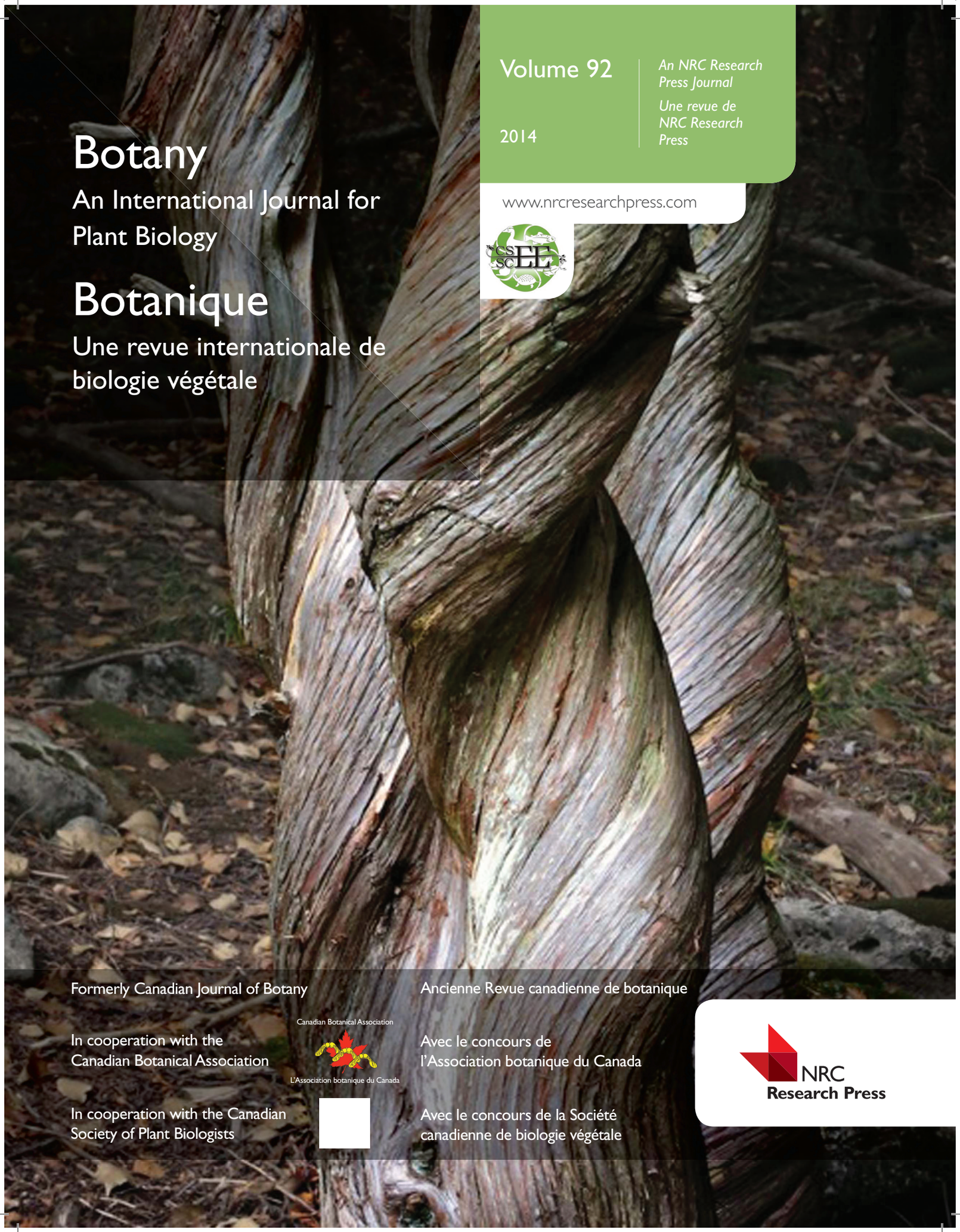

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NOTE

Low reproductive success of hay-scented fern (*Dennstaedtia punctilobula*) regardless of inbreeding level or time since disturbance

Kathryn M. Flinn, Matthew M. Loiacono, and Hannah E. Groff

Abstract: Self-fertilization can facilitate the colonization of new habitats because it allows a single individual to found a population. Here we investigated the relationship between mating systems and colonization in hay-scented fern (*Dennstaedtia punctilobula* (Michx.) T.Moore). Throughout eastern North America, this species has been called a "native invasive" for its tendency to dominate forest understories disturbed by logging, inhibiting tree regeneration. Thus, it is important to understand the mechanisms of its spread. We hypothesized that if populations were founded through selfing, then populations disturbed more recently would retain higher selfing ability; this pattern would demonstrate an important link between mating systems and colonization. For four populations logged at different times in the past, we compared the sporophyte production of gametophytes at different levels of inbreeding (intragametophytic selfing, intergametophytic selfing, and outcrossing) using laboratory crosses. Across all treatments, only 9.8% of gametophytes formed sporophytes (N = 400 gametophytes). Neither inbreeding level nor time since disturbance affected sporophyte production. Selfing ability did not differ across populations logged at different times; there was no interaction between inbreeding level and time since disturbance. The low reproductive success of *D. punctilobula*, regardless of inbreeding level or time since disturbance, suggests that population establishment and expansion via sexual reproduction may be relatively rare in this clonal species.

Key words: inbreeding depression, invasive species, mating systems, reproductive assurance, self-fertilization.

Résumé : L'auto-fertilisation peut faciliter la colonisation de nouveaux habitats car elle permet à un seul individu de fonder une population. Les auteurs ont examiné ici la relation entre les systèmes de croisement et la colonisation de la fougère odorante (*Dennstaedtia punctilobula* (Michx.) T.Moore). À travers tout l'est de l'Amérique du Nord, cette espèce a été identifiée comme indigène envahissante à cause de sa tendance à dominer les sous-étages forestiers perturbés par l'exploitation forestière, inhibant la régénération des arbres. Il est alors important de comprendre les mécanismes de sa dissémination. Les auteurs ont émis l'hypothèse que si des populations étaient fondées par autofécondation, alors les populations perturbées plus récemment pourraient conserver plus longtemps leur capacité d'autofécondation; ce patron démontrerait un lien important entre les systèmes de croisement et la colonisation. Les auteurs ont comparé chez quatre populations exploitées à différentes périodes dans le passé, la production sporophyte des gamétophytes à différents niveaux d'endogamie (autofécondation intra-gamétophyte, autofécondation inter-gamétophyte et croisement éloigné) à l'aide de croisements en laboratoire. À travers tous les traitements, 9.8 % des gamétophytes seulement formaient des sporophytes (N = 400 gamétophytes). Aucun niveau d'endogamie, ni le temps écoulé depuis la perturbation n'affectait la production de sporophytes. La capacité d'autofécondation ne différait pas d'une population à l'autre, exploitées à des périodes différentes; il n'y avait pas d'interaction entre le niveau d'endogamie et le temps écoulé depuis la perturbation. Le faible succès reproducteur de *D. punctilobula*, peu importe le niveau d'endogamie ou le temps écoulé depuis la perturbation, suggère que l'établissement et l'expansion de la population par la reproduction sexuée peut être relativement rare chez cette espèce clonale. [Traduit par la Rédaction]

Mots-clés : dépression d'endogamie, espèce envahissante, systèmes de croisement, assurance reproductive, auto-fertilisation.

Introduction

Self-fertilization should facilitate plant invasions by providing reproductive assurance in new habitats, where mates or pollinators may be scarce (Darwin 1876; Baker 1955). In fact, introduced species often have a higher selfing ability than their native relatives (Harmon-Threatt et al. 2009; Burns et al. 2011; Vervoort et al. 2011). Also, among South African irises, introduced species that became naturalized had a higher selfing ability than introduced relatives that did not become naturalized (van Kleunen et al. 2008), and among European plants in North America, species capable of selfing had larger ranges (van Kleunen and Johnson 2007). This recent evidence shows that mating systems may indeed play a key role in plant invasions. If plants have the ability both to self and to outcross, then the founding of populations could act as a filter, removing individuals that do not self-fertilize and leaving young populations composed of plants with high selfing ability (Baker 1955; Pannell and Barrett 1998). Outcrossing rates might then increase over time as additional individuals colonized a site and mating opportunities expanded, especially if selfed progeny showed inbreeding depression. This process would lead to another testable prediction of the hypothesis that selfing facilitates invasions — that recently founded populations should have higher selfing ability than long-established populations. Some studies have upheld the prediction that recently founded populations have

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Table 1. Characteristics of four forested sites in Lancaster County, Pennsylvania, USA, from which *Dennstaedtia punctilobula* spores were collected.

Site name	Time since last logging (years)	Latitude, longitude
Governor Dick Park	4	40°14′04″N, 76°27′33″W
Steinman Run Nature Preserve	33	39°54′18″N, 76°17′05″W
Ray's Woods Nature Preserve	~50	39°53′31″N, 76°15′56″W
Fishing Creek Nature Preserve	~60	39°48′23″N, 76°14′26″W

higher selfing ability; for example, an introduced tobacco had a higher selfing ability on a Channel Island colonized ~30 years before than on an island colonized ~100 years before, or at longer-occupied California mainland sites (Schueller 2004).

Here we tested the prediction that populations disturbed more recently would have higher selfing ability in the invasive homosporous fern *Dennstaedtia punctilobula* (Michx.) T.Moore (hay-scented fern). Though native to eastern North America, this species is considered invasive or weedy because it spreads aggressively in logged forests (Hughes and Fahey 1991; Hill and Silander 2001; Aikens et al. 2007) and interferes with tree regeneration (Horsley and Marquis 1983; de la Cretaz and Kelty 2002; Fei and Steiner 2008). Dense understories formed by *D. punctilobula* can thus alter the structure, diversity, and composition of forests (George and Bazzaz 1999; Royo and Carson 2006). This problem has been exacerbated by deer overpopulation because deer browse tree saplings but find *D. punctilobula* relatively unpalatable (Horsley and Marquis 1983). In addition, dense fern cover can provide a haven for small mammals that prey on tree seeds and seedlings (Royo and Carson 2008). Understanding how *D. punctilobula* spreads and establishes new populations would aid efforts to control it; the extent to which the invasiveness of *D. punctilobula* depends on selfing — and the mating system of this species in general — remain unknown.

To assess selfing ability, we compared the sporophyte production of gametophytes at different levels of inbreeding for four populations of *D. punctilobula* from forests in Lancaster County, Pennsylvania, USA, that were logged at different times in the past. We hypothesized that if populations were founded through selfing, then populations disturbed more recently would retain higher selfing ability. In fact, populations may have formed or grown by sexual reproduction near the time of logging, or they may have simply expanded vegetatively, as *D. punctilobula* is strongly clonal via rhizomes (Flora of North America 2014). It is reasonable to think that sexual reproduction may have taken place because *D. punctilobula* forms a spore bank in forest soils (Penrod and McCormick 1996), and the soil disturbance associated with logging stimulates spore germination, gametophyte growth, and sporophyte establishment via sexual reproduction (Groninger and McCormick 1992). Homosporous ferns can potentially reproduce by intragametophytic selfing, between gametes from the same gametophyte, which has no analog in seed plants and yields fully homozygous progeny; intergametophytic selfing, between gametophytes from the same sporophyte, which is analogous to selfing in seed plants; or outcrossing, between gametophytes from different sporophytes (Klekowski 1969). We performed laboratory crosses between gametophytes to compare sporophyte production across four populations disturbed at different times and across these three inbreeding levels.

Materials and methods

Spores for this experiment came from four *D. punctilobula* populations in forested sites in Lancaster County, Pennsylvania, USA (Table 1). We determined the time since last logging for each forest through personal communications (Governor Dick Park: A. Wells, Governor Dick Park, 2013; Steinman Run Nature Preserve: T. Stahl, Lancaster Conservancy, 2013) or field evidence including tree species and diameter at breast height (Fishing Creek Nature Preserve, Ray's Woods Nature Preserve: K.M. Flinn, personal observations, 2013). We collected fertile fronds from 20 individuals in each population in September 2013. Fronds were collected at least 5 m apart to increase the probability that they came from different genets. The fronds were sealed in glassine envelopes and dried in ovens at 30 °C for 3 days to promote spore release.

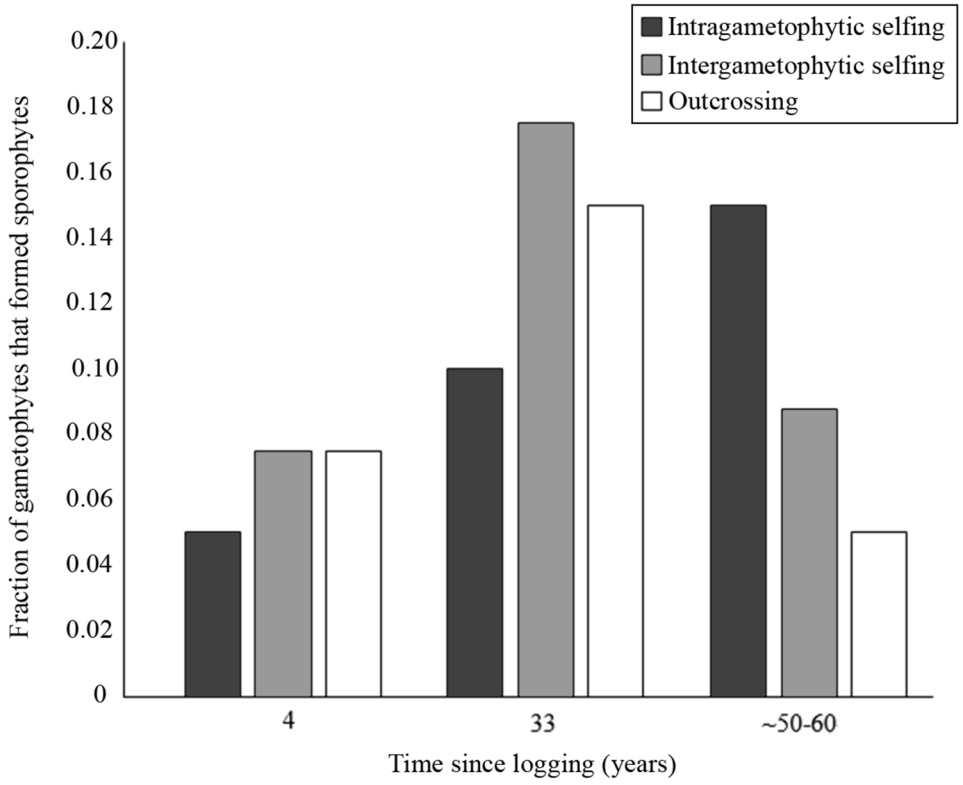
We sowed spores in 60 mm × 15 mm petri dishes on nutrient medium containing Parker's macroelements and Thompson's microelements solidified with 1% agar (Klekowski 1969). We autoclaved the medium at 121 °C for 15 min and added the fungicide nystatin at 50 mg·L^{-1}. The dishes were sealed with Parafilm (American National Can, Chicago, Illinois, USA) and placed under continuous illumination. After 3 weeks, we transplanted gametophytes into fresh dishes for three inbreeding-level treatments: intragametophytic selfing (isolated), intergametophytic selfing (paired with another gametophyte from the same sporophyte, 1 cm apart), and outcrossing (paired with another gametophyte from a different sporophyte from the same population, randomly chosen, 1 cm apart). These treatments represented potential levels of inbreeding, as isolated gametophytes could only reproduce through intragametophytic selfing, whereas paired gametophytes could either self-fertilize or outcross. Thus, the experiment involved three inbreeding levels, four populations with different times since disturbance, and 20 individuals per population, for a total of 240 crosses involving 400 gametophytes. Sample sizes for individual treatments were: 4 years since logging, intragametophyic selfing, $N = 20$; 4 years since logging, intergametophyic selfing, $N = 40$; 4 years since logging, outcrossing, $N = 40$; 33 years since logging, intragametophytic selfing, $N = 20$; 33 years since logging, intergametophytic selfing, $N = 40$; 33 years since logging, outcrossing, $N = 40$; ~50–60 years since logging, intragametophytic selfing, $N = 40$; ~50–60 years since logging, intergametophytic selfing, $N = 80$; ~50–60 years since logging, outcrossing, $N = 80$. We watered the gametophytes weekly to facilitate fertilization, and examined them for the presence of sporophytes every week for 23 weeks.

For analysis, we pooled data for the Ray's Woods and Fishing Creek populations because they were logged at similar times (~50 and ~60 years ago, respectively; Table 1). We analyzed the effects of inbreeding level, time since disturbance, and their interaction on the probability of forming sporophytes by the end of 23 weeks using log-linear analysis of a three-way contingency table (Lowry 2014). Specifically, we performed three G tests of independence to test whether inbreeding level (intragametophytic selfing, intergametophytic selfing, or outcrossing), time since disturbance (4, 33, or ~50–60 years), or the interaction between inbreeding level and time since disturbance affected the probability of forming sporophytes. We also conducted a two-way factorial analysis of variance (ANOVA), with the proportion of gametophytes forming sporophytes as the response variable and inbreeding level, time since disturbance, and the interaction between inbreeding level and time since disturbance as predictor variables, using a generalized linear model in R (R Core Team 2014).

Results

Gametophytes had low reproductive success overall; only 9.8% of gametophytes formed sporophytes (Fig. 1). Sporophyte production

Fig. 1. Fraction of *Dennstaedtia punctilobula* gametophytes that formed sporophytes at different inbreeding levels (intragametophytic selfing, intergametophytic selfing, outcrossing) and from populations in forests logged at different times in the past (N = 400 gametophytes).



did not differ across inbreeding levels ($G_{[2]}$ = 0.84, P = 0.6570). Time since disturbance also had no effect on the probability of forming sporophytes ($G_{[2]}$ = 4.06, P = 0.1313). Selfing ability did not differ across populations logged at different times; there was no interaction between inbreeding level and time since disturbance ($G_{[12]}$ = 8.12, P = 0.7757). Similarly in the two-way factorial ANOVA, neither inbreeding level, time since disturbance, nor their interaction had any effect on the proportion of gametophytes forming sporophytes (all P > 0.10).

Discussion

The reproductive success of *D. punctilobula* in this experiment falls at the low end of the range reported for other fern species under similar conditions (Table 2). This could indicate that *D. punctilobula* indeed has lower rates of sporophyte production than many other fern species, that standard laboratory conditions are less conducive to sporophyte production in *D. punctilobula* than in many other fern species, or both. Fern gametophytes can have different patterns of sexual development at low density and on agar than in natural populations on soil (Rubin and Paolillo 1983; Rubin et al. 1985; Ranker and Houston 2002). A low rate of sexual reproduction is perhaps not surprising for a strongly clonal, long-lived perennial plant, and it would make *D. punctilobula* similar to *Pteridium aquilinum* (L.) Kuhn, another clonal, long-lived, and sometimes weedy fern in which sexual reproduction occurs very infrequently and in association with disturbance (Marrs and Watt 2006).

Dennstaedtia punctilobula also showed no differences in sporophyte production among intragametophytic selfing, intergametophytic selfing, and outcrossing treatments. This is in contrast to the many fern species that had higher rates of sporophyte production when gametophytes were paired rather than isolated (13 of 19 species tested; Table 2). This suggests that genetic load or any adaptations to promote outcrossing are not the primary obstacles to sexual reproduction in this species. If nearby individuals in fact belonged to the same or a closely related genet, this could also have reduced any difference between the two paired treatments, because in this case the intergametophytic selfing and outcrossing treatments would have involved similar levels of inbreeding. In fact, in the extreme case that the entire population resulted from a single spore that underwent intragametophytic selfing to produce a sporophyte that expanded clonally, then the intragametophytic selfing, intergametophytic selfing and outcrossing treatments would be genetically identical. This situation is thought to have occurred in several ferns that colonized recently planted forests on Dutch polders (de Groot et al. 2012).

The lack of association between selfing ability and colonization also contrasts with previous findings for other fern species. For example, Hawaiian *Sadleria* species frequently found on newly formed lava flows had higher selfing ability than species of late-successional habitats (Holbrook-Walker and Lloyd 1973). Lott et al. (2003) found high selfing ability in two invasive ferns in Florida. Among three native ferns in central New York, species more frequent in post-agricultural forests had higher reproductive success and selfing ability than species more frequent in forests that were never cleared for agriculture (Flinn 2006). A few other studies have also compared selfing ability within species, among populations with different disturbance histories. Geographically disjunct, solitary *Asplenium platyneuron* (L.) Oakes plants on recent coal spoils had greater selfing ability than plants from dense populations in the center of the species' range (Crist and Farrar 1983). de Groot et al. (2012) found that fern populations on a Dutch polder recently reclaimed from the sea had higher selfing ability than mainland populations. However, within the three native fern species in central New York, populations in post-agricultural forests and in forests never cleared for agriculture had similar rates of sporophyte production across inbreeding levels (Flinn 2006). For *D. punctilobula*, the lack of variation in selfing ability

Table 2. Sporophyte production by fern gametophytes grown on agar-solidified nutrient medium in the laboratory, either isolated or paired; species are listed in order of increasing sporophyte production of isolated gametophytes.

Species	Sporophyte production of isolated gametophytes	Sporophyte production of paired gametophytes	Reference
Elaphoglossum callifolium (Blume) T.Moore	0	0.21	Chiou et al. 2002
Elaphoglossum crassifolium (Gaudich.) W.R.Anderson & Crosby	0	0.17	Chiou et al. 2002
Stenochlaena tenuifolia (Desv.) T.Moore	0	1.0	Klekowski 1969
Sadleria pallida Hook. & Arn.	0.0049	Not tested	Ranker et al. 1996
Athyrium filix-femina (L.) Roth	0.010	Not tested	Peck et al. 1990
Cryptogramma stelleri (S.G.Gmel.) Prantl	0.010	Not tested	Peck et al. 1990
Dryopteris marginalis (L.) A.Gray	0.020	Not tested	Peck et al. 1990
Sadleria cyatheoides Kaulf.	0.036	Not tested	Ranker et al. 1996
Sadleria pallida Hook. & Arn.	0.040	0.20	Holbrook-Walker and Lloyd 1973
Pseudodrynaria coronans (Wall. ex Mett.) Ching	0.050	Not tested	Singh and Roy 1977
Cystopteris bulbifera (L.) Bernh.	0.060	Not tested	Peck et al. 1990
Asplenium scolopendrium L.	0.10	0.22	Wubs et al. 2010
***Dennstaedtia punctilobula* (Michx.) T.Moore**	**0.11**	**0.094**	**This study**
Polystichum acrostichoides (Michx.) Schott	0.11	0.30	Flinn 2006
Dryopteris cristata (L.) A.Gray	0.26	Not tested	Peck et al. 1990
Thelypteris simulata (Davenport) Nieuwl.	0.26	Not tested	Peck et al. 1990
Dryopteris intermedia A.Gray	0.30	0.32	Flinn 2006
Polypodium virginianum L.	0.32	Not tested	Peck et al. 1990
Thelypteris palustris Schott	0.32	Not tested	Peck et al. 1990
Microsorum punctatum (L.) Copel.	0.36	Not tested	Peck et al. 1990
Adiantum pedatum L.	0.38	Not tested	Peck et al. 1990
Cibotium glaucum (Sm.) Hook. & Arn.	0.40	Not tested	Srivastava et al. 2008
Osmunda regalis L.	0.41	Not tested	Klekowski 1970
Asplenium trichomanes L. subsp. *quadrivalens* D.E.Mey.	0.56	0.72	Suter et al. 2000
Sadleria cyatheoides Kaulf.	0.56	1.0	Holbrook-Walker and Lloyd 1973
Sadleria souleyetiana (Gaudich.) T.Moore	0.56	0.96	Holbrook-Walker and Lloyd 1973
Cystopteris tenuis Desv.	0.59	Not tested	Peck et al. 1990
Dicranopteris linearis (Burm.f.) Underw.	0.63	Not tested	Lloyd 1974
Dryopteris carthusiana (Vill.) H.P.Fuchs	0.63	0.46	Flinn 2006
Phegopteris decursive-pinnata Fée	0.73	Not tested	Masuyama 1979
Woodsia obtusa (Spr.) Torrey	0.80	Not tested	Peck et al. 1990
Pteridium aquilinum (L.) Kuhn	0.82	0.58	Klekowski 1971
Asplenium platyneuron (L.) Oakes	0.86	0.91	Crist and Farrar 1983
Onoclea sensibilis L.	0.87	1.0	Klekowski and Lloyd 1968
Acrostichum danaeifolium Langsd. & Fisch.	0.90	Not tested	Lloyd and Gregg 1975
Ceratopteris thalictroides (L.) Brongn.	0.90	Not tested	Singh and Roy 1977
Phymatosorus scolopendria (Burm. f.) Pic.Serm.	0.93	Not tested	Lloyd 1974
Woodwardia radicans (L.) Sm.	0.96	1.0	Klekowski 1969
Ceratopteris thalictroides (L.) Brongn.	0.98	Not tested	Lloyd and Warne 1978
Pityrogramma calomelanos (L.) Link	0.98	Not tested	Singh and Roy 1977
Doodia aspera R.Br.	1.0	1.0	Klekowski 1969
Doodia caudata (Cav.) R.Br.	1.0	1.0	Klekowski 1969
Woodwardia fimbriata Sm.	1.0	1.0	Klekowski 1969
Woodwardia virginica (L.) Sm.	1.0	1.0	Klekowski 1969
Nephrolepis exaltata (L.) Schott	1.0	Not tested	Lloyd 1974

Note: The numbers presented are the fractions of gametophytes that formed sporophytes.

with time since disturbance could suggest that populations established or expanded through clonal rather than sexual reproduction, or that they established or expanded through sexual reproduction but not selfing. It is also possible that field conditions affecting gametophyte development and mating patterns vary with disturbance history, so that field populations could differ in effective inbreeding rates even though their gametophytes behaved similarly under common laboratory conditions.

This is, to our knowledge, the first quantitative study of sexual reproduction in *D. punctilobula*, so many questions remain unanswered. Though Conard (1908) described gametophytes of this species as "practically always dioecious" (p.35), quantitative observations of sexual development would be useful. In addition, *D. punctilobula* gametophytes were shown to respond to the antheridiogen produced by *P. aquilinum*, a hormone released by female gametophytes that induces maleness in neighboring gametophytes (Näf 1979). However, it is unknown whether *D. punctilobula* typically secretes and responds to its own antheridiogen, or to what extent this mechanism may facilitate outcrossing. Population genetic studies could quantify genetic diversity within *D. punctilobula* populations to elucidate the relative importance of selfing, outcrossing, and sexual reproduction in general, as opposed to vegetative spread. The data reported here suggest that population establishment and expansion via sexual reproduction may be relatively rare in this clonal species.

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References

Aikens, M.L., Ellum, D., McKenna, J.J., Kelty, M.J., and Ashton, M.S. 2007. The effects of disturbance intensity on temporal and spatial patterns of herb colonization in a southern New England mixed-oak forest. For. Ecol. Manage. **252**: 144–158. doi:10.1016/j.foreco.2007.06.031.

Baker, H.G. 1955. Self-compatibility and establishment after "long-distance" dispersal. Evolution, **9**: 347–349. doi:10.2307/2405656.

Burns, J.H., Ashman, T.-L., Steets, J.A., Harmon-Threatt, A., and Knight, T.M. 2011. A phylogenetically controlled analysis of the roles of reproductive traits in plant invasions. Oecologia, **166**: 1009–1017. doi:10.1007/s00442-011-1929-9. PMID: 21328010.

Chiou, W.-L., Farrar, D.R., and Ranker, T.A. 2002. The mating systems of some epiphytic Polypodiaceae. Am. Fern J. **92**: 65–79. doi:10.1640/0002-8444(2002)092[0065:TMSOSE]2.0.CO;2.

Conard, H.S. 1908. The structure and life-history of the hay-scented fern. Carnegie Institution of Washington, Washington, D.C.

Crist, K.C., and Farrar, D.R. 1983. Genetic load and long-distance dispersal in *Asplenium platyneuron*. Can. J. Bot. **61**(6): 1809–1814. doi:10.1139/b83-190.

Darwin, C. 1876. The effects of cross- and self-fertilization in the vegetable kingdom. John Murray, London, U.K.

de Groot, G.A., Verduyn, B., Wubs, E.R.J., Erkens, R.H.J., and During, H.J. 2012. Inter- and intraspecific variation in fern mating systems after long-distance colonization: the importance of selfing. BMC Plant Biol. **12**: 3. doi:10.1186/1471-2229-12-3. PMID:22217252.

de la Cretaz, A.L., and Kelty, M.J. 2002. Development of tree regeneration in fern-dominated forest understories after reduction of deer browsing. Restor. Ecol. **10**: 416–426. doi:10.1046/j.1526-100X.2002.02037.x.

Fei, S., and Steiner, K.C. 2008. Relationships between advance oak regeneration and biotic and abiotic factors. Tree Physiol. **28**: 1111–1119. doi:10.1093/treephys/28.7.1111. PMID:18450575.

Flinn, K.M. 2006. Reproductive biology of three fern species may contribute to differential colonization success in post-agricultural forests. Am. J. Bot. **93**: 1289–1294. doi:10.3732/ajb.93.9.1289. PMID:21642193.

Flora of North America. 2014. *Dennstaedtia punctilobula*. New York. [Online.] Available from http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=200003214 [accessed 13 June 2014].

George, L.O., and Bazzaz, F.A. 1999. The fern understory as an ecological filter: growth and survival of canopy-tree seedlings. Ecology, **80**: 846–856. doi:10.1890/0012-9658(1999)080[0846:TFUAAE]2.0.CO;2.

Groninger, J.W., and McCormick, L.H. 1992. Effects of soil disturbance on hay-scented fern establishment. North. J. Appl. For. **9**: 29–31.

Harmon-Threatt, A.N., Burns, J.H., Shemyakina, L.A., and Knight, T.M. 2009. Breeding system and pollination ecology of introduced plants compared to their native relatives. Am. J. Bot. **96**: 1544–1550. doi:10.3732/ajb.0800369. PMID:21628299.

Hill, J.D., and Silander, J.A., Jr. 2001. Distribution and dynamics of two ferns: *Dennstaedtia punctilobula* (Dennstaedtiaceae) and *Thelypteris noveboracensis* (Thelypteridaceae) in a northeast mixed hardwoods-hemlock forest. Am. J. Bot. **88**: 894–902. doi:10.2307/2657041. PMID:11353714.

Holbrook-Walker, S.G., and Lloyd, R.M. 1973. Reproductive biology and gametophyte morphology of the Hawaiian fern genus *Sadleria* (Blechnaceae) relative to habitat diversity and propensity for colonization. Bot. J. Linn. Soc. **67**: 157–174. doi:10.1111/j.1095-8339.1973.tb01736.x.

Horsley, S.B., and Marquis, D.A. 1983. Interference by weeds and deer with Allegheny hardwood reproduction. Can. J. For. Res. **13**(1): 61–69. doi:10.1139/x83-009.

Hughes, J.W., and Fahey, T.J. 1991. Colonization dynamics of herbs and shrubs in a disturbed northern hardwood forest. J. Ecol. **79**: 605–616. doi:10.2307/2260656.

Klekowski, E.J., Jr. 1969. Reproductive biology of the Pteridophyta. III. A study of the Blechnaceae. Bot. J. Linn. Soc. **62**: 361–377. doi:10.1111/j.1095-8339.1969.tb01973.x.

Klekowski, E.J., Jr. 1970. Populational and genetic studies of a homosporous fern–*Osmunda regalis*. Am. J. Bot. **57**: 1122–1138. doi:10.2307/2441278.

Klekowski, E.J., Jr. 1971. Evidence against genetic self-incompatibility in the homosporous fern *Pteridium aquilinum*. Evolution, **26**: 66–73. doi:10.2307/2406983.

Klekowski, E.J., Jr., and Lloyd, R.M. 1968. Reproductive biology of the Pteridophyta. I. General considerations and a study of *Onoclea sensibilis* L. Bot. J. Linn. Soc. **60**: 315–324.

Lloyd, R.M. 1974. Mating systems and genetic load in pioneer and non-pioneer Hawaiian Pteridophyta. Bot. J. Linn. Soc. **69**: 23–35. doi:10.1111/j.1095-8339.1974.tb01611.x.

Lloyd, R.M., and Gregg, T.L. 1975. Reproductive biology and gametophyte morphology of *Acrostichum danaeifolium* from Mexico. Am. Fern J. **65**: 105–120. doi:10.2307/1546343.

Lloyd, R.M., and Warne, T.R. 1978. The absence of genetic load in a morphologically variable sexual species, *Ceratopteris thalictroides* (Parkeriaceae). Syst. Bot. **3**: 20–36. doi:10.2307/2418530.

Lott, M.S., Volin, J.C., Pemberton, R.W., and Austin, D.F. 2003. The reproductive biology of the invasive ferns *Lygodium microphyllum* and *L. japonicum* (Schizaeaceae): implications for invasive potential. Am. J. Bot. **90**: 1144–1152. doi:10.3732/ajb.90.8.1144. PMID:21659214.

Lowry, R. 2014. VassarStats: website for statistical computation. [Online.] Available from http://vassarstats.net/abc.html [accessed 5 May 2014].

Marrs, R.H., and Watt, A.S. 2006. Biological flora of the British Isles: *Pteridium aquilinum* (L.) Kuhn. J. Ecol. **94**: 1272–1321. doi:10.1111/j.1365-2745.2006.01177.x.

Masuyama, S. 1979. Reproductive biology of the fern *Phegopteris decursive-pinnata*. I. The dissimilar mating systems of diploids and tetraploids. Bot. Mag. Tokyo, **92**: 275–289.

Näf, U. 1979. Antheridiogens and antheridial development. *In* The experimental biology of ferns. *Edited by* A.F. Dyer. Academic Press, London, U.K. pp. 435–470.

Pannell, J.R., and Barrett, S.C. 1998. Baker's law revisited: reproductive assurance in a metapopulation. Evolution, **52**: 657–668. doi:10.2307/2411261.

Peck, J.H., Peck, C.J., and Farrar, D.R. 1990. Influences of life history attributes on formation of local and distant fern populations. Am. Fern J. **80**: 126–142. doi:10.2307/1547200.

Penrod, K.A., and McCormick, L.H. 1996. Abundance of viable hay-scented fern spores germinated from hardwood forest soils at various distances from a source. Am. Fern J. **86**: 69–79. doi:10.2307/1547640.

R Core Team. 2014. R: a language and environment for statistical computing, Vienna, Austria. Available from http://www.r-project.org [accessed 25 August 2014].

Ranker, T.A., and Houston, H.A. 2002. Is gametophyte sexuality in the laboratory a good predictor of sexuality in nature? Am. Fern J. **92**: 112–118. doi:10.1640/0002-8444(2002)092[0112:IGSITL]2.0.CO;2.

Ranker, T.A., Gemmill, C.E.C., Trapp, P.G., Hambleton, A., and Ha, K. 1996. Population genetics and reproductive biology of lava-flow colonising species of Hawaiian *Sadleria* (Blechnaceae). *In* Pteridology in perspective. *Edited by* J.M. Camus, M. Gibby, and R.J. Johns. Royal Botanic Gardens, Kew, U.K. pp. 581–598.

Royo, A.A., and Carson, W.P. 2006. On the formation of dense understory layers in forests worldwide: consequences and implications for forest dynamics, biodiversity, and succession. Can. J. For. Res. **36**(6): 1345–1362. doi:10.1139/x06-025.

Royo, A.A., and Carson, W.P. 2008. Direct and indirect effects of a dense understory on tree seedling recruitment in temperate forests: habitat-mediated predation versus competition. Can. J. For. Res. **38**(6): 1634–1645. doi:10.1139/X07-247.

Rubin, G., and Paolillo, D.J., Jr. 1983. Sexual development of *Onoclea sensibilis* on agar and soil media without the addition of antheridiogen. Am. J. Bot. **70**: 811–815. doi:10.2307/2442930.

Rubin, G., Robson, D.S., and Paolillo, D.J., Jr. 1985. Effects of population density on sex expression in *Onoclea sensibilis* L. on agar and ashed soil. Ann. Bot. (Lond.), **55**: 205–215.

Schueller, S.K. 2004. Self-pollination in island and mainland populations of the introduced hummingbird-pollinated plant, *Nicotiana glauca* (Solanaceae). Am. J. Bot. **91**: 672–681. doi:10.3732/ajb.91.5.672. PMID:21653422.

Singh, V.P., and Roy, S.K. 1977. Mating systems and distribution in some tropical ferns. Ann. Bot. (Lond.), **41**: 1055–1060.

Srivastava, R., Srivastava, J., Behera, S.K., and Khare, P.B. 2008. In vitro studies on development of gametophyte, sex-ontogeny and reproductive biology of a threatened fern, *Microsorum punctatum* (L.) Copel. Ind. J. Biotechnol. **7**: 266–269.

Suter, M., Schneller, J.J., and Vogel, J.C. 2000. Investigations into the genetic variation, population structure, and breeding systems of the fern *Asplenium trichomanes* subsp. *quadrivalens*. Int. J. Plant Sci. **161**: 233–244. doi:10.1086/314258.

van Kleunen, M., and Johnson, S.D. 2007. Effects of self-compatibility on the distribution range of invasive European plants in North America. Conserv. Biol. **21**: 1537–1544. doi:10.1111/j.1523-1739.2007.00765.x. PMID:18173477.

van Kleunen, M., Manning, J.C., Pasqualetto, V., and Johnson, S.D. 2008. Phylogenetically independent associations between autonomous self-fertilization and plant invasiveness. Am. Nat. **171**: 195–201. doi:10.1086/525057. PMID: 18197772.

Vervoort, A., Cawoy, V., and Jacquemart, A.-L. 2011. Comparative reproductive biology in co-occurring invasive and native *Impatiens* species. Int. J. Plant Sci. **172**: 366–377. doi:10.1086/658152.

Wubs, E.R.J., de Groot, G.A., During, H.J., Vogel, J.C., Grundmann, M., Bremer, P., and Schneider, H. 2010. Mixed mating system in the fern *Asplenium scolopendrium*: implications for colonization potential. Ann. Bot. (Lond.), **106**: 585–590.