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GENECOLOGICAL STUDIES OF HAWAIIAN FERNS:
REPRODUCTIVE BIOLOGY OF PIONEER AND
NON-PIONEER SPECIES ON THE ISLAND OF HAWAII

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ABSTRACT

Sporophytes from natural populations of ferns occupying pioneer lava and mature rainforest habitats on the island of Hawaii, Hawaiian Islands, were investigated to determine their mating system and frequency of recessive lethal genes (genetic load). Species dominant in pioneer lava habitats were found to have intragametophytic mating systems and to be devoid of lethal genotypes. Species from intermediate and mature rainforest habitats exhibited complex intergametophytic mating systems and higher levels of genetic load. It is suggested that natural selection has favored intragametophytic mating and homozygosity in species of less diverse and less competitive pioneer habitats and intergametophytic mating and heterozygosity in species of more mature habitats.

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INTRODUCTION

Homosporous ferns are characterized by free-living gametophytes
which normally produce both antheridia and archegonia and can be self-
fertilized. As these gametophytes result from a single haploid spore
by mitotic divisions the genotype of both gametangia and gametes is
identical. Thus, sporophytes produced by selfing can be considered to
be genetically homozygous. Although most gametophytes have the propen-
sity for hermaphroditism there are several factors relative to their
reproductive biology which vary the probabilities of self-fertilization
occurring. These factors include: time of maturation of archegonia
relative to antheridia and length of the archegoniate or antheridiate
stage before hermaphroditism is attained; orientation of archegonial
necks either toward or away from antheridia; presence of an antheridogen
or other condition which increases the number of unisexual male gameto-
phytes in a population; density and spatial arrangement of gametophytes;
and presence of recessive genes which are lethal in the homozygous
diploid zygotic condition (genetic load as here defined) (Klekowski and
Lloyd, 1968; Klekowski, 1969a, 1971; Lloyd, 1974).

As a result of the above factors, two basic levels of mating can be
recognized in homosporous ferns, intragametophytic and intergametophytic.
Intragametophytic mating is by definition self-fertilization within
single gametophytes with the resulting intralocus genetic homozygosity.
Intergametophytic mating results in various levels of heterozygosity
depending on the origin of gametes. Gametes originating from separate
gametophytes but these in turn from spores of the same parental sporophyte
(intergametophytic selfing) yield levels of heterozygosity less than
the parental sporophyte; gametes originating from gametophytes from two
different sporophytes (intergametophytic crossing) result in maintained

or increased levels of heterozygosity. The latter two categories are analogous respectively to inbreeding and outbreeding in flowering plants, whereas intragametophytic selfing is unique to homosporous ferns and many Bryophyta.

By studying gametophyte ontogeny and testing for the presence of genetic load it is possible to analyze species of homosporous ferns and determine their basic mating system operative in nature and the degrees of sporophytic heterozygosity for lethal genes. By isolating single gametophytes intragametophytic selfing is obligate and leads to homozygous gene combinations in resulting sporophytes. If deleterious genes are present in the organism they can be expressed either at syngamy or in subsequent stages of sporophyte development. These expressions of lethality usually take the form of abortive zygotes, abortive embryos which appear as swollen tissue within the archegonium, small abnormal sporophytic tissue, or abnormalities in the production of the first few fronds or roots (Ganders, 1972). These types of data have been obtained on several species of ferns to date, including Osmunda regalis (Klekowski, 1970a, 1973); Ceratopteris thalictroides (Klekowski, 1970b); Pteridium aquilinum (Klekowski, 1972); Onoclea sensibilis (Klekowski and Lloyd, 1968; Ganders, 1972; Saus, 1974); and Sadleria spp. (Holbrook-Walker and Lloyd, 1973).

Although mating system information is known in several species, correlation of this information with the habitat and the ecological success of a species has been attempted in only a few cases (Holbrook-Walker and Lloyd, 1973; Lloyd, 1973; Klekowski, 1973). In these studies hypotheses relative to population size and age, habitat diversity, and propensities for colonization have been formulated.

This paper describes the mating system and genetic load of the dominant species of ferns found in varying habitats in the Hawaiian Islands. These islands offer a unique biological laboratory in which to study changes in pteridophyte reproductive biology relative to changes in habitat. The youngest island, Hawaii, is volcanically active. Fresh volcanic materials are being deposited continually and in turn provide pioneer substrates for establishment of plant species. As volcanic flows have been produced periodically since/the original formation of the island it is possible to study habitats and vegetation types of varying ages and to document the characteristics of reproductive biology of the primary fern species found therein. Species investigated in this study include: Nephrolepis exaltata (L.) Schott (Davalliaceae), the primary vascular plant pioneer on new volcanic lava along the Puna Coast; Polypodium pellucidum Kaulf. (Polypodiaceae), commonly found on lava in the Saddle district between Mauna Kea and Mauna Loa; Microsorium scolopendria (Burm.) Copel. (Polypodiaceae), an adventive species which only rarely occurs on lava; Dicranopteris linearis (Burm.) Underw. (Glecheniaceae), a common species in young open forest; and Cibotium glaucum (Sm.) H & A. (Cyatheaceae), the dominant substory species in mature Acacia koa rain forest at mid-elevations.

MATERIALS AND METHODS

Spore samples were collected from sporophytes chosen at random in natural populations on the island of Hawaii at the following locations: 1855 lava flow, 19.8 miles west of route 19 on route 20 (Polypodium pellucidum); 1750 lava flow, Puna District, 0.85 miles north of route 137 on route 13 (Nephrolepis exaltata, 4644; Microsorium scolopendria,

4645, 4719); 3.4 miles south of road to Opihikao on route 13

(Dicranopteris linearis, 4647); 3.1 miles south of road to Opihikao on

route 13 (D. linearis, 4648); 1.8 miles south of road to Opihikao on

route 13 (N. exaltata, 4651); 1.4 miles south of road to Opihikao on

route 13 (D. linearis, 4653); Kilauea Forest Reserve (IBP study area),

about 7 miles NNW of Volcano (Cibotium glaucum).

Spores from individual sporophytes were sown on inorganic nutrient

medium solidified with 1% agar (Table 1; Klekowski, 1969b) in 100 x 15 mm

petri dishes. Gametophytes were grown under continuous illumination from

white fluorescent and incandescent lamps at an intensity of 220 ft. c.

at about 24° C. Prior to the attainment of hermaphroditism gametophytes

were selected at random for breeding tests. These tests were constructed

utilizing 20 or 30 isolated gametophytes from each sporophyte grown on

nutrient agar in 60 x 15 mm petri dishes. Isolate cultures were watered

twice weekly with sterile distilled water to facilitate fertilization.

Morphological observations were made on living material grown on

agar medium as well as sterile soil medium and from gametophytes mounted

in Hoyer's medium (Beeks, 1955) mixed with acetocarmine.

RESULTS

1. Nephrolepis exaltata (L.) Schott

Nephrolepis exaltata is a variable, ill-defined pantropical species

which is apparently indigenous to the Hawaiian Islands. It is found

commonly on the island of Hawaii where it is the dominant vascular plant

in the initial sere of plant succession on volcanic substrates along

the Kona and Puna Coasts. Near Kilauea Volcano, N. exaltata was observed

TABLE 1: Chemical Composition of nutrient medium utilized in

gametophytic studies.

Formula for standard stock solutions:

A. NH_4NO_3...Ammonium nitrate..................2.5 grams/100ml Dist. HOH

B. KH_2PO_4...Potassium phosphate..............2.0 grams/100ml Dist. HOH

C. $MgSO_4$..7HOH...Magnesium sulfate...........1.0 grams/100ml Dist. HOH

D. $CaCl_2$...Calcium chloride...................1.0 grams/100ml Dist. HOH

Formula for micronutrient stock solutions:

E. NaFe...Sequestrene (13% Fe)...............1.4 grams/100ml Dist. HOH

F. H_3BO_3...Boric acid.......................11.42 grams/liter Dist. HOH

G. $ZnSO_4$..7HOH...Zinc sulfate................8.82 grams

   $CuSO_4$..5HOH...Copper sulfate..............1.57 grams

   $MnCl_2$..4HOH...Manganous chloride..........1.44 grams

   H_2Cl_2..HOH...Molybdic acid................0.7455 grams

   $Co(NO_3)_2$..6HOH...Cobalt nitrate..........0.49 grams

   Acidified water...1 ml H_2SO_4 + liter dist. HOH

Formula for agar mixture: (pH 5.8)

Standard Stock Solution	Amount
A.	5 ml
B.	25 ml
C.	12 ml
D.	2 ml
E.	1 ml
F.	1 ml
G.	1 ml
Agar	10 grams
Distilled HOH	to 1 liter

on lava produced four months earlier. On more mature substrates, such
as the lava flow of 1950, the species occurs frequently, occupying
scattered crevices throughout the flow.   Young volcanic flows
in the Puna District are also colonized by other vascular plants, including
Microsorium scolopendria, Pityrogramma calomelanos (L.) Link, Psilotum
nudum (L.) Griseb., Pteris vittata L., Sadleria cyatheoides Kaulf., and
Metrosideros collina (Forst.) Gray ssp. polymorpha (Gaud.) Rock.

Initial establishment on lava by N. exaltata is by spores from
plants from surrounding flows and areas of vegetation left uncovered.
Due to rampant growth of lateral stolons, individual plants, once
established, can vegetatively rapidly produce numerous plantlets
throughout the length of a crevice. As lava substrate ages N. exaltata
increases in size and abundance, as is found on the flow of 1750 in
the Puna District. This trend continues until the habitat changes
sufficiently and the species is eliminated.

Sporophytes from the flow of 1750 (4644 A - E) exhibit 64 spores
per sporangium. These spores germinate when fresh between four and
seven days following sowing on agar medium. Gametophyte growth is
relatively rapid and gametangia initiation occurs 47 days following
germination with the appearance of antheridia initially along the central
posterior cushion and later on wing tissue immediately adjacent to the
cushion. The antheridiate stage lasts between four and 30 days before
gametophytes become hermaphroditic. Archegonia are produced in the
anterior cushion region contiguous with antheridia. Necks of most
archegonia are oriented posteriorly toward the antheridia (Table 2).

TABLE 2: Sequential sex expression: length of time between initial production of gametangia and attainment of hermaphroditism (in days).

Species	Ontogenetic sequence	
	Male to hermaphrodite	Female to hermaphrodite
Nephrolepis exaltata	4 - 30	
Dicranopteris linearis	21 - 24	
Polypodium pellucidum (soil)	17	
Cibotium glaucum	7 - 18	
Cibotium glaucum		4 - 10
Polypodium pellucidum (agar)		0 - 9*
Microsorium scolopendria		14 - 79
Sadleria pallida		29 - 78**

* Most gametophytes remain unisexual and female on agar medium.

** Gametangia ontogeny is female to male with the hermaphrodite stage of less than 4 days.
Date from Holbrook-Walker & Lloyd, 1973.

Gametophytes remained hermaphroditic until the termination of the experiment following sporophyte production. This ontogenetic sequence, from male to hermaphroditic, occurs in all gametophytes of both isolate and crowded cultures on both agar and soil mediums.

The male to hermaphroditic ontogenetic sequence has been described by Klekowski (1969a) as one with the highest probabilities for intra-gametophytic selfing. It has been reported in many of the homosporous ferns (Atkinson and Stokey, 1964) as well as Lycopodiaceae and Psilotaceae (Freeberg and Wetmore, 1957; Parihar, 1965). In this sequence, as viable sperm are produced continuously prior to and throughout archegonia maturation, the probability is high that self-fertilization will occur due to proximity of sperm to archegonia and the timing of development of sperm and egg. One factor which can affect negatively this probability is the presence of recessive gene combinations which are lethal in the homozygous condition, thus preventing zygote formation following self-fertilization. To test this phenomenon, breeding tests were devised for all plants studied utilizing 20 gametophytes from each sporophyte. These were individually isolated in agar culture. Results from these tests are given in Table 3.

Sporophyte production from isolated gametophytes was uniformly 100% in all parental sporophytes tested. It can be concluded, therefore, that recessive deleterious genes are lacking in these plants. As ontogeny of gametangia favors intragmaetophytic selfing and as lethal genes are lacking it is strongly suggested that the primary mode of reproduction in this species is by intragametophytic mating, with

TABLE 3: Breeding tests for <u>Nephrolepis exaltata</u>, <u>Microsorium</u>

scolopendria, and <u>Dicranopteris linearis</u>: Percent of

sporophyte production from isolate cultures.

Parent Plant	No. Gametophytes Studied	No. Sporophytes Produced	Percent of Gametophytes Not Producing Sporophytes
<u>Nephrolepis exaltata</u>			
4644 - A	20	20	0 %
B	20	20	0
C	20	20	0
D	20	20	0
E	20	20	0
<u>Microsorium scolopendria</u>			
4645 - A	20	18	10.
B	20	18	10.
C	20	19	5.
D	20	20	0
4719 - A	30	27	10.
<u>Dicranopteris linearis</u>			
4647	20	15	25.
4648	20	7	65.
4653	20	16	20.

recessive lethal genes being eliminated when they arise and with the resulting genetic homozygosity in the sporophytes produced.

Sporophytes of N. exaltata sampled from an open Metrosideros forest (4651-A) produce gametophytes which initiate sporophytes apogamously. Although gametophyte ontogeny is similar to sexual plants, archegonia frequently fail to develop or are at most few in number. Sporophyte production from within the cushion is preceded by the appearance of vascular tissue. Sporangia in these plants exhibit 32 spores per sporangium and these spores are five to ten microns larger in size than those of sexual plants.

2. Microsorium scolopendria (Burm.) Copel.

Microsorium scolopendria is widely distributed throughout the Old World tropics from Africa to Ceylon, Malaysia, Australia, and Polynesia. Wagner (1950) suggests that the species was introduced to Hawaii and began to appear in native vegetation about 1920. It is now common in lowland wet secondary woods and can be found near steam vents and other hot areas in Hawaii National Park. Along the Puna Coast, very small populations of M. scolopendria were observed in crevices on the flow of 1950 and on rock surfaces on the flow of 1750. Five plants, forming the entire population, were sampled in this study from the 1750 flow.

Sporangia of M. scolopendria exhibit 64 spores per sporangium. These spores germinate when fresh five to six days following sowing on agar medium. Gametophyte growth and gametangia initiation is slower than in N. exaltata and initial production of archegonia on cordate

gametophytes occurs 62 days following germination. Gametangia ontogeny
is from archegoniate to hermaphroditic. Archegonia are produced on
the anterior cushion region immediately behind the apical meristem and
their necks are oriented posteriorly. Antheridia appear between 14 and
79 (mean = 40) days following initial appearance of archegonia (Table 2).
These are produced initially along the extreme posterior margins of the
prothallus; later, they appear on lower wing tissue adjacent to the
margin. The location of gametangia in widely separated areas of the
gametophyte and the long archegoniate stage allows for fertilization of
mature viable archegonia by sperm from adjacent gametophytes, assuming
they are spatially arranged to allow for transference of such sperm.
This type of mating would probably be prevalent in all gametophyte
populations with the exception of those produced by initial spore
dissemination in which case gametophytes are likely to be widely
scattered. In addition, the ability of gametophytes to become
hermaphroditic with age indicates that intragametophytic selfing is
possible in isolated prothalli.

Breeding test data for this species support the above hypothesis
as to mating system (Table 3). These data indicate that 80% of the
sporophytes tested are heterozygous for recessive lethal genes, a con-
dition maintained by intergametophytic mating. However, although
heterozygosity is present in a high majority of sporophytes, the
frequency of lethal spore genotypes as expressed in isolate cultures is
low, varying from five to ten percent. Interpretation of these data
is somewhat difficult especially when the mating system data is con-
sidered. In this case, population size may be a controlling factor.

It is not unlikely that this population of only five sporophytes was initially established by a single spore or a very few spores. Due to the obligate intragametophytic selfing necessary in a single gametophyte the initial sporophyte (or sporophytes) established in this location would be devoid of lethal genes. Although the initial sporophyte produced by intragametophytic selfing would exhibit intralocus homozygosity and thus would produce identical spore genotypes (barring homoeologous chromosome pairing), subsequent gametophyte populations undergoing intergametophytic mating would tend to preserve recessive lethals brought about by mutation. Thus, over a period of time, these lethals would become more common in a population as it increased in size. As the present population consists of a low number of individuals it should be anticipated that the number of recessive lethals would also be low and this is supported by breeding data. Similar trends in genetic load and population size have been noted in studies on Ceratopteris thalictroides (Klekowski, 1970b, and pers. comm.) and Osmunda regalis (Klekowski, 1973).

3. Polypodium pellucidum Kaulf.

Polypodium pellucidum is an endemic species to the Hawaiian Islands. It is commonly found near Kilauea Volcano and on lava flows in the Saddle District between Mauna Loa and Mauna Kea on Hawaii. Due to the growth of its prostrate rhizome the species frequently forms small mats over large areas of substrate surface, especially on lava of the a-a type (chunky lava with sharp angular blocks and rough fragments).

Sporangia exhibit 64 spores per sporangium. Gametophyte and gametangia ontogeny is variable depending on culture techniques. On agar medium, gametophytes initially produce archegonia and remain unisexual indefinitely (Table 2). On soil culture, the ontogeny of gametangia is from male to hermaphroditic. Antheridia appear on very young gametophytes after the apical meristem is produced but before the appearance of the cushion. Antheridia initially appear in the posterior region amongst the rhizoids but later can be found in central and anterior regions of the cushion. Archegonia appear 17 days following initial appearance of antheridia. These are located in the anterior cushion immediately behind the apical notch. Necks of archegonia are uniformly pointed back toward the antheridia. Although breeding tests were not conducted due to the lack of hermaphroditic gametophytes in agar culture, the male to hermaphroditic gametangia sequence exhibited on soil culture and the contiguous zones of male and female gametangia are characteristics which have been interpreted as offering maximum probabilities for intragametophytic mating (Klekowski, 1969a).

4. Dicranopteris linearis (Burm.) Underw.

Dicranopteris linearis is morphologically variable and widely distributed in the tropics and subtropics of the Old World. On the island of Hawaii it is a scandant weedy fern, frequently found in sunny disturbed habitats and in open Metrosideros forest, where it forms large and dense thickets by means of rapid and prolonged frond growth. In some locations, D. linearis can be found covering entire

open hillsides, reproducing in these open habitats not only by vegetative growth but also by profuse spore and gametophyte production. Although this species is sometimes found in more mature and closed vegetations it is most appropriately considered as a colonizing fern.

Sporangia exhibit 64 spores per sporangium. Gametophyte ontogeny is uniformly from male to hermaphroditic in all gametophytes tested. Antheridia are initiated between 40 and 50 days following sowing of spores. Gametophytes become hermaphroditic with the production of archegonia between 21 and 24 days after initial antheridia production (Table 2). All gametophytes remained hermaphroditic until termination of experiments. Initial production of antheridia is on the central and posterior regions of the asymmetrical gametophytes. With further growth of the gametophyte antheridia become very numerous over most of the wing tissue. Archegonia are produced in the immediate notch area and their long necks are oriented toward the meristem and away from the antheridia.

Breeding tests indicate that recessive lethal genes in a hetero-zygous condition are found in all sporophytes tested (Table 3). Gametophyte genotypes from individual sporophytes which are lethal in the homozygous condition were found in 20 to 65% of the gametophytes tested. All gametophytes not producing sporophytes were observed and found to be hermaphroditic and bore numerous abortive sporophytic embryos, indicating that fertilizations had occurred repeatedly but that growth of sporophytic tissue was terminated at an early stage.

Gametangia ontogeny in D. linearis, from male to hermaphroditic, is one which favors intragametophytic selfing and the resulting

homozygosity. However, in this study all sporophytes tested were found to be heterozygous for lethal genes, indicating that intergametophytic crossing is occurring frequently enough to maintain deleterious genotypes in the population. Other factors which relate to mating system include gametophyte density and spatial arrangement and orientation of archegonial necks. In this species archegonial necks are oriented away from antheridia. Although the significance of neck orientation has not been tested it has been hypothesized to have some influence on the mating system (Klekowski, 1969a).

5. _Cibotium glaucum_ (Sm.) H. & A.

The mature rain forest on the island of Hawaii is dominated by an association of _Acacia koa_, _Metrosideros collina_ and _Cibotium glaucum_. In the Kilauea Forest Reserve, _C. glaucum_ is the major substory species and it forms a dense layer below the tree cover.   Due to its frequency and density it can be considered as the major stabilizing factor within the rain forest. In addition to _C. glaucum_, a second species, _C. chamissoi_, is also found, as well as 22 other epiphytic and terrestrial ferns. Spore samples were collected from ten individuals of _C. glaucum_ for this study.

Sporangia in all individuals exhibit 64 spores per sporangium. Gametangia ontogeny is variable in gametophytes from different sporophytes (Table 4). Initial production of gametangia in isolate culture occurs between 46 and 58 days following sowing. Ontogeny varies from male to hermaphroditic, female to hermaphroditic, male to hermaphroditic to female, and initially dioecious with male and female gametophytes,

TABLE 4: Sequential sex expression in _Cibotium glaucum_.

Parent plant	Crowded culture	Isolate culture
A	male to hermaphroditic	male to hermaphroditic
B	male to hermaphroditic	female to hermaphroditic
C	male to hermaphroditic	female to hermaphroditic
D	male to hermaphroditic	male female to hermaphroditic
E	male to hermaphroditic	male to hermaphroditic
F	male to hermaphroditic	female to hermaphroditic
G	male to hermaphroditic	male female to hermaphroditic
H	male to hermaphroditic	female to hermaphroditic
I	– – – –	male to hermaphroditic to female
J	? to hermaphroditic to female	female to hermaphroditic

both of which ultimately become hermaphroditic. In crowded cultures
(more than 100 gametophytes per culture dish) nearly all individuals
exhibit gametangia ontogeny from male to hermaphroditic, a response
either to physical crowding or to biochemical control of precocious
antheridia production (antheridogen). In one individual (J) initial
stages of ontogeny were not observed although later stages indicated
that in crowded culture hermaphroditic gametophytes ceased production
of antheridia while continuing production of archegonia.

Breeding tests on gametophyte populations from each sporophyte
produced variable results although most expressed relatively high
percentages of lethal genes. Sporophyte production from gametophyte
families in isolate cultures varied from a high of 73.4% in individuals
E and I to a low of 10% in individual G. Mean production of sporo-
phytes for all individuals tested was 41.0% (Table 5). Observation of
those gametophytes not producing sporophytes indicated that all were
hermaphroditic or were hermaphroditic at one stage in development.
Observed visible lethals in the form of abortive sporophytic embryos
were observed in five of the gametophyte families. Abortive sporophytes
per gametophyte varied in number from 1 to 9 (mean = 4).

The pairs/isolates test devised by Klekowski (1971) was utilized
to determine a genetic base for the lack of sporophyte production in
isolated gametophytes. Those gametophytes not producing sporophytes
were sectioned, with two-thirds of the original gametophyte tissue of
each being maintained in isolate culture. The one-third section remaining
was placed with a similar section from a gametophyte of a separate

TABLE 5: Breeding tests for _Cibotium glaucum_ population: percent of sporophyte production from isolate cultures.

Parent Plant	No. gametophytes studied	No. sporophytes produced	Percent of gametophytes NOT producing sporophytes	observed lethals
A	20	14	30.0%	
B	20	12	40.0	
C	20	7	65.0	+
D	30	5	83.7	+
E	30	22	26.6	
F	30	5	83.7	+
G	30	3	90.0	+
H	30	12	60.0	
I	30	22	26.6	
J	30	7	76.7	+
Mean	--	--	59.0%	

gametophyte family. In this way it was possible to test two different gametophyte genotypes in a single test. Gametophytes tested originated from families A, B, C, D, F, and G. Cultures were allowed to regenerate gametangia and were then watered to allow for fertilization. Sporophyte production was measured in both isolate as well as paired cultures. In all cases, gametophytes in isolate culture failed to produce sporophytes, whereas both gametophyte in all pair cultures produced normal sporophytes (Table 6). As gametophytes from separate sporophytes probably possess non-allelic genetic lethals, fusion of sperm and egg from separate sources in pair cultures can be interpreted as evidence for the genetic control of lack of sporophyte production due to homozygous lethal gene combinations in isolate gametophytes.

Although all gametophyte families tested were high in recessive lethals (26.6 to 90.0%) there is a direct correlation between gametangia ontogeny and percent of recessive lethals present in parental sporophytes. Those sporophytes which produce gametophytes with a gametangia ontogeny from male to hermaphroditic exhibit the lowest levels of genetic load, 26.6 to 30.0 (mean = 27.8)%. Sporophytes which produced gametophytes with gametangia ontogeny from female to hermaphroditic exhibit genetic loads varying from 40.0 to 83.7 (mean = 65.1)%. Highest levels of genetic load are exhibited by sporophytes which produce gametophytes which are initially dioecious, 83.7 to 90.0 (mean = 86.9)%. These data can be interpreted as direct confirmation of the effect of gametangia ontogeny in controlling mating system with the male to hermaphroditic sequence favoring intragametophytic selfing,

TABLE 6: Pair cultures of recessive lethal-bearing gametophytes of

Cibotium glaucum.

Gametophytic pairs tested	Result of pairs	Result of isolates
A x B	both viable	both lethal
C x G	both viable	both lethal
D x F	both viable	both lethal

therefore eliminating recessive lethals from the population, and the female to hermaphroditic and initially dioecious sequences favoring intergametophytic mating and the maintenance of recessive lethal genotypes.

The uniform production of gametangia ontogeny from male to hermaphroditic exhibited by gametophytes grown in crowded cultures indicates that a biochemical antheridogen effect may be operating in this species. Therefore, the mating system of C. glaucum appears to be unique in its complexity when compared to species of ferns which have been studied to date (see Lloyd, 1974, for review). In general, C. glaucum can be characterized as a highly heterozygous species, both genetically and morphologically. These factors may relate to the current taxonomic difficulties in circumscribing species of the genus in Hawaii.

DISCUSSION

From an evolutionary standpoint the optimum reproductive system for a homosporous fern is one which is initially adapted for intergametophytic mating with the capacity through time for intra-gametophytic selfing (Klekowski, 1969a). Intergametophytic mating provides for maintained or increased levels of genetic heterozygosity in resulting sporophytes, whereas intragametophytic selfing allows for single gametophytes to produce sporophytes. This latter aspect is especially important in that it allows for colonization of disturbed or newly formed habitats by single spores. The optimum gametangia sequence which facilitates the above reproductive sequence is from

female to hermaphroditic, with the archegoniate stage being somewhat prolonged to increase probabilities of intergametophytic mating in more dense gametophyte populations, but with ultimate hermaphroditism to facilitate self-fertilization. This type of reproductive system has been well-documented in Onoclea sensibilis, a common weedy fern in the eastern United States and Asia (Saus, 1974).

Variations from the above reproductive system can be interpreted as due to selective pressures brought about by ecological conditions. For example, species which occur in habitats with a short growing season would not under normal situations be able to reproduce due to the necessary prolonged gametophyte growth before the hermaphroditic condition is attained. This aspect of gametangia ontogeny can sometimes be circumvented by the production of antheridogen which precociously initiates antheridia on young asexual gametophytes in a population. The optimum shortening of the gametophyte generation is reached in apogamous taxa, which do not require sex organ production before sporophytes can be formed. Indeed, many desert species, where rains are infrequent, reproduce apogamously.

Species investigated in this study with more optimum reproductive systems are Cibotium glaucum and Microsorium scolopendria. In addition, previous work by Holbrook-Walker and Lloyd (1973) has indicated that Sadleria cyatheoides Kaulf. (Blechnaceae), a component of the more open seral Acacia-Metrosideros-Cibotium forest as well as a wide variety of other habitats including new volcanic substrate, has a similar reproductive system. All of these species can sometimes be found as pioneers

on new volcanic substrates or in early seres of plant succession. In addition, C. glaucum and S. cyatheoides are major components of mature vegetation types. Microsorium scolopendria, although initially appearing as an adventive only about the year 1920, has become an important and conspicuous plant in understory vegetation at lower elevations on all of the major islands of the Hawaiian chain. It is apparent from this study that the success of these species in colonization as well as in mature vegetation is due primarily to their reproductive system which allows for maximum amounts of outcrossing with resulting heterozygosity as well as the ability ultimately to undergo intragametophytic selfing.

Species investigated with less optimum reproductive systems include Nephrolepis exaltata, Polypodium pellucidum, and Dicranopteris linearis. Additional species which can be added to this group include Sadleria pallida and S. squarrosa (Holbrook-Walker and Lloyd, 1973). These species are all characterized by gametangia sequences other than female to hermaphroditic. The gametangia sequence in S. pallida is from female to male and breeding tests performed on this species indicate that sporophyte production in isolate cultures does not or only rarely occurs. Gametophytes of S. squarrosa grow normally only under very low light intensities; gametangia sequence in this species is probably from male to hermaphroditic.

Certain intriguing relationships can be made between the reproductive system that most of these species exhibit and their habitat and distribution. Studies on N. exaltata indicate that this species

normally reproduces by intragametophytic selfing, vegetative stolons, or apogamy. In any case, new plants produced are relatively homozygous and bear the same genotype as the parental sporophyte (with the exception of non-lethal mutations). The habitat of this species is one which is relatively harsh and uniform but which is more or less continuously being produced by frequent lava flows. It is interesting to speculate that selection has been for a reproductive system which perpetuates specific genotypes which are optimum for pioneering new substrate. Thus, homozygosity has been favored over heterozygosity and this is expressed phenotypically by the uniformity in sporophyte morphology within these populations as well as by the lack of ability of this species to diversify into more variable habitats and mature vegetation in the near vicinity of the populations studied here.

Sadleria squarrosa and to a lesser extent S. pallida, exhibit restricted distribution patterns. The former species is found only rarely on dark steep wet banks at upper elevations in rain forest. The latter species is more common but is restricted in distribution to relatively stable mature forest in dense populations and is rarely found as a colonizer, a factor undoubtedly controlled by its lack of ability to form hermaphroditic gametophytes.

There are major differences in mating system and degrees of heterozygosity in the dominant species of ferns found in the habitats studied. Dominant species found in pioneer habitats appear to be highly homozygous and recessive lethal genes are rare. In contrast, dominant species found in mature habitats appear to be highly heterozygous with numerous recessive lethal genotypes (Tables 7, 8). From this study it appears that there is a gradual increase

TABLE 7: Genetic load: mean percent of gametophytes bearing recessive lethal genes per population.

Species	Number gametophytes studied	Percent bearing lethal genes
Nephrolepis exaltata	100	0%
Microsorium scolopendria	110	7.2
Sadleria cyatheoides*	75	44.0
Dicranopteris linearis	60	36.0
Sadleria souleyetiana*	75	44.0
Sadleria pallida*	75	96.0**
Cibotium glaucum	270	59.0

* From Holbrook-Walker & Lloyd, 1973.

** Lack of sporophyte production probably due to lack of synchronization in sex organ production.

TABLE 8: Genetic load relative to habitat.

Habitat	Genetic Load	
	Range (%)	Mean (%)
Pioneer Volcanic Flows	0 - 44%	17.0%
Open Intermediate Age Forest	10 - 59	37.2
Mature Rainforest*	44 - 59	51.5

* Does not include S. pallida.

in heterozygosity and lethal genes as the habitat matures. As maturing habitats bring, in turn, more species and more competition for available niches, it can be argued that selection is favoring increased heterozygosity for increased competitive viability.

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