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Comparative study of the sympatric ferns *Culcita macrocarpa* and *Woodwardia radicans:* Sexual phenotype

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Abstract

The sexual phenotypes of 1152 gametophytes from four populations of *Culcita macrocarpa* and *Woodwardia radicans* were monitored over a 1-year period. Gametophytes were maintained under three experimental conditions: (1) isolated, (2) pairs from the same sporophyte, or (3) pairs from different sporophytes. The frequencies of the sexual phenotypes did not vary significantly among these three conditions, and although there were some quantitative differences between populations, the sexual-phenotype sequences observed were species-specific. Gametophytes of *C. macrocarpa* were first male and then hermaphrodite: this sequence, together with the absence of antheridiogens, favours intragametophytic selfing. Natural populations of *C. macrocarpa* are presumably androdioecious. Gametophytes of *W. radicans* were first female and then hermaphrodite: this sequence and antheridiogen activity favour intergametophytic and even xenogamous mating. Despite these laboratory findings, populations of *W. radicans* are probably trioecious (because of the effects of antheridiogen). Few sporophytes of *W. radicans* were obtained in the present study, and none of *C. macrocarpa*: this is attributable to limiting illumination or substrate.

Keywords: Antheridiogen; Breeding system; Homosporous fern; Dichogamy; Sexual phenotype; Spain

Introduction

The gametophytes of homosporous ferns are potentially bisexual, making possible all breeding systems described in embryophytes (Cruden and Lloyd, 1995; Klekowski, 1979; Lloyd, 1974). Automixis (i.e. intragametophytic selfing) is the most extreme form of endogamy, since the sporophytes produced are completely homozygous; this system allows new populations to be established from a single spore. Autogamy (i.e. intergametophytic selfing) occurs between gametophytes derived from two different meiotic cells of a single sporophyte; heterozygosity is markedly reduced, but theoretically not entirely absent as in automixis. Xenogamy (intergametophytic crossing) occurs between gametophytes derived from different sporophytes. Between xenogamy and the two types of endogamy are mixed systems in which selfing and crossing have varying importances.

For a long time it was thought that automixis was the predominant system in natural populations of homosporous ferns (Klekowski, 1979; Klekowski and Baker, 1966). This view is inconsistent with numerous studies

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based on electrophoretic methods, which allow testing for random mating, and even estimation of rates of automixis and autogamy (e.g. Haufler, 1987; Ritland et al., 1990; Soltis and Soltis, 1992). In addition, in experimental cultures, relatively few species show high frequencies of automixis (see Lott et al., 2003, and references therein).

There are various mechanisms by which the gametophytes of homosporous ferns may avoid automixis. For example, the female and male gametangia may mature at different times. In addition, in ferns of various families it has been shown that gametophytes with archegonia produce pheromones that induce nearby immature gametophytes to produce antheridia (e.g. Yamane, 1998). Consequently, these compounds, together known as antheridiogens, stimulate intergametophytic reproduction.

Culcita macrocarpa C. Presl is the only European member of the family Dicksoniaceae, while *Woodwardia radicans* (L.) Sm. is one of only two European members of the family Blechnaceae, together with *Blechnum spicant* (L.) Roth. The principal populations of both ferns are located in northern coastal regions of Spain, and in the Azores, Madeira and Canary Islands. The ideal habitat for these species is mature riparian woodland on north-facing slopes of enclosed valleys, close to the coast (Amigo and Norman, 1995). Both species typically show strong clonal growth leading to patch formation. In many locations in which both species are present, their patches may be contiguous.

In addition to overlap in distribution and ecology, *C. macrocarpa* and *W. radicans* show great similarity in various life history characteristics, despite their phylogenetic remoteness. Both species have very large fronds, often over 2 m long, which persist throughout the winter. The period during which frond expansion occurs is the same in both species (unpublished own data). Spore release likewise occurs during the same period. Finally, the spores of both species are highly sensitive to desiccation (Quintanilla et al., 2002).

The present study investigated whether *C. macrocarpa* and *W. radicans* have adopted similar sex expression strategies. The gametophyte of *C. macrocarpa* has been studied previously by Stokey (1930), Rezende-Pinto (1943) and Mukherjee and Sen (1986). Although these studies described the sequence of appearance of gametangia, their central focus was on morphology. In contrast, there have been studies of the reproductive system in *W. radicans* (Klekowski, 1969b), but no studies of gametophyte morphology. Furthermore, it is difficult to compare the findings of these studies, because of differences in the culture methods used.

In the present study, we cultured isolated and paired gametophytes of *C. macrocarpa* and *W. radicans*. Our specific aims were (1) to compare the sequence of appearance of the gametangia of each species, (2) to determine whether there is among-population variation in sex expression, and (3) to assess whether gametangia sequence can be affected by antheridiogens. We also utilize this laboratory information and some field data to infer the likely breeding systems and population sex structure in nature.

Materials and methods

Plant material

We selected two populations of *C. macrocarpa* and two populations of *W. radicans* in northwest Spain (Table 1), the northernmost limit of the ranges of these species. We have reported data on the germination of spores from these populations in a previous study (Quintanilla et al., 2000); Table 1 maintains the acronyms used in this study. In addition, the two populations in the Eume watershed (C3 and W5) were included in a previous study comparing different methods of spore conservation (Quintanilla et al., 2002). From each population we collected spores from four individuals assumed to be genetically distinct: specifically, we collected spores from four ramets each separated by at least 10 m. All spores were collected in March 2000.

Experimental conditions

Spores of each individual were sown onto a 5.5-cm-diameter Petri dish with mineral agar (see Dyer 1979, p. 282). To minimize the risk of fungal contamination, the culture medium contained nystatin (100 U/ml). The dishes were sealed with Parafilm (American National

Table 1. Populations from which spores were obtained

Species	Acronym	Location (river valley)	Latitude	Altitude (m a.s.l.)
Culcita macrocarpa	C2	Seixo	43°41′N	280
	C3	Eume	43°24′N	90
Woodwardia radicans	W5	Eume	43°24′N	80
	W6	Xubia	43°30′N	250