Density Affects Gametophyte Growth and Sexual Expression of *Osmunda cinnamomea* (Osmundaceae: Pteridophyta)

YAO-MOAN HUANG\(^1\), HSUEH-MEI CHOU\(^1\) and WEN-LIANG CHIOU\(^2\,*

\(^1\)Department of Biology, National Taiwan Normal University, Taipei 106, Taiwan and \(^2\)Division of Forest Biology, Taiwan Forestry Research Institute, Taipei 100, Taiwan

Received: 14 January 2004 Returned for revision: 18 March 2004 Accepted: 20 April 2004 Published electronically: 30 June 2004

**Key words:** Gametophyte density, *Osmunda cinnamomea*, sexual expression.

**INTRODUCTION**

To understand eggplant reproduction, it is necessary to understand sexual expression and the factors that affect it. Although homosporous ferns have the potential to be hermaphroditic, many factors may affect their growth and sexual expression. Although homosporous ferns have the potential to be hermaphroditic, many factors may affect their growth and sexual expression. As density increased, gametophytes became significantly smaller and more slender. Female and asexual gametophytes dominated in populations of low and high densities, respectively. At intermediate population densities, hermaphroditic and male gametophytes were dominant. Female gametophytes were larger than gametophytes of all other types. Hermaphroditic gametophytes were larger than male gametophytes, which were larger than asexual gametophytes. Large gametophytes were wide-cordate, whereas smaller ones tended to be narrow-spatulate.

**Conclusions** Gametophyte size of *O. cinnamomea* is negatively related to the population density, which significantly affects gametophytes' sexual expression. The presence of unisexual and bisexual gametophytes at intermediate densities indicates that both intergametophytic and intragametophytic selfing may occur.

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**Key results** One-year-old multispore cultures of the fern *Osmunda cinnamomea* and to provide information on the density of growth needed to favour successful reproduction.

**MATERIALS AND METHODS**

Fresh spores of *Osmunda cinnamomea* L. were obtained from a plant growing in a marsh at Tsao-Pi (24°45′N, 121°33′E), Ilan County, in north-eastern Taiwan. Spores were sown on eight sheets of membrane filter (pore 0.45 μm, 47 mm in diameter; Gelman Laboratory). Each sheet lay on 2–4 cm deep moist *Sphagnum* moss in a plastic box (114 × 86 × 65 mm). All cultures were maintained under white fluorescent illumination at about 24 μmol m⁻² s⁻¹, 12 h d⁻¹ and the temperature was between 20 and 28 °C. Because spores disperse randomly in nature, instead of being controlled at specific densities as in most experiments, spores in this study were sown randomly and gametophytes were sampled randomly in the culture. One year later, gametophytes growing at different population densities (1, 2, 3, 6, 10, 12, 14, 32, 37, 41, 66, 92, 131 and 253 individuals cm⁻²) were sampled. The length and width of each gametophyte was measured (Fig. 1), and their genders were recorded. A stereomicroscope (Leica, Wild M8) and a compound light microscope (Leitz, Dialux 20) were used to work out the density and to examine the morphology, sizes and genders of the gametophytes.
The chloroplast-bearing spores germinated within 1 week of sowing. A typical, mature \textit{Osmunda cinnamomea} gametophyte was cordate and without trichomes. Initiation of antheridia and archegonia did not occur until 6 and 9 months after sowing, respectively. Antheridia formed on the dorsal and ventral surfaces of the wing, while archegonia formed on the ventral surface of the anterior cushion. Sporophytes were produced 1 year after the spores were sown.

One year after spores were sown, hermaphroditic, male, female and asexual gametophytes appeared in the culture synchronously. Of 17 hermaphroditic gametophytes examined, nine had antheridia containing antherozoids and eight had empty antheridia. At the density of 1, 2 and 3 individuals cm$^{-2}$ all gametophytes were female, but at 41 individuals cm$^{-2}$ there were no female gametophytes (Fig. 2). In contrast, the frequency of asexual gametophytes increased with density. At densities over 66 individuals cm$^{-2}$, only asexual gametophytes were produced. Male gametophytes occurred at densities between 12 and 41 individuals cm$^{-2}$, and hermaphroditic gametophytes occurred at densities between 6 and 37 individuals cm$^{-2}$ (Fig. 2).

Female gametophytes were much larger, in all respects, than other types of gametophytes (Table 1). Hermaphroditic gametophytes were larger than male gametophytes, which were larger than asexual gametophytes. Gametophyte size could be estimated by the regression equation:

$$A = 175.45D^{-1.4247}, \quad r^2 = 0.95, \quad P < 0.05$$

where $A$ = width $\times$ length (mm$^2$) and $D$ = individuals cm$^{-2}$. This equation clearly shows that gametophyte size decreased significantly as density increased. When the population density was more than 16 individuals per cm$^2$, the size of all gametophytes did not exceed 3.38 mm$^2$ (Fig. 3). Large, usually wide, cordate gametophytes occurred in low-density populations and were female or hermaphroditic. Small, usually slender-spathulate gametophytes occurred in high-density populations and were asexual (Fig. 4). The mid-sized male gametophytes appeared in populations of intermediate density.

**DISCUSSION**

Gametophyte growth and sexual expression are affected by many factors, including spore size, germination time, nutrition source, growth density and antheridiogen (Schedlbauer, 1976; Nester and Schedlbauer, 1981; Korpelainen, 1994). The density of cultured gametophytes has been shown to modify sexual expression in several species of homosporous ferns (Cousens and Horner, 1970; Rashid, 1970; Lloyd and Gregg, 1975; Cousens, 1979; Rubin et al., 1985; Warne and Lloyd, 1987; Greer, 1993). In this study, it was found that population density affected the sexual expression of \textit{Osmunda cinnamomea}. Gametophyte densities above a specific level yield relatively constant sex ratios (Warne and Lloyd, 1981). In this study, solely asexual gametophytes were produced when the population density exceeded 66 individuals cm$^{-2}$. The high asexual/sexual percentage (74/26) of the laboratory-cultured gametophytes and the low asexual/sexual percentage (20/80) of field gametophytes of \textit{Sadleria cyatheoides} and \textit{S. pallida} (Ranker and Houston, 2002) may partly be explained by the influence of density as demonstrated in this study.

Female and hermaphroditic gametophytes are usually larger than male and asexual gametophytes (Korpelainen, 1994). This was true of \textit{O. cinnamomea} gametophytes. In this observation, density affected gametophyte size and shape, and size was closely related to gametophyte gender. Miller (1968) stated that weak vegetative growth favours the formation of male and asexual gametophytes, whereas vigorous growth favours the formation of female and hermaphroditic gametophytes. When gametophytes are sparse, each gametophyte can obtain more resources than those gametophytes in dense populations. This may explain, in part, why the gametophyte size was significantly negatively correlated with population density.

Antheridium formation may consume energy and reduce gametophyte growth (Miller, 1968; Nör, 1979). Antheridiogen, a hormone promoting antheridium formation, affects size and size-influenced sexual expression in \textit{Dryopteris filix-mas} (Korpelainen, 1994). Potential vegetative growth is diverted to antheridium formation by antheridiogen in polypodiaceous ferns (Nör, 1956). However, further studies are needed to determine whether antheridiogen is present in \textit{O. cinnamomea}. Chemicals other than antheridiogen may also affect gametophyte growth. For example, the gametophytes of some ferns in the Polypodiaceae may produce growth inhibitors (Chiou and Farrar, 1997). Reciprocal allelopathy has been demonstrated between \textit{O. cinnamomea} and \textit{Dryopteris intermedia}, but gametophyte growth was not retarded by spraying with the supernatant of the same species (Petersen and Fairbrothers, 1980). However, in this study, it is unclear whether the growth of gametophytes in high-density populations was inhibited by allelochemicals.
Different patterns of sexual expression may provide evidence for different mating systems (Klekowski, 1972; Pajaron et al., 1999). Unisexual gametophytes favour out-crossing, whereas bisexual ones provide the opportunity for intragametophytic selfing (Schneller et al., 1990; Haufler, 2002). The occurrence of unisexual (female and male) and bisexual gametophytes of O. cinnamomea indicates that both intergametophytic and intragametophytic selfing may produce sporophytes, although possible limitations of genetic load on the latter have not been tested. In nature, most fern spores are dispersed only a limited distance, with very few or even only single spores travelling longer distances (Peck et al., 1990). The habitat in this study is fully covered by a Sphagnum mat. Dispersed spores of O. cinnamomea may either gather on the Sphagnum mat or float further and separate to various densities. Gametophytes growing in different densities will express different sexualities and may produce sporophytes through different mating systems accordingly.

These results were derived from a single plant and thus may not represent the species as a whole. However, this study does indicate the potential that ex situ conservation of this species may be achievable by sowing spores sparsely, as it will lead to subsequent development of gametophytes of both sexes and promote successful sporophyte production.

**Table 1. Growth characteristics of Osmunda cinnamomea gametophytes of different genders (average ± s.d.)**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Asexual</th>
<th>Male</th>
<th>Female</th>
<th>Hermaphroditic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>647</td>
<td>40</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>0.33 ± 0.39</td>
<td>1.19 ± 0.40</td>
<td>4.36 ± 1.53</td>
<td>2.74 ± 0.98</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>0.55 ± 0.33</td>
<td>1.48 ± 0.36</td>
<td>4.86 ± 2.50</td>
<td>2.46 ± 1.05</td>
</tr>
<tr>
<td>Width × length</td>
<td>0.29 ± 0.65</td>
<td>1.83 ± 0.81</td>
<td>24.45 ± 20.40</td>
<td>7.44 ± 5.57</td>
</tr>
</tbody>
</table>

**Fig. 2.** Gender frequencies of Osmunda cinnamomea grown at different gametophyte densities.

**Fig. 3.** The relationship between Osmunda cinnamomea population density and average gametophyte size after 1 year in culture. A, size; D, density.

$A = 175 - 45D^{1.427}$

$r^2 = 0.95 \ P < 0.05$
FIG. 4. Two types of Osmunda cinnamomea gametophytes: (A) female gametophytes are wide-cordate; (B) asexual gametophytes are spatulate.

ACKNOWLEDGEMENTS

We thank Drs Donald R. Farrar, Tom A. Ranker and Liz Sheffield for their valuable comments. The assistance of Dr Alan Warneke in editing the manuscript is gratefully acknowledged.

LITERATURE CITED


