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THE REPRODUCTIVE BIOLOGY OF THE INVASIVE FERNS *LYGODIUM MICROPHYLLUM* AND *L. JAPONICUM* (SCHIZAEACEAE): IMPLICATIONS FOR INVASIVE POTENTIAL[1]

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The effect of culture system and population source on sexual expression and sporophyte production was examined for two invasive fern species in Florida, USA, *Lygodium microphyllum* and *L. japonicum* (Schizaeaceae). Both species are currently spreading through Florida. Long-distance dispersal of ferns is thought to rely on successful intragametophytic selfing. Given the rate of spread observed in both *Lygodium* species, we hypothesized that both species are capable of intragametophytic selfing. To test this hypothesis, gametophytes of both species were grown in vitro as isolates, pairs, and groups. Both species were capable of intragametophytic selfing; 78% of *L. microphyllum* isolates produced sporophytes and over 90% of the *L. japonicum* isolates produced sporophytes. *Lygodium microphyllum* also displayed the ability to reproduce via intergametophytic crossing, facilitated by an antheridiogen pheromone. Sporophyte production was rapid across mating systems for both species, an advantage in Florida's wet and dry seasonal cycles. The high intragametophytic selfing rate achieved by both species has likely facilitated their ability to colonize and spread through Florida. The mixed mating system observed in *L. microphyllum* appears to give this species the ability to invade distant habitats and then adapt to local conditions.

Key words: Florida; invasive species; *Lygodium*; reproductive biology; Schizaeaceae; selfing.

Homosporous ferns have two free-living generations: a haploid gametophyte and a diploid sporophyte. Because homosporous ferns have bisexual gametophytes, intragametophytic selfing (i.e., the union of egg and sperm from the same gametophyte) was long held to be an important mode of reproduction in populations of homosporous ferns (Soltis et al., 1988). However, in controlled growth studies to examine reproductive strategies in homosporous ferns, only a few species had high intragametophytic selfing rates (e.g., Crist and Farrar, 1983; Korpelainen, 1996, 1997). The majority of studied fern species reproduce through intergametophytic crossing (Hedrick, 1987; Soltis and Soltis, 1992; Korpelainen and Kolkkala, 1996; Hooper and Haufler, 1997). Mixed mating has only been observed in a few species, including *Onoclea sensibilis* (Klekowski, 1982) and *Dryopteris expansa* (Soltis and Soltis, 1987). Therefore, both intragametophytic selfing and outcrossing may represent stable mating systems in homosporous ferns (e.g., Crist and Farrar, 1983; Peck et al., 1990; Korpelainen, 1996). In addition, homosporous ferns have evolved both morphological (e.g., asynchronous maturation of gametophytes) and physiological mechanisms (e.g., the pheromone antheridiogen) to promote outcrossing (e.g., Tryon and Vitale, 1977; Haufler and Welling, 1994). Antheridiogens are pheromones that promote outcrossing in fern gametophytes (e.g., Döpp, 1950; Haufler and Welling, 1994). These compounds are typically secreted by meristematic female gametophytes and trigger precocious antheridial formation on neighboring smaller, less mature gametophytes (e.g., Hamilton and Lloyd, 1991; Haufler and Welling, 1994).

This study examines mating systems in *L. microphyllum* (Cav.) R. Br. (Schizaeaceae) and *L. japonicum* (Thunb.) Swartz. The genus *Lygodium* is relatively small, comprised of up to 40 species, including one, *L. palmatum* (Bernh.) Sw., native to North America (Pemberton, 1998). *Lygodium* is mostly found in tropical regions of the world. The native range of *L. microphyllum* extends into moist habitats throughout the tropical Old World. *Lygodium japonicum* also has a large native range occurring in both temperate and tropical Asia (Pemberton, 1998).

Both species have a climbing habit and are nonindigenous and invasive in Florida, USA. Currently, *L. microphyllum* is expanding its range in southern Florida, while *L. japonicum* is expanding its range in northern Florida (Schmitz et al., 1997; Pemberton and Ferriter, 1998). For example, the total area infested by *L. microphyllum* was estimated to have expanded from approximately 11 200 hectares in 1993 to approximately 43 300 hectares in 1999 (Pemberton and Ferriter, 1998; A. Ferriter, South Florida Water Management District, personal communication). Both species are capable of smothering and displacing native understory vegetation, and in extreme infestations, shrub and canopy vegetation. This is particularly notable in *L. microphyllum*, which can form rachis mats up to a meter thick, effectively eliminating most understory vegetation.

Several reproductive life history characteristics in plants have been proposed that may facilitate the greater competitive ability of nonindigenous plant species, such as self- or wind pollination (fertilization), rapid growth to reproductive age or size, high and continuous seed (spore) production, adaptations for short- and long-distance seed (spore) or vegetative dis-

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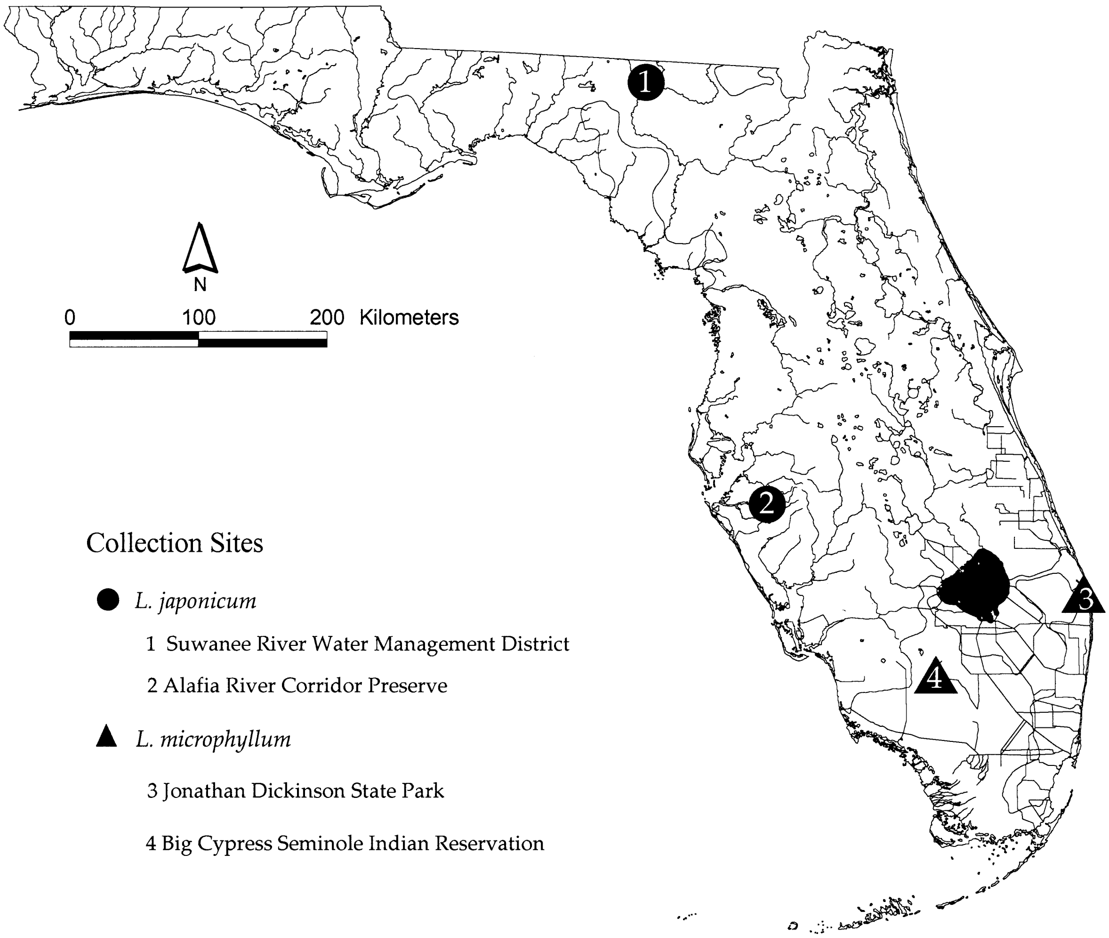

Fig. 1. Location of collection sites of spores of *Lygodium microphyllum* and *L. japonicum* in Florida, USA.

persal, and vegetative as well as sexual reproduction (Baker, 1974; Newsome and Noble, 1986; Roy, 1990; Reichard and Hamilton, 1997; Sakai et al., 2001). In homosporous ferns, the ability to reproduce through intragametophytic selfing promotes long-distance dispersal (Crist and Farrar, 1983; Peck et al., 1990). As discussed by Peck et al. (1990), spores transported long distances would be unlikely to establish gametophytes in close enough proximity to allow for intergametophytic selfing or crossing. Therefore, long-distance dispersal and colonization may be dependent upon the successful establishment of sporophytes by single spores. This ability to reproduce through intragametophytic selfing would promote the naturalization of introduced fern species, such as *Lygodium*.

Both *Lygodium* species are unusual among ferns in Florida in that they can climb into tree canopies. *Lygodium microphyllum*, in particular, has been observed overtopping tree canopies among tree islands in the Arthur R. Marshall Loxahatchee National Wildlife Refuge, Boynton Beach, Florida. This vine-like growth should theoretically promote the long-distance dispersal of these species because spores released at or above the tree canopy could potentially be carried a considerable distance by prevailing winds. Given these factors, we have hypothesized that both *Lygodium* species are capable of intragametophytic selfing.

MATERIALS AND METHODS

Plant material—Fertile fronds from *L. microphyllum* were collected in the field in October 1999 and May 2000, and *L. japonicum* fronds were collected in June and October 2000. Fertile fronds of *L. microphyllum* were collected from Jonathan Dickinson State Park and the Big Cypress Seminole Indian Reservation, located in Martin and Hendry counties, respectively, Florida, USA (Fig. 1). *Lygodium japonicum* fronds were collected from Suwannee River Water Management District land in Suwannee County and at the Alafia River Corridor Preserve in Hillsborough County (Fig. 1). Fertile fronds were collected from at least four individual plants from each population.

Fronds of both species were stored in sealed plastic bags and brought back to the laboratory. The bags were opened and stored at ambient temperatures and allowed to dry for several weeks, allowing the spores to be released. Each bag was kept separated from the others to prevent cross contamination. After the spores were released, they were sieved to remove leaf material. Following sieving, spores were sterilized in a 1% bleach solution and rinsed in sterile, distilled water. While in solution, the spores were sown with a pipette onto 100×20 mm plastic petri dishes containing modified Parker/Thompson's basal nutrient medium, containing both macro- and micronutrients, and solidified with 1% agar (Klekowski, 1969). The petri dishes were placed in a growth chamber under cool-white fluorescent illumination of approximately 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux with a photoperiod of 13/11 hours and a temperature of 25°/20°C, light/dark, respectively. After approximately 14 d, individual and pairs of asexual gametophytes were transferred using a dissecting microscope and a scalpel onto fresh medium in 65×10 mm plastic petri dishes.

Experimental design—To investigate the reproductive biology of both *Lygodium* species, a series of experiments was conducted. Experiments 1–3 were designed to test the three potential mating types, intragametophytic selfing, intergametophytic selfing, and intergametophytic crossing. A fourth experiment used groups of gametophytes to test for the presence of antheridiogen pheromones. To test for sporophyte production through intragametophytic selfing (Experiment 1), 60 gametophytes from each population were transferred into individual petri dishes. To test for either intragametophytic selfing or intergametophytic selfing, 30 pairs of gametophytes from each population were transferred to individual petri dishes (Experiment 2). Gametophyte pairs originated from the same sporophyte. To test for intergametophytic crossing (Experiment 3), 30 pairs of gametophytes from each population, with one gametophyte originating from separate sporophytes, were transferred to individual petri dishes. To test for the presence of antheridiogen pheromones (Experiment 4), 15 gametophytes were placed in individual petri dishes. For this fourth experiment, additional spores were placed in each of the 15 dishes, with four gametophytes maintained in each dish. In each of the four experiments, intragametophytic selfing was also possible. When grown together in pairs or groups (Experiments 2–4), gametophytes were maintained approximately 1 cm from each other.

Gametophytes were watered weekly to promote fertilization. The sexual status of each gametophyte was determined under a compound microscope (40× or 100× magnification) every 4 wk over a 16-wk period. Sexual status was determined by examining each gametophyte for the presence of antheridia and archegonia. Gametophytes were examined weekly through week 20 for the presence of sporophytes. The size of each gametophyte was measured at weeks 8 and 16 by measuring the length through the apical notch.

Data analysis—The CATMOD maximum-likelihood analysis was used to examine the effects of population, culture system, and their interaction on sexual status and sporophyte production (SAS Version 8, SAS Institute, Cary, North Carolina, USA). This method was also used to examine the effect of population on sexual expression and sporophyte production within groups. For analysis of sexual expression, pre-sexuals and hermaphrodites were grouped to reduce the number of zero data points in the analysis. These two sexual types were chosen because they typically were not present in large numbers at the same time period. A general linear model was used to examine the effects of mating system, population, and sexual type on gametophyte size (Minitab 13.1, Minitab, State College, Pennsylvania, USA).

RESULTS

Sexual expression (Experiments 1–3)—Consistent with Brown (1984), spore germination began in both species within 6 d of sowing. Growth of the gametophytes was typically biplanar, and recongnizable heart-shaped prothalli were observed within 7 d of germination.

The results of the experiments with isolates (Experiment 1) and pairs (Experiments 2 and 3) of *L. microphyllum* gametophytes indicate sexual expression was affected by the culture systems employed (Fig. 2). Within the isolates, antheridial formation was delayed. For instance, archegonia were observed within 10 d of germination, although antheridia were not seen frequently until 50 d after germination. In contrast, for Experiments 2 and 3, male and female gametophytes were commonly found together among pairs in both the intergametophytic selfing and crossing plates through week 8 (Fig. 2). The culture system significantly affected sexual expression through week 12 ($P < 0.05$, $P < 0.001$, Table 1). By week 16, gametophytes were predominately hermaphroditic in all three culture systems. This behavior in sexual expression was consistent between both the Jonathan Dickinson and Big Cypress populations. A significant difference in sexual expression ($P < 0.001$) was observed between the two populations at week 4, which resulted from the slower development of the Big Cypress population and the larger numbers of pre-sexuals observed at week 4 (Table 2). However, by week 8 there were no significant differences between the two *L. microphyllum* populations (Table 2).

In contrast, both archegonia and antheridia developed nearly simultaneously for *L. japonicum* on all gametophytes across the isolate and pair cultures (Experiments 1–3) (Fig. 3). Neither culture system nor population source affected sexual expression at any time interval (Table 1). Gametophytes from the Alafia River Corridor were found to develop slightly faster than the Suwannee River population. However, by week 8 nearly 100% of the gametophytes were hermaphroditic in both populations.

Reproductive success—Reproductive success was high for both species across all three culture system experiments. In *L. microphyllum*, sporophyte production began sooner among gametophyte pairs (Experiments 2 and 3) when either intergametophytic selfing or intergametophytic crossing was possible and continued at a more rapid rate through week 8 (Fig. 4). Culture system significantly affected sporophyte production in weeks 8 and 12 (Table 2). This difference in sporophyte production correlated with female-hermaphrodite and male pairs observed in the pair experiments. Female gametophytes located in the pair experiments were the first to produce sporophytes. Because isolated gametophytes were predominately female through week 8, sporophyte production did not begin among the isolates until after this time. However, once intragametophytic selfing began, sporophyte production continued at a rate similar to that observed in the pairs. Intragametophytic selfing was likely facilitated by the location of antheridia. In both *Lygodium* species, antheridia were intermixed with the archegonia behind the apical notch on bisexual gametophytes. By week 16, significant differences were no longer observed across the culture systems. The pattern of sporophyte production was consistent between the Jonathan Dickinson and Big Cypress populations. However, due to the slower maturation of gametophytes observed in the Big Cypress population, a significant difference ($P < 0.01$) in sporophyte production between populations was observed at week 8 (Table 2).

For *L. japonicum*, because gametophytes quickly developed into hermaphrodites, sporophyte production was similar between culture systems across time and was faster than *L. microphyllum* sporophyte production (Fig. 4). By week 20, sporophyte production was remarkably consistent across culture systems and between the two populations of *L. japonicum* (Fig. 4). A significant difference ($P < 0.01$) in sporophyte production across culture systems was observed at week 8. As a result of the rapid rate of sporophyte production between weeks 4 and 8 in the Alafia River Corridor population, a significant difference in sporophyte production between the two populations was also seen at weeks 8 and 12 (Table 2). This difference disappeared by week 16, at which time sporophyte production in the Suwannee River population equaled that of the Alafia River Corridor.

Size—Gametophyte sizes were measured at weeks 8 and 16 (Table 3). For *L. microphyllum*, sizes differed considerably among the sexes at week 8 ($P < 0.001$). Males were approximately half the size of either females or hermaphrodites (Table 3). By week 16, the difference was no longer observed because the majority of gametophytes were hermaphrodites. Differences were not seen across culture systems or between populations. In contrast, significant differences in gametophyte size for *L. japonicum* across culture systems and between populations was observed at both weeks 8 and 16 ($P < 0.001$). Differences between sexes were not observed because game-

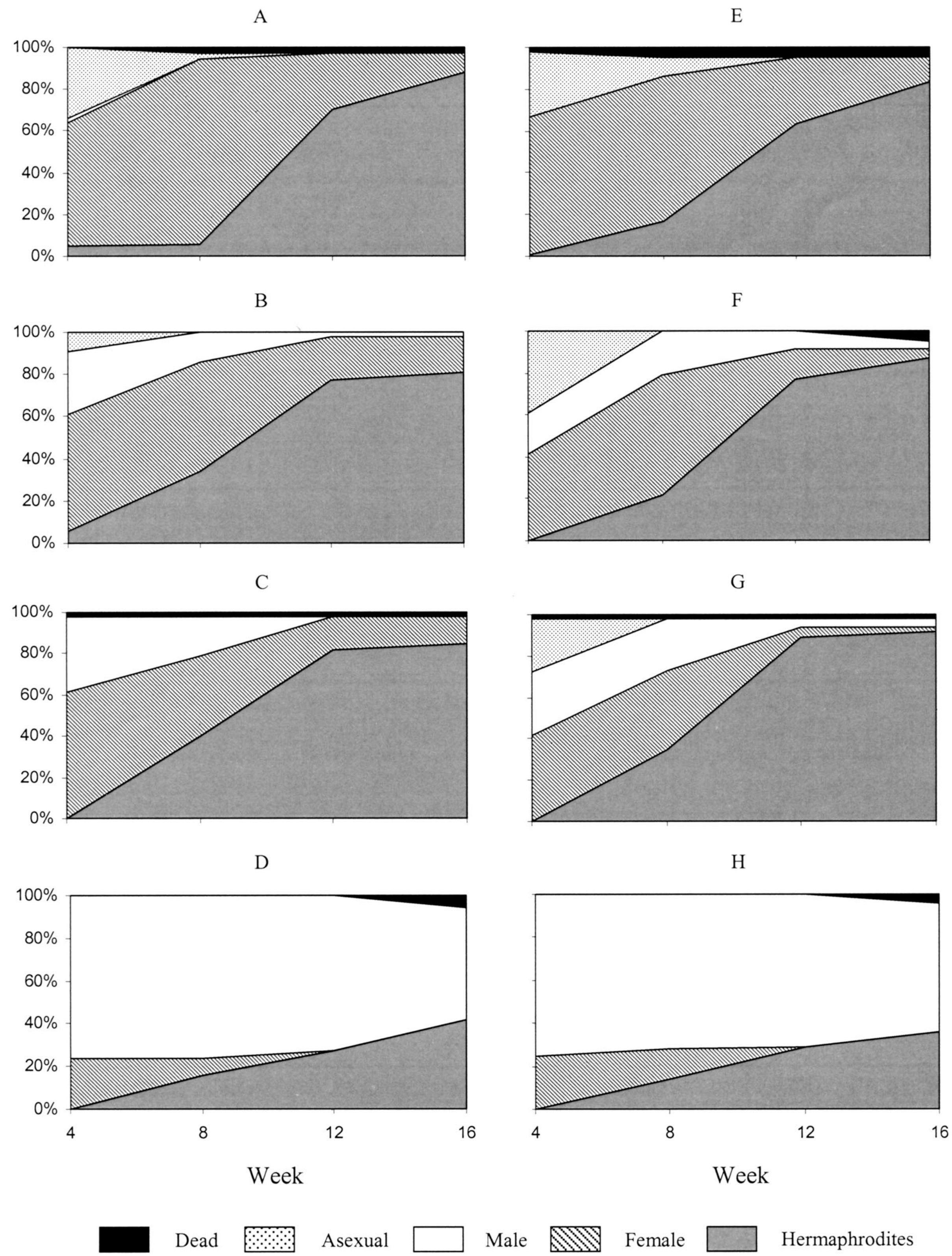

Fig. 2. Proportion of sexual types and dead gametophytes across mating systems in *Lygodium microphyllum* (Jonathan Dickinson Park, A–D; Big Cypress Seminole Reservation, E–H), where A and E = intragametophytic selfing, B and F = intergametophytic selfing, C and G = intergametophytic crossing, and D and H = groups.

TABLE 1. Significance levels for the effects of population and culture system on sexual status in two invasive ferns.

	Effect					
	Population		Mating system		Population × mating system	
Week	χ^2	*P*	χ^2	*P*	χ^2	*P*
Lygodium microphyllum						
4	22.10	<0.001	19.19	<0.05	10.21	NS
8	1.43	NS[a]	38.31	<0.001	8.51	NS
12	4.15	NS	18.59	<0.001	4.27	NS
16	5.61	NS	2.51	NS	8.32	NS
Lygodium japonicum						
4	2.30	NS	2.48	NS	0.48	NS
8	0.82	NS	1.69	NS	0.29	NS
12	0.82	NS	1.69	NS	0.29	NS
16	0.82	NS	1.69	NS	0.29	NS

[a] NS = not significant.

TABLE 2. Significance levels for the effects of population and culture system on sporophyte production in two invasive ferns.

	Effect					
	Population		Mating system		Population × mating system	
Week	χ^2	*P*	χ^2	*P*	χ^2	*P*
Lygodium microphyllum						
4	0.93	NS[a]	1.44	NS	0.22	NS
8	10.42	<0.01	7.61	<0.05	2.18	NS
12	0.06	NS	22.38	<0.001	6.86	<0.05
16	0.48	NS	2.85	NS	17.30	<0.001
20	1.48	NS	2.71	NS	10.13	NS
Lygodium japonicum						
4	1.49	NS	2.22	NS	0.33	NS
8	33.82	<0.001	11.64	<0.01	6.36	<0.05
12	14.73	<0.001	1.75	NS	3.76	NS
16	2.34	NS	0.11	NS	2.94	NS
20	0.16	NS	1.88	NS	0.04	NS

[a] NS = not significant.

tophytes became hermaphrodites quickly throughout the experiments.

Groups (Experiment 4)—The growth of *L. microphyllum* gametophytes in groups had a dramatic affect on sexual expression. Gametophytes germinating next to females universally became male (Fig. 2). Antheridial formation was precocious and abundant, starting shortly after germination. Unlike the pair experiments, 50% of the gametophytes remained males at week 16 (Fig. 2). This effect was less dramatic within groups of *L. japonicum* (Fig. 3). Male gametophytes of this species, however, had become largely hermaphroditic by week 16. Sexual expression did not differ significantly between populations in the group study in either species.

DISCUSSION

Intragametophytic selfing in some homosporous ferns promotes successful colonization (Klekowski, 1982; Suter et al., 2000). Together with long-distance dispersal of spores, intragametophytic selfing is a common trait in colonizing species such as *Asplenium platyneuron*; *A. trichomanes* subsp. *quadrivalens*, a widespread European subspecies; and *Onoclea sensibilis*, a species with a wide global distribution (Klekowski, 1982; Crist and Farrar, 1983; Suter et al., 2000). Both *Lygodium* species examined in this study have wide native geographic distributions. Indeed, we discovered that populations of both *Lygodium* species in Florida are capable of intragametophytic selfing, thus supporting our original hypothesis.

In Florida, *L. microphyllum* was discovered to have naturalized in the 1960s on the east coast of south Florida (Beckner, 1968; Nauman and Austin, 1978) and has rapidly spread reaching the west coast and as far north as Hillsborough County, in west central Florida (Pemberton and Ferriter, 1998). *Lygodium japonicum* was first observed in north Florida in 1932 (Gordon and Thomas, 1997) and has spread south into central Florida (FLEPPC, 2001). The ability to reproduce by intragametophytic selfing, as observed in this study, would facilitate such rapid spread. In fact, selfing rates for *L. japonicum* averaged 95%, while the average rate for *L. microphyllum* was 78%. The higher genetic load observed in *L. microphyllum* may be the result of its promotion of outcrossing through development of an initial female phase before becoming hermaphroditic. In addition, genetic load may increase with time as new spores colonize the area and a gradual increase in outcrossing occurs (Crist and Farrar, 1983).

The results of the experiments using pairs and groups of *L. microphyllum* gametophytes indicate the presence of an antheridiogen system. Antheridiogen systems have been previously reported in the genus *Lygodium* (Yamauchi et al., 1996; Wynne et al., 1998), including *L. japonicum* (Yamane et al., 1979, 1988) and *L. microphyllum* (Kurumatani et al., 2001). Antheridiogens have also been reported in members of the Polypodiaceae (Chiou and Farrar, 1997). Kurumatani et al. (2001) described *L. microphyllum* as a high antheridiogen-producing fern. This supports the precocious and abundant antheridia produced on males within the pairs and group cultures. The production and persistence of males in the group culture also suggests activity of an antheridiogen system in *L. japonicum*. However, in contrast, there was no evidence of an antheridiogen system for *L. japonicum* within the mating system experiments. The lack of an observable antheridiogen system in these experiments may have been because the distance between the gametophytes was too great for an antheridiogen system to be effective or the similar ages of the gametophytes may have left them sensitive or insensitive at the same time (Korpelainen, 1996). On the other hand, because antheridial and archegonial formation was nearly simultaneous on *L. japonicum* gametophytes within all three mating system experiments, it appears unlikely that outcrossing is a primary reproductive strategy in the populations studied. The populations of *L. japonicum* in Florida might have originated from relatively few plants. In this situation, with a limited chance for successful outcrossing, selfing variants would be unconditionally favored (Holsinger, 1990; Korpelainen, 1996). Establishment of a selfing variant of *L. japonicum* free of recessive sporophytic lethals would allow the spread of the mutation (Soltis and Soltis, 1992). As a result, intragametophytic selfing may be a stable mating system in the Florida population. Further study utilizing electrophoretic techniques would be necessary to test this hypothesis.

The ability of *L. microphyllum* to both self and outcross suggests that a mixed mating system may occur in Florida populations. Because outcrossing may be dependent on the density of gametophytes (Soltis and Soltis, 1987), intragametophytic selfing would likely be the primary mode of repro-

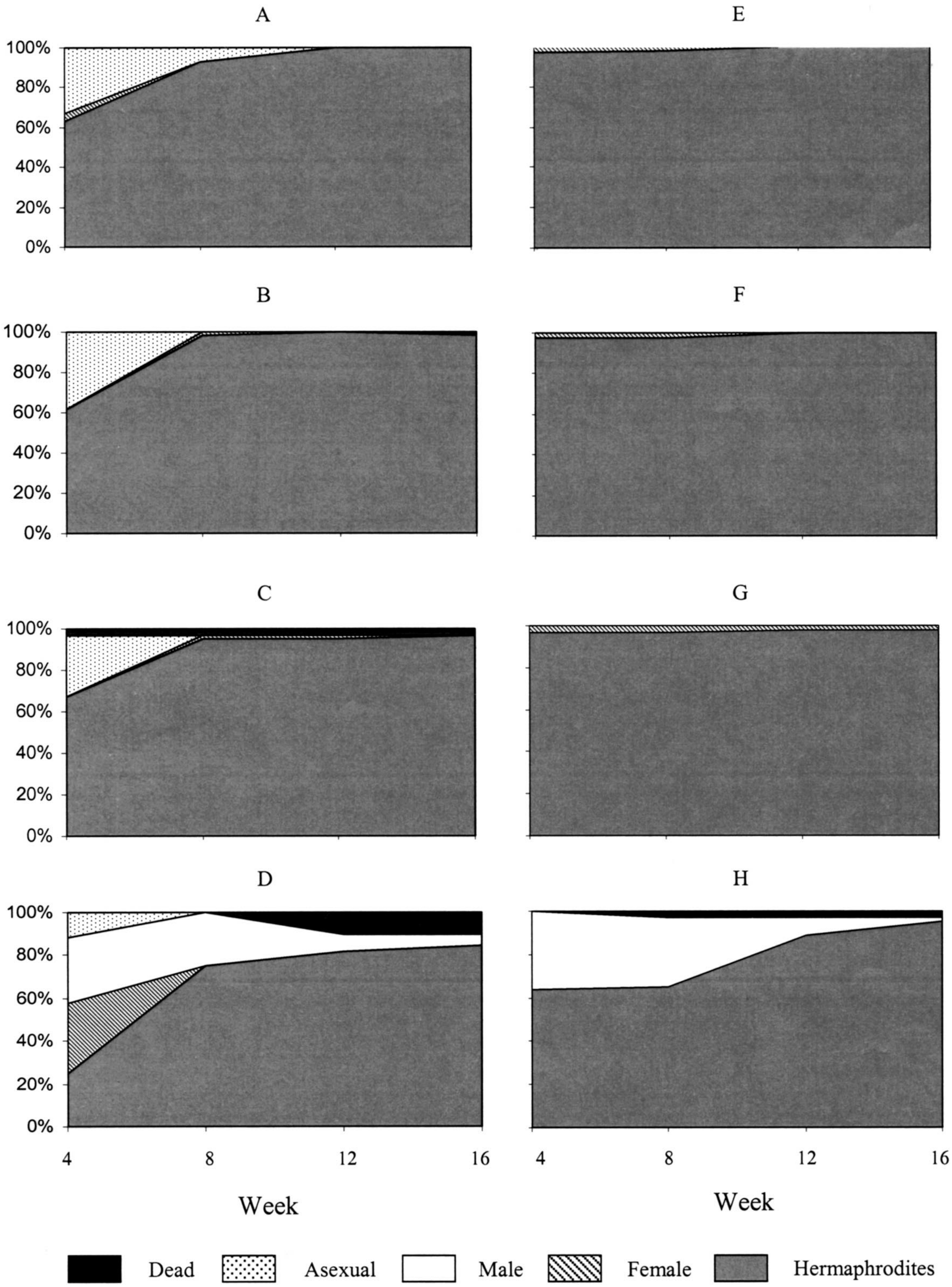

Fig. 3. Proportion of sexual types and dead gametophytes across mating systems in *Lygodium japonicum* (Suwannee River Water Management District, A–D; Alafia River Corridor Preserve, E–H), where A and E = intragametophytic selfing, B and F = intergametophytic selfing, C and G = intergametophytic crossing, and D and H = groups.

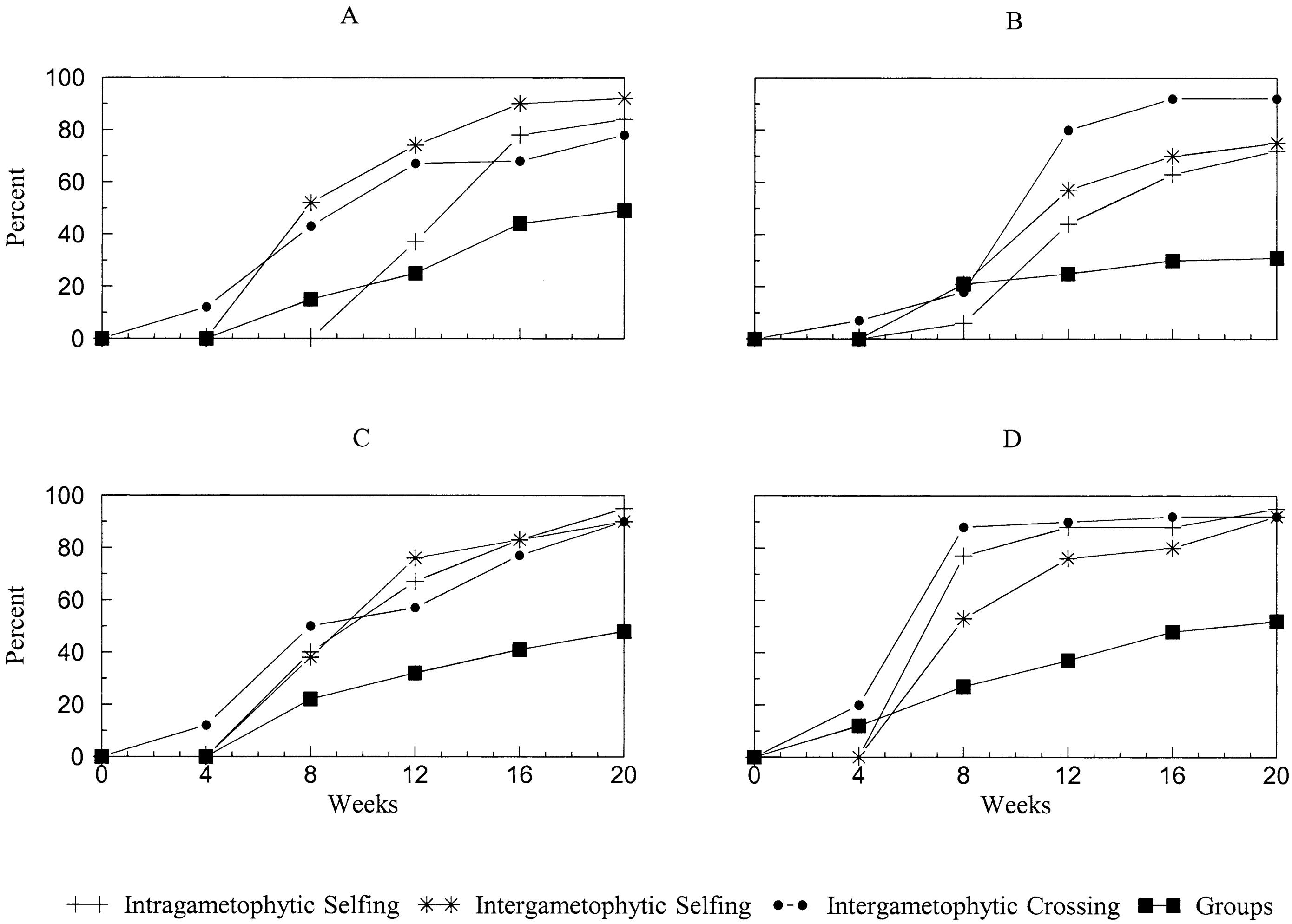

Fig. 4. Sporophyte production across mating systems and groups for two populations of *Lygodium microphyllum* (A–B) and *L. japonicum* (C–D), where A = Jonathan Dickinson State Park, B = Big Cypress Seminole Indian Reservation, C = Suwannee River Water Management District, and D = Alafia River Corridor Preserve. Data presented as percentage of gametophytes producing sporophytes.

duction at the beginning of a new infestation. As sporophyte densities increase over time, opportunities for outcrossing would be greater as the number of spores and resulting gametophytes increase. The potential presence of an antheridiogen system may support increased gametophyte densities within an infestation since they have been shown to induce dark germination of spores (Weinberg and Voeller, 1969; Schneller et al., 1990; Haufler and Welling, 1994). The promotion of spore germination would lead to an increase in gametophyte densities, particularly of males (Greer and McCarthy, 1999).

The Everglades in southern Florida are considered among the top locations in the United States in the severity of nonindigenous plant invasion (Loope, 1992; Gordon, 1998). In this region, both the insularity and the subtropical climate strongly contribute to successful establishment of nonindigenous species (Simberloff, 1994; Austin, 1999). In addition, natural disturbances, such as hurricanes and fires, are relatively common periodic events in Florida and provide ideal opportunities for the establishment of invader species (Horvitz et al., 1995; Simberloff et al., 1997). The environments encountered by *Lygodium* within Florida are also highly variable, particularly with regard to seasonal cycles of drought and rainfall (and local flooding). Under stressful environmental conditions, gametophytes may have a shorter life span. Fern gametophytes that reach sexual maturity quickly would have the greatest chance to produce sporophytes before dying (Greer and McCarthy, 1999). Sexually mature gametophytes of *L. microphyllum* and *L. japonicum* were observed within 5 wk of germination. Once sexual maturity was reached, sporophyte production began and continued rapidly through week 12. This ability to reproduce quickly would be an advantage in the habitats encountered in Florida. For example, *L. microphyllum* commonly invades cypress communities, and gametophytes and young sporophytes are typically found on cypress knees and trunks of trees above the level of recent inundation. These communities are often inundated during the summer rainy season and can dry down during the winter dry season or during periods of extensive drought. Therefore, reaching sexual maturity at a young age would allow for the production of a greater number of offspring during favorable conditions.

The plasticity of mating systems observed in *L. microphyllum* may partially explain the vast geographic range of the species and its appearance in diverse habitats (Pemberton, 1998). Although outcrossing was not observed in the pair culture experiments with *L. japonicum*, the presence of males in

TABLE 3. Average size (in millimeters) and standard error of gametophytes of two invasive ferns at weeks 8 and 16. The number of gametophytes measured is in parentheses.

Species	Population	All	Male	Female	Hermaphrodites
Week 8					
L. microphyllum					
Isolates[a]	Jonathan Dickinson SP[a]	4.7 ± 2.5 (60)	1.9 (1)	4.8 ± 2.6 (57)	4.1 (1)
	Seminole Reservation	4.3 ± 2.9 (56)	—	4.7 ± 1.3 (50)	4.0 ± 0.4 (6)
Selfing Pairs	Jonathan Dickinson SP	5.0 ± 3.6 (58)	2.8 ± 1.4 (8)	5.8 ± 4.4 (31)	4.8 ± 2.7 (19)
	Seminole Reservation	4.7 ± 3.7 (56)	2.1 ± 1.9 (12)	5.9 ± 2.3 (31)	4.3 ± 3.5 (13)
Outcrossing	Jonathan Dickinson SP	4.7 ± 3.7 (58)	2.5 ± 1.9 (12)	5.6 ± 2.5 (23)	5.1 ± 3.3 (23)
	Seminole Reservation	5.0 ± 4.0 (54)	3.4 ± 2.2 (14)	6.0 ± 2.5 (21)	5.2 ± 2.8 (19)
L. japonicum					
Isolates	Suwannee River	5.8 ± 3.6 (60)	—	—	5.9 ± 3.5 (56)
	Alafia River	6.7 ± 1.7 (60)	—	7.0 ± 0.0 (2)	6.7 ± 1.7 (58)
Selfing Pairs	Suwannee River	5.1 ± 3.5 (60)	—	5.0 (1)	5.1 ± 3.5 (59)
	Alafia River	7.8 ± 1.4 (58)	—	6.0 (1)	7.8 ± 1.4 (57)
Outcrossing	Suwannee River	5.3 ± 1.5 (56)	—	4.0 (1)	5.4 ± 1.4 (55)
	Alafia River	5.6 ± 2.2 (60)	—	3.9 ± 0.5 (2)	5.6 ± 1.4 (58)
Week 16					
L. microphyllum					
Isolates	Jonathan Dickinson SP	7.4 ± 2.6 (60)	—	7.1 ± 1.3 (6)	7.4 ± 2.6 (54)
	Seminole Reservation	6.6 ± 4.4 (56)	—	3.8 ± 3.4 (8)	7.1 ± 1.5 (48)
Selfing Pairs	Jonathan Dickinson SP	5.6 ± 3.8 (58)	1.8 (1)	5.9 ± 2.5 (10)	5.6 ± 3.8 (47)
	Seminole Reservation	5.5 ± 3.7 (55)	2.0 ± 0.1 (2)	4.8 ± 0.8 (2)	5.6 ± 3.4 (53)
Outcrossing	Jonathan Dickinson SP	5.4 ± 3.4 (58)	—	6.0 ± 3.6 (8)	5.3 ± 3.3 (50)
	Seminole Reservation	5.8 ± 4.4 (55)	2.1 ± 0.7 (2)	4.4 (1)	6.0 ± 2.6 (52)
L. japonicum					
Isolates	Suwannee River	6.7 ± 2.9 (60)	—	—	6.7 ± 2.9 (56)
	Alafia River	7.0 ± 3.0 (60)	—	—	7.0 ± 3.0 (58)
Selfing Pairs	Suwannee River	6.5 ± 4.1 (58)	—	—	6.5 ± 4.1 (58)
	Alafia River	8.0 ± 2.4 (58)	—	—	8.0 ± 2.4 (57)
Outcrossing	Suwannee River	6.3 ± 2.7 (54)	—	—	6.3 ± 2.7 (54)
	Alafia River	6.0 ± 2.0 (58)	—	4.0 (1)	6.1 ± 1.9 (57)

[a] SP = State Park.

the group experiment and the identification of antheridiogen in other studies suggest that outcrossing does occur within this species (e.g., Yamane et al., 1988). As discussed by Schneller et al. (1990), sexual strategies can be variable both within and among populations of a fern species. This plasticity of reproductive strategies may allow fern species to colonize a diversity of habitats (Schneller et al., 1990). The ability to reproduce by intragametophytic selfing would allow the establishment of distant populations, while increased genetic diversity obtained through outcrossing would theoretically allow increased adaptability. In the case of *L. microphyllum* and *L. japonicum*, this may occur both in Florida and globally.

In summary, this study demonstrates that both *L. microphyllum* and *L. japonicum* share life history characteristics that facilitate their invasiveness, suggesting both species will continue to spread in Florida. The significant differences in sexual expression and sporophyte production observed in pairs of *L. microphyllum* gametophytes were likely the result of an antheridiogen system. Similar differences between isolates and pairs were not observed in *L. japonicum*, for which all of the gametophytes rapidly became hermaphroditic, and individual males were not observed. Both species displayed the ability to reproduce through intragametophytic selfing. This ability to reproduce through intragametophytic selfing supports the hypothesis that reproductive strategies partially explain the colonization and spread of these two species in Florida and the potential to invade suitable areas in other countries in the region.

In addition to the *Lygodium* species examined in this study, numerous other fern species have naturalized in Florida (Wunderlin, 1998). Several of these species have also invaded Florida's ecosystems, including *Nephrolopesis multiflora*, *N. cordifolia*, and *Tectaria incisa* (Florida Exotic Pest Plant Council Category 1, 2001), while other species such as *Pteris vitatta* are very widespread (now pantropical) but have not become invasive. Similarly, 32 species have naturalized in Hawaii (Wilson, 2002). Additional research would provide information on whether these successful invaders share mating system strategies similar to those observed in *Lygodium microphyllum* and *L. japonicum*. Perhaps intragametophytic selfing or mixed mating are traits common in invasive fern species.

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