

LIFE-HISTORY DIFFERENTIATION AND THE MAINTENANCE OF MONOECY AND DIOECY IN *SAGITTARIA LATIFOLIA* (ALISMATACEAE)

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Abstract.—The existence of monoecious and dioecious populations within plant species is rare. This limits opportunities to investigate the ecological mechanisms responsible for the evolution and maintenance of these contrasting sexual systems. In *Sagittaria latifolia*, an aquatic flowering plant, monoecious and dioecious populations exist in close geographic proximity but occupy distinct wetland habitats differing in the relative importance of disturbance and competition, respectively. Life-history theory predicts contrasting evolutionary responses to these environmental conditions. We propose that the maintenance of monoecy and dioecy in *S. latifolia* is governed by ecological selection of divergent life-history strategies in contrasting habitats. Here we evaluate this hypothesis by comparing components of growth and reproduction between monoecious and dioecious populations under four conditions: natural populations, a uniform glasshouse environment, a common garden in which monoecious and dioecious populations and their F₁ progeny were compared, and a transplant experiment using shaded and unshaded plots in a freshwater marsh. Plants from dioecious populations were larger in size and produced heavier corms in comparison with monoecious populations. Monoecious populations flowered earlier and produced more flowers, clonal ramets, and corms than dioecious populations. The life-history differences between the sexual systems were shown to have a quantitative genetic basis, with F₁ progeny generally exhibiting intermediate trait values. Survival was highest for each sexual system in field plots that most closely resembled the habitats in which monoecious (unshaded) and dioecious (shaded) populations grow. These results demonstrate that monoecious and dioecious populations exhibit contrasting patterns of investment in traits involved with growth and reproduction. Selection for divergent life histories between monoecious and dioecious populations of *S. latifolia* appears to be the principal mechanism maintaining the integrity of the two sexual systems in areas of geographic overlap.

Key words.—Ecological selection, gender dimorphism, life-history strategies, local adaptation, sexual systems.

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All species possess traits that allow them to survive and reproduce in the habitats of their recent ancestors. These attributes jointly comprise the life-history strategy of an organism (Roff 1992; Stearns 1992). Life-history strategies evolve in response to variation in environmental conditions, including disturbance and competition and their effects on survival and fertility (Salisbury 1942; MacArthur and Wilson 1967; Levins 1968; Harper 1977; Grime 1979). For example, frequent disturbance is expected to result in an evolutionary response involving greater investment in traits determining numerical increase and reduced investment in those governing competition and survival (Bolker and Pacala 1999; Ronce et al. 2000; Crowley and McLetchie 2002). This may occur as a trade-off between investment in current growth and reproductive effort at the expense of investment in storage organs and future propagation (Reznick 1983; Silvertown et al. 2001), or as a trade-off between the number and size of propagules (Harper et al. 1970; Jakobsson and Eriksson 2000; Stuefer et al. 2002). Divergence in life-history traits through ecological selection is expected to result in occupation of different habitats and may therefore promote reproductive isolation and speciation (Schluter 2000).

Investigations of the evolution of life-history strategies in seed plants have largely focused on traits associated with growth, survival, and reproduction (Gadgil and Solbrig 1972; Law et al. 1977; Kittelson and Maron 2001). Less attention has been paid to the joint evolution of life-history strategies and reproductive systems (but see Zhang and Wang 1994; Morgan et al. 1997; Pannell 1997a). This is surprising given the diversity of reproductive systems in plants, the well-known associations between longevity, size, and mating pat-

terns (Stebbins 1957; Baker 1955; Maynard Smith 1978; Barrett et al. 1996), and the ecological correlates of dioecy (Renner and Ricklefs 1995; Sakai and Weller 1999). Because both life history and mode of reproduction influence response to variation in ecological and demographic conditions, reproductive success in a given habitat will necessarily depend on functional linkages between them.

The interaction between ecological factors and reproductive systems can help to explain the evolutionary maintenance of combined versus separate sexes. For example, if periodic disturbance leads to cycles of extinction and colonization, unisexual individuals of gender dimorphic species may be at a selective disadvantage compared to hermaphroditic, self-compatible individuals because these require only one individual to successfully colonize open habitats (Baker 1955; Pannell and Barrett 1998). However, in less disturbed but more competitive habitats, large unisexual individuals may benefit from enforced outbreeding and efficient pollen dispersal whenever hermaphrodites experience substantial geitonogamous selfing, inbreeding depression, and pollen discounting (Charlesworth and Charlesworth 1978; Harder and Barrett 1995; Eckert 2000; De Jong 2000; Reusch 2001).

We were interested in evaluating evidence for associations between sexual system and life-history traits in plants with combined versus separate sexes. A major obstacle for examining such associations within an experimental framework is the rarity of species possessing both hermaphroditic and dioecious populations. However, in *Sagittaria latifolia* (Alismataceae), both hermaphroditic (monoecious) and dioecious populations are commonly found, often in close geographic proximity (Wooten 1971; Sarkissian et al. 2001; Dor-

ken et al. 2002). In spite of their proximity, gene flow between monoecious and dioecious populations is limited, and the sexual systems of *S. latifolia* are largely reproductively isolated from one another (Dorken et al. 2002). Because the progeny of crosses between monoecious and dioecious populations of *S. latifolia* are fully fertile (see below), reproductive isolation between the sexual systems appears instead to be governed by divergent life histories. If F_1 progeny of crosses between sexual systems have intermediate values for life-history traits, as is usually the case for quantitative traits (Mazer and Lebuhn 1999), they may be poorly adapted to the habitats in which monoecious or dioecious populations grow, particularly when these differ.

In southern Ontario, Canada, where our study took place, monoecious and dioecious populations of *S. latifolia* occupy contrasting wetland habitats. Monoecious populations primarily colonize ephemeral aquatic environments such as roadside ditches, farm ponds, and stream and lake edges. These habitats are typically open and unshaded, with an inorganic substrate, and the monoecious populations within these habitats seem likely to be short-lived because of frequent disturbance (Sarkissian et al. 2001; Dorken et al. 2002; and see below). In contrast, dioecious populations commonly inhabit large, stable marshes associated with the southern Great Lakes and the major river systems that drain into them. Dioecious habitats are characterized by deep, organic substrates and are typically dominated by tall emergent aquatic macrophytes, particularly *Typha latifolia*. These habitats are highly productive, competitive environments with considerable shading from neighbors. Because differences in the frequency of disturbance and the intensity of competition are expected to favor different suites of life-history characteristics, we predicted that traits involved with growth and reproduction would differ between monoecious and dioecious populations of *S. latifolia*.

There are several life-history traits that influence the ability of *S. latifolia* to avoid disturbance and tolerate competition. Because monoecious populations are found in more disturbed habitats than dioecious populations, we predicted that plants from these populations would have greater investment in traits associated with colonization and numerical increase, including the production of flowers, clonal ramets, and corms. In contrast, we predicted that plants from dioecious populations should delay flowering relative to monoecious populations and be larger in size to avoid shading by neighbors. In *S. latifolia*, corm size is associated with emergence success (Garbisch and McIninch 1994) and is therefore likely to affect competitive ability. We therefore predicted larger corms in dioecious populations than monoecious populations. Although trait differences between monoecious and dioecious populations could arise from phenotypic plasticity, and indeed, *S. latifolia* is well known for displaying considerable phenotypic modification of both vegetative and reproductive traits in response to environmental conditions (Sculthorpe 1967; Sarkissian et al. 2001), we predicted that any differences revealed by our investigations would be more likely to result from genetic differentiation. Comparisons of monoecious and dioecious populations under uniform growing conditions enabled us to distinguish between these alternative hypotheses.

Here we compare the life-history strategies of monoecious and dioecious populations of *S. latifolia* by investigating components of growth and reproduction under contrasting environmental conditions. Because ecological differentiation is a ubiquitous feature of plant populations, regardless of their sexual system, we took special effort to sample a large number of populations over a large geographical area and to replicate our results under a variety of environmental conditions. Therefore, any associations between sexual system and life-history traits revealed by our comparisons would be unlikely to result from the effects of particular experimental conditions or limited population sampling. First, we quantified gender variation in 32 natural populations and examined whether two key traits, plant height and flower production, differed between monoecious and dioecious populations. Second, to validate whether observed phenotypic differences in the field reflect genetic differences, we grew 16 monoecious and 11 dioecious populations under uniform glasshouse conditions. We determined whether plant height and the production of ramets, flowers, and corms differed between the sexual systems. Third, we performed intra- and intersexual system crosses to generate F_1 progeny, and then grew these in a common garden. In this way, we assessed the life-history traits of F_1 progeny and examined the prediction that reproductive isolation between the sexual systems is promoted by intermediate trait values in intersexual system crosses for key components of the life history. Finally, we transplanted plants from nine monoecious and nine dioecious populations into the field using a split-plot experimental design in which plants were grown in either manipulated shaded or unshaded plots. We assessed differences in survival, plant height, ramet, and flower production between monoecious and dioecious populations and examined the interaction between sexual system and the shading treatment. We predicted that monoecious and dioecious populations would survive best in the unshaded and shaded plots, respectively, because these treatments most closely resemble the conditions of their natural habitats.

MATERIALS AND METHODS

Study Species

Sagittaria latifolia is a clonal aquatic that grows in a variety of wetland habitats throughout eastern North America that reproduces by both sexual and clonal reproduction. Regeneration by seed is an important means of propagation, especially in monoecious populations in which seedlings are commonly observed. Plants grow as a rosette of emergent leaves and propagate clonally via the production of ramets during the growing season and corms toward the end of the season. Ramets can be either vegetative or reproductive. Both clonal ramets and corms are produced at the terminal ends of axillary stolons. Corms are the sole perennating structures in this species; however, because of their relatively large size are less likely than seeds to be dispersed to other wetlands. Throughout this study, we define "plant" as a single ramet or shoot, excluding clonal ramets produced by the plant via stolons during the season. In southern Ontario, *S. latifolia* flowers between July and September, producing racemes with three unisexual flowers at each node. In monoecious popu-

lations, female flowers grow at basal nodes of the inflorescence with male flowers at upper nodes. The single-day flowers are visited by a variety of insect pollinators, including bees, flies, wasps and butterflies (Muenchow and Delesalle 1994; M. E. Dorken, pers. obs.).

Field Comparisons

To investigate the relation between gender variation and life-history traits we located 22 monoecious and 10 dioecious populations of *S. latifolia* along a 550-km transect across southern Ontario (see Appendix 1 for locality information). We sampled flowering ramets at a minimum distance of 2 m to avoid repeated sampling of genets, although in some populations this may have been unavoidable. There was no difference in the mean number of plants sampled between monoecious (mean \pm SE = 29.0 \pm 2.3) and dioecious populations (mean \pm SE = 29.4 \pm 3.4). For each ramet, we measured the length of the inflorescence between the proximal and distal nodes of the inflorescence, the number of flowers per inflorescence, and the length of the largest leaf subtending the inflorescence. Because leaf length was measured from the base of the petiole at substrate level to the tip, it represents a good surrogate of overall plant size (see Sarkissian et al. 2001).

We calculated standardized phenotypic gender for each reproductive shoot following Lloyd (1980): $G_i = d_i/(d_i + l_iE)$, where d_i and l_i are the numbers of female and male flowers per inflorescence for all plants in a population, respectively, and $E = \Sigma d_i/\Sigma l_i$ per population. To summarize variation in standardized phenotypic gender within and among populations, we used an index of standardized phenotypic gender that describes the departure from equal allocation to female and male flower production relative to the other plants in a population:

$$2 \times |G_i - 0.5|. \quad (1)$$

Therefore, plants that are phenotypically female or male receive a score of 1, and hermaphroditic plants receive a value greater than or equal to 0 and less than 1.

We evaluated differences in growth and reproduction between monoecious and dioecious populations using nested analysis of variance (ANOVA). Sexual system was treated as a fixed effect, with population nested within sexual system as a random effect. Tests of significance for random effects in this study were evaluated using log-likelihood ratio tests (Sokal and Rohlf 1995). Where appropriate, we transformed data to meet the assumptions of ANOVA. These transformations, and those performed for the analyses described below, had no qualitative effect on the outcome of any analysis presented in this study. However, where applicable, we report the results of analyses of transformed data. We used a square-root transformation for inflorescence length, and a log transformation for flower production per inflorescence. Differences between gender classes (i.e., hermaphrodites and phenotypic males in monoecious populations and females and males in dioecious populations) were evaluated by including gender, nested within sexual system as a factor in our analyses. Gender was included as a nested effect because previous research on size-dependent sex expression in *S. latifolia* in-

dicates that phenotypic males (110 of 638 plants) in monoecious populations are not functionally equivalent to males in dioecious populations (Sarkissian et al. 2001). In addition, females and males in dioecious populations of *S. latifolia* bear unequal costs of reproduction (Sarkissian et al. 2001), indicating that allocation to life-history traits is likely to vary between the sexes in these populations as well. Because we simultaneously examined multiple response variables, we held experimentwise type I error rates constant at 5% using the sequential Bonferroni method (Sokal and Rohlf 1995) for all comparisons.

To investigate ecological parameters relevant to competition and disturbance, we characterized five habitat variables in a subset of the populations investigated in this study (listed in Appendix 1). At each site, we randomly selected five focal plants and measured the height of the nearest plant growing within one meter and if the common emergent aquatics *Typha latifolia* or *T. angustifolia* were present. We also determined whether focal plants were rooted in productive organic soils versus sand, using methods described by Lee et al. (1998). Finally, to evaluate the degree of disturbance at each site we recorded whether ramets of *S. latifolia* emerged from flowing water and were therefore subject to flooding and erosion, and whether populations were locally extirpated between 1998, when they were first sampled, and the 2000 growing season. We evaluated whether differences in these ecological parameters were evident between monoecious and dioecious populations using ANOVA and chi-square tests.

Glasshouse Experiment

To compare life-history traits between monoecious and dioecious populations, we grew plants under uniform glasshouse conditions during 1999. We sampled a total of 199 plants from 16 monoecious populations and 175 plants from 11 dioecious populations from southern Ontario and western Pennsylvania. We followed the same sampling procedure outlined above to reduce the likelihood of repeated sampling of genets. All plants were sampled in May as small emergent shoots and were transferred to four-inch pots and placed into three randomized blocks, with glasshouse benches serving as blocks. To take potential carryover effects into account, we measured the fresh weight and height of all shoots before the start of the experiment. Plants were grown in trays to maintain saturated soil. Trays were monitored daily to ensure adequate water levels. Fertilizer was added to trays regularly using 250 ml of a 1% 20:20:20 N:P:K nutrient fertilizer solution.

We measured plant height and the number of leaves and clonal ramets produced by the focal plant every two weeks throughout the growing season. Height was measured from the substrate surface to the tip of the tallest leaf and we recorded the date of first flowering for each plant. The number of inflorescences and female and male flowers were counted for all ramets in each pot. Because the glasshouse was maintained pollinator-free, plants produced no seed. The experiment was continued until all plants had senesced (mid-September 1999), at which time all corms in each pot were washed, counted, and weighed to determine their fresh weight. The senescence of ramets corresponded to the natural timing of this event under field conditions.

As in the field comparisons, we evaluated differences in growth and reproduction between monoecious and dioecious populations using nested ANOVA. We also evaluated block effects, tray effects, and interactions between block and sexual system and population nested within sexual system. We considered variation among trays a random effect in our analyses. All plants from monoecious populations produced a combination of female and male flowers. Therefore, our analyses of gender effects in this experiment include only differences between females and males from dioecious populations. There was a difference in initial shoot height between monoecious and dioecious populations (mean \pm SE for monoecious populations = 14.7 ± 1.0 cm, dioecious populations = 19.4 ± 1.2 cm; ANOVA of population means: $F_{1,25} = 9.5$, $P < 0.05$). We controlled for this difference using initial shoot height as the covariate in the analyses presented below. However, for the analyses of average corm mass and the number of corms produced per genet, we used total corm mass as the covariate to control for variation among plants in total corm mass. To meet ANOVA assumptions, we used square-root transformations on the following variables: the number of clonal ramets per plant, flowers per genet, corms per genet, and total and average fresh weight of corms. To avoid variation in corm mass that might arise due to differential drying among samples, we collected and weighed corms directly following excavation from uniformly saturated soils. We also investigated correlations among all traits using principal components analysis to determine whether groups of traits differed between monoecious and dioecious populations. Finally, we examined the potential for a trade-off between corm size and number, holding total corm mass constant using partial correlation of population means.

Crossing Experiment

To confirm that life-history differences between the sexual systems have a genetic basis, we conducted a series of crosses within and between monoecious and dioecious populations in August 2000 in a pollinator-free glasshouse. All plants used in crosses had been grown under uniform glasshouse conditions in 1999 (see above). We used plants from six monoecious and six dioecious populations as maternal parents, and plants from each of five of these monoecious and dioecious populations as paternal parents. Crosses performed within sexual systems included both within and between population crosses. However, there was no significant difference between these crosses, or interactions between this effect and the main effects described below. Accordingly, these crosses are pooled in the analyses described below. In May 2001, we grew a total of 464 plants of which 116 were from crosses among plants from monoecious populations ($M \times M$), and 116 plants were from crosses among plants from dioecious populations ($D \times D$). The remaining 232 plants were from crosses between the sexual systems with equivalent numbers representing $M \times D$ and $D \times M$ crosses. Seeds from crosses were stored dry at 5°C and prior to germination they were placed for three weeks at 5°C in vials containing distilled water. The hydrated seeds were then placed on the surface of saturated, sandy soil and germination occurred approximately three weeks later. All seedlings were transplanted to

four-inch pots that were individually placed in five-liter buckets and arranged into four randomized blocks outside in a common garden at the University of Toronto. Once per week, we added 200 ml of a 1% 20:20:20 nutrient fertilizer solution to each bucket.

After plants ceased flowering at the end of August, we measured the height of the tallest ramet per genet, and counted the number of ramets and flowers produced per genet. We then allowed the plants to senesce naturally and counted the number of corms per genet and calculated the total and average fresh weights of the corms produced. For each of these variables, we evaluated overall differences between the four cross types, the effect of maternal population nested within cross type, block effects, and the interaction between cross type and block effects using nested ANOVA. We treated the effect of maternal population as random, and evaluated all other components of the model as fixed effects. To meet ANOVA assumptions, we square-root transformed the number of corms per genet, average and total corm mass, and log transformed the number of flowers per genet. We used least squares contrasts to test for significant differences in life-history traits between $M \times M$ versus $D \times D$ crosses and also to examine the potential for maternal effects by comparing $M \times D$ versus $D \times M$ crosses. As in the previous experiment, for evaluating differences in corm production and average corm mass we used total corm mass as the covariate to control for variation among plants in this variable.

Field Transplant Experiment

To examine interactions between habitat conditions, sexual system and life-history traits in *S. latifolia* we grew plants from nine monoecious and nine dioecious populations under contrasting shade treatments in the field. We subjected transplants to unshaded and shaded conditions to simulate the growth conditions typically found in monoecious versus dioecious populations, respectively. This allowed us to test the prediction that performance for each sexual system would be highest in the plots that most closely resemble the types of habitats in which monoecious and dioecious populations exist.

In May 1999, we collected emerging shoots from natural populations and measured each plant's height and fresh weight. We then transferred them into four-inch pots and placed them in a glasshouse. In June, these plants were transplanted into a marsh at the University of Toronto field station at Jokers Hill, near Newmarket, Ontario, at which *S. latifolia* was absent prior to the beginning of the experiment. The marsh is characterized by an exposed, saturated organic substrate dominated by dense stands of *Typha latifolia* in the interior portions of the marsh. At the margins of the marsh, *T. latifolia* is absent, the vegetation is of low stature, and there are areas of exposed organic substrate. We transplanted 36 plants (two from each monoecious and dioecious population) into each of 12 plots using a split-plot experimental design. Half of the plots were placed in areas dominated by *T. latifolia*. In these plots, the vegetation was left intact. We placed the remaining plots in adjacent areas where *T. latifolia* was absent and trimmed all vegetation to within 5 cm of the surface of the substrate, including a one-meter border to pre-

TABLE 1. Habitat differences between monoecious and dioecious populations of *Sagittaria latifolia* measured from a subsample of the populations used in this study. The values shown are the average height of neighboring plants (\pm SE) from 15 monoecious and 11 dioecious populations, the number of populations in which *S. latifolia* plants emerge from flowing water, the number of sites at which *Typha* spp. were present, the number of sites at which *S. latifolia* plants were rooted in primarily organic substrates, and whether populations were locally extirpated between 1998, when they were first sampled, and 2000, when the data included in this table were collected. For qualitative comparisons, we indicate the sample size in parentheses. Values with different letter superscripts are significantly different, based on one-way analysis of variance of population means for quantitative data, and chi-square tests for qualitative data.

Sexual system	Neighbor height (cm)	Flowing water	<i>Typha</i> spp.	Organic substrate	Extirpated
Monoecious	56.7 (\pm 10.9) ^b	12 (15) ^a	7 (15) ^b	2 (15) ^b	6 (19) ^a
Dioecious	142.4 (\pm 10.9) ^a	1 (11) ^b	11 (11) ^a	9 (11) ^a	0 (13) ^b

vent edge effects. For the remainder of the growing season, these plots were trimmed weekly to ensure that *S. latifolia* was not shaded by surrounding vegetation. Within each plot, we spaced plants at a distance of 60 cm from one another in a 480 \times 300-cm grid.

We sampled plants on five occasions during the growing season. On each visit, we measured the height of the plant from the substrate surface to the tip of the tallest leaf, the number of leaves, the number of clonal ramets produced, and the length of the stolons connecting plants to their daughter ramets for each focal plant in the experiment. We identified each ramet's clonal parent by uncovering the stolon connecting the two plants. We marked the most recently unfurled leaf on each focal plant to allow us to count the number of leaves produced between censuses. Because these marks were occasionally lost to herbivory, to maintain sample sizes we used the number of new leaves produced during the three-week period 19 July to 11 August as the measure of leaf turnover. In addition, the site was visited regularly following the onset of flowering to record the date of initiation of flowering per focal plant.

We evaluated differences between monoecious and dioecious populations, and interactions between sexual system and the shading treatment using ANOVA for a split-plot experimental design (Neter et al. 1996). As in the previous analyses, we included population nested within sexual system as a random effect. We used ramet height at the start of the experiment as the covariate in our analyses. We conducted analyses of continuous variables using restricted maximum likelihood procedures in JMP (ver. 4.0.4; SAS Institute 2002) and analyses of categorical variables (survival and flowering

in the field transplant experiment) by fitting linear models to a logistic response function using the maximum likelihood routine in JMP. We tested for significance in these analyses using log-likelihood-ratio tests. Finally, to evaluate evidence for genetically based differences in life history among the 18 populations in this experiment, we compared trait values to those obtained in the glasshouse using bivariate regression.

RESULTS

Field Comparisons

The habitats occupied by populations of *S. latifolia* with contrasting sexual systems differed significantly with respect to the ecological variables examined (Table 1). Plants in dioecious populations had significantly taller neighbors and were more likely to be found in sites occupied by *Typha* spp. than plants in monoecious populations. Plants in dioecious populations were also more likely to be rooted in deep organic substrates than plants in monoecious populations, which were usually growing in sand, silt, or rocky substrates. Monoecious populations were more frequently found in habitats with flowing water subject to natural or human disturbance. Accordingly, we recorded a significantly higher rate of local extirpation of monoecious populations compared to dioecious populations. All dioecious populations persisted over the two-year observation period whereas one third of the monoecious populations had been destroyed and were absent from the site.

We detected substantial variation in gender among populations of *S. latifolia*; this largely reflects the existence of monoecious and dioecious populations (Fig. 1). However, the

TABLE 2. Nested analysis of variance for plant height, inflorescence length, and the number of flowers per inflorescence measured in 22 monoecious and 10 dioecious populations of *Sagittaria latifolia*. Population nested within sexual system was tested using likelihood-ratio tests (see Materials and Methods). Sexual system means are the least-square means for each variable, back-transformed where applicable (\pm SE; standard errors for back-transformed means were calculated by adding and subtracting the standard errors to the means before backtransforming, and are therefore not equal to one another).

	Plant height	Inflorescence length ¹	Flowers/inflorescence ²
Sexual system	$F_{1,30} = 8.3^{**}$	$F_{1,30} = 31.0^{***}$	$F_{1,30} = 0.2$
Population [sexual system]	$\chi^2 = 594.7^{***}$	$\chi^2 = 351.5^{***}$	$\chi^2 = 224.3^{***}$
Gender [sexual system]	$F_{3,775} = 13.3^{***}$	$F_{3,852} = 23.2^{***}$	$F_{3,897} = 11.9^{***}$
Sexual system means			
Monoecious	41.9 (\pm 3.8)	6.3 (+0.8, -0.8)	6.8 (+0.7, -0.6)
Dioecious	60.5 (\pm 5.2)	15.2 (+1.6, -1.5)	7.2 (+0.8, -0.7)

** Significant, Dunn-Sidak; *** $P < 0.001$.

¹ Square-root transformed.

² Log-transformed.

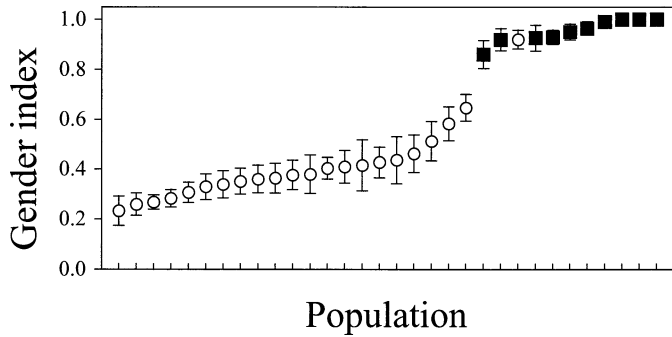


FIG. 1. Variation in mean standardized gender among 22 monoecious (open circles) and 10 dioecious (closed squares) populations of *Sagittaria latifolia* measured in natural populations. The index of phenotypic gender is the population's mean (\pm SE) deviation from equal allocation to male and female sex function (see Materials and Methods).

presence of hermaphroditic and male ramets (hereafter phenotypic males) also contributes to gender variation in monoecious populations. As predicted, gender variation was associated with significant differences in plant size between monoecious and dioecious populations. These size differences were reflected in terms of both plant height and inflorescence length (Table 2). Plants in dioecious populations were on average 47% taller than plants in monoecious populations (Table 2). Inflorescences were more than twice the length in dioecious compared to monoecious populations (Table 2). However, there was no difference in the number of flowers per inflorescence between the two sexual systems (Table 2).

Glasshouse Experiment

As in natural populations, we detected significant differences in plant height between monoecious and dioecious populations. On average, plants from dioecious populations were 24% taller than plants from monoecious populations (Table 3, Fig. 2a). Plants from monoecious populations produced 17% more leaves (Fig. 2b) and produced those leaves at a 10% faster rate (Fig. 2c) than plants from dioecious populations. Contrary to our

expectations there were no significant differences in the production of clonal ramets, the timing of the onset of flowering, or the number of flowers produced per genet between monoecious and dioecious populations (Fig. 2d-f).

We predicted different patterns of investment in perennating structures in monoecious versus dioecious populations of *S. latifolia*. The results from our glasshouse comparisons supported these predictions. There was no overall difference in total corm mass between monoecious and dioecious populations (Fig. 2g); however, the two sexual systems had divergent patterns of investment in the size versus the number of corms produced. Average corm mass at the end of the growing season was 47% greater among plants from dioecious compared to monoecious populations (Fig. 2h). In contrast, plants from monoecious populations produced 41% more corms than plants from dioecious populations (Fig. 2i).

Principal components analysis revealed that traits related to size versus propagation differed between monoecious and dioecious populations (Fig. 3). Traits involved in propagation had, for the most part, contrasting patterns of association with the first axis compared to features of plant size. With the exception of the number of flowers produced, the number of clones and corms, total number of leaves, and the number of new leaves produced per focal plant per day all had negative eigenvectors for the first axis (0.08, -0.36, -0.23, -0.25, -0.28, respectively). In contrast, plant height, average corm mass, and total corm mass had positive eigenvectors (0.51, 0.53, 0.27, respectively). Scores along the first axis, which explained 29% of total variation, differed significantly between dioecious (mean \pm SE = 0.71 \pm 0.30; one-way ANOVA of population means: $F_{1,25} = 19.3$, $P = 0.0002$) and monoecious populations (mean = -0.98 \pm 0.25 SE). Further evidence for size-number trade-offs was reflected in the negative partial correlation between corm size and number ($r = -0.91$, $n = 27$, $P < 0.0001$; Fig. 4).

Crossing Experiment

Comparisons of F_1 crosses within and between sexual systems in *S. latifolia* confirm the genetic basis for life-history trait differentiation between monoecious and dioecious popu-

TABLE 3. Analysis of covariance for components of growth and reproduction for 16 monoecious and 11 dioecious populations of *Sagittaria latifolia* grown under uniform glasshouse conditions. For each focal plant, we analyzed the maximum plant height, maximum number of leaves, average number of new leaves produced per day, total number of clones produced, and number of days elapsed before flowering. Analyses of the total number of flowers produced, total corm mass, number of corms produced, and average corm mass include values from each focal plant plus all daughter ramets. Random effects (tray nested within block, and population nested within sexual system) were tested using likelihood-ratio tests (see Materials and Methods). Initial plant height and the square root of total corm mass per genet were used as covariates. Means are in Figure 2.

	Plant height	Leaves/plant	New leaves/plant/day	Clones/plant ¹
Block	$F_{2,81} = 4.2^*$	$F_{2,81} = 2.2$	$F_{2,81} = 7.2^{**}$	$F_{2,81} = 1.5$
Tray [block]	$\chi^2 = 19.4^{***}$	$\chi^2 = 0.0$	$\chi^2 = 51.0^{***}$	$\chi^2 = 18.4^{***}$
Sexual system	$F_{1,25} = 8.1^{**}$	$F_{1,25} = 16.7^{***}$	$F_{1,25} = 7.8^{**}$	$F_{1,25} = 4.9^*$
Population [sexual system]	$\chi^2 = 121.1^{***}$	$\chi^2 = 39.3^{***}$	$\chi^2 = 20.5^{***}$	$\chi^2 = 31.6^{***}$
Gender [sexual system]	$F_{1,243} = 1.2$	$F_{1,243} = 0.0$	$F_{1,243} = 0.2$	$F_{1,243} = 1.2$
Block \times sexual system	$F_{2,243} = 1.1$	$F_{2,243} = 2.4$	$F_{2,243} = 0.5$	$F_{2,243} = 2.4$
Initial plant height	$F_{1,179} = 15.7^{***}$	$F_{1,191} = 19.1^{***}$	$F_{1,243} = 0.5$	$F_{1,243} = 11.5^{***}$
Total corm mass ¹	—	—	—	—

* $P < 0.05$ (marginal); ** significant, Dunn-Sidak; *** $P < 0.001$.

¹ Square-root transformed.

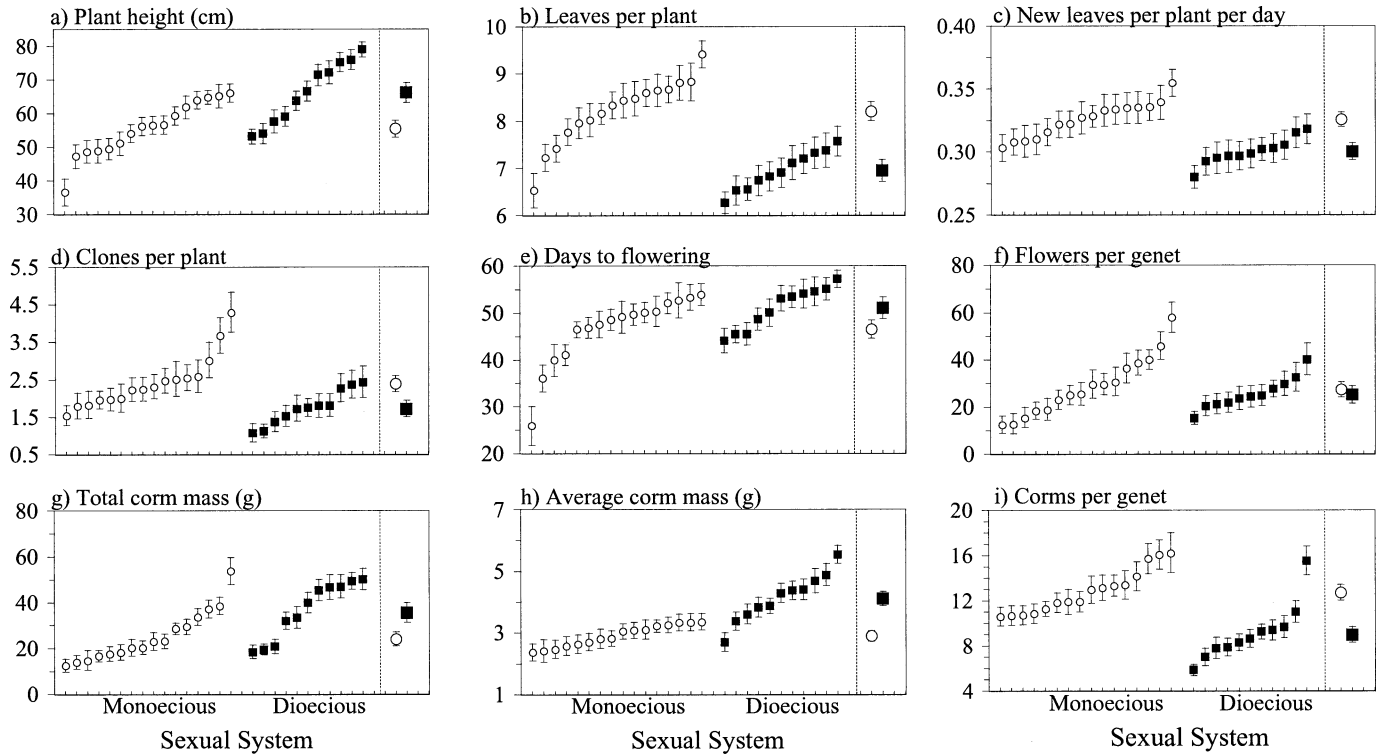


FIG. 2. Variation in components of growth and reproduction among 16 monoecious (open circles) and 11 dioecious (closed squares) populations of *Sagittaria latifolia* grown under common glasshouse conditions. Values shown are least-square means (\pm SE) for plant height (a), the number of leaves per plant (b), the number of new leaves produced per plant per day (c), the number of clones produced per plant (d), the number of days elapsed before flowering (e), the number of flowers produced per genet (f), total corm mass (g), average corm mass (h), and the number of corms produced per genet (i). Populations are grouped by sexual system and in ascending order of their values for each trait. Sexual system means (\pm SE) are shown at the right of each panel.

ulations. Trait values for the two intersexual system crosses were generally intermediate between the two intrasexual system crosses, consistent with quantitative inheritance. Cross type had a significant effect on all components of growth and reproduction in the crossing experiment (Fig. 5). Differences among cross types resulted primarily from crosses performed within sexual systems (i.e., M \times M vs. D \times D). We detected significant differences between these crosses for all components of growth and reproduction (Fig. 5). Plants from dioecious populations were 43% taller than plants from monoecious populations (Fig. 5a) and produced 54% larger corms (Fig. 5b), results entirely consistent with our findings from field and glasshouse comparisons. Plants from monoecious

populations produced more than twice as many corms (Fig. 5c) and flowers (Fig. 5d) compared to plants from dioecious populations. In contrast to the results of the glasshouse experiment, we found significant differences between the sexual systems for ramet production and total corm mass. Plants from crosses among dioecious populations produced 69% more total corm mass than plants from monoecious crosses (Fig. 5e), and plants from monoecious crosses produced 40% more ramets than plants from dioecious crosses (Fig. 5f). With the exception of total corm mass, there were no significant differences between the reciprocal intersexual system crosses. We found that plants from M \times D crosses had 68% more total corm mass than plants from D \times M crosses.

TABLE 3. Extended.

Days to flowering	Flowers/genet ¹	Total corm mass ¹	Average corm mass ¹	Corms/genet ¹
$F_{2,81} = 4.6^*$	$F_{2,81} = 2.6$	$F_{2,81} = 2.8$	$F_{2,81} = 0.3$	$F_{2,81} = 0.4$
$\chi^2 = 11.4^{***}$	$\chi^2 = 0.9$	$\chi^2 = 14.9^{***}$	$\chi^2 = 1.4$	$\chi^2 = 0.9$
$F_{1,25} = 2.4$	$F_{1,25} = 0.2$	$F_{1,25} = 5.1^*$	$F_{1,25} = 18.1^{***}$	$F_{1,25} = 13.7^{**}$
$\chi^2 = 48.2^{***}$	$\chi^2 = 25.7^{***}$	$\chi^2 = 35.7^{***}$	$\chi^2 = 40.7^{***}$	$\chi^2 = 57.5^{***}$
$F_{1,201} = 15.3^{***}$	$F_{1,243} = 36.6^{***}$	$F_{1,238} = 0.4$	$F_{1,238} = 0.4$	$F_{1,238} = 0.0$
$F_{2,201} = 2.0$	$F_{2,243} = 0.6$	$F_{2,238} = 0.1$	$F_{2,238} = 4.4^*$	$F_{2,238} = 3.9^*$
$F_{1,201} = 11.8^{***}$	$F_{1,243} = 23.8^{***}$	$F_{1,238} = 34.9^{***}$	—	—
—	—	—	$F_{1,238} = 108.7^{***}$	$F_{1,238} = 291.2^{***}$

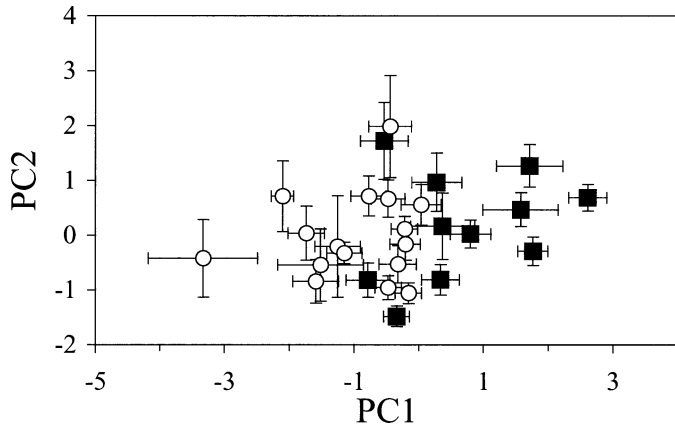


FIG. 3. Bivariate plot of the first two principal components from an analysis of variation in life-history traits among monoecious (open circles) and dioecious (closed squares) populations of *Sagittaria latifolia*. Population means (\pm SE) are shown. PC1 and PC2 explain 29% and 23% of the variation, respectively.

Field Transplant Experiment

In the field experiment, we detected significant differences between monoecious and dioecious populations for all components of growth and reproduction (Table 4). These differences were consistent with our predictions of greater investment in size versus numerical increase in dioecious compared to monoecious populations. Averaging across the shading treatments, plants from dioecious populations were 36% taller (Fig. 6a) compared to plants from monoecious populations. In addition, stolon length among plants from dioecious populations was, on average, 28% longer than plants from monoecious populations (Fig. 6b). Although plants from monoecious populations were shorter, they produced 12% more leaves (Fig. 6c) at a rate 21% higher than plants from

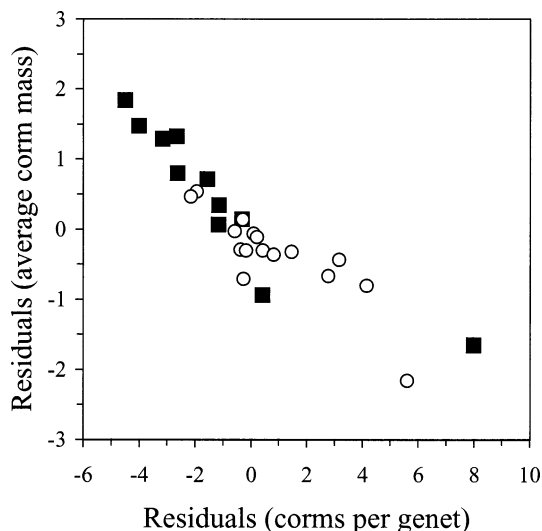


FIG. 4. Relation between the number of corms produced per genet and average corm mass for 16 monoecious (open circles) and 11 dioecious populations (closed squares) of *Sagittaria latifolia* grown under common glasshouse conditions, holding variation among plants in total corm mass per genet constant using partial correlation.

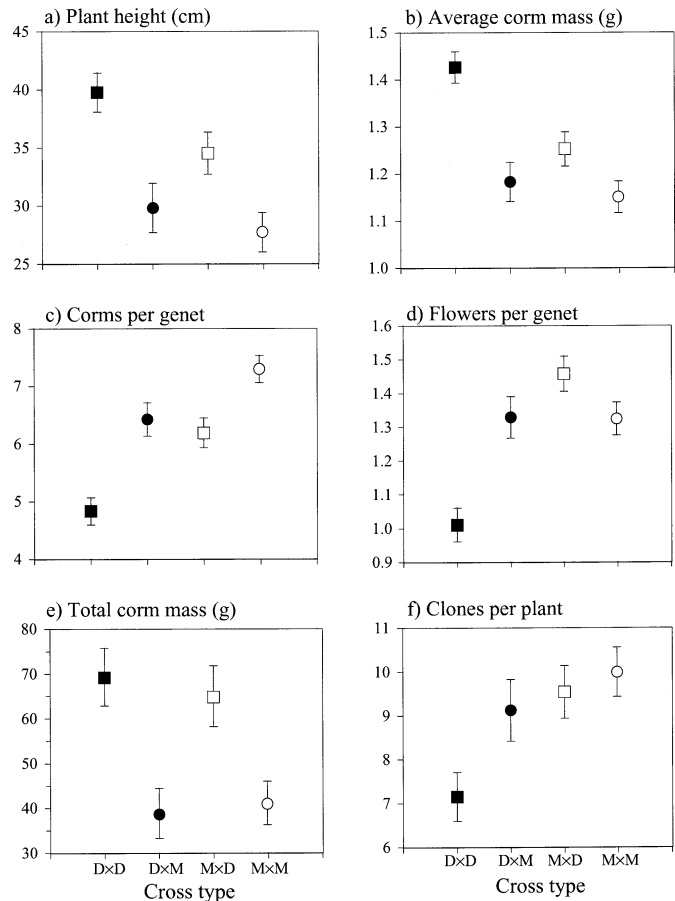


FIG. 5. Variation in components of growth and reproduction for *Sagittaria latifolia* plants derived from four types of crosses: plants from dioecious populations used as maternal parents in crosses with dioecious (D \times D) and monoecious populations (D \times M), and plants from monoecious populations used as maternal parents in crosses with dioecious (M \times D) and monoecious populations (M \times M). Values shown are means for each cross type (\pm SE) for plant height (a), average corm mass (b), the number of corms produced per genet (c), the number of flowers produced per genet (d), total corm mass (e), and the number of clonal ramets produced per genet (f).

dioecious populations, a finding similar to the results of our glasshouse experiment. Monoecious populations had greater investment in traits involved with numerical increase, producing more than twice as many clonal ramets (Fig. 6d), and more than 2.5 times as many flowers per plant (Fig. 6e). Moreover, monoecious populations flowered on average 17 days earlier than individuals from dioecious populations (Fig. 6f), and 73% more plants in the experiment flowered in total (the proportion of plants from dioecious populations that flowered was 0.38 ± 0.06 SE compared to 0.66 ± 0.05 SE in monoecious populations; Table 5).

Averaging across the shading treatments, survival did not differ between monoecious and dioecious populations. However, we detected a significant interaction between sexual system and the shading treatment for the proportion of individuals that survived to the end of the experiment (Table 5). In the shaded plots, survival was greater among dioecious populations compared to monoecious populations. This interaction was in the direction predicted based on differences

TABLE 4. Analysis of covariance for components of growth and reproduction for nine monoecious and nine dioecious populations of *Sagittaria latifolia* transplanted into two shade treatments in the field. Variables analyzed are maximum plant height, the maximum number of leaves, leaf turnover rate, total number of clonal ramets, average stolon length, number of days elapsed before flowering, and the total number of flowers produced for each focal plant. The random effects population, treatment by population, and block by population were tested using likelihood-ratio tests (see Materials and Methods). Means are in Figure 5.

	Plant height	Leaves/plant	Leaf turnover	Clones/plant	Stolon length	Days to flowering	Flowers/plant
Treatment	$F_{1,16} = 0.8$	$F_{1,16} = 55.5^{***}$	$F_{1,16} = 193.4^{***}$	$F_{1,16} = 297.3^{***}$	$F_{1,258} = 7.7^{**}$	$F_{1,178} = 1.4$	$F_{1,16} = 110.4^{***}$
Subplot [treatment]	$F_{10,160} = 2.6^{**}$	$F_{10,160} = 1.3$	$F_{10,319} = 4.0^{***}$	$F_{10,160} = 1.7$	$F_{10,258} = 0.3$	$F_{10,178} = 1.5$	$F_{10,370} = 3.8^{***}$
Sexual system	$F_{1,16} = 22.1^{***}$	$F_{1,16} = 11.6^{**}$	$F_{1,16} = 20.4^{***}$	$F_{1,16} = 35.2^{***}$	$F_{1,16} = 13.8^{**}$	$F_{1,16} = 23.3^{***}$	$F_{1,16} = 24.7^{***}$
Population [sexual system]	$\chi^2 = 2.6$	$\chi^2 = 4.7^*$	$\chi^2 = 0.5$	$\chi^2 = 1.4$	$\chi^2 = 10.1^{***}$	$\chi^2 = 22.1^{***}$	$\chi^2 = 6.5^{**}$
Treatment \times sexual system	$F_{1,16} = 3.1$	$F_{1,16} = 2.8$	$F_{1,16} = 1.1$	$F_{1,16} = 40.0^{***}$	$F_{1,258} = 16.9^{***}$	$F_{1,178} = 0.1$	$F_{1,16} = 9.0^*$
Treatment \times population	$\chi^2 = 5.8^{**}$	$\chi^2 = 6.3^{**}$	$\chi^2 = 0.3$	$\chi^2 = 0.0$	—	—	$\chi^2 = 0.0$
Sexual system \times subplot	$F_{10,160} = 1.3$	$F_{10,160} = 0.6$	$F_{10,319} = 0.8$	$F_{10,160} = 2.6^{**}$	—	$F_{10,178} = 1.0$	$F_{10,370} = 1.4$
Subplot [treatment] \times population	$\chi^2 = 75.9^{***}$	$\chi^2 = 6.3^{**}$	—	$\chi^2 = 0.0$	—	—	—
[sexual system]	$F_{1,210} = 18.8^{***}$	$F_{1,210} = 1.5$	$F_{1,319} = 0.1$	$F_{1,210} = 1.1$	$F_{1,258} = 5.9^*$	$F_{1,178} = 2.4$	$F_{1,370} = 4.7^*$
Initial plant height							

* $P < 0.05$ (marginal); ** significant, Dunn-Sidak; *** $P < 0.001$.

in habitat occupancy of dioecious and monoecious populations. Survival was 14% higher for plants from monoecious (0.86 ± 0.04) compared to dioecious populations in the unshaded plots (0.74 ± 0.06). This pattern was reversed for the shaded plots, in which survival was 8% higher for plants from dioecious (0.85 ± 0.04) compared to monoecious populations (0.79 ± 0.05).

Clonal ramet production of monoecious and dioecious populations also depended on the shading treatment, with a greater reduction in the shaded compared to the unshaded plots in monoecious populations. The shading treatment therefore reduced the performance of monoecious populations to a greater extent than dioecious populations. On average, plants from monoecious populations produced six fewer ramets in the shaded plots, compared to less than three in dioecious populations (Fig. 6d). We also detected a significant sexual system-by-shading treatment interaction for the length of stolons between focal plants and their clonal ramets. Stolons were 22% longer in shaded compared to unshaded plots in dioecious populations. In contrast, stolons were 6% shorter in shaded compared to unshaded plots in monoecious populations.

The shading treatment had a significant effect on five components of growth and reproduction, independently of its effect on sexual system. These included the number of leaves per plant, the leaf turnover rate, the number of flowers and inflorescences per ramet, and the proportion of plants flowering (Table 4). In each case, the values obtained were lower in the shaded compared to the unshaded plots.

Comparison of Populations in the Field versus Glasshouse

We detected significant correlations between life-history traits among the same populations measured in the field trans-plant and glasshouse experiments (plant height: $r = 0.74$, $n = 18$, $P < 0.001$, Fig. 7a; ramet production: $r = 0.50$, $n = 18$, $P < 0.05$, Fig. 7b; days to flowering: $r = 0.30$, $n = 18$, $P < 0.05$, Fig. 7c; flower production: $r = 0.50$, $n = 18$, $P < 0.05$, Fig. 7d). In general, these correlations reflected the overall differences in life-history traits between the sexual systems. However, within sexual systems, these correlations were often significant, particularly for dioecious populations. Among dioecious populations we found significant correlations between our measures of plant height, ($r = 0.79$, $n = 9$, $P < 0.05$, Fig. 7a), ramet production ($r = 0.79$, $n = 9$, $P < 0.05$, Fig. 7b) and the number of days to flowering ($r = 0.69$, $n = 9$, $P < 0.05$, Fig. 7c). In contrast, among monoecious populations, only the correlation between flower production was significant in the field and glasshouse ($r = 0.69$, $n = 9$, $P < 0.05$, Fig. 7d).

DISCUSSION

The role of ecology as a driving force in the evolution and maintenance of reproductive systems is widely recognized, yet there have been few experimental investigations of the relations between ecological differentiation and intraspecific variation in reproductive systems in plants (Costich 1995; Case and Barrett 2001; Fausto et al. 2001; O'Connell and Eckert 2001). Instead, much of our knowledge of ecological associations with reproductive systems comes from compar-

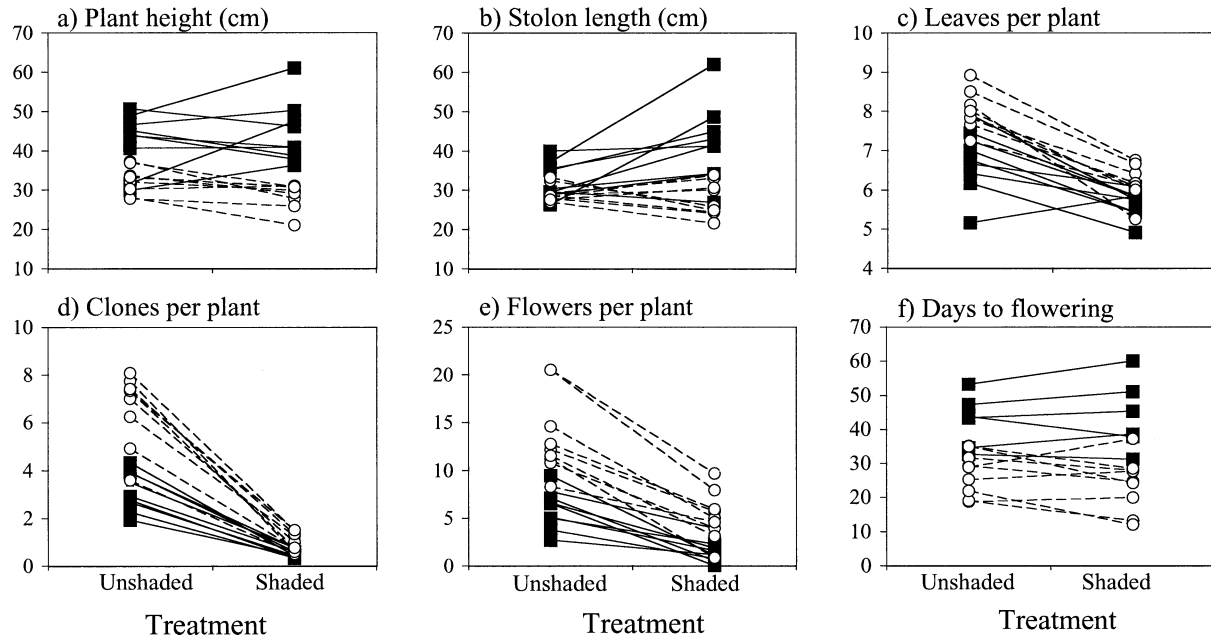


FIG. 6. Variation in components of growth and reproduction among nine monoecious (open circles) and nine dioecious populations (closed squares) of *Sagittaria latifolia* grown in shaded and unshaded growth treatments in a split-plot field transplant experiment. Population means are joined by dashed (monoecious populations) and solid (dioecious populations) lines. Values shown are population means for plant height (a), stolon length (b), the number of leaves produced per plant (c), the number of clones produced per plant (d), the number of flowers produced per plant (e), and the number of days elapsed before the onset of flowering (f).

ative analyses at the interspecific level (e.g., Renner and Ricklefs 1995; Barrett et al. 1996). Our study of a large sample of monoecious and dioecious populations of *S. latifolia* contrasted under a range of conditions represents the most extensive intraspecific investigation of ecological traits associated with the existence of combined versus separate sexes in plants. The results provide the first experimental evidence of extensive life-history differentiation between monoecious and dioecious plant populations. The differences we observed are in accord with theoretical expectations for the evolution of life histories in the competitive versus disturbed habitats in which dioecious and monoecious populations exist, respectively. Below, we discuss our findings and evaluate the hypothesis that the evolutionary maintenance of two distinct sexual systems in *S. latifolia* results from their divergent life histories.

Life-History Variation in Sagittaria latifolia

Theories for the evolution of adaptive life histories are built around assumptions of trade-offs between allocation to

growth, reproduction, and survival. Because plants are modular, these trade-offs may arise via the balance between the number of modules produced and the size of individual modules (Weis et al. 1987; Simons and Johnston 2000). The results of our glasshouse experiment provide two lines of evidence for such trade-offs. First, principal components analysis revealed a negative association between investment in size versus propagation. Plant height and corm mass each had positive loadings on the first principal axis, whereas the number of clones and corms had negative loadings. Second, holding total investment in corm mass constant, we found a strong negative correlation between the number and size of corms in the glasshouse experiment. Together, these results indicate that there is a trade-off between allocation to size versus propagation in *S. latifolia*. These different patterns of allocation to module size and propagation observed in dioecious versus monoecious populations likely reflect divergent selection on patterns of size versus propagation in the contrasting wetland habitats in which they typically exist.

Trade-offs between allocation to size versus propagation

TABLE 5. Analysis of survival to the end of the experiment and the proportion of individuals flowering for nine monoecious and nine dioecious populations of *Sagittaria latifolia* transplanted into two shading treatments in the field. Values shown are *P*-values from likelihood-ratio tests using logistic regression.

Effect	Survival	Proportion flowering
Treatment	<i>P</i> = 0.98	<i>P</i> = 0.0000
Subplot[treatment]	<i>P</i> = 0.001	<i>P</i> = 0.0005
Sexual system	<i>P</i> = 0.98	<i>P</i> = 0.0000
Population[sexual system]	<i>P</i> = 0.03	<i>P</i> = 0.0009
Treatment × sexual system	<i>P</i> = 0.004	<i>P</i> = 0.15
Treatment × population[sexual system]	<i>P</i> = 0.06	<i>P</i> = 0.08

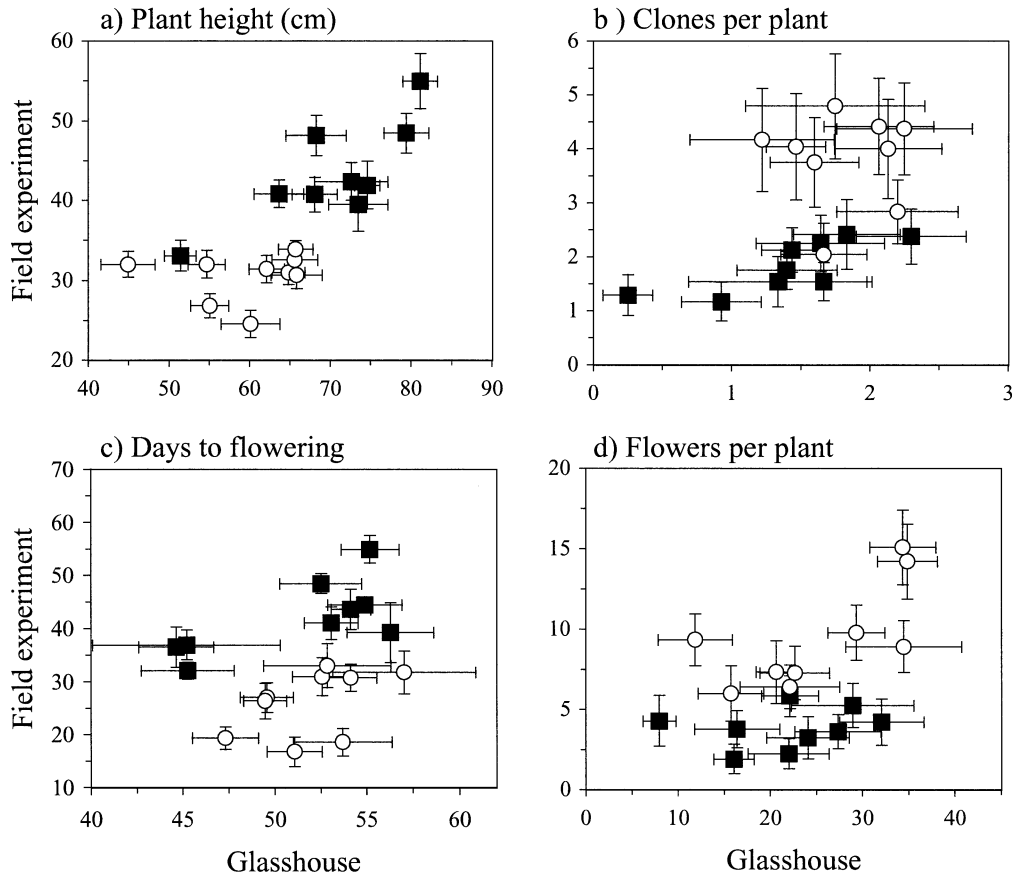


FIG. 7. Relations between components of growth and reproduction obtained in the glasshouse and field transplant experiments. Values shown are population means (\pm SE) for plant height (a), the number of clones produced per plant (b), the number of days elapsed before the onset of flowering (c), and the number of flowers produced per plant (d) for the nine monoecious (open circles) and nine dioecious (closed squares) populations used in both experiments.

in plants have important consequences for resource capture and emergence success (Rees and Westoby 1997; Stuefer et al. 2002). Increased allocation to ramet size is likely to maximize fitness in more competitive habitats where opportunities for horizontal expansion are limited. In addition, if competition from neighbors reduces emergence success, increased allocation to corm size, and thus the height of growing shoots, should be favored (Keddy et al. 1994; Gaudet and Keddy 1995; Wetzel and Van der Valk 1998). Our survey of populations demonstrated that plants from dioecious populations generally had taller neighbors (Table 1). Accordingly, we found increased investment in ramet and corm size in dioecious compared to monoecious populations. In natural populations, the glasshouse, common garden, and field transplant experiment, dioecious ramets were 44%, 24%, 43% and 36% taller than monoecious ramets, respectively, and produced 47% and 54% larger corms by mass in the glasshouse and crossing experiments, respectively. Greater allocation to ramet and corm size at the expense of propagation in dioecious populations is likely to have important implications for the balance between sexual and clonal reproduction in the permanent wetlands in which dioecious populations exist. For example, because corm size is positively associated with emergence success in *S. latifolia* (Garbisch and McIninch 1994), large corm size is likely to favor the persistence of

genets over seedling recruitment. Extensive unisexual patches of plants are typical of many dioecious populations, indicating that genet persistence may often preclude opportunities for seedling recruitment. Indeed, clone sizes can be very large in the stable wetlands occupied by dioecious populations (M. E. Dorken and S.C.H. Barrett, unpubl. data).

Life-history theory predicts that in open, disturbed habitats, increased allocation to reproductive activities should be favored (MacArthur and Wilson 1967; Roff 1992; Stearns 1992). In this study, we found that plants from monoecious populations had higher investment in both flowering and clonal propagation than dioecious populations. This occurred via increased production of clonal ramets and corms. In the glasshouse, monoecious plants produced 41% more corms, and more than twice as many in the common garden experiment. Moreover, in the crossing and field transplant experiments, monoecious plants produced 40% and 106% more clonal ramets in comparison with dioecious plants, respectively. Increased allocation to clonal propagation in monoecious compared to dioecious populations is undoubtedly associated with survival in the highly disturbed habitats in which monoecious populations are commonly found. An increase in the spatial distribution of genets can reduce the probability that periodic disturbance will destroy entire genets (Crowley and McLetchie 2002). Furthermore, high corm

production is likely to increase vegetative dispersal if disturbance occurs during dormant periods, such as the annual early spring flooding of streams and ditches that characterizes populations of *S. latifolia* in Ontario. Ecological differences between the habitats occupied by monoecious and dioecious populations (Table 1) suggest that flooding and local population extirpation are more common in monoecious populations, supporting the hypothesis that increased corm production in monoecious populations is a strategy for tolerating periodic disturbance.

Disturbance also affects optimal schedules of reproduction if disturbance occurs before seed maturation (Harper 1977; Grime 1979). Frequent disturbance should therefore lead to more rapid development times (Lewontin 1965). Although monoecious plants did not flower significantly earlier in the glasshouse, where regular application of fertilizer probably delayed flowering, under field conditions monoecious plants flowered, on average, more than two weeks earlier than plants in dioecious populations. Because inflorescences are initiated approximately two to three weeks prior to first flowering (M. E. Dorken, pers. obs.), flowering time differences are unlikely to have resulted from carryover effects from the previous season's growth (also see below). The delayed flowering of dioecious populations, and subsequent later seed maturation, may restrict opportunities for establishment in the disturbed habitats occupied by monoecious populations. Also, delayed flowering is probably associated with the larger size of dioecious plants. In general, later flowering is associated with continued vegetative growth, resulting in increased plant size and greater competitive ability (Harper 1977; Dorn and Mitchell-Olds 1991).

Because flowering phenology determines opportunities for gene flow between populations, differences in flowering time between monoecious and dioecious populations also have consequences beyond responses to environmental heterogeneity. Indeed, the divergent flowering phenologies of sympatric hermaphroditic and dioecious populations of *Wurmbea dioica* have been invoked as a potential mechanism promoting reproductive isolation between these sexual systems (Case and Barrett 2001). However, reproductive phenology is unlikely to play an important role in maintaining distinct sexual systems in *S. latifolia*. Although monoecious ramets flower earlier than dioecious ramets in the field, they continue to flower for as long as dioecious ramets, providing opportunities for hybridization between plants with different sexual systems in the rare instances in which mixed populations exist.

The observed differences in life-history traits between monoecious and dioecious populations are unlikely to result from carryover effects from the previous season's growth in the vegetative shoots used in the field and glasshouse comparisons. Similar patterns of life-history differentiation have remained in three subsequent years of growth under uniform conditions in the glasshouse (M. E. Dorken and S. C. H. Barrett, unpubl. data). More importantly, these patterns were also evident when plants in the common garden study were grown from seed under uniform conditions, ruling out the possibility that the observed life-history differentiation between the sexual systems is environmentally induced. Finally, any possible carryover effects associated with differences in initial shoot

size were controlled for in our population comparisons using analysis of covariance.

Much of the variation in life-history traits observed in the glasshouse and field transplant experiments occurred among populations within the two sexual systems. This is not surprising because variation among populations in life-history traits is commonly detected whenever multiple populations are compared (reviewed in Mazer and Leubhn 1999). In *S. latifolia*, this variation most likely reflects the adaptation of populations to local conditions across the 550-km transect that we sampled. Indeed, the positive correlations of trait values we observed among populations when we contrasted performance in glasshouse and field transplant experiments support this interpretation. Interestingly, trait values among monoecious populations were less likely to be correlated in the glasshouse versus field experiment, implying greater phenotypic plasticity of life-history traits in monoecious compared to dioecious populations. Greater plasticity in monoecious populations seems likely to represent an adaptive response to the disturbed environments in which these populations exist.

Origin and Maintenance of Sexual Systems

Evolution involves both the origin and maintenance of adaptive traits. Models for the origin of dioecy from hermaphroditism highlight the importance of selfing and inbreeding depression in the spread of unisexual variants (reviewed in Charlesworth 1999). However, these models, which are primarily genetic in nature, do not consider the ecological context under which increased rates of selfing and inbreeding depression become important agents of selection. Large plant size and mass flowering in hermaphroditic populations can lead to increased selfing rates because of the greater opportunities for geitonogamous pollen transfer (Eckert 2000). Several workers have proposed that such size-dependent selfing could act as a stimulus for the evolution of dioecy (Barrett 1984; Webb 1999; de Jong 2000; Reusch 2001) although empirical support for this hypothesis has been difficult to obtain.

In sites occupied by *S. latifolia* where disturbance is less frequent, clone sizes in monoecious populations can become quite large, resulting in increased rates of selfing through interinflorescence geitonogamy. Under these conditions, inbreeding depression and pollen discounting may favor the spread of unisexual individuals. Indeed, in monoecious populations of *S. latifolia*, selfing rates and inbreeding depression can occasionally exceed the threshold ($s \times \delta > 0.5$; Charlesworth and Charlesworth 1978) above which male sterility mutations are favored (Dorken et al. 2002). Thus, according to this hypothesis, the proximate ecological factor favoring the origin of dioecy in *S. latifolia* is habitat stability and its influence on clone size and selfing rates. However, although we have comparative evidence based on *cpDNA* variation that dioecy is likely to be derived from monoecy in *S. latifolia* (M. E. Dorken and S. C. H. Barrett, unpubl. data) the results from this study do not provide unequivocal evidence for the selective mechanism(s) involved. This is because large size in dioecious populations could be a consequence of dioecy rather than a causal factor driving increased selfing rates in

ancestral monoecious populations. Free from the constraints of geitonogamy, clones in dioecious populations may attain much larger sizes than are possible in the disturbed environments occupied by monoecious populations. In general, historical inferences concerning past selective factors responsible for the origin of reproductive adaptations are often uncertain, even if phylogenetic information is available.

Determining the contemporary selective forces maintaining variation in sexual systems may be feasible if consistent associations between ecology and trait variation are evident within a species. Our finding of significant life-history differentiation between monoecious and dioecious populations of *S. latifolia* strongly suggests that ecological factors associated with habitat sorting maintain the integrity of the two sexual systems in regions of sympatry. Evaluating this hypothesis requires demonstrating a role for divergent selection between monoecious and dioecious populations and showing that the factors maintaining population differentiation also promote reproductive isolation (reviewed in Schluter 2001). Evaluating the first requirement involves distinguishing divergent selection from other agents of evolutionary divergence, the most obvious of which in plants are polyploidization and genetic drift. Although evolutionary divergence due to polyploidization occurs in several other plant groups with combined versus separate sexes (*Isotoma*, McComb 1969; *Mercurialis*, Pannell 1997b; *Wurmbea*, A. L. Case and S. C. H. Barrett, unpubl. data), it is clearly not involved in the maintenance of distinct sexual systems in *S. latifolia*, since populations with these different sexual systems are diploid and fully interfertile.

Genetic drift is unlikely to result in the patterns of life-history differentiation we observed between the two sexual systems of *S. latifolia*. We surveyed a large number of populations across a substantial geographic transect and grew plants from these populations under common environmental conditions. Because genetic drift is not expected to act in a uniform fashion among populations, it cannot be the primary cause of life-history differentiation between monoecious and dioecious populations. The consistent differences in life-history traits between monoecious and dioecious populations across the transect suggest that the patterns of differentiation we revealed are likely to be a general feature of *S. latifolia* populations with contrasting sexual systems. Indeed, our investigations of the associations between sexual systems and life-history differentiation in populations from other parts of the geographical range of this species generally support this conclusion (M. E. Dorken and S. C. H. Barrett, unpubl. data).

Reproductive isolation between the sexual systems of *S. latifolia* will largely be governed by whether monoecious and dioecious populations can establish and persist in each other's habitats. Our manipulations of growth conditions in the field transplant experiment indicate that such habitat switching may be quite infrequent. The relative performance of each sexual system was greatest in the plots that most closely resembled the types of habitats in which each typically grows. Monoecious populations performed relatively poorly in shaded plots compared to unshaded plots, with reduced survival and clonal propagation. In contrast, dioecious populations were less affected by the different growing conditions, with survival and clonal propagation less influenced by the con-

trasting levels of above ground competition (see also Costich 1995). However, unisexual individuals from dioecious populations are unlikely to colonize and persist in the disturbed habitats characteristic of monoecious populations because seed production may be constrained by mate availability, and because slower development to reproductive maturity and lower rates of clonal propagation are likely to limit population growth. Reciprocal transplant experiments could be used to further evaluate the hypothesis of adaptive ecological differentiation between the sexual systems.

Isolation between populations with contrasting sexual systems may be further reinforced if the genetic factors governing life-history differentiation cause reduced fitness of hybrids in parental habitats (Turelli et al. 2001; Via 2001). In *S. latifolia*, the infrequent existence of populations composed of mixtures of hermaphroditic and truly unisexual individuals (Sarkissian et al. 2001; Dorken et al. 2002) indicate that only occasional opportunities for gene flow between sexual systems occur. However, crosses between populations with divergent life-history traits are likely to have reduced fitness relative to their parents because, as we have shown, the progeny of these crosses have intermediate life-history phenotypes and these may be maladaptive in the divergent habitats of their parents (Anderson 1948; Allan et al. 1997; Epperson et al. 2001).

So far, we have not considered the spatial scale or location(s) in which evolutionary divergence between monoecious and dioecious populations of *S. latifolia* may have occurred. If evolutionary divergence arose in sympatry, how did it start? If divergence arose in allopatry, in which portions of the species range was one sexual system excluded and why? Theoretical studies have shown that evolutionary divergence can occur in sympatry if gene flow is not too high (reviewed in Turelli et al. 2001; Via 2001). However, sexual-system differentiation in *S. latifolia* need not have occurred primarily in sympatry. Certainly, allozyme evidence from Ontario populations indicating distinct clustering of monoecious versus dioecious populations would appear to rule out divergence of sexual systems in this region (Dorken et al. 2002). The present distribution of sexual systems in Ontario seems more likely to reflect secondary contact between monoecious and dioecious populations, the life histories of which may have diverged in allopatry, perhaps in geographically disjunct glacial refugia. We are currently evaluating this hypothesis using molecular markers.

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APPENDIX

General information on the 50 populations of *Sagittaria latifolia* sampled in this study. M, monoecious populations; D, dioecious populations. The specific experiments in which these populations were used are also indicated with a single letter (F, field observations; E, ecological data; G, glasshouse experiment; C, crossing experiment; T, field transplant experiment).

Population	Sexual system	Experiment	Latitude (N)	Longitude (W)	Estimated population size (ramets)	Habitat
1 Acton	M	G,C,T	43°38'	80°03'	400	shallow creek
2 Asphodel	M	F	44°21'	78°00'	450	shallow creek
3 Avonton	M	F,E	43°21'	81°05'	400	river margin
4 Belfast	M	F	43°53'	81°34'	300	ditch
5 Brampton	M	F,E,G,C,T	43°45'	79°50'	500	shallow creek
6 Cayer	M	F,G	45°07'	75°22'	225	ditch
7 Cayuga	M	E,G,C,T	42°57'	79°52'	525	river margin
8 Davis Lock	M	E,G	44°36'	76°17'	750	ditch
9 Dwyer Hill	M	F,G	45°05'	75°59'	300	stream margin
10 Ellisville	M	F,E	44°29'	76°12'	750	ditch
11 Elmira	M	F,E	43°36'	80°33'	275	stream margin
12 Fernbank	M	F,E	43°37'	80°49'	300	shallow creek
13 Guelph	M	F,E,G,C,T	43°33'	80°13'	2500	ditch
14 Kingston	M	F,E	44°16'	76°30'	1250	ditch
15 Lakefield	M	G	44°26'	78°16'	150	stream margin
16 London	M	F	43°01'	81°09'	75	ditch
17 Luther Lake	M	G	43°58'	80°24'	75	lake margin
18 Mount Forest	M	F	43°54'	80°44'	650	stream margin
19 Paugh Lake	M	F,E,G	45°36'	77°45'	450	lake margin
20 Port Hope	M	E,G,T	43°57'	78°18'	80	shallow creek
21 Rockwood	M	G,C,T	43°37'	80°08'	3500	shallow creek
22 Rothsay	M	F	43°48'	80°43'	1500	stream margin
23 Round Lake	M	F	45°39'	77°35'	425	lake margin
24 Sky Lake	M	G	44°48'	81°17'	225	lake margin
25 Sprucedale	M	E,G,C	45°29'	79°28'	35	bog edge
26 St. Jacobs	M	F,E	43°32'	80°33'	250	river margin
27 Thamesford	M	F,E,G,T	43°04'	81°00'	3000	river margin
28 Toronto	M	E,G,T	43°36'	79°28'	300	freshwater marsh
29 Uxbridge	M	F	44°12'	79°10'	1200	river margin
30 Varna	M	F	43°30'	81°40'	25	ditch
31 Villanova	M	E,G,T	42°56'	80°13'	75	shallow creek
32 Whitechurch	M	F	43°55'	81°26'	500	shallow creek
33 Wicklow Beach	M	F,E	43°59'	77°58'	300	freshwater marsh
34 Downey's	D	F,E,G,C,T	43°28'	80°13'	350	bog edge
35 Erinsville	D	F,E	44°29'	77°08'	75	freshwater marsh
36 Gary's Loch	D	F,E	45°15'	74°46'	125	freshwater marsh
37 Hartstown, PA	D	G	41°33'	80°28'	500	freshwater marsh
38 Heart Lake	D	F,E,G,C,T	43°45'	79°48'	3000	freshwater marsh
39 Kingston Mills	D	F,E	44°16'	76°30'	1750	freshwater marsh
40 Long Point	D	F,E,G,C,T	42°36'	80°28'	10,000+	freshwater marsh
41 Long Point Marina	D	G,T	42°35'	80°27'	150	roadside ditch
42 Mississauga	D	E,G,C,T	43°31'	79°36'	2550	freshwater marsh
43 Hillier	D	G,C,T	43°58'	77°27'	300	freshwater marsh
44 Outlet	D	F,E	44°29'	76°03'	200	freshwater marsh
45 Pembroke	D	F,E,G	45°49'	77°07'	500	freshwater marsh
46 Petawawa	D	F,E,G	45°54'	77°20'	150	freshwater marsh
47 Plaisance	D	F,E	45°35'	75°04'	1500	freshwater marsh
48 Severn Falls	D	G,T	44°52'	79°36'	125	swamp
49 Stromness	D	E,G,C,T	42°53'	79°33'	1500	freshwater marsh
50 Whitby	D	E,G,T	43°51'	78°54'	75	freshwater marsh