The reproductive ecology of island populations of distylous *Menyanthes trifoliata* (Menyanthaceae)

Faye L. Thompson, Luise A. Hermanutz, and David J. Innes

Abstract: *Menyanthes trifoliata* L. is a distylous, clonal aquatic macrophyte found in shallow bogs and river margins throughout the boreal ecosystem, including the island of Newfoundland. A combination of long-distance dispersal and colonization after deglaciation, and pollen limitation resulting from reduced pollinator diversity and abundance documented on islands, predicts the breakdown of heterostyly to favour the establishment of self-compatible homostyles on islands. To test if self-fertilizing homostyles have been selected, variation in floral characters and compatibility relationships were examined in *M. trifoliata* populations from the Avalon Peninsula of Newfoundland. Morph ratio and its effect on fruit and seed set were examined in nine populations. Of the seven dimorphic populations, morphs occurred in a 1:1 ratio in four populations and deviated significantly from a 1:1 ratio in three populations. The two populations monomorphic for either pin or thrum morphs set few fruits or seeds (<15%). A strictly reciprocal arrangement of stigma height and anther length was not observed between pin and thrum morphs in the majority of populations studied. Stigma–anther separation showed a bimodal distribution with few intermediate “homostylous” flowers, rather than the discreet bimodal distribution typical of distylous species. Fruit and seed set were high (>60%) in equal morph ratio populations and were not significantly correlated to stigma–anther separation, indicating that there was no selective advantage of being homostylous. All three populations tested were highly self-incompatible, confirming that there has not been a breakdown of heterostyly on the island of Newfoundland. A reduced pollinator fauna typical of island environments may have relaxed stabilizing selection for strict herkogamy between floral morphs, resulting in the observed lack of reciprocity.

Key words: *Menyanthes trifoliata*, distyly, homostyle, reciprocal herkogamy, clonal aquatic macrophyte, island of Newfoundland.

Résumé : Le *Menyanthes trifoliata* L. est une macrophyte aquatique clonale distyle qu’on retrouve dans les marais peu profonds et au bord des rivières partout dans l’écosystème boréal, incluant l’île de Terre-Neuve. Une combinaison de dispersion à distance et de colonisation après le départ du glacier accompagnées d’une limitation de la dispersion du pollen liée à une faible diversité des pollinisateurs ainsi que l’abondance observée sur des îles, permettent de prédire la disparition de l’hétérostylie en faveur de l’établissement d’homostyles compatibles, sur les îles. Afin de vérifier si des plantes homostyles auto-fertiles ont été sélectionnées, les auteurs ont examiné la variation des caractères floraux et les relations de compatibilité dans des populations de *M. trifoliata* situées sur la péninsule d’Avaléon, dans l’île de Terre-Neuve. Ils ont examiné le rapport des morphes et son effet sur la mise à fruits et à graines dans neuf populations localisées sur la péninsule d’Avaléon. Sur les sept populations dimorphes, le rapport des morphes est de 1 : 1 dans quatre cas et il dévie significativement dans deux populations. Les deux autres populations monomorphes pour l’une ou l’autre des morphes produisent des fruits et des graines (<15%). Il n’a pas été possible d’observer un arrangement réciproque strict de la hauteur du stigma par rapport à la longueur des anthères entre les deux morphes, dans la majorité des populations étudiées. La séparation stigma–anthère montre une distribution bimodale avec peu de fleurs << homostyles >> intermédiaires, plutôt que la distribution bimodale discrète typique des espèces distyles. La mise à fruits et à graines est élevée (>60%) dans les populations à rapports de morphes égaux, et n’est pas significativement corrélée à la séparation anthère–stigma, ce qui indique qu’il n’y a pas d’avantage sélectif dans l’homostylie. L’ensemble des trois populations testées sont fortement auto-compatibles, ce qui confirme qu’il n’y a pas eu de chûte de l’hétérostylie dans l’île de Terre-Neuve. La présence d’une faune pollinisatrice réduite, typique des environnements insulaires, peut avoir relâché la sélection stabilisatrice pour l’herkogamie entre les morphes florales, conduisant ainsi à l’absence de réciprocité observée.

Mots clés : *Menyanthes trifoliata*, distylie, homostyle, herkogamie réciproque, macrophyte aquatique clonale, île de Terre-Neuve.

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Introduction

The colonization of islands by plants with heterostylous breeding systems may have several important consequences. As many heterostylous species possess self-incompatibility mechanisms to ensure disassortative mating among floral morphs (Ganders 1979; Lloyd and Webb 1992), multiple individuals of both morphs would have to successfully colonize

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new islands. Alternatively, the colonization process may select for self-fertilizing homostylous variants to ensure propagule production, an example of Baker's rule (Baker 1967; Barrett 1996; Olesen 1987). In addition, the effects of island establishment on plant breeding systems is particularly important for insect-pollinated species. Many island environments have a lower diversity and abundance of pollinating insects than do continental areas, such that pollen limitation results in low levels of seed production (Barrett 1996; Spears 1987). One potential consequence of pollen limitation is the breakdown of heterostyly (Barrett 1992; Lloyd and Webb 1992). A shift to a less diverse pollinator fauna and (or) unreliable pollinator service can also lead to the selection of self-pollinating homostylous variants (Washitani et al. 1994). Homostyles have anthers and stigmas at the same height within the flower and may arise by crossing over in the supergene that controls distyly (Shore and Barrett 1985). Reproductive assurance under conditions of suboptimal pollinator levels is achieved as homostyles are both self-compatible and self-pollinating (Barrett and Richards 1990).

The increased occurrence of homostyles on islands is exemplified on the Caribbean Islands (Barrett 1985, 1992, 1996). Self-compatible, homostylous variants of the distylous weed, *Turnera ulmifolia* L. were favoured following island colonization (Barrett and Shore 1987). Pollen limitation due to the absence of specialized long-tongued pollinators on the island of Jamaica resulted in the establishment of a self-pollinating, semi-homostylous variant of the midstyled morph in the tristylous aquatic plant *Eichhornia paniculata* (Spreng.) Solms. (Glover and Barrett 1986).

Newfoundland is a large island (111 390 km^2), separated from mainland Canada by the Cabot Strait in the south (Cape Breton, N.S.; 105 km), and Strait of Belle Isle (Labrador; 16–24 km) in the north. Although coastal nunataks may have existed during the Wisconsinan glaciation (Grant 1989), whether they supported angiosperm vegetation is controversial (J. Brown Macpherson, personal communication). Therefore, peak recolonization of the island by angiosperm taxa originating from mainland sources began as more land freed of ice and appropriate habitats became available, approximately 10 000 ^{14}C years BP (Anderson and Macpherson 1994).

The pollinator fauna of insular Newfoundland is not well documented, but comparisons of species number of the major pollinator groups (Apoidea: Kevan et al. 1993, and Syrphidae: Vockeroth 1992) with those found in the continental boreal ecosystem indicate a depauperate fauna (Constantine 1986). Populations of important pollinators such as bumblebees are small, a consequence of the cool, wet weather (D. Larson, personal communication). Therefore, on the island of Newfoundland, long-distance colonization from mainland sources, combined with reduced pollinator diversity and densities, predicts that self-compatible homostylous forms of heterostylous species may be selected.

Menyanthes trifoliata L. (Menyanthaceae) is a distylous aquatic macrophyte of bogs, fens, and pond and river margins, with a circumpolar distribution between 40°N and the Arctic Circle (Hewett 1964). A small-flowered variety, *M. trifoliata* var. *minor* Raf. occurs east of the Rocky Mountains to the Atlantic Ocean (Fernald 1950) and has been found throughout the island of Newfoundland and Labrador (Rouleau and Lamoureux 1992). There have been conflicting reports in the literature as to the existence of homostyly in *M. trifoliata*. Warming (1886) described homostylous specimens from Greenland, which were used by Baker (1955, 1967) as examples of Baker's rule. But Olesen (1987), after surveying single populations in Greenland, Iceland, and Denmark, attributed homostyly to the extremely variable flower morphology of the species. None of these studies tested the levels of self-compatibility.

This study examines the variation in floral characters and compatibility relationships of *M. trifoliata* from the island of Newfoundland to (*i*) determine whether the establishment of self-compatible homostylous variants has occurred and document variability of the distylous syndrome in 10 populations; (*ii*) examine morph ratios and population density; and (*iii*) determine the relationship between fruit and seed set and between floral characters associated with distyly and pollen limitation.

Materials and methods

Study species

Menyanthes trifoliata var. *minor* reproduces vegetatively by clonal fragmentation and sexually by seed. Individual ramets produce a raceme of 6–31 white tubular flowers (Hewett 1964). Anther filaments attach to the actinomorphic corolla tube, which is 4–5 mm deep. At anthesis, the anther sacs reflex away from the stigma becoming perpendicular to the filament. Flowers are insect pollinated (Proctor and Yeo 1973). On the island of Newfoundland, plants bloom from mid-June to early July and ripen fruits 3–4 weeks later (F. Thompson and L. Hermanutz, personal observation). Ten populations of *M. trifoliata* from the Avalon Peninsula of Newfoundland were examined from May to August 1995. Study populations were found in four regions: Flatrock (FL), Salmonier (SL), Bauline Line (BL), and the Oxen Pond Botanical Garden, St. John's (OP) (Fig. 1). Populations were located in shallow bogs or at pond margins.

Population structure

Population structure was examined at all study populations with the exception of FR-3. At each of these populations, flowering ramet density and total ramet density were estimated in 0.25-m^2 quadrats placed at intervals along transects to approximate equal sampling intensity among populations (Table 1). For each population, the flowering ramets present in each quadrat were classified as pin or thrum by visually assessing the style length of their flowers. Morph ratio in each population, and heterogeneity among populations were tested for a 1:1 ratio using a *G* test (Sokal and Rohlf 1995). The area of each population was determined, and the total number of ramets was estimated by multiplying mean ramet density per square metre by population area.

Floral measurements

To examine the variation in floral characters associated with distyly, flowering ramets from each population were haphazardly chosen from spatially separated, single morph clumps, marked, and designated as genets. The synchronous flowering of ramets within each clump was used to confirm genet membership. Marked ramets were classified as pin, thrum, or homostyle, and the number of flowers per inflorescence was counted. At the onset of anther dehiscence, a single flower was harvested from the base of the rachis of each flowering ramet and kept on ice until measured. For each flower sampled, flower size (measured as corolla tube + petal length), stigma, and anther height were measured from the base of the ovary using a dissecting microscope equipped with an ocular micrometer, and the number of ovules per ovary was counted. Morph-specific differences in floral measurements (flower size, flower number per inflorescence, ovule

Fig. 1. Location of study populations of *Menyanthes trifoliata* on the Avalon Peninsula of Newfoundland. See Materials and methods for an explanation of population names.

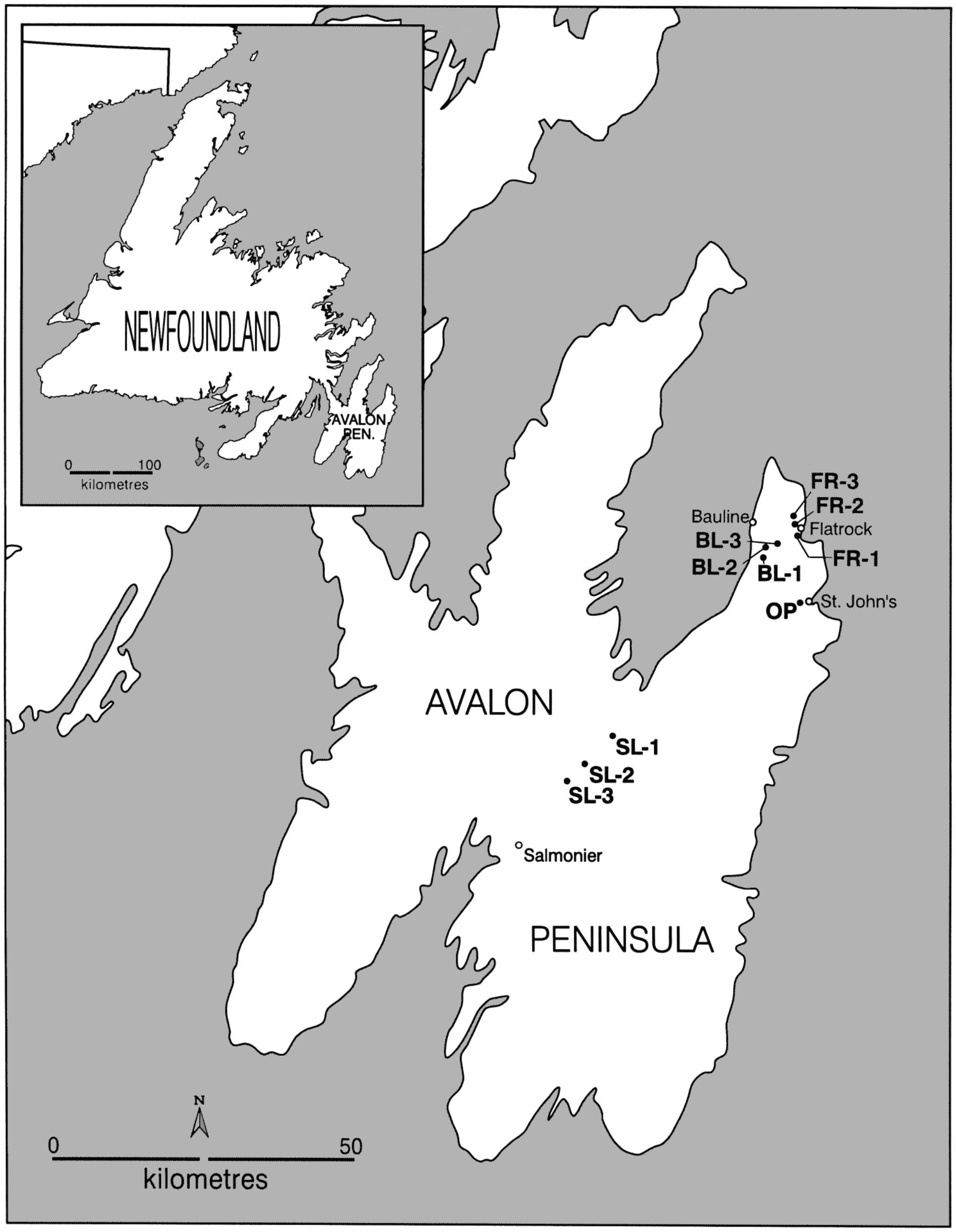


Table 1. Population structure of 10 study populations of *Menyanthes trifoliata*.

Population	Area (m^2)	No. of quadrats sampled	Ramet density (no. per 0.25 m^2)	Flower density (no. per 0.25 m^2)	Estimated population size (no. of ramets)
BL-1	75	14	5.0±0.9	3.0±0.5	1 408
BL-2	104	16	5.0±0.8	5.0±0.9	2 247
BL-3	229	7	12.0±2.7	11.0±2.5	10 729
FR-1	127	15	14.0±2.5	11.0±2.3	7 473
FR-2	227	27	6.0±0.7	6.0±0.7	5 442
FR-3	38	—	—	—	—
SL-1	38	10	8.0±3.8	6.0±3.7	1 245
SL-2	282	14	18.0±3.1	9.0±2.5	19 819
SL-3	743	14	12.0±1.8	6.0+1.4	35 058
OP	16	—	—	—	15

Note: Values for ramet and flower density are mean ± SE. Quadrat data were not collected from populations FR-3 and OP. See Materials and methods for population names.

number per ovary, stigma height, anther height, and stigma–anther separation) were tested using a nested analysis of variance (SAS GLM; SAS Institute Inc. 1994) with type III sum of squares. To remove the allometric effects of flower size on organ variation, flower size was used as covariate in testing stigma and anther heights and separation. The α value was adjusted for the number of comparisons using the Bonferroni procedure (Sokal and Rohlf 1995). Residuals were tested for normality (PROC UNIVARIATE; SAS Institute Inc. 1994) and examined for independence by plotting them against predicted values, to screen for model robustness (Draper and Smith 1981). Each analysis tested population and morph type as the main effects, genet nested within population, and ramet nested within genet. Population × morph interaction was used as an error term for morph.

In addition, each population was visually screened for the occurrence of homostyles, which if detected were harvested, with no genet affiliation designated, and then measured. These were not included in the statistical tests, as no genet affiliation was known.

To compare reciprocity between the long (L; pin styles vs. thrum anther heights) and short (S; thrum styles vs. pin anther heights) organ levels of the two morphs among populations, and to other distylous species, the relative reciprocity ratio for each organ level was calculated as follows (Richards and Koptur 1993):

$$\text{Relative reciprocity ratio} = \frac{\text{anther height} - \text{reciprocal stigma height}}{\text{anther height} + \text{reciprocal stigma height}}$$

Differences within the L and S organs in each population were tested using *t* tests, and the α value was corrected for multiple tests as above.

To examine pollen size dimorphism between morphs, pollen from one flower of randomly chosen thrum (five) and pin (four) inflorescences from population BL-3 was mounted on aluminum stubs, sputter coated with gold, and examined using a Hitachi S570 scanning electron microscope. The longest visible axis was measured on photographed pollen grains (108 pin, 169 thrum), using vernier calipers. Morph-specific differences in pollen size were tested with a one-tailed *t* test after establishing homogeneity of variance between morphs using the $F_{\max}$ test (Sokal and Rohlf 1995).

Compatibility

Self-incompatibility in *M. trifoliata* was examined in three populations (BL-3, FR-2, and OP). Ten inflorescences (five pin, five thrum) from the dimorphic populations BL-3 and FR-2, and three inflorescences (thrum) from the monomorphic population OP, were chosen using the criterion that each inflorescence had at least two buds that would open on the day of treatment. Each inflorescence was bagged with insect netting prior to anthesis to exclude pollinators. As buds opened, thrum flowers were emasculated and flowers were randomly assigned to one of two pollination treatments: (*i*) cross, receiving intermorph pollen from the opposite morph type, or (*ii*) self, receiving intramorph or self pollen from the same morph type. Within each inflorescence, one to three flowers received cross pollen from two or three pollen donors from population BL-2, and one or two flowers received self or intramorph pollen. Treated flowers were labeled with colored string to differentiate treatment types. Pollen was applied to stigmas at anthesis and 24 h post-anthesis to ensure successful pollination, and inflorescences were rebagged until seeds were set. At maturity, fruit and seed set were recorded for each pollinated flower. Fruit set was calculated as the percentage of flowers per ramet that appeared visibly swollen at maturity. Seed set per fruit was calculated as the percentage of ovules forming viable seeds.

As pollinator service may be depressed in island populations, pollen limitation was assessed in populations BL-3 and FR-2 by recording percent fruit and seed set of one to three flowers on each treatment inflorescence that had been exposed to be naturally pollinated before inflorescences were bagged. For each population, fruit and seed set were compared between cross and intermorph, and between cross and natural pollination treatments with Fisher's exact test (Sokal and Rohlf 1995).

Table 2. Population morph ratios of *M. trifoliata*.

Population	Proportion P:T[a]	Sample size P	Sample size T	G_{adj}	Morph ratio 1:1?
BL-1	0.86:0.14	36	6	23.50	No
BL-2	0.53:0.47	42	37	0.31	Yes
BL-3	0.51:0.49	40	39	0.01	Yes
FR-1	1:0	327	0	—	No
FR-2	0.43:0.57	44	58	1.92	Yes
SL-1	0.45:0.55	13	16	0.31	Yes
SL-2	0:1[b]	0	123	—	No
SL-3	0.12:0.88	10	79	60.49	No
OP	0:1	0	15	—	No

[a] P, pin; T, thrum.

[b] A few pin ramets were detected outside of sampled quadrats.

The capacity for fruit production in the absence of pollinators (autogamy) was examined at population BL-3. Ten inflorescences (four pin, six thrum), each with a large portion of their flowers at the bud stage, were randomly chosen and bagged to exclude pollinators. For those flowers that bloomed while each inflorescence was bagged, fruit set was recorded at maturity.

Fruit set and seed set

Natural levels of fruit set per ramet, and seed set per fruit were recorded for the marked ramets. To increase sample size, additional ramets adjacent to marked ramets (and assigned to the same genet), were harvested, and included in fruit- and seed-set calculations. Morph-specific differences in percent fruit and seed set were tested using nested analysis of variance (SAS GLM; SAS Institute Inc. 1994) after arcsine square root transformation in each population. The α value was adjusted for the number of comparisons as above. Residuals from each model were tested as above. Each analysis tested main effects (morph and genet) and nested effects (ramet nested within genet). A small proportion of fruits had started to disperse seeds prior to harvest; therefore, only infructescences with at least 85% of capsules intact were used for the analysis of seed set.

Spearman's rank correlation was used to determine the relationship between fruit set and degree of herkogamy, anther height, and stigma height. As morph ratio affects fruit set (see Results), populations with equal morph ratios were pooled for the analysis. The adjusted α was set at 0.017 to reflect the three tests.

Results

Population structure

Populations of *M. trifoliata* varied in area (16–743 m^2) and in size (15 – 35 000 ramets), with a high proportion and density of flowering ramets (Table 1). Six of the nine populations in which morph ratio was determined were dimorphic (Table 2). Two of the six dimorphic populations deviated from a 1:1 morph ratio (Table 2), and there was significant heterogeneity ($G = 81.21$, df = 5, $p < 0.001$) in morph ratio among the six populations. Equal morph ratios were detected in four of the dimorphic populations (BL-2, BL-3, FR-2, SL-1; Table 2) with no significant heterogeneity ($G = 2.15$, df = 3) among these populations. Among the two dimorphic study populations with unequal morph frequencies, BL-1 was predominately pin while SL-3 was predominately thrum (Table 2). Although only thrum ramets were found when SL-2 was sampled, a small number (20) of pin ramets were detected outside

Table 3. Comparison of mean floral characters of pin and thrum morphs in 10 populations of *M. trifoliata*.

Population		Flower size (mm)	Ovule no. per flower	Stigma height (mm)	Anther height (mm)	Stigma–anther separation (mm)	Flower no. per inflorescence (mm)
BL-1	P	14.1±0.42 (6)	17±2.3 (6)	13.5±0.49 (6)	8.6±0.29 (6)	4.9±0.39 (6)	19.7±0.92 (6)
	T	14.8±0.83 (6)	21±1.7 (5)	9.4±0.27 (6)	10.9±0.23 (6)	–1.5±0.21 (6)	17.6±0.65 (7)
BL-2	P	14.8±0.31 (6)	18±3.0 (6)	12.1±0.82 (6)	8.5±0.24 (6)	3.5±0.71 (6)	21.4±1.03 (8)
	T	15.0±0.38 (6)	22±2.1 (6)	9.6±0.13 (6)	12.0±0.41 (6)	–2.4±0.51 (6)	20.4±1.56 (7)
BL-3	P	17.0±0.47 (9)	19±1.3 (9)	14.9±0.35 (9)	9.2±0.31 (9)	5.7±0.25 (9)	17.4±0.99 (18)
	T	15.4±0.68 (8)	17±1.3 (7)	10.0±0.21 (8)	11.4±0.34 (8)	–1.3±0.33 (8)	19.6±1.42 (11)
FR-1	P	15.5±0.52 (9)	15±0.8 (10)	12.3±0.19 (9)	6.8±0.13 (9)	5.4±0.24 (9)	14.8±1.41 (13)
	T	—	—	—	—	—	—
FR-2	P	15.1±0.90 (6)	18±1.3 (6)	12.5±0.67 (6)	8.4± 0.54 (6)	4.2±0.51 (6)	15.4±1.31 (9)
	T	15.1±0.63 (6)	16±2.3 (6)	8.4±0.14 (6)	12.2±0.64 (6)	–3.8±0.67 (6)	16.7±0.53 (9)
FR-3	P	15.3±0.24 (10)	20±1.7 (10)	12.5±0.20 (10)	6.9±0.23 (10)	5.5±0.26 (10)	15.4±0.91 (8)
	T	16.6±0.63 (10)	17±1.5 (10)	8.6±0.18 (10)	11.8±0.30 (10)	–3.1±0.27 (10)	17.1±0.81 (8)
SL-1	P	14.3±0.72 (8)	20±1.6 (8)	11.5±0.66 (8)	8.3±0.33 (8)	3.2±0.63 (8)	20.0±2.36 (9)
	T	13.8±0.53 (17)	16±0.8 (16)	9.6±0.14 (17)	12.2±0.28 (17)	–2.6±0.19 (17)	14.9±1.22 (18)
SL-2	P	13.4±0.53 (4)	23±5.5 (4)	12.1±0.26 (4)	7.7±0.54 (4)	4.3±0.75 (4)	19.4±1.21 (8)
	T	12.4±0.39 (4)	23±1.6 (14)	8.2±0.19 (14)	10.3±0.40 (14)	–2.0±0.32 (14)	24.4±0.89 (24)
SL-3	P	12.5±0.44 (23)	22±1.4 (23)	12.6±0.31 (23)	6.7±0.20 (23)	5.9±0.20 (23)	19.9±1.42 (19)
	T	13.8±0.52 (17)	19±0.9 (15)	8.7±0.28 (17)	10.7±0.39 (17)	–2.1±0.23 (17)	20.1±1.23 (14)
OP	P	—	—	—	—	—	—
	T	13.8±0.53 (5)	18±1.2 (5)	7.9±0.22 (5)	10.7±0.25 (5)	–2.8±0.16 (5)	20.8±1.10 (9)

Note: Values are mean ± SE, with sample size given in parentheses. P, pin; T, thrum.

the quadrats, and in the remaining analyses this populations was considered dimorphic. Two of the populations examined were monomorphic, containing thrum (OP) or pin (FR-1) ramets.

Floral measurements

Of the floral characters studied, only flower size showed significant differences among populations (Tables 3 and 4). The number of flowers per inflorescence, the number of ovules per flower, and flower size showed no significant difference between morphs (Tables 3 and 4). Consistent with other distylous species, thrum pollen was significantly larger than pin pollen (pin = 52.8 ± 0.4 μm (mean ± SE); $N = 108$; thrum = 60.9 ± 0.3 μm; $N = 169$; $t = -15.8$, df = 275, $p < 0.0001$).

Both anther and stigma heights showed considerable variation within and among populations (Fig. 2, Table 3), but differences among populations or genets were not significant (Table 4). The majority of dimorphic populations exhibited two distinct morphs with some degree of overlap in both anther and (or) stigma heights, but only two populations (FR-2 and FR-3) showed 2 discreet, non-overlapping morphs (Fig. 2). Morphological “homostyles” were recorded in four populations, but upon measurement the anthers and stigmas were found to be unequal (Fig. 2). Pooling populations, both stigma and anther heights were bimodal, with some overlap in organ heights (Figs. 3*a* and 3*b*). The distinctness of the morphs was confirmed by significant morph-specific differences in both anther and stigma heights, but the magnitude of difference in anther and stigma heights between morphs varied among populations (Table 4).

Stigma–anther separation showed a bimodal distribution, with a small number of flowers with equal organ heights (Fig. 3*c*). The degree of herkogamy exhibited between morph types was not symmetrical (Fig. 3*c*). Stigma–anther separation in the pin morph was significantly greater than in the thrum morph (Tables 3 and 4). A significant interaction (Table 4) confirms that herkogamy varied among populations. Within populations, genets varied significantly in stigma–anther separation (Table 4).

Within populations, a perfectly reciprocal arrangement of stigma and anther heights between morphs was evident in only two populations (FR-2, SL-2; Fig. 4). Although the remaining populations showed asymmetry, the asymmetric organ level was not consistent. In two populations (BL-1, BL-3) the long organs were not reciprocal, while in three populations (BL-2, SL-1, FR-3) the short organs were not reciprocal, with SL-3 having both organ levels asymmetrical (Fig. 4). In all but SL-1, stigma height was greater than anther height of the opposite morph.

Fruit set was independent of degree of herkogamy ($r_s = 0.16$, $p = 0.30$), as well as anther height ($r_s = -0.24$, $p = 0.127$) and stigma height ($r_s = 0.34$, $p = 0.03$; $\alpha = 0.017$), in populations with equal morph frequencies.

Compatibility

In all three populations tested, *M. trifoliata* was highly self-incompatible (Table 5). Flowers treated with self pollen set significantly fewer fruits and seeds than did flowers treated with cross pollen. The monomorphic pin population OP showed the highest seed set among cross-pollination treatments. Fruit set at populations BL-3 and FR-2, and seed set at population BL-3, were not pollinator limited (Table 5). A significantly higher seed set in cross- than in natural-pollination treatments was observed at population FR-2 (Table 5B). However, both cross- and natural-pollinated seed set in treatment flowers at this population were unusually low, indicating that some other experimental or environmental variable, such as high level of fungal infection (Thompson 1996), and not pollinator limitation explain the observed results. It is obvious that *M. trifoliata* relies on insects to transfer intermorph pollen, as only a single fruit was set in the absence of pollinators (0% thrum, $N = 50$ flowers; 1.8% pin, $N = 56$ flowers).

Table 4. Morph and population differences for floral characters in 10 populations of *M. trifoliata*.

Character and source	df	MS	*F*
Flower no. per inflorescence			
Population	9	84.84	3.54ns
Morph	1	20.11	0.99ns
Genet (population)	12	23.97	2.99ns
Ramet (genet)	32	8.02	0.42ns
Population × morph	7	20.22	1.05ns
Flower size			
Population	9	27.25	4.65*
Morph	1	0.093	0.02ns
Genet (population)	13	5.86	1.85ns
Ramet (genet)	25	3.17	1.12ns
Population × morph	7	5.17	1.83ns
Ovule no. per flower			
Population	9	60.85	1.79ns
Morph	1	26.78	0.62ns
Genet (population)	13	34.08	1.57ns
Ramet (genet)	25	21.66	0.86ns
Population × morph	7	42.97	1.71ns
Stigma height			
Population	9	4.98	2.44ns
Morph	1	341.18	91.74*
Genet(population)	13	2.04	2.08ns
Ramet (genet)	25	0.98	1.90ns
Population × morph	7	3.72	7.16*
Anther height			
Population	9	4.97	5.69ns
Morph	1	305.06	156.33*
Genet(population)	13	0.87	2.21ns
Ramet (genet)	25	0.4	0.89ns
Population × morph	7	1.95	4.37*
Stigma–anther separation			
Population	9	7.45	1.95ns
Morph	1	1291.5	290.82*
Genet (population)	13	3.82	4.87*
Ramet (genet)	25	0.78	1.09ns
Population × morph	7	4.44	6.17*

Note: Flower size was used as a covariate in the analysis of stigma and anther height, and stigma–anther separation. ns, not significant; *, $p < 0.008$ ($\alpha/6$).

Natural fruit set and seed set

A nonlinear relationship between morph ratio and fruit and seed set was detected in populations of *M. trifoliata*. In populations with unequal morph ratio, the rarer morph had greater fruit and seed set (Figs. 5*a* and 5*b*), although the differences were significant only in population SL-2 (fruit set: $F_{[1,5]} = 147.6$, $p < 0.0001$; seed set: $F_{[1,83]} = 32.6$, $p < 0.0001$). There were no significant differences in fruit and seed set between morphs in populations with equal morph frequency (all others $F < 6.25$, $p > 0.05/7 = 0.007$; Figs. 5*a* and 5*b*). Fruit and seed set in the two monomorphic populations was low (Figs. 5*a* and 5*b*). A higher fruit set was observed in population FR-1 (12.6%) than in population OP (1.4%), while seed set in both populations was equal (9.9%).

Discussion

Contrary to earlier studies of *M. trifoliata* (Warming 1886; Sjörs 1988), the present study found no evidence of a breakdown of distyly to favor self-fertilizing homostyles on the island of Newfoundland. Flowers with little to no stigma–anther separation were detected, but these did not show an obvious increase in fertility usually associated with breakdown of heterostyly (Barrett 1992; Washitani et al. 1994). This study suggests that multiple colonization events, combined with extensive clonal growth and the lack of pollen limitation, successfully maintained the distylous breeding system in Newfoundland.

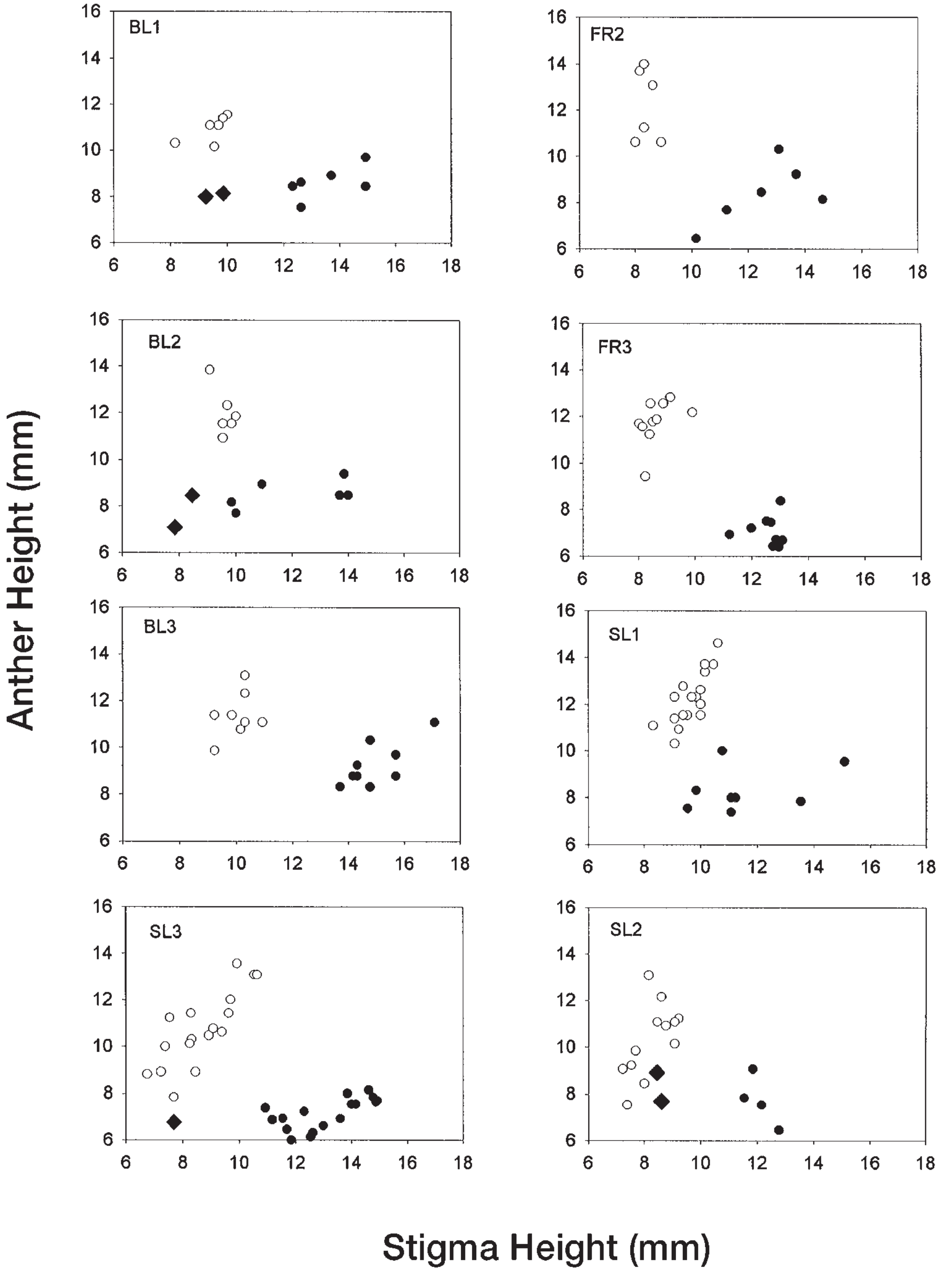
Population structure

Although self-incompatible, distylous species are predicted to reach 1:1 morph ratios in one generation (Barrett 1992), this study and others have reported populations of *M. trifoliata* with unequal morph frequencies (Nic Lughadha and Parnell 1989; Barrett 1992). Anisoplethy is typical of plants that rely heavily upon clonal propagation (Ganders 1979; Barrett 1980; Eckert and Barrett 1993). In other heterostylous species (e.g., *Eichhornia crassipes* (Mart.) Solms-Laubach), unequal morph frequencies have been attributed to a differential fitness between morphs caused by the higher reproductive capacity, or the superior cloning ability of one morph type over the other (Barrett and Forno 1982). Among populations of *M. trifoliata* with unequal morph frequencies, neither morph was consistently dominant suggesting no obvious superiority in cloning ability, nor was there evidence for differential reproductive fitness between the morphs. Dominance of a particular morph within a population is most likely the result of historical precedence (Eckert and Barrett 1995), with waterfowl acting as important vectors in the spread of *M. trifoliata* seed over long (from continental sites) and short distances (Olesen 1987).

Factors affecting natural levels of fruit and seed set

In Newfoundland populations, once morph frequency exceeds approximately 15%, fruit and seed set attain a relatively stable plateau (Fig. 5), which may indicate legitimate pollen was no longer limiting. In addition to morph ratio, the spatial distribution and the size of morph patches can affect levels of fruit and seed set (Wyatt and Hellwig 1979). In our study, quadrats contained an abundance of a single morph (Thompson 1996), indicating spatially clumped distribution of morph clones, common in aquatic plants with extensive clonal growth (Barrett et al. 1993). With dense monomorphic patches and large distances between compatible genets, it was expected that illegitimate pollen layered on the stigma would interfere with fruit and seed set (Wyatt and Hellwig 1979). Fruit set was high (>60%) in most populations suggesting intermorph movements by the pollinators deposited sufficient legitimate pollen to stimulate fruit development. Hicks et al. (1985) found very high levels of fruit set (>85%) despite spatial segregation of clones in distylous *Mitchella repens* L. However, the incomplete fertilization of all ovules within de-

Fig. 2. Scatter plots of stigma and anther heights for dimorphic populations of *Menyanthes trifoliata* from Newfoundland. See Fig. 1 for population locations. By projecting the range of observations onto each axis, the degree of overlap for anther and stigma heights are illustrated. ○, thrum; ●, pin; ◆, homostyles.



veloping fruits (i.e., seed set <50%) may be the result of the combined effects of illegitimate pollen clogging and (or) insufficient legitimate pollen. This is consistent with Nic Lughadha and Parnell's (1989) finding of less than 10 legitimate pollen grains on most *M. trifoliata* stigmas and high numbers of illegitimate grains.

Environmental factors may also affect fruit and seed set. At populations BL-1 and SL-3, the majority of the infructescences were broken off or had been removed during the period between flowering and seed dispersal. Examination of the stem remains suggested moose browsing or loss to insect stem borers was responsible for the large proportion of infructescences lost (Thompson 1996).

Floral variability

There was no evidence of a breakdown of heterostyly in *M. trifoliata*, either on the island of Newfoundland or in Greenland and Danish populations (Olesen 1987). The species exhibited traits typical of distylous species, such as bimodal distributions of anther and stigma heights, reciprocal herkogamy, self-incompatibility, and pollen dimorphism (Ganders 1979). Consistent with the findings of Nic Lughadha and

Parnell (1989), thrum pollen was larger than pin pollen in *M. trifoliata*. Flower size was similar for both pin and thrum morphs. There were no differences in ovule number per ovary, or in fruit or seed set between morphs, indicating a lack of gender specialization, with no evidence of evolution towards dioecy.

What appears to be atypical of distyly in *M. trifoliata* is the degree of overlap in the distributions of anthers, stigmas, and anther–stigma separation, as well as the lack of strict reciprocal herkogamy in many of the populations studied. Obviously distylous species segregate into two distinct groups (morphs), with little overlap in anther and stigma heights (Opler et al. 1975; Barrett 1978; Barrett and Richards 1990; Richards and Koptur 1993), while other less obviously distylous species, such as *Guettarda scabra* L. (Rubiaceae) (Richards and Koptur 1993), show various degrees of overlap in anther and (or) stigma heights (Barrett and Richards 1990; Barrett 1992). Our measurements indicate that *M. trifoliata* is intermediate between these two extremes, with overlap in both organ distributions. Stigma–anther separation (herkogamy) ultimately defines the degree of difference between morphs, and in clearly distylous species there are two distinct modes (Richards and Koptur 1993). In *M. trifoliata*, morphs exhibit significantly different separations, but flowers with similar organ heights occurred, as in *Guettarda scabra* (Richards and Koptur 1993). Again *M. trifoliata* displays greater variation than other obviously distylous species.

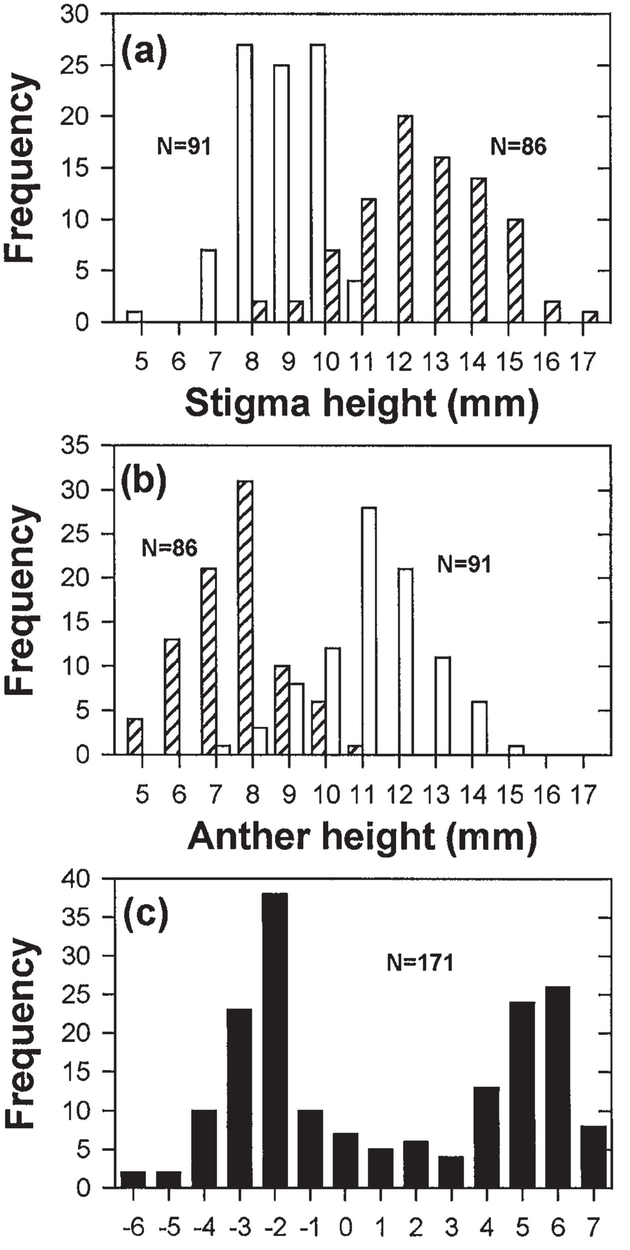
Reciprocal herkogamy is one of the defining characters of the distylous syndrome and is thought to have evolved to promote efficient pollen transfer and outcrossing between morphs (Darwin 1877; Barrett 1992; Lloyd and Webb 1992). Reciprocity in *M. trifoliata* is not symmetrical in most populations (Fig. 4). Greenland and Danish populations sampled by Olesen (1987) show a similar lack of reciprocity (Fig. 4). Compared with other distylous species (Richards and Koptur 1993, Fig. 11), *M. trifoliata* shows obvious asymmetry between morphs. Recent evidence confirms that reciprocal herkogamy can vary greatly (Barrett 1992).

Compatibility

Menyanthes trifoliata exhibited self-incompatibility typical of other heterostylous members of the family Menyanthaceae (*Nymphoides indica* (Thwaites) O. Kuntze, Barrett 1980; *Villarsia* spp., Ornduff 1986), confirming no trend towards self-compatibility on the island of Newfoundland. Seed set following incompatible pollination was low (<3%) in the three populations tested, while Nic Lughadha and Parnell (1989) found a complete lack of seed set after illegitimate pollination in a single Irish population of *M. trifoliata* they tested. Thus, it is highly unlikely that the colonization and spread of *M. trifoliata* on the island of Newfoundland involved self-fertility.

The compatibility of "homostylous" flowers was not explicitly tested, but the lack of correlation of fruit set with degree of anther separation or organ heights suggests that the homostyly is morphological rather than functional. As well, these flowers comprised a small proportion of the flowers sampled. Knowledge of whether these flowers represent homostylous genets should be confirmed (Olesen 1987) and deposition of self-pollen quantified to determine decrease in fruit and seed set (Lloyd and Webb 1992).

Fig. 3. Pooled frequency distribution of (*a*) stigma height, (*b*) anther height, and (*c*) stigma–anther separation of *M. trifoliata* from Newfoundland. □, thrum; ▨, pin.



The most obvious insect visitors to *M. trifoliata* flowers on the Avalon Peninsula included bumblebees (*Bombus sandersonii* Franklin, *Bombus terricola* Kby., and *Bombus borealis* Kby.) and syrphid flies, but many other visitors, especially flies, were observed. During the summer of 1995, floral visitors were abundant, and natural fruit and seed set were high in populations where adequate legitimate pollen was available. Fruit set was not pollinator limited at populations FR-2 and BL-3, where high levels of natural fruit set were observed in experimental pollination treatments. Seed set may have been pollen limited as a result of insufficient pollen applied to crossed stigmas (BL-3), while the low seed set of naturally pollinated flowers in FR-2 was the result of fungal infection.

Fig. 4. Relative reciprocity ratios for the long (L) organ level (pin stigmas vs. thrum filaments) versus the short organ level (pin filaments vs. thrum stigmas) for each dimorphic population of *M. trifoliata*. See Materials and methods for definition of ratio. Significant differences within levels are indicated as follows: ○, both L and S levels equal; ◑, L unequal and S equal heights; ◐, S unequal and L equal heights; ●, both L and S levels unequal. Danish and Greenland populations (◆) of *M. trifoliata* are plotted for comparison (Olesen 1987).

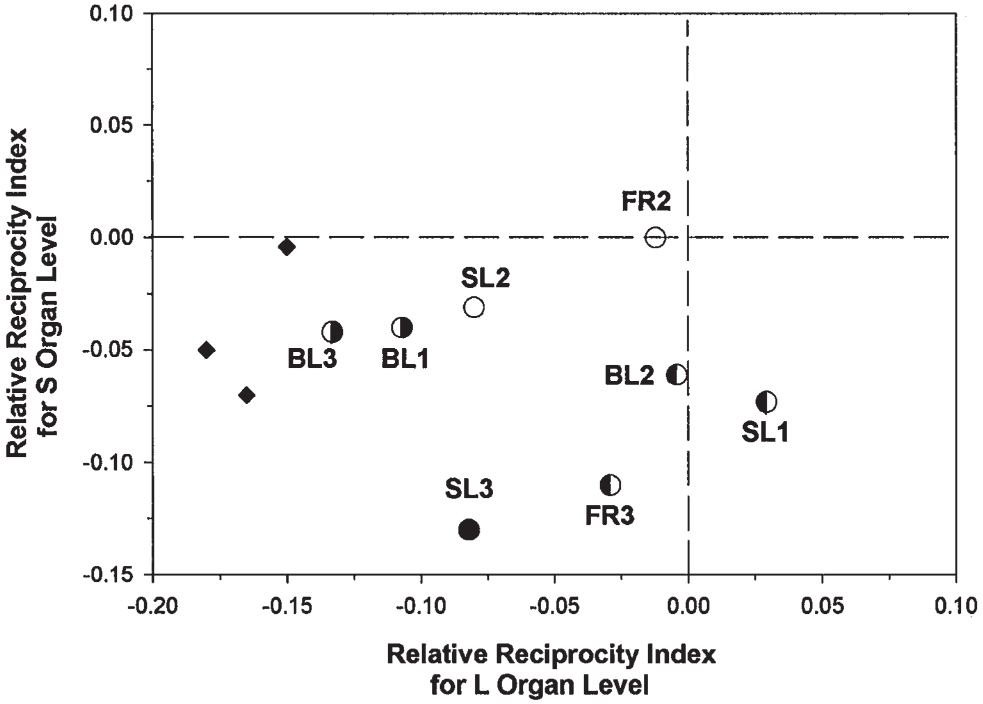


Table 5. Percentage of fruit and seed set of pollination treatments at three populations of *M. trifoliata*.

(A) Fruit set.

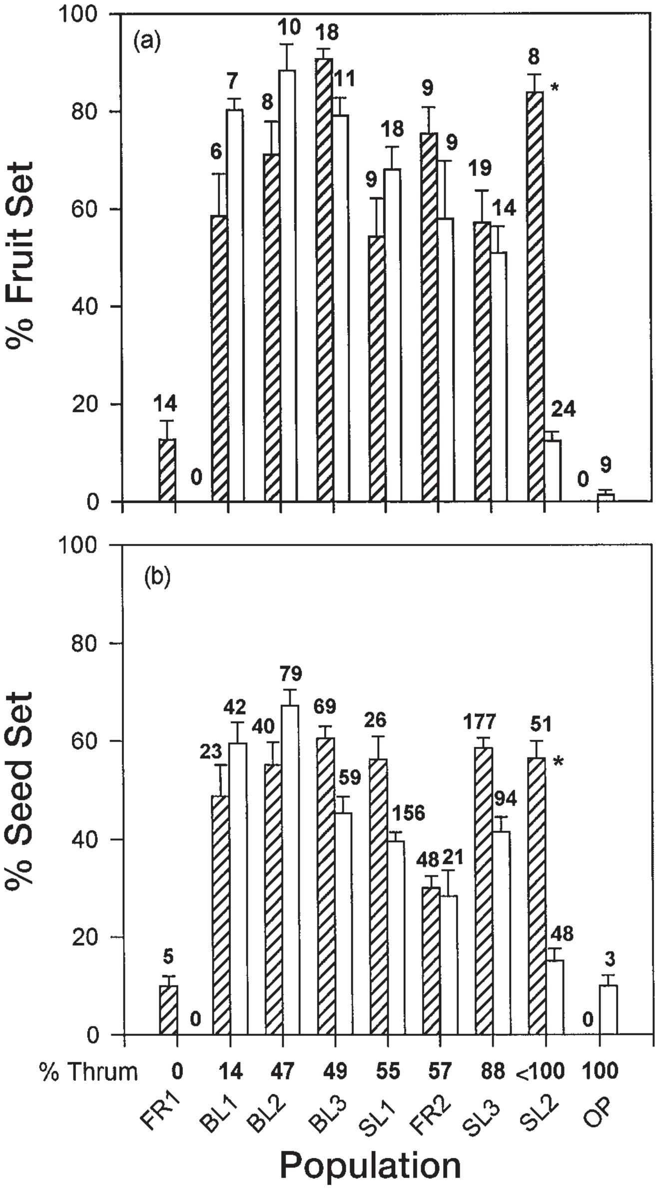
Population	Pollination treatment	*N*	Fruit set (%)	*P* Cross vs. self pollinations	 Cross vs. natural pollinations
BL-3	Autogamy	106	0.9±0.9	<0.01	ns
	Cross	10	80.0±13.3		
	Self	15	13.3±9.1		
	Natural	18	100.0±0.0		
FR-2	Cross	12	83.3±11.2	<0.01	ns
	Self	9	11.1±11.1		
	Natural	12	83.3±11.2		
OP	Cross	6	100.0±0.0	<0.05	—
	Self	4	25.0±25.0		
	Natural	—	—		

(B) Seed set.

Population	Pollination treatment	*N*	Seed set (%)	*P* Cross vs. self pollinations	 Cross vs. natural pollinations
BL-3	Cross	10	42.7±11.3	<0.001	<0.001
	Self	15	2.7±2.0		
	Natural	18	64.0±6.8		
FR-2	Cross	12	22.5±4.9	<0.001	<0.05
	Self	9	1.7±1.7		
	Natural	12	14.3±4.4		
OP	Cross	6	80.6±2.5	<0.001	—
	Self	4	1.8±1.8		
	Natural	—	—		

Note: Probabilities were determined using Fisher's exact test, df =1.

Fig. 5. Percentage (*a*) fruit set and (*b*) seed set in the nine populations of *M. trifoliata*. In each panel, populations are arranged by the proportion of thrum ramets present. The vertical line over each bar is the standard error, and numbers are sample sizes. An asterisk indicates a significant difference in fruit or seed set between morphs. □, thrum; ▨, pin.



Variable herkogamy and the asymmetry documented in *M. trifoliata* suggest a relaxation in stabilizing selection between the floral morphs of *M. trifoliata*. Selection is maintained by appropriate pollinators, which maximize legitimate intermorph pollen transfer by precise placement of pollen within each organ level (Wolfe and Barrett 1989). The pollinator fauna found on islands is usually a small subset of that found in mainland locations and is comprised of more generalist species such as flies (Spears 1987; Barrett 1996). Although detailed observation of the floral visitors to flowers of *M. trifoliata* was not done in this study, the impoverished nature of the pollinator fauna on the island of Newfoundland is clear. Insular Newfoundland has only about 40% of the Apoidea (34 of 97 species) and Syrphidae (36 of 86 species) species diversity found in mainland boreal (northern Ontario) sites where *M. trifoliata* is common (Constantine 1986; Vockeroth 1992; Kevan et al. 1993). In addition to the three species of bumblebees, a variety of visitors are commonly found foraging on *M. trifoliata* flowers, including syrphid and other species of flies, butterflies, and rarely, honey bees. The variation in size, behaviour, and morphology of potential pollinators is enormous, suggesting that the placement of pollen on the insects will be variable (Lloyd and Webb 1992; Eckert and Mavraganis 1996). In addition, the relatively open actinomorphic corolla allows short-tongued "generalist" visitors to be effective pollinators, similar to the findings in island populations of *Turnera* (Belaoussoff and Shore 1995). Fruit set within isoplethic populations is comparable with other outcrossing herbaceous species (Hicks et al. 1985), suggesting the loss in precision of pollen placement has not compromised the reproductive potential of *M. trifoliata*. The lack of correlation between stigma–anther separation and fruit set indicates that legitimate pollination is successful in the absence of strict reciprocal herkogamy between floral morphs. With a lack of pollinator precision in donation and receipt of compatible pollen, and no apparent loss of reproductive output, selection for perfect reciprocal symmetry would be relaxed, explaining the observed variation in stigma–anther separation and the wide range of stigma and anther heights observed in study populations. Under conditions of self-incompatibility, the degree of asymmetry may be limited by selection to minimize deposition of illegitimate intramorph pollen and pollen wastage, thus maintaining distinct morphs (Lloyd and Webb 1992).

In summary, to test the effect of asymmetry documented in island populations of *Menyanthes trifoliata* on morph-specific fitness, patterns of inter- and intra-morph pollen flow in flowers with variable organ levels and differential levels of reciprocity should be carried out (Lloyd and Webb 1992). In addition, future studies must compare pollen loads and placement of pollen on pollinators of varying sizes, to understand patterns of fruit and seed set (Wolfe and Barrett 1989; Barrett 1992).

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