity to sites suitable for gametophytes of other species (and large numbers of spores present) no gametophytes of *Athyrium angustum* were observed.
Similar observations were made by Farrar and Gooch (1975). Furthermore, Peck (1980) did not locate any small sporophytes suggestive of recent sexual production of sporophytes, but did find abundant evidence of asexual expansion of clonal apices.

In contrast, Schneller (1979) reported that gametophytes of *Athyrium felix-femina* were found in unexposed places on bare soil or, more rarely, on rotten wood. Furthermore, he found that suitable habitats were invariably fully colonized. Schneller (1979) did not indicate whether naturally occurring gametophytes were producing sporophytes or if natural populations of sporophytes showed a range of size classes which might indicate additions to populations through sexual reproduction.

Schneller (1979) demonstrated an antheridogen effect by showing dark germination and precocious antheridia formation for spores sown on fresh media supplemented with old media.

Although Schneller (1979) reported sexual development from multispore cultures, he did not indicate the number of cultures he examined nor the method of sampling used to determine sex ratios. Gametophytes were grown for 60 days. The first males were noted just prior to 30 days and the first females shortly afterwards. This may be due to the antheridogen effect that Schneller demonstrated. Bisexual gametophytes were rare, comprising approximately 3% of the gametophytes at 40 days and 1% at 60 days. By 60 days, males were estimated to account for nearly 50% of all
ganetophytes; females accounted for approximately 40%. The low proportion of bisexuels observed by Schneller (1979) may simply be due to the early age at which the cultures were terminated.

Schneller (1979) concurred with Masuyama (1975a, 1975b) in finding that prothallial width (measured perpendicular to the direction of growth) correlated with gametophyte sexual status. In measurements of gametophytes from 10 to 60 days old, he found that mean width increased from neuter to male, to bisexual to female. He also found that this relationship was present in naturally occurring gametophytes. The assumption advanced by Masuyama, that relative size also indicated sexual sequence, was not demonstrated. Females at 30 days were as narrow or narrower than males of any age. Only older females were distinctly wider.

Peck (1980) studied Athyrium angustum from the Woodman Hollow population. He found that by 33 days after sowing in multispore culture, 40% of the gametophytes had become sexual. Both males and females were present. This correlates well with the timing observed by Schneller (1979).

In regard to sexual sequence, Schneller (1979) reported that cultured females were cordate and later became bisexual. Large old females formed antheridia on separate outgrowths. Males were non-cordate. Some of the latter became cordate and bisexual, then female, and finally bisexual again.

Sexuality in isolate culture, according to Schneller (1979),
depended upon developmental state at the time of isolation. Isolated male gametophytes soon became bisexual; whereas, isolated sterile gametophytes or isolated spores developed first into females.

In natural populations of gametophytes, Schneller (1979) found both male and female gametophytes. Males predominated, being two to four times the number of females. A small percentage of bisexual gametophytes were present in only two of five populations examined. Although he indicated that the prothalli in natural populations were of different ages, the two populations containing bisexual gametophytes also had the fewest neuter gametophytes, possibly indicating that these populations were older or growth conditions were more favorable than in the other three.

From his observations of naturally occurring gametophytes, Schneller (1979) concluded that gametangia of bisexual prothalli were situated in different regions and separated by a sterile zone. The archegonia grew near the initial cells on young tissue; the antheridia grew in the rhizoid region on older tissue. The necks of the archegonia were turned towards the rhizoid region. In many of the bisexual prothalli, the antheridia were empty when archegonia were present. The kind of old and big gametophytes found in cultures, bearing archegonia in the center and antheridia on special outgrowths or wings, were never observed in nature.
Schneller did not indicate whether ameristic males were present in nature.

Genetic load of *Athyrium felix-femina* was examined by Schneller (1979) using a pairs and isolates test. He tested 15 plants from seven populations. He tested 200 isolates (114 gametophytes from six plants of one population; 32 gametophytes from four plants of one population; 9 to 16 gametophytes for a single plant of five populations). He did not give load estimates separately for the first two populations, nor did he indicate within population variation. Average load was 73% (range of 90-44.4%).

Schneller (1979) established pairs of three types (from the same sporophyte, from different sporophytes from the same population, and from different sporophytes from different populations). The three types of pairs, respectively, produced 79%, 96.9%, and 100% sporophytes. The results indicated that inbreeding depression was present within sporophytes and within populations.

When Schneller (1979) cultivated the sporophytes produced in these experiments, a higher percentage of sporophyte deaths and abnormalities occurred among sporophytes produced by isolates (42-20%) than among sporophytes produced from pairs. Pairs from the same sporophyte produced sporophytes of which 32% died and 6% were abnormal; whereas, pairs from different sporophytes produced
sporophytes of which only 7% died and none were abnormal.

Results

Experimental results for Athyrium angustum are presented in Tables 3, 4, and 5. Of 125 spores sown, 90 (72%) produced mature gametophytes by 16 weeks (Tables 3 and 4). Of these gametophytes, 99% were single cordate lobes; 1% were multilobed (Table 33). Sampling for sexual status at this time revealed that only 24% of the gametophytes had attained bisexual status; the remainder were female. Only one sporophyte was produced. After six weeks for additional growth and reproduction, isolates (Table 5) failed to produce additional sporophytes. The sexual composition of gametophytes at 22 weeks (Table 5) was now predominantly bisexual (70%) with the remaining gametophytes female. The sporophyte was transplanted, but failed to survive the first year.

Discussion

Athyrium angustum, from all observations to date, appears to be a species in which an antheridogen is present, causing a high percentage of males to form in gametophyte populations. Failure to observe males in isolate culture corresponds to Schneller's (1979) observations that isolated spores became female first. Males present in multispore cultures (Schneller, 1979; Peck, 1980) apparently occurred only through antheridogen induction. The intrinsic developmental pattern of the gametophyte is protogynous, since in isolation all gametophytes become female first.
Table 3. Sexual development and sporophyte production at 16 weeks for gametophytes from 5 sporophyte plants of *Athyrium angustum* from Woodman Hollow, Webster Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Spores Sown</th>
<th>Mature Prothalli</th>
<th>Sporophytes Produced by Mature Prothalli</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>#(%)</td>
<td>#(%)</td>
<td>n</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>16(64)</td>
<td>0(0)</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>21(84)</td>
<td>0(0)</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>23(92)</td>
<td>1(4)</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>17(68)</td>
<td>0(0)</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>13(52)</td>
<td>0(0)</td>
<td>5</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>90(72)</td>
<td>1(1)</td>
<td>33</td>
</tr>
</tbody>
</table>
Table 4. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants of *Athyrium angustum* from Woodman Hollow, Webster Co., Iowa. Data taken at 16 weeks and expressed as percentages; U denotes an undefined numerical value.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination-Development Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>50</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2</td>
<td>84</td>
<td>29</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>92</td>
<td>36</td>
<td>12</td>
<td>88</td>
<td>4</td>
<td>4</td>
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<tr>
<td>4</td>
<td>68</td>
<td>0</td>
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<td>U</td>
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<tr>
<td>5</td>
<td>52</td>
<td>0</td>
<td>U</td>
<td>U</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>23</td>
<td>4</td>
<td>95</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 5. Sexual development and sporophyte production at 22 weeks for gametophytes of 5 sporophyte plants of *Athyrium angustum* from Woodman Hollow, Webster Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>New Sporophytes Produced from Week 16 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample n</td>
<td>Sexual Condition #(#%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0(0)</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0(0)</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0(0)</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0(0)</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>0(0)</td>
</tr>
<tr>
<td>Totals</td>
<td>20</td>
<td>0(0)</td>
</tr>
</tbody>
</table>
Based on multispore culture, Schneller (1979) reported a small percentage (1-3%) of bisexual gametophytes at 40-60 days. Although in the present study bisexuals did occur in isolate cultures, the species exhibited a relatively poor bisexual potential (Table 4). By week 16, 23% of isolates were bisexual. Even at 22 weeks, 30% of isolates remained female. *Athyrium angustum* ranked ninth of ten species evaluated in regard to bisexual potential (Table 34). The single-lobed morphology displayed by the isolates may have generally failed to produce antheridia because no antheridogen sensitive tissue, in the form of separate lobes or proliferations, was present.

Because relatively few bisexual gametophytes were produced, the number of gametophytes tested for genetic load was relatively low. Average genetic load (Table 4) for bisexual gametophytes was 95%. This is somewhat higher than the average of 73% reported by Schneller (1979) for *A. felix-femina*, but both are quite high.

Thus, for *Athyrium angustum* both low bisexual potential and high genetic load contributed to the lowest isolate potential of all tested species (Table 32). Consideration of Schneller's observations of sexuality and genetic load, suggests that low bisexual potential and high genetic load may be widespread in this taxon. If that is generally true, *Athyrium angustum* may be a species which is generally restricted to expanding its range along a broad front, initiating new sporophyte populations by
intergametophytic mating within populations of gametophytes. Because this type of reproduction does not eliminate genetic lethals, all populations, even newly established ones, could maintain some level of genetic load.

Conditions at Woodman Hollow today are apparently not suitable for effective sexual production of new *A. angustum* sporophytes. Since an estimated 26 billion spores (Table 35) are released into Woodman Hollow each year (Peck, 1980), it seems unlikely that spores have not reached suitable sites with favorable microenvironments, if they exist within Woodman Hollow. In the absence of sexual reproduction, mutational load may build up in long-lived individuals and clones, thus, increasing the levels of genetic load in the population.

The low isolate potential (Table 4) and high genetic load observed for all 5 plants tested strongly support the idea that this population has been in Woodman Hollow for a very long time and that sexual reproduction by selfing has not cleansed the genotype of lethals within the recent past. There is little evidence to indicate the length of time required to build up such a high level of genetic load.

**Cryptogramma stelleri**

**Background**

*Cryptogramma stelleri* is a small fern with a soft, fragile, creeping rhizome which bears a few, small, spaced fronds. It
typically grows on cool, moist, shaded ledges of limestone or calcareous sandstone in northeastern and northwestern North America (Wherry, 1961; Mickel, 1979).

**Cryptogramma stelleri** is rare in Iowa, where it is known from 15 counties, mostly in the northeastern portion of the state. The disjunct population at Woodman Hollow is the most westerly station (Peck, 1976). There, Peck (1980) found *Cryptogramma* restricted to the lower north-facing slope. In contrast to many of the other Woodman Hollow ferns, this species does not frequent upland mesic woods (Peck, 1980).

Its abundance at the site was low (100–500 plants) and vegetative reproduction from rhizome proliferations was evident. It produced far fewer spores per plant (54,000) than any other Woodman Hollow species and released fewer than half (35%) of those in a favorable year.

Peck (1980) never observed gametophytes growing in the study area, although high spore germination (79–99%, mean 90%) and adequate gametophyte establishment on a variety of natural substrates from Woodman Hollow were demonstrated in the laboratory.

In contrast, successful sexual reproduction of *Cryptogramma stelleri* was frequently observed in the driftless area of Iowa, Wisconsin, and Minnesota (Peck, 1982), 180 km to the east and north of Woodman Hollow.
**Cryptogramma stelleri** reached sexual maturity in 65 days (40% of the gametophytes), making it and **Polypodium virginianum** the two slowest growing of the Woodman Hollow species, as observed in multisporophyte culture (Peck, 1980).

**Results**

Experimental results for *Cryptogramma stelleri* are presented in Tables 6, 7, and 8. In the present study, germination-development potential of isolated spores was 84% (Tables 6 and 7). All gametophytes were multilobed (Table 33). The sexual status of isolated gametophytes at 16 weeks was 0% neuter, 4% male, 51% female, and 45% bisexual. Males were not uniformly abnormal or retarded in growth. Average size of unisexual males and females was the same (7 mm diameter); bisexuals were slightly larger (7.7 mm).

Although after four months of growth, nearly one-half of the gametophytes had the sexual potential to produce a sporophyte, only one demonstrated the ability to do so. Of the 118 mature gametophytes, one sporophyte was produced at the 16 week sampling date, giving an isolate potential of 0.84% (Table 7). Thus, genetic load was measured at 98%.

Continued culture of gametophytes for another six weeks (Table 8) resulted in no additional sporophytes being formed. Consequently, it may be concluded that sufficient time was provided at the 16 week sampling date. At 22 weeks, no male
Table 6. Sexual development and sporophyte production at 16 weeks for gametophytes of 5 sporophyte plants of *Cryptogramma stelleri* from Woodman Hollow, Webster Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Spores Sown</th>
<th>Mature Prothalli</th>
<th>Sporophytes Produced by Mature Prothalli</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>#(%)</td>
<td>#(%)</td>
<td>Sample n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N M F B</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>24(96)</td>
<td>1(4)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0(0) 0(0) 5(71) 2(29)</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>24(96)</td>
<td>0(0)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0(0) 0(0) 3(50) 3(50)</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>25(100)</td>
<td>0(0)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0(0) 0(0) 6(86) 1(14)</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>23(92)</td>
<td>0(0)</td>
<td>10</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0(0) 1(10) 3(30) 6(60)</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>22(88)</td>
<td>0(0)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0(0) 4(29) 0(0) 10(71)</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>118(95)</td>
<td>1(1)</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0(0) 5(11) 17(39) 22(50)</td>
</tr>
</tbody>
</table>
Table 7. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants of *Cryptogramma stelleri* from Woodman Hollow, Webster Co., Iowa. Data taken at 16 weeks and expressed as percentages

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination-Development Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>96</td>
<td>32</td>
<td>13</td>
<td>87</td>
<td>4</td>
<td>4</td>
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<td>5</td>
<td>88</td>
<td>71</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>50</td>
<td>2</td>
<td>98</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 8. Sexual development and sporophyte production at 22 weeks for gametophytes of 5 sporophyte plants of *Cryptogramma stelleri* from Woodman Hollow, Webster Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>New Sporophytes Produced from Week 16 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>n</td>
<td>Sexual Condition</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>0(0)</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0(0)</td>
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<td>3</td>
<td>6</td>
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<tr>
<td>4</td>
<td>5</td>
<td>0(0)</td>
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<td>5</td>
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<td>0(0)</td>
</tr>
<tr>
<td>Totals</td>
<td>26</td>
<td>0(0)</td>
</tr>
</tbody>
</table>
gametophytes remained, suggesting that either with time they became bisexual, or that transfer influenced sexuality.

Discussion

Considering the habitat affinity of this species for coolness, moisture, and low light levels, it is probably desirable to assay this species under growth chamber conditions more closely approximating natural conditions for this species. This might result in data not much different than portrayed here, since 118 of 125 gametophytes reached maturity, suggesting that minimally favorable conditions were met.

Cryptogramma stelleri has a limited ability to reproduce by single spores. Males were observed in isolate culture (Table 6), possibly as a result of strong light or high temperatures, excessive for this species' normal growth. However, it should be noted that the male gametophytes were of the same size as those which were female. Low isolate potential and high genetic load in this species favors reproduction only as multispore populations.

Cryptogramma's habitat is disjunct, especially in the Midwest. Either its habitat was previously more continuous or it must have been able to reach somewhat distant suitable sites and establish itself by single spores.

The portrait thus far derived of Cryptogramma stelleri at Woodman Hollow is that of a plant of very low reproductive potential. This is probably an indication that, whatever the
origin of the Woodman Hollow population, *Cryptogramma*’s immigration there has not been a recent one, and that vegetative reproduction has been the mode of continuation of this species at Woodman Hollow.

**Cystopteris bulbifera**

Background

*Cystopteris bulbifera* is a medium sized fern with a short-creeping rhizome bearing clumped fronds. The taxon is reported to be diploid (n=42) (Cranfill, 1980). It frequents outcrops, especially those composed of limestone rocks or calcareous sandstones. *C. bulbifera* occurs across eastern North America, except for the Coastal Plain, and terminates in the Great Plains, with disjunct populations occurring in Arizona. Mickel (1979) reports it to be common.

In Iowa, it occurs frequently in the eastern one-half of the state (Peck, 1976). Woodman Hollow, Webster Co., Iowa, is its most westerly known station. This population is disjunct; the species is not known from adjacent counties. Within Woodman Hollow, it is found on the lower north-facing and south-facing slopes. It is not an upland, mesic species in Iowa (Peck, 1980). Within Woodman Hollow, there are 500 to 1,000 plants. The plants expand vegetatively through branching rhizomes and by specialized bulblets produced on the leaves.

Each plant produced approximately six million spores. Peck
(1980) found that 75-99% of the spores are released, depending upon conditions. The species ranked tenth out of the 14 Woodman Hollow species in spore production and release. It produced 0.4% of the annual spore crop. Farrar (1976) found that unreleased spores were released the following spring. Peck (1980) trapped spores of *C. bulbifera* at two of four stations within ten meters of sporophyte plants, indicating that some spores may escape the immediate vicinity of the parent plant.

Gametophytes of this species were never observed at Woodman Hollow. In the laboratory, spore germination was 81-95% with an average of 91% among plants tested (Peck, 1980). Farrar (1976) found a substantial drop in viability (from 62% to 6%) for spores collected in December compared to those that had overwintered on the plant until March. This was the only Woodman Hollow species that exhibited such a significant drop.

In multispore culture, sex organs were observed on the majority of cultured gametophytes by the end of the first month (Peck, 1980).

**Results**

Experimental results for *Cystopteris bulbifera* are presented in Tables 9, 10, and 11. Because germination-development was poor (26%), only 33 gametophytes were available for study (Table 10). All gametophytes first exhibited a cordate morphology, followed by proliferation of multiple lobes. After 16 weeks, 44% were neuter,
Table 9. Sexual development and sporophyte production at 16 weeks for gametophytes from 5 sporophytes of *Cystopteris bulbifera* from Woodman Hollow, Webster Co., Iowa

<table>
<thead>
<tr>
<th>Plant Sown</th>
<th>Mature Prothalli</th>
<th>Sporophytes Produced by Mature Prothalli</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sample n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>7(28)</td>
<td>0(0)</td>
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<tr>
<td>2</td>
<td>25</td>
<td>11(44)</td>
<td>0(0)</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>5(20)</td>
<td>1(20)</td>
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<tr>
<td>4</td>
<td>25</td>
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</tr>
<tr>
<td>5</td>
<td>25</td>
<td>5(20)</td>
<td>1(20)</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>32(26)</td>
<td>2(6)</td>
</tr>
</tbody>
</table>
Table 10. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants of *Cystopteris bulbifera* from Woodman Hollow, Webster Co., Iowa. Data taken at 16 weeks and expressed as percentages; U denotes an undefined numerical value.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination-Development Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>0</td>
<td>U</td>
<td>U</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>0</td>
<td>U</td>
<td>U</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>10</td>
<td>100</td>
<td>0</td>
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</tr>
<tr>
<td>4</td>
<td>16</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>100</td>
<td>20</td>
<td>80</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>17</td>
<td>38</td>
<td>62</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 11. Sexual development and sporophyte production at 22 weeks for gametophytes of 5 sporophyte plants of *Cystopteris bulbifera* from Woodman Hollow, Webster Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>New Sporophytes Produced from Week 16 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
<td>Sexual Condition (#, %)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0(0)</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0(0)</td>
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<tr>
<td>3</td>
<td>1</td>
<td>0(0)</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0(0)</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0(0)</td>
</tr>
<tr>
<td>Totals</td>
<td>8</td>
<td>0(0)</td>
</tr>
</tbody>
</table>
22% were male, 22% were female, and 11% were bisexual. Of the bisexual gametophytes at this date, 38% produced sporophytes (Table 9). This gives a gametophyte isolate potential of 6% and 1.6% spore isolate potential (Table 10).

After six additional weeks of growth and watering, no neuter or male gametophytes were present (Table 11). Females accounted for 32% of the gametophytes; the remaining 68% were bisexual. No additional sporophytes were formed during this period.

Discussion

The low number of gametophytes produced at 16 weeks makes interpretations tentative. The specialized vegetative reproductive capabilities of this species may allow it to be less dependent on sexual reproduction. Bulblets may allow effective dispersal over short distances, replacing or compensating in the local habitat for reduced spore or gametophyte potentials. However, the long distance limitations of these plants would not be overcome by bulblets.

The high percentage of neuter gametophytes is much greater than observed by Peck (1980) for multispore cultures. It is not clear whether the low germination and high percentage of neuter and male gametophytes is related to poor growth in isolation or simply poor potential for this spore collection. The low percentage of bisexual gametophytes at 16 weeks compared to 22 weeks suggests that 16 weeks was insufficient for maturity of this
species in isolation. Alternatively, transfer may have stimulated sexual maturation.

Overall, the gametophyte maturation was low, attainment of bisexuality was slow, isolate potential was low, and genetic load was high. Although low isolate potential was primarily a result of failure to attain bisexuality, these aspects together suggest that these plants have not recently undergone intragametophytic selfing. Therefore, the population at Warren Co., Missouri has probably not been recently established by long distance dispersal. Its presence within local habitats probably has been facilitated by vegetative proliferation from rhizomes and vegetative reproduction from bulblets. The failure of this species to reproduce sexually at Woodman Hollow over the last 10 years may reflect genetic and reproductive conditions. Long term reliance on vegetative reproduction may have resulted in the accumulation of sufficient genetic load to limit effective sexual reproduction.

**Cystopteris tenuis**

**Background**

*Cystopteris tenuis* has a short-creeping rhizome with clumped, small to medium sized fronds. It occurs on cliffs and in moist woods across the northern and southwestern United States (Mickel, 1979). This species, formerly known as *C. fragilis* (L.) Bernh. var. *mackeyi* Laws. in the Iowa fern literature (Peck, 1982), was recently corrected by Lellinger (1981). The species is tetraploid
with $n=84$ (Wagner and Hagenah, 1956). Moran (1983) suggested that it is an autotetraploid, based on its morphological distinctness. This species is widespread in Iowa, with scattered populations occurring in over 40 counties (Peck, 1976). *C. tenuis* is particularly well-suited to rocky outcrops and associated mineral soil slumps (Peck, 1980, 1982).

In his study of *C. tenuis* from Woodman Hollow, Peck (1980) reported a large population (1,000-10,000 plants). It is one of the three most common plants at the site (along with *Woodsia obtusa* and *Adiantum pedatum*). A single plant produced an average of 10 million spores per year. Nearly all of these spores (88-99%) were released from the plant. The species ranked third of 14 in numbers of spores produced at Woodman Hollow, but because of the size and location of the fronds, most spores were released into the herbaceous layer. Some spores were able to escape into the local environment, where they were trapped at three of eight spore traps within 10 m of *C. tenuis* sporophytes.

In laboratory studies, Peck (1980) reported a range of 90-99% spore germination with a mean of 95% germination, among all plants tested. He found that in multispore cultures of *C. tenuis* gametophytes, the majority reached sexual maturity by the age of one month.

Peck (1980) observed gametophytes of *C. tenuis* in 18 of 54 gametophyte populations he studied at Woodman Hollow, suggesting
that this species undergoes abundant sexual production of new sporophytes.

Farrar and Gooch (1975) examined the sexual status of a single natural population of gametophytes growing at Woodman Hollow. These gametophytes were collected in November, five months after maximum spore release for this species. At this time, they reported most gametophytes were mature and sporophyte production was evident. Of the 75 gametophytes examined, 11% were neuter, 28% were male, and 61% were bisexual. They stated that gametophytes at this time occurred in populations of about 75-100 individuals within areas of 10 cm x 10 cm or less. They did not indicate whether gametophytes were predominantly clumped or were relatively evenly spaced.

Results

Experimental results for *Cystopteris tenuis* are presented in Tables 12, 13, and 14. In the present study, 54% of 125 isolated spores germinated and produced mature gametophytes (Table 13). Isolate morphology was highly proliferative; only three gametophytes expressed single-cordate-lobe morphology (Table 33).

Of the 66 gametophytes surviving to 16 weeks, sampling indicated that 21% were neuter, 12% were male, none were female, and 67% were bisexual (Table 12). Although the smallest gametophytes were neuter, inability to form sex organs was not strictly associated with retarded growth. Many large (up to 18 mm
Table 12. Sexual development and sporophyte production at 16 weeks for gametophytes of 5 sporophyte plants of *Cystopteris tenuis* from Woodman Hollow, Webster Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Spores Sown</th>
<th>Mature Prothalli</th>
<th>Sporophytes Produced by Mature Prothalli</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>#(%)</td>
<td>#(% )</td>
<td>Sample n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>18(72)</td>
<td>11(61)</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>18(72)</td>
<td>14(78)</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>14(56)</td>
<td>5(36)</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>7(28)</td>
<td>4(57)</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>9(36)</td>
<td>5(56)</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>66(53)</td>
<td>39(60)</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 13. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants of *Cystopteris tenuis* from Woodman Hollow, Webster Co., Iowa. Data taken at 16 weeks and expressed as percentages.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination-Development Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>61</td>
<td>100</td>
<td>0</td>
<td>61</td>
<td>44</td>
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<td>2</td>
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<tr>
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<td>56</td>
<td>52</td>
<td>69</td>
<td>31</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>57</td>
<td>100</td>
<td>0</td>
<td>57</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>78</td>
<td>71</td>
<td>29</td>
<td>56</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>67</td>
<td>88</td>
<td>12</td>
<td>59</td>
<td>31</td>
</tr>
</tbody>
</table>
Table 14. Sexual development and sporophyte production at 22 weeks for gametophytes of 5 sporophyte plants of *Cystopteris tenuis* from Woodman Hollow, Webster Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>New Sporophytes Produced from Week 16 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample n</td>
<td>Sexual Condition #(%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N  M  F  B</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0(0) 0(0) 0(0) 2(100)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0(0) 0(0) 0(0) 1(100)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0(0) 0(0) 0(0) 2(100)</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0(0) 0(0) 0(0) 1(100)</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0(0) 0(0) 0(0) 1(100)</td>
</tr>
<tr>
<td>Totals</td>
<td>7</td>
<td>0(0) 0(0) 0(0) 7(100)</td>
</tr>
</tbody>
</table>
(15 mm) neuter gametophytes were also present.

All sporophytes were formed on bisexual, cordate gametophytes. Thirty-nine sporophytes were produced. This is an isolate potential of 31%, based on the number of spores sown, or 57%, based on the number of surviving gametophytes (Table 13). Of bisexual gametophytes, 81% produced sporophytes. Therefore, genetic load was 19% or less (Table 13).

Although all gametophytes had become bisexual after six additional weeks of growth, no additional sporophytes were produced (Table 14).

Discussion

Although germination-development was relatively low and morphology was highly proliferative, above average bisexual potential and modest genetic load combined to give this species the second highest isolate potential of all species tested.

At 16 weeks, a high percentage of males were present; females were absent. In this species, maleness was not associated with retarded growth or extreme abnormal morphology. The size and morphology of male gametophytes was well within the size range for the bisexual isolated gametophytes in this study. Since all gametophytes became bisexual by 22 weeks, this species may be protandrous with males becoming bisexuals, or females may have become bisexual more rapidly than did males. In either case, this differs from observations of other species in this investigation.
The presence of large neuter gametophytes suggests that failure to produce sex organs was not entirely due to immaturity or poor growth conditions, but rather that some innate physiological or genetic problem exists.

Sex types present were the same as and sexual ratios were similar to the natural populations observed by Farrar and Gooch (1975). Bisexuality was very similar. The increase in males over neuters might be attributed to antheridogen or enhancement of sexuality in populations as compared to isolated gametophytes. This species needs to be tested for antheridogen production and for the formation of protogynous gametophytes, which have, thus far, been universally associated with antheridogen systems.

The possibility also exists that the Farrar-Gooch population was of such low density (75-100 gametophytes/10 cm x 10 cm area) that the gametophytes were effectively isolated.

Given the high reproductive success at 16 weeks, the reproductive failure of gametophytes by 22 weeks is either caused by gametophyte age or by inherent genetic problems, since bisexuality was attained. The shift to bisexuality of these gametophytes may have been influenced by media enrichment (new media) or transfer shock, but bisexuality alone was not sufficient to result in new sporophytes.

The low genetic load and high isolate potential of this species, estimated from plants at Woodman Hollow, suggests little
about its history at Woodman Hollow. It could either be a relatively recent addition, or have arrived long ago, maintained low genetic load by frequent sexual reproduction of isolated gametophytes, and persisted by constant sexual replacement. The observations of Peck (1980) and Farrar and Gooch (1975) indicating high bisexuality and frequent sexual reproduction in nature indicate that the latter hypothesis is a viable alternative.

At Woodman Hollow, *Cystopteris tenuis* is not a strong vegetative species; few instances of multiple apices were evident (Peck, 1980). Although the species has acquired some genetic load, load varies from plant to plant. This low vegetative capacity and the variation in genetic load among plants emphasize the reliance upon sexual reproduction within the population. Its strong sexual reproduction and low vegetative reproduction make this species similar to *Woodsia obtusa* with which it shares many gametophytic reproductive traits.

**Dryopteris cristata**

*Dryopteris cristata*, a large fern with erect fronds, is typical of marshes, bogs, and swamps of northern and eastern North America with peripheral populations in Iowa (Carlson and Wagner, 1982). It is considered to be an allotetraploid species with n=82 (Britton, 1961). Specimens for study were gathered from the only sphagnum bog in Iowa, at Pilot Knob State Preserve, Hancock, Co.,
Results

Experimental results for *Dryopteris cristata* are presented in Tables 15, 16, and 17. Because only four plants were available as spore sources, only 100 gametophytes of this species were sown. Of these, 82 had formed mature gametophytes by 16 weeks (Table 15).

Although all four morphological types were expressed, single-lobe, cordate morphology was predominant (86%) (Table 33). Only those gametophytes which developed as a normal, single two-dimensional plate with an apical notch formed sex organs.

Observations of young, isolated gametophytes 4-5 weeks after sowing showed that many were already sexual. Sampling at 16 weeks indicated that 17% of the gametophytes were neuter, 50% were female, and 33% were bisexual (Table 15). All bisexual gametophytes formed sporophytes. No genetic load was expressed (Table 16).

Twelve isolates (Table 17) were allowed to grow for an additional six weeks. Watering was continued on a weekly basis. At 22 weeks, all gametophytes were bisexual except for one female. Three new sporophytes had formed.

Discussion

Although germination–development was relatively high, the presence of large neuter gametophytes (up to 24 mm x 11 mm at 16
Table 15. Sexual development and sporophyte production at 16 weeks for gametophytes of 4 sporophyte plants of *Dryopteris cristata* from Pilot Knob, Hancock Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Spores Sown</th>
<th>Mature Prothalli</th>
<th>Sporophytes Produced by Mature Prothalli</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>#(% )</td>
<td>#(% )</td>
<td>Sample n</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>1</td>
<td>25</td>
<td>22(88)</td>
<td>6(27)</td>
<td>7</td>
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<tr>
<td>2</td>
<td>25</td>
<td>21(84)</td>
<td>6(29)</td>
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</tr>
<tr>
<td>3</td>
<td>25</td>
<td>19(76)</td>
<td>4(21)</td>
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<td>4</td>
<td>25</td>
<td>20(80)</td>
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</tr>
<tr>
<td>Totals</td>
<td>100</td>
<td>82(82)</td>
<td>21(26)</td>
<td>27</td>
</tr>
</tbody>
</table>
Table 16. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants of *Dryopteris cristata* from Pilot Knob, Hancock Co., Iowa. Data taken at 16 weeks and expressed as percentages.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination-Development Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88</td>
<td>27</td>
<td>100</td>
<td>0</td>
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<td>24</td>
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<td>2</td>
<td>84</td>
<td>28</td>
<td>100</td>
<td>0</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>21</td>
<td>100</td>
<td>0</td>
<td>21</td>
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<td>36</td>
<td>70</td>
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<tr>
<td>Total</td>
<td>82</td>
<td>27</td>
<td>92</td>
<td>8</td>
<td>26</td>
<td>21</td>
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</tbody>
</table>
Table 17. Sexual development and sporophyte production at 22 weeks for gametophytes of 4 sporophyte plants of *Dryopteris cristata* from Pilot Knob, Hancock Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sample n</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>New Sporophytes Produced from Week 16 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sexual Condition (#(%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>M</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
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</tr>
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<td>2</td>
<td>3</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>Totals</td>
<td>12</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>
weeks) suggests that there are some developmental problems, perhaps relating to the culture conditions, which must be very different than those afforded in the acid, sphagnum bog, where these samples were collected.

The pattern of sexual development presented by this species indicated that isolated gametophytes uniformly became female first (suggesting that male gametophytes may form only in response to antheridogen stimulus). As would be expected of a recent colonizer, the female gametophytes were able to develop into bisexual gametophytes and a high percentage of those bisexual gametophytes produced sporophytes. The presence of females at 16 weeks, but not at 22 weeks, suggests that the gametophytes may not have been fully mature at 16 weeks, or that transfer facilitated sexual maturity. The high bisexual attainment at 22 weeks would strongly favor reproduction by this species, whether in a population or as single spores.

The pattern of sporophyte formation demonstrated that there were no genetic barriers to sporophyte production. These results suggest that the plants of Dryopteris cristata at Pilot Knob are either recently arrived by long distance dispersal (see maps in Peck, 1982; Carlson and Wagner, 1982) or represent reproduction by isolated spores and gametophytes from older plants at the bog.

The failure of many of the 22 week old bisexual gametophytes to form sporophytes may have been the result of culture
conditions, developmental conditions (such as the age of the gametophyte or archegonia), or insufficient time (although the latter seems less likely).

Time may be a less critical factor for species in a habitat with constant water supply than for species in more xeric environments (Whittier, 1968, 1970). Constant availability of water would also favor fertilization for gametophytes in wetland environments. This contrasts to the situation reported by Peck (1980) for Woodman Hollow. There, availability of water for fertilization was limiting for sexual reproduction within gametophyte populations.

Since the bog may have been present for as long as 9,000 years, occupation of the bog by this species may have occurred at any time. Acidic wetlands were probably much more widespread over the upper Midwest in the early postglacial period. Today bog habitats are widely scattered south of northern Minnesota and northern and central Wisconsin (Transeau, 1935), where this species also occurs in acid, moist habitats (Peck, 1982). To have recently occupied these sites, long distance single spore dispersal would have been required.

**Dryopteris marginalis**

**Background**

*Dryopteris marginalis*, a large fern with erect fronds, is common on rocky wooded slopes of northeastern North America
Peripheral populations occur in the Driftless Area of Iowa (Peck, 1982) and along the Iowa River in Hardin Co., Iowa (Peck, 1976). The latter populations have been interpreted as disjunct relicts (Pammel, 1905; Conard, 1952).

**Results**

Experimental results for *Dryopteris marginalis* are presented in Tables 18, 19, and 20. Four weeks after sowing, 85% of 125 isolated spores had germinated. None of these gametophytes appeared to develop according to the typical pattern of an initial uniseriate filament forming a terminal two-dimensional plate. Rather, these plants appeared to have very early formed two- and three-dimensional masses of cells, which then gave rise to numerous two-dimensional cell plates. More plates were produced as the gametophyte developed. The overall shape of the gametophyte was a hemisphere, composed of many plates radiating from a central core (Table 33).

Eighty-nine percent of the original gametophytes survived sixteen weeks after sowing; 71% of the initial spores had produced surviving gametophytes (Table 19). At this stage, both male (21%) and female (28%) unisexual gametophytes were present. Although 51% of all gametophytes were bisexual (Table 18), only 4% of the bisexuals (2% of all gametophytes) produced sporophytes. This is an isolate potential of only 2% (Table 19).
Table 18. Sexual development and sporophyte production at 16 weeks for gametophytes of 5 sporophyte plants of *Dryopteris marginalis* from Iowa Co., Wisconsin

<table>
<thead>
<tr>
<th>Plant</th>
<th>Spores Sown</th>
<th>Mature Prothalli</th>
<th>Sporophytes Produced by Mature Prothalli</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>(#(%))</td>
<td>#(%))</td>
<td>Sample</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>N</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>23(92)</td>
<td>0(0)</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>22(88)</td>
<td>1(4)</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>21(84)</td>
<td>0(0)</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>14(56)</td>
<td>0(0)</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>9(36)</td>
<td>1(11)</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>89(71)</td>
<td>2(2)</td>
<td>28</td>
</tr>
</tbody>
</table>
Table 19. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants *Dryopteris marginalis* from Iowa Co., Wisconsin. Data taken at 16 weeks and expressed as percentages; U denotes undefined numerical value.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination-Development Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>51</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>88</td>
<td>46</td>
<td>10</td>
<td>90</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>84</td>
<td>75</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>0</td>
<td>11</td>
<td>U</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>56</td>
<td>20</td>
<td>80</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>51</td>
<td>4</td>
<td>96</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 20. Sexual development and sporophyte production at 22 weeks for gametophytes of 5 sporophyte plants of *Dryopteris marginalis* from Iowa Co., Wisconsin

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>New Sporophytes Produced from Week 16 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample n</td>
<td>Sexual Condition #(%): N M P F B</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0(0) 0(0) 3(60) 2(40)</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0(0) 0(0) 2(40) 3(60)</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0(0) 0(0) 0(0) 4(100)</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0(0) 0(0) 3(75) 1(25)</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0(0) 0(0) 0(0) 2(100)</td>
</tr>
<tr>
<td>Totals</td>
<td>20</td>
<td>0(0) 0(0) 8(40) 12(60)</td>
</tr>
</tbody>
</table>
Isolated gametophytes grown for an additional six weeks produced no additional sporophytes (Table 20). By this time, the sex ratio had changed to 33% female and 67% bisexual.

Discussion

It might have been hypothesized that the peculiar gametophyte morphology observed for *Dryopteris marginalis* would enhance bisexuality, because it effectively formed a small population of gametophyte lobes with relatively little tissue connection. The connections might easily have been broken. This might have allowed an antheridogen producing female lobe to induce other lobes to become male (Emigh and Farrar, 1977). It also might be suggested that such morphology was simply abnormal and such gametophytes might be unable to produce sex organs and/or viable sporophytes. However, the unusual gametophyte morphology was not associated with a failure to produce sex organs, nor did it appear to strongly favor bisexuality and sporophyte production.

At sixteen weeks, male gametophytes (average 5 mm) were somewhat smaller than females (7.75 mm), which in turn were smaller than bisexual gametophytes (11.36 mm). Smaller gametophytes tended to be isodiametric; larger ones tended to have a few larger lobes which altered gametophyte shape.

The changes in sexuality from 16 to 22 weeks suggest that gametophytes, which were earlier male, became bisexual, while initially female gametophytes tended to remain unisexual.
The plants of this species expressed low isolate potential and high genetic load. In part, this may have been confounded by gametophyte immaturity, since the strongly bisexual composition of the gametophytes at 22 weeks contrasted with the unisexual status at 16 weeks. This may have been from media enrichment, transfer shock, or innate development at a slower than expected rate. However, since gametophyte morphology was highly abnormal, a genetic basis might be suggested.

The sporophyte populations tested are, therefore, probably not good at producing new sporophytes. They may have been at their location for a long time and have not recently undergone intragametophytic selfing. They are probably limited to outcrossing reproduction. In either case, they do not appear to have either recently arrived from long distance dispersal by single spores or to have reproduced by single spore at the site for some time.

**Dryopteris spinulosa**

**Background**

*Dryopteris spinulosa* is a large fern with erect fronds typically occurring in swamps and wetland woods of northern North America (Mickel, 1979). It occurs in peripheral populations on moist sandstone cliffs in Woodman Hollow, Webster Co., Iowa (Peck, 1980). Carlson (1979) reported that in the Michigan habitats he studied, *D. spinulosa* was most common in swamps; occurred less
frequently in low wet forests, seepage areas, and on ravine slopes; and that marginal habitat included beech-sugar maple-basswood stands, transition slopes, pine stands, and pine-poplar-oak stands. It is a tetraploid sexual species (n=82). Wagner (1970) has hypothesized that the species is of hybrid origin, the parents being D. intermedia and an undiscovered species, D. "semicristata". Hickel (1979) lists D. spinulosa as common throughout its range, but Peck (1980) reported it as infrequent in Iowa, where it occurs in 21 counties, mostly to the north and east of Woodman Hollow.

At Woodman Hollow, approximately 100 plants are present (Peck, 1980). Dryopteris spinulosa has a tipped-up rhizome that probably spreads vegetatively only as the result of injury. Peck (1980) found no evidence of vegetative reproduction at Woodman Hollow. Here, it was found on the lower north-facing slope and on the floodplain. Each plant produced an average of 150 million spores, but because of the low number of fertile plants present, this represented only 1% of all spores produced at Woodman Hollow. These spores were readily released from the plant (95% and 99% in a wet and dry year, respectively).

Peck (1980) reported that in multispore cultures germination was high (84–99% with 96% mean). By day six, 40% of all spores had germinated. It was not until day 50 that 40% of the gametophytes had produced sex organs. Both antheridia and
archegonia were present.

D. spinulosa was represented in 2 of 54 gametophyte populations studied by Peck (1980) at Woodman Hollow. Small sporeling plants and small sporophyte plants provided ample evidence of continuing sexual reproduction in this population.

Results

Germination of spore samples for this species was so low that it was not considered for the isolate potential assay. At five weeks, only 29% of the spores had germinated. Most of those were still quite small (27% were a few cells, 33% were 0.05 cm long, 20% were 0.1 cm, 14% were 0.2 cm, and 6% were 0.3-0.5 cm in length).

The developmental pattern appeared to be one of early formation of a three dimensional mass of cells that then gave rise to one or sometimes several two-dimensional plates. Frequently rhizoids were not evident. What appeared to be bacterial contamination was common around spores and small gametophytes.

Because of the poor germination and development in these cultures, they were removed from the lighted culture benches and set aside in another part of the room where light levels were considerably lower. Later, when it was discovered that there were numerous fully developed gametophytes present, it was decided to at least determine the sex ratios of these isolates.

Although some of the original gametophytes had died, five
additional spores had germinated and produced mature gametophytes. At the time of census, there were 37 gametophytes. There were 1 neuter, 4 male and 32 bisexual gametophytes. Two sporophytes were produced. The sexual status of the gametophytes producing sporophytes was not determined. The young sporophytes were transferred to soil in peat pots that were then enclosed in clear plastic vegetable crispers with water in the bottom. Both sporophytes were alive at the end of the first year.

Discussion

The sex ratios observed indicate that Dryopteris spinulosa has the genetic potential to produce a high percentage of bisexual gametophytes from isolated spores. The presence of males demonstrates that protandrous or male gametophytes could be produced in this species without the influence of antheridogen. Low germination and low growth rate suggest that this species was not performing well under culture conditions. These factors plus the low light levels during gametophyte growth may have favored the formation of male gametophytes. The absence of females suggests that either they were not formed or that they had already become bisexual.

Because these gametophytes were not watered to effect fertilization, no speculation can be made regarding genetic load. The formation of two viable sporophytes indicates some potential for reproduction by isolated spores, even without adequate
watering regime. These sporophytes may have been produced through apogamy or water from condensation may have been sufficient for fertilization. The observation that this species was actively reproducing at Woodman Hollow (Peck, 1980) combined with a high bisexual potential leads to the prediction that selfing may be frequent and thus, genetic load would be low. However, no conclusions could be drawn regarding this species and its reproductive potential as the experimental protocol was not possible. This species should be tested again to test these predictions.

Polypodium virginianum

Background

Polypodium virginianum is a small fern with a long-creeping rhizome with clustered fronds. It typically occurs on shaded rocks or moist mineral soil embankments of eastern and central North America. Key (1982) reported that in Missouri Polypodium grows on moist shaded sandstone.

Diploid, triploid and tetraploid races have been reported (Manton, 1951, 1957; Shivas, 1961a, 1961b; Manton and Shivas, 1953). It has been suggested that the triploid is a hybrid between the diploid and tetraploid which has overcome hybrid sterility through the occasional production of unreduced spores and apogamous sporophytes (Evans, 1970). Although the geographic ranges are incompletely known, all three cytotypes are widespread,
occurring from southern U.S. (Evans, 1970; Cranfill, 1980), to Canada (Manton, 1957; Kott and Petersen, 1974; Kott and Britton, 1982) and from the East (Manton, 1957) to the Mid-west (Evans, 1970). Kott and Britton (1982) found tetrapoids to be widespread over Ontario with diploids and triploids less frequent. Evans (1970) suggested that the tetraploid may be more northerly, whereas, the triploid has a more southerly distribution similar to the diploid.

The morphological characters which can be used to separate the three cytotypes have been summarized by Kott and Britton (1982).

Peck (1976) found Polypodium to be rare in Iowa. In Missouri, Key (1982) reported Polypodium from "Salem Plateau, St. Francois Mountains and a few eastern counties north of the Missouri River." It is not reported from the western half of Missouri.

At Woodman Hollow, frond morphology resembled the diploid type (Peck, 1980). Peck (1980) found relatively few plants (100–500) at Woodman Hollow. These were restricted to the upper and lower north-facing slopes. With the exception of Cryptogramma stelleri, Polypodium produced fewer spores per plant (350,000) than any other fern at Woodman Hollow and released the lowest percentage of those spores (7%–18% in wet and dry years respectively).

Peck (1980) reported high germination in multisporic cultures (87–98% with a mean of 93%). Kott and Petersen (1974) reported
similar germination percentages for tetraploid *Polypodium virginianum* (85-97%), but much lower percentages for the diploid cytotype (40-60%). The diploid also exhibited some sensitivity to the medium, achieving only 20% germination when Whittier's medium was used rather than Bold's.

Kott and Petersen (1974) reported a low level of spore viability for triploids. From 1 in 62 to 1 in 203 spores were found to be viable. DeBenedictis (1969, as cited in Kott and Petersen 1974) also reported a few percent of viable spores for the triploid.

Although spore germination was high in the laboratory and fertile *Polypodium* plants were within 10 meters of 19 studied gametophyte populations, no *Polypodium* gametophytes were observed at Woodman Hollow (Peck, 1980).

Typical cordate morphology was reported for diploids and tetraploids in culture (Kott and Petersen, 1974; Peck, 1980), but triploid gametophytes exhibited highly irregular morphology. They formed masses of cells with no rhizoids, masses of filaments, or multilobed mounds. Growth was very slow and no sex organs developed. Triploids on soil were more regular in form, but all grew slowly and many died prematurely. It is not clear whether any reached sexual maturity (Kott and Petersen, 1974).

Peck (1980) found that *Cryptogramma* and *Polypodium* took longer to reach sexual maturity than any other Woodman Hollow
species (65 days for 40% of the gametophytes).

In comparisons of diploid and tetraploid forms, Kott and Petersen (1974) observed even longer times to sexual maturity. The tetraploids required 90–95 days, the diploids 105–110 days when cultured on agar. They found soil cultures to mature considerably more rapidly (45–50 days for tetraploids and 65 days for diploids).

However, it is not clear whether the differences were due to culture medium rather than growth conditions. Agar cultures were grown in growth chambers under low light conditions (150 ft-c). Culture dishes were inverted, which may have reduced the light further. Soil cultures were grown at room temperature under fluorescent lights of unspecified intensity. The low light intensities for the agar cultures may also explain the considerable difference from Peck's report.

Kott and Petersen (1974) suggested that the more rapid development (approximately three weeks advantage) of the tetraploid may have given it an advantage in establishing a more extensive and northerly range. They interpreted the greater sensitivity of the diploid to the growth medium used as an indication of a less adaptable physiology which might tend to restrict distribution.
Results

Experimental results for *Polypodium virginianum* are presented in Tables 21, 22, and 23. The five plants used in this study came from three different populations. Plant number one was growing on a roadside bluff in Warren County, Missouri. Plants number two and three came from a ravine in the same county. Plants four and five were from Hardin County, Iowa. The response of isolated spores from these plants were distinct for each of the three sites (Tables 21 and 22).

For plant one, although germination was high and 100% of bisexual gametophytes produced sporophytes at 16 weeks, isolate potential was only 4% due to low bisexual potential. No genetic load was indicated, but because only one gametophyte was bisexual, genetic load was not adequately measured.

For plants two and three, the zero isolate potential is entirely attributable to low germination and zero bisexual potential at 16 weeks. Genetic load was untested for these plants due to absence of bisexuals.

For plants four and five, germination was high and no genetic load was expressed. The only moderate bisexual potential at 16 weeks is the sole source of the reduction in gametophyte isolate potential.

At 16 weeks, sampling indicated 74% female and 26% bisexual gametophytes (Table 21). All plants demonstrated an increased
Table 21. Sexual development and sporophyte production at 16 weeks for gametophytes from 5 sporophyte plants of *Polypodium virginianum* (Plants #1-#3 from Warren Co., Missouri, and Plants #4-#5 from Hardin Co., Iowa)

<table>
<thead>
<tr>
<th>Plant Sown</th>
<th>Spores Sown</th>
<th>Mature Prothalli #</th>
<th>Prothalli Produced by Mature Prothalli #(#%)</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>Sample n</th>
<th>Sexual Condition #(#%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>N M F B</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>22(88)</td>
<td>1(4)</td>
<td>12</td>
<td>12</td>
<td>0(0) 0(0) 12(100) 0(0)</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>8(32)</td>
<td>0(0)</td>
<td>5</td>
<td>5</td>
<td>0(0) 0(0) 5(100) 0(0)</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>12(48)</td>
<td>0(0)</td>
<td>3</td>
<td>3</td>
<td>0(0) 0(0) 3(100) 0(0)</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>25(100)</td>
<td>13(52)</td>
<td>4</td>
<td>4</td>
<td>0(0) 0(0) 4(100) 0(0)</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>17(68)</td>
<td>10(59)</td>
<td>2</td>
<td>2</td>
<td>0(0) 0(0) 2(100) 0(0)</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>74(59)</td>
<td>24(32)</td>
<td>26</td>
<td>26</td>
<td>0(0) 0(0) 26(100) 0(0)</td>
</tr>
</tbody>
</table>
Table 22. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants of *Polypodium virginianum* (Plants #1-#3 from Warren Co., Missouri and Plants #4-#5 from Hardin Co., Iowa). Data taken at 16 weeks and expressed as percentages; U denotes undefined numerical value.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination-Development Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88</td>
<td>4</td>
<td>100</td>
<td>0</td>
<td>4</td>
<td>4</td>
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<td>2</td>
<td>32</td>
<td>0</td>
<td>U</td>
<td>U</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>0</td>
<td>U</td>
<td>U</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>52</td>
<td>100</td>
<td>0</td>
<td>52</td>
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<td>5</td>
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<td>59</td>
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<td>Total</td>
<td>59</td>
<td>32</td>
<td>100</td>
<td>0</td>
<td>32</td>
<td>19</td>
</tr>
</tbody>
</table>
Table 23. Sexual development and sporophyte production at 22 weeks for gametophytes from 5 sporophyte plants of *Polypodium virginianum* (Plants #1 through #3 from Warren Co., Missouri, and Plants #4 and #5 from Hardin Co., Iowa)

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>New Sporophytes Produced from Week 16 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample n</td>
<td>Sexual Condition #(%): N M F B</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0(0) 0(0) 2(67) 1(33)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0(0) 0(0) 0(0) 1(100)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0(0) 0(0) 0(0) 3(100)</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0(0) 0(0) 1(33) 2(67)</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0(0) 0(0) 0(0) 2(100)</td>
</tr>
<tr>
<td>Totals</td>
<td>12</td>
<td>0(0) 0(0) 3(25) 9(75)</td>
</tr>
</tbody>
</table>
bisetual potential by 22 weeks (Table 23).

Discussion

Although the cytotypes of these plants were not determined, the germination was too high and the growth too regular to suspect that any were triploids. However, the possibility that some were diploids and others were tetraploids cannot be eliminated.

The relatively late attainment of bisexuality may be a reflection of the slow sexual development as observed by Peck (1980) and Kott and Petersen (1974).

The sequence of sexual development was consistent for all plants. No males were observed at either sampling date. The high number of females observed at the early sampling date indicates a female to bisexual ontogeny for isolated gametophytes. Evidence to date indicates that ferns in the genus Polypodium neither produce nor respond to any known antheridogens (Naf, 1969, 1979). If this is accurate, male gametophytes would not be expected in populations either.

Whether absence of an antheridogen system would favor intragametophytic selfing is not certain. This would depend primarily upon bisexual potential. Although genetic load was not adequately measured for all plants, no genetic load was expressed by any of the plants. Isolate potential was reduced solely by a combination of low germination potential and low bisexual potential. If no load is present, this would suggest that
intragametophytic selfing may be a frequent reproductive mode for this species.

The high level of variation among plants from different populations suggests that isolate potential may not be a species characteristic, but may vary from population to population.

**Thelypteris noveboracensis**

**Background**

*Thelypteris noveboracensis* is a medium sized fern of eastern North America. It forms extensive colonies in acid, humic soils of woods and thickets (Wherry, 1961; Mickel, 1979). This species acts as a very aggressive weed in the northeastern United States and Appalachians. This fern was chosen for examination because of its relationship to *T. simulata* and *T. palustris* and occurrence with them in eastern North America. Its range is less that that of *T. palustris*, but greater than that of *T. simulata*.

Iowa State University plant morphology class graduate student reports by J. O'Keefe and Y. Paisooksantivatana on *T. noveboracensis* from Giles, Co., Virginia, indicated low and slow germination. Many of the gametophytes which were formed died within the first two months. Incidence of abnormal morphology was high. Bisexual gametophytes were not observed. (These project reports were made available by Dr. D. R. Farrar, Iowa State University, Botany Department.)
Results

Five plants were transplanted from Ricketts Glen, Luzerne Co., Pennsylvania to the greenhouse at Iowa State University, where they grew new and fertile fronds the following year. Spores from these plants were used for isolate and multispore cultures. After 16 weeks, only 27 spores had germinated and 12 gametophytes had survived. These gametophytes were highly abnormal in growth. The isolate and genetic load assays were not conducted, and cultures were abandoned. Germination in multispore culture was equally poor. Spores from plants one through four produced no gametophytes; 36% of the spores from plant five germinated, but 56% of these were achlorophyllous and the remainder displayed abnormal morphology.

Discussion

Considering the rank colonies this species forms and the vigor of the plants in the greenhouse, it was surprising to find such difficulties in culturing spores and gametophytes of this species. Although there may have been some environmental or medium factor which affected their growth, long-lived clones of *T. noveboracensis* may have built up sufficient genetic load that germination and gametophyte morphology have been affected. The earlier observations by O'Keefe and Paisooksantivatana, based on spores from plants growing in their natural habitat at a site
quite distant from the spore source for the present study, suggest that the poor germination and aberrant morphology may be common in this species. The species warrants additional efforts to study its reproductive potential.

**Thelypteris palustris**

**Background**

*Thelypteris palustris* is a medium sized fern typical of shaded and exposed wetlands of eastern North America (Mickel, 1979) with peripheral populations in the North Central States (Peck, 1982). It is a member of the Thelypteridaceae, with 1,000 species, and specifically of the section *Cyclosorus*, which is typified by a chromosome base number of 36 (Smith, 1971). *Thelypteris palustris* is reported to be a diploid taxon (n=35) (Wagner, 1963).

This species has previously been studied for genetic load. Ganders (1972) presented data for 167 isolated and 12 paired gametophytes from six plants of a single small population. Average genetic load was 60% (range of 30–92%).

**Results**

Experimental results for *Thelypteris palustris* are presented in Tables 24, 25, and 26. Of 125 spores sown, 113 (90%) developed into mature gametophytes (Tables 24 and 25). At 16 weeks, most gametophytes displayed normal morphology (Table 33). One-fourth, mostly from plant number five, were multilobed. Some
Table 24. Sexual development and sporophyte production at 16 weeks for gametophytes from 5 sporophyte plants of *Thelypteris palustris* from Pine Barrens, New Jersey

<table>
<thead>
<tr>
<th>Plant</th>
<th>Spores Sown</th>
<th>Mature Prothalli</th>
<th>Sporophytes Produced by Mature Prothalli</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>#(#%)</td>
<td>#(#%)</td>
<td>Sample n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>23(92)</td>
<td>1(4)</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>19(76)</td>
<td>15(79)</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>22(88)</td>
<td>3(14)</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>25(100)</td>
<td>8(32)</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>24(96)</td>
<td>9(38)</td>
<td>6</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>113(90)</td>
<td>36(32)</td>
<td>28</td>
</tr>
</tbody>
</table>
Table 25. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants of Tlielypteris palustris from the Pine Barrens, New Jersey. Data taken at 16 weeks and expressed as percentages

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination-Development Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>89</td>
<td>5</td>
<td>95</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
<td>100</td>
<td>89</td>
<td>21</td>
<td>79</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>88</td>
<td>88</td>
<td>16</td>
<td>84</td>
<td>14</td>
<td>12</td>
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<tr>
<td>4</td>
<td>100</td>
<td>100</td>
<td>32</td>
<td>68</td>
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<tr>
<td>5</td>
<td>96</td>
<td>100</td>
<td>38</td>
<td>62</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>95</td>
<td>33</td>
<td>66</td>
<td>32</td>
<td>29</td>
</tr>
</tbody>
</table>
Table 26. Sexual development and sporophyte production at 22 weeks for gametophytes from 5 sporophyte plants of *Thelypteris palustris* from Pine Barrens, New Jersey

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>New Sporophytes Produced from Week 16 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
<td>Sexual Condition #(#%)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0(0)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0(0)</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0(0)</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0(0)</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0(0)</td>
</tr>
<tr>
<td>Totals</td>
<td>19</td>
<td>0(0)</td>
</tr>
</tbody>
</table>
proliferations were beginning to develop on most gametophytes by this time.

Sampling indicated that 2% of the gametophytes were male and 98% were bisexual. No neuter or female gametophytes were ever observed (Table 24). Of the two male gametophytes sampled, one had died after growing under the agar, and the second was small and abnormally developed.

At 16 weeks, 36 of 113 gametophytes had produced sporophytes. Gametophyte isolate potential was 33%; spore isolate potential was 29% (Table 25).

From the non-sporophyte producing gametophytes, 17 isolates were grown and watered for an additional six weeks (Table 26). Gametophytes were all bisexual at the end of this period. Twelve percent of these isolated gametophytes produced additional sporophytes.

Discussion

For this population, different plants displayed different isolate potentials. At 16 weeks, the range was from 4% to 79%. This variation among plants was not associated with the morphological variation noted for gametophytes from different sporophytes. Gametophytes from plant number one, which had produced only one sporophyte by 16 weeks, produced no additional sporophytes from gametophytes maintained until 22 weeks. From plant number two, all gametophytes grown to 22 weeks produced
sporophytes. This correlated with a high isolate potential (79%) at 16 weeks. Thus, for the plants with the highest and lowest isolate potentials, no change in relative reproduction was observed.

Because of the rapid formation of bisexual gametophytes in this species, little can be said about sexual development. The presence of male gametophytes, which might suggest that in this species male gametophytes may be formed without the influence of antheridogen, is clouded by the fact that the only male gametophytes observed were morphologically abnormal. Because all of the remaining gametophytes were bisexual by the first sampling date, nothing can be inferred about the sequence of sexual development. However, the rapidity with which bisexuality was attained would tend to enhance isolate potential.

That the plants of a strongly vegetative, clone-forming species have a varied genetic load suggests that the population tested is not entirely of vegetative origin and/or that divergence due to somatic mutations has occurred within the population. Ganders (1972) also found a high level of variability among plants of this species, suggesting that this may be a widespread and typical structure for populations of this species.
Thelypteris simulata

Background

*Thelypteris simulata* is a medium sized fern with upright fronds that frequents acidic coniferous or blueberry swamps of the upper Eastern Coastal Plain of the United States and Canada with disjunct populations in Wisconsin (Hartley, 1965; Mickel, 1979). Because *T. simulata* (n=64 for Maryland material) is somewhat intermediate between *T. palustris* (n=35) and *T. noveboracensis* (n=27), Wagner (1955, 1963) suggested it might have formed by hybridization followed by two aneuploid changes.

Smith (1971), and Tryon and Tryon (1974) questioned the hybrid origin of this species, suggesting that it shows closer affinity to Asian species. Smith (1971) reported for *Thelypteris* (s.l.) that chromosome base numbers of 36, 35, 34, 31, 30, 29, and 27 (26?) are known. He found the chromosome numbers of both *T. noveboracensis* and *T. simulata* to be somewhat anomalous for the New World and suggested affinity with Asian species, possibly within *Parathelypteris*. He felt that doubling of a base number of 32 is a simpler hypothesis for *T. simulata* than that offered by Wagner. Smith (1971) indicated that numbers of 31 and 32 have also been reported for Asian members of *Parathelypteris*.

The species was first collected at Seabrook, New Hampshire in 1880. By 1948, it had been collected in Wisconsin (Peck, 1982).
Hartley (1965) published the first report of disjunct populations of *T. simulata* in Wisconsin. He found an abundance of this species growing in low sandy woods in the old bed of Glacial Lake Wisconsin in Jackson County, Wisconsin. Collections were made in 1958 and 1960 at two localities about eight miles apart. The chromosome number has been reported to be "very close to n=64" (Hartley, 1965). Tryon and Tryon (1974) reported 2n=128 for mitotic preparations from root tips of Wisconsin material.

Hartley (1965) suggested three possible origins for the Wisconsin populations: 1) independent hybrid origin, 2) relictual origin from a possible late or post-pleistocene distribution along the great lakes from the Atlantic Coastal Plain, or 3) a recent long distance dispersal. Hartley thought long distance dispersal less likely, due to the prevailing winds from the west and the closed nature of the Wisconsin woodlands.

Peck (1982) reported 34 populations of the species from five Wisconsin counties, greatly expanding extent of the Wisconsin disjunct portion of the range. Moran (1980) reported a plant growing just above ground level in a crevice of a sandstone cliff at Castle Mound State Park, Jackson Co., Wisconsin. He believed this plant must have been the result of single spore reproduction. Moran (1981) has also noted a southern disjunct of this species at Newfound Gap, Tennessee, further attesting to its ability to occupy areas beyond its typical range in the extreme northeastern
United States. Study material for this investigation came from 10 disjunct populations in Wisconsin (Peck, 1982).

**Results.**

Experimental results for *Thelypteris simulata* are presented in Tables 27, 28, and 29. From population number one (Monroe Co., Wisconsin, 25 spores were sown for each of five plants to assay reproductive potentials. At 16 weeks (Table 27), only 50% of the spores had formed mature gametophytes.

The species exhibited all four classes of morphological development (Table 33). With 12% calluslike and 9% bushy gametophytes mounds, this species was the most abnormal, except for *Dryopteris marginalis*, of all species tested. Although nearly 30% of the gametophytes produced sporophytes, the sexuality of the gametophytes suggested some sexual immaturity was present. The most abnormal gametophytes were neuter or male. It appeared that normal gametophytes first became female and then bisexual. These plants were limited by a low isolate potential (26% gametophyte and 13% spore). Values were similar for the five plants (Table 28). At 22 weeks, isolated gametophytes had produced no additional sporophytes (Table 29).

A survey of plants from 10 different populations was also conducted. At four months after sowing, a test of 4,250 isolated *Thelypteris simulata* spores produced 2.9% sporophytes. At the same age, and following the same watering regime, 30 (20%) of 150
Table 27. Sexual development and sporophyte production at 16 weeks for gametophytes from 5 sporophyte plants of *Thelypteris simulata* from Monroe Co., Wisconsin

<table>
<thead>
<tr>
<th>Plant</th>
<th>Spores Sown</th>
<th>Mature Prothalli</th>
<th>Sporophytes Produced by Mature Prothalli</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>Sample n</th>
<th>Sexual Condition #(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>#(#%)</td>
<td>#(#%)</td>
<td>Sample n</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sexual Condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>16(64)</td>
<td>4(25)</td>
<td>3</td>
<td>0(0)</td>
<td>1(33)</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>17(68)</td>
<td>4(24)</td>
<td>5</td>
<td>0(0)</td>
<td>1(20)</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>16(64)</td>
<td>5(31)</td>
<td>4</td>
<td>2(50)</td>
<td>0(0)</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>11(44)</td>
<td>4(36)</td>
<td>3</td>
<td>0(0)</td>
<td>1(33)</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>2(8)</td>
<td>1(50)</td>
<td>1</td>
<td>1(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>62(50)</td>
<td>18(29)</td>
<td>16</td>
<td>3(19)</td>
<td>3(19)</td>
</tr>
</tbody>
</table>
Table 28. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants of *Thelypteris simulata* from Monroe Co., Wisconsin. Data taken at 16 weeks and expressed as percentages

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination-Development Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
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<td>67</td>
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<tr>
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<td>64</td>
<td>66</td>
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<td>52</td>
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<td>4</td>
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<td>79</td>
<td>46</td>
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<td>8</td>
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<td>100</td>
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<tr>
<td>Total</td>
<td>50</td>
<td>67</td>
<td>38</td>
<td>62</td>
<td>26</td>
<td>13</td>
</tr>
</tbody>
</table>
Table 29. Sexual development and sporophyte production at 22 weeks for gametophytes of 5 sporophyte plants of *Thelypteris simulata* from Monroe Co., Wisconsin

<table>
<thead>
<tr>
<th>Plant</th>
<th>Sexual Status of Non-sporophyte Producing Prothalli</th>
<th>New Sporophytes Produced from Week 16 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
<td>Sexual Condition #(%)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>N</td>
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<tr>
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<tr>
<td>3</td>
<td>3</td>
<td>0(0)</td>
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<tr>
<td>4</td>
<td>2</td>
<td>1(50)</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0(0)</td>
</tr>
<tr>
<td>Totals</td>
<td>11</td>
<td>1(9)</td>
</tr>
</tbody>
</table>
multispore gametophyte populations produced one or more sporophytes. A high percentage of these gametophytes, both as isolates and in multispore culture, displayed aberrant development and morphology.

Discussion

The limited gametophyte and spore isolate potentials of these plants, coupled with significant abnormal gametophyte development, suggest that genetic limitations would significantly restrict these populations. This suggests that the plants that were sampled had not recently undergone intragametophytic selfing. They are not recent arrivals in Wisconsin. Their strong vegetative expansion by rhizomes into large colonies may explain why variation within populations was small. That similar values were obtained in all 10 populations (0-14% with 2.9% average), suggests that a similar scenario exists for all 10 populations.

The results from the present study provide evidence bearing upon the possible origins of the Wisconsin populations of Thelypteris simulata as proposed by Hartley (1965). The uniformly low isolate potential, including relatively high genetic load, preclude the possibility that these populations have resulted from recent long distance single spore dispersal.

Several factors mitigate against separate hybrid origin. First, the hybrid origin proposed by Wagner (1955, 1963) may not be the most likely origin considering the similarity to Asian
species and the frequency of Asian–eastern North American distributions. Second, the present study suggests that one of the putative parents, Thelypteris noveboracensis, may have a reproductive potential so low as to call into question its involvement in frequent hybridization events. Separate hybrid origin of the Wisconsin Thelypteris simulata would not have been a recent occurrence, since the present distribution of T. noveboracensis does not extend to Wisconsin. Thus, the separate hybrid origin hypothesis is also a relictual hypothesis.

The results of the present study support a relictual hypothesis for T. simulata in Wisconsin. That there are a fairly large number of populations distributed over several counties and that these populations all have a low isolate potential favors the possibility of an expanded post–pleistocene distribution for the species. The present populations are a poor source for single spore colonization, and the low isolate potential for all populations indicates they may all be equally old.

It would be of interest to test the isolate potential of the Castle Rock plant and the Tennessee population as well as populations from the center of the range.

**Woodsia obtusa**

**Background**

Woodsia obtusa, a small fern with a short–creeping rhizome, is a common inhabitant of shaded cliffs and rock ledges in eastern
North America (Mickel, 1979). The species is reported to be diploid (n=41) (Cranfill, 1980).

The Woodman Hollow population from Webster Co., Iowa, used for this study, is at the northwestern periphery of this range (Peck, 1976). Here, plants occur on upper and lower north-facing and south-facing slopes (Peck, 1980).

There have been two important studies of this species. Gametophyte morphology was studied by Kaur and Chandra (1973). Peck (1980) made an extensive study of this species under natural conditions at Woodman Hollow and in multispore culture.

Peck (1980) reported that *Woodsia obtusa* was one of the five most abundant pteridophyte species present in Woodman Hollow (1,000-10,000 apices). Evidence of sexual reproduction was commonly observed, but *Woodsia* did not appear to reproduce vegetatively at Woodman Hollow.

Estimates of spore production indicated that *W. obtusa*, with large numbers of plants present (ten thousand) and fairly large numbers of spores per plant (sixty million), was the largest spore producer among the 14 fern species in the valley. Its approximately 600 billion spores per season accounted for 42% of spore production at Woodman Hollow. Spore release was nearly complete (90-99% depending on moisture conditions). Farrar (1976) also reported 1-10% spore retention for this species at Woodman Hollow.
Summer spore release with a June-July maximum was typical. In wet years, which favored late season frond production, a second spore release period occurred in late autumn or early spring. During both periods canopy cover was present, a condition favoring retention of spores in the local environment. Some spores were found to escape the immediate vicinity of the plant. Five of nine spore traps within 10 meters of Woodsia plants trapped a total of 38 spores during the year. However, no spores were trapped at stations beyond ten meters from fertile plants (Peck, 1980).

Germination was reported to occur within 10-15 days after spores were sown (Kaur and Chandra, 1973). Peck (1980) found germination to be high (mean 96%) for Woodsia spores from plants at Woodman Hollow. Viability was not reduced after 15 months refrigerated storage. Farrar (1976) tested the viability of spores retained on overwintering fronds. Woodsia spore germination dropped from 82% in December to 59% in March. Because increases of this magnitude were as frequent as decreases, Farrar attributed these differences to variation among plants rather than to a genuine reduction in viability.

Kaur and Chandra (1973) reported germination was of the Vittaria type and gametophyte development was of the Aspidium type. They noted that in their multispore cultures, some germ filaments branched. They also noted that on young and old gametophytes, cells subtending marginal hairs sometimes grew out
from the margin as uniseriate filaments which then gave rise to secondary cordate prothalli. In his comparisons of field grown gametophytes to cultured gametophytes, Peck (1980) found field gametophytes to be far more uniform in shape, margins, and location of sex organs.

In 54 gametophyte populations studied, *Woodsia* gametophytes were found in 86% of gametophyte populations within 10 meters of *Woodsia* sporophyte plants (Peck, 1980). This was nearly twice as high as the next most frequent species of gametophyte.

Farrar and Gooch (1975) reported that by early November most gametophytes in natural populations at Woodman Hollow were mature and sporophyte production was evident. This was approximately four months after the initiation of spore release as reported for that year.

Kaur and Chandra (1973) reported that gametophytes were mature 10-12 weeks after germination, but they do not indicate the criteria used to determine maturity. They reported that sex organs occurred only on the undersurface of the midrib with antheridia developing much later than archegonia.

In multispore culture (Peck, 1980), 40% of *Woodsia* gametophytes were found to be sexually mature approximately one month after germination. Based on samples taken at monthly intervals, Peck (1980) found an early predominance of males (74% at 2 months) with later dominance of bisexuals (44% at 4 months,
74% by 9 months). Females, never were predominant (high of 18% at 3 months), and later declined to a low percentage (2% by 3 months):

In culture, Peck (1980) demonstrated an antheridogen effect for *Woodsia*. In antheridogen treated cultures, 11% males were present as early as seven days. At 21 days, when 3% males had formed in untreated multispore culture, 99% of treated gametophytes were male. In untreated cultures, a large fraction of the gametophytes later developed as males, 43% by day 28, the last day of the experiment. Males in treated populations were dwarfed and ameristic, such as those typically described as the product of early antheridogen influence. Males in untreated populations were cordate. Kaur and Chandra (1973) reported ameristic gametophytes in untreated multispore cultures.

In 15 *Woodsia* gametophyte populations sampled in the fall of 1975, Peck (1980) also found a high percentage of male gametophytes (72–85%). In spite of the high percentage of males, Peck (1980) reported that he could not unequivocally ascribe this to antheridogen effects because the percentage of male gametophytes did not increase with increased population density, nor were the male gametophytes dwarfed or ameristic. He also examined 100 naturally isolated gametophytes and found all of these to be male, suggesting that factors other than antheridogen may favor the development of males.
The same gametophyte populations, sampled the following spring, were found to be predominantly bisexual (93% mean). The percentage of male gametophytes dropped correspondingly (82% to 3%), while percent females and neuter declined only slightly (6% to 4% and 1% to 0.3%). This suggests that males became bisexual; whereas, females tended to remain female.

Even though abundant males were present, Peck (1980) found that sporophytes were formed by bisexual gametophytes, compared to female gametophytes, at a much greater frequency than would be expected according to their relative frequency within the population.

Results

Experimental results for *Woodsia obtusa* are presented in Tables 30 and 31. In the present study, isolated gametophytes were examined four months after spores were sown. Germination-development potential was high; 82.4% of spores produced gametophytes surviving to maturity (Table 31). Gametophytes uniformly developed as a single cordate lobe (Table 33). At 16 weeks, some proliferations were beginning to develop. Of the surviving gametophytes, 80% were bisexual; 20% were female (Table 30). In isolate culture, all bisexual gametophytes produced a sporophyte. With 100% sporophyte production, no genetic load was demonstrable from the plants selected for study (Table 31). At the end of one year, 51% of these sporophytes had survived.
Table 30. Sexual development and sporophyte production at 16 weeks for gametophytes from 5 sporophyte plants of Woodsia obtusa from Woodman Hollow, Webster Co., Iowa

<table>
<thead>
<tr>
<th>Plant</th>
<th>Spores Sown</th>
<th>Mature Prothalli</th>
<th>Sporophytes Produced by Mature Prothalli</th>
<th>Sexual Status pf Non-sporophyte Producing Prothalli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>#(#)</td>
<td>#(#)</td>
<td>Sample</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>23(92)</td>
<td>14(61)</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>25(100)</td>
<td>17(68)</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>21(84)</td>
<td>19(90)</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>21(84)</td>
<td>19(90)</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>13(52)</td>
<td>13(100)</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>103(82)</td>
<td>82(80)</td>
<td>21</td>
</tr>
</tbody>
</table>
Table 31. Reproductive potential of gametophytes sown from 25 spores of 5 sporophyte plants of *Woodsia obtusa* from Woodman Hollow, Webster Co., Iowa. Data taken at 16 weeks and expressed as percentages.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Germination Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>61</td>
<td>100</td>
<td>0</td>
<td>61</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>68</td>
<td>100</td>
<td>0</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>3</td>
<td>84</td>
<td>90</td>
<td>100</td>
<td>0</td>
<td>90</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>90</td>
<td>100</td>
<td>0</td>
<td>90</td>
<td>76</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>80</td>
<td>100</td>
<td>0</td>
<td>80</td>
<td>66</td>
</tr>
</tbody>
</table>
Discussion

Woodsia obtusa's high germination, uniform normal development, high percentage of bisexual isolated gametophytes, and absence of genetic load all should favor success of isolated Woodsia obtusa spores in establishing new populations. According to Peck's (1980) data, these factors should also contribute to successful sexual reproduction in the local habitat, since his data suggested that most new sporophytes may be produced by intragametophytic selfing, even in populations of gametophytes with sex ratios which would appear to favor outcrossing.

The high numbers of sporophytes observed at Woodman Hollow combined with low capacity for vegetative reproduction suggests that this species is capable of active sexual reproduction within a population. However, this gives no indication of isolate potential or the potential for long distance dispersal. Long distance dispersal of spores is the necessary precursor to disjunct population establishment. The success of Woodsia in producing large local populations, and thus, large numbers of spores, enhances its relative potential for long distance spore dispersal.

However, the only factor cited by Peck (1980), for which Woodsia did not have an advantage over all of the other 13 Woodman Hollow ferns, was ability to distribute spores away from the
immediate vicinity of the plant. Only about half the time could Peck demonstrate that spores were distributed up to 10 m away from a potential spore source and in no instance could he demonstrate spores dispersed more than 10 m from a spore source. This does not necessarily mean spores never escape the local environment.

Woodsia obtusa has demonstrated the ability to colonize new sites at some distance from a spore source. Woodsia has colonized Klein Mine coal spoils, Marion Co., Iowa, a site available for less than 40 years (Crist and Farrar, 1983). There are nearby spore sources along the Des Moines River valley at least 10 km away.

In summary, Woodsia obtusa at Woodman Hollow appears to be a species relatively well-adapted to establish new populations from single spores, if its spores can escape their local environment and become lodged in suitable sites. There is evidence that Woodsia obtusa has been able to establish new populations far enough from parent spore sources to assume that they were established by a single spore.

The proliferative morphologies described by Kaur and Chandra (1973) contrast with the highly regular development observed in the present study. This may be evidence of interpopulation variation. Another possibility is that the high light levels (600 ft-c), or other unreported cultural parameters, used in their study influenced development.
Lack of males in isolate culture indicates that males may form only in response to antheridogen; without antheridogen influence all gametophytes are first female. A second possibility is that males rapidly became bisexual, while females remained female. This seems less likely. The distribution of sexes was distinctly different from sex ratios observed by Peck (1980) in both natural and cultured populations. At four months, he observed 2% neuter, 48% male, 6% female, and 44% bisexual gametophytes in multispore cultures. These differences point to a factor in populations that increased maleness, an intergametophytic antheridogen. However, the lack of a correlation between increasing percentage of males with increasing population density and the occurrence of only male, isolated gametophytes in Peck's (1980) data remains unexplained by an antheridogen hypothesis.

The higher percentage of bisexuals in isolated culture at four months may be due to differences in initial sexual development. Induced males in multispore culture may be slower to become bisexual. Alternatively, isolated culture may have provided more favorable growth conditions, allowing isolated gametophytes to be larger by four months and, thus, to be able to attain bisexual status sooner.

Demise of sporophytes may be more attributable to culture conditions than to genetic defects. The .51% survival does
indicate that a large number of viable sporophytes were produced.

The high isolate potential does not indicate whether the population at Woodman Hollow is relictual or recent. It only demonstrates that high bisexual potential favors frequent intragametophytic selfing and that frequent intragametophytic selfing has maintained a load free population.
RESULTS AND DISCUSSION II: REPRODUCTIVE PHENOMENA

Introduction

The following section compares variations observed among species relative to germination, morphology, sexual development, genetic load, and isolate potential. Experimental protocol is evaluated and suggestions for future studies are presented. To aid in this discussion, summary values for gametophyte reproductive potentials for all species are presented in Table 32. Table 33 presents percent occurrence of four morphological classes for the species studied. Table 34 presents sexual status for all species at sixteen weeks. Comparative spore and gametophyte reproductive data are presented in Table 35. Correlation coefficients (r) were calculated to quantify the strength of associations between the values from Tables 32, 33, 34, and 35. Resultant r values were tested for significance and are presented in Table 36.

Germination Potential

The percentage of spores which gave rise to mature gametophytes (germination potential) ranged from virtually 0% for *Thelypteris noveboracensis* to lows of 26% for *Cystopteris bulbifera* and 30% for *Dryopteris spinulosa* to highs of 90% for *Thelypteris palustris* and 94% for *Cryptogramma stelleri* (Table 32).

There was no relationship between presence of a perispore and
Table 32. Summary table of comparative gametophytic reproductive potentials for the species surveyed

<table>
<thead>
<tr>
<th>Species</th>
<th>Germination Potential</th>
<th>Bisexual Potential</th>
<th>Selfing Potential</th>
<th>Genetic Load</th>
<th>Gametophyte Isolate Potential</th>
<th>Spore Isolate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. angustum</td>
<td>72</td>
<td>23</td>
<td>4</td>
<td>95</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C. stelleri</td>
<td>94</td>
<td>50</td>
<td>2</td>
<td>98</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C. bulbifera</td>
<td>26</td>
<td>17</td>
<td>38</td>
<td>62</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>C. tenuis</td>
<td>53</td>
<td>67</td>
<td>88</td>
<td>12</td>
<td>59</td>
<td>31</td>
</tr>
<tr>
<td>D. cristata</td>
<td>82</td>
<td>28</td>
<td>92</td>
<td>8</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>D. marginalis</td>
<td>71</td>
<td>51</td>
<td>4</td>
<td>96</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>P. virginianum</td>
<td>59</td>
<td>32</td>
<td>100</td>
<td>0</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>T. palustris</td>
<td>90</td>
<td>95</td>
<td>33</td>
<td>66</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>T. simulata</td>
<td>50</td>
<td>68</td>
<td>38</td>
<td>62</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>W. obtusa</td>
<td>82</td>
<td>80</td>
<td>100</td>
<td>0</td>
<td>80</td>
<td>66</td>
</tr>
</tbody>
</table>
Table 33. Survey of gametophyte morphology, contrasting the study species with the percentage of normal (single cordate lobe) with the percentage of abnormal (calluslike, hairy or bushy mounds, and multilobed) gametophytes.

<table>
<thead>
<tr>
<th>Species</th>
<th>single cordate lobe</th>
<th>calluslike</th>
<th>hairy or bushy mounds</th>
<th>multilobed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. angustum</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>C. stelleri</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>C. bulbifera</td>
<td>78</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>C. tenuis</td>
<td>5</td>
<td>0</td>
<td>33</td>
<td>62</td>
</tr>
<tr>
<td>D. cristata</td>
<td>86</td>
<td>1</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>D. marginalis</td>
<td>2</td>
<td>0</td>
<td>96</td>
<td>2</td>
</tr>
<tr>
<td>P. virginianum</td>
<td>96</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>T. palustris</td>
<td>73</td>
<td>0</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>T. simulata</td>
<td>42</td>
<td>13</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>N. obtusa</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 34. Summary of gametophyte sexuality at 16 weeks of isolate culture for sporophyte bearing plus non-sporophyte bearing gametophytes expressed as a percent of the population being neuter, male, female, or bisexual.

<table>
<thead>
<tr>
<th>Species</th>
<th>Neuter</th>
<th>Male</th>
<th>Female</th>
<th>Bisexual</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. angustum</td>
<td>0</td>
<td>0</td>
<td>77</td>
<td>23</td>
</tr>
<tr>
<td>C. stelleri</td>
<td>0</td>
<td>11</td>
<td>39</td>
<td>50</td>
</tr>
<tr>
<td>C. bulbifera</td>
<td>42</td>
<td>21</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>C. tenuis</td>
<td>21</td>
<td>12</td>
<td>0</td>
<td>67</td>
</tr>
<tr>
<td>D. cristata</td>
<td>22</td>
<td>0</td>
<td>50</td>
<td>28</td>
</tr>
<tr>
<td>D. marginalis</td>
<td>0</td>
<td>21</td>
<td>28</td>
<td>51</td>
</tr>
<tr>
<td>D. spinulosa</td>
<td>3</td>
<td>11</td>
<td>0</td>
<td>86</td>
</tr>
<tr>
<td>P. virginianum</td>
<td>0</td>
<td>0</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>T. palustris</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>T. simulata</td>
<td>14</td>
<td>14</td>
<td>4</td>
<td>68</td>
</tr>
<tr>
<td>W. obtusa</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th># Spores Produced (millions)</th>
<th>% Spore Release</th>
<th>SIP Isolate Competent Spores (1,000's)</th>
<th>Spores/m² Woodman Hollow (1,000's)</th>
<th>Spores/m² Iowa (Iowa)</th>
<th>Sq Meters per Spore (Iowa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. obtusa</td>
<td>600,000</td>
<td>99</td>
<td>392,000,000</td>
<td>1,500,000</td>
<td>2.7</td>
<td>0.37</td>
</tr>
<tr>
<td>C. tenuis</td>
<td>100,000</td>
<td>99</td>
<td>30,700,000</td>
<td>117,000</td>
<td>.21</td>
<td>4.76</td>
</tr>
<tr>
<td>A. angustum</td>
<td>26,000</td>
<td>99</td>
<td>257,000</td>
<td>984</td>
<td>.00177</td>
<td>565</td>
</tr>
<tr>
<td>C. bulbifera</td>
<td>6,000</td>
<td>99</td>
<td>119,000</td>
<td>463</td>
<td>.000833</td>
<td>1,200</td>
</tr>
<tr>
<td>C. stelleri</td>
<td>16</td>
<td>35</td>
<td>56</td>
<td>0.21</td>
<td>.000000378</td>
<td>265,000</td>
</tr>
</tbody>
</table>
Table 36. Correlation coefficients for the extent of association between 16 variables taken two at a time with respect to gametophyte reproductive potentials, gametophyte sexuality, and gametophyte morphology. Critical values were determined from Rohlf and Sokal (1981).

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gm Pt (#16)</td>
<td>.398</td>
<td>.168</td>
<td>-.534</td>
<td>-.733*</td>
<td>-.012</td>
<td>.119</td>
<td>-.036</td>
</tr>
<tr>
<td>BP (#15)</td>
<td>.999**</td>
<td>-.729*</td>
<td>-.064</td>
<td>-.410</td>
<td>-.115</td>
<td>-.216</td>
<td>.075</td>
</tr>
<tr>
<td>Gn Pt (#14)</td>
<td>.096</td>
<td>.040</td>
<td>-.475</td>
<td>.192</td>
<td>-.015</td>
<td>-.268</td>
<td>-.265</td>
</tr>
<tr>
<td>GL (#13)</td>
<td>-.098</td>
<td>.042</td>
<td>.479</td>
<td>-.194</td>
<td>.008</td>
<td>.269</td>
<td>.268</td>
</tr>
<tr>
<td>GIF (#12)</td>
<td>.585</td>
<td>-.403</td>
<td>-.403</td>
<td>-.062</td>
<td>.103</td>
<td>-.111</td>
<td>-.180</td>
</tr>
<tr>
<td>SIP (#11)</td>
<td>.664*</td>
<td>-.462</td>
<td>-.378</td>
<td>-.103</td>
<td>.161</td>
<td>-.137</td>
<td>-.163</td>
</tr>
<tr>
<td>Normal (#10)</td>
<td>-.242</td>
<td>.435</td>
<td>-.661*</td>
<td>.023</td>
<td>.448</td>
<td>-.694*</td>
<td>-.616</td>
</tr>
<tr>
<td>Abnormal (#9)</td>
<td>.242</td>
<td>-.435</td>
<td>.661*</td>
<td>-.023</td>
<td>-.448</td>
<td>.694*</td>
<td>.616</td>
</tr>
<tr>
<td>Callus (#8)</td>
<td>.227</td>
<td>-.347</td>
<td>.224</td>
<td>.087</td>
<td>-.409</td>
<td>.110</td>
<td>-.074</td>
</tr>
<tr>
<td>Bushy (#7)</td>
<td>.073</td>
<td>-.183</td>
<td>.565</td>
<td>-.123</td>
<td>-.330</td>
<td>-.130</td>
<td>.999**</td>
</tr>
<tr>
<td>Multi (#6)</td>
<td>.215</td>
<td>-.342</td>
<td>.302</td>
<td>.066</td>
<td>.213</td>
<td>.999**</td>
<td></td>
</tr>
<tr>
<td>Common (#5)</td>
<td>-.121</td>
<td>.233</td>
<td>-.400</td>
<td>.030</td>
<td>.999**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuter (#4)</td>
<td>-.406</td>
<td>-.272</td>
<td>.453</td>
<td>.999**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (#3)</td>
<td>-.065</td>
<td>.489</td>
<td>.999**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (#2)</td>
<td>.731</td>
<td>.999**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bisexual (#1)</td>
<td>.999**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Critical values based on n = 10, df = 2, \( v = 8 \), significance at \( P = 0.05 \), if \( r > \pm 0.632 \), denoted with *
** significance at \( P = 0.01 \), if \( r > \pm 0.765 \), denoted with **
nonsignificant values not denoted
<table>
<thead>
<tr>
<th></th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.299</td>
<td>0.040</td>
<td>-0.034</td>
<td>0.281</td>
<td>0.071</td>
<td>0.121</td>
<td>-0.122</td>
<td>0.487</td>
<td>0.999**</td>
</tr>
<tr>
<td>2</td>
<td>0.216</td>
<td>0.245</td>
<td>-0.243</td>
<td>0.663*</td>
<td>0.585</td>
<td>-0.095</td>
<td>0.093</td>
<td>0.999**</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-0.101</td>
<td>-0.404</td>
<td>0.405</td>
<td>0.584</td>
<td>0.799**</td>
<td>-0.999**</td>
<td>0.999**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.103</td>
<td>0.407</td>
<td>-0.407</td>
<td>-0.586</td>
<td>-0.799**</td>
<td>0.999**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-0.006</td>
<td>-0.216</td>
<td>0.218</td>
<td>0.907**</td>
<td>0.999**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-0.062</td>
<td>-0.230</td>
<td>0.235</td>
<td>0.999**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-0.136</td>
<td>-0.999**</td>
<td>0.999**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.134</td>
<td>0.999**</td>
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high germination potential for isolated spores, as was suggested by Bell (1958). Cryptogramma stelleri and Polypodium virginianum, the two species lacking a perispore, exhibited the highest and the seventh highest germination potentials of the 11 species surveyed.

Differences in germination of the magnitude observed in this study could contribute to significant differences in species reproductive success. However, differences as measured under conditions of this study may not reflect true differences among species. Although the ability to germinate and produce viable gametophytes undoubtedly has a genetic component, other variables, such as environmental effects during spore formation, variation in spore maturity at time of collection, and variation in effects of storage, may be large enough to generally mask or confound any inherent genetic differences.

Furthermore, it may be difficult to distinguish among causes for poor germination. To assess the relative importance of these factors would require a study comparing germination in single spore and multispore culture for spores of individual plants collected at different times, sown at different ages, and grown on different substrates.

In contrast to the isolate germination potentials observed in this study, Peck (1980) demonstrated germination percentages greater than 90% in multispore culture for all Woodman Hollow species at the time of collection and after 15 months of storage.
The wide range of germination percentages observed for isolated spores of the same species in the present study could be due to differential effects of isolation among species. It has been observed that spores in isolation may germinate less well than those in multispore cultures (Nester, 1979). However, from the data available, environmental differences as cited above cannot be discounted. No definite conclusions can be drawn without more detailed surveys of isolated spore germination potential, including comparisons with germination in multispore cultures.

Morphology

Of the 10 species studied, nine formed single cordate gametophytes, nine formed multilobed gametophytes, five formed hairy or bushy mounds, and three species formed calluslike growths (Table 33). Distribution of the four morphological types among the study species was not uniform. Six species (Athyrium angustum, Cystopteris bulbifera, Dryopteris cristata, Polypodium virginianum, Thelypteris palustris, and Woodsia obtusa) displayed predominantly normal morphology (one to two large cordate lobes). Two species (Cryptogramma stelleri and Cystopteris tenuis) were predominantly multilobed. Dryopteris marginalis gametophytes were predominantly hairy or bushy mounds. Although no species exhibited predominantly calluslike morphology, a significant proportion of Thelypteris simulata gametophytes displayed this morphology.
Previous reports have suggested an increased incidence of morphological aberrations in isolate versus multispore cultures (Bell, 1958; Nester, 1979). In the present study, a high percentage of the gametophytes were morphologically abnormal. Even though isolated gametophytes of all species were grown under uniform conditions, morphological differences among species were apparent. The percentages of abnormal gametophytes as well as the relative numbers of different types of abnormal gametophytes differed widely among the species tested. Although more natural growing conditions (less diffusion, presence of fungi, stronger polarity cues from the environment) might modify the percentages, the range of response shown by the species under uniform conditions of this study show that isolation alone was not sufficient to induce abnormal growth in all species. Rather, species or populations of gametophytes differed in their potential to produce normal gametophytes in isolation.

Additional support for the existence of inherent species' tendencies comes from comparison of morphological development in isolate versus multispore culture. Multispore culture of *Thelypteris simulata* sown from 10 different populations all exhibited a high rate and degree of morphological irregularity. All of the morphological types observed in isolate culture were also observed in populations. This suggests that the abnormalities are inherent within the species and not only a
response to conditions of culture as isolated spores.

The environment may also play a role in determining gametophyte morphology. For example, if concentrations of certain metabolites are a critical factor in normal development, the relatively large volume of agar per gametophyte (four ml) in isolate culture may tend to reduce concentrations of diffusable substances in and around the gametophyte. This might tend to increase the number of abnormal gametophytes, as suggested by Bell (1958), by influencing the minimally competent gametophytes to develop abnormally.

Presence of other gametophytes in multispore cultures may provide or increase concentrations of growth regulating substances, minimizing medium influences. Lower frequencies of aberrations in multispore cultures may be evident because normal gametophytes in populations secrete sufficient metabolites into the medium which are able to compensate for growth regulators or other substances absent in the potentially abnormal gametophyte, and thus, allow normal development to proceed. In isolate culture, gametophytes cannot overcome any genetic defects, and thus, the true percentage of such abnormalities becomes apparent.

Effects of diffusion and lack of polarity cues from the uniform agar medium may be increased if the spore or young gametophyte begins development under the agar surface or within a droplet or pool of liquid on the agar surface. These
circumstances may be more frequent for spores placed on the agar surface with a needle than for spores sprinkled on the surface of the medium.

Polarity is evidently important in the initiation of normal morphological development (Kotenko, 1983). Natural gradients in light, moisture, and perhaps other factors in the environment of the spore, which may be important in establishing and maintaining polarity within the developing gametophyte, are undoubtedly much weaker under typical culture conditions than in nature.

Morphological development in isolate culture may differ from gametophyte development in nature. Reports of the morphology of naturally occurring gametophytes have stressed the high degree of uniformity and absence of aberrant development (Cousens, 1979, 1981; Schneller, 1975; Peck, 1980). In nature, diffusion of metabolites into the environment would probably be reduced in comparison to diffusion in agar culture.

In nature, the presence of other organisms may alter the morphological development of the gametophyte compared to that observed in culture. For example, most reports suggest that fungi enhance gametophyte growth and normal development (Smith and Robinson, 1969; Hutchinson and Fahim, 1958). Although detailed observations on the relationship of fungi to morphology in isolate and multispore cultures were not made in this investigation, minimal fungal contamination was observed in isolate culture. In
nature, where fungi are ubiquitous, the complex interactions among microorganisms and among fungi could potentially improve the growth rate and produce a higher percentage of gametophytes with normal morphology. Moderate damage from insects or overwintering could initiate proliferative morphology in nature. The factors cited above would tend to enhance normal development in nature, as compared with development in agar culture.

In considering the general absence of aberrant gametophytes in nature, the significant question to ask is whether gametophytes displaying abnormal morphology in culture would (1) develop normally in nature, or whether they would (2) fail to develop or be selected against in nature. In the first situation, the proportion of gametophytes which have aberrant morphology in culture might be expected to behave in nature similarly to species with gametophytes that behaved normally in culture. For example, if normal morphology was associated with sporophyte production, then potentially sporophyte producing gametophytes would be expected to increase under natural conditions. If bisexuality was associated with proliferative morphology, however, then unisexual gametophytes would be increased and isolate potential decreased in nature.

In the second situation, aberrant gametophytes fail to develop or are selected against in nature. This may reduce natural isolate potential in proportion to the extent that
aberrant gametophytes in culture produced sporophytes. On the other hand, any explanation that relates lessened adaptive value to aberrant morphology must be stated carefully. Hairy or bushy mound morphology, for example, which is aberrant for fern gametophytes, is the typical form of *Equisetum* gametophytes in culture and in nature. The adaptive significance of varied gametophyte morphology has not been adequately examined.

**Gametophyte Sexuality**

The present study provides some initial comparative data on the sexual development of homosporous fern gametophytes grown in the absence of intergametophytic interactions (Table 34). All four types of sexual condition were observed at 16 weeks. Sexual expression differed among species. Five of 10 study species had some neuter gametophytes; seven of 10 had males; eight of 10 had females; and all 10 had bisexual gametophytes.

The presence of neuter and unisexual gametophytes reduced the bisexual potential and contributed to lower isolate potential of the study species. Slow growth and poor germination contributed to the presence of neuters. Males were not expected, if they were initiated only in response to antheridogen activity. Stress or disruption of development may have contributed to the presence of males. Females were indicative of the expected female to bisexual developmental pattern in isolation. Presence of bisexuals indicated that unisexual gametophytes were not an inherent or
obligatory developmental pattern.

In isolate culture, gametophytes of some species developed protogynously and others developed protandrously, becoming initially male, even in the absence of antheridogen influence from other gametophytes. Either initial sex expression is determined in the spore or a factor not controlled in this experiment influenced sexual development. As a result, the species differed in the relative numbers of female to male gametophytes produced.

Although most gametophytes studied did become bisexual, there was variation among species. Over the time of the study, it appeared that males of some species became bisexual while females remained unisexual. Isolated gametophytes of all species studied ultimately produced a high percentage of bisexual gametophytes over the course of 16-22 weeks. This indicates that given appropriate conditions these species all have the potential to form bisexual gametophytes, at least in culture.

There are factors other than antheridogens which influence sexuality among gametophytes and determine the initial sexual expression of gametophytes. Before the discovery of antheridogens, early workers noted that crowded conditions, low light levels, and possibly poor nutrition favored maleness. Antheridogen effects may explain those observations. More recently, Warne and Lloyd (1980) found that sex ratios in populations of *Matteuccia struthiopteris* gametophytes varied with
temperature. Factors such as these might partially explain some of the results noted in isolate culture under present conditions of culture.

Age, size, proliferation, and damage followed by regeneration have been associated with the conversion of female antheridiogen producing gametophytes to bisexual ones. Natural gametophytes apparently are much slower growing and much more regular in form than are cultured gametophytes. Therefore, it could be expected that conversion to bisexuality would require a longer time period in nature, perhaps longer than the time available for growth in the habitat in one year. On the other hand, moderate damage from insects and overwintering could cause subsequent proliferations and, thus, favor bisexuality under natural conditions.

Independent formation of males and females in isolate culture is clearly possible. Additional studies using isolate culture are needed to examine factors influencing sex expression of homosporous fern gametophytes.

 Genetic Load

For the species tested, genetic load ranged from 0% to 98% (Table 32). This range of values is similar to that reported by Holbrook-Walker and Lloyd (1973) and Lloyd (1974b) for ferns from different habitats in Hawaii. They associated successional stage of the habitat to mating system and mating system to genetic load. Colonizing species had mating systems which favored inbreeding and
had low genetic load. Species in mature, stable habitats had
outbreeding systems and high genetic load. They reasoned that
habitat selected for mating system which selected for genetic
load. According to their hypothesis, one would expect the ferns
surveyed in the present study to all have outbreeding systems and
high genetic load, since they came from mature habitats. The
data, however, does not fit such a hypothesis. An alternative
explanation is required.

Klekowski (1984) suggested that old clones or populations of
sporophytes may build up substantial load through the accumulation
of somatic mutations. Morphological mutations of this type may be
one source of morphological aberrations observed in isolate and
multispore cultures. Klekowski (1984) suggested that chromosomal
aberrations and mutations affecting meiosis may also accumulate in
old clonal populations. These would have no effect on persistence
or vegetative proliferation of the sporophyte generation, but
would cause increased failure of spore formation and or production
of spores with chromosomal abnormalities. These mutations may be
another source of observed morphological abnormalities in
gametophytes.

Accumulation of genetic load will be modified to the extent
that sexual reproduction has taken place within the population
following initial establishment. The degree of modification is
dependent on the mutational rate, the level of inbreeding, and the
extent of heterosis. The higher the level of inbreeding, the more readily the deleterious mutations will be eliminated or reduced in the population. The higher the extent of heterosis, the selective advantage of heterozygotes, the more load that would be maintained in the population.

The hypothesis of mutational load seems a much more satisfying explanation than heterotic load for the largest proportion of load expressed in organisms with potential generation times of hundreds or even thousands of years. On the other hand, relatively short-lived plants, those with generations times of a decade or less, which give evidence of regular sexual reproduction in the local environment, probably will have a low mutational load and show low genetic load in isolate studies. Most load present in such plants would be heterotic, and the level of load would be highly dependent on breeding system. Consequently, it is the rate of mutation relative to the age of the plants in the habitat which will determine whether genetic load is a useful measure of the age or relictualness of a plant and its population.

Although environmental factors that influence morphology in isolate culture may be unrelated to genetic load, it would be expected that intrinsic genetically determined morphological differences due to mutational load and meiotic abnormalities accumulated by long-lived clonal populations, as observed by
Klekowski (1984), would be correlated with high genetic load of sporophytic lethals as well.

Disjunct populations or populations at the edge of the species' range may be growing under conditions marginal or unfavorable for growth and reproduction. These conditions may favor the persistence of one generation over the other. Farrar (1967, 1974, 1978) has presented persuasive evidence for the occurrence and relictual nature of perennial, vegetatively persistent gametophyte populations which have lost the ability to produce sporophytes. An analogous situation may occur in marginal sporophyte populations. Conditions which favor persistence and vegetative expansion of sporophyte clones may be entirely unsuitable for sexual reproduction. Over time, somatic mutations could accumulate to levels which preclude sexual reproduction under any conditions.

The presence of high genetic load may be viewed as evidence supporting relictual rather than recent origin for disjunct populations. Thus, the high levels of very aberrant gametophytes and the high levels of genetic load observed for *Thelypteris simulata* and *Dryopteris marginalis* favor a relictual hypothesis for the disjunct populations of these species examined in the present study.

Of the Woodman Hollow species tested, genetic load was low for *Woodsia obtusa* and *Cystopteris tenuis* and very high for
**Cryptogramma stelleri** and **Athyrium angustum**. The low load species are relatively short-lived plants which Peck (1980) and Farrar and Gooch (1975) observed undergoing abundant sexual reproduction in Woodman Hollow. Peck's study of gametophytes of *Woodsia obtusa* suggested that sporelings are most often produced by intragametophytic selfing. Sporophytes so produced would be load-free. Relatively short-lived species with frequent establishment of new sporophytes by selfing can thus maintain load-free or low load populations. The low load of these populations, however, is not necessarily indicative of a young age of the population in the local habitat.

Species with such a high reproductive potential are capable of establishing new sporophytes within the local environment and establishing new distant populations by long distance dispersal of isolated spores. The plants examined of these species are limited only by their ability to disperse spores to suitable habitats.

In contrast, gametophytes of *Athyrium angustum* and *Cryptogramma stelleri* were not observed in Woodman Hollow (Peck 1980). Sexual reproduction was not providing new plants for replacement of the population. These species were maintained by persistence of old apices and vegetative expansions. In these long-lived clonal populations, somatic mutations accumulate over time, and represent a genetic load.

A load of the magnitude measured for these species (95–98%),
clearly indicates that these plants have not been recently established by single spores. Two alternative hypotheses about the origins of these ferns, may be suggested. Because populations with an outbreeding mating system may shelter a certain amount of genetic load, initial establishment could have occurred through intergametophytic mating within a once more widespread population of plants with some genetic load. Later as relictual populations, these plants no longer reproduced sexually, tending to increase genetic load through accumulation of somatic mutations. A second possibility would allow establishment by a single spore to produce a population initially free of genetic load, followed by accumulation of genetic load as the plants and population aged.

With the present evidence, one cannot distinguish between these two possibilities. Observations made on these species, from populations in other parts of the range, might be enlightening as to the significance of the present results.

The value of genetic load build up as a genetic clock to assess the time since the last selfing event will become clearer only with more studies that suggest the length of time or number of generations required to express high levels of inbreeding depression (Lande and Schemske, 1985; Schemske and Lande, 1985). If the populations have existed in the habitat for a longer period of time than this, then the value of genetic load to suggest or refute alternative hypotheses about recent versus relict
population origins will be minimal.

Isolate Potential

Three classes of isolate potential were noted in the species studied (Table 32). Isolate potential greater than 50% was noted for Cysopteris tenuis and Woodsia obtusa, 20-30% for Dryopteris cristata, Polypodium virginianum and Thelypteris simulata, and <10% for Athyrium angustum, Cryptogramma stelleri, Cysopteris bulbifera, Dryopteris marginalis, Dryopteris spinulosa, and Thelypteris palustris. Total range of isolate potential was from 1-80%. In other words, for isolated spores that reach a suitable habitat, Woodsia obtusa has 80x the chance of Athyrium angustum or Cryptogramma stelleri to produce a sporophyte.

The isolate potentials from monospore culture are not directly comparable to the isolate potentials which might be inferred from sexual status and sporophyte production reported in previous genetic load studies. Those studies generally established isolates by transferring morphologically normal, presexual gametophytes from multispore culture. The selection of morphologically normal gametophytes may bias the study. Transfer itself may alter the sexual status, facilitating a higher bisexual status of the cultures, and thus, increase the apparent isolate potential. The isolate potential measured in the present study is a better indication of true isolate potential.

In order to assess the biological relationship of isolate
potential to other reproductive factors, spore production data presented by Peck (1980) for Woodman Hollow was combined with isolate potential as measured in the present study (Table 35). These differences in isolate potential combined with differences among species in local abundance (due to related factors of vegetative reproductive capacity and within population sexual reproductive capacity), spore production, spore release and dispersal, and spore viability to give species a wide range of relative potentials for long distance dispersal and establishment.

For Woodman Hollow populations, the combination of these factors widened the overall differences in reproductive potential estimated by Peck (1980) for Woodsia obtusa and Cryptogramma stelleri. Considering spore production at Woodman Hollow and isolate potential, Woodsia obtusa has seven million times the isolate reproductive potential of Cryptogramma stelleri. From Woodman Hollow alone, if spores were evenly distributed across the state of Iowa, Woodsia obtusa could have approximately four spores per square meter; Cryptogramma stelleri would have one spore per hectare. Considering isolate potential, 2.7 spores per square meter of Woodsia obtusa would produce sporophytes compared to one spore per 100 hectares for Cryptogramma stelleri.

Associations Between Reproductive Phenomena

Significant correlations were demonstrated between the following reproductive measures and phenomena: germination
potential and percent neuter gametophytes (-0.733), bisexual potential and percent female gametophytes (-0.729), bisexual potential and spore isolate potential (0.663), genetic potential and gametophyte isolate potential (0.799), genetic load and gametophyte isolate potential (-0.799), gametophyte isolate potential and spore isolate potential (0.907), spore isolate potential and percent bisexual gametophytes (0.664), percent normal morphology and percent males (-0.661), percent normal morphology and percent multilobed gametophytes (-0.694), percent abnormal morphology and percent males (0.661), percent abnormal morphology and multilobed (0.694), and percent female gametophytes with percent bisexual gametophytes (0.731), as presented in Table 36.

The strong negative association between germination potential and percent neuter gametophytes may reflect an overall poor reproductive potential for spores from the plants of the species which were tested. Species with poor germination tended to have a higher percentage of gametophytes which were unable to form sex organs by 16 weeks. These species also tended to have more unisexual males, but the correlation was not statistically significant.

Early workers associated maleness with poor growing conditions, crowding, low light levels, and lack of nutrients. Many of these observations may be better explained as the effects
of an antheridogen system in multispore culture. Whether some unfavorable conditions for growth also strongly promote male gametophyte development in isolate culture remains to be tested. The associations demonstrated among poor germination, abnormal development, and maleness suggest the possibility that conditions unfavorable to spore germination and gametophyte development, including innate conditions, may favor maleness.

Maleness was significantly correlated with abnormal morphology. This may partially reflect the failure or inability of the more filamentous bushy gametophytes to produce three-dimensional archegonial cushions. It may also suggest that maleness may not be part of the normal developmental pattern for these species.

Although no significant correlation was found between morphology and bisexuality, species with normal morphology were less likely to be bisexual than were those with high levels of abnormal morphology. Unisexual gametophytes in species with normal morphology tended to be female rather than male.

There was a slight correlation (0.448) between species commonness and normal gametophyte morphology, but it was not statistically significant. This kind of correlation may indicate that species with a high percentage of single-lobed gametophytes reproduce more successfully under natural conditions than do those species with high levels of abnormal gametophytes.
The presence of aberrant morphology was not necessarily correlated with low bisexual potential or low isolate potential in culture. However, the degree of aberration may be significant. *Woodsia obtusa* had the second highest bisexual potential and the highest isolate potential. All of its isolated gametophytes developed as a single, cordate lobe. This demonstrated that for some species physically separated lobes of gametophyte tissue are not required for bisexuality.

For *Cystopteris tenuis*, which also had a high gametophyte isolate potential, only 5% of the isolates had simple cordate morphology. Most (62%) were multilobed. The remainder (33%) were highly proliferative, forming bushy mounds of gametophyte tissue. However, none were calluslike.

*Thelypteris simulata* and *Dryopteris marginalis*, on the other hand, had low isolate potential and a high percentage of aberrant gametophytes, including numerous gametophytes that were small with calluslike morphology. However, for these two species, low isolate potential was the result of high genetic load as much or more than it was due to low bisexual potential.

*Athryrium angustum*, *Polypodium virginianum*, *Dryopteris cristata*, and *Cystopteris bulbifera*, which ranked second to fifth respectively in percent of normal gametophytes, ranked seventh to tenth in bisexual potential. This relationship conforms to expectations based upon antheridogen production combined with
insensitivity of contiguous gametophyte tissue to its effects.

If low bisexual potential indicates an outbreeding mating system, low bisexuality would be expected to be associated with high genetic load. This expectation was not supported by the results of this study. These data suggest that genetic load is independent of mating system. This suggests the possibility that load in these ferns may not be predominantly heterotic in nature.

Protocol Evaluation

In this study, more gametophytes were examined per plant and per populations than were examined for the few earlier studies of isolated gametophytes or for most genetic load studies. For some species, the methodology used in this study gave a fairly clear picture of sexual development and reproductive potential. For other species, interpretation of results was confounded by low germination, morphological aberrations, low numbers of bisexual gametophytes, and high variability among plants. While cultural conditions were apparently adequate for a number of species, there were indications that light and temperature levels may have been too high for optimal growth and reproduction of a few species.

The protocol utilized in this study is probably adequate for species that do well under these cultural conditions. When conditions favor high germination, survival, and normal development, then the numbers of gametophytes available at the time data is recorded will be adequate for analysis. Woodsia
obtusa at Woodman Hollow, for example, is clearly a highly reproductive fern with a high reproductive potential in multispore and isolate culture. Similarly, confidence in the validity of the observations on Cryptogramma stelleri is enhanced by the high germination rate and robust gametophyte development, even though its eventual reproductive success was low. However, for species which had poor germination, aberrant morphology, slow development, few bisexuals and high variability among plants, such as for Thelypteris noveboracensis, more observations of gametophytes need to be made to be equally confident of measures of reproductive phenomena.

It is known that different species have different environmental optima (Hill, 1971). It may also be true that growth conditions are not as critical for multispore cultures as they appear to be for monospore cultures. Isolate potential for a species may change as conditions vary from the optimal for that species. Thus, some of the differences observed among species may be related to how closely experimental conditions approximated optimal conditions for the different species.

Genetic load may be studied more efficiently if it is studied separately from isolate development, particularly for species which perform poorly in culture. In isolate developmental studies, it is important to distinguish intergametophytic influences on developmental patterns, gametophyte morphology, and
sexual development from intrinsic differences and effects of environmental variables. In genetic load studies, it is important to obtain sufficient bisexual gametophytes, irrespective of how they are obtained. Future isolate studies should be based on more spores per plant and more plants per population. With this approach, species with low reproductive potentials or with aberrant morphology will be more adequately assayed for isolate potential. To do this, perhaps 100-200 spores per plant and 10-20 plants per population need to be tested for poor performers.

Comparisons with Other Studies

In the present study using isolate culture, the individual gametophyte was not limited by competition for nutrients, light, or space. Gametophytes became very large, filling the volume of the culture container. Bushy growth, branching, and/or marginal or surficial outgrowths were almost universally present for each species.

It is unlikely that development of gametophytes in nature would result in a similar sized gametophyte in a similar time period. Each isolate was provided with adequate nutrients and space without contaminants and competition. In addition, continuous light provided in culture allowed photosynthetic time two or three times longer than would be available to gametophytes in nature over the same four month period. Under these conditions, gametophytes of Woodman Hollow species grew 10-20x
larger than gametophytes of comparable age from multispore cultures, grew two to three times larger than equal-aged field-grown gametophytes, and were even larger than gametophytes that had overwintered and proliferated the following spring at Woodman Hollow (Peck, 1980).

Comparisons of reproductive data from different studies are subject to serious limitations. Different researchers have used different media, different light and temperature regimes, and different gametophyte densities. All too frequently critical culture procedures or growth conditions are not specified.

For example, gametophytes have generally been transferred to establish isolate and pair cultures. Some workers have failed to report the method of isolate establishment. Often experimental procedures and conditions are simply inadequately described. The source and number of spore parent plants and whether results are reported separately for separate plants or whether plants differ may not be reported.

It cannot be assumed that these conditions or differences are irrelevant. Various reports indicate that many of these factors may indeed affect gametophyte morphology and ultimate sexual expression. Thus, because there is no standardization of procedures, there may not be a valid basis for comparisons.
CONCLUDING REMARKS

Studies of fern gametophytes grown as isolated plants can provide new and useful information regarding fern reproductive biology. Significant differences among species were observed for all reproductive features examined. Germination-development potential ranged from less than 10% to 94%; bisexual potential from 17% to 95%; selfing potential from 2% to 100%; genetic load from 0% to 98%; spore isolate potential from 1% to 66%; and gametophyte isolate potential from 1% to 80%. Isolate potential (the ability of isolated gametophytes to produce sporophytes) provides a summary value of spore and gametophyte reproductive potentials. As such, isolate potential and its components are important factors to consider when discussing the biology and geography of ferns. Isolate potential may aid in interpreting past changes in distribution and in predicting future changes.

For example, evidence of low isolate potential for *Athyrium angustum*, *Cryptogramma stelleri*, *Dryopteris marginalis*, and *Thelypteris simulata* support the hypothesis that the populations investigated in the present study may be post-Pleistocene relicts rather than recent colonizers. It can be predicted that long-distance species dispersal from these populations will be unlikely. The high isolate potential of *Woodsia obtusa* and *Cystopteris tenuis* is probably related to current sexual reproduction involving a high degree of intragametophytic mating.
within the study population. Thus, for these species high isolate potential is not necessarily indicative of recent population establishment. Their relative potential for successful long distance dispersal is high compared to the former species. Based on the survey conducted in this investigation, additional and expanded studies of isolated gametophytes promise to aid in unraveling the reproductive history, population structure, reproductive potential, and distribution of fern species.

Consideration of fern distribution only in terms of suitable habitat availability (Tryon, 1970; 1972) is probably inadequate. Intrinsic species specific differences in factors which contribute to successful species dispersal (sporophyte reproduction in the local habitat; spore production, release, and dispersal; germination-development potential; bisexual potential; selfing potential or genetic load) may be equally important for many species. Since these biological factors may vary from species to species, population to population, and plant to plant, each species' reproductive success is a factorial expression of all of these factors in all populations.

Failure to demonstrate significant correlation between bisexual potential and genetic load in the present study suggests that these factors must be considered independently. Because of this and because of the high levels of variation among species in all components of reproductive success, it is clear that
individual fern species need to be considered individually. The unique biology of a species must be studied and understood before accurate interpretations can be made relative to its distributional history and to its current local and long distance reproductive potential. Clearly, patterns among the reproductive profiles of fern species are more subtle, complex, and varied than previously stated (Lloyd, 1974b).

The results of this study have brought us to the same conclusion for ferns that was reached by Cushing (1965) for the distribution patterns of wind-pollinated flowering-plant species; post-Pleistocene range changes must be considered individualistically, one species at a time. Similarly, ferns, in spite of having wind dispersed spores, which may have the potential to initiate new populations in distant habitats, cannot be considered to be equal in dispersability. The differences among species, as demonstrated and discussed in the present study, are of sufficient magnitude that species cannot be assumed to be reproductively equivalent, even over geological time spans. These differences in reproductive potentials are the result of interactions among a variety of factors including morphology, physiology, genetics, and environmental conditions.

In order to gain an understanding of the unique biology of fern species, they must be studied in the laboratory and in the field, species by species, population by population, and plant by
plant before adequate interpretations can be made. Such an empirical species biology approach is quite laborious, but will allow hypotheses to be considered on a more rigorous basis or at least on a more biologically relevant basis.

Current discussions of fern reproduction in nature have been highly deductive, constructed upon a woefully inadequate data base. Although limited, the empirical bases for statements about reproduction within populations of fern gametophytes are greater than for isolated fern gametophytes. At present, the relative reproductive success of isolated gametophytes, compared to gametophyte populations, within local sporophyte populations is not known. An understanding of the development and reproductive behavior of isolated and grouped gametophytes in this situation may be the best approach to understanding the differences, if any, between local and long distance reproduction. Ideally, such investigations would include: 1) autecological study of the local population, 2) isozyme analysis of population structure, and 3) laboratory study of germination, development, sexuality, and reproductive biology (antheridigen influence, genetic load, inbreeding depression, and isolate potential) for both isolated gametophytes and gametophyte populations.
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