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Sexual reproduction and population genetics of the clonal dioecious macrophyte Vallisneria americana Michx.

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Sexual Reproduction and Population Genetics of the Clonal Dioecious Macrophyte *Vallisneria americana* Michx.

by

Cynthia Lokker

A Dissertation
Submitted to the College of Graduate Studies and Research
through the Department of Biological Sciences
in Partial Fulfilment of the Requirements for
the Degree of Doctor of Philosophy
University of Windsor

Windsor, Ontario, Canada

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Abstract

Populations of clonal species are predicted to be genetically depauperate compared to non-clonal species, as clonal growth tends to be the primary mode of reproduction in these populations. *Vallisneria americana* is a dioecious, clonal aquatic macrophyte that regularly flowers and produces seed. Sites from the Great Lakes (20) and Florida (5) were assayed for allozyme polymorphisms. The sites were found to be genetically variable (mean H_{exp}=0.216), and multiclonal (D range 0.744 - 0.967). Little genetic differentiation was detected among Great Lakes sites (F_{ST}=0.038); Florida sites were somewhat differentiated (F_{ST}=0.122) corresponding to geographical disjunction, and were significantly different from Great Lakes sites (F_{ST}=0.124). This lack of differentiation among sites within a water system, coupled with high levels of genetic variation and clonal diversity, suggest that sexual reproduction and gene flow are significant evolutionary processes in *V. americana* populations.

Studies of sexual reproduction in *V. americana* showed that male and female shoots did not differentially compete with each other, nor were they allelopathic. Nutrient regimes, sediment composition and light differences were not correlated with flowering, seed production and biomass at three sites in the Huron-Erie corridor. In controlled pollinations, outcrossing and inbreeding effects were not detected in seed production or seedling vigour measures in crosses between nearby mates nor between distant mates. Differences in seedling performance were, however, associated with maternal identity. *Vallisneria*

americana populations in the Huron-Erie corridor were found to produce significant numbers of seeds, and maintain a seed bank.

Despite an efficient mode of clonal reproduction, sexual reproduction appears to play a significant role in populations of *V. americana,* a finding similar to that for a range of clonal plant and animal species. This role, and the potential for significant gene flow among sites are considered in the context of population genetics and evolutionary processes in populations of *V. americana*.

Dedication

To Jamie,

with thanks for what has passed,

and to

Jamie, Sabine, Lauren, and 'Baby Kate'

with hope for what is yet to come.

Acknowledgements

I would like to thank Dr. Lesley Lovett-Doust for her supervision and input throughout the course of my graduate studies. Her patience, understanding and support allowed me to follow my heart and concentrate on my children when they needed me most, and to get back to work when time permitted. Drs. Jon Lovett-Doust and Michael Petras provided helpful advice as my dissertation evolved. I would like to thank them, and my other committee members, Drs. Iain Samson and Joe Leach for their encouragement, advice and patience.

I would like to thank Dr. P. M. Catling and the Canadian Journal of Plant Sciences for permission to reproduce their illustration of *Vallisneria americana* (Canadian Journal of Plant Science 74: 884), and the Botanical Society of America for permission to incorporate as Chapter 2.3, the article:

Lokker, C., L. Lovett-Doust and J. Lovett-Doust. 1997. Seed output and the seed bank in *Vallisneria americana* (Hydrocharitaceae). American Journal of Botany 84: 1420-1428.

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Table of Contents

Appendix D

List of Tables

List of Figures

Chapter 1

General Introduction

Vallisneria americana

Vallisneria americana, most commonly known as American wildcelery, is a submerged aquatic macrophyte native to North America, and has been extensively described by Catling *et al.* (1994) and Korschgen and Green (1988). The species is a dioecious perennial which can reproduce both sexually and vegetatively. It has ribbon-like leaves which, in the Huron-Erie corridor, emerge from winter buds (turions) in late May. In this area (83°W, 43°N), the species produces ramets throughout the growing season, reaching peak biomass in mid-August. Flowering occurs from July to mid-September, with seed maturation being achieved by October. Turions, the overwintering buds, are formed in mid- to late September. The leaves and reproductive structures senesce completely by the end of October. Seasonal changes in biomass have been described by Titus and Stephens (1983).

Vallisneria americana has been placed in synonymy with *V. neotropicalis* and *V. gigantea*, which grows in east and southeast Asia, Oceania and Australia (Lowden, 1982). There are two *V. americana* varieties, *V. americana* var. *americana* and *V. americana* var. *biwaensis* (Lowden, 1982; Catling *et al.*, 1994); however the latter variety has not been reported in Canada (Catling *et al.*, 1994). Within *V. americana* var. *americana*, there are narrow- and broad-leaved

variants that differ in their distributions; the narrow-leafed varieties tend to be found in inland freshwater ways, lakes, and lagoons, whereas broad-leafed plants are found in coastal freshwater inlets where brackish waters are common at high tide (Catling *et al.*, 1994). The narrow-leafed variant is found in the Great Lakes (C. Lokker, personal observation), while both variants have been observed in Florida (D. Sutton, University of Florida, personal communication). A closely related species is *V. spiralis* in which both staminate and pistillate flowers are different from those of *V. americana*. This species has also not been reported in Canada (Lowden, 1982; Catling *et al.*, 1994). Manitoba specimens of *V. americana* var. *americana* had a diploid chromosome number of 20 (Löve, 1981), 2n=40 was reported for *V. americana* var. *biwaensis* (Jørgensen, 1921). All *Vallisneria* spp. are distributed widely around the world, but their native ranges are not known (Cook, 1985).

Vallisneria americana is an important component of aquatic ecosystems (Catling *et al.* 1994; Korschgen and Green, 1988). In general, the species provides food, shelter and shade for fish, insects, and other benthic invertebrates. In addition, it provides spawning habitat for fish, and the turions are an important food source for many migratory ducks, especially Canvasbacks (*Aythya valisneria*). The presence of rooted *V. americana* increases rates of sedimentation, thereby increasing nutrient availability and water clarity, plays a role in micro- and macro-element cycling, and attenuates water currents (cf Catling *et al.*, 1994; Fischer and Claflin, 1995). Its usefulness as a biomonitor of

organic (Lovett-Doust *et al.*, 1993; Lovett-Doust *et al.*, 1994; Biernacki *et al.*, 1996; 1995 a, b; Biernacki and Lovett-Doust,1997) and metal contaminants (Hudon, 1998) has been established. Under some field conditions, *V. americana* has been shown to successfully resist the invasion of exotic, weedy invading species such as *Hydrilla verticillata* (Smart and Doyle, 1994) and *Myriophyllum spicatum* (Doyle and Smart, 1994).

From another perspective, *V. americana* is considered a problem species in Southern Ontario and Quebec, where it impedes boat traffic and other recreational activities (Catling *et al.*, 1994). It also assists in the spread of adult zebra mussels attached to drifting shoots (Horvath and Lamberti, 1997). *Vallisneria americana* has also been reported to act as a sink for heavy metals (Hudon, 1998) and organic contaminants (Biernacki *et al.*, 1995 a, b, Biernacki and Lovett-Doust, 1997; Lovett-Doust *et al.*, 1997). These contaminants may then be biomagnified in the food web, or transported and redistributed downstream as rosettes senesce, detach, disperse and decompose in the fall.

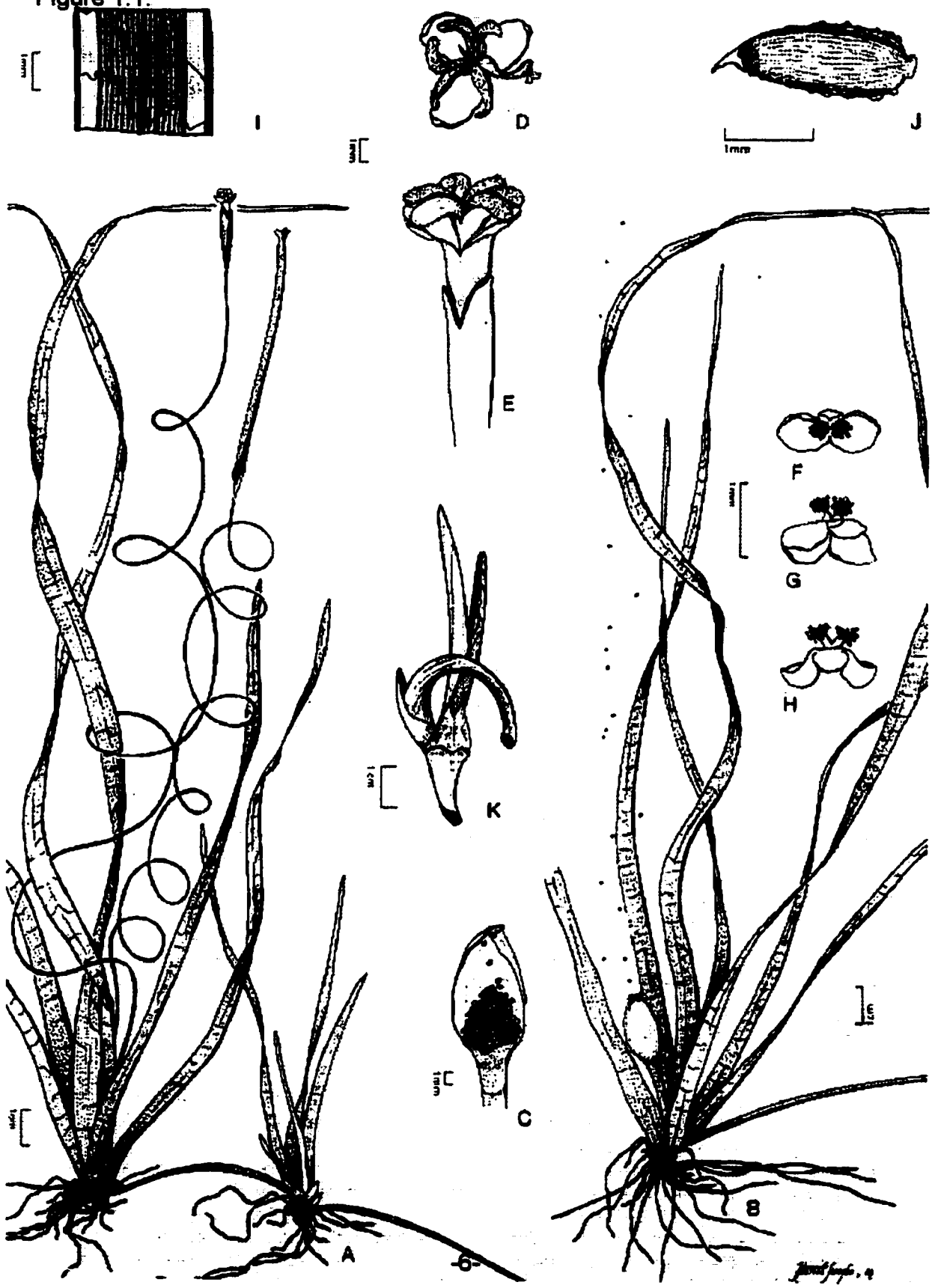
Vallisneria americana grows in various substrates to depths of 0.3 - 7 m, generally at pH>6.0 (Catling *et al.*, 1994). It is common in quiet waters in southern Canada and the Dakotas, and its range extends south to Florida and Texas. It has recently been introduced to the Pacific Northwest, Washington, Oregon and British Columbia (Catling *et al.*, 1994). The species shows remarkable halotolerance for a freshwater plant (Kraemer *et al.*, 1999). This may explain its presence in estuaries of Florida and along the eastern coast of the

United States. *Vallisneria americana* has recently been reported as the most abundant submersed macrophyte species in the Huron-Erie corridor of the Great Lakes (Schloesser and Manny,1986, 1990; Schloesser *et al.*, 1985; Leach, 1991), having rebounded from a drastic 72% decline in turion numbers in the Detroit River between 1950-1985 (Schloesser and Manny,1986, 1990; Schloesser *et al.*, 1985). A comparable population decline and recovery is reported for Put-in-Bay, Ohio (Stuckey and Moore, 1995).

The complex and intricate pollination process is epihydrophilous; stigmas are raised to the water surface, to which floating male flowers are carried by wind/water currents, or surface tension (Wylie, 1917; Sculthorpe, 1967; Cook, 1988). Pollination has been described as occurring in a 2-dimensional environment in that pollen travels at the air-water interface, and its delivery is affected by the capillary behaviour of the water surface (Cox and Knox, 1989; Laushman, 1993). The pistillate flowers are solitary and borne on a peduncle that elongates to the water surface (Figure 1.1). Staminate flowers are numerous and originate from a spathe on a basal stalk, near the sediment-water interface (Figure 1.1). The spathe opens to release the male flowers, which float to the water surface. The male flowers, termed 'boats', which remain dry throughout, are moved about in groups by wind and water currents (Wylie, 1917; Cook, 1988; Titus and Hoover, 1991). It is at the water surface that pollination occurs. The female flower forms a slight bubble on the surface, into which the male 'boats' slide (Svedelius, 1932; Cook, 1982). The stamens then rub against the

Figure 1.1. Schematic diagram of *Vallisneria americana* shoots and flowers: A, pistillate plant with pistillate flower and a young fruit; B, staminate plant with a spathe; C, dissected spathe showing numerous staminate flowers; D-E, top and side view of the pistillate flower, showing the slender tube, three petals, three divided stigmas and a small staminate flower attached on the stigma; F-G-H, top and side views of the staminate flower showing calyx three-parted and the two stamens; I, portion of the leaf showing venation; J, detached overwintering stolon bud (turion). Reprinted from P. M. Catling, K. W. Spicer, M. Biernacki and J. Lovett-Doust 1994. The biology of Canadian weeds. 103. *Vallisneria americana* Michx. Canadian Journal of Plant Science 74: 884. with permission from P. M. Catling and the Canadian Journal of Plant Science.

Figure 1.1.



stigmas, fertilizing the female flower. *Vallisneria* species are unique in that the female peduncle forms a spiral and retracts after fertilization (Lowden, 1982), pulling the developing seed pod underwater.

Resource allocation to flowering in *V. americana* is generally low, ranging from 1-10% of biomass (Titus and Stephens, 1983; Donnermeyer and Smart, 1985; Lovett-Doust and LaPorte, 1991; Madsen, 1991). In the field, not all *V. americana* rosettes flower in a given season; reported flowering percentages range from 5% to 72%, with sites often having highly biased tertiary sex ratios (Lovett-Doust and LaPorte, 1991; Laushman, 1993; Lokker *et al.*, 1994). A laboratory study has shown that 88% of female plants flower if they achieved a threshold dry mass size of 0.75 g; for males there was no size threshold for flowering (Titus and Hoover, 1991). In the field, however, females did not show a size threshold for flowering (Titus and Hoover, 1991). Lovett-Doust and LaPorte (1991) determined that approximately 50% of (male) pollen grains were sterile. Pollen dispersal is confined to a single water body since it never becomes airborne (Les, 1988). Sullivan and Titus (1996) found that pollination success was related to male density and pollen retention time.

Seeds are produced in cylindrical pods, embedded in a gelatinous matrix (Catling *et al.*, 1994; Ferasol *et al.*, 1995). This matrix keeps the seeds wet, and may also aid in dispersal of seeds by adhesion to animals, mostly waterfowl (Korschgen and Green, 1988). Dispersal may also be assisted by water currents and sediment movement (Korschgen and Green, 1988; Catling *et al.*, 1994,

McFarland and Rogers, 1998), although Kaul (1978) reported that seeds tend to settle close to their parents. Lovett-Doust and LaPorte (1991) recorded seed production in natural populations in the Lake Huron-Lake Erie corridor, and found a range of 167-288 seeds per pod. These seeds had high viability (93-98%). Laushman (1993) reported an average of 318±85.6 seeds per pod at Put-in-Bay, Lake Erie. In contrast to these high levels of fecundity, naturally-occurring seedlings have rarely been observed (Titus and Hoover, 1991; Lokker *et al.*, 1997; C. Lokker, personal observation; and see Chapter 3.4), and their demographic characterization would require careful, repeated tracking during peak germination and establishment time to be adequately assessed. Titus and Hoover (1991), in a small seedling establishment study, observed the survival of 16 out of 24 *V. americana* seedlings which had been planted in a monitored site; these survivors reached a mean size of 1.9 g dry mass and 6.3 rosettes per plant over 4 months. Kimber *et al.* (1995) suggested that beds of *V. americana* decimated by flooding events in Chesapeake Bay may be re-established from seedlings.

Vegetative reproduction is accomplished through the production of ramets and turions in *V. americana*. Ramets or vegetative rosettes are produced along stolons throughout the growing season. Korschgen and Green (1988) reported the production of as many as 20-40 rosettes from one individual (parent plant) in a season. At the end of the growing season, most rosettes produce buried turions which act as dormant overwintering structures. These turions form at the

end of stolons; each rosette may thereby produce numerous turions that may act as dispersal units in the fall or in the spring. In the spring, turions germinate and produce rosettes, which then resume clonal growth via further stolon production. There has been no evidence to suggest that gender is labile in *V. americana*, ie., shoots or ramets of a clone express the same gender, if they flower, of the mother ramet (Korschgen and Green, 1988; Catling *et al.*, 1994).

The aquatic environment

The aquatic environment has long been characterized as more stable than the terrestrial environment, given the potential for physical and chemical properties of water to buffer and moderate environmental influences (Sculthorpe, 1967; Hutchinson, 1975). This perception is not as widely held today (e.g. Laushman, 1993; Rea and Ganf, 1994 a, b, c), yet a large body of literature is based on this assumption. On a small scale, Cook (1987 b) describes the aquatic environment as a mosaic, similar to the heterogeneity recognized in the terrestrial environment, an opinion shared by Laushman (1993) and Rea and Ganf (1994 a, b, c).

Growth of aquatic macrophytes in relation to environmental parameters has long been studied. Some factors which have been shown to impact on growth and morphology in a variety of aquatic species include: sediment texture and organic matter content (Barko, 1982; Barko and Smart, 1986; Chambers and Kalff, 1987), pH (Grisé *et al.;* 1986; Titus *et al.*; 1990; Overath *et al.*; 1991, Titus

and Hoover, 1993), CO_2 (Titus *et al.*, 1990; Vadstrup and Madsen, 1995), light (Titus and Adams, 1979; Barko and Smart, 1981; Carter and Rybicki, 1985; Chambers and Kalff, 1987), herbivory (Carter and Rybicki, 1985), dissolved inorganic carbon content of the water column (Overath *et al.*, 1991), nutrients (Barko, 1982; Duarte, 1995), temperature (Barko and Smart, 1981; Barko *et al.*, 1986), organic contamination (Lovett-Doust *et al.*, 1994; Biernacki *et al.*, 1996, 1995 a, b; Biernacki and Lovett-Doust, 1997), metal contamination (Hudon, 1998) and overall site quality characterized by a number of the above factors.

Recent studies of aquatic macrophyte populations invoke drought (Kimber *et al.*, 1995), water level fluctuations (Rea and Ganf, 1994 b), turbidity (Carter and Rybicki, 1985; Crowder and Painter, 1991; Carter *et al.*, 1994; Fischer and Claflin, 1994; Rogers, 1994; Stuckey and Moore, 1996) and pollution (Schloesser and Manny, 1990) as environmental causes for declines in macrophyte populations.

Light penetration and attenuation in aquatic environments depend on water depth, flow and colour, which are in turn affected by shading and the concentrations of suspended organic and inorganic particles and phyto- and zoo-plankton (Sculthorpe, 1967; Hutchinson, 1975). Light is a key variable determining the distribution and abundance of aquatic macrophytes (Barko *et al.*, 1986; Chamber and Kalff, 1987; Kautsky, 1988; Carter *et al.*, 1996; Korschgen *et al.*, 1997).

Water can buffer temperature changes, compared with the aerial

environment (Sculthorpe, 1967; Hutchinson, 1975), but temperature regimes, interacting with light, can limit aquatic macrophyte growth and abundance (Barko and Smart, 1981; Barko *et al.*, 1982; Duarte, 1991).

Rooted submersed plants obtain nutrients from the water column and the sediment substrate (cf Barko *et al.*, 1986; Gopal, 1990). The major ions required for plant growth are potassium, calcium, magnesium, iron, nitrate, sulphate, ammonium, chloride, phosphate and bicarbonate (Sculthorpe, 1967; Galston *et al.*, 1980; Stern, 1985). Nutrients may often limit macrophyte growth (e.g. Barko and Smart, 1986; Kautsky, 1988; Rogers *et al.*, 1992; van Lent *et al.*, 1995).

Rates of vegetative and sexual reproduction in aquatic macrophytes are also affected by environmental parameters. Low pH has been shown to reduce both the size and numbers of turions in *Vallisneria americana* (Grisé *et al.*, 1986; Titus and Hoover 1993). In *Hydrilla verticillata*, Miller *et al.* (1993) found that turion production increased with decreasing daylength (<12 hours), and decreased with increasing plant density. Nitrogen enrichment prompted a significant increase in flowering in *Zostera marina* (van Lent *et al.*, 1995), whereas increasing organic contamination caused a reduction in flowering in *V. americana* (Biernacki *et al.*, 1995 a, b). Titus *et al.* (1990) and Titus and Hoover (1991) found that flowering frequency in *V. americana* was correlated with plant biomass, suggesting that factors limiting growth may also limit reproduction. As for all plant species, the environment within which aquatic macrophytes grow is a key factor affecting growth, reproduction and distribution.

Reproduction in aquatic vascular plants

In many aquatic plants reproduction can be both sexual and vegetative, although vegetative propagation is thought to predominate (Sculthorpe, 1967; Hutchinson, 1975; Barrett *et al.*, 1993). Vegetative increase is hypothesized to be more suitable in the aquatic environment because this mode allows well-adapted genotypes to proliferate in what is considered by some to be a stable environment (Sculthorpe, 1967; Hutchinson, 1975; Les, 1988; but see Laushman, 1993). Sexual reproduction is often presumed to be less important in population growth and maintenance (Sculthorpe, 1967; Hutchinson, 1975; Les, 1988), yet has the advantage of increasing genetic variation (Hutchinson, 1975; Watkinson and Powell, 1993) and effective population size (Hartl and Clark, 1989; Barrett et al., 1993).

A demographic distinction has been made in plant communities comprising sexually derived individuals and clonally produced ramets because they essentially represent two levels of population structure (Harper, 1977). These levels are described as the number of genets in a population defined as N (i.e., the number of genetically different individuals), and the number of ramets represented as Nn (i.e., sub-individual units [Harper, 1977; Lovett-Doust, 1981]). This distinction is made since the demographics of a population are affected by changes in either the number of genets and/or the number of ramets (Harper, 1977). Yet, clonal subunits (ramets) are not to be counted as equal to the genets in a population; the genet is the summation of its constituent ramets (Harper,

1977; Lovett-Doust, 1981). Evolutionary fitness is measured in terms of sexually-produced progeny, and not the size of the genetic individual. So, while having many ramets increases the number of opportunities a genet has for successful sexual reproduction and high evolutionary fitness, the ramets are not progeny in an evolutionary sense.

From a population genetics standpoint, the effective population size (N_e) differs from the total number of individuals (N) in a population because it is determined by the number of individuals contributing to the following generation (Ayala, 1982), and not on the number of individuals in a population. N_e is a more meaningful measure in the assessment of evolutionary processes (Husband and Barrett, 1992; Barrett et al., 1993). Estimates of N_e represent the number of individuals in an 'ideal' population with the same variance in allele frequencies or level of inbreeding as observed in the actual population (Hartl and Clark, 1989; Barrett et al., 1993). Among factors that result in deviations of N_e from N are fluctuating population size, gene flow, biased sex ratios, non-random mating, the number of fecund individuals and overlapping generations (Hartl and Clark, 1989; Barrett et al., 1993). The specific effect of clonal growth on N_e is not well understood (Barrett et al., 1993), but has been reported to result in a decrease in N_e (Mayes et al., 1998). Estimates of N_e can be based on population data (numbers of breeding individuals, sex ratio, mating system and variation in reproductive output; Husband and Barrett, 1992) or genetic data (Hartl and Clark, 1989; Barrett et al., 1993). Where estimates of N_e are reported, their

values are in general significantly lower than the value of N (cf Barrett et al., 1993). Sexual reproduction and random mating result in values of N_e approaching N, thereby increasing the number of individuals relevant to evolutionary processes.

Les (1988) proposes that the prevalence of dicliny (the separation of male and female flowers) in hydrophilous (wind-pollinated) species may be a relict characteristic. He bases this on findings that genera with a majority of hydrophilous species are species poor (i.e., have undergone little speciation) and such species also tend to show low levels of genetic diversity (i.e., rely on vegetative propagation). Les (1988) further states that few extant hydrophilous species meet the criteria for outcrossing, which are sexual reproduction, xenogamy, genetic differences between parents and survival of offspring. He hypothesizes that traits associated with hydrophilous species represent an adaptive shift to asexual reproduction as a means of preserving well-adapted gene complexes. These traits include inefficient pollen transfer, reduced flowering, widespread clonal growth and reduced seed production (Les, 1988).

Although *V. americana* is not strictly hydrophilous, its pollination mechanism mimics hydrophily in that pollen is moved above the water surface (Les, 1988). For a number of clonal aquatic macrophytes, especially *V. americana* extensive flowering and seed set have been observed (Lovett-Doust and LaPorte, 1991; Laushman, 1993; Sullivan and Titus, 1996; Lokker *et al.*, 1997). Successful sexual reproduction may be considered rare in clonal aquatics

for a number of practical reasons: rarity of suitable germination coinciding with seed set, seedlings being small and overlooked, and establishment events being rare and patchy (Rea and Ganf, 1994 b). Although recruitment from seed is rare or infrequent in most clonal plant species (Eriksson, 1992), a seedling recruitment rate of 3% is sufficient to maintain genetic and clonal diversity within a population (Watkinson and Powell, 1993). As a result, irregular establishment events over extended time scales need to be considered as they may significantly impact vegetation and populations (Rea and Ganf, 1994 b).

Aquatic macrophytes have evolved several structural modifications related to reproduction in the aquatic environment (Sculthorpe, 1967). Peduncles, leaves or lateral shoots are able to present flowers above the waters surface, although the flowers themselves are very similar to those of terrestrial plants (Cook, 1982). Pollen vectors include insects, wind and water (Sculthorpe, 1967). Surface-pollinated dioecious plants tend to have females borne on flexible peduncles with freely-floating male flowers (Sculthorpe, 1967; Hutchinson, 1975).

Clonal growth structures in aquatic macrophytes are similar to those observed in terrestrial species, and include rhizomes, runners, stolons, tubers and turions (Barrett *et al.*, 1993). For many species, clonal growth is key for colonization (Cook, 1987 a) and population maintenance. *Posidonia oceanica* (Procaccini and Mazzella, 1998), *Phragmites australis* (Pellegrin and Hauber, 1999), *Hydrilla verticillata* (Ryan, 1989), and *Podostemum ceratophyllum*

(Philbrick and Crow, 1992) are among the species in which some populations appear to be maintained by clonal growth alone. This process can effectively extend the longevity of a genetic individual, slowing the loss of genetic diversity (Widén *et al.*, 1994; Mayes *et al.*, 1998).

Clonal growth strategies among species are varied, but the two extremes of rapid, linear growth (guerrilla strategy) and slow advancement of clumped ramets (phalanx strategy) have extensively been used to characterize species (Lovett-Doust, 1981). These different strategies influence the population genetic structure and genetic processes within populations. Genets of species with phalanx cloning patterns are expected to be clumped (Widén *et al.*, 1994). A guerilla cloning strategy is expected to result in mosaic genet distributions, especially if the species grows in a heterogenous environment (Widén *et al.*, 1994). Genet mapping has been carried out by few other authors; in *Glechoma hederacea* clones are found in clumped arrangements reflecting a phalanx clonal growth strategy (Widén *et al.*, 1994). Field observations of stolon growth and shoot emergence patterns of *V. americana* are consistent with a guerrilla strategy (C. Lokker, personal observation).

Dispersal of aquatic macrophytes usually occurs by water, with wind and animals as secondary but significant vectors (Cook, 1987 a). Both sexual (pollen and seeds) and clonal propagules (specialized buds such as bulbils, hibernacula, and turions), including vegetative shoot fragments, can be dispersed by these modes (Cook, 1987 a).

Population genetics of aquatic vascular plants

Recent advances in molecular techniques have allowed population biologists to assess allelic and genotypic variability in organisms based on variation observed at the level of allozymes and DNA (Allard and Kahler, 1971; Allard *et al.*, 1975; Loveless and Hamrick, 1984; Hamrick and Godt, 1989; Barrett *et al.*, 1993; Waycott, 1998). Allele and genotype frequency data allow for genetic variability to be estimated. Based on these values, the distribution of genetic variability can be assessed within and among populations of a species (Wright, 1940, 1943, 1946,1951 ; Hartl and Clark, 1989).

Estimates of genetic differentiation can also be made in terms of the genetic distance between populations (Nei, 1972, 1978), the number of migrants per generation (Slatkin and Maruyama, 1975; Slatkin, 1981), and outcrossing rates (Hedrick, 1985; Waycott and Sampson, 1997). These estimates are used to describe mating systems and to infer such population processes as gene flow, migration and differentiation (Hartl and Clark, 1989; Barrett *et al.*, 1993).

Life history and mating system are key factors influencing genetic variation and differentiation in populations of terrestrial plant species (Loveless and Hamrick, 1985; Hamrick and Godt, 1989). Species which are outcrossed, wind-pollinated, and/or long-lived perennials tend to have higher genetic diversity within populations and lower genetic differentiation among populations (Loveless and Hamrick, 1985; Hamrick and Godt, 1989). On the other hand, species which frequently inbreed, are insect pollinated, and/or are annuals, tend

to show lower genetic diversity within populations and higher differentiation among populations as a result of genetic drift (Loveless and Hamrick, 1985; Hamrick and Godt, 1989).

High phenotypic plasticity, prolific clonal growth, potentially limited sexual reproduction, a pollination mechanism similar to hydrophily, and water-dispersed diaspores are characteristics distinctive to aquatic plants (Barrett *et al.*, 1993). The potential genetic consequences of these traits vary from limiting genetic variability to providing opportunities for local and long-distance dispersal (Barrett *et al.*, 1993).

Most studies on aquatic plants show low genetic variability, high allele fixation rates and low sexual reproduction (Les, 1988; Ryan, 1989; Philbrick and Crow, 1992; Laushman, 1993; Waycott, 1998; Pellegrin and Hauber, 1999). In contrast, however, other studies have found significant levels of sexual reproduction at some sites (Harrison and Durance, 1991; Laushman, 1993; Lokker *et al.*, 1994), and significant levels of genetic and clonal diversity (Laushman, 1993; Lokker *et al.*, 1994; Procaccini and Mazzella, 1998).

The relative importance of clonal and sexual reproduction has repeatedly been found to be a significant factor influencing population genetic variability and clonal structure in species reproducing by both of these modes (Watkinson and Powell, 1993; Holderegger *et al.*, 1998; Chaplin and Ayre, 1997; Shufran *et al.*, 1997).

Objectives

This dissertation addresses questions concerning sexual reproduction in the clonal, dioecious, submerged aquatic macrophyte, *Vallisneria americana* and the potential impact this mode of reproduction has on the population genetic processes occurring in natural populations.

Chapter 2 introduces the electrophoretic technique used to assess genetic variability in the species, and to characterize variation observed at a number of levels. The variability (1) between adjacent ramets at a site, (2) within and among local sites, (3) between sites arrayed along the Great Lakes system, and (4) between these Great Lakes sites and sites of the same species growing in Florida is described and analysed.

Chapter 3 contains studies that quantify and characterize sexual reproduction in *V. americana*. The effects of ramet density and nutrient availability on flowering and reproduction were investigated. The effect of outcrossing on seed production and seedling growth was tested experimentally. Finally, the presence and size of the "seed bank" reserve of the species was estimated and related to sexual reproduction in the previous season.

Chapter 4 briefly synthesizes the new knowledge of sexual reproduction and population genetic variability in *V. americana* in the context of plant population genetic processes.

Chapter 2

Population Genetic Structure in *Vallisneria americana*

Introduction

Genetic diversity in plants has been shown to be related to the geographic range, and breeding system of a species (Loveless and Hamrick, 1985; Hamrick and Godt,1989). Predominant outcrossers, and species with wind pollination have been found to have higher levels of genetic diversity within populations, and lower levels between populations than do species which self or those with insect pollination (Loveless and Hamrick, 1984; Hamrick and Godt, 1989). Thus the capacity of a species for gene flow is an important determinant of genetic structure within and among plant populations (Loveless and Hamrick, 1984; Hamrick and Godt, 1989).

Clonal growth is often assumed to be the predominant mode of propagation in aquatic macrophytes (Sculthorpe, 1967; Les, 1988; Barrett *et al.*, 1993), however, recent reviews considering populations of highly clonal species have revealed populations which are typically genetically diverse and multiclonal (Ellstrand and Roose, 1987; Hamrick and Godt, 1989, Widén *et al.*, 1994). Such diversity can be maintained primarily through sexual reproduction.

Les (1988) hypothesized that hydrophilous aquatic plants, in particular, would have low levels of genetic diversity due to their potentially inefficient

pollination system, and their propensity to reproduce clonally. There was some support for this hypothesis in recent studies of *V. americana* in Ohio (Laushman, 1993), although high genetic diversity was reported for a population of *V. americana* in the Detroit River (Lokker *et al.*, 1994).

Objectives

The population genetics of *V. americana* was assessed systematically at a number of levels: shoot-to-shoot and year-to-year (Section 2.1); among sites within the same water system, and among distant sites (Section 2.2).

The objectives of the survey of local sites (Section 2.1) were to estimate and describe genetic variation and clonal structure within and among three local sites and to test the hypotheses that (1) *V. americana* sites were multiclonal; and (2) gene flow occurred between nearby sites within a water system .

In the extended geographic survey (Section 2.2) the objectives were to estimate and describe genetic variation within and among distant sites. The hypotheses tested were that (1) genetic distance between sites increased with geographic distance; (2) geographically distant sites were genetically differentiated from each other; and (3) genetic diversity increased downstream as a result of the downstream nature of dispersal. Based on the findings of the population genetic structure studies, the significance of gene flow and sexual reproduction as evolutionary processes in *V. americana* populations was examined.

These objectives were achieved by sampling and assessing the genetic composition of *V. americana* sites at varying spatial and temporal scales. First, at three local sites all adjacent plants along linear transects were sampled to describe plant-to-plant genetic variation (Section 2.1). Comparisons of genetic variation within and between sites within the same water system with the genetic variability within and between distant sites were made by surveying 25 sites in the Great Lakes and in Florida (Section 2.2).

Methods

Electrophoretic methods

Allard *et al.* (1975) in an early review of isozyme studies in plant population genetics outlined four main advantages to gel electrophoresis: (1) many single gene characteristics become available for study, (2) individual alleles at each locus are revealed, (3) banding phenotype corresponds to genotype since most banding patterns are co-dominant, and (4) the methodologies are adaptable to many animal and plant species. Isozyme studies can be used to assess mating systems, selection and multilocus spatial structure of populations (Allard *et al.*, 1975).

Cellulose acetate gel electrophoresis

Cellulose acetate gel electrophoresis was used to assay genetic variability in allozymes in *V. americana*. Cellulose acetate has a number of advantages over other commonly used gel media such as starch and acrylamide. Gel run times are much shorter, often only 10 minutes in duration, allowing a larger number of samples to be assayed (Hebert and Beaton, 1989). Also, the quantity of reagents required is considerably reduced and, as a number of authors have reported, the technique requires as little as 0.5-2 μL of sample, thereby providing greater sensitivity and excellent resolution (Hebert and Beaton, 1989, Yang *et al.*, 1999).

Protocol

Procedures for allozymic analysis of *V. americana* were first developed by Drs. Lesley Lovett-Doust and David Susko in 1991, with 21 enzyme systems investigated in a preliminary survey. Of these, six enzymes showed consistently clear resolution and staining intensity. These were phosphoglucomutase (PGM), malic enzyme (ME), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6PGDH), malate dehydrogenase (MDH), and phosphoglucose isomerase (PGI) (Table 2.0.1). Stolon material, the underground structures connecting ramets, provided the best tissue for results. The specific reagents, quantities and protocols are given in Appendix A.

Table 2.0.1. List of enzymes assayed in *V. americana* with their E.C. number. Locus refers to putative loci identified for *V. americana*.

Enzyme	Locus	E.C. number
Phosphoglucomutase	PGM-1,2	2.7.5.1
Malic enzyme	ME-1,2	1.1.1.40
Isocitrate dehydrogenase	IDH-1	1.1.1.42
6-phosphogluconate dehydrogenase	6PGDH-1	1.1.1.44
Malate dehydrogenase	MDH-1,2	1.1.1.37
Phosphoglucose isomerase	PGI-1,2	5.3.1.9

Band interpretation

Based on the quaternary structure of enzymes, theoretical banding patterns can be predicted for multi-allelic loci. Figure 2.0.1 depicts the theoretical expectations of two-allele loci for monomeric, dimeric and tetrameric enzymes. Often electrophoretic analysis is unable to uncover all bands, especially if different enzyme subunits have similar electrophoretic mobility. In such instances a working hypothesis is developed that best accounts for the observed banding patterns (Richardson *et al.*, 1986).

For the patterns observed in *V. americana*, working hypotheses were developed for the loci PGM-2, ME-1, IDH-1, 6PGDH-1, MDH-2 and PGI-1; all loci which were consistently interpretable and well-resolved (Table 2.0.2). A number of putative loci were genetically uninterpretable and were therefore not included in the formal population genetic measures. These included PGM-1, ME-2, MDH-3 and PGI-2. These loci were, however, included in an analysis of allozyme patterns, where clonal diversity based on 11 characters was assessed.

When more than one putative locus (isozyme) was detected for an enzyme, they were numbered sequentially, with the isozyme travelling the least (i.e., remaining closest to the cathode) labelled 1, and all subsequent loci numbered sequentially. Allelic variation at a locus was coded alphabetically, with A representing the band closest to the cathode, and subsequent bands being labelled B, C, etc. sequentially. In accordance with standard analytical procedures, any allele with a population frequency lower than 0.05 was not

Figure 2.0.1. Theoretically expected banding patterns for mono-, di- and tetra-meric enzymes in a simple one locus, two allele system (Richardson *et al.*, 1986). Gray bands at the top represent the origin (site where sample extracts are applied). Black bands for heterozygous di- and tetra-meric enzymes (enzymes with 2 and 4 subunits) differ in size to reflect differences in staining intensity. Thinner bands reflect a lower concentration of enzyme composed of homozygous subunits, whereas thicker bands reflect higher concentrations of enzyme composed of heterozygous subunits. In enzymes composed of more than one subunit, all combinations of subunits associated with each allele are possible.

Figure 2.0.1

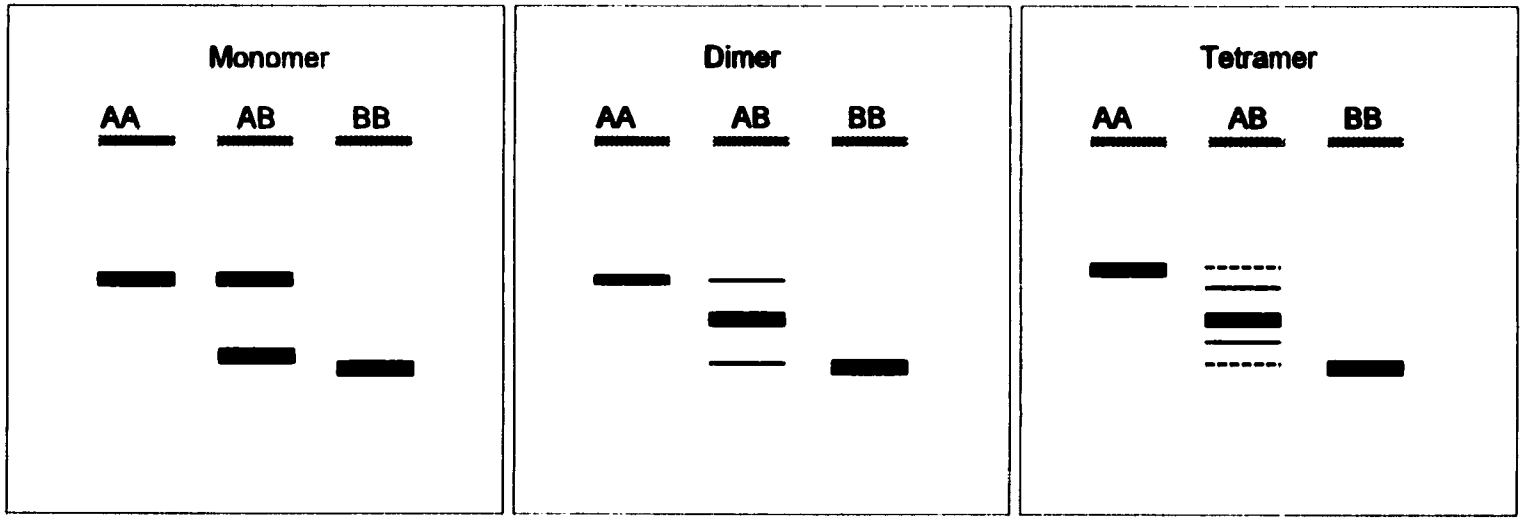


Table 2.0.2. Enzyme systems assayed, quaternary structure, working hypothesis of its genetic nature based on banding patterns observed and allelic designations. Loci that were not genetically interpretable, but were used in allozyme banding pattern analysis, are included below.

Locus	Structure	Observations	Hypothesis	Allele designation
PGM-2	monomer	-2 bands, both present	1 allele, represented by 2 bands	A
		-or none		
ME-1	tetramer	- 4 different bands	3 alleles in Canada	A, B, C
		- combinations of 1, 2, or none	3 alleles in Florida	A, B, D
		- one band only in Canada,		
		- another band only in Florida		
IDH-1	monomer	- 2 bands	2 alleles	A, B
	or dimer	- combinations of 1 or 2		
6PGDH-1	dimer	- 2 bands	2 alleles	A, B
		- combinations of 1 or 2		
MDH-2	dimer	- 2 bands	2 alleles	A, B
		- combinations of 1 or 2		
PGI-1	dimer	- 3 bands	3 alleles	A, B, C
		- combinations of 1 or 2		

Uninterpretable Locus	Observations	Banding patterns recognized
PGM-1	- 3 bands - combinations of 1, 2, 3 or none	11, 12, 13, 123, 33, 22, 0
ME-2	- 3 bands - combinations of 1, 2, 3 or none	11, 12, 22, 123, 0
MDH-3	- 2 bands - combinations of 1, 2, or none	11, 12, 0
PGI-2	- 2 bands - combinations of 1, 2, or none	11, 12, 22, 0

included in the genetic data analysis (Hartl and Clark, 1989).

Electrophoresis

All stolon samples were placed in 1.5 ml eppendorf tubes immediately after sampling and kept on dry ice or in liquid nitrogen until they were returned to the lab. There they were stored in an ultracold freezer at -80 C until they underwent electrophoresis. Running conditions for the six enzyme systems are outlined in Table 2.0.3. Appendix A describes the specifics of the cellulose acetate gel electrophoresis protocol, the equipment used, and reagents and quantities for the various stains and buffers used.

Genetic data analysis

Since *V. americana* propagates both vegetatively and sexually, any site will contain a certain number of genets (genetic individuals) and each of these will comprise a certain number of ramets (individual shoots). In order to properly designate the products of clonal growth, most genetic data were analysed in two distinct ways. First, genetic parameters were estimated treating each ramet collected as a separate individual. Second, these same parameters were estimated for genets (putative clones). Assignment of clonal identity was based on gender and banding patterns at the six genetically interpretable loci. Multi-locus allozyme phenotypes (hereafter termed allelomorphs) were then assigned for each individual that showed readable results for all six loci. The allelomorph

Table 2.0.3. Running conditions for the six enzyme systems investigated here.

Enzyme	Buffer	Voltage	Running Time
PGM	Tris Glycine, pH 8.5	100 V	45 min
ME	Tris Glycine, pH 8.5	100 V	45 min
IDH	Tris Citrate, pH 7.0	100 V	45 min
6PGDH	Tris Citrate, pH 7.0	100 V	45 min
MDH	Tris Citrate, pH 7.0	100 V	45 min
PGI	Citric Acid Aminopropyl Morpholine (CAAPM), pH 6.0	100 V	90 min

therefore comprised seven characteristics (six genetic loci + gender). Any individuals within a site that shared an allelomorph designation were classified as members of the same putative genet. Each putative genet was then described in terms of its constituent genotypic and allelic frequencies, allowing genetic parameters to be estimated for putative genets as well as for individual ramets, as described above. For each of the three local sites, maps of the sampled transects were produced to show the extent of all genets using the above allelomorph designations.

These two approaches to analysis of the data yield two distinct perspectives on population genetic parameters. The ramet-based values will include both sexually- and vegetatively- produced units, therefore inferred levels of inbreeding will likely be exaggerated. On the other hand, the genet-based values may underestimate genetic diversity, since any individuals sharing allelomorphs for the loci studied here may be genetically different at other loci which were not surveyed (type II statistical error).

The data for the three local sites were also assessed in a third way, whereby allelomorphs were assigned to individuals having readable banding patterns at all 10 putative loci described in Table 2.0.2, despite the lack of an explanation for the banding patterns observed. Those individuals which lacked bands at a particular locus, including the interpretable PGM-2 and ME-1 (see Table 2.0.2), were included. This was done under the assumption that a lack of enzyme response could reflect a genetic difference. Since the genetic basis of a

number of these loci was uninterpretable, these banding pattern distinctions could not be described in terms of genotype and allele frequencies. Rather, only clonal diversity and evenness statistics were estimated based on banding patterns for these 11-character allelomorphs.

For the extended geographic survey, hierarchical analysis was achieved by assigning sites to specific survey areas. These broad survey areas included Lake Huron, the Huron-Erie corridor, western Lake Erie, northern Lake Ontario and northern Florida. All Great Lakes sites were then combined into a value for the entire study region. Finally, the Great Lakes and Florida sites were all used to estimate overall species measures. Combining sites does not, however, mean pooling of individual plant data. Rather, each site was treated as a subpopulation within the 'total' population (i.e., over each survey area, over the Great Lakes and overall).

General genetic diversity measures, including mean percent polymorphic loci (P), mean number of alleles (A) and mean expected and observed heterozygosity (H), were estimated for the various areas and combinations of sites (equations and definitions given in Appendix B, Table B1).

For both the ramets and the genets (i.e., putative clones) based on six genetically interpretable loci, the following statistics were estimated (equations and definitions given in Appendix B, Table B1). Deviations from Hardy-Weinberg Equilibrium (HWE), for each locus at each site, were tested with χ^2 tests on Wright's inbreeding co-efficient ($F= (H_{exp}-H_{obs})/H_{exp}$; Li and Horvitz, 1953). Nei's

measures of partitioning of the observed genetic diversity within and among sites (H_I, H_T, H_S, D_{ST}, G_{ST}) were estimated for each locus within each survey area (Nei, 1973; Hartl and Clark, 1989). Sites were treated essentially as subpopulations in relation to the total population (i.e., encompassing the survey area). Heterogeneity of allele and genotype frequencies among sites and between years was assessed using χ^2 following the methods of Workman and Niswander (1970; see equations Appendix B, Table B1). Wright's F-statistics, F_{IS}, F_{IT} and F_{ST} were estimated using the Cockerham-Weir ANOVA estimation method which takes into account sample variance and sample size. This analysis was performed using the computer program Genetic Data Analysis (GDA; Lewis and Zaykin, 2000). Confidence intervals (95%) for F_{IS}, F_{IT} and F_{ST} were obtained using the bootstrapping function in GDA. If both upper and lower limits for the F-statistic confidence intervals proved to be above (or below) zero, than the value for the F-statistic was determined to be significantly greater (or less) than zero. Migrant numbers were approximated using F_{IS} (the inbreeding coefficient) following $Nm= (1/F_{ST})-1)/4$ (Hartl and Clark, 1989).

Nei's genetic distances and identities between each pair of sites were estimated (Nei, 1972). Regression analysis was used to assess the relationship between genetic and geographic distances, and cluster analysis (unweighted pairwise groups method using arithmetic average (UPGMA) included in Systat) was used to separate sites based on genetic distances. Differences in genetic diversity statistics between ramets and clones were assessed using paired t-

tests. Among-area differences were tested with Kruskal-Wallis One-Way-ANOVA.

Clonal diversity measures were calculated separately for the seven- and ten-locus allelomorphs. These estimates were determined following the methods of Eckert and Barrett (1993, see Appendix B, Table B1 for equations and definitions).

2.1 Survey of local sites

Sampling

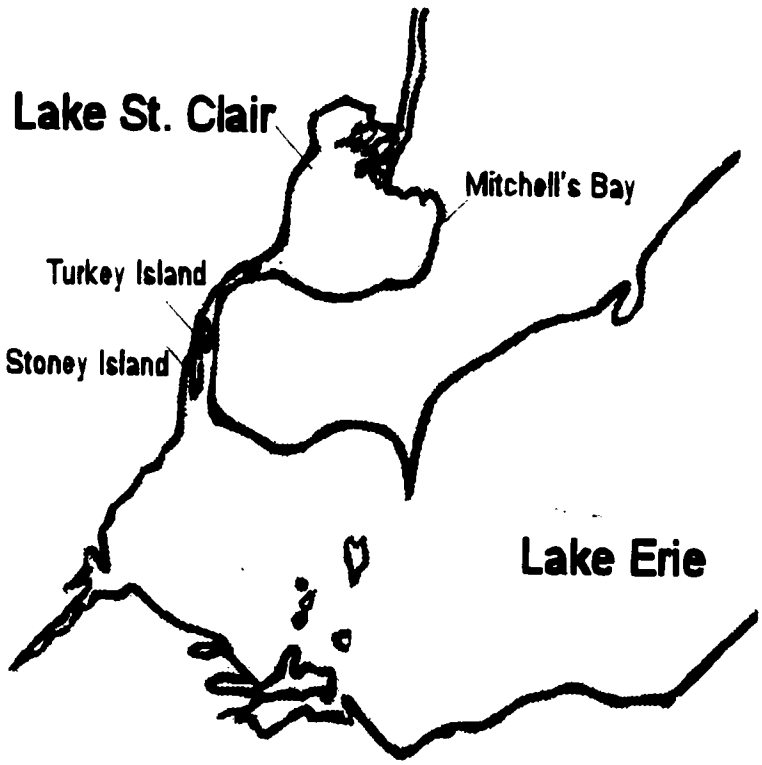
To investigate small scale population variation three sites in the Huron-Erie corridor of the Great Lakes were sampled: Mitchell's Bay in Lake St. Clair, and Turkey and Stoney Islands in the Detroit River (Figure 2.1.1). Turkey Island was initially sampled in 1991, Mitchell's Bay and Stoney Island were sampled in 1992, and all three sites were sampled again in 1993.

Sampling consisted of establishing 20 cm wide transects and excavating the first 400 contiguous plants. Transect length varied from 3-5 m depending on shoot density. Whole plants were removed because tissue from stolons (underground connections between ramets) gave the best results for electrophoretic analysis. At each site, transects were sampled at similar depths, c. 40 and 80 cm. At Mitchell's Bay and Stoney Island only the shallower (40 cm) transects were sampled again in 1993, whereas the Turkey Island site was sampled again at both depths. Samples (n=400) taken in 1993 were from adjacent and parallel transects to those transects sampled initially. At sampling, the flowering status of each ramet was recorded as either flowering female, flowering male or vegetative (non-flowering).

All 1991 and 1992 samples were analyzed electrophoretically, while a random subsample of 150 plants (determined by random number generation in Systat) from each transect was analyzed for the 1993 samples.

Figure 2.1.1. Map of the Huron-Erie corridor, with the location of the three sites surveyed: Mitchell's Bay in Lake St. Clair, and Turkey and Stoney Islands in the Detroit River.

Figure 2.1.1.


Lake St. Clair
Mitchell's Bay
Turkey Island
Stoney Island
Lake Erie

Results

Allele and genotype variation

The ramet genotype and allele frequencies of the six interpretable loci for all of the transects sampled at Mitchell's Bay, Turkey Is., and Stoney Is. are presented in Appendix B (Tables B2 and B3, respectively). Allele and genotype frequencies based on putative genets (clones) sampled in 1993 are presented in Appendix B (Table B4). Note that the third alleles for ME-1 and PGI-1 (see Table 2.0.1) were not represented in any of these sites (Appendix B., Tables B3, B4). PGM-2, PGI-1 and MDH-1 do not add information regarding genetic diversity and structure in this survey as they were monomorphic at the study sites.

For each polymorphic locus (ME-1, IDH-1 and 6PGDH-1) deviations from HWE expectations based on transect ramet allele frequencies were analysed with χ^2 tests (Li and Horvitz, 1953). For 6PGDH-1 and ME-1, 5 of the10 sampled transects did not fit with expectations (Appendix B, Table B3). These deviations resulted mostly from greater expected heterozygosity than was observed (80% of the time for ME and 60% for 6PGDH). Eight of ten transects deviated for IDH-1, with six of those deviations resulting from greater observed heterozygosity than was expected (Appendix B, Table B3). Few clear site or year patterns were evident; the transects sampled at Stoney Island in 1992 (40 cm depth) and Turkey Island in 1991 (80 cm depth) deviated from HWE for each of the polymorphic loci. Overall, the high variability in locus-to-locus behaviour was

suggestive of a system not at equilibrium.

Differences between paired transects sampled at Mitchell's Bay, Stoney Is. and Turkey Is. were determined by performing X^2 tests on (a) ramet allele frequencies and (b) ramet genotype frequencies (following Workman and Niswander, 1970). The pair of transects sampled at each of Turkey Island in 1991 and at Stoney Island 1992 differed significantly in allele and genotype frequencies for each of the polymorphic loci ME-1, IDH-1 and 6PGDH-1 (Table 2.1.1). Allele frequencies between pairs of transects sampled at Turkey Island 1993 and Mitchell's Bay 1992 did not differ significantly (Table 2.1.1). Genotype frequencies, however, did show significant differences for ME-1 (Turkey Is.) and 6PGDH-1 (Turkey Is. and Mitchell's Bay).

Similar tests were performed to determine if between-year differences in genotype and allele frequencies existed at each of these three sites. Between years, all three of the polymorphic loci (ME-1, IDH-1 and 6PGDH-1) differed in genotype frequencies at all three sites (Table 2.1.2). Allele frequencies were often, but not consistently, significantly different among years (Table 2.1.2).

Genetic diversity among three sites

Genetic diversity statistics were estimated using only 1993 shallow transect samples as these transects were sampled at each of the three sites. Based on these three transects and all six loci, the percentage of polymorphic loci (P_P) was 50, mean number of alleles (A) was 1.50 ±0.121, with the mean

Table 2.1.1. Results of χ^2 comparing ramet allele (df=1) and ramet genotype frequencies (df=2) **between two transects** sampled at different depths within the same site within the same year (*p<0.05, **p<0.01, ***p<0.001). For monomorphic loci (PGM-2, MDH-2 and PGI-1) df=0, therefore no χ^2 values were determined.

Locus	Mitchell's Bay		Turkey Is.		Turkey Is.		Stoney Is.	
	1992		1991		1993		1992	
	Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype
PGM-2	--	--	--	--	--	--	--	--
ME-1	3.011	5.493	12.84***	37.56***	0.7420	8.854*	114.6***	117.5***
IDH-1	0.8932	4.913	27.55***	58.50***	0.0103	1.015	62.19***	123.3***
6PGDH-1	0.1004	15.10***	45.78***	62.52***	0.1002	13.36**	77.30***	98.71***
MDH-2	--	--	--	--	--	--	--	--
PGI-1	3.149	4.049	--	--	--	--	--	--

Table 2.1.2. Results of X^2 comparing ramet allele (df=1) and ramet genotype frequencies (df=2) between adjacent transects sampled in **two different years** at Turkey Is., Stoney Is., and Mitchell's Bay (*p<0.05, **p<0.01, ***p<0.001). For monomorphic loci (PGM-2, MDH-2 and PGI-1) df=0, therefore no χ^2 values were determined.

Locus	Mitchell's Bay, shallow		Turkey Is., shallow		Turkey Is., mid		Stoney Is., shallow	
	Allele	Genotype	Allele	Genotype	Allele	Genotype	Allele	Genotype
PGM-2	--	--	--	--	--	--	--	--
ME-1	15.35***	46.18***	13.81***	23.25***	0.0730	14.92***	0.9103	55.59***
IDH-1	1.814	10.04**	0.6513	11.57**	30.71***	177.0***	6.694**	17.47***
6PGDH-1	4.692*	7.355*	6.375*	9.114*	15.13***	96.03***	0.0302	20.80***
MDH-2	--	--	--	--	--	--	--	--
PGI-1	--	--	--	--	--	--	--	--

effective number of alleles (A_e) estimated to be 1.32 ±0.082. For ramets, mean expected heterozygosity (H_{exp}) at all six loci was 0.195 ±0.018, which was slightly lower, but not statistically different from mean observed heterozygosity (H_{obs}) of 0.228 ±0.036 (t=-1.562, df=34, p=0.149).

Chi-squared tests for heterogeneity of allele frequencies among sites (Workman and Niswander, 1970) revealed significant differences among sites in ramet allele frequencies for the three variable loci, ME-1, IDH-1 and 6PGDH-1, but no differences among sites were detected for genets (Table 2.1.3).

Nei's measures of genetic diversity were estimated to assess partitioning of genetic diversity among the three sites. Levels of genetic diversity for the polymorphic loci were quite high for ramets, with H_T ranging from 0.376-0.413, and significantly higher for genets, with a range of 0.459-0.481 (Table 2.1.3). Total genetic diversity was partitioned mostly within populations/sites (H_S), with more than 97% of the variation observed apparent within sites (Table 2.1.3). Values for H_S were significantly different for genets and ramets (t=6.571, df=4, p=0.003). There was very little genetic partitioning among the sites (D_{ST} values <0.010), with mean G_{ST} values not significantly different from zero, indicating that gene flow occurred between the sites indicating panmixia. Again, variability in locus behaviour was detected.

Ramets and genets had similar F_{IS} values (t=-0.9525, df=4, p=0.395 [Table 2.1.4]). Based on the confidence intervals obtained (see Appendix B, Table B5), mean F_{IS} for ramets and clones were significantly less than 0. A

Table 2.1.3. Nei's genetic diversity measures for each genetically interpretable allozyme locus over the three sites surveyed in 1993; Turkey Is., Stoney Is., and Mitchell's Bay. Sites are treated as subpopulations of the total Huron-Erie corridor. Values based on ramet allele frequencies and putative genet allele frequencies are presented. H_I is the mean observed heterozygosity at the three sites, H_T is a measure of the total genetic diversity over all sites, H_S is the amount of genetic diversity partitioned within sites, D_{ST} is the amount of genetic diversity partitioned among the sites, while G_{ST} is a measure of the proportion of genetic diversity accounted for by differences among the sites. Allele frequency heterogeneity among sites was tested with $\chi 2$ (Workman and Niswander, 1970). *p<0.05, **p<0.01, ***p<0.001. Paired t-test results comparing ramet and genet values are given at the bottom of the table. T-tests were performed to determine if mean G_{ST} was equivalent to zero.

Ramet	H_I	H_T	H_S	D_{ST}	G_{ST}	χ^2	**df**
Values							
ME-1	0.402	0.376	0.368	0.008	0.022	18.23***	2
IDH-1	0.559	0.413	0.407	0.007	0.017	13.65**	2
6PGDH-1	0.401	0.387	0.383	0.004	0.009	7.861*	2
mean	0.454	0.392	0.386	0.006	0.016^{ns}		
±SE	±0.014	±0.011	±0.011	±0.001	±0.004		
Genet Values							
ME-1	0.452	0.472	0.470	0.002	0.005	0.6437	2
IDH-1	0.573	0.459	0.458	0.001	0.003	0.4267	2
6PGDH-1	0.397	0.481	0.475	0.006	0.013	1.937	2
mean	0.474	0.471	0.468	0.003	0.007^{ns}		
±SE	±0.052	±0.006	±0.005	±0.002	±0.003		
t	0.2764	6.198	6.571	-1.715	-1.850		
df	4	4	4	4	4		
p	0.756	0.003**	0.003**	0.162	0.138		

Table 2.1.4. Wright's F-statistics for the shallow transects sampled at three sites, Turkey Is., Stoney Is., and Mitchell's Bay in 1993, for ramets and genets (i.e., putative clones). The F-statistics were estimated using the Cockerham-Weir ANOVA method by Lewis (2000) Genetics Data Analysis program. Nm represents the estimated number of migrants (Nm; Equations in Appendix B, Table B1). T-test results comparing ramet and genet values are given at the bottom of the table; note all differences are non-significant ($p>0.05$). Asterisks indicate mean F values that differed significantly from zero based upon 95% confidence intervals generated by bootstrapping (Lewis, 2000).

Ramet Values:	F_{IS}	F_{ST}	F_{IT}	Nm
ME-1	-0.0653	0.0331	-0.0300	7.303
IDH-1	-0.3728	0.0222	-0.3424	11.01
6PGDH-1	-0.0439	0.0145	-0.0288	16.99
mean	-0.1607*	0.0233*	-0.1337*	11.77
±SE	±0.1062	±0.0054	±0.1043	±2.822
Genet Values:				
ME-1	-0.4471	0.0017	-0.4447	146.8
IDH-1	-0.3195	-0.0029	-0.3233	
6PGDH-1	-0.1219	0.1898	0.0910	1.067
mean	-0.2962*	0.0629	-0.2257	73.93
±SE	±0.0946	±0.0635	±0.1622	±72.87
t	-0.9525	0.6216	-0.4768	
df	4	4	4	
p	0.3948	0.5679	0.6584	

negative F_{IS} value results from outcrossing effects and an excess of heterozygotes. Note that, in a two allele system, Wright's fixation index F_{ST}(Table 2.1.4) is equivalent to G_{ST}, Nei's fixation index (Table 2.1.3)[Hartl and Clark, 1989]. In the present study, F_{ST} was calculated using the Cockerham-Weir ANOVA method, and is a more precise measure of the fixation index, taking into account sample size and variance (Weir, 1990). Sampling effects on F_{ST} are most clearly seen when F_{ST} estimates are compared with G_{ST} (Tables 2.1.3 and 2.1.4). In the case of genets, mean F_{ST} was 0.063 and was quite a bit higher than genet G_{ST} (0.007); ramet F_{ST} and G_{ST} values, which dealt with a greater number of samples, were more similar (0.023 and 0.016 respectively). Despite these differences in ramet and genet values, their means were not different based on t-tests (Table 2.1.4). The ramet F_{ST} value was significantly greater than zero (Appendix B, Table B5), indicating that population differentiation was detected at that level.

Wright's inbreeding coefficient (F_{IT}) was negative for all of the polymorphic loci at the ramet level, with an average of -0.134 ±0.104 (Table 2.1.4) which was significantly less than zero (Appendix B, Table B5). This indicated greater heterozygosity than expected, as was evident in a number of individual transects in terms of negative and often significant F_{IT} values (see Appendix B, Table B3). For genets, the overall mean F_{IT} was not different from zero (-0.226 ±0.162), and was consistent with random mating. Ramet and genet means were not significantly different based on t-tests (Table 2.1.4).

An estimate of the number of migrants (Nm) between the sites showed a mean of 11.77 ±2.82 migrants per generation for ramets and 73.93 ±72.87 for genets (Table 2.1.4).

Measures of Nei's genetic distance among pairs of sites (Table 2.1.5) were quite low, ranging from 0.0021-0.0089 for ramets, and were lower, but not significantly so, for genets (0.0019-0.0093; t=-0.9831, df=4, p=0.381). These low values provide further evidence for gene flow between the sites. Regression and cluster analysis were not performed on the data from the three sites due to lack of power. Mitchell's Bay and Stoney Is. were closest genetically with respect to ramets and genets, but most distant based on geographic distance. The ranking of genetic distance was the same between the ramet and genet analysis.

Clonal diversity

Clonal diversity measures based on allelomorph differences among the sites (Table 2.1.6) indicate a significantly higher proportion of distinguishable allelomorphs when 11 characters were used (0.44-0.74) than when seven characters were used (0.16-0.25; t=4.452, df=4, p=0.011). All of the sites showed consistently high genotypic diversity and evenness, with diversity values being consistently significantly lower for seven-character allelomorphs than for 11-character allelomorphs (t=-4.700, df=4, p<0.01). At Stoney Is., the site was less even when more characters were used, but overall eveness measures were similar between analyses (Table 2.1.6).

Table 2.1.5. Nei's genetic distance D, based on six loci, for ramets (above the diagonal, in boldface) and genets (below the diagonal) between pairs of sites sampled in 1993. The ramet mean (0.0053±0.002) did not differ significantly from genet mean (0.0056±0.002) based on a t-test (t=-0.9831, df=4, p=0.381).

	Mitchell's Bay	**Turkey Is.**	**Stoney Is.**
Mitchell's Bay		**0.0089**	**0.0021**
Turkey Is.	0.0037		**0.0049**
Stoney Is.	0.0043	0.0016	

Table 2.1.6. Estimates of clonal diversity for putative clones based on six genetically interpretable loci plus gender and analysis at 10 putative loci plus gender at Mitchell's Bay, Turkey Is., and Stoney Is. in 1993. Number of ramets (Nr) with full composite allelomorphs, the number of distinct allelomorphs observed (G), the maximum number of allelomorphs possible (G_{max}), the proportion of distinct genotypes (G/Nr), clonal (genet) diversity (D), and genotypic evenness (E) (Eckert and Barrett, 1993). G_{max} is equal to Nr when Nr is lower than the theoretical G_{max}. T-test results comparing ramet and genet values are given at the bottom of the table; *p<0.05, **p<0.01.

	Nr	G	G_{max}	G/Nr	D	E
Seven character analysis:						
Mitchell's Bay	108	27	108	0.2500	0.9069	0.8810
Turkey Is.	144	23	144	0.1597	0.8655	0.8563
Stoney Is.	101	21	101	0.2079	0.9097	0.9135
11 character analysis:						
Mitchell's Bay	133	98	133	0.7368	0.9931	0.9386
Turkey Is.	147	66	147	0.4490	0.9572	0.8859
Stoney Is.	119	85	119	0.7143	0.9849	0.8561
t				4.452	4.700	0.3395
df				4	4	4
p				0.011*	0.009**	0.751

The three sites all shared 15 seven-character, and seven 11-character allelomorphs (Table 2.1.7). Based on 11-character analysis, each site had >60% unique allelomorphs, while for the seven-character analysis, unique allelomorphs were rare (<20%). The seven-character allelomorphs which were shared among all three sites (e.g., aaa, abb, bbb) tended to be more common within sites, and comprised a greater number of ramets (Table 2.1.8).

Allelomorph (seven-character) maps of the transects sampled in 1993 are presented in Figure 2.1.2 a-d. All transects were multi-clonal, with some genets extending along the entire length. The more common genotypes tended to be the ones shared among the sites (i.e., aaa, abb, baa, bbb); these allozyme genotypes were often present as both male, female and vegetative ramets (Figure 2.1.2, Table 2.1.8).

Discussion

Allele and genotype variation

Systematic sampling of all ramets along a transect allows genetic diversity to be analyzed on a smaller scale than is commonly used by plant population geneticists. Such a study allows for the assessment of spatial location of these sessile individuals. Even more importantly, this survey allows insights into the relative contributions of sexual reproduction and clonal growth to population (ramet) numbers.

Year-to-year differences in genotype frequencies were detected for the

Table 2.1.7. Breakdown of number (and percentage) of shared and unique allelomorphs among sites for seven character and 11 character analysis. The number of ramets assayed is included for comparison.

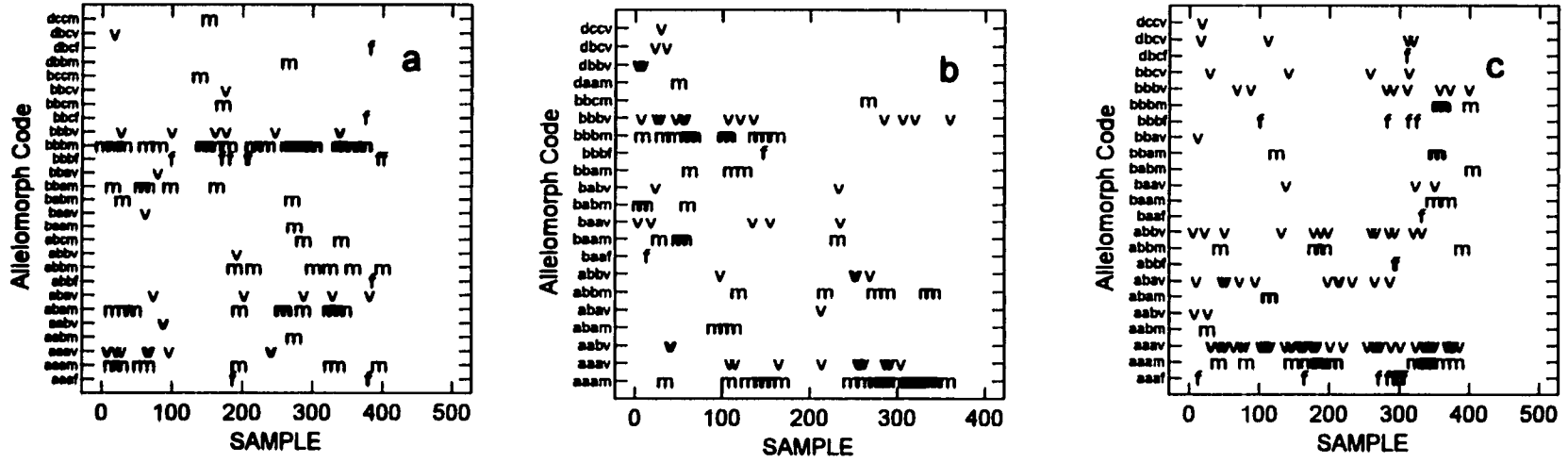
	Seven character analysis			11 character analysis		
	Mitchell's Bay	Turkey Is.	Stoney Is.	Mitchell's Bay	Turkey Is.	Stoney Is.
No. Ramets	108	144	101	133	147	119
No. Allelomorphs:						
Total	27	23	21	98	66	85
Unique	5	0	3	71	42	59
	(18.5)	(0)	(14.3)	(72.4)	(63.6)	(69.4)
Shared (%):	(81.5)	(100)	(85.7)	(27.6)	(36.4)	(30.6)
M-T-S	15	15	15	7	7	7
M-T	6	6		9	9	
M-S	1		1	11		11
T-S		2	2		8	8

Table 2.1.8. Seven-character allelomorph frequencies based on ramets for the three sites. Allelomorphs separated by gender; f=female, m=male, v=vegetative. Allelomorphs code for genotype of the three polymorphic loci ME-1, IDH-1 and 6PGDH-1, where 'a' represents homozygous slow, 'b' heterozygous and 'c' homozygous fast.

Allelomorph	Mitchell's			Stoney			Turkey		
	f	m	v	f	m	v	f	m	v
aaa	3	8	9		20	11	9	15	46
aab		1	2			3		1	2
aba		12	5		3	1		2	11
abb	1	6	1		6	4	2	4	13
abc		2							
baa		1	1	1	4	5	1	2	3
bab		2			3	2		1	
bba		5	1		3			3	1
bbb	7	26	7	1	12	14	5	5	8
bbc	1	1	1		1		4		
bcc		1							
caa					1				
cbb		1				3			
cbc	1		1			2	1		4
ccc		1				1			1
totals	13	67	28	2	53	46	22	33	89

Figure 2.1.2. Allelomorph maps of transects sampled in 1993 at a) Mitchell's Bay, b) Stoney Island, c) Turkey Island. Allelomorph designation is based on banding patterns at the 6 loci studied, where ME-1, IDH-1 and 6PGDH-1 were polymorphic. Gender is indicated as the last letter of the allelomorph code and the symbol plotting ramets along the transect. Sample represents location of ramet along the 20 cm wide transect, and is based on the sample number along the transect of 400 ramets.

Figure 2.1.2.



three sites at all three polymorphic loci. However, due to the sampling strategy employed, this study does not adequately address year-to-year shifts in site allele or genotype frequencies. With sampling of adjacent transects, temporal and spatial differences were detected. Given the differences observed between transects sampled within the same site and year (see Table 2.1.1), it is not surprising that an adjacent transect sampled the following year would be different as well (Table 2.1.2).

That being said, differences in allele and genotype frequencies were detected among transects, both within a year and between years, and may reflect *V. americana's* guerilla clonal habit. This is characterized by a meandering and branching form of ramet production, with variable inter-ramet lengths and clonal fragmentation (Lovett-Doust, 1981). Based on physical measurements, clonal expansion in a given season has been estimated at 1.66 m ± 1.42 (Laushman,1993). Stolons from different ramets often overlap, and new shoot growth of neighbouring ramets occurs in unoccupied spaces (C. Lokker, personal observations). A guerrilla growth strategy is also expected to maintain higher local genetic diversity since ramets of other genets may enter spaces between shoots (Hutchings and Bradbury, 1986).

Shifts and differences in genetic structure over time and space may also reflect differential clonal growth as ramets emerge from turions formed the previous fall (Laushman, 1993). Turion production occurs in close proximity to the parent plant, thereby tending to limit spatial genet expansion; the number of

turions produced, and subsequent turion germination success could readily affect the allele and genotype arrangements in the same location between years. Given that the scale of the study is small, and the differences between years may be a result of the growth form of *V. americana*, similar differences might be detected between adjacent transects sampled concurrently, or adjacent transects sampled at different times during the growing season (Laushman, 1993).

Spatial and temporal changes in allele and genotype frequencies can also indicate other population processes, such as selection, mutation, gene flow and genetic drift (Hartl and Clark, 1989). For instance, the lack of individuals homozygous for the B allele of IDH-1 suggests that the locus may not be neutral; selection may be acting against this genotype, resulting in the observed excess of heterozygotes. The observed variability and significant deviation from zero for F_{IT} values and deviations from HWE suggest that the *V. americana* sites sampled were not at equilibrium. If the sites were in flux, and subject to factors such as selection, then the estimates of population genetic parameters need to be cautiously interpreted as these are based upon models of populations at equilibrium.

Genetic diversity

Biological characteristics associated with high genetic diversity include outcrossed breeding system, dioecy, sexual reproduction, wind pollination, seed

dormancy, long-lived lifespan, widespread distribution and large population size (Loveless and Hamrick, 1984). Clonal aquatic macrophytes are generally predicted to be genetically depauperate as a result of effective clonal strategies coupled with infrequent successful sexual reproduction (Les, 1988; Triest, 1991; Barrett *et al.*, 1993).

Levels of genetic diversity in *V. americana* detected at the three sites studied here are higher or comparable with estimates for other clonal species; the ramet mean H_{exp} was 0.195 for all six loci. In their literature review, Hamrick and Godt (1989) reported a mean H_{exp} for clonal terrestrial species of 0.103. Recently H_{exp} for *Quercus havardii,* a clonal, wind-pollinated woody perennial was estimated at 0.289 (Mayes *et al.*, 1998), and Montalvo *et al.* (1997) found an H_s (expected heterozygosity under HWE) value of 0.443 for another clonal oak species, *Quercus chrysolepsis*, ramet values similar to that reported here for *V. americana* (ramet mean 0.388, Table 2.1.3). However, *V. americana* has a number of attributes that would lead one to expect higher levels of genetic diversity. The species is widespread and dioecious, it flowers and sets fruit regularly, and its pollination mechanism is epihydrophilous, with pollen movement affected by wind and water currents.

Laushman (1993), reported a low mean ramet H_{exp} of 0.085 in 12 populations of *V. americana* sampled in lakes and rivers in Ohio (using 16 loci). In his study, Laushman sampled 48 plants, each at 2 m intervals along a 100 m transect. He also sampled more extensively the *V. americana* population at Put-

in-Bay, Ohio where 4 transects of 48 m length were spaced 30 m apart and sampled at 1 m intervals. At Put-in-Bay, H_{exp} was 0.268 (Laushman, 1993). The wide range of H_{exp} estimated by Laushman (1993) indicates that sampling strategy should be taken into account when comparing studies; finer-scale studies can detect finer-scale processes and patterns (Laushman, 1993).

Wright's F-statistics measure departure from expectation of panmictic proportions. For *V. americana* in the present study F values were variable (indicative of disequilibrium). Excess heterozygotes resulted in significantly negative F_{IS} values for ramets and genets (Table 2.1.4). Heterozygote advantage may account for the observed excess, which would mean that selection was a factor at the sites. Another explanation for the excess heterozygosity observed lies in 'isolate breaking" whereby subdivided populations fixed for different alleles become united through interbreeding resulting in the production of heterozygotes (Hartl and Clark, 1989; Mitton, 1997). This is especially possible in the Detroit River populations which have recently rebounded from dramatic declines in population size (Schloesser and Manny, 1990). These possibilities, in combination with variable locus behaviour, point to the system not having established equilibrium. However, based on the genetic data amassed for *V. americana* , it would appear that there was high genetic variability within sites, and low differentiation among sites. At the genet level, panmixis was indicated by non-significant F_{ST} and F_{IT} values. Further, estimated values for Nm, although questionable based on the non-equilibrium

scenario, were generally greater than 4, a value above which populations are believed to behave as a single panmictic unit (Kimura and Maruyama, 1971).

The effect on genetic diversity statistics of having both clonal and sexual propagation can be assessed by comparing population genetic variables between ramets and genets. In a previous study, Lokker *et al.* (1994) reported a significant reduction in inbreeding-like effect at Turkey Island when ramets were attributed to putative genets. In the current study, significant differences in Wright's F-statistics were not detected with t-tests (Table 2.1.4). Yet, genet values for F_{ST} and F_{IT} were not different from zero, while ramet values were (Table 2.1.4). Since mean ramet F_{IT} was negative, excess heterozygotes were detected. This difference between ramets and genets could be a function of ramets over-representing common (ie., heterozygote) genotypes through vegetative propagation. Assessing putative genets in the present study resulted in a reduction of an outcrossing effect. This difference may also be attributable a reduction in sample size when genets were used in the analysis.

Kumar and Rogstad (1998) found that clonality had little effect on population genetic characters in *Quercus gambelii*, an outcrossing, wind-pollinated, clonal woody perennial which experienced high gene flow and low population subdivision. For a population of *Quercus havardii*, Mayes *et al.* (1998) treated clones as individuals, and found that a weak genetic substructure existed. Despite rare evidence of successful sexual reproduction, characteristics associated with an interbreeding population were also detected (Mayes *et al.*,

1998) . The ability to identify putative genets based on matching allozyme banding patterns should allow researchers to more precisely measure the effects of breeding system without the influence of clonality.

Clonal diversity

Mapping ramets along the sampled transects allows characterization of local clonal structure in space. Several studies have demonstrated that clonal plant populations are indeed multiclonal and more diverse than originally predicted. Ellstrand and Roose (1987) and Widén *et al.* (1994) surveyed the literature on diversity in clonal plant species and reported that the majority (74%) of genotypes were local rather than widespread (26%). Also, clonal diversity and evenness in clonal plant populations tended to be intermediate (Ellstrand and Roose, 1987). That is, populations were not usually dominated by a few genotypes (low E), nor were they composed of a large number of equally frequent genotypes (high D, high E).

Clearly, transects of *V. americana* at the three sites were multiclonal, and genets inter-mingle along their length. Also, a large proportion of unique allelomorphs was found for 11-character analysis (>60%), but when seven characters were used, only 20% of the allelomorphs were unique. Diversity and evenness were both high, indicating that there were a high number of 'genotypes', and they were generally represented in equal frequencies. Increasing the number of characters to determine genet composition results in

detection of more allelomorphs, a greater proportion of which are unique.

Lokker *et al.* (1994) also reported a mosaic of genet distributions for six transects within the Turkey Island population of *V. americana*. A guerilla cloning strategy is expected to result in mosaic genet distributions, especially if the species grows in a heterogenous environment (Widén *et al.*, 1994). Genet mapping has been carried out by few other authors; in *Glechoma hederacea* genets were found in clumped arrangements indicating a phalanx clonal growth strategy (Widén *et al.*, 1994). Limited seed dispersal generated fine-scale substructuring in the oak *Quercus chrysolepsis* (Montalvo *et al.*, 1997).

A number of studies comparing analytical methodologies have shown that increasing sample size (Hebert *et al.*, 1988), the number of characters (i.e., allozymes; Ellstrand and Roose, 1987; Hebert *et al.*, 1988; Widén *et al.*, 1997) and the use of RAPD (random amplified polymorphic DNA) and ISSR (inter-simple sequence repeat) technology vastly increases the detection of new allelomorphs (Waycott, 1998; Esselman *et al.*, 1999). In the present study, there was an increase in the number of allelomorphs detected when 11 characters were used as opposed to seven characters (Table 2.1.6), with a concomitant increase in the number of unique allelomorphs (see Table 2.1.7). Increasing the number of putative loci clearly results in the detection of more distinct genotypes, both unique and shared. Increasing sampling intensity, however, does not appear to result in as dramatic change in clone detection as does increasing the number of characters used to ascertain genetic identity (Ellstrand

and Roose, 1987, Widén *et al.*, 1994).

The data for *V. americana* suggest that as few as zero of the allelomorphs present at the sites were unique, or only detected at one site, with a range as high as 72% (Table 2.1.7). Generally, these unique allelomorphs were represented by only one or two ramets (see Table 2.1.8). Unique allelomorphs reflect novel genotypes at the sites whose origins may lie in mutation, migration or sexual processes. Any number of the allelomorphs detected may have arisen by these processes, but the unique allelomorphs represent individuals who are uncommon within a site (comprise few clonal ramets), and not yet shared among sites. These allelomorphs are therefore likely to be recent seedling recruits, turion or shoot migrants or mutants.

Evolutionary processes

Some interesting questions remain. What is the origin of the observed genetic variation? How is it maintained?

Genetic variation is generated by somatic mutation, gametic mutation or recombination as a result of sexual reproduction. Mutation rates are generally considered to be low enough to be negligible (Hartl and Clark, 1989). Variation can be maintained by ongoing sexual reproduction and gene flow of both sexual and asexual propagules. Processes such as selection and drift can result in the decay of genetic variability. Selection, however, can also increase genetic variability.

The amount of genetic variability observed for *V. americana* in the current study and in Lokker *et al.* (1994) is considerable; and these estimates likely under-report the "true" level of genetic diversity. However, these findings are unexpected because some Detroit River populations of *V. americana* have in recent years rebounded (Lovett-Doust and LaPorte, 1991; C. Lokker, personal observation) from a dramatic decrease in turions that occurred from 1950-1985 (Schloesser and Manny, 1990). Schloesser and Manny (1990) interpreted the decline as a consequence of increased water pollution over that period.

Following such a decline in numbers, populations often go through a "bottleneck", and allelic diversity and levels of heterozygosity within them is often reduced (Hartl and Clark, 1989). The subsequent increase in heterozygosity in recovering populations, however, depends on how severe the bottleneck was and on the speed of population restoration (Hartl and Clark, 1989). In the present study, ramet H_{obs} (0.228 ±0.036) is slightly, but not significantly, greater than H_{exp} (0.195 ±0.018), and reflects that the bottleneck was not very severe (i.e., a number of individuals survived), that population recovery must have been fairly quick, and that extensive outcrossing has occurred within the affected areas during the recovery period. Genetic diversity in the extant population may also be derived from colonizers or migrants from upstream (i.e., gene flow), which would further explain the low level of differentiation among sites in the water system (especially for genets).

For pollination systems such as that of *V. americana,* the observed high

levels of variation within and low levels of variation between sites are to be expected (Loveless and Hamrick, 1984). The lack of subdivision among sites (low or non-significant F_{ST} values) indicates a significant amount of gene flow is occurring. Certainly, given that successful fertilization of a great many seed pods is observed in the species (Lokker *et al.*, 1997; Sullivan and Titus, 1996; Lokker *et al.*, 1994; Laushman, 1993; Lovett-Doust and LaPorte, 1991), and the fact that a seed bank of *V. americana* is present in the Huron-Erie corridor (Lokker *et al.*, 1997; and see McFarland and Rogers 1998, Kimber *et al.*, 1995), some downstream dispersal of new genets must exist.

The question of how much gene flow is necessary to maintain the observed levels of variation remains. A small amount of gene flow is sufficient to counteract the effects of selection and genetic drift and maintain diversity in populations (cf Hartl and Clark, 1989). In fact, populations are believed to behave as a single panmictic unit as long as Nm, the number of migrants per generation, is greater than 4 (Kimura and Maruyama, 1971), as is the case in the present study. Although, since F values were variable and sites show evidence of disequilibrium, Nm values should be treated as only a guide since the model assumes equilibrium. Gene flow could, however, be a significant process for *V. americana* sites in the Huron-Erie corridor where low differentiation among sites was found (G_{ST}<0.020). The three sites sampled here lie within the same water system, and pollen, seeds or clonal fragments (shoots and turions) could readily migrate downstream, possibly in a "stepping-stone" manner. There is also a

possibility of upstream gene flow, mediated by man and fowl. Upstream gene flow, however, would certainly be less common than downstream gene flow. Laushman (1993), found a significant amount of subdivision among populations of *V. americana* sampled in Ohio (G_{ST}=0.457), where sites were sampled from isolated water systems.

Shared allelomorph data suggest that gene flow between the study sites would explain the lack of differentiation detected among the sites (Table 2.1.7). For the seven-character analysis, 56-71% of detected allelomorphs were found to be present at all three of the sites; 7-11% for the 11-character analysis. These common allelomorphs may have migrated as clonal fragments from upstream sites where they are well-established. The other possibility is that the common allelomorphs possess 'all-purpose' genotypes that have selective advantages in a variety of habitats. A study tracking allelomorph composition of adjacent sites over a number of seasons would be valuable in testing the validity and magnitude of stepping-stone migration.

Conclusions

In summary, the present study reports higher than expected levels of genetic variation at six loci. An H_T of 0.392 ±0.011 (ramet value) was found for *V. americana* , which is generally thought to reproduce primarily through vegetative means. This value is high compared to a mean population H_T of 0.103 ±0.013 for species reproducing both sexually and asexually (based on studies of 56

terrestrial species [Hamrick and Godt, 1989]).

The three sites had high genetic diversity, and were multi-clonal. Gene flow was detected based on estimates of number of migrants and the low level of genetic differentiation among sites, and would appear to be a significant process contributing to the origin and maintenance of the observed genetic diversity. This is further supported by the high fecundity in the species, with many filled fruits observed each year, which suggests that sexual reproduction, too, has an important role in the generation of genetic diversity. The importance of sexual reproduction will be further investigated in Chapter 3, Sections 3.3 and 3.4.

2.2 Extended geographic survey

Sampling

In 1992, seven sites along the Huron-Erie corridor and Lake Erie were sampled, with 400 plants being collected along each of two transects, for a total of 800 plants per site. A random subsample of 300 plants per site were analyzed electrophoretically. The sites were: Clay Creek in the St. Clair River; Walpole Island and Mitchell's Bay in Lake St. Clair; Peche and Stoney Islands in the Detroit River; and Rondeau and Long Point Bays in Lake Erie (Figure 2.2.1, Table 2.2.1).

In 1994 the geographic scale of the survey was extended to include a number of sites from Lake Huron to Lake Ontario (Figure 2.2.2, Table 2.2.1) and five sites from northern Florida (Figure 2.2.3, Table 2.2.1; see Appendix C for detailed site descriptions). This represents a large cross section of the north-south range of *V. americana* in North America. The Great Lake sites included: Thessalon R., Blind R., Serpent R., Spanish Harbour, Bayfield Inlet, Key R., Big Chute, Put-in-Bay, Niagara R., Brighton, Adolphustown, Howe Is., and South Lancaster (Table 2.2.1). The northern Florida sites included: Blue Springs, Chassahowitza, Homosassa, St. Marks R., and Wakulla R.

For the Great Lakes sites, quadrat samples were taken in order to obtain a good estimate of overall diversity across the entire site. Eight to ten .25 m^2 quadrats, spread apart to sample the full extent of each site, were taken. In Florida, due to the nature of the sites and field conditions, plants could not be

Figure 2.2.1. Map of Great Lakes area encompassing the Huron-Erie corridor (St. Clair River, Lake St. Clair, Detroit River) and Lake Erie, depicting site locations sampled in August 1992.

Figure 2.2.1.

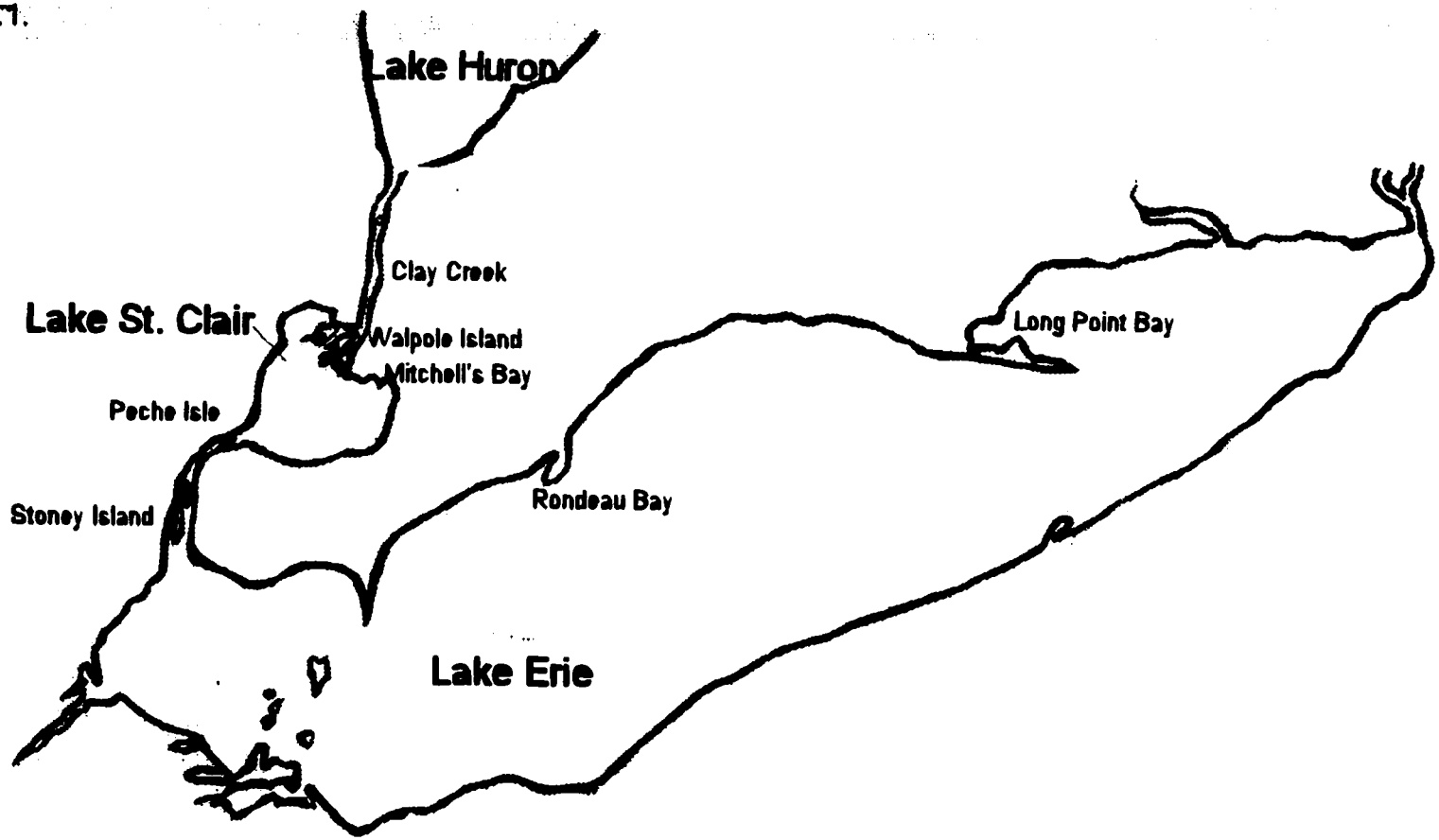

Lake Huron
Clay Creek
Lake St. Clair
Walpole Island
Mitchell's Bay
Peche Isle
Stoney Island
Rondeau Bay
Long Point Bay
Lake Erie

Table 2.2.1. Sites sampled in the Great Lakes and Florida, with their associated water bodies, the county and province (state) they are in and survey areas indicated. The year sampling was performed is included. Isolation refers to whether the site was directly along the shore of a Great Lake or connecting channel (n) or within a tributary or embayment and therefore somewhat isolated from the mass-flow of Great Lakes waters (y).

Site	Year	Abbrev.	Survey Area	Water body	County, Prov. (State)	Isolated
Thessalon	1994	Thes	Lake Huron	Lake Huron	Algoma District, ON	n
Blind R.	1994	Blind	Lake Huron	Lake Huron	Algoma District, ON	y
Serpent R.	1994	Serp	Lake Huron	Lake Huron	Algoma District, ON	n
Spanish Harbour	1994	Span	Lake Huron	Lake Huron	Algoma District, ON	y
Bayfield Inlet	1994	Bayf	Lake Huron	Lake Huron	Parry Sound District, ON	y
Key R.	1994	Key	Lake Huron	Lake Huron	Parry Sound District, ON	y
Big Chute	1994	Big C	Lake Huron	Trent/Severn	Simcoe Cty., ON	y
Clay Creek	1992	Clay	Huron-Erie	St. Clair River	Lambton Cty., ON	n
Walpole Island	1992	Walp	Huron-Erie	Lake St. Clair	Lambton Cty., ON	n
Mitchell's Bay	1992	Mitch	Huron-Erie	Lake St. Clair	Chatham-Kent, ON	n
Peche Isle	1992	Pech	Huron-Erie	Detroit River	Essex Cty., ON	n
Stoney Island	1992	Ston	Huron-Erie	Detroit River	Wayne Cty., MI	n
Rondeau Bay	1992	Rond	Lake Erie	Rondeau Bay	Chatham-Kent, ON	y

Site	Year	Abbrev.	Survey Area	Water body	County, Prov. (State)	Isolated
Long Point Bay	1992	Long	Lake Erie	Long Point Bay	Haldimand-Norfolk, ON	y
Put-in-Bay	1994	Put	Lake Erie	Put-in-Bay	Ottawa Cty., OH	y
Niagara R.	1994	Niag	Lake Ontario	Lake Ontario	Niagara Region, ON	n
Brighton	1994	Brigh	Lake Ontario	Lake Ontario	North Humberland Cty., ON	n
Adolphustown	1994	Adol	Lake Ontario	Lake Ontario	Prince Edward Cty., ON	n
Howe Island	1994	Howe	Lake Ontario	Lake Ontario	Frontenac Cty., ON	n
South Lancaster	1994	SLan	Lake Ontario	Lac St. Louis	Stormont, Dundas and Glengarry, ON	n
Blue Springs	1994	Blue	Florida	Santa Fe R.	Alachua Cty., FL	
Chassahowitzka	1994	Chass	Florida	Gulf of Mexico	Citrus Cty., FL	
Homosassa	1994	Hom	Florida	Gulf of Mexico	Citrus Cty., FL	
St. Marks R.	1994	StM	Florida	Gulf of Mexico	Wakulla Cty., FL	
Wakulla R.	1994	Wak	Florida	St. Marks R.	Wakulla Cty., FL	

Figure 2.2.2. Map of sites sampled in the Great Lakes from Lake Huron to the St. Lawrence River. Sites indicated were sampled in August 1994.

Figure 2.2.2.

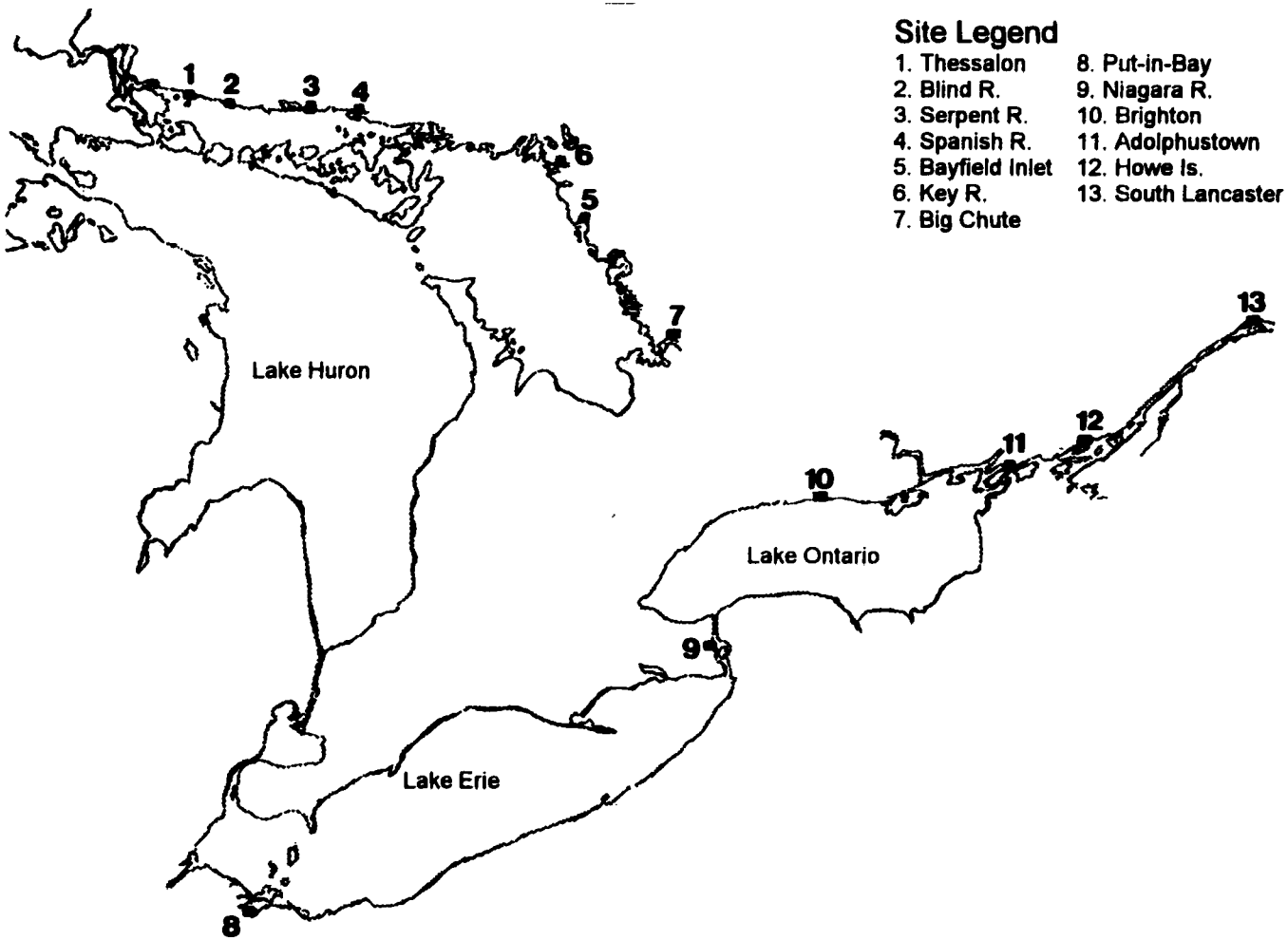
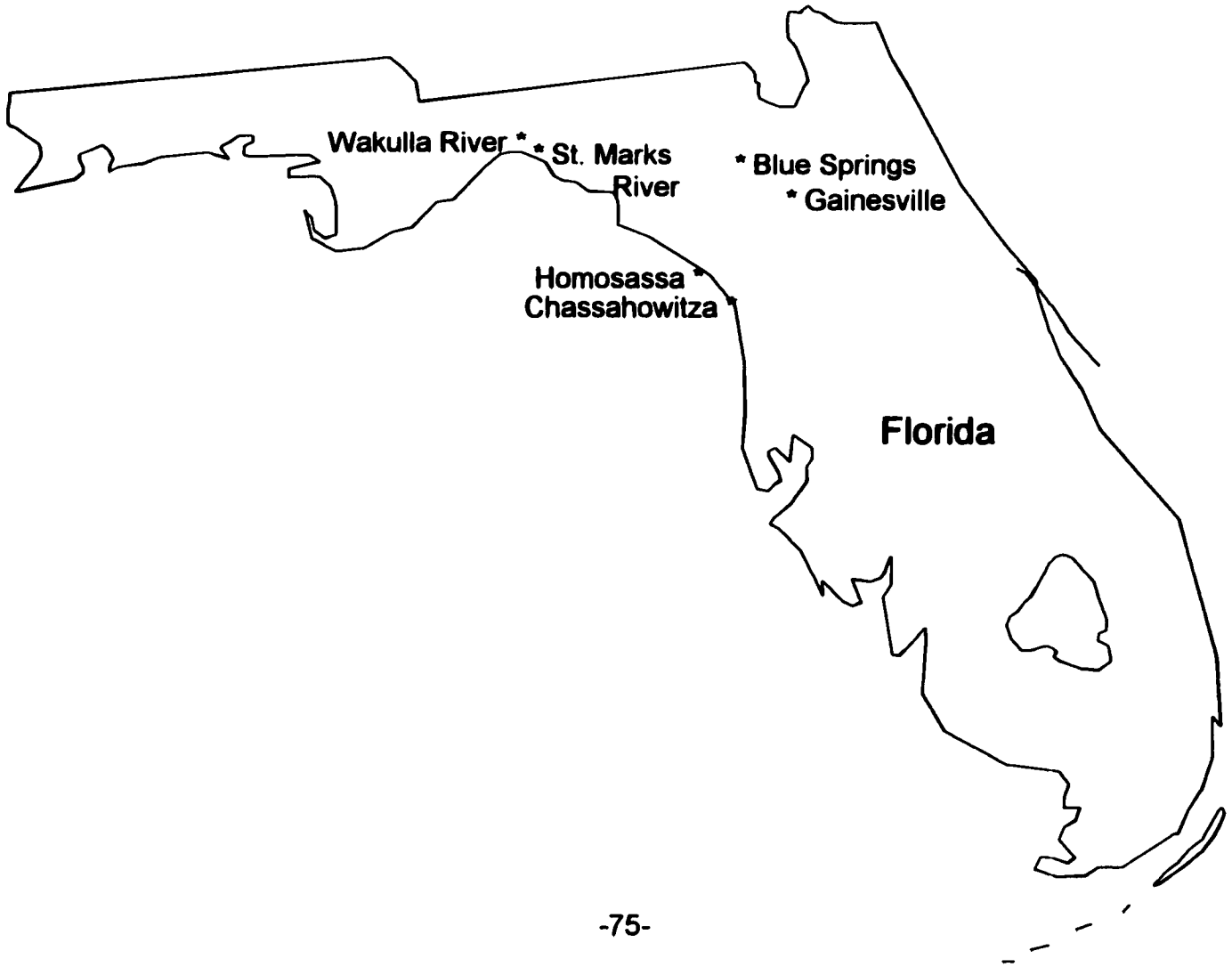

Site Legend
1. Thessalon
2. Blind R.
3. Serpent R.
4. Spanish R.
5. Bayfield Inlet
6. Key R.
7. Big Chute
8. Put-in-Bay
9. Niagara R.
10. Brighton
11. Adolphustown
12. Howe Is.
13. South Lancaster
Lake Huron
Lake Erie
Lake Ontario

Figure 2.2.3. Map showing field sites in Florida. Sites were sampled in September 1994.

Figure 2.2.3.


Wakulla River
St. Marks River
Blue Springs
Gainesville
Homosassa
Chassahowitza
Florida

sampled within distinct quadrats. Instead, "grabs" of plants were taken at random microsites dispersed across the extent of each site. A total of 160 plant samples (stolon tissue) were collected per site. For each sample at all of the sites, the flowering status of each ramet was recorded.

The genetic measures and statistics are presented for the survey areas partitioned into Lake Huron (7 sites), the Huron-Erie corridor (5 sites), Lake Erie (3 sites), Lake Ontario (5 sites), the Great Lakes sites combined (20 sites, total), Florida (5 sites) and Great Lakes/Florida combined (25 sites, total) (Table 2.2.2). For the statistics, sites are treated as subpopulations, while the survey area is treated as the total population. Equations for statistics are given in Appendix B, Table B1, definitions for terms are included in the glossary, Appendix D.

Results

Allele and genotype variation

The overall proportion of polymorphic loci (P, based on the number of loci which showed any polymorphism) increased as a greater number of sites was surveyed, from 50% in the Lake Erie (n=3) and Huron-Erie (n=5) surveys, to 83.3% when Florida and the Great Lakes were pooled (n=25) to produce a species value (Table 2.2.2). Regional means over sites for P (polymorphic loci) ranged from 50-67%. Across survey areas, the mean number of alleles per polymorphic locus per site increased from 2.0 to 2.33, and the mean number of alleles for all loci increased from 1.5 to 1.83 over the increase in geographic

Table 2.2.2. General genetic diversity statistics based on ramets for the various survey areas, the pooled Great Lakes and the species as a whole in 1994. Means ±(SE) reflect mean values of the parameter over sites within a survey area. $P_{overall}$ is based on the number of loci polymorphic in any site over the survey area, P_p is the mean percentage of polymorphic loci ±(SE), A_p is the mean number of alleles per polymorphic locus, while A is the mean number of alleles over all loci, A_e is the mean number of effective alleles, H_{obs} and H_{exp} are the mean observed and expected heterozygosities over the sites within the survey areas (see Appendix B, Table B1 for equations). Comparisons were made with species with wind pollination,, which reproduce both sexually and asexually and those with widespread distributions, as well as with species whose life history traits are different from *V. americana* , and which result in low genetic diversity (taken from Hamrick and Godt, 1989). The top row indicates species values while the bottom line represents population values. The values presented are based on the six genetically interpretable loci; PGM-2, ME-1, IDH-1, 6PGDH-1, MDH-2, PGI-1.

Area	n	$P_{overall}$	P_p	A_p	A	A_e	H_{obs}	H_{exp}
Lake Huron	7	66.7	66.7±2.4	2.00±0.00	1.67±0.024	1.46±0.016	0.347±0.021	0.245±0.007
Huron-Erie	5	50	50.0±0.0	2.00±0.00	1.50±0.00	1.34±0.026	0.239±0.035	0.201±0.093
Lake Erie	3	50	50.0±0.0	2.00±0.00	1.50±0.00	1.29±0.039	0.199±0.048	0.182±0.019
Lake Ontario	5	66.7	66.7±3.3	2.00±0.00	1.67±0.034	1.46±0.013	0.310±0.022	0.246±0.027
Great Lakes	20	66.7	66.7±1.1	2.00±0.00	1.67±0.012	1.41±0.019	0.289±0.019	0.225±0.007
Florida	5	66.7	66.7±9.1	2.33±0.076	1.83±0.033	1.28±0.054	0.195±0.045	0.183±0.030
All Sites	25	88.3	66.7±1.9	2.33±0.020	1.83±0.019	1.38±0.020	0.270±0.018	0.216±0.009
(Species)								

Characteristic	Level	n	P_p	A	A_e	H_{exp}
Outcrossing/						
Wind	species	105	66.1±2.7	2.40±0.13	1.21±0.02	0.162±0.009
pollinated	pop	102	49.7±2.6	1.79±0.06	1.19±0.02	0.148±0.009
Selfing	species	123	41.8±2.9	1.69±0.09	1.18±0.02	0.124±0.011
	pop	113	20.0±2.3	1.31±0.05	1.10±0.03	0.074±0.010
Sexual	species	66	43.8±3.7	1.69±0.08	1.20±0.03	0.138±0.016
/asexual	pop	56	29.4±3.3	1.47±0.06	1.14±0.02	0.103±0.013
Sexual	species	407	51.6±1.57	2.00±0.05	1.21±0.01	0.151±0.006
only	pop	413	34.9±1.3	1.53±0.03	1.16±0.01	0.114±0.005
Widespread	species	105	58.9±3.1	2.29±0.16	1.31±0.03	0.202±0.015
	pop	85	43.0±3.3	1.72±0.07	1.23±0.02	0.159±0.013
Endemic	species	81	40.0±3.2	1.80±0.08	1.15±0.04	0.096±0.010
	pop	100	26.3±2.1	1.39±0.03	1.09±0.01	0.063±0.006

scale (i.e., Lake Erie to pooled Great Lakes and Florida) and number of sites surveyed (i.e., n=3 to n=25). The effective number of alleles (A_e) was lowest in Florida and Lake Erie (1.28 and 1.29 respectively) and highest in the Lake Ontario and Lake Huron (1.46, Table 2.2.2).

Within surveys, observed heterozygosity was consistently higher than expected heterozygosity, but t-tests show that this difference was only significant at Lake Huron ($t=-4.65$, $df=12$, $p<0.001$), Lake Ontario ($t=-2.91$, $df=8$, $p=0.020$) and the Great Lakes pooled ($t=-3.23$, $df=38$, $p=0.003$). In general, expected heterozygosity, or gene diversity, was high or similar when compared to the statistics of Hamrick and Godt (1989) for species with a range of life history characteristics (seeTable 2.2.2).

The mean number of alleles per locus (Table 2.2.2) observed for *V. americana* (1.50-1.83) fell within the range reported for species with a range of life history traits (1.31-2.40), but the effective number of alleles was greater (1.38 versus a range of 1.09-1.31; Hamrick and Godt, 1989). The percentage of polymorphic loci was also generally greater for *V. americana* than for species with both similar and dissimilar attributes (Table 2.2.2).

Ramet genotype values for the surveys are given in Appendix B, Table B6. These values were used to calculate allelic frequencies and heterozygosity (Appendix B, Table B7). New alleles, not previously observed for sites within the Great Lakes, were detected at the ME-1 locus in Lake Ontario, and at the PGI-1 locus in the Huron-Erie corridor and in Lakes Erie and Ontario (Appendix B,

Tables B6 and B7). In all of these cases, the frequency of the new allele was less than 0.05, and following accepted population genetics procedures, the loci continued to be classified as monomorphic (Hartl and Clark, 1989, p. 18). A new allele was also found at each of PGI-1 and ME-1 in Florida in frequencies greater than 0.05 (Appendix B, Tables. B6 and B7).

Significant departures from HWE, determined from X^2 analysis of Wright's inbreeding co-efficient F (analogous to F_{IT}) were more commonly detected among the ramets (Appendix B, Table B7) than among the genets (Appendix B, Table B8). Where significant deviations among ramets were detected, F values were most often negative for ME-1, IDH-1 and 6PGDH-1and resulted from an excess of heterozygotes (Appendix B, Table B7). For ME-1 and 6PGDH-1 genet F values were rarely significant (Appendix B, Table B8). Some geographic patterns were discernable; for ramets Huron-Erie and Lake Erie had positive F values for ME-1 (i.e., deficiency of heterozygotes), while a series of sites from Thessalon to Walpole had negative values (i.e., excess heterozygotes). Florida sites also had positive values for ME-1. Most F values were negative for IDH-1 (i.e., excess heterozygotes), and the majority, except those between Mitchell's Bay and Long Point, were negative for 6PGDH-1 (Appendix B, Table B6). A number of Great Lakes sites (9) had significant negative F values for IDH-1 for genets (Appendix B, Table B8). The variability in locus behaviour indicates that other forces were possibly acting at certain loci. As in Chapter 2.1, heterozygote excess was frequently observed, and may be a function of selection favouring

heterozygotes, or a case of isolate breaking (Hartl and Clark, 1989; Mitton, 1997) most notably in the case of IDH-1. As such, the system studied may not be in equilibrium.

Tests for heterogeneity of allele frequencies among the sites within each survey area were performed following Workman and Niswander (1970). Differences in ramet allele frequencies among the sites were detected in all survey areas for ME-1, for all but Lake Huron for IDH-1, for 6PGDH-1 in Huron-Erie and Lake Ontario, for PGI in Lake Huron, Lake Ontario and Florida, and MDH-1 in Florida (Table 2.2.3). Most individual survey areas did not have heterogeneous allele frequencies for the genets. The only exceptions were MDH-1 in Lake Huron, 6PGDH-1 in Lake Ontario and IDH-1 and MDH-1 in Florida (Table 2.2.4). Pooled Great Lakes, and species data showed heterogeneity among sites.

Genetic diversity

Values for genetic diversity (H_T) tended to be lower for ramets (Table 2.2.3) than for genets (Table 2.2.4), but not significantly so, based on t-tests (Table 2.2.5). H_T values fell within the range of 0-0.5. The polymorphic loci tended to show great genetic diversity, with values >0.25. For the various Great Lakes surveys PGI-1 and MDH-2 invariably had lower values than the more variable ME-1, IDH-1 and 6PGDH-1. The Florida survey had the opposite pattern, with 6PGDH-1 having a low H_T, and PGI-1 and MDH-2 having larger values. Values among surveys were not statistically different based on

Table 2.2.3. Nei's genetic diversity measures for ramets from sites sampled in the Great Lakes and Florida, grouped by water body and then combined within Great Lakes and overall for the species. Heterogeneity of allele frequency among sites in each grouping tested with $\chi 2$ (Workman and Niswander, 1970). Loci considered monomorphic (alleles in frequency <0.05) lacked df to perform test. Means were calculated using only polymorphic loci. Kruskal-Wallis analysis was performed to test differences in diversity measures among surveys. T-tests were performed to determine if mean G_{ST} was equivalent to zero. ns=not significant, *p<0.05, **p<0.01, ***p<0.001, n=number of sites per survey area.

Zone	Locus	H_I	H_S	H_T	D_{ST}	G_{ST}	χ^2	df
Lake Huron	PGM-2	0	0	0	0	0		0
n=7	ME-1	0.6369	0.4684	0.4869	0.0185	0.0379	***	6
	IDH-1	0.6831	0.4472	0.4619	0.0147	0.0318	ns	6
	6PGDH-1	0.7063	0.4943	0.4956	0.0013	0.0030	ns	6
	MDH-2	0.0061	0.0135	0.0136	0.0001	0.0074		0
	PGI-1	0.0502	0.0493	0.0526	0.0033	0.0633	***	6
	mean	0.4165	0.2945	0.3021	0.0729	0.0283^{ns}		
	±SE	±0.1591	±0.1078	±0.1101	±0.0644	±0.0111		
Huron-Erie	PGM-2	0	0	0	0	0		0
n=5	ME-1	0.2954	0.3314	0.3363	0.0049	0.0147	***	4
	IDH-1	0.6433	0.4303	0.4495	0.0192	0.0428	***	4
	6PGDH-1	0.4819	0.4267	0.4398	0.0131	0.0297	***	4

Zone	Locus	H_I	H_S	H_T	D_{ST}	G_{ST}	χ^2	df
	MDH-2	0	0	0	0	0		0
	PGI-1	0.0131	0.0168	0.0170	0.0002	0.0103		0
	mean	0.3584	0.3013	0.3107	0.0098	0.0242*		
	±SE	±0.1353	±0.0976	±0.1012	±0.0039	±0.0075		
Lake Erie	PGM-2	0	0	0	0	0		0
n=3	ME-1	0.4156	0.3533	0.3674	0.0139	0.0386	***	2
	IDH-1	0.3183	0.2748	0.2797	0.0049	0.0173	***	2
	6PGDH-1	0.4436	0.4242	0.4248	0.0006	0.0021	ns	2
	MDH-2	0	0	0	0	0		0
	PGI-1	0.0182	0.0377	0.0380	0.0003	0.0080		0
	mean	0.2989	0.2725	0.2775	0.0050	0.0164^{ns}		
	±SE	±0.0974	±0.0840	±0.0852	±0.0032	±0.0081		
Lake Ontario	PGM-2	0	0	0	0	0		0
n=5	ME-1	0.5111	0.4451	0.4594	0.0143	0.0312	***	4
	IDH-1	0.6790	0.4825	0.4874	0.0049	0.0101	**	4
	6PGDH-1	0.6108	0.4851	0.4950	0.0099	0.0199	***	4

Zone	Locus	H_I	H_S	H_T	D_{ST}	G_{ST}	χ^2	df
	MDH-2	0.0144	0.0141	0.0143	0.0002	0.0140		0
	PGI-1	0.0440	0.0468	0.0486	0.0018	0.0374	***	4
	mean	0.3718	0.2947	0.3009	0.0062	0.0224*		
	±SE	±0.1425	±0.1082	±0.1103	±0.0026	±0.0052		
Great Lakes	PGM-2	0	0	0	0	0		0
n=20	ME-1	0.4869	0.411	0.4387	0.0277	0.0631	***	19
	IDH-1	0.6174	0.4260	0.4503	0.0243	0.054	***	19
	6PGDH-1	0.5868	0.4646	0.4794	0.0148	0.031	***	19
	MDH-2	0.0060	0.0080	0.0080	0	0		0
	PGI-1	0.0346	0.0388	0.0407	0.0020	0.0464	***	19
	mean	0.3463	0.2697	0.2835	0.0138	0.0413**		
	±SE	±0.1349	±0.1010	±0.1060	±0.0056	±0.0090		
Florida	PGM-2	0	0	0	0	0		0
n=5	ME-1	0.2261	0.3211	0.3357	0.0147	0.0437	***	8
	IDH-1	0.2490	0.2065	0.2860	0.0795	0.2779	***	4
	6PGDH-1	0.0234	0.0285	0.0288	0.0003	0.0104		0

Zone	Locus	H_I	H_S	H_T	D_{ST}	G_{ST}	χ^2	df
	MDH-2	0.3419	0.2326	0.3119	0.0793	0.2544	***	4
	PGI-1	0.2938	0.271	0.2881	0.0171	0.0593	***	8
	mean	0.2268	0.2119	0.2501	0.0382	0.1287^{ns}		
	±SE	±0.0546	±0.0498	±0.0561	±0.0171	±0.0568		
All sites	PGM-2	0	0	0	0	0		0
n=25	ME-1	0.4374	0.3930	0.4270	0.0340	0.0796	***	48
	IDH-1	0.5437	0.3821	0.4266	0.0445	0.1044	***	24
	6PGDH-1	0.4741	0.3774	0.4365	0.0591	0.1354	***	24
	MDH-2	0.0730	0.0531	0.0805	0.0274	0.3402	***	24
	PGI-1	0.0864	0.0852	0.0964	0.0111	0.1154	***	48
	mean	0.3229	0.2582	0.2934	0.0352	0.1550*		
	±SE	±0.1008	±0.0774	±0.0837	±0.0081	±0.0472		
Kruskal-		2.390	2.685	2.668	9.262	16.07		6
Wallis (p)		(0.881)	(0.847)	(0.849)	(0.159)	(0.013)*		

Table 2.2.4. Nei's genetic diversity measures for genets (i.e., putative clones) from sites sampled in the Great Lakes and Florida, grouped by water body and then pooled within Great Lakes and overall. Heterogeneity of allele frequency among sites in each grouping was tested with $\chi 2$ (Workman and Niswander, 1970). Means were calculated using only polymorphic loci. Kruskal-Wallis analysis was performed to test differences among surveys. T-tests were performed to determine if mean G_{ST} was equivalent to zero. ns=not significant, *p<0.05, **p<0.01, ***p<0.001, n=number of sites per survey area.

Zone	Locus	H_I	H_S	H_T	D_{ST}	G_{ST}	χ^2	df
Lake Huron	PGM-2	0	0	0	0	0		0
n=7	ME-1	0.5637	0.4829	0.4942	0.0113	0.0229	ns	6
	IDH-1	0.6136	0.4342	0.4439	0.0097	0.0220	ns	6
	6PGDH-1	0.4899	0.4910	0.4950	0.0041	0.0083	ns	6
	MDH-2	0.0451	0.0578	0.0596	0.0018	0.0300	0	6
	PGI-1	0.1849	0.2170	0.2185	0.0015	0.0069	ns	12
	mean	0.3794	0.3366	0.3422	0.0057	0.0180*		
	±SE	±0.1119	±0.0857	±0.0872	±0.0020	±0.0045		
Huron-Erie	PGM-2	0	0	0	0	0		0
n=5	ME-1	0.3911	0.4839	0.4912	0.0073	0.0149	ns	4
	IDH-1	0.5169	0.4320	0.4370	0.0051	0.0116	ns	4
	6PGDH-1	0.2583	0.4930	0.4963	0.0033	0.0066	ns	4

Zone	Locus	H_I	H_S	H_T	D_{ST}	G_{ST}	χ^2	df
	MDH-2	0	0	0	0	0		0
	PGI-1	0.1819	0.2762	0.2812	0.0050	0.0178	ns	8
	mean	0.3370	0.4213	0.4264	0.0052	0.0127*		
	±SE	±0.0739	±0.0502	±0.0502	±0.0008	±0.0024		
Lake Erie	PGM-2	0	0	0	0	0		0
n=3	ME-1	0.5280	0.4568	0.4604	0.0037	0.0079	ns	2
	IDH-1	0.5342	0.4023	0.4081	0.0058	0.0142	ns	2
	6PGDH-1	0.3163	0.4750	0.4914	0.0164	0.0334	ns	2
	MDH-2	0	0	0	0	0		0
	PGI-1	0.1147	0.2217	0.2236	0.0019	0.0086	ns	4
	mean	0.3733	0.3890	0.3959	0.0069	0.0160^{ns}		
	±SE	±0.1000	±0.0578	±0.0599	±0.0032	±0.0060		
Lake Ontario	PGM-2	0	0	0	0	0		0
n=5	ME-1	0.5214	0.4888	0.5087	0.0198	0.0390	ns	8
	IDH-1	0.6039	0.4630	0.4769	0.0139	0.0292	ns	4
	6PGDH-1	0.5049	0.4520	0.4769	0.0249	0.0522	**	4

Zone	Locus	H_I	H_S	H_T	D_{ST}	G_{ST}	χ^2	df
	MDH-2	0	0.0292	0.0301	0	0.0296	ns	4
	PGI-1	0.1056	0.2132	0.2158	0.0026	0.0123	ns	8
	mean	0.4339	0.4042	0.4196	0.0153	0.0332**		
	±SE	±0.1116	±0.0641	±0.0683	±0.0048	±0.0084		
Great Lakes	PGM-2	0	0	0	0	0		0
n=20	ME-1	0.5046	0.4807	0.4946	0.0139	0.0282		38
	IDH-1	0.5751	0.4360	0.4475	0.0115	0.0256		19
	6PGDH-1	0.4097	0.4793	0.4916	0.0122	0.0249		19
	MDH-2	0.0158	0.0275	0.0287	0.0012	0.0418		19
	PGI-1	0.1538	0.2316	0.2348	0.0032	0.0138		38
	mean	0.3318	0.3310	0.3394	0.0084	0.0269**		
	±SE	±0.1065	±0.0887	±0.0912	±0.0026	±0.0045		
Florida	PGM-2	0	0	0	0	0		0
n=5	ME-1	0.3505	0.5069	0.5724	0.0656	0.1146	ns	8
	IDH-1	0.3473	0.3136	0.3669	0.0533	0.1453	***	4
	6PGDH-1	0.0350	0.0512	0.0526	0.0014	0.0266		0

Zone	Locus	H_I	H_S	H_T	D_{ST}	G_{ST}	χ^2	df
	MDH-2	0.6778	0.2622	0.3628	0.1006	0.2772	***	4
	PGI-1	0.2728	0.1347	0.1367	0.0020	0.0147	ns	4
	mean	0.3367	0.2537	0.2983	0.0446	0.1157^{ns}		
	±SE	±0.1029	±0.0784	±0.0923	±0.0191	±0.0475		
All sites	PGM-2	0	0	0	0	0		0
n=25	ME-1	0.4738	0.4859	0.5180	0.0321	0.0619		72
	IDH-1	0.5295	0.4116	0.4343	0.0228	0.0525	***	24
	6PGDH-1	0.3348	0.3937	0.4571	0.0634	0.1386		24
	MDH-2	0.1482	0.0745	0.1115	0.0371	0.3323	***	24
	PGI-1	0.1776	0.2122	0.2160	0.0038	0.0176		48
	mean	0.3328	0.3156	0.3474	0.0318	0.1206^{ns}		
	±SE	±0.0764	±0.0752	±0.0780	±0.0097	±0.0565		
Kruskal-		1.279	3.057	1.843	7.777	14.39		6
Wallis (p)		(0.938)	(0.802)	(0.934)	(0.255)	(0.026)*		

Table 2.2.5. Values for t, resulting from t-tests comparing mean diversity statistics between ramets and genets. None of the tests were significant.

Survey area	df	H_I	H_S	H_T	D_{ST}	G_{ST}	F_{IS}	F_{ST}	F_{IT}	Nm
Huron	8	0.1904	-0.3502	-0.2856	1.044	0.8594	0.5599	-1.838	0.0738	-1.817
Huron-Erie	6	0.1387	-1.094	-1.025	1.156	1.455	-0.0500	0.1696	-0.2914	0.8141
Erie	6	-0.5330	-1.143	-1.136	-0.4208	0.0370	-0.6604	-0.0670	-0.7010	-0.0610
Ontario	7	-0.2387	-0.8110	-0.8551	-1.667	-1.141	1.023	1.198	1.076	-1.829
Great Lakes	8	0.0844	-0.4558	-0.3999	0.8656	1.434	0.3194	-0.9827	0.1803	-0.8084
Florida	8	-0.9430	-0.4499	-0.4461	-0.2502	0.1758	0.7491	-0.5059	0.6479	-1.603
Species	8	-0.0780	-0.5324	-0.4718	0.2675	0.4677	1.216	-0.3773	0.6230	-0.8571

Kruskal-Wallis analysis (Tables 2.2.4, 2.2.5).

Since values for H_S were not much smaller than H_T, the majority of the genetic diversity detected was partitioned within sites (relatively high H_S) rather than between sites (low D_{ST}). H_S ranged from 0-0.5, and was greater for the polymorphic loci ME-1, IDH-1 and 6PGDH-1 than for PGI-1 and MDH-2. Again, the pattern for the loci was different in Florida, with PGI-1 and MDH-2 having lower values than ME-1, IDH-1 and 6PGDH-1. Furthermore, in comparison to other geographic areas, the Florida survey had lower H_S values, and subsequently greater D_{ST} values, suggesting that the sites within this survey experienced less gene flow and greater genetic isolation from the other sites. The differences among survey areas were, however, not significant based on Kruskal-Wallis analysis (Tables 2.2.3, 2.2.4). Overall, ramets (Table 2.2.3) and genets (Table 2.2.4) followed the same trends, and were not statistically different based on t-tests (Table 2.2.5).

The proportion of genetic diversity which was attributable to differences among sites (G_{ST}) was consistently low for the Great Lakes surveys (<4%), but greater for the Florida survey (13%) and for the species considered as a whole (>16%). The differences among surveys were significant (Kruskal-Wallis analysis, Tables 2.2.4, 2.2.5). Tests of whether mean G_{ST} values deviated from zero showed that mean ramet G_{ST} was equivalent to zero for Lakes Huron and Erie and for Florida. For genets, mean G_{ST} was equivalent to zero for Lake Erie, Florida and all sites. So, despite the trend of greater genetic divergence among

Florida sites, the mean divergence was not necessarily significant due to wide variation of G_{ST} at each locus (from near zero for many loci in the Great Lakes, to >25% for IDH-1 and MDH-2 in Florida alone). The loci which contributed the most to the mean G_{ST} values for all sites were MDH-2 (>30%), 6PGDH-1 (>13%) and PGI-1 (11.5% for ramets, 2% for clones). Genet G_{ST} values (Table 2.2.4) tended to be slightly, but not significantly, lower than ramet G_{ST} values (Table 2.2.3).

G_{ST} values are equivalent to F_{ST} values in a simple two-allele system (Hartl and Clark, 1989). In the present study, these values are not equivalent since F statistics were calculated using the Cockerham-Weir ANOVA method which takes into account sample size and variance (Weir, 1990; Lewis, 2000).

The measures of F_{ST} across loci showed departures from zero for all survey areas for ramets (Table 2.2.6; Appendix B, Table B9 for confidence intervals), and all but Lake Huron and the Huron-Erie corridor for genets (Table 2.2.7, Appendix B, Table B9 for confidence intervals). Based on Kruskal-Wallis analysis, Florida and all sites measures of mean ramet F_{ST} were greater than all other survey areas (Table 2.2.6), but mean genet F_{ST} values were not significantly different over survey areas (Table 2.2.7). These results indicate that there was a significant, although relatively low (generally <0.100), amount of genetic differentiation among sites within survey areas, and for ramets, these levels were greater in Florida and when all sites were combined. Mean ramet F_{IS} values differed from zero and were negative for Lake Huron, Lake Ontario, the

Table 2.2.6. Wright's F-statistics and migration rate (Nm) based on Nei's statistics for ramets surveyed at Great Lakes and Florida sites. The F-statistics were estimated using the Cockerham-Weir ANOVA method by Lewis (2000) Genetics Data Analysis program. Nm represents the estimated number of migrants (Nm; Equations in Appendix B, Table B1). Asterisks indicate mean F values that differed significantly from zero based upon 95% confidence intervals generated by bootstrapping (Lewis, 2000). Kruskal-Wallis analysis was performed to test differences among survey areas; results are given at the bottom of the table.

Zone	Locus	F_{IS}	F_{ST}	F_{IT}	Nm
Lake	PGM-2	0	0	0	
Huron	ME-1	-0.3639	0.0349	-0.3163	6.913
n=7	IDH-1	-0.5325	0.0330	-0.4820	7.326
	6PGDH-1	-0.4393	0.0010	-0.4380	249.8
	MDH-2	0.5683	0.0010	0.5688	178.3
	PGI-1	-0.0180	0.0697	0.0530	3.337
	mean	-0.1309*	0.0233*	-0.1024*	74.27
	±SE	±0.1663	±0.0114	±0.1620	±45.16
Huron-Erie	PGM-2	0	0	0	
n=5	ME-1	0.2164	0.0080	0.2229	30.24
	IDH-1	-0.2975	0.0634	-0.2150	3.693
	6PGDH-1	-0.0410	0.0546	0.0160	4.329
	MDH-2	0	0	0	
	PGI-1	0.1803	0.0116	0.1898	21.3
	mean	0.0097	0.0230*	0.0355	9.927
	±SE	±0.0751	±0.0116	±0.0645	±5.194
Lake Erie	PGM-2	0	0	0	
n=3	ME-1	-0.0631	0.0472	-0.0102	5.047
	IDH-1	-0.1723	0	-0.1720	624.8

Zone	Locus	F_{IS}	F_{ST}	F_{IT}	Nm
	6PGDH-1	0.0408	0.0010	0.0420	249.8
	MDH-2	0	0	0	
	PGI-1	0.3695	-0.0010	0.3689	
	mean	0.0291	0.0079*	0.0377	293.2
	±SE	±0.0746	±0.0079	±0.0728	±180.2
Lake	PGM-2	0	0	0	
Ontario	ME-1	-0.1476	0.0362	-0.1061	6.656
n=5	IDH-1	-0.4031	0.0099	-0.3892	25
	6PGDH-1	-0.2567	0.0220	-0.2290	11.11
	MDH-2	-0.0176	0.0137	0	18
	PGI-1	0.0651	0.0421	0.1044	5.688
	mean	-0.1266*	0.0206*	-0.1039*	11.08
	±SE	±0.0727	±0.0066	±0.0733	±3.711
Great	PGM-2	0	0	0	
Lakes	ME-1	-0.0640	0.0622	0	3.769
n=20	IDH-1	-0.3595	0.0602	-0.2777	3.903
	6PGDH-1	-0.1628	0.0467	-0.1085	5.103
	MDH-2	0.3244	0.0117	0.3323	21.12
	PGI-1	0.0884	0.0483	0.1324	4.926
	mean	-0.0289*	0.0382*	0.0134	6.470
	±SE	±0.0945	±0.0106	±0.0848	±3.052
Florida	PGM-2	0	0	0	
n=5	ME-1	0.2463	0.0487	0.2830	4.883
	IDH-1	-0.1860	0.3111	0.1830	0.5563
	6PGDH-1	0.1850	0.0045	0.1886	55.31

Zone	Locus	F_{IS}	F_{ST}	F_{IT}	Nm
	MDH-2	-0.4601	0.2961	-0.0277	0.5943
	PGI-1	-0.0992	0.0716	-0.0205	3.242
	mean	-0.0523	0.1220*	0.1011	10.76
	±SE	±0.1056	±0.0585	±0.0545	±8.942
All sites	PGM-2	0	0	0	
n=25	ME-1	-0.0362	0.0670	0.0332	3.476
	IDH-1	-0.3479	0.0875	-0.2299	2.607
	6PGDH-1	-0.1594	0.1029	-0.0400	2.182
	MDH-2	-0.3595	0.3588	0.1283	0.4468
	PGI-1	-0.0247	0.1286	0.1071	1.694
	mean	-0.1546*	0.1242*	-0.0002	1.734
	±SE	±0.0669	±0.0502	±0.0528	±0.5373
Kruskal-Wallis		4.375	**12.66**	4.381	**15.82**
(p) df=6		(0.6234)	**(0.0488)**	(0.6252)	**(0.0147)**

Table 2.2.7. Wright's F-statistics and migration rate (Nm) based on Nei's statistics for genets (i.e., putative clones) surveyed in the Great Lakes and Florida. Means were calculated using only polymorphic loci. Kruskal-Wallis analysis was performed to test differences among surveys.

Zone	Locus	F_{IS}	F_{ST}	F_{IT}	Nm
Lake Huron	PGM-2	0	0	0	
n=7	ME-1	-0.1477	0.0127	-0.1332	19.44
	IDH-1	-0.3893	0.0172	-0.3654	14.28
	6PGDH-1	-0.1716	-0.0060	-0.1787	
	MDH-2	0.5305	0.0082	0.0543	30.24
	PGI-1	0.0979	-0.0125	0.0867	
	mean	-0.0134	0.0005	-0.0894	21.32
	±SE	±0.1282	±0.0049	±0.0697	±3.641
Huron-Erie	PGM-2	0	0	0	
n=5	ME-1	-0.0610	0.0187	-0.0412	13.12
	IDH-1	-0.1715	0.0042	-0.1666	59.27
	6PGDH-1	0.0089	0.1339	0.1416	1.617
	MDH-2	0	0	0	
	PGI-1	0.1341	0.0059	0.1392	42.12
	mean	0.0054	0.0271*	0.0122	19.36
	±SE	±0.0412	±0.0215	±0.0476	±10.35
Lake Erie	PGM-2	0	0	0	
n=3	ME-1	-0.1152	0.0005	-0.1147	499.8
	IDH-1	-0.3170	0.0111	-0.3024	22.27
	6PGDH-1	0.1138	0.0268	0.1376	9.078
	MDH-2	0	0	0	
	PGI-1	0.1000	-0.0056	0.0949	

Zone	Locus	F_{IS}	F_{ST}	F_{IT}	Nm
	mean	-0.0364	0.0073	-0.0308	63.96
	±SE	±0.0655	±0.0043	±0.0650	±177.0
Lake Ontario	PGM-2	0	0	0	
n=5	ME-1	-0.0281	0.0400	0.0130	5.185
	IDH-1	-0.2806	0.0268	-0.2463	9.078
	6PGDH-1	-0.0557	0.0594	0.0071	0.1709
	MDH-2	-0.0257	0.0145	-0.0108	1.474
	PGI-1	0.2384	-0.0062	0.2337	
	mean	-0.0253	0.1343*	-0.0006	3.977
	±SE	±0.0674	±0.0946	±0.0621	±2.005
Great Lakes	PGM-2	0	0	0	
n=20	ME-1	-0.0884	0.0183	-0.0685	13.41
	IDH-1	-0.2855	0.0177	-0.2628	13.87
	6PGDH-1	-0.0464	0.0791	0.0364	2.911
	MDH-2	0.3538	0.0227	0.3684	10.76
	PGI-1	0.1413	-0.0041	0.1378	
	mean	0.0125	0.0223*	0.0352	10.24
	±SE	±0.0886	±0.0122	±0.0861	±2.537
Florida	PGM-2	0	0	0	
n=5	ME-1	0.3292	0.1006	0.3967	2.235
	IDH-1	-0.0761	0.1413	0.0760	1.519
	6PGDH-1	0.3938	-0.0138	0.3854	
	MDH-2	-0.3501	0.2892	0.0404	0.6145
	PGI-1	0.0801	-0.0173	0.0642	

Zone	Locus	F_{IS}	F_{ST}	F_{IT}	Nm
	mean	0.0628	0.0833*	0.1604*	1.456
	±SE	±0.1117	±0.0492	±0.0737	±0.4061
All sites	PGM-2	0	0	0	
n=25	ME-1	-0.0340	0.0398	0.0072	6.031
	IDH-1	-0.2658	0.0316	-0.2259	7.661
	6PGDH-1	-0.0380	0.1669	0.1355	1.248
	MDH-2	-0.1006	0.3418	0.2756	0.4814
	PGI-1	0.1366	-0.0044	0.1328	
	mean	-0.0503	0.0959*	0.0542	3.856
	±SE	±0.0538	±0.0554	±0.0696	±1.765
Kruskal-		1.756	6.143	6.081	**15.97**
Wallis (p)		(0.9407)	(0.4074)	(0.4141)	**(0.0139)**
df=6					

Great Lakes combined and for all sites combined (Table 2.2.6; Appendix B, Table B9). A negative F_{IS} is indicative of excess heterozygotes resulting from such processes as significant outcrossing or selection. Genet F_{IS} values were not different from zero (Table 2.2.7; Appendix B, Table B9), and were consistent with sites undergoing random mating. The overall inbreeding co-efficient, F_{IT} was different from zero for Lake Huron and Lake Ontario ramets where it was negative and indicative of

outcrossing effects, and for Florida genets where it was positive and indicative of inbreeding effects. In general, however, values of zero for F_{IT} reflect random mating and low divergence of sites within survey areas.

The number of migrants per generation (Nm) varied greatly among loci within survey areas (Tables 2.2.6, 2.2.7). In the cases where G_{ST} or F_{ST} were equivalent to zero, values for Nm convey little information as the sites within those areas act as a single panmictic unit. Furthermore, variability in Nm values reflects the variability observed in locus behaviour, and again is suggestive of a system not at equilibrium. Nm should therefore be treated as an indicator of gene flow, but not an absolute measure. Kruskal-Wallis tests for differences among survey areas indicated that mean ramet Nm was lower for Florida and all sites combined than for the other survey areas. These were also lower for genets, as was mean genet Nm for Ontario.

In order to test the effect of geographic isolation on genetic diversity, Great Lakes sites were categorized as either isolated or not, depending on

whether their water flow was directly associated with Great Lakes mass-flow (Table 2.2.1 for categorization). A t-test on mean H_{EXP} for these two groups was non-significant (t=0.1599, df=18, p=0.875). To determine if sites downstream experienced greater or reduced genetic variability, a further test of non-isolated sites regressing mean H_{EXP} with site position downstream was performed, and was non-significant (F=0.3787, df=1,10, p=0.5521).

Genetic distance

Estimates of Nei's genetic distance between pairs of sites, based on ramets, ranged from 0.005-0.0313 for the individual surveys in the Great Lakes, and 0.002-0.0716 when these sites were considered all together (Table 2.2.8). For all survey areas but Lake Erie, genetic distance for both ramets and genets were significantly greater than zero (Table 2.2.8). For genets in comparison with ramets, the ranges and means were somewhat smaller for Lake Huron and the Huron-Erie corridor, but greater for Lake Erie and Lake Ontario (Table 2.2.8). Overall, for the 20 Great Lakes sites together, mean genet genetic distance between pairs of sites (0.100±0.006) was smaller than mean ramet genetic distance (0.0153±0.0010; Table 2.2.8, t=5.064, df=290, p<0.001). Based on Scheffé's *post hoc* test the mean genetic distance between pairs of Florida sites was greater than mean survey values found in the Great Lakes, with the exception of pairs of Lake Erie sites (F=124.57, df=6, 293, p<0.001). These values were even greater than the mean genetic distances among the combined

Table 2.2.8. Mean Nei's genetic distance ±(SE) for ramets and genets based on values for each possible pair of sites, and mean geographic distance between all pairs of sites for the surveys of the Great Lakes and Florida. T-tests were performed to determine if mean genetic distances were greater than zero. T-tests were also performed to determine if ramet and genet genetic distance differed. Results of regression analysis of genet genetic distance with geographic distance are given in the last two columns. *p<0.05, **p<0.01, ***p<0.001.

Area		Genetic distance Ramets	Genetic distance Genets	t-test between ramet and genet	Geographic distance (km)	Genet regression F(p)
Lake Huron	n	21	21	t=1.191	21	6.415*
	mean±SE	0.0106±0.0025***	0.0073±0.0012***	df=40	163.8±21.56	(0.0203)
	range	0.0005-0.0313	0.0018-0.0192	p=0.2405	30-370	
Huron-Erie	n	10	10	t=1.928	10	0.0005
	mean±SE	0.0096±0.0016***	0.0059±0.0011***	df=18	50.0±8.85	(0.9834)
	range	0.0036-0.0200	0.0008-0.0126	p=0.0699	5-105	
Lake Erie	n	3	3	t=-0.8766	3	2.792
	mean±SE	0.0058±0.0024	0.0092±0.0031	df=4	170.0±44.44	(0.3433)
	range	0.0032-0.0105	0.0051-0.0152	p=0.4302	105-255	

Area		Genetic distance Ramets	Genetic distance Genets	t-test between ramet and genet	Geographic distance (km)	Genet regression F(p)
Lake	n	10	10	t=-1.758	10	0.5143
Ontario	mean±SE	0.0086±0.0025**	0.0176±0.0045**	df=18	228.0±41.39	(0.4937)
	range	0.0007-0.0270	0.0055-0.0512	p=0.0958	70-500	
Great	n	190	190	t=5.064	190	10.35**
Lakes	mean±SE	0.0153±0.0010***	0.0100±0.0006***	df=290	570.5±28.20	(0.0016)
1994	range	0.0002-0.0716	0.0006-0.0512	p<0.001***	5-1560	
Florida	n	10	10	t=-1.045	10	2.875
	mean±SE	0.0486±0.0081***	0.0604±0.0077***	df=18	148.0±27.40	(0.1284)
	range	0.0091-0.0736	0.0168-0.0914	p=0.3097	10-240	
Species	n	300	300	t=-0.7733	300	1.691
	mean±SE	0.0397±0.0023***	0.0381±0.0025***	df=198	910.4±35.33	(0.1965)
	range	0.0002-0.1815	0.0006-0.1987	p=0.4403	5-1930	

Table 2.2.9. Nei's genetic distances between pairs of sites: for ramets above diagonal (in bold), for genets (putative clones) below diagonal.

	Lake Huron							Huron-Erie Corridor				
	Thes	Blind	Serp	Span	Bayf	Key	Big C	Clay	Walp	Mitch	Pech	Ston
Thes	-	**0.000**	**0.004**	**0.004**	**0.003**	**0.004**	**0.030**	**0.005**	**0.010**	**0.010**	**0.019**	**0.023**
Blind	0.003	-	**0.002**	**0.001**	**0.003**	**0.001**	**0.020**	**0.005**	**0.018**	**0.014**	**0.024**	**0.029**
Serp	0.010	0.004	-	**0.021**	**0.004**	**0.001**	**0.029**	**0.004**	**0.010**	**0.011**	**0.022**	**0.021**
Span	0.002	0.004	0.010	-	**0.006**	**0.001**	**0.030**	**0.010**	**0.012**	**0.013**	**0.025**	**0.026**
Bayf	0.003	0.003	0.010	0.003	-	**0.005**	**0.023**	**0.001**	**0.016**	**0.007**	**0.010**	**0.016**
Key	0.002	0.004	0.010	0.003	0.007	-	**0.026**	**0.005**	**0.015**	**0.013**	**0.024**	**0.029**
Big C	0.012	0.017	0.013	0.019	0.013	0.015	-	**0.029**	**0.072**	**0.053**	**0.050**	**0.060**
Clay	0.005	0.010	0.010	0.010	0.010	0.010	0.010	-	**0.011**	**0.004**	**0.010**	**0.012**
Walp	0.002	0.002	0.010	0.001	0.004	0.003	0.021	0.010	-	**0.010**	**0.020**	**0.014**
Mitch	0.002	0.001	0.010	0.003	0.004	0.004	0.019	0.010	0.002	-	**0.004**	**0.090**
Pech	0.001	0.005	0.010	0.004	0.010	0.002	0.010	0.005	0.004	0.005	-	**0.010**
Ston	0.010	0.010	0.010	0.013	0.010	0.011	0.010	0.001	0.013	0.010	0.010	-
Rond	0.003	0.010	0.015	0.003	0.010	0.005	0.025	0.016	0.001	0.003	0.010	0.016
Long	0.002	0.004	0.010	0.005	0.002	0.010	0.010	0.002	0.010	0.004	0.003	0.003
Put	0.010	0.010	0.014	0.010	0.003	0.017	0.016	0.011	0.112	0.010	0.013	0.010

	Lake Huron							Huron-Erie Corridor				
	Thes	Blind	Serp	Span	Bayf	Key	Big C	Clay	Walp	Mitch	Pech	Ston
Niag	0.012	0.012	0.010	0.014	0.022	0.005	0.026	0.016	0.084	0.012	0.010	0.020
Brigh	0.010	0.006	0.014	0.010	0.010	0.015	0.027	0.014	0.158	0.010	0.015	0.014
Adol	0.010	0.003	0.005	0.010	0.004	0.010	0.016	0.010	0.059	0.010	0.010	0.010
Howe	0.020	0.015	0.023	0.016	0.010	0.028	0.031	0.024	0.070	0.017	0.025	0.021
SLan	0.010	0.010	0.010	0.004	0.010	0.010	0.034	0.019	0.003	0.010	0.010	0.022
Blue	0.111	0.103	0.100	0.103	0.084	0.122	0.104	0.106	0.112	0.108	0.117	0.097
Chass	0.102	0.079	0.073	0.085	0.072	0.098	0.120	0.103	0.084	0.081	0.103	0.095
Hom	0.158	0.145	0.123	0.143	0.122	0.156	0.149	0.150	0.158	0.152	0.164	0.139
StM	0.061	0.052	0.058	0.056	0.040	0.074	0.063	0.060	0.059	0.057	0.068	0.053
Wak	0.064	0.054	0.050	0.067	0.044	0.076	0.042	0.048	0.070	0.061	0.066	0.037

	Lake Erie			Lake Ontario				
	Rond	Long	Put	Niag	Brigh	Adol	Howe	SLan
Thes	0.030	0.020	0.010	0.010	0.004	0.004	0.005	0.004
Blind	0.036	0.023	0.012	0.010	0.004	0.002	0.010	0.010
Serp	0.036	0.022	0.013	0.010	0.001	0.000	0.010	0.004
Span	0.040	0.026	0.016	0.010	0.002	0.000	0.010	0.004
Bayf	0.018	0.010	0.004	0.017	0.004	0.004	0.003	0.010
Key	0.037	0.025	0.015	0.005	0.002	0.001	0.010	0.005
Big C	0.057	0.042	0.022	0.028	0.037	0.032	0.038	0.050
Clay	0.016	0.010	0.004	0.019	0.003	0.003	0.002	0.010
Walp	0.035	0.024	0.025	0.030	0.010	0.010	0.010	0.005
Mitch	0.012	0.010	0.010	0.033	0.010	0.010	0.003	0.010
Pech	0.002	0.001	0.010	0.050	0.016	0.020	0.010	0.018
Ston	0.016	0.010	0.011	0.054	0.018	0.020	0.010	0.021
Rond	-	0.004	0.010	0.070	0.030	0.030	0.010	0.030
Long	0.007	-	0.003	0.050	0.018	0.020	0.006	0.022
Put	0.015	0.010	-	0.033	0.013	0.013	0.005	0.018
Niag	0.014	0.019	0.036	-	0.012	0.009	0.027	0.015

	Lake Erie			Lake Ontario				
	Rond	Long	Put	Niag	Brigh	Adol	Howe	SLan
Brigh	0.011	0.010	0.010	0.029	-	**0.001**	**0.005**	**0.002**
Adol	0.014	0.010	0.010	0.020	0.010	-	**0.006**	**0.003**
Howe	0.024	0.015	0.003	0.051	0.010	0.010	-	**0.007**
SLan	0.006	0.014	0.018	0.011	0.011	0.010	0.023	-
Blue	0.124	0.096	0.070	0.161	0.083	0.084	0.060	0.117
Chas	0.101	0.094	0.067	0.114	0.064	0.059	0.053	0.066
Hom	0.173	0.141	0.114	0.199	0.132	0.126	0.107	0.150
StM	0.070	0.050	0.027	0.106	0.036	0.038	0.018	0.066
Wak	0.084	0.046	0.029	0.103	0.048	0.037	0.030	0.083

	Florida				
	Blue	Chas	Hom	StM	Wak
Thes	0.099	0.082	0.131	0.078	0.071
Blind	0.118	0.097	0.140	0.101	0.080
Serp	0.113	0.078	0.130	0.096	0.076
Span	0.123	0.085	0.142	0.105	0.085
Bayf	0.083	0.074	0.111	0.068	0.052
Key	0.123	0.093	0.142	0.105	0.086
Big C	0.132	0.149	0.146	0.120	0.086
Clay	0.083	0.068	0.112	0.068	0.050
Walp	0.100	0.042	0.129	0.081	0.072
Mitch	0.074	0.056	0.110	0.058	0.046
Pech	0.049	0.061	0.090	0.037	0.024
Ston	0.049	0.031	0.082	0.036	0.023
Rond	0.044	0.079	0.090	0.034	0.020
Long	0.044	0.060	0.084	0.034	0.019
Put	0.062	0.072	0.093	0.050	0.030
Niag	0.172	0.116	0.182	0.152	0.128

	Florida				
	Blue	Chas	Hom	StM	Wak
Brigh	**0.106**	**0.068**	**0.132**	**0.088**	**0.071**
Adol	**0.111**	**0.070**	**0.133**	**0.093**	**0.074**
Howe	**0.067**	**0.054**	**0.102**	**0.050**	**0.041**
SLan	**0.107**	**0.064**	**0.133**	**0.088**	**0.078**
Blue	-	**0.067**	**0.045**	**0.009**	**0.018**
Chas	0.079	-	**0.067**	**0.064**	**0.067**
Hom	0.063	0.057	-	**0.074**	**0.064**
StM	0.017	0.055	0.087	-	**0.012**
Wak	0.055	0.074	0.091	0.026	-

Great Lakes/Florida sites (genet value: 0.0381 ±0.0025; ramet value: 0.0397 ±0.0023; Table 2.2.8). This may have been due to the greater representation of Great Lakes pairs in comparison with Florida pairs and Great Lakes/Florida pairs. Genetic distance between each pair of sites surveyed is presented in Table 2.2.9.

Generally a positive relationship between genetic distance and geographic distance is observed (see Hartl and Clark, 1989). Geographic distances between Great Lakes sites were measured as water distance, and distances between Florida sites and between Florida and Great Lakes sites were estimated using most direct land distances. Genet genetic distances and ramet genetic distances were very strongly correlated (R^2 =0.758, F=935.074, p<0.001, df=1,298), enabling genet distances to be used to represent both ramet and genet distances in subsequent regression analyses. Results of regression analysis were highly significant when all Great Lakes and Florida sites were analyzed (R^2 =0.476, F=270.960, p<0.001, df=1,298; Figure 2.2.4 a). The relationship showed a sharp incline in rate of change of genetic distance at approximately 1000 km. This corresponded to using only the Great Lakes sites in the analysis, and here the relationship between genetic and geographic distance was again significantly positive (R^2 =0.051, F=10.018, p<0.01, df=1,188; Figure 2.2.4b). Graphically, the change is consistent with sites showing clinal changes with distance, with a more dramatic cline at about 500 km (Richardson *et al.*, 1986). When regression of genetic distance with

Figure 2.2.4. Regression relationships between clonal genetic and geographic distances for a) all sites, b) Great Lake sites and c) Florida sites. Regressions were significant for all sites (R^2 =0.476, F=270.960, p<0.001, df=1, 298) and for the Great Lakes (R^2 =0.051, F=10.018, p<0.01, df=1, 188), but not for Florida (R^2 =0.264, F=2.875, p=0.128, df=1, 8). Lines were smoothed with the LOWESS method (Systat, 1997).

Figure 2.2.4

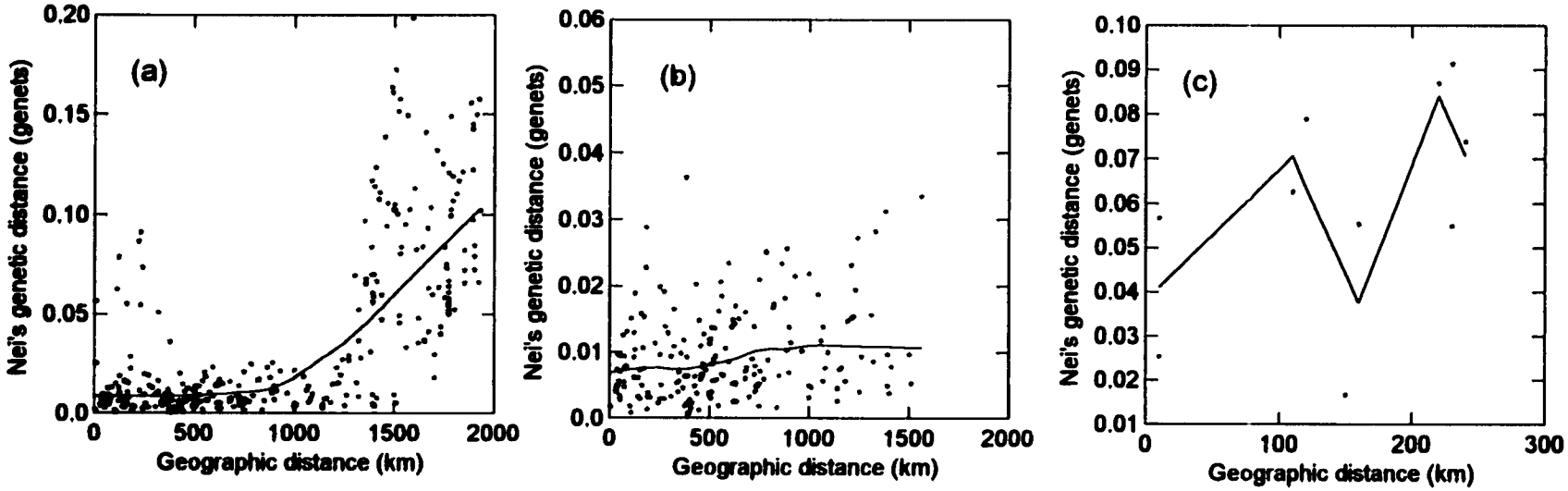
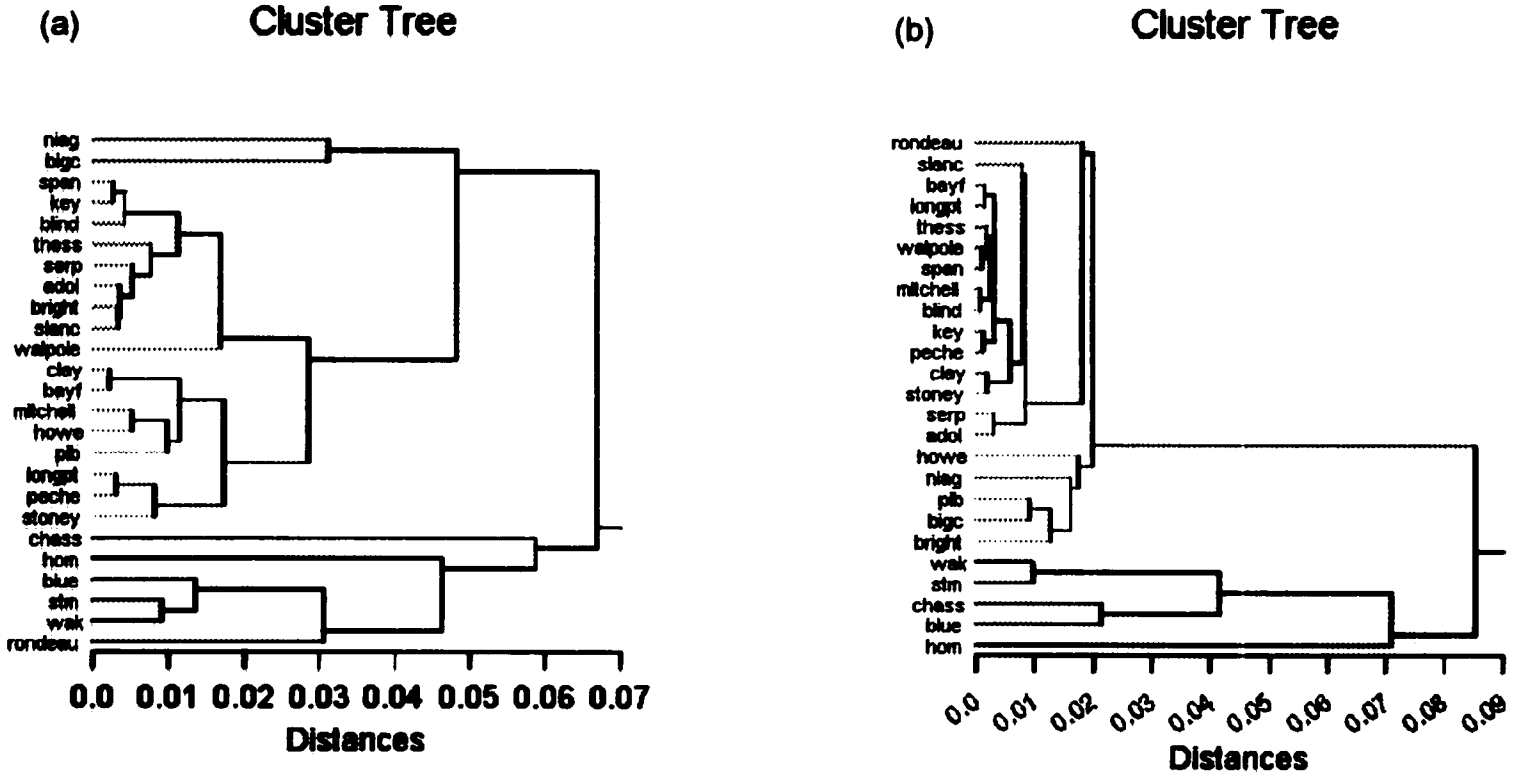

(a)
Nei's genetic distance (genets)
0.20
0.15
0.10
0.05
0.0
0
500
1000
1500
2000
Geographic distance (km)
(b)
Nei's genetic distance (genets)
0.06
0.05
0.04
0.03
0.02
0.01
0.0
0
500
1000
1500
2000
Geographic distance (km)
(c)
Nei's genetic distance (genets)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
100
200
300
Geographic distance (km)

Figure 2.2.5. Cluster analysis (UPGMA) dendograms of genetic distance clustering sites based on a) ramet and b) genet values for Nei's genetic distance.

Figure 2.2.5


(a)
Cluster Tree
niag
bigc
span
key
blind
thess
serp
edol
bright
slanc
walpole
clay
bayf
mitchell
howe
plb
longpt
peche
stoney
chess
hom
blue
stm
wak
rondeau
0.0 0.01 0.02 0.03 0.04 0.05 0.06 0.07
Distances
(b)
Cluster Tree
rondeau
slanc
bayf
longpt
thess
walpole
span
mitchell
blind
key
peche
clay
stoney
serp
edol
howe
niag
plb
bigc
bright
wak
stm
chess
blue
hom
0.0 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08 0.09
Distances

geographic distance was performed separately for survey areas, Lake Huron was the only Great Lake area which showed a significant positive relationship (R^2 =0.252, F=6.415, p=0.0203, df=1,19). Florida sites on their own did not show a significant relationship between genetic and geographic distance (R^2 =0.264, F=2.875, p=0.128, df=1,8; Figure 2.2.4c). This lack of a relationship may be due to the small sample size (5 sites), as well as the relative geographic isolation of the Florida sites. The Florida sites generally did not share water systems (except Wakulla R and St. Marks R.) as compared to the Great Lakes sites, which were all arrayed along the Great Lakes-St. Lawrence Seaway.

The UPGMA phenograms of Nei's genetic distance for ramets and genets show the Florida sites clustered apart from the Great Lakes sites at genetic distances of 0.066 and 0.085 respectively (Figures 2.2.5a, 2.2.5b). The Great Lakes sites were clustered in a pattern generally consistent with their geographic locations, reflecting low genetic distances between geographically- close sites, with Niagara and Big Chute diverging from the geographical pattern (Figure 2.2.5a). For the genets, Big Chute, which is in Georgian Bay of Lake Huron, clustered with the Lake Ontario sites; Rondeau Bay was an outlier, and the other Great Lakes sites were clustered at genetic distances <0.01 (Figure 2.2.5b). The genetic distance at which Florida sites branched from the Great Lakes sites was generally greater than the genetic distance at which Great Lakes sites clustered from each other.

Table 2.2.10. Measure of clonal diversity for the Great Lake-Florida survey, based on putative clones identified using only the six genetically interpretable loci plus gender. Number of ramets (Nr) with full composite allelomorphs, the number of distinct allelomorphs observed (G), the maximum number of allelomorphs possible (Gmax), the proportion of distinct genotypes (G/Nr), clonal diversity (D), and genotypic evenness (E) (Ellstrand and Roose, 1989). Gmax is equal to Nr when Nr is lower than the theoretical G_{max}.

Area	Site	Nr	G	Gmax	G/Nr	D	E
Lake Huron	Thessalon	110	25	110	0.227	0.907	0.892
	Blind R.	96	30	96	0.313	0.923	0.883
	Serpent R.	91	24	91	0.264	0.878	0.827
	Spanish Harbour	117	25	117	0.214	0.864	0.827
	Bayfield Inlet	109	26	109	0.239	0.900	0.875
	Key R.	79	28	79	0.354	0.915	0.848
	Big Chute	65	32	65	0.492	0.962	0.912
Huron-Erie	Clay Creek	125	28	125	0.224	0.909	0.893
	Walpole Is.	235	33	235	0.140	0.913	0.915
	Mitchell's Bay	297	48	297	0.162	0.928	0.921
	Peche Is.	177	41	177	0.232	0.925	0.904
	Stoney Is.	494	42	494	0.090	0.803	0.786
Lake Erie	Rondeau Bay	213	36	213	0.169	0.907	0.897
	Long Point Bay	198	43	198	0.217	0.912	0.884
	Put-in-Bay	76	25	76	0.329	0.932	0.908
Lake Ontario	Niagara R.	101	44	101	0.436	0.967	0.937
	Brighton	105	24	105	0.229	0.887	0.861
	Adolphustown	107	23	107	0.215	0.873	0.845
	Howe Is.	118	28	118	0.237	0.929	0.923
	South Lancaster	92	28	92	0.304	0.945	0.936
Florida	Blue Spring	55	21	55	0.382	0.901	0.814
	Chassahowitza	70	17	70	0.243	0.915	0.928

Area	Site	Nr	G	Gmax	G/Nr	D	E
	Homosassa Spr.	87	24	87	0.276	0.915	0.892
	St. Mark's R.	107	15	107	0.140	0.743	0.715
	Wakulla R.	46	10	46	0.217	0.744	0.688

Clonal diversity

The proportion of seven character allelomorphs which was distinguishable (G/Nr; Table 2.2.10, see Appendix B., Table B1 for equations) was calculated as the number of allelomorphs detected at each site divided by the number of individuals yielding readable results for the seven characters, rather than the theoretical maximum number of allelomorphs possible (see Eckert and Barrett, 1993); the theoretical maximum number of allelomorphs is quite high based on seven characters, each having 2 or more alternate patterns, in all possible combinations. The proportion distinguishable was generally consistent within surveys, with site values ranging from 0.0850 at Stoney Is. to 0.4923 at Big Chute (Table 2.2.10). Allelomorph (seven-character) diversity and evenness values were generally greater than 0.85 and 0.80, respectively, over all of the survey areas. This suggests that the allelomorphs were present in fairly equal abundances, with no one particular, or small group of allelomorphs dominating. Three sites, Stoney Is., St. Marks and Wakulla, were exceptions; each showed lower diversity and evenness than the rest of the sites, with values <0.80 for both parameters (Table 2.2.10). These three sites, then, had lower numbers of genotypes in relation to the number of ramets surveyed, and had a more uneven distribution of genets than did sites with higher evenness values.

The percentage of unique seven-character allelomorphs was generally low among sites within each of the Great Lakes surveys (0-19%; Table 2.2.11), while it was highly variable in the Florida survey (0-62%). This result echos the

Table 2.2.11. Unique (present only at one site) and shared allelomorphs (between pairs of sites sampled). Total allelomorphs detected per site is noted below each site name. Based on genets (putative clones) composed of 6 loci plus gender, a total of 203 different allelomorphs were detected, 89 of which were unique to single sites. The numbers of unique allelomorphs per site are presented on the diagonal (in bold).

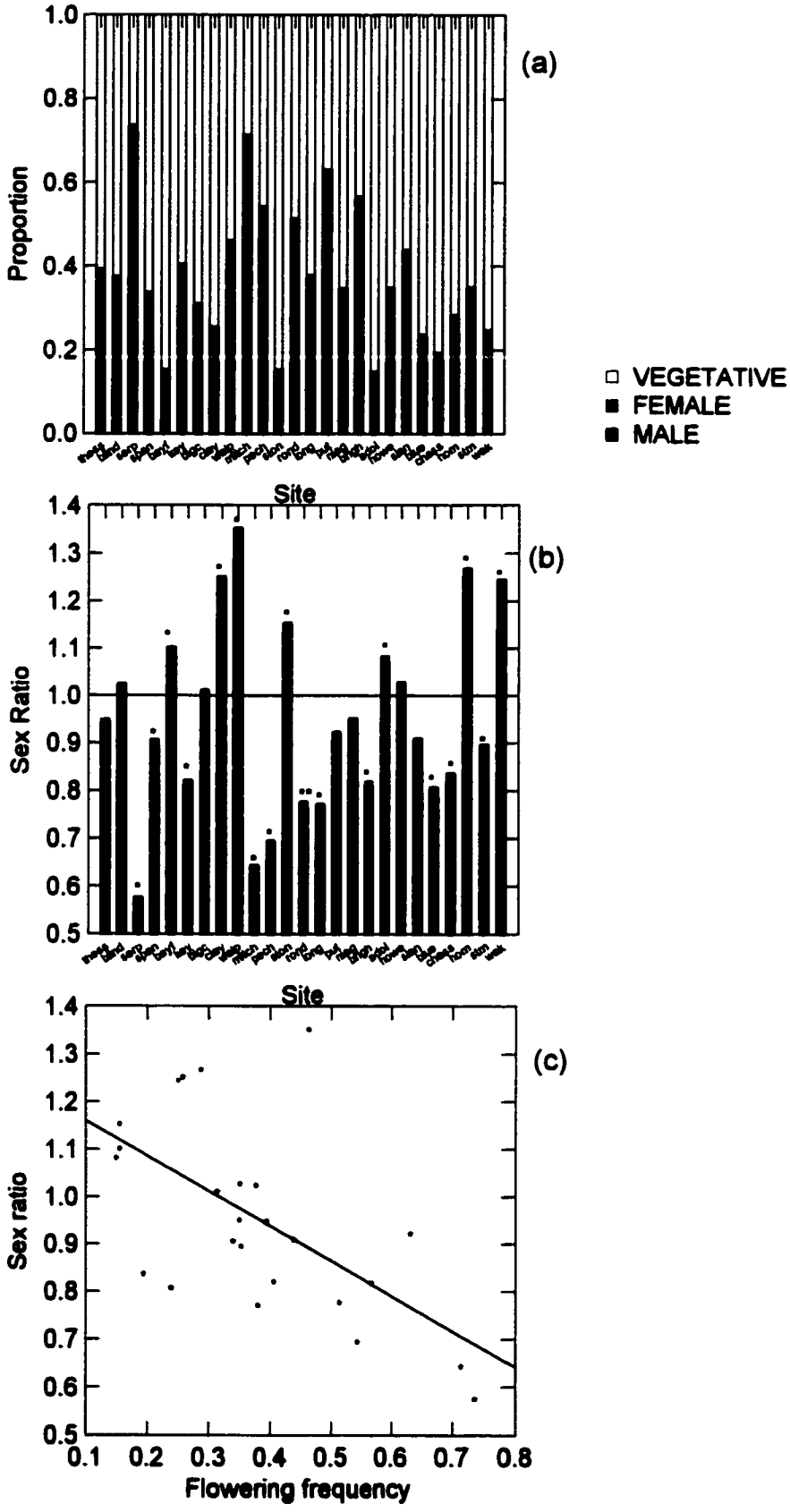
	Lake Huron							Huron-Erie Corridor				
	Thes	Blind	Serp	Span	Bayf	Key	Big C	Clay	Walp	Mitch	Pech	Ston
	25	30	24	25	26	28	32	28	33	48	41	42
Thes	**0**											
Blind	15	**0**										
Serp	12	15	**4**									
Span	17	15	11	**1**								
Bayf	17	15	9	15	**4**							
Key	16	15	13	14	11	**3**						
Big C	14	12	8	13	15	9	**6**					
Clay	9	11	4	11	12	6	12	**3**				
Walp	12	13	7	13	13	6	12	18	**4**			
Mitch	18	19	14	16	12	18	12	14	22	**6**		
Pech	13	17	14	12	11	13	15	13	18	25	**4**	
Ston	10	13	5	13	11	9	16	22	20	20	17	**5**
Rond	15	16	11	16	14	15	11	13	17	24	18	16

	Lake Huron								Huron-Erie Corridor			
	Thes	Blind	Serp	Span	Bayf	Key	Big C	Clay	Walp	Mitch	Pech	Ston
	25	30	24	25	26	28	32	28	33	48	41	42
Long	16	16	13	15	16	16	15	15	17	25	24	22
Put	14	14	12	14	13	12	11	13	13	18	16	12
Niag	16	17	15	17	12	15	12	15	18	24	21	18
Brigh	10	11	9	9	9	7	7	8	10	11	11	8
Adol	8	11	6	10	10	5	10	12	12	12	12	17
Howe	11	13	9	12	14	8	12	13	15	15	16	15
SLan	11	11	9	11	11	10	7	9	13	16	13	11
Blue	2	2	2	0	3	2	2	1	2	3	4	1
Chas	0	3	3	2	0	1	1	1	2	3	3	2
Hom	0	1	1	1	0	0	0	1	1	1	1	1
StM	3	9	7	4	4	5	3	3	6	7	7	4
Wak	1	2	1	1	2	1	4	3	5	5	4	7

	Lake Erie			Lake Ontario					Florida				
	Rond	Long	Put	Niag	Brigh	Adol	Howe	SLan	Blue	Chas	Hom	StM	Wak
	36	43	25	44	24	23	28	28	21	17	24	15	10
Rond	**3**												
Long	24	**2**											
Put	18	15	**0**										
Niag	25	13	17	**2**									
Brigh	12	14	13	14	**4**								
Adol	11	15	11	15	10	**0**							
Howe	15	18	19	16	14	14	**3**						
SLan	15	16	14	18	11	12	17	**3**					
Blue	3	3	2	3	1	0	2	2	**9**				
Chas	1	3	2	3	2	2	3	3	4	**9**			
Hom	0	1	0	1	1	1	1	3	5	5	**15**		
StM	6	7	6	7	4	4	8	7	5	4	1	**2**	
Wak	1	5	3	3	1	4	4	2	2	2	0	4	**0**

Figure 2.2.6. a) Proportion of male, female and vegetative ramets per site. b) Ramet sex ratios per site. Asterisks identify sites with sex ratios significantly different from 1. c) Regression relationship between sex ratio and flowering frequency, which was significant (R^2=0.3668, F=13.32, p<0.01, df=1, 23).

Figure 2.2.6.


(a)
Proportion
1.0
0.8
0.6
0.4
0.2
0.0
VEGETATIVE
FEMALE
MALE
Site
(b)
Sex Ratio
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
Site
(c)
Sex ratio
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Flowering frequency

genetic substructuring pattern identified using Nei's measures of genetic diversity, where greater partitioning of genetic diversity was seen among the Florida sites than among the Great Lakes sites. The three Florida sites with high proportions of unique allelomorphs were those that were more physically isolated from one another (i.e., site Blue Springs, Chassahowitza, and Homosassa were in separate water systems).

Seven-character allelomorphs that were shared between pairs of sites arepresented in Table 2.2.11. There is no systematic geographic pattern, except that relatively few allelomorphs were shared between Great Lakes and Florida sites. Overall, 203 different seven-character allelomorphs were detected, 89 (44%) of which were unique to a site, while the remainder were present in 2 or more sites. All seven-character allelomorphs detected in the Great Lakes and Florida, and their frequencies are presented in Appendix B, Tables B10 and B11 respectively).

Flowering

Flowering frequency ranged from about 15% of shoots at Bayfield Inlet, Stoney Island and Adolphustown to 74% at Serpent River (Figure 2.2.6a, Appendix C, Table C1). Flowering males were generally more abundant than females; eleven sites had significantly male-biased flowering sex ratios, seven were significantly female-biased, and the remaining seven sites did not depart from a flowering sex ratio of 1 (Figure 2.2.6b). Sex ratios were calculated as

(frequency of females+1) / (frequency of males+1). There was no apparent geographic trend in the shoot sex ratio. However, with increasing flowering frequency, the sex ratio declined significantly; that is, there was a change in sex ratio from female- to male-biased frequencies (R^2=0.3668, F=13.32, p<0.01, df=1, 23, Figure 2.2.6c).

Discussion

Genetic diversity

Given that *V. americana* is epihydrophilous, flowers regularly, and produces seed by obligate outcrossing, and that a previous investigation at Turkey Is. showed high levels of genetic diversity (Lokker et al., 1994), sites were expected to be genetically variable, despite the species' ability to reproduce vegetatively. Genetic differentiation among sites was expected to be higher as distances between sites increased; most notably Florida sites were expected to be genetically disjunct from those sampled in the Great Lakes .

The previous study of *V. americana* at Turkey Island in the Detroit River, found levels of ramet and genet genetic variability to be high (Lokker *et al.* 1994). The results of the present study support this finding, and indicate high genetic variation calculated for both ramets and genets in the Great Lake basin as a whole, and in Florida.

Levels of genetic diversity found in this geographically extensive survey were higher than or comparable to other values reported for clonal species,

ranging from a mean H_{exp} of 0.182 for Lake Erie, to 0.246 for Lake Ontario (Table 2.2.2). Looking at discrete genets of the outcrossing, wind-pollinated *Quercus havardii*, Mayes *et al.* (1998) found an H_{exp} of 0.289. Using clones as individuals in the clonal aquatic *Posidonia oceanica*, Procaccini and Mazzella (1998) reported population H_{exp} to range from 0.190-0.380 based on ISSR (DNA inter-simple sequence repeat) analysis. Although values for *V. americana* genetic diversity were high, they were not out of line with values determined for other species having similar life-history traits (Mayes *et al.*, 1998; Procaccini and Mazzella, 1998)

According to assumptions of the HWE, in the absence of other factors operating, following one generation of random mating, allele frequencies should reach equilibrium values (cf Ayala, 1982; Hartl and Clark, 1989). For the sites sampled in the current study, tests of Hardy-Weinberg Equilibrium (HWE) often indicated statistically significant departure from expected genotype frequencies for ramets, but rarely for genets. Most often these deviations were a result of an excess of heterozygotes. Similarly, based on heterogeneity χ^2 tests, allele frequencies within survey areas were generally not different among sites for genets whereas they tended to be for ramets. The ability to distinguish between ramets and putative genets allowed for the effect of clonal propagation of successful genets to be separated from the genet composition of a population. As such, the genet populations showed characteristics consistent with successful sexual random mating. This was further supported by non-significant

F values for genets. The deviations observed for the ramets may reflect selective advantages for the heterozygotes, resulting in their excess. Such selective advantages may be realized as a higher rate of ramet production for the heterozygotes, and warrants further study. Such effects, however, also lead to the conclusion that the system under study was not at equilibrium.

In cases where significant HWE deviations were detected for genets, heterozygotes were usually found in excess. The PGI-1 locus was the exception to this, exhibiting significant deviations from HWE with heterozygote deficiencies corresponding to inbreeding effects. Ordinarily, such inbreeding would result in heterozygote deficiency at all loci (Richardson *et al.*, 1896; Hartl and Clark, 1989; Mayes *et al.*, 1998). This variability in locus behaviour, and the variability in F values suggest that the sites studied were not in equilibrium. As such, the measures based on assumptions of equilibrium (Nei's genetic diversity statistics, Wright's F statistics and Nm) must be interpreted cautiously.

Levels of genetic variability in the various surveys of *V. americana* in the Great Lakes were high, as indicated by high H_T values (Tables 2.2.3, 2.2.5). Genetic divergence among the sites was quite low as indicated by very low D_{ST} and G_{ST} values. Yet based on high locus-to-locus variability, only mean ramet G_{ST} for Lakes Huron and Erie and for Florida and genet G_{ST} values Lake Erie, Florida and all sites were in fact equivalent to zero. Significant (although low) genetic differentiation using G_{ST} as a measure was therefore detected among sites in most survey areas. F_{ST} values differed from zero for all ramet survey

areas, and all but Lakes Huron and Erie for genets (Tables 2.2.4, 2.2.6). Again, this suggests that a significant, but fairly low level, of population differentiation did exist in most survey areas. For ramets in Florida and all sites combined, genetic differentiation among sites was significantly greater than that detected within the other survey areas. Despite the F_{ST} values being significantly different from zero based on bootstrapping, their absolute value is low, indicating that considerable gene flow was present among sites within survey areas. Furthermore, values for F_{IS} and F_{IT} equivalent to zero (which was the case for genets for all survey areas but Florida F_{IT}) reflect sites undergoing random mating with low divergence, or sites acting as a panmictic unit.

The degree to which genetic variability is partitioned among sites depends largely on the species' capacity for gene flow, with outcrossing and wind-pollinated terrestrial species having greater within-population variation than selfing or insect-pollinated terrestrial species (Loveless and Hamrick, 1984; Hamrick and Godt, 1989; Barrett *et al.*, 1993). A number of authors have detected low levels of population differentiation and high genetic diversity despite a lack of evidence of successful sexual reproduction in a number of taxa. Jonsson *et al.* (1996) found H_{exp}=0.167 and G_{ST}=0.055 for the rhizomatous *Carex bigelowii* at sites where seedlings had not been observed for five years. Mayes *et al.* (1998) reported a similar trend for *Quercus havardii*. For the wind-pollinated, sexual *Plantago cordata*, Mymudes and Les (1993) found the opposite trend; G_{ST} was high (0.865), while total diversity was low (H_T=0.0916)

partly as a result of low reproductive output. Low genetic subdivision (G_{ST}) values, ranging from 0-0.0862 have been reported for *V. americana* genets within the total population at Turkey Is. At this site, significant seed production and seedling germination has been observed, though levels of seedling recruitment have not been determined (Lokker *et al.*, 1994).

Laushman (1993) examined twelve populations of *V. americana* in lakes and rivers of northern Ohio, and around islands in western Lake Erie. Laushman's results, based on analysis of 5 enzymes, indicated low levels of variation within populations (H_{exp}=0.085) and high levels of differentiation among populations (G_{ST}=0.457). He suggested that the lack of variation he observed may have been due to rapid clonal expansion of a few successful genotypes in the sites he studied. The populations of *V. americana* which he studied were multiclonal and he attributed the differentiation among the populations to geographic isolation since samples were taken from lakes and isolated river populations (Laushman, 1993), between which little gene flow would be anticipated (Hamrick and Godt, 1989).These patterns are consistent with observations for Florida sites, which were also isolated geographically from each other and experienced greater genetic subdivision. These sites also tended to have fewer putative clones than comparable sites in the Great Lakes; however, they did show higher genetic diversity and lower genetic differentiation than Laushman's *V. americana* populations.

Overall, the genetic diversity data indicated that there was a low amount

of significant genetic differentiation (G_{ST}) among the sites in all survey areas, and a greater amount of differentiation between Florida sites and between all sites. Despite rare observations of seedling recruitment, the species is highly variable genetically, and shows evidence of regular outcrossing (as indicated by HWE and non-significant F_{IS} values for genets). There is also evidence that sites in the Great Lakes are genetically similar, a finding which implicates gene flow as an important population process in this species. Similar selective forces in the Great Lakes could also contribute to the genetic similarly observed.

Clonal diversity

Many terrestrial clonal plant species are thought to rarely reproduce sexually, nevertheless they have repeatedly been shown to form multiclonal populations, composed of a number of different genotypes (see Ellstrand and Roose, 1987; Widén *et al.* 1994 for reviews). Such populations tend to show intermediate clonal diversity and eveness, reflecting clonal composition which is neither dominated by a few genotypes, nor equally represented by all (Ellstrand and Roose, 1987; Widén *et al.* 1994). Widén *et al.* (1994) reported that genotypes tend to be unique (mean 62%) rather than shared (20%) among populations, and that increased sample size typically results in increased probability of sampling a new genotype. The general dearth of common genotypes has been interpreted as suggesting that gene flow is not of major importance to the clonal composition and genetics of clonal species in general

(Ellstrand and Roose, 1987). Populations of *V. americana* were expected to be multiclonal following the trend of other clonal species and the results reported for Turkey Is. (Lokker et al., 1994).

Clonal diversity and evenness were generally high, corresponding to many, well-represented allelomorphs. A large proportion of the allelomorphs (56%) were shared by at least two sites, lending support to the inference of gene flow among sites. Florida sites again showed genetic differentiation with few allelomorphs shared between the Florida group and the Great Lakes sites.

A number of aquatic plant populations have been found to be either monoclonal or comprising only a few genets. *Phragmites australis* populations invading the Gulf Coast of Texas and Florida were found to be monoclonal, all sampled shoots (n=4) sharing the same genotype characterized in terms of 17 loci (Pellegrin and Hauber, 1999). The pattern of low enzymatic variability found in the invasive *Hydrilla verticillata* in the United States suggested two independent introductions of a few individuals, followed exclusively by clonal expansion (Ryan, 1989). The river weed *Podostemum ceratophyllum* appears to be a recent colonizer to northern U. S. where populations are monoclonal, although each population is represented by a different clone, representing independent colonizing events (Philbrick and Crow, 1992). Waycott (1998) found that populations of *Posidonia australis* in Australia varied in clonal diversity based on allozyme analysis, with some populations being monomorphic. However, these same populations, showed high polymorphism when re-

analyzed using RAPDs (randomly amplified polymorphic DNA; Waycott, 1998). Similarly, for the rare *Calamagrostis porteri* spp. *insperata*, Esselman *et al.* (1999) detected distinct genotypes using RAPD and ISSR markers, while allozyme analysis indicated monomorphism. Allozyme monomorphism, therefore, need not necessarily mean material has been clonally propagated.

For the tristylous, clonal emergent *Decodon verticillatus*, Eckert and Barrett (1993), using three loci and style morph as genetic characteristics, found a large range of values for clonal diversity (D) from 0-0.93, where populations with D=0 appeared to be a single clone/genet. Such site-to-site variation in clonal diversity likely reflects the relative importance of reproductive modes, clonal and sexual, in different populations (Eckert and Barrett, 1993). Similarly, Harrison and Durance (1991) found that clonal diversity differed among *Zostera marina* sites; a newly colonized site had higher clonal diversity consistent with successful sexual reproduction and seedling establishment, while older, more established sites were clonally depauperate. *Quercus chrysolepis* populations had a mean D=0.95, with the majority of the clones being unique to a site, suggesting a significant role for sexual reproduction in a species considered to reproduce mainly by cloning (Montalvo *et al.*, 1997). Clonal diversity measures for *Posidonia australis* ranged from 0-1, and were higher with RAPD than allozymic analysis (Waycott, 1998). Three populations of the sedge *Carex bigelowii* had a mean D=0.98 signifying high clonal diversity (Jonsson *et al.*, 1996). For *V. americana*, clonal diversity measures were similar at all sites, with

a relatively narrow range of D (0.7434-0.9615). These values of D are high, indicating an important role for sexual reproduction at all sites. Further, the high number of shared genotypes implies gene flow occurs among the sites.

Findings of clonal diversity are similar for parthenogenetic organisms from the animal kingdom. Multiclonal populations of moths, *Alsophila pometaria*, (Mitter *et al.*, 1979), weevils, *Otiorrhynchus scaber* (Saura *et al.*, 1976), freshwater ostracods, *Candonocypris novaezelandiae* (Chaplin and Ayre, 1997), aphids, *Sitobion avenae* (Sunnucks *et al.*, 1997), and crustaceans, *Daphnia pulex* (Hebert and Crease, 1983; Hebert *et al.*, 1988; Weider *et al.*, 1999 a, b) have been reported. However, in some studies of facultative asexual animals, clonal diversity is low (eg. the earthworm *Octolasion tyrtaeum* [Jaenike *et al.*, 1980], the rock lizard *Lacerta unisexualis* [Fu *et al.*, 1998], the crustacean *Halopedium gibberum* [Thier, 1994] and the snail *Campeloma decisa* [Selander *et al.*, 1978]).

In *Daphnia pulex*, 68% of clones were unique to a site, and on average a site shared only 13% of its clones; a pattern which suggests low levels of gene flow among populations (Hebert *et al.*, 1988, Weider *et al.*, 1999 b). Populations comprising few clones tended to deviate from HWE and were found to reproduce by obligate parthenogenesis, while populations characterized by multiple clones and HWE had a mixed reproductive system (cyclic parthenogenesis). Sporadic sexual reproduction in some *D. pulex* populations in the holarctic could permit such populations to serve as diversity-dispersal

centres to other parts of the region, with some genotypes showing up in populations over 1000 km apart (Weider *et al.*, 1999 b). Periodic sex also provides a mechanism by which parthenogenetic greenbugs *Schizaphis graminum* (Shufran *et al.*, 1997) and the crustacean *Halopedium gibberum* (Thier, 1994) generate and maintain unexpectedly high levels of genetic variability and clonal diversity. Clearly then, sporadic sexual reproduction can produce and maintain significant amounts of genetic variability in populations of asexual/clonal species. For *V. americana* then, the often substantial sexual reproduction observed in the field could readily result in the genetic and clonal diversity detected in the present study.

Detection of genotypes depends not only on sample size, but on the number of characteristics (including loci) studied. With the addition of more loci, clonal identity can be more reliably established (Ellstrand and Roose, 1987; Hebert *et al.*, 1988; Widén *et al.* 1994). In populations where sexual reproduction occurs, more loci are required to detect distinct clones (Hebert *et al.*, 1988). Type II errors are more likely when fewer characteristics are used to determine clonal identity (Rogers, 1999). Such errors would occur if ramets of different genotypes were incorrectly allocated to the same clone. The estimates of clonal diversity for *V. americana* are likely to be underestimates since they are based on only seven characters.

Genetic effect of clonal growth

Clonality adds an extra layer of information in the study of basic processes occurring within populations of species that also reproduce sexually. The delineation of an individual is less clear, as are genetic neighbourhoods and the approximate characterization of effective population size. Lokker *et al.* (1994) demonstrated that genet values of population genetic parameters deviated from ramet values. In the present study, deviations from HWE as well as some population genetic parameters are significantly different when genets represent individuals as opposed to ramets. Genet identification, therefore, is absolutely essential for useful studies of genetic structure and mating systems in facultative clonal/sexual species (see also Rogers, 1999).

Geographic trends

A correlation between genetic distance and geographic distance is not necessarily to be expected for species with significant dispersal ability (Hartl and Clark, 1989), as is the case for the epihydrophilous, water dispersed *V. americana*. When all of the *V. americana* study sites were included, a significant positive correlation between genetic and geographic distance, however, was observed, with a pattern consistent with population disjunction occurring at approximately 1000 km distance (equivalent to the 'distance' between Florida and Great Lake sites [Richardson *et al.*, 1986]). At distances below 1000 km,

panmixia was indicated by the asymptotic relationship between geographic and genetic distances (Richardson *et al.*, 1986). When the relationships among Great Lakes sites were focussed on (Figure 2.2.4 b), the trend at distances less than 1000 km was clinal rather than strictly asymptotic.

Although clinal trends were evident, the actual magnitude of genetic distance among *V. americana* sites in the Great Lakes was quite low, although values for all survey areas but Lake Erie were significantly greater than zero. Nei's D ranged from 0.0132-1.279 for *Phragmites australis*, showing geographical clustering of sites in the southern U. S. from sites associated with the Mississippi river (Pellegrin and Hauber, 1999). Procaccini and Mazzella (1998) used a measure of genetic distance suitable for microsatellite analysis; the six populations of *Posidonia oceanica* which they studied in the Western Mediterranean, clustered along a latitudinal gradient, with genetic disjunction between the two most northern populations and the four south-central populations.

Geographic disjunction results in greater genetic distances as a result of gene flow barriers (land, salt water). Isolated populations independently undergo genetic differentiation through random genetic drift and selection processes (Hartl and Clark, 1989). The genetic distances between paired Florida sites reflected the geographic distribution of these sites, as they tended to represent different water systems. On average, fewer allelomorphs were found at these Florida sites, a greater number of which were unique (see Table 2.2.11). There

results fit with the lack of physical connectivity among the sites which sites were often close to springs and associated with rivers such as the Santa Fe (Blue Springs), or the Gulf of Mexico (St. Marks, Chassahowitza, Homosassa). The Wakulla R. joins the St. Marks R., and based on Nei's genetic distances these two rivers clustered together (Figure 2.2.5). The waters from the springs were quite warm and generally flowed quickly, and are fed by the Floridan aquifer, a regional aquifer system (Mattson *et al.*, 1995).

Water flow in the Great Lakes, however, is far more connected, with mass-flow of the water body occurring from Lake Superior through to the St. Lawrence River (Schweiger, 1999). Water retention times for some of the connecting waters of the Great Lakes have been determined to be 21 h for St. Clair River, 5-7 days for Lake St. Clair and 19 h for the Detroit River. Water flow patterns at specific sites were not determined. A number of the sites were located in large embayments (e.g., Rondeau, Long Point, Put-in-Bay) or along rivers that empty into the associated lake (e.g., Blind, Spanish, Key), and therefore they may be isolated compared to the sites exposed to the broad water current. A comparison of these isolated sites with sites directly associated with Great Lakes waters showed no difference in levels of genetic diversity. Models to explain genetic diversity patterns along downstream courses have not been developed as yet for aquatic plants (Barrett *et al.*, 1993). It is not clear that more or less diversity should be expected downstream. In the present study, no significant pattern of genetic diversity downstream was detected among sites

influenced by the mass-flow of Great Lakes waters. However, populations with high numbers of migrants should approximate panmixis, as was observed for *V. americana* at the Great Lakes sites where migrant numbers (although questionable) were high and genetic differentiation among all sites was low, though significantly greater than zero (see Tables 2.2.4, 2.2.6).

Environmental factors can significantly influence the population genetics and clonal structure of a species. The effect of organic contamination on clonal diversity was also studied in red raspberry ([*Rubus idaeus*] Keane *et al.*,1998). Results showed that although levels of heterozygosity and genetic differentiation among contaminated and uncontaminated sites did not differ significantly, contaminated sites had fewer private (or unique) alleles, signifying a loss of genetic variation (Keane *et al.*, 1998). For *V. americana* no clear differences in genetic or clonal diversity among sites in the Great Lakes were detected, but contamination levels at the sites studied was not assessed. However, extensive studies on the suitability of *V. americana* as a biomonitor of contamination have been carried out by Lesley Lovett-Doust and Jon Lovett-Doust (e.g., Lovett-Doust *et al.*, 1993, 1994; Biernacki *et al.* 1996, 1995 a, b). Biernacki and Lovett-Doust (1997) demonstrated that some clones were resistant to contaminant levels experienced at heavily polluted regions in the Huron-Erie corridor, and that contaminant levels were correlated with reduced rates of clonal growth, flowering and turion production. Studies designed specifically to examine genetic variation and clonal diversity in relation to contaminant levels could be

valuable, especially if populations were tracked before, during and after implementation of 'Remedial Action Plans' for 'Areas of Concern' in the Great Lakes (See International Joint Commission, 1998). Such studies could determine if genetic diversity is indeed lost due to heavy contamination, and if remediation of the site can result in an increase in population genetic diversity (and evolutionary potential).

Florida

Florida sites, as expected, showed greater genetic disjunction from the Great Lakes, and to some extent from each other (i.e., higher F_{ST} values; Tables 2.2.4, 2.2.6), This is signified by low gene flow between Florida and the Great Lakes sites and among Florida sites. The Florida sites were mainly composed of unique allelomorphs, sharing few with the Great Lake sites, and Nei's genetic distances between pairs of sites were greater than distances among more widely spaced sites in the Great Lakes system.

Based on field observations, plants sampled from Florida were much larger than those from the more temperate sites. Leaf widths were greater than 2 cm, and stolon diameters greater than 1 cm (compared with leaf widths of 1-2 cm and shoot diameters < 1 cm in Great Lakes sites). Some narrow-leaved variants have, however, been found in Lake Okeechobee, Florida (David Sutton, University of Florida, personal communication). In some regions of Florida the plants do not senesce in winter nor do they produce turions (Dawes and

Lawrence, 1989; Bartodjiej and Leslie, 1992; David Sutton, University of Florida, personal communication).

Summers in Florida extend to more than 5 months and winters last only about 1.5 months based on air mass classification (Cheng and Kalkstein, 1997). This effectively eliminates the typical seasonal cycle observed in the temperate zone. Such climactic differences could have profound effects on population processes for *V. americana*. With reduced investment in turion production, and a longer growing season, more resources would be available for vegetative growth via stolons, or sexual reproduction via flowering. Flowering frequencies in Florida at sampling were on the lower end of the spectrum observed in our study (range of 19-35% vs 15-74% for Great Lakes; Appendix C, Table C1). It is possible that the flowering season in Florida is more extensive and peaks later as a result of climactic differences. As such, flowering frequency in the present study may have been underestimated. Unfortunately little published data exists on the general life history characteristics of the species in Florida (see Dawes and Lawrence, 1989). However, based on observations of lower clonal diversity and reduced flowering, it is possible that population growth and maintenance is achieved primarily through vegetative growth of *V. americana* at Florida sites.

Evolutionary processes

The intriguing questions raised in Section 2.1 occur again here. What is the origin of the genetic variation observed in *V. americana* and how is it

maintained?

Genetic variability is generated by somatic mutation (considered rare) and recombination, it can be maintained by ongoing sexual reproduction, gene flow and selection, while selection and genetic drift can result in its decay (Hartl and Clark, 1989). The potential influence of a number of these processes will be considered further.

Sexual reproduction

A number of findings indicate that sexual reproduction is an important influence in *V. americana* populations. High clonal and genetic diversity, low genetic distances between sites, high estimates of number of migrants, the high frequencies of flowering shoots and successful seed set all contribute to this view.

Levels of successful sexual reproduction need not be high to maintain genetic diversity within clonal species (Holderegger *et al*, 1998). Soane and Watkinson (1979) and Watkinson and Powell (1993) reported that a 3% seedling recruitment rate would be sufficient to maintain high clonal diversity within *Ranunculus repens* populations. In the absence of recurrent seedling recruitment, a computer simulation showed that genet loss would be high, and a few clones would come to dominate populations (Watkinson and Powell, 1993). Past, rather than current, sexual reproduction or recent colonization from seed have also explained observed genetic variation (e.g., Harrison and Durance,

1991), but in the absence of sexual recruitment, variation would be expected to decline over time as a result of selection and genetic drift.

Despite historic perceptions of aquatic macrophytes as predominately clonal, recent studies indicate evidence for significant occurrence of sexual reproduction. A number of studies have also reported seed production in *V. americana* (Lovett-Doust and LaPorte 1991; Titus and Hoover 1991; Laushman 1993; Lokker *et al.*, 1994; Stuckey and Moore, 1995; Sullivan and Titus, 1996) and seed banks have been investigated and quantified in the Detroit (Lokker *et al.*, 1997) and Mississippi (Kimber *et al.*, 1995) Rivers.

Findings of successful seed production are mitigated by the paucity of direct observations of recruitment from seed, an event which generally tends to be rare in clonal populations (Lovett-Doust, 1981; Cook, 1983; Eriksson, 1989, 1992); however this probably has more to do with the difficulty of detecting rare demographic events than suggesting they do not occur. Potential effects of sexual reproduction and gene flow on *V. americana* population genetics will be considered below.

Gene flow

In the present study, the low levels of genetic subdivision and low genetic distance estimates among often widely separated Great Lakes populations can be attributable to such gene flow that panmixia is achieved. Migration, the movement of individuals among populations, has the effect of homogenizing

variation among populations (Hartl and Clark, 1989). A value of Nm (number of migrants per generation) greater than four in theory represents populations which behave as a single panmictic unit (Kimura and Maruyama, 1971). Nm values in the present study exceed this threshold, and indicate high numbers of migrants (Nm), suggesting a tendency towards panmixis. Looking at individual Great Lakes, the number of migrants within these boundaries are very large, ranging from a ramet mean of 9.927 in the Huron- Erie corridor to 104.9 in Lake Erie. When boundaries were expanded to include all of the Great Lakes sites, the mean dropped to 6.470, a value similar to the mean for Florida's isolated populations. The geographic scale of study influences these values as expected; fewer migrants would be predicted to move over a greater space, or between physically disjunct populations than between closer, more connected sites.

In a number of other clonal plant species, lower values of Nm have been found. Genetic disjunction between North and South Central populations of *Posidonia oceanica* in the Mediterranean corresponded with few migrants between these regions ([Nm=1.55] Procaccini and Mazzella, 1998). For the clonal oak tree, *Quercus chrysolepsis*, Montalvo *et al.* (1997) estimated Nm to be 9.5 among six sites along the San Bernardino Mountains in California.

F_{ST} values, from which gene flow has been estimated, can however be affected by other population processes. Most notably, selection that spatially or temporally alters genotypic and allelic frequencies can bias these values (Cabe and Alstad, 1994). Estimates of migrant numbers are therefore suspect if

selection pressures are present. Furthermore, Cockerham and Weir (1993) caution that reduced differentiation among populations is presumed to be caused by gene flow despite no direct observations. Nm is considered by Ennos (1994) as a measure of the effectiveness of gene flow in preventing divergence through drift rather than an absolute measure of gene flow. Any inferences made from Nm are limited to the assumptions of the model that alleles are neutral and that populations have attained equilibrium (Cockerham and Weir 1993), a caution repeated by Boileau *et al.* (1990) who found that gene frequencies were often not in equilibrium.

The values of Nm found for *V. americana* were variable and quite high in comparison to estimates for other species (e.g., Montalvo *et al.*, 1997; Procaccini and Mazzella, 1998; Chung, 1999) and have been measured as significant over a large geographic range. It would not be reasonable to presume that Florida populations are panmictic with Great Lake populations just because the mean Nm estimated in the present study is greater than 4. In fact, there is a great amount of variation between loci which may be a result of selection of heterozygotes, and the system not being in equilibrium. The use of private (or unique) alleles and data from a larger number of polymorphic loci to calculate Nm would provide a more accurate estimate. Despite this, based on the loci used here, the genetic diversity data, and the distribution of genetic diversity among sites, all suggest that *V. americana* sites although genetically variable were not highly disjunct from each other genetically (ie., the effect of genetic drift

was not great).

Dispersal

If gene flow as a significant evolutionary process is pursued, a consideration of migration need not be limited to the dispersal of vegetative fragments (shoots, turions), but would include pollen and seeds as well. Indeed, *Vallisneria americana* may be transported downstream, and less often upstream, as seed pods, ramet fragments or shoots, and turions as well as pollen (Korschgen and Green, 1988; Laushman, 1993; Catling *et al.*, 1994). Viable *V. americana* shoots have been reported to drift up to 300 m (Horvath and Lamberti, 1997), and seeds have been found much greater distances downstream of established *V. americana* beds in the Upper Mississippi (McFarland and Rogers,1998). These additional modes of dispersal differ in their influence on gene flow and population genetic structure since pollen flow would involve paternal nuclear genes, while seeds include these and maternal nuclear and cytoplasmic (organellar) genes (Ennos, 1994).

Dispersability of the various propagules will also differ based on their buoyancy and longevity. Cook (1985, 1987 a, b) has extensively considered the dispersal of aquatic plant diaspores. Water-dispersed diaspores, such as *V. americana* seeds and floating turions, rely primarily on water currents, but over large water bodies, wind may also play a significant role (Cook, 1987 a). Without biotic intervention (animal, human), dispersal tends to be downstream

(Barrett *et al.*, 1993) and confined to single bodies of water (Cook, 1987 b). Zonal dispersal may also occur when water currents deposit propagules along shores and in shallow areas, resulting in a non-random pattern of gene flow (Laushman, 1993). Similarly, 'detrital windrows' can 'capture' drifting macrophytes, while some will move through the water system intact (Edwards *et al.*, 1989). Zonal dispersal of *V. americana* pollen, seed pods, turions and shoots have been observed in the field, where the dispersing structures have accumulated along sand banks and along shore (C. Lokker, personal observations). Turion and seed pods have also been observed floating downstream in the fall, entangled with *V. americana* shoots which have become separated and dislodged during senescence (C. Lokker, personal observations).

Barrett *et al.*, (1993) consider hydrophily to be analogous, in terms of dispersal efficiency, to wind pollination. *Vallisneria americana* is not truly hydrophilous, but has a greater capacity for gene flow than hydrophilous species in that its pollination mechanism is two-dimensional. According to the model of Cox and Knox (1989), two-dimensional pollination systems, in which male flowers form aggregates, that they termed 'search vehicles', upon the water surface have greater efficiency due to the increased surface area that the search vehicles can cover. The male flowers thereby have a greater probability of encountering a receptive female stigma.

The potential for gene flow via pollen for *V. americana* is therefore considerable. Male flowers move along the water in aggregates (C. Lokker

personal observation, Titus and Hoover, 1991; Sullivan and Titus, 1996), limited only by the margins of the water body. Sullivan and Titus (1996) found pollination success to increase with male plant density and a higher pollen retention time, which was contingent upon water and wind currents. Downstream gene flow of pollen, seeds or shoots could readily provide sufficient gene flow to maintain genetic variability within a population, and 'homogenize' variability among populations. Further studies on pollen longevity and its physical potential for long-distance movement would be helpful in obtaining parameters to model this process.

It is unlikely that *V. americana* pollen from Thessalon was transported to South Lancaster, but a 'stepping-stone' model might provide an explanation for the similarities between distant populations. Initially, such a model would predict that genetic diversity would be greater 'downstream' than 'upstream', until an equilibrium was reached. There is no indication in the present study of increasing diversity with increasing distance downstream.

Water generally flows from north to south, and west to east in the Great Lakes with discharges increasing in spring and summer and decreasing in fall and winter (Lee and Sydor, 1999). Weed growth, including that of *V. americana,* in late summer can reduce water flow in the Great Lakes (Lee and Sydor, 1999), which coincides with peak flowering in *V. americana* (C. Lokker, personal observation). The reduced water flow at that time would therefore affect pollen and seed dispersal.

Kudoh and Whigham (1997) developed three alternate gene flow models for the wetland macrophyte *Hibiscus moscheutos*, an insect-pollinated perennial with water-dispersed seeds. A model relating population location to the tidal stream best fit their findings of moderate differentiation (F_{ST}=0.062) and significantly different allele frequencies among sites, and indicated the importance of hydrochory (seed dispersal by water) to the spatial genetic structure of the species. For the wind-pollinated, outcrossing *Quercus gambelii*, Kumar and Rogstad (1998) found that a low F_{ST}=0.023 corresponded with high gene flow associated with animal dispersal.

A small amount of gene flow is sufficient to counteract selection and genetic drift by contributing to the genetic variation within a population (Hartl and Clark 1989). After all, a 3% seedling recruitment rate can maintain population genetic variation and clonal diversity (Soane and Watkinson,1979; Watkinson and Powell 1993). Studies on realized seedling recruitment would provide valuable tests of the role played by sexual reproduction and gene flow at *V. americana* sites; however such studies would require intensive demographic monitoring routines covering a large area in a difficult environment.

Founder effects and bottlenecks

A genetic bottleneck may have been experienced by some Great Lake sites as a result of declines attributed to pollution and turbidity. Detroit River populations of *V. americana* have rebounded in recent years (Lovett-Doust and

LaPorte, 1991; C. Lokker, personal observation) from a dramatic decrease in turions occurring from 1950-1985 (Schloesser and Manny, 1990). Similar changes have been reported for Put-in-Bay, Lake Erie (Stuckey and Moore, 1995).

The magnitude of the reduction in allelic diversity following a bottleneck depends upon the severity of the decrease in the size of the population, as well as the speed at which population restoration occurs (Nei *et al.*, 1974; Hartl and Clark, 1989). The effect of a single bottleneck is presumed to be small unless populations are reduced to one or two individuals (Frankel and Soulé, 1981). A low level of diversity was not detected in the present study for *V. americana* , which could indicate that rapid restoration has already occurred or that sufficient numbers of individuals survived the bottleneck.

Since these sites were not sampled throughout the turbulent years, the dynamics of the regrowth of *V. americana* has not been documented demographically. The origin of shoots at re-established sites is also unknown, but could result from germination of local dormant seeds or turions, or from migrants from other sites upstream. In the Detroit River, Schloesser and Manny (1990) reported a reduction, but not elimination, of turions, which likely formed a source pool at the recovering sites. Lokker *et al.* (1997) reported seed germination from seed bank samples that occurred over a 15 month period, with repeated sediment agitation required. This suggests that *V. americana* seeds are capable of long term dormancy, thereby providing a further source for

recovering sites. Downstream gene flow of propagules may also have contributed to the diversity observed through the introduction of novel genotypes and/or as a consequence of released heterozygosity whereby individuals fixed for different alleles produce heterozygote progeny (Mitton, 1997).

Tracking sites over time, and determining if levels of diversity increase or decrease would show whether genetic variation was being maintained by processes such as gene flow and successful recruitment, or whether random genetic drift and selection were acting to deplete genetic diversity. Either way, observed diversity is the net consequence of these opposing forces, and all of these processes are likely to be occurring to a greater or lesser degree. Changes in such factors as the level of water contamination or rates of water flow (related to changing lake levels) might have predictable effects on genetic diversity in *V. americana.*

Clonal growth

For some clonal species, vegetative growth plays a crucial role in colonization and population maintenance. Examples include *Posidonia oceanica* (Procaccini and Mazzella, 1998), *Phragmites australis* (Pellegrin and Hauber, 1999), *Hydrilla verticillata* (Ryan, 1989), and *Podostemum ceratophyllum* (Philbrick and Crow, 1992). Populations of a number of these species are characterized by low F_{ST} and low clonal diversity, suggesting that the same few clones have colonized a number of populations (Ryan, 1989; Pellegrin and

Hauber 1999). Alternately, northern populations of *P. ceratophyllum* had low clonal diversity with extremely high F_{ST}, reflecting different colonizing events for each isolated population (Philbrick and Crow, 1992). In all of these cases, clonal growth appears to be the only detectable mode of reproduction following colonization. This is common among invading aquatic plants since a frequent consequence of dispersal is that only one sex or incompatible mating type colonizes a new area (Cook, 1987 b).

Older populations of clonal species are often less diverse than young ones as genet death results in reduced diversity (Widén *et al.*, 1994). Alternately, clonal growth can also extend the longevity of a genet, thereby retarding the loss of genetic diversity (Widén *et al.*, 1997; Mayes *et al.*, 1998).

Mutation, selection and drift

Mutation, selection and drift are other processes which influence population genetic variability and structure. Genetic drift is a stochastic process which results in the progressive reduction of genetic diversity (Ayala, 1982; Hartl and Clark, 1989). It tends to be significant in small, isolated populations (Ayala, 1982; Hartl and Clark, 1989), and therefore becomes a particular concern in endangered species. Genetic drift leads to divergence of subdivided populations. Such populations are characterized by low genetic diversity and high fixation of alleles (Hartl and Clark, 1989). None of these tendencies are evident in the present study of *V. americana*. However, prior genetic drift effects

and allele fixation in populations which experienced declined growth and subsequent re-growth(see Schloesser and Manny, 1990; Stuckey and Moore, 1995) could have lead to isolate breaking. This is characterized by excess heterozygosity resulting from breeding between once subdivided populations fixed for different alleles (Hartl and Clark, 1989; Mitton, 1997), and is a possible scenario explaining the excess heterozygosity observed for *V. americana.*

Selection occurs when genetic variants have differential reproductive success or fitness (Ayala, 1982; Hartl and Clark, 1989). It often results in a reduction of genetic diversity, except when heterozygotes are favoured (Ayala, 1982). Measuring selection pressures requires detection of fitness differences among genotypes, sexes, gametes etc., and is difficult to do because of the biological and genetic complexity of fitness (Hartl and Clark, 1989). For example, in the present study the lack of homozygous B individuals at the IDH-1 locus could be a result of selection against this genotype, however, a full study of fitness differences among genotypes was not carried out. There is evidence that environmental factors can exert selection pressures on *V. americana* clones, in terms of differential survival of cloned biomonitors deployed by Biernacki and Lovett-Doust (1997) at contaminated sites in the Huron-Erie corridor. However, direct relationships between allozyme genotypes and fitness remain to be identified.

Mutations generate genetic variability (Ayala, 1982). Most mutations are lethal or non-adaptive, and these are removed by selection. Rates of mutation,

however, are considered to be small, resulting in slow changes in allele frequencies (Ayala, 1982; Hartl and Clark, 1989). In clonal species, somatic mutations can become widely established quite rapidly through vegetative reproduction. Lushal *et al.* (1998) followed 32 generations of the facultative parthenogenetic grain aphid in the lab and documented population genetic changes based on somatic mutations and one germ line mutation (rate of mutation?). In the clonally depauperate rock lizard, Fu *et al.* (1998) found only three clones, two of which were each represented by one individual variable at only one locus. The origin of each of these two clones was therefore attributed to a somatic mutation at the polymorphic locus (Fu *et al.*, 1998).

Ploidy changes are a special case of mutation which are fairly common in plants (Briggs and Walters, 1984), and also found in some clonal animals (e.g., ostracods *Prionocypris glacialis* and *Candona rectangula*, Little and Hebert, 1997). These changes can involve whole sets of chromosomes (euploidy) or a number of chromosomes less than a whole set (aneuploidy, Tamarin, 1991). Sterility problems with euploids can be overcome in clonal plants by chromosome doubling in both male and female gametes.

Although the ploidy level of *V. americana* in the study area has not been investigated, Manitoba specimens of *V. americana* var. *americana* had chromosome number of 2n=20 (Löve, 1981). Jørgensen (1921) reports 2n=20 for *V. spiralis*, and 40 for *V. gigantea*, suggesting that the latter is a tetraploid of the former, with the size differences between the species typical of polyploidy.

Variable aneuploid counts are reported for *V. spiralis* (16, 18, 24), with aneuploid and euploid counts for *V. spiralis* as 16, 18, 24 and 20, 30, 40 respectively (from Les and Philbrick, 1993). Since larger size is a common effect of increased ploidy level, it would be interesting to determine chromosome numbers of Great Lake plants, and the large and small variants reported in Florida (David Sutton, University of Florida, personal communication).

Conclusions

Vallisneria americana is a highly genetically variable species. Sites within a water system, such as the Great Lakes, were not highly genetically differentiated based on allozyme assays (G_{ST}=0.0269). Isolated Florida sites were differentiated from the Great Lake sites (G_{ST}=0.1206), and to a lesser degree, from each other (G_{ST}=0.1157). Based on the fact that genets were in HWE, the low inter-site differentiation and high estimates of migrant numbers, outcrossing and gene flow appear to be important forces determining genetic variability within, and the genetic similarity among, sites throughout the Great Lakes, and separately, sites in northern Florida.

Chapter 3

Sexual Reproduction in *Vallisneria americana*

Introduction

The importance of identifying genets and their constituent ramets has been described in Chapter 2. The entire genetic individual needs to be considered when assessing evolutionary fitness and population genetic processes. As such, allozyme studies are critical in determining the extent of clonal propagation, but information on sexual reproduction is required to augment population genetic studies in clonal species such as *V. americana*. By determining the extent and success of sexual processes (flowering, pollination, seed production and dispersal, seedling recruitment), the possibility of sexual origin of new genotypes, and the significance of sexual reproduction to population genetic structure can be deduced.

Although aquatic perennials such as *V. americana* have efficient modes of clonal propagation, many researchers have observed significant amounts of flowering and seed production in this species (e.g., Laushman, 1993; Titus and Hoover, 1991; Lovett-Doust and LaPorte, 1991), and in other highly clonal aquatic plants (Rea and Ganf, 1994 b). Historically, aquatic macrophytes have been thought to be able to sustain populations largely through clonal propagation based on the argument that the aquatic environment is considered

fairly stable (Sculthorpe, 1967; Hutchinson, 1975; Cook, 1987; Les, 1988). By this argument, ecological selective pressures would be constant, favouring established successful genotypes. Thus, well adapted genotypes would be expected to proliferate and dominate the population. Genetic variability generated through sexual reproduction could possibly disrupt these adaptive combinations. Of course, on the other hand, variability would be adaptive during periods of environmental change.

Some recent studies indicate that sexual reproduction plays an important role in the persistence of macrophyte populations through adverse environmental conditions such as drought (Kimber *et al.*, 1995) and fluctuations in water level (Rea and Ganf, 1994 b). The question of how much sexual reproduction contributes to population growth, maintenance and genetic variability remains since few studies have concentrated on the success of sexual reproduction in aquatic macrophytes.

In 1991, Titus and Hoover outlined ontogenic and ecological stages in the production and fate of sexual and vegetative propagules of *V. americana*. They identified potential limiting factors affecting each of these modes of reproduction and to predict reproductive success.

The sequential stages of sexual propagule production and fate included: flower induction and production, anthesis, pollen transport, pollen tube germination and growth, fertilization, embryogenesis and fruit maturation (Titus and Hoover, 1991). Evolutionary fitness can be indirectly estimated as fertility,

however a true fitness measure requires tracking of the subsequent stages of seed survival, seedling recruitment, survival to sexual maturity, production of 'grandchildren', and benefits from a survey of genetic diversity within populations and progenies.

In the present chapter, I hope to amplify Titus and Hoover (1991), with the ultimate goal of combining information on sexual reproduction in *V. americana* with data on the population genetic structure of the species at various population levels described in Chapter 2.

Assessing the success of sexual reproduction in *V. americana* can be very challenging in the field. The aquatic habitat is not conducive to meticulous demographic observations, and seedlings are very small and easily overlooked (Rea and Ganf, 1994 b). Indirect and laboratory-based measures of seedling production and success were therefore used to compliment field studies.

Floral induction, the initial stage of sexual reproduction in aquatic plants, has been associated with nutrient levels and photoperiod (cf Sculthorpe, 1967). Early observations of three sites in the Huron-Erie corridor with varying flowering frequencies, sex ratios and ramet densities (C. Lokker, personal observation) generated hypothesis that led to the investigation of environmental variables which may serve as important factors determining flowering in *V. americana*. Possible associations between environmental variables (nutrient levels, light, and sediment type) and flowering, growth and seed output were examined through field studies and an *in situ* reciprocal transplant experiment was carried

out among the three *V. americana* sites to investigate effects of site on flowering variables (see Section 3.2).

A laboratory experiment assessing the effects of density and gender of the nearest neighbour on flowering and growth in *V. americana* was designed to explore possible associations among these variables (see Section 3.1). It had been observed that flowering male ramets predominated in shallow water, and females at deeper water, with greater ramet density in the deeper sites (Lokker *et al.*, 1994).

Pollination, the next step in the sexual reproduction process, was investigated in a greenhouse pollination experiment as a function of the male parent (pollen donor) source site distance from the source site of female plants (see Section 3.3). In this manner, inbreeding and outcrossing effects on seed production, maturation and subsequent seedling growth could be assessed.

The fate of seeds is difficult to track *in situ*. An indirect measure of seed fate was determined based on assessment of extensive seed bank and seed deposition samples from the same three sites. The association of the characteristics of the seed bank with measures of sexual reproduction in the previous season was determined (see Section 3.4); this provided an indication of the relative success of seeds that would survive to the next developmental stage. Germination information was obtained from the seed bank study (see Section 3.4) as well as from the pollination study (see Section 3.3).

3.1 Flowering shoot density and sex of genets

Objective

In 1991, spatial segregation of male and female flowering ramets of *V. americana* was observed at Turkey Island, with males predominating in shallow water and females in the deeper water (Table 3.1.1). Ramet densities were also 2-3 times greater at water depths of 3 m than they were in the shallower areas (< 1m). Based on these preliminary observations, the following experiment was designed to assess the effect of population density and the presence/absence of ramets of the opposite sex, on flowering and growth in male and female ramets of *V. americana*, and to assess potential differences in growth patterns of males and females under these conditions. In this experiment, water depth was controlled, and the initial density of ramets and the initial sex ratio of ramets were varied.

Segregation of male and female ramets may reflect differential competition for habitat, the presence of only one gender at the depth extremes or different environmental flowering cues required for each of the sexes (Les, 1988). Spatial and temporal partitioning among the sexes has been documented for a number of terrestrial plant species (Freeman *et al.*, 1976; Cox, 1981; Bierzychudek and Eckhart, 1988), and has been attributed to sex-associated traits such as differential growth rates, mortality or attractiveness to herbivores (Onyekwelu and Harper, 1979; Lovett-Doust and Lovett-Doust, 1985) and to abiotic environmental conditions such as moisture and salinity gradients, light or

Table 3.1.1. Population parameters for *V. americana* at Turkey Island in 1991. Transects 1-3 were sampled parallel to shore, while transects 4-6 were perpendicular. Flowering frequencies were based on ramet flowering status. The number of ramets sampled is represented by n. Data previously published by Lokker *et al.* (1994).

Transect	n	Density ($/m^2$)	Mean depth (cm)	Percent male	Percent female	Percent vegetative
1	241	241	41.5	9.10	0.500	90.4
2	233	233	40.5	12.5	0	87.5
3	305	272	37.0	4.90	0	95.1
4	339	339	52.3	16.2	0	83.8
5	401	401	77.0	23.9	6.50	69.6
6	575	676	306	0	14.1	85.9

nutrient availability (Bierzychudek and Eckhart, 1988; Ramadan *et al.*, 1994).

Density-dependent effects tend to regulate population size, often by reducing the probability or amount of reproduction (Weiner, 1988). Reproduction can be affected by a change in allocation of resources, timing of reproduction, reproductive mode from sexual to asexual, and change in mating behaviour (Weiner, 1988). Male and female reproductive responses vary for some species.

The primary question addressed in this study is: Do male and female ramets of *V. americana* show differential growth and reproduction under contrasting treatment conditions (variable density and initial sex ratio), at a fixed water depth?

Methods

Experimental design

Shoots of known sex were collected from Turkey Island in late September 1992, and were allowed to overwinter in tubs of Turkey Island sediment in the greenhouse. In mid-April, both germinated and ungerminated turions were excavated and planted in the 4-inch experimental pots. The fresh mass of each turion was noted before transplanting. Four pots containing well-mixed samples of Turkey Island sediment were placed in each of 12 aquaria (60 L). Each aquarium contained 4 pots, each pot planted with 2, 4, 8 or 16 turions (which germinated into new shoots). These densities translate into a range of 52-421 shoots/m^2, matching the typical range of densities which had been observed in

natural populations. The position of each pot within the aquarium was randomized. The pots contained combinations of all male, all female or 1:1 male and female shoots. There were four replicates of each sex ratio at each of the four densities.

The aquaria were monitored weekly for flowering, production of new ramets and death of previously-measured ramets. The plants were harvested in late September, 1993. Measures taken for each initial shoot and its daughter ramets included final number of ramets, cumulative stolon length and genet dry mass (the genet consisting of all ramets present at the end of the experiment). Measures taken for each individual ramet at the end of the study included the length of the longest leaf; number of leaves, flowers, and turions; and dry mass of leaves, roots, flowers, and turions. Few ramets produced turions (n=3) or flowers (n=2); these measures are therefore not included separately in the analysis, although their masses were added to the total genet mass. Measures of percent ramet mortality per pot, final ramet density per pot and the percent increase in density per pot were calculated. Ramet mortality was based upon the absence at harvest of any ramet measured at a previous census.

Statistical analysis

ANOVAs were performed to ensure there was no effect due to replicate tanks. Normality was assessed, and Bartlett's test of homogeneity of variances was performed for all measures, in all cases indicating that the variances were

equal. ANOVAs on initial nutrient levels (nitrate, total phosphate and potassium) showed no significant differences among the 20 pots randomly sampled.

To assess the effects of density, sex ratio and their interaction on reproduction and growth, a step-down analysis (Tabachnick and Fidell, 1989) was performed with mean genet mass, mean ramet number, percent increase in density and percent mortality per pot as the dependent variables. This analysis involved an ANOVA with the most important dependent variable, followed by a number of ANCOVAs whereby the dependent variable from the previous step became a covariate in the subsequent steps, and lower ranked parameters were used as the dependent variable. The order in which the parameters were placed in the step-down analysis was based on univariate F-test results. This analytical procedure was used due to the detection of correlations among the dependent variables. To achieve an overall $p<0.05$, probability was Bonferroni adjusted to $p<0.004$ for twelve independent tests. Significant main effects were tested using Scheffés comparisons of means.

The effect of genet sex on ramet-specific growth patterns was also assessed using step-down analysis with density as a covariate throughout, and genet sex as the independent factor. The dependent variables (leaf number; root, ramet and leaf mass) were ranked based on univariate F-tests. Probability was Bonferroni adjusted $p<0.0125$ for four independent tests.

To determine if the carrying capacity of the pots had been reached, regression analysis was performed on final ramet density versus initial ramet

density.

Results

During the course of the experiment, only two ramets produced flowers and three ramets produced turions. A male ramet in an all-male, 16-density pot produced two spathes, and a female ramet in an all-female, 4-density pot produced one flower. Turions were produced by females in a 2- and 4-density pot, and by a male in an 8-density pot (number of turions=4, 3, 2 respectively). All of the above pots originally contained the 50:50 ratio of males and females. Due to the low sample size, the effect of density and sex ratio on flowering and turion production could not be statistically evaluated.

Univariate F-tests (Bonferroni adjusted $p<0.004$ for 12 independent tests) and Pillai's Trace on reproduction and survival data were significant for density, but not for sex ratio or the interaction between sex ratio and density (Table 3.1.2). Since the dependent variables were correlated (Table 3.1.3), step-down analysis was performed, with the variables ranked in the order genet mass, percent increase in density, ramet number, and percent mortality (Table 3.1.4). Based on this analysis, density did have a significant effect on genet mass and sex ratio had no effect on any growth and survival measures. No further variation, due to the main factors, density and sex ratio, was accounted for by the other measures, as demonstrated by the lack of significance of these factors and the significance of higher ranked covariates in subsequent steps (see

Table 3.1.2. MANOVA results used to obtain ranking of correlated dependent variables for subsequent step-down analysis to determine the effect of density, sex ratio and their interaction on reproduction and survival. Due to 12 independent F-tests, probability was Bonferroni adjusted to $p<0.004$. Probability for multivariate statistics is therefore maintained at $p<0.05$. *$p<0.01$, **$p<0.001$.

Univariate F		**Density**	**Sex ratio**	**Interaction**	**Error**
Measure	df	3	3	3	36
Mean ramet	SS	40.54	2.471	3.193	45.25
number	F	10.8	0.983	0.423	
	p	**<0.001****	**0.384**	**0.858**	
Genet mass	SS	3.809	0.0180	0.1890	3.005
(g)	F	15.2	0.109	0.378	
	p	**<0.001****	**0.897**	**0.888**	
Mortality (%)	SS	0.1270	0.0090	0.0750	0.292
	F	5.23	0.572	1.54	
	p	**0.004***	**0.569**	**0.195**	
Increase in	SS	50.63	2.681	3.466	47.88
density (%)	F	12.7	1.00	0.434	
	p	**<0.001****	**0.375**	**0.851**	
Multivariate statistics					
Pillai's Trace		0.675	0.154	0.448	
	df	12, 105	8, 68	24, 144	
	F	2.540	0.707	0.758	
	p	**0.006***	**0.684**	**0.783**	

Table 3.1.3. Pearson correlation coefficients for reproduction and survival variables used in step-down analysis assessing the effects of density, sex ratio and their interaction. n=48; *p<0.01, **p<0.001.

	Mean ramet number	**Genet mass (g)**	**Mortality (%)**
Genet mass (g)	0.831**		
Mortality (%)	-0.402*	-0.496**	
Increase in density (%)	0.987**	0.852**	-0.537**

Table 3.1.4. Step-down analysis of the effect of density and sex ratio treatments on reproduction and survival parameters. The ranking of the correlated variables (dependent variable = DV) are based upon univariate F-tests. The dependent variable of the previous step becomes a covariate in the subsequent step (specified by COV). Treatment variable are represented by IV (independent variable). Probability was Bonferroni adjusted to $p<0.0125$ for four independent tests. *$p<0.001$.

DV	IV and COV	SS	df	F	p
Genet mass	density (d)	3.81	32	15.2	**<0.001***
(g)	sex ratio (sr)	0.018	63	0.109	**0.897**
	d*sr	0.189	6	0.378	**0.888**
	error	3.01			
Increase in	density (d)	1.75	32	0.865	**0.469**
density (%)	sex ratio (sr)	1.59	61	1.178	**0.320**
	d*sr	1.74	35	0.428	**0.855**
	genet mass (COV)	24.2		35.8	**<0.001***
	error	23.7			
Mean ramet	density (d)	0.118	32	0.799	**0.503**
number	sex ratio (sr)	0.067	61	0.675	**0.516**
	d*sr	0.419	13	1.41	**0.238**
	genet mass (COV)	0.015	4	0.295	**0.590**
	% increase (COV)	22.3		452	**<0.001***
	error	1.68			

DV	IV and COV	SS	df	F	p
Mortality (%)	density (d)	0.001	32	0.256	**0.857**
	sex ratio (sr)	0.001	61	0.850	**0.437**
	d*sr	0.004	11	0.770	**0.593**
	genet mass (COV)	0.001	33	0.589	**0.448**
	% increase (COV)	0.247		328	**<0.001***
	ramet no.(COV)	0.236		314	**<0.001***
	error	0.025			

Tabachnick and Fidell, 1989). Scheffé's test of density in the first step of the analysis indicated that an initial density of two was significantly different from initial densities of four, eight and sixteen ramets for genet mass (with $p < 0.05$ since only one multiple comparison was performed; Figure 3.1.1a).

Figures 3.1.1 (a-d) show the trends of genet mass, ramet number, percent increase in density and percent mortality across the four density treatments. Although the latter three variables were not significantly affected by density when correlations were taken into account, univariate F-tests were significant for density for each of the variables (Table 3.1.2) and the trends consistently showed an increase in performance with a decrease in initial density (Figure 3.1.1).

Genet sex did not have an effect on ramet-specific growth responses, based on Univariate F-tests (Bonferroni adjusted $p<0.0125$ for four independent tests) and Pillai's Trace (Table 3.1.5) with the significant effect of density removed by using it as a covariate. Again due to correlations among the growth variables (Table 3.1.6), step-down analysis was performed with density as a covariate, since it was shown to have an effect on growth in the earlier analysis. Even accounting for correlations among the variables, no effect of sex was detected, but density was a significant factor in the second step of the analysis (Table 3.1.7). This suggests that the variance in mean root mass, as a function of density, was not fully accounted for by the variance in mean leaf number. The variance in these two measures, however, accounted for all variance as a result

Figure 3.1.1. Reproduction and survival parameters at harvest for the four experimental densities. (a) mean plant mass, (b) mean number of ramets per plant, (c) percent increase in density per pot and (d) percent mortality per pot. Each of the measures were significantly affected by density in univariate F-tests, but when correlations among them were taken into account, no new variance was added to the latter three parameters by removing the effect of density treatment.

Figure 3.1.1.

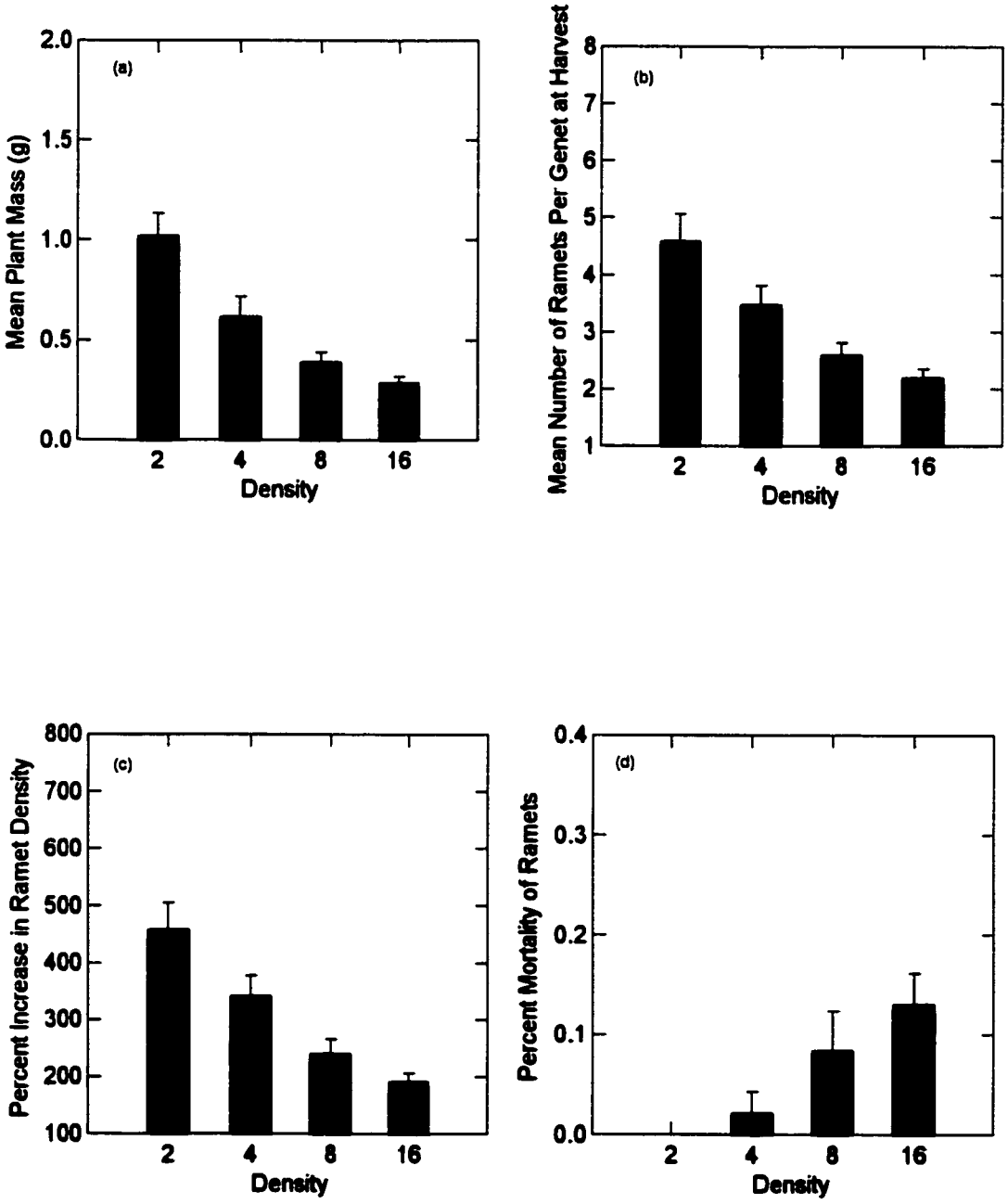

(a)
Mean Plant Mass (g)
2.0
1.5
1.0
0.5
0.0
2
4
8
16
Density
(b)
Mean Number of Ramets Per Genet at Harvest
8
7
6
5
4
3
2
1
2
4
8
16
Density
(c)
Percent Increase in Ramet Density
800
700
600
500
400
300
200
100
2
4
8
16
Density
(d)
Percent Mortality of Ramets
0.4
0.3
0.2
0.1
0.0
2
4
8
16
Density

Table 3.1.5. MANOVA results used to obtain ranking of correlated dependent variables for subsequent step-down analysis to determine the effect of genet sex on ramet growth parameters. Due to four independent F-tests, probability was Bonferroni adjusted to $p<0.0125$.

Univariate F		**Genet sex**	**Error**
Measure	df	1	323
Mean ramet	SS	0.001	4.05
mass (g)	F	0.112	
	p	**0.730**	
Mean leaf	SS	0.717	658
number	F	0.352	
	p	**0.554**	
Mean leaf	SS	0.001	2.58
mass (g)	F	0.022	
	p	**0.882**	
Mean root	SS	0.001	0.416
mass (g)	F	0.273	
	p	**0.601**	
Multivariate statistics			
	Pillai's Trace	0.062	
	df	4, 320	
	F	0.187	
	p	**0.945**	

Table 3.1.6. Pearson correlation coefficients for ramet growth variables used in step-down analysis assessing effects of genet sex. n=325; *p<0.001.

	Mean leaf number	**Mean leaf mass (g)**	**Mean root mass (g)**
Mean leaf mass (g)	0.583*		
Mean root mass (g)	0.316*	0.606*	
Mean ramet mass (g)	0.506*	0.910*	0.793*

Table 3.1.7. Step-down analysis of the effect of genet sex on ramet growth parameters. The ranking of the correlated variables (DV) are based upon univariate F-tests. The dependent variable of the previous step becomes a covariate (specified by COV) in the subsequent step. Density was maintained as a covariate throughout the analysis to remove the effect it had on the parameters. Probability was Bonferroni adjusted to $p<0.0125$ for four independent tests. *$p<0.01$, **$p<0.001$.

DV	IV and COV	SS	df	F	p
Mean leaf	genet sex	0.717	113	0.352	**0.554**
number	density (COV)	0.826	23	0.405	**0.525**
	error	658			
Mean root	genet sex	0.001	111	0.125	**0.724**
mass (g)	density (COV)	0.011	32	9.84	**0.002***
	leaf number (COV)	0.041	2	35.4	**<0.001****
	error	0.375			
Mean ramet	genet sex	0.001	111	0.130	**0.719**
mass (g)	density (COV)	0.001	13	0.306	**0.581**
	leaf number (COV)	0.299	2	77.8	**<0.001****
	root mass (COV)	1.78	1	461	**<0.001****
	error	1.24			

Mean leaf	genet sex	0.001	1.1e+07	0.147	**0.701**
mass (g)	density (COV)	0.001		0.751	**0.387**
	leaf number (COV)	0.033		33.9	**<0.001****
	root mass (COV)	0.084		86.1	**<0.001****
	ramet mass (COV)	0.909		926	**<0.001****
	error	0.314			

of density, thus density became non-significant in subsequent steps. For steps two to four, the previous dependent variables were significant covariates, reflecting their strong correlations. Overall, the pattern of growth in ramets of the two sexes was not different (Figure 3.1.2).

Final densities in the pots ranged from 5-43 shoots/pot (corresponding values of 131-1131 shoots/m^2). Results of regression analysis and a comparison of initial versus final densities suggest that even the most densely populated pots had not reached carrying capacity (Figure 3.1.3). Nevertheless, the decrease in ramet production per pot in the higher density treatments (Fig. 3.1.1b) and the decrease in biomass production per pot (Fig. 3.1.1a) suggest that competition was occurring.

Discussion

Spatial segregation of the sexes

Sex ratio bias can influence population growth rates (Johnson, 1994), and can reduce the effective population size (Hartl and Clark, 1989; Barrett *et al.*, 1993; Thomas and LaFrankie, 1993). Clonal increase can lead to biased sex ratios (Putwain and Harper, 1972), and can lead to the observation of spatial segregation of the sexes (Iglesias and Bell, 1989).

Lokker *et al.* (1994) and Laushman (1993) have noted somewhat greater rates of flowering of *V. americana* at greater depths, and that greater flowering, in turn, was associated with an increase in the relative abundances of flowering

Figure 3.1.2. Partitioning of biomass among ramet structures in all male and female plants studied. Sex ratio treatments were pooled as they had no significant effect on plant growth. There were no significant differences between male and female plants in terms of the biomass in leaf, root, or stolon.

Figure 3.1.2.

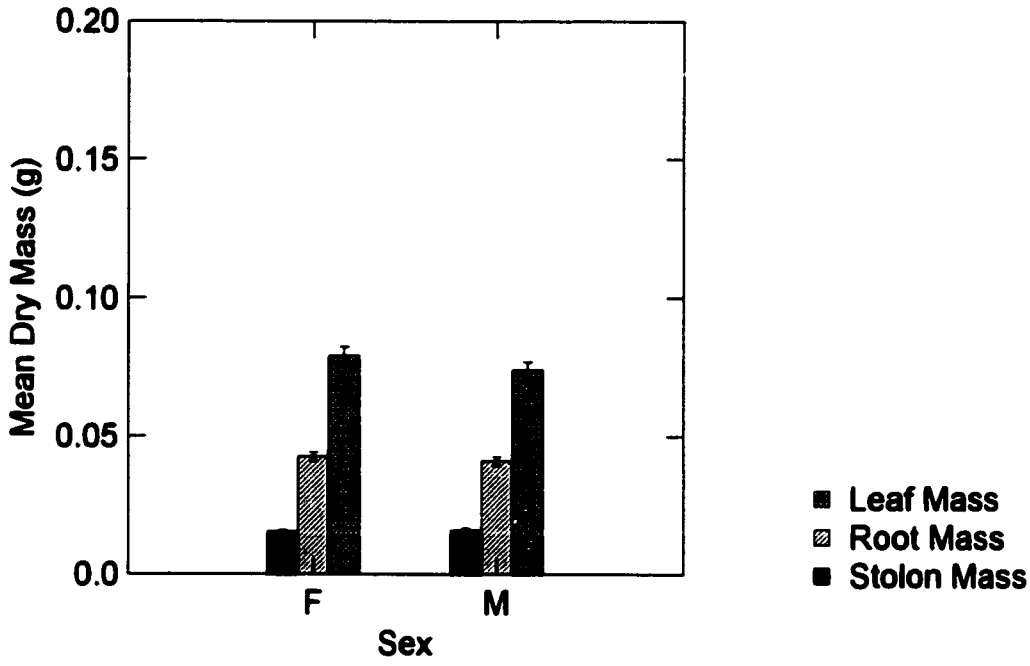
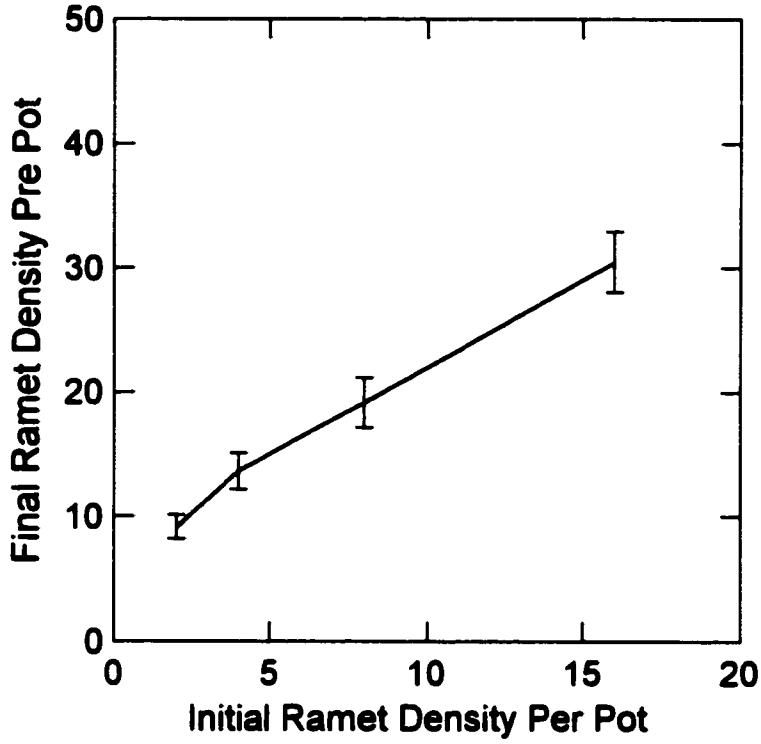

0.20
0.15
0.10
0.05
0.0
Mean Dry Mass (g)
F
M
Sex
Leaf Mass
Root Mass
Stolon Mass

Figure 3.1.3. Initial ramet density per pot versus final ramet density. No asymptote was reached within this experiment, even at the highest densities, suggesting that the carrying capacity of pots had not been reached.

Figure 3.1.3.


Final Ramet Density Pre Pot
50
40
30
20
10
0
0
5
10
15
20
Initial Ramet Density Per Pot

females. A female bias is commonly associated with an increase in the proportion of plants that are flowering (Les, 1988).

Although biased sex ratios and apparent spatial segregation of the sexes were observed for *V. americana* in the field, the presence of the opposite sex did not influence growth of *V. americana* in the current study. This suggests that the male and female ramets did not compete differentially for resources when they shared pots in the experiment. No clear allelopathic effects were detected, since the presence of the opposite sex did not result in reduced growth of the alternate sex. Allelopathy refers to the reduction in growth of one sex (or species) as a result of toxic metabolites produced by the opposite sex (or other species) (Begon and Mortimer, 1986). Concluding that allelopathic mechanisms cause reduced growth, however, can be difficult in laboratory studies since other factors may be responsible (Harper, 1977). Flowering response could not be tested in the current study due to a lack of flower production.

Quinn (1991) found no evidence of differential competitive success or niche partitioning in populations of the dioecious, perennial grass *Buchloe dactyloides,* where males and females appeared to be spatially distributed by gender. In Quinn's (1991) study, male and female plants did not differ in vegetative characteristics, total biomass or reproductive output, and no correlations with environmental factors such as soil topography or moisture were detected (Quinn, 1991).

The sex ratio pattern that was observed for *V. americana* in the field could

well be an artifact of clonality. Iglesias and Bell (1989) illustrated that clonality results in members of the same gender occupying adjacent space as a function of their ramet connections; such a mosaic would therefore not necessarily reflect microenvironmental heterogeneity. The presence of non-flowering ramets of *V. americana* makes it difficult to know the true ramet sex ratio at sites. The gender of vegetative neighbours in the field is therefore largely unknown. It is possible that only one gender occupies a large swath of a site simply as a function of clonal growth (i.e., in deeper waters where only female flowers are typically observed). However, based on genet maps depicted in Figure 2.1.2, where male and female ramets form a mosaic with vegetative ramets in the shallower waters, this does not appear to be the case for *V. americana*.

The flowering sex ratio, however, is conditional upon flowering cues being present. Titus and Hoover (1991) have laboratory-based evidence which suggests that female ramets of *V. americana* need to attain a certain size threshold before flowering. The presence of females in the deeper waters may be a result of greater water depth being available for leaf growth.

Water depth is associated with other environmental factors such as attenuation of light and its spectral characteristics, sedimentation rates and water flow. Any of these correlates of water depth may also affect the sex ratio either through differential mortality of males and females, or acting via distinct flowering cues for male and female ramets.

Density influence on growth and flowering

Density dependent effects are greater when a greater number of competitors are present. These effects can influence reproduction by altering resource allocation patterns, timing of reproduction, mode of reproduction or mating behaviour (Weiner, 1988). In this study, *V. americana* showed reduced plant mass as a result of increasing ramet density, and although not significant, there was a trend toward reduced ramet production and an increase in percent mortality as density increased.

De Kroon and Kwant (1991) found that shoot production was negatively density dependent; with increasing density, fewer shoots were produced by the clonal grass species *Brachypodium pinnatum* and *Carex flacca*. *Carex* also showed density dependent mortality (de Kroon and Kwant, 1991). These authors also showed that under increased density, the plants controlled overproduction of new shoots, but not necessarily as a result of physiological shoot integration.

Onyekwelu and Harper (1979) observed that density differentially affected growth of male and female plants of *Spinacia oleracea*, with females faring better in lower densities.

A switch in resource allocation from sexual reproduction to clonal growth under conditions of increasing density has been predicted by Abrahamson (1980). Based on his studies of *Rubus* and *Fragaria*, Abrahamson (1980) predicted that in stable habitats, allocation to sexual and asexual reproduction should be more flexible than in heterogenous habitats. At low densities, clonal

growth would be favoured as a mechanism to colonize and propagate successful genotypes. As density increases, sexual reproduction would be favoured as a means of dispersal and the production of novel genotypes.

This scenario would appear to be similar to the findings of greater density associated with greater flowering in female plants of *V. americana* in deeper waters (see Lovett-Doust and LaPorte, 1991; Lokker *et al.*, 1994). It is possible that in the field greater resources are directed to flowering in deeper waters where growth was more vigorous and shoot density, and competition, were greater (Lokker *et al.*, 1994).

3.2 Flowering: Environmental factors

Objective

Dioecious species such as *V. americana* are obligate outcrossers, thus it is important to determine factors that influence and in particular that may limit flowering, reproduction and seed set in this species. Titus and Hoover (1991) have suggested that floral induction or pollen transport are possible factors limiting sexual reproduction in *V. americana.*

Although the proximate cues for floral induction in *V. americana* are not known, a laboratory study by Titus and Hoover (1991) suggested that flowering of female shoots occurred if the shoot attained a threshold size of 0.75 g, a relationship which did not, however, hold up in the field. Yet, a number of other authors have found positive correlations between plant biomass and flowering (Titus and Hoover, 1990; Biernacki *et al.*, 1995 a) or seed set (Rogers *et al.*,1992) .

Given the important role of environmental factors in growth and reproduction of aquatic macrophytes (Barko *et al.*, 1986), this study examined physical and chemical environmental variables which may influence the likelihood that a ramet of *V. americana* will flower. Factors such as irradiance (Carter *et al.*, 1996), pH (Titus and Hoover, 1993), nutrient availability (Rogers *et al.*, 1992; vanLent *et al.*, 1995) and anthropomorphic organic contamination (Biernacki *et al.*, 1995 a, 19995 b, 1996) have been shown to affect flowering and seed set in *V. americana* and other aquatic plant species. In most cases,

these factors impact on the growth of the study species, which in turn influences the probability of flowering. Other factors which have been reported to affect aquatic macrophyte growth, but that have not been studied in terms of reproduction, include organic matter content (Overath *et al.*, 1991), and substrate composition (Barko *et al.*, 1986; Biernacki *et al.*, 1995 b).

In 1992, *V. americana* at three sites in the Huron-Erie corridor showed differing flowering frequencies, shoot sex ratios and densities. Flowering frequency and sex ratios may greatly influence the success of sexual reproduction in this outcrossing species, especially since female-biased sex ratios tend to coincide with greater overall flowering frequency (Les, 1988; Lokker *et al.*, 1994). The associations between sediment nutrient concentrations, sediment composition measured as texture (particle size distribution) and organic content, pH and water transparency and the amount of sexual and asexual reproduction occurring at three sites were assessed. In addition, a reciprocal transplant experiment among the three sites studied was carried out to test the effects of plant, sediment and water origin on growth and flowering.

The questions addressed here are: what is the extent of flowering and seed production at the three *V. americana* sites? Do nutrient regimes, sediment composition and light differ among these sites? What is the relationship between flowering, seed production and seed biomass and selected environmental parameters?

Methods

Study sites

Three sites in the Huron-Erie corridor were studied: Mitchell's Bay in Lake St. Clair, and Turkey and Stoney Islands in the Detroit River (Figure 3.2.1). These sites were selected based on previous observations of contrasting flowering frequencies and shoot sex ratios (Table 3.2.1), which were determined from detailed study of ramets excavated along transects at each site. Transects were 20 cm wide, and ranged in length from 2.8 m to 6.3 m. Transect lengths varied since the primary objective of the collection was to sample 400 ramets with stolons suitable for electrophoretic analysis (see Chapter 2). Two transects were sampled at Stoney Is. and Mitchell's Bay in 1992, while six were sampled at Turkey Is. in 1991. Mitchell's Bay had a high proportion of flowering ramets (69.3%), with a preponderance of male ramets (a tertiary or flowering sex ratio of 0.710). Turkey Is. had, overall, a sex ratio of unity (close to 1.000), and a low frequency of flowering (19.6%). As reported by Lokker *et al.* (1994) for Turkey Is., within each transect there was a gradual change in the tertiary sex ratio, with males predominating in shallow water (<1 m) and females predominating in deeper water (>3 m). Stoney Is. also had a low overall frequency of flowering (18.4%), with female ramets present, but no males (Table 3.2.1). The three sites therefore displayed a range of flowering frequencies and sex ratios.

The Mitchell's Bay site is a narrow navigational channel (20 m wide) leading to Bass Haven off the open Bay, with beds of *V. americana* growing

Figure 3.2.1. Map of Huron-Erie corridor, indicating the location of the three sites surveyed: Mitchell's Bay in Lake St. Clair, and Turkey and Stoney Islands in the Detroit River.

Figure 3.2.1.

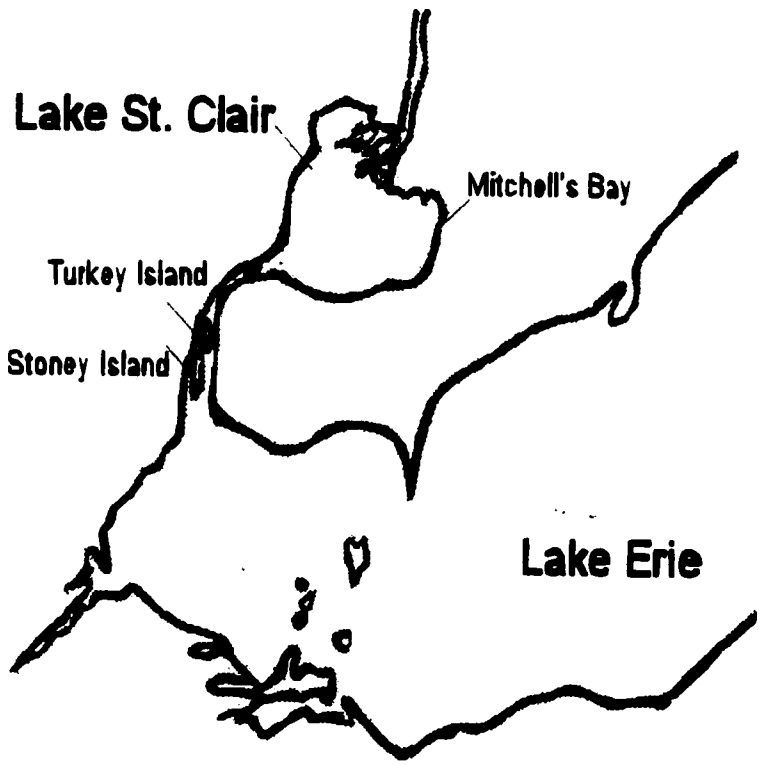

Lake St. Clair
Mitchell's Bay
Turkey Island
Stoney Island
Lake Erie

Table 3.2.1. Population parameters based on transects sampled in 1991 (Turkey Is.) and 1992 (Mitchell's Bay and Stoney Is.). Three transects were sampled at Turkey Is., two at Stoney Is. and Mitchell's Bay. Ramets were summed over the transects prior to the calculation of the site parameters; the measures are therefore overall site values. It should be noted that the Turkey Is. transects were separated more widely than those at other sites since the population was more extensive. This site is represented both by overall values (data from Lokker *et al.*, 1994), and by values from the subset of transects sampled within the same depth range as the present study.

Site	Total no. of shoots	% ramets flowering	% ramets male	% ramets female	Sex Ratio
Mitchell's Bay	1062	69.3	57.5	11.8	0.71
Stoney Is.	1164	18.4	0	18.4	1.2
Turkey Is. (overall)	1315	19.6	11.5	8.1	0.97
Turkey Is. <3 m	740	23.9	20.4	3.5	0.86

along each side, at depths ranging from 20 cm to 2 m. The water is generally slow-moving, but there is periodic wave disturbance associated with intermittent boat traffic. Turkey Is. has a large stand of *V. americana* on its eastern shore, extending into the river approximately 300 m to a depth of 3-7 m, though growing mostly in the shallow range of 0.8-1.3 m. A swift north to south current exists. The Stoney Is. site is situated within a bay which, to the west, has a narrow passage to the major shipping lane in the Detroit River, and an opening on the east side that faces Grosse Isle, MI. Water flows quickly through this bay from west to east. The bay is shallow, with a maximum depth < 2 m.

Sampling procedures

In 1993, sediment samples were collected to assess the amount of nitrate, potassium ion, total phosphate, pH and organic matter content in the sediments at the three sites, and to classify sediment texture based on the proportion of sand, silt and clay. Samples for nutrients and pH were taken three times during the season: late June, mid-August, and early October. Samples for sediment texture were collected in June. Organic matter analysis was determined for sediment samples collected in both June and October.

At each site, sampling was carried out every 10 m along 3 transects ranging from 60-100 m long. Three replicate samples were taken at each sampling point. At Turkey and Stoney Islands, the transects ran perpendicular to the shore and approximately 50 m apart, encompassing variations in water depth

and the vast *V. americana* beds. At Mitchell's Bay, due to the linear distribution of *V. americana* along the sides of a dredged channel, transects were taken parallel to the shore and 1.5 m apart. Secchi depth was taken at each sampling point in August and October (the water being too rough to allow reading of the disk when sampling was done in June). Sediment samples of 250 ml were collected and 2.5 ml of boric acid was added as a preservative to each sample.

During September, 0.25 m^2 quadrats were excavated at five randomly determined sediment sampling stations along each of the three transects per site (n=15 per site). The flowering status of each ramet within the quadrats was assessed. Any seed pods produced within the quadrats were stored in water in a cold room (5° C) for three months. Seeds within each pod were counted and classified according to the following categories: mature (firm, brown) and immature (white but filled, see Ferasol *et al.*, 1995). Estimates of flowering frequency, shoot sex ratio, seed production, ramet density and fresh mass were therefore obtained for each quadrat.

Nutrient and pH determination

Sediment nitrate (NO_3), potassium (K) and pH levels were analysed the day following collection. Total phosphate (PO_4) and sand/silt/clay were analysed 1-2 weeks later, giving sediments adequate time to dry out before being sieved. Nitrate and potassium concentrations were determined using specific ion electrodes. Samples were first solubilized with distilled water at 20:1 (by

volume) and mixed for 20 minutes (Moore and Chapman, 1986). The supernatant was then divided equally between NO_3 and K^+ test beakers. Appropriate amounts of N-ISA (Nitrate-Ionic Strength Adjuster), nitrate suppressor and K-ISA (Potassium-Ionic Strength Adjuster) were added to the supernatant of the samples following set procedures from Orion. Samples were measured on an Accumet 950 pH/ion meter, in mol/l. Supernatant pH was measured after 20 min stirring (2:1 sediment to water mixture by volume, Moore and Chapman, 1986).

Total phosphate content by volume was estimated using the 'molybdenum blue' reaction following Moore and Chapman (1986), with transparency measured at 700 nm on a Spec 20 spectrophotometer. Concentrations (mg PO_4 / l) were estimated based on a standard curve of transparency and mg PO_4/l. Total phosphate measures are reported in μg PO_4/l.

Sediment composition

Organic matter content was measured for August and October samples using the loss on ignition technique, following Moore and Chapman (1986). Preweighed dried samples were placed in a muffle furnace at 550°C for 2 hours, and were then re-weighed. The proportion of sample mass ignited (lost) was calculated.

Sand, silt and clay composition of the sediments was determined using the sedimentation method and LaMotte's soil texture kits. Sample preparation

followed Foth (1970).

Transplant experiment

A reciprocal transplant experiment, factoring plant origin, sediment and water column, was initially set up in late summer 1993. At each site, 36 tubs (46 cm X 33.5 cm X 11 cm) were placed at a depth of approx. 80 cm (for a total of 108 tubs among the three sites). Twelve tubs of each sediment type were placed at each site (i.e., in each water column). Of each sediment-set of twelve tubs, four contained plants from each of the sites. Nine shoots were placed in each tub in late August, representing 3 known male, 3 known female and 3 previously vegetative shoots. Per site, the design included four replicates of the combinations of: plant origin (3 sites) X sediment origin (3 sites) X water column (3 sites). Unfortunately, shoot survival was very low, and the experiment was re-established with the same grid of sediments, water column, plant source and replication using turions of unknown sex in the fall of 1994. Therefore the sex of the plants in each tub was unknown.

From May 1995-September 1995, monthly measurements were made of germinated turions and their ramets. The plants were harvested in September 1995, at which time final counts of leaf number and leaf length, dry mass and flowering status were recorded for each ramet. Due to limited turion survival, sample sizes for each plant X sediment X water combination were reduced, so tubs were treated as (4) replicates, rather than ramets within a tub.

Statistical analysis

To assess the effects of site, month and their interaction on nutrient and pH levels, a step-down analysis (Tabachnick and Fidell, 1989) was performed. This analysis involved an ANOVA with the most important dependent variable, followed by a number of ANCOVAs in which the dependent variable from the previous step became a covariate in the subsequent steps, and lower-ranked parameters were then used as the dependent variable. The order in which the parameters were placed in the step-down analysis was based on the magnitude of correlation coefficients and univariate F-test results. This analytical procedure was used because there were significant correlations among the dependent variables. Probability was Bonferroni adjusted to $p<0.0125$ for four independent tests. Significant main effects were tested using Scheffés test for comparison of means.

A similar step-down analysis was used to test the effect of site on sediment composition. Based upon the correlation patterns, ANOVA of the percent sand was performed first, followed by MANCOVA of percent silt and clay with percent sand as covariate and probability Bonferroni adjusted to $p<0.025$ for two independent tests.

ANCOVA was used to analyse secchi depth, with sampling depth as covariate. A separate analysis was performed due to a lack of data for the month of June (see methods above). Similarly, a separate MANOVA was performed for organic matter content, with site and month as factors, since June samples were

not analysed.

Due to non-normality that could not be corrected by transformation, the data for growth and reproduction measures were analysed using Kruskal-Wallis one-way analysis of variance (ANOVA; Zar, 1984) to test for differences among sites in measures of sexual reproduction, seed production and ramet densities. *A posteriori* multiple comparisons of significant Kruskal-Wallis tests were performed using a non-parametric Tukey-like test (Zar, 1984). Means and standard errors given here are based on non-transformed data.

Sex ratios were calculated by adding 1 to the proportion of males and females, then dividing the female frequency by that of males. A ratio of 1 indicates an equal sex ratio, less than 1 is male-biased and a ratio greater than 1 is female-biased.

The association between growth and reproduction and the environmental and physical variables was assessed using Systat's canonical correlation analysis (Tabachnick and Fidell, 1989). Due to a limited sample size (45), only measures from one month could be used while still maintaining statistical power. June was chosen as the time that best represented the resources that would be available for the coming season (August was used for Secchi depth and organic matter). Variables were selected based on Pearson correlation coefficients. Those variables least correlated with other variables, and considered more biologically significant, were added to the analysis. The analysis was performed on non-transformed and rank-transformed data, both giving the same results.

Due to small sample size and some empty cells, a means model test was employed to analyse the transplant experiment (Milliken and Johnson, 1984). Number of ramets and flower number proved to be correlated with a number of the other variables studied, and were therefore identified as biologically important dependent variables. Following Milliken and Johnson (1984) the three-way interaction model was first tested. If this proved non-significant each two-way interaction was tested and then individual effects were tested.

Results

Nutrients

Since the total sediment nutrient sample size was 264, with greater than 20 df in each site x month cell, normality of data was not required (Tabachnick and Fidell, 1989). Homogeneity of variances among groups was verified using the F_{max} test. Correlations among NO_3, K and pH were significant; PO_4 was correlated with K (Table 3.2.2). Univariate F-tests for the interaction between site and month (Table 3.2.3., probability adjusted to $p<0.004$) provided rankings of the dependent variables for the step-down analysis (Table 3.2.4). Significance levels were Bonferroni adjusted to $p<0.0125$ to achieve an overall $p<0.05$. Nutrient concentrations were significantly dependent upon the site X month interaction in all steps of the analysis, even after covariates were taken into account (Table 3.2.4). Significance of the main effects of site and month was therefore masked. The measures were clearly dynamic over time and site

Table 3.2.2. Pearson correlation coefficients for sediment nutrient concentrations and pH. *p<0.001.

n=264	NO_3	K	PO_4
K	0.349*		
PO_4	0.052	0.330*	
pH	-0.291*	-0.540*	-0.077

Table 3.2.3. MANOVA results used to obtain ranking of correlated dependent variables for subsequent step-down analysis, assessing the effect of site and month on sediment nutrient concentrations and pH. Probability was Bonferroni adjusted to $p<0.004$ due to multiple independent tests. *$p<0.001$. Univariate F-tests for each nutrient and pH are given first, followed by results of multivariate statistic for site, month and their interaction.

Univariate F		**Site**	**Month**	**Interaction**	**Error**
	df	2	2	4	255
K	SS	20.31	14.06	75.77	115.4
	F	22.44	15.53	41.84	
	p	**<0.001***	**<0.001***	**<0.001***	
NO_3	SS	73.16	123.3	57.17	137.9
	F	36.62	114.0	26.42	
	p	**<0.001***	**<0.001***	**<0.001***	
PO_4	SS	0.8325×10^7	0.2168×10^7	0.6961×10^7	0.308×10^8
	F	34.48	8.981	14.41	
	p	**<0.001***	**<0.001***	**<0.001***	
pH	SS	1.108	3.460	3.281	7.022
	F	20.12	62.82	29.79	
	p	**<0.001***	**<0.001***	**<0.001***	
Multivariate statistics for MANOVA					
Pillai's Trace		0.556	0.748	1.05	
	df	8, 506	8, 506	16, 1020	
	F	24.34	37.79	22.73	
	p	**<0.001***	**<0.001***	**<0.001***	

Table 3.2.4. Step-down analysis of the effects of site and month on nutrient concentrations. The ranking of the correlated dependent variables (DV) are based upon univariate F-tests (see Table 3.2.3). The dependent variable in the previous step becomes a covariate in subsequent steps (specified by COV). IV represents independent variable. Due to multiple independent F-tests, probability was Bonferroni adjusted to $p<0.0125$. *$p<0.05$, **$p<0.001$.

DV	IV and COV	SS	df	F	p
K	site	20.3	224	22.4	<0.001**
	month	14.1	255	15.5	<0.001**
	s*m	75.8		41.8	<0.001**
	error	115			
pH	site	0.781	224	14.7	<0.001**
	month	2.64	125	49.7	<0.001**
	s*m	1.38	4	13.0	<0.001**
	K (COV)	0.275		10.4	<0.001**
	error	6.75			
NO_3	site	45.6	224	43.4	<0.001**
	month	82.8	112	78.8	<0.001**
	s*m	57.4	53	27.3	<0.001**
	K (COV)	2.86		5.48	0.020*
	pH (COV)	1.27		2.42	0.121
	error	132			

PO_4	site	0.366 x10^7	224	19.2	<0.001**
	month	0.241 x10^7	111	12.7	<0.001**
	s*m	0.914 x 10^7	252	24.0	<0.001**
	K (COV)	0.583 x 10^7		61.2	<0.001**
	pH (COV)	0.259 x 10^6		2.72	0.100
	NO_3 (COV)	0.463 x 10^6		4.86	0.028*
	error	0.239 x10^6			

(Figures 3.2.2 a-c). Although no clear patterns were present, typically variation within a site between months was greater than between sites.

Sediment character

Measures of sand, silt and clay composition are not independent as they are each proportions of total sediment. Since these measures are clearly related, an ANOVA was performed on sand, followed by MANCOVA performed with silt and clay as dependent variables and sand as covariate. Bonferroni adjusted $p<0.001$ (Table 3.2.5). Scheffé's tests indicated that Mitchell's Bay and Turkey Island had a significantly greater proportion of sand than Stoney Island. Site and percent sand significantly affected percent silt and clay, based upon significant Pillai's trace values. Stoney Is. appeared to have a siltier and more clay-based substrate compared with the sandy nature of sediment at Turkey Is. and Mitchell's Bay (Figure 3.2.3).

The results of the MANOVA of organic matter data indicate that there was no interaction between site and month ($F=1.847$, $P=0.159$), but both site and month significantly affected organic matter content ($F=140.775$, $p<0.001$; $F=4.379$, $p<0.05$, respectively). Table 3.2.6 shows the means ±(SE) for organic matter content for the three sites in June and October. Based on Tukey's HSD test, Turkey Is. had greater organic content than Stoney Is., which was greater than Mitchell's Bay. Also, October organic matter levels were significantly lower for Mitchell's Bay and Turkey Is. than those found in June (Table 3.2.6).

Figure 3.2.2. Nutrient and pH levels over the season at the three sites (a) nitrate x 10^5 mol/L, (b) phosphate μg/L (c) potassium ion x 10^5 mol/L and (d) pH.

Figure 3.2.2.

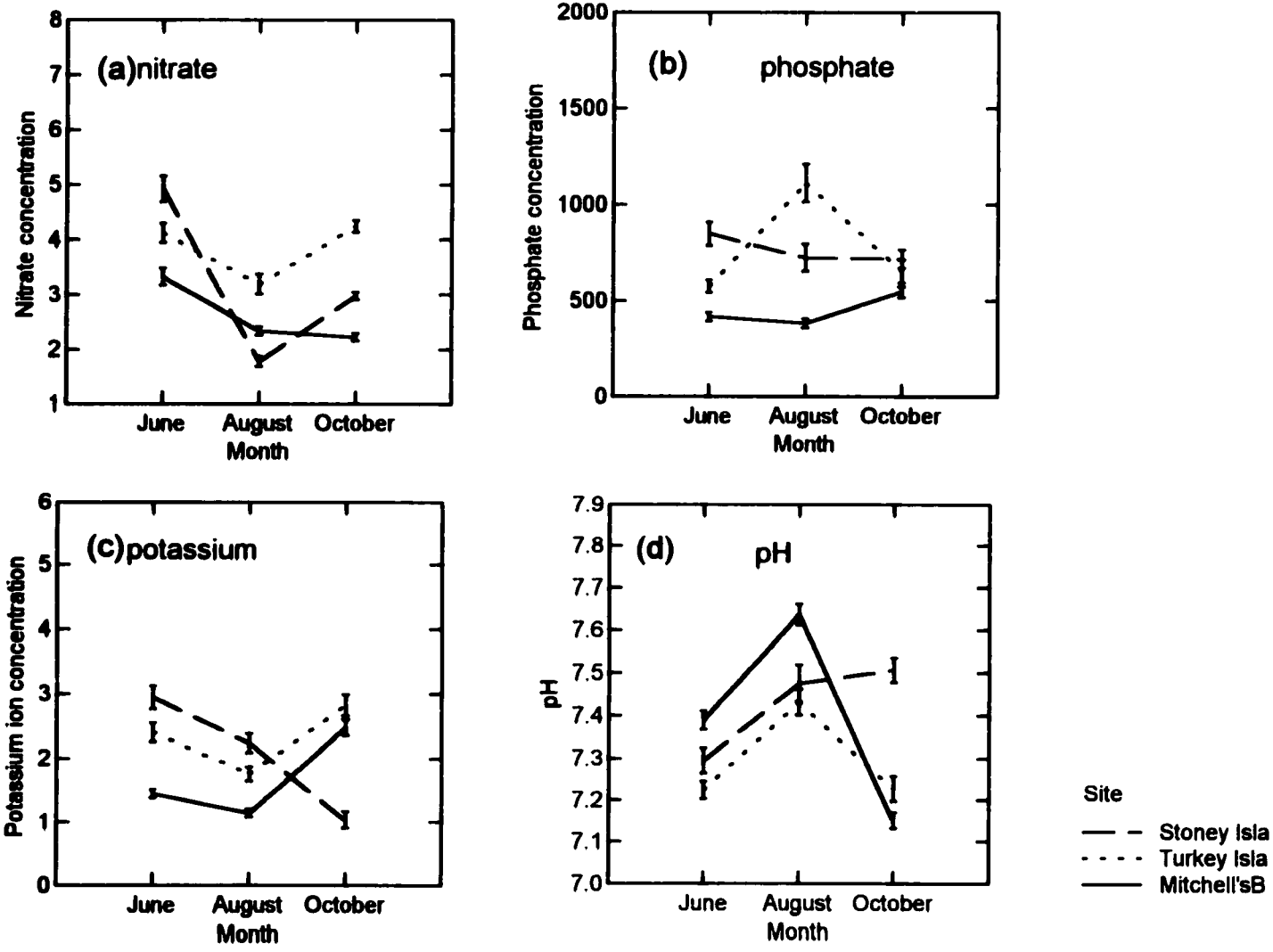

(a)nitrate
Nitrate concentration
8
7
6
5
4
3
2
1
June
August
October
Month
(b) phosphate
Phosphate concentration
2000
1500
1000
500
0
June
August
October
Month
(c)potassium
Potassium ion concentration
6
5
4
3
2
1
0
June
August
October
Month
(d) pH
pH
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
June
August
October
Month
Site
Stoney Isla
Turkey Isla
Mitchell'sB

Table 3.2.5. MANOVA results used to obtain ranking of correlated dependent variables for subsequent step-down analysis to determine differences in sediment composition among the sites. Due to multiple independent F-tests, probability was Bonferroni adjusted to $p<0.001$. ***$p<0.001$. Univariate F-tests for each variable are given first, followed by results of multivariate statistic for site and percent sand.

Univariate F		**Site**	**Percent sand**	**Error**
Measure	df	2	1	85/84
Percent	SS	0.7372		0.95
sand	F	33.03		
	p	**<0.001***		
Percent	SS	0.4072	0.2540	0.49
silt	F	34.98	43.59	
	p	**<0.001***	**<0.001***	
Percent	SS	0.0141	0.0763	0.10
clay	F	8.415	90.74	
	p	**<0.001***	**<0.001***	

Multivariate statistics			
Pillai's Trace	0.479	0.666	
df	4, 168	2, 83	
F	13.24	82.84	
p	**<0.001***	**<0.001***	

Figure 3.2.3. Sediment character as proportions of sand, silt and clay. Significant differences in percent sand are represented by lower-case letters above the error bars.

Figure 3.2.3.

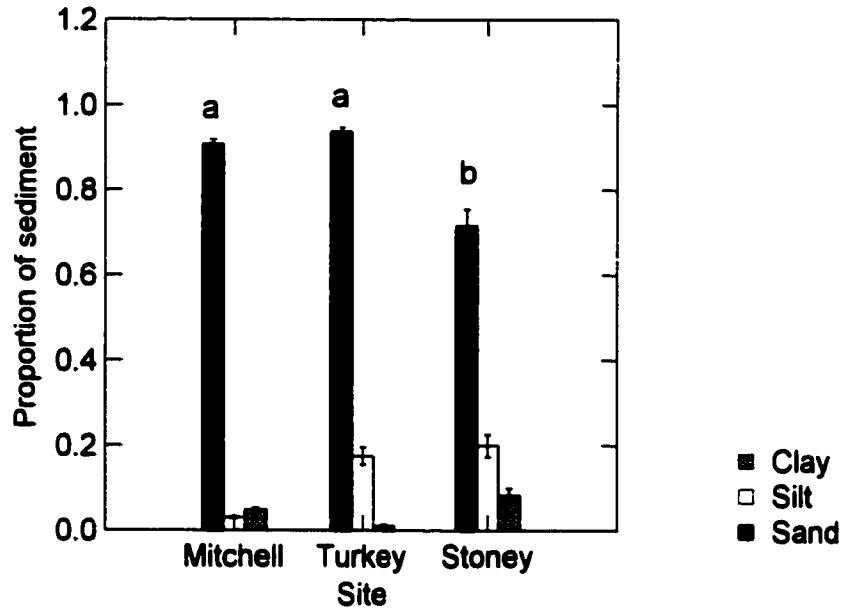

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Proportion of sediment
a
a
b
Mitchell
Turkey
Stoney
Site
Clay
Silt
Sand

Table 3.2.6. Mean ±SE (n) proportion of sediment sample ignited at 550°C, as a measure of organic matter content in the sediments of the three sites in June and October. Site ($F=140.775$, $p<0.001$) and month ($F=4.379$, $p<0.05$) significantly affected organic matter content. Based on Tukey's HSD test, in both June and October, Turkey Is. organic content was greater than Stoney Is., which was greater than Mitchell's Bay. Between months, October organic levels were significantly lower than June's. Lower case letters indicate significant differences in means among sites, within months based on Tukey's HSD test..

Site	June	October
Mitchell's Bay	0.027 ±0.001 [a]	0.016 ±0.001 [a]
	(100)	(98)
Stoney Is.	0.045 ±0.002 [c]	0.045 ±0.005 [c]
	(69)	(69)
Turkey Is.	0.166 ±0.014 [b]	0.133 ±0.011 [b]
	(101)	(102)

Light

A separate ANCOVA of Secchi depth (Table 3.2.7) indicated that after sampling depth was accounted for ($F=681.7$, $p<0.001$), Secchi depth differed significantly among sites ($F=16.51$, $p<0.001$) and between August and October ($F=4.367$, $p=0.038$). There was also a highly significant interaction between site and month ($F=18.99$, $p<0.001$). Figure 3.2.4a indicates that Mitchell's Bay tended to have lower values for Secchi depth, significantly so in October, based on Scheffé's test for the site by month interaction. The multiple comparison also showed Turkey Is. to have changed significantly in Secchi depth between the census periods, and to be significantly different from Stoney Is. in August (Figure 3.2.4b).

Growth and reproduction

A total of 536, 740 and 934 ramets were excavated from the fifteen 0.25 m^2 quadrats at Mitchell's Bay, Stoney Is. and Turkey Is., respectively. Measures of flowering at the three sites are presented in Table 3.2.8. Kruskal-Wallis analysis of variance on the proportion of ramets flowering indicated that Mitchell's Bay had significantly more flowering ramets than did Stoney Is. ($q=4.62$, $p<0.05$). The mean proportions of flowering female ramets among the sites were not significantly different (Table 3.2.8). Mitchell's Bay and Turkey Is., however, had a significantly greater proportion of flowering male ramets than did Stoney Is., based on the Tukey-like non-parametric multiple comparison test

Table 3.2.7. ANCOVA results for secchi depth using sampling depth as a covariate (COV). Probability was maintained at $p<0.05$. *$p<0.05$, **$p<0.001$.

DV	IV and COV	SS	df	F	p
Secchi	site (s)	2989	2e+	16.51	<0.001**
depth	month (m)	395.3	06	4.367	0.038*
	s*m	3439		19.00	<0.001**
	depth(COV)	0.6171×10^5		681.8	<0.001**
	error	0.1530×10^5			

Figure 3.2.4. (a) Secchi and (b) sampling depths for the three sites in August and October.

Figure 3.2.4.

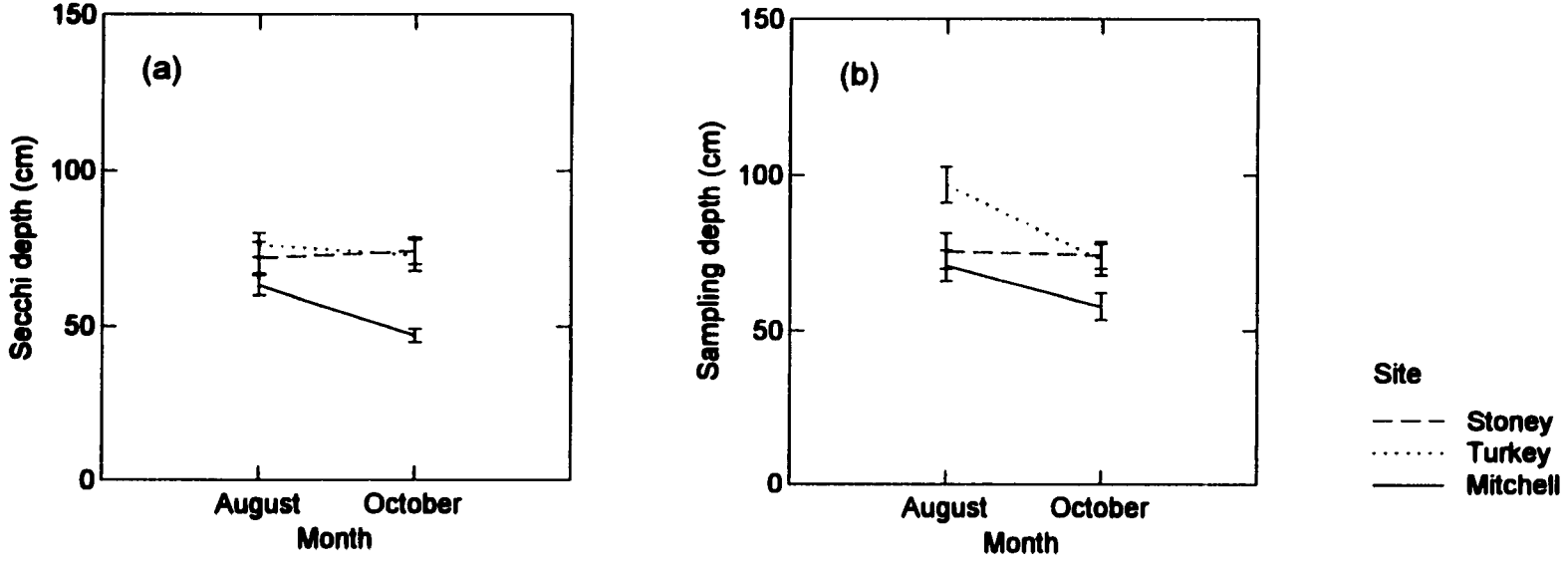

150
100
50
0
(a)
Secchi depth (cm)
August
October
Month
150
100
50
0
(b)
Sampling depth (cm)
August
October
Month
Site
Stoney
Turkey
Mitchell

Table 3.2.8. Flowering frequencies and results of Kruskal-Wallis one-way ANOVA for the three sites, for plants excavated from the fifteen quadrats at each site (1993 data). Means (±SE of non-transformed data are given in parenthesis), n=15. Results of *a posteriori* multiple comparisons among sites and within a column are indicated by lower case letters. Similar superscript letters in a column indicate that means are not significantly different; dissimilar letters indicate a significant difference. *p<0.01, ***p<0.001.

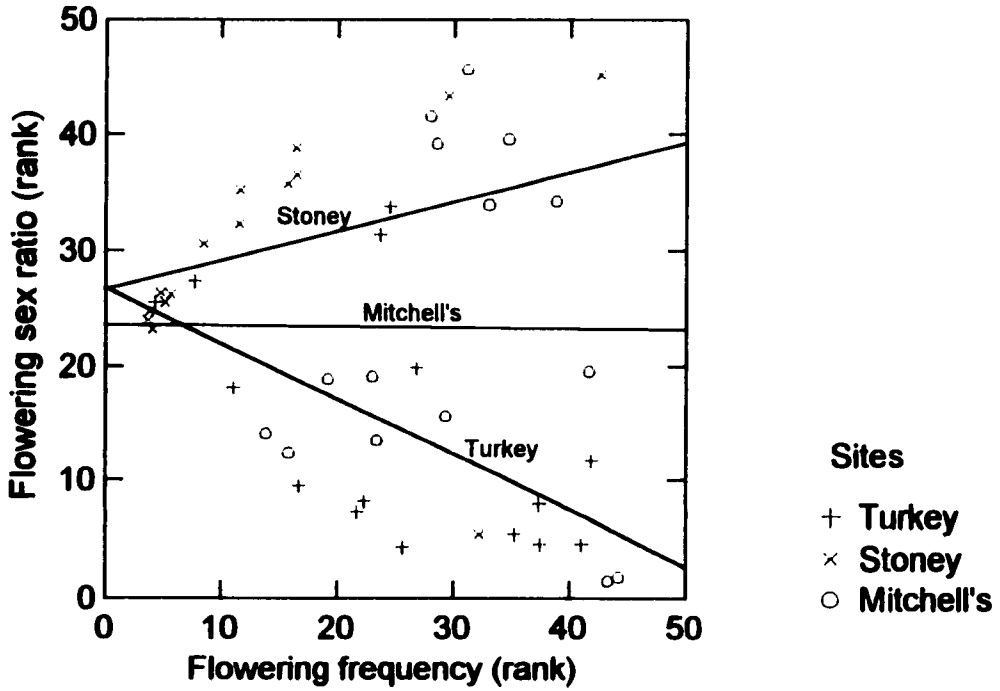
Site	Proportion of ramets flowering	Proportion of ramets: male	Proportion of ramets: female	Sex ratio per quadrat
Mitchell's Bay	0.603[a]	0.327[a]	0.276	1.03[ab]
	(0.045)	(0.076)	(0.063)	(0.097)
Stoney Island	0.275[b]	0.043[b]	0.204	1.21[a]
	(0.070)	(0.042)	(0.067)	(0.077)
Turkey Island	0.497[b]	0.402[a]	0.095	0.813[b]
	(0.052)	(0.061)	(0.036)	(0.059)
Kruskal-Wallis	11.27	21.15	3.964	10.50
p	<0.01*	<0.001**	0.138	<0.01*
df	2	2	2	2

(q_{m-s} =4.816, $p<0.05$; q_{s-t}=6.035, $p<0.05$; where subscripts m, s and t denote the three sites; Mitchell's Bay, Stoney Is. and Turkey Is. respectively). Shoot sex ratios differed among sites, with Stoney Is. having a higher sex ratio (more female-biased) than Turkey Is. ($q=4.561$, $p<0.05$). The relationship between increasing frequency of flowering, and changes in the sex ratio at the three sites was tested by ANCOVA (a single statistical outlier quadrat with a male bias at Stoney Is., was removed from the analysis; Figure 3.2.5). The ANCOVA indicated a significant interaction between site and flowering frequency ($F=5.097$, $p<0.05$). Individually, Turkey Is. and Stoney Is. had significant regression lines ($F=6.547$, $p<0.05$, $R^2=0.335$ and $F=60.630$, $p<0.001$, $R^2=0.835$ respectively), while Mitchell's Bay did not ($F=0.001$, $p=0.976$, $R^2=0.000$).

Chi-squared tests were performed to detect departure from a 1:1 sex ratio within each site. Both Stoney Is. and Turkey Is. had biased sex ratios, X^2 =13.68 and 13.27 respectively. Mitchell's Bay had a unitary sex ratio (X^2 =0.267). Stoney Is. was female-biased, with a mean sex ratio greater than 1, while Turkey Is. was male-biased, with a mean sex ratio less than 1 (Table 3.2.8). The sex ratios observed in 1993 differed only slightly from those observed at these sites in previous years (see Table 3.2.1), where the overall sex ratios were: Mitchell's Bay 0.71, Turkey Is. 0.97 and Stoney Is. 1.18. The sex ratio for the subset of transects at comparable depths in the 1991 survey at Turkey Is. (<3 m) was 0.86. Only Mitchell's Bay showed a major change between years in the overall sex ratio, switching from male-bias in 1992 to a unitary sex ratio in the following

Figure 3.2.5. Ranked flowering frequencies in relation to ranked sex ratio at three sites. One outlying data point for Stoney Is., which was the only male-biased quadrat at that site, was removed from the analysis. ANCOVA results on ranked data indicate a significant interaction between site and flowering frequency ($F=5.097$, $p<0.05$). The individual regression equations for the three sites are: Stoney Is.: sex ratio = 24.770 + 0.586(flowering)*** ($F=60.630$, ***$p<0.001$); Mitchell's Bay: sex ratio = 23.304 + 0.012(flowering) ($F=0.001$, $p>0.05$); Turkey Is.: sex ratio = 26.942 + 0.479(flowering)* ($F=6.547$, *$p<0.05$). Based on the best fitting linear plot, sex ratio became increasingly male-biased as the proportion of flowering ramets increased at Turkey Is.; it became increasingly female-biased at Stoney Is., and there was no significant relationship at Mitchell's Bay.

Figure 3.2.5.


Flowering sex ratio (rank)
Flowering frequency (rank)
0
10
20
30
40
50
Stoney
Mitchell's
Turkey
Sites
Turkey
Stoney
Mitchell's

season. Turkey Island became slightly more male-biased.

Based on Kruskal-Wallis one-way ANOVA, the numbers of seed pods, mature and immature seeds per quadrat did not differ significantly among sites (Table 3.2.9). Stoney Is., however, tended to have greater seed production and a higher level of variation in terms of the above three measures. Density, measured as number of ramets per quadrat, and fresh mass of *V. americana* did not differ significantly among sites (Table 3.2.9).

At each site, the number of pods produced per unit area correlated strongly with the number of mature (R^2>0.96) and immature (R^2>0.78) seeds produced per unit area so pod production could be used to characterize the relationship between seed production and flowering measures. Step-down multiple regression was used (N=45), with site (T=-1.483, p=0.146), ramet number (T=4.619, p<0.001), flowering frequency (T=3.357, p=0.002) and sex ratio (T=-1.609, p=0.546) as independent variables in the first step (F=9.588, df=4,40, p<0.001). Independent variables were not correlated with each other, based on Pearson correlation coefficients. Site and flowering frequency were removed from the second step as they were not significant, resulting in pod production being defined as: pod production = - 4.741 + 0.048 (ramet number) *** + 4.737 (sex ratio) *** with F=19.560***, df=2,42, (***p<0.001). Sex ratio and ramet density, together, accounted for 48.2% of the variation in seed pod production.

Table 3.2.9. Mean ± (SE) measures of plant reproduction and growth, with results of Kruskal-Wallis one-way ANOVA for three sites in the Huron-Erie corridor. Means and SE are based on non-transformed data, and are per 0.25 m^2 quadrat, n=15. Differences between sites are, in all cases, non-significant.

Site	Number of pods	Number of mature seeds	Number of immature seeds	Number of ramets	*V. americana* fresh mass (g)	Seeds per ramet
Mitchell's Bay	1.73	484.5	14.40	35.73	769.0	60.55
	(0.431)	(132.9)	(4.411)	(8.037)	(52.76)	(22.03)
Stoney Island	4.07	859.7	80.80	50.73	424.3	34.83
	(1.48)	(303.7)	(33.78)	(14.52)	(118.0)	(14.75)
Turkey Island	1.53	329.1	26.13	61.87	499.6	11.29
	(0.551)	(116.4)	(11.35)	(7.262)	(76.90)	(3.675)
Kruskal-Wallis	0.261	0.384	0.003	4.28	4.18	2.32
p	0.877	0.825	0.999	0.117	0.124	0.314
df	2	2	2	2	2	2

Factors influencing growth and reproduction

Based upon correlations among physical and growth variables (Table 3.2.10), representative environmental variables used in the canonical correlation were K^+, percent sand, Secchi depth and organic matter. Flowering, growth and reproduction were represented by flowering frequency, sex ratio, ramet number, seeds/m^2 and fresh biomass of *V. americana*. The distribution of the canonical variate scores was normal and linear, enabling non-transformed data to be used in the analysis. The first canonical correlation was 0.727 (52.8% of variance). The second, third and fourth canonical correlation coefficients were 0.310, 0.230 and 0.145, respectively, and accounted for 9.6%, 5.3% and 2.1% of the remaining variance. With all four canonical correlations included, $\chi^2_{(20)} = 32.246$, $p<0.05$. Since this was the only significant χ^2, only the first of the four variates derived from canonical correlation was significant. Canonical correlation statistics between growth and environmental measures for the first canonical variate are given in Table 3.2.11. The following trends can be discerned from the correlations between the variables and the first variate: quadrats with lower flowering frequency (correlation with first variate= -0.798) and increased seed production (0.585) tended to also have lower K^+ (-0.762) and a higher percent sand (0.851).

Table 3.2.10. Correlations among flowering variables and among growth and reproduction variables on non-transformed data. Least correlated variables were used in canonical correlation analysis relating environmental parameters to growth and reproduction. *p<0.05, **p<0.001.

n=45	**Percent female**	**Percent male**	**Flowering frequency**
Percent male	-0.514**		
Flowering frequency	0.334*	0.637**	
Sex ratio	0.897**	-0.824**	-0.099
n=45	Flowering frequency	Seed set /0.25m^2	Ramet number
Seed set/0.25m^2	-0.332*		
Ramet number	0.128	-0.089	
V. americana fresh biomass	-0.067	0.135	0.222

Table 3.2.11. First variate results of canonical correlation on measures of growth and reproduction with environmental parameters. Correlations between variables and first variate, standardized canonical coefficients, canonical correlation ($X^2_{(20)}$ =32.246, $p<0.05$), percent of variance and redundancies.

	Correlation with first variate	**Standardized canonical coefficient**
Growth measures		
Flowering frequency	-0.798	-0.601
Sex Ratio	0.367	0.308
Ramet number	-0.360	0.333
Fresh mass	0.332	0.320
Seeds	0.585	0.310
Percent of variance	27.07	
Redundancy	14.31	
Environmental Set		
K^+ concentration	-0.762	-0.721
Percent sand	0.851	0.453
Secchi depth	-0.280	-0.073
Organic matter	0.117	0.379
Percent of variance	34.92	
Redundancy	18.46	
Canonical correlation	0.727	

Transplant experiment

Turion survival was low in the transplanted tubs, with a maximum of 18% for plants growing in Mitchell's Bay water and a minimum of 2% for plants growing in Stoney Is. water (Table 3.2.12). As a result tubs, rather than ramets within the tubs, were treated as replicates in the statistical analysis, with sample sizes (number of tubs) shown in Table 3.2.13. Due to the low sample sizes and some empty cells, a means model was performed (Milliken and Johnson, 1984) to assess the effects of water, sediment and plant origin (Table 3.2.14). Results of the means model for turion survival indicated that water and water-by-sediment effects were significant (Table 3.2.14). Post hoc Fisher's HSD tests suggest that Turkey Is. and Mitchell's Bay had similar survivorship, and survival at both sites was significantly greater than at Stoney Is.

Analysis for the number of ramets showed that there were no significant interactions, but water column on its own was a significant factor (Table 3.2.14). The number of ramets at Turkey Island was significantly different from that at Mitchell's Bay based on a post hoc Fisher's LSD test. For flower number, neither interactions nor individual factors proved significant. Means ±(SE) for number of ramets and number of flowers, calculated by location (water column), sediment origin and plant origin separately, are presented in Table 3.2.12.

Table 3.2.12. Mean, (SE) and *n* for turion survival per tub, and mean ramet number and flower number per surviving turion in the reciprocally transplanted tubs at Mitchell's Bay, Turkey Is., and Stoney Is. Means and SE were calculated by water column, sediment origin and plant origin. (see also Table 3.2.14).

Factor	**Site**	**Initial turion survival / tub**	**Number of ramets / tub**	**Number of flowers / ramet**
Water	Mitchell	0.181	9.33	1.71
column		(0.034)	(0.926)	(0.178)
		36	*22*	*22*
	Turkey	0.122	17.1	0.153
		(0.027)	(2.204)	(0.109)
		36	*18*	*18*
	Stoney	0.021	26.4	1.10
		(0.015)	(8.625)	(1.100)
		36	*2*	*2*
Sediment	Mitchell	0.069	11.5	0.788
origin		(0.023)	(0.121)	(0.299)
		36	*12*	*12*
	Turkey	0.111	15.3	1.04
		(0.029)	(3.112)	(0.291)
		36	*13*	*13*
	Stoney	0.142	13.4	1.16
		(0.032)	(1.700)	(0.263)
		36	*17*	*17*

Factor	Site	Initial turion survival / tub	Number of ramets / tub	Number of flowers / ramet
Plant origin	Mitchell	0.153 (0.033) *36*	16.1 (2.115) *19*	1.15 (0.264) *18*
	Turkey	0.083 (0.021) *36*	11.7 (2.095) *14*	0.851 (0.255) *14*
	Stoney	0.087 (0.028) *36*	10.7 (2.368) *9*	0.971 (0.339) *9*

Table 3.2.13. Design and surviving sample sizes (number of tubs out of an initial 4) at the end of the reciprocal transplant experiment.

Plant origin	Sediment origin	Water origin (location of tubs)		
		Mitchell's	Stoney	Turkey
Mitchell's	Mitchell's	1	0	3
	Stoney	4	1	3
	Turkey	3	1	3
Stoney	Mitchell's	1	0	2
	Stoney	3	0	0
	Turkey	1	0	2
Turkey	Mitchell's	3	0	2
	Stoney	4	0	2
	Turkey	2	0	1

Table 3.2.14. Results of means model analysis (Milliken and Johnson, 1984) of the transplant experiment data. The results for each factor and dependent variable are from independent tests. ANOVA F-test results are presented (p in brackets). To maintain an overall p=0.05 for the 21 independent tests, significance level was set at p<0.0024. * indicates significance , all other results were non-significant.

Factor	Turion survival	Number of ramets	Number of flowers
plant X sediment X water	0.702	0.997	0.666
	(0.689)	(0.496)	(0.745)
plant X sediment	1.64	0.278	1.30
	(0.171)	(0.891)	(0.317)
plant X water	1.69	1.97	0.817
	(0.159)	(0.096)	(0.531)
sediment X water	2.73	1.34	0.604
	(0.035)*	(0.263)	(0.665)
plant	2.55	1.76	0.427
	(0.085)	(0.185)	(0.659)
sediment	2.23	0.124	0.653
	(0.115)	(0.884)	(0.531)
water	10.8*	3.59	1.88
	(<0.001)*	(0.037)	(0.185)

Discussion

Physical and chemical properties of sediments over time and sites

Levels of the three nutrients analysed and pH varied by site and month (Fig. 3.2.2 a-d). These variables are known to be affected by the phenology of growth of aquatic macrophytes (Wigand *et al.*, 1997; Crowder and Painter, 1991; Gopal, 1990; Wetzel, 1979), by temperature changes through the season, and other environmental factors. There does not, however, appear to be a consistent pattern among the three sites over the course of the summer.

Vallisneria americana, like other rooted, submersed macrophytes, utilizes nutrients from both sediments and water (cf Gopal, 1990). Large standing crops of macrophytes in the Great Lakes have been shown to reflect relatively large sediment and atmospheric nutrient pools (Edwards *et al.*, 1989). Nitrogen and phosphorous are typically taken up through the roots, while potassium is taken up predominately through the shoots; the primary sources of these nutrients being the sediments and water columns respectively (cf Barko *et al.*, 1986; Overath *et al.*, 1991). Some sediments provide sufficient potassium for root uptake, yet *Hydrilla verticillata* grew better when additional potassium was added to a variety of sediments (Barko, 1982). Fourqurean *et al.* (1992) reported that sediment porewater is the most important source of nutrients for seagrass growth, and in the subtropical environment where seagrasses grow, phosphorus availability is the limiting factor to growth.

Although some studies have indicated that nutrients do not limit growth of

macrophytes in moderately eutrophic systems (Barko *et al.*, 1986; Gopal, 1990), Barko and Smart (1986) found that additions of some nutrients (notably P and Fe) resulted in enhanced growth of *Hydrilla verticullata* and *Myriophyllum spicatum*. Also, additions of N promoted growth of *V. americana* from Lake Onalaska, WI (Rogers *et al.*, 1992) and *Zostera marina* from the Netherlands (vanLent *et al.*, 1995). Overath *et al.* (1991) correlated reduced growth of *V. americana* with low nutrient levels and extremely high organic matter content in three upstate New York lakes, suggesting nutrient limitation to growth. Nitrogen availability, affected by sediment accretion and ground water influx, was critical for *V. americana* population re-establishment and maintenance in the Upper Mississippi River (Rogers, 1994). It is not clear if the nutrients assayed in this study limit *V. americana* growth at the sites studied.

Low levels of organic matter are thought to stimulate aquatic plant growth as a result of increased ionic exchange properties and increased nutrient content of the sediments (Barko *et al.*, 1986). The proportion of organic matter in the sediments at the three sites in this study were quite low, and ranged from less than 2% at Mitchell's Bay to 16% at Turkey Island (Table 3.2.6). Levels were significantly higher in June than October (Table 3.2.6). Greater wet biomass of *V. americana* was evident at Mitchell's Bay despite the significantly lower sediment organic matter content (Table 3.2.9). Barko and Smart (1986) found declines in growth of *H. verticullata* and *M. spicatum* as organic matter increased to 20% of sediment dry mass, but Titus and Stephens (1983) studied

the effects of neighbouring plants on *V. americana* growth, and found that the site with an organic content of near 50% in the top 10 cm layer of sediment also had greater *V. americana* growth.

Sediment organic content and texture are important factors affecting growth of species on different sediments (Barko and Smart, 1986). Sandy substrates are known to be nutritionally poor (Barko *et al.*, 1986), and can limit the distribution of many aquatic macrophytes. *Vallisneria americana* is most often found on silty to sandy sediments (Korschgen and Green, 1988, Catling *et al.*, 1994). Nichols (1992) and Nichols and Yandell (1995) found *V. americana* presence to be positively correlated with 'hard' substrates (gravel and sand). Sediment texture analysis showed Mitchell's Bay to have sediment that was more sandy than both Turkey and Stoney Islands (Fig. 3.2.3); this was associated with generally lower nutrient levels (Fig. 3.2.2 a-c). Despite these findings, biomass production was greater at Mitchell's Bay (Table 3.2.9). This may be attributable to agricultural and urban inputs in the south-eastern basin of Lake St. Clair (Leach, 1991). These inputs, in addition to a more stable water body (Leach, 1991) are responsible for higher nutrient concentrations in the water column of this area of Lake St. Clair (Leach, 1991). Increased sand content can also alleviate the effects of organochlorine pollution on *V. americana* growth as shown in laboratory studies of Biernacki *et al.* (1995 b).

Titus and his colleagues have extensively studied the effects of low pH on the growth and morphology of *V. americana* (Titus and Stone, 1982; Grisé *et al.*,

1986; Titus *et al.*, 1990; Titus, 1992; Titus and Andorfer, 1996). Their studies indicate that growth and reproduction decline significantly at pH<5.0, but that an increased availability of dissolved inorganic carbon at such a low pH can offset the decline. Also, as pH increased from 7 to 8, a 61% decline in growth of *V. americana* was observed by Titus and Stone (1982), with a concomitant decrease in plant weight, number of rosettes and number of buds.

The pH at the sites studied here range from seven-to-eight and are not low enough to cause a severe reduction in growth and reproduction in *V. americana*, and the observed fluctuations may reflect alkalinity changes resulting from photosynthesis which peaks with *V. americana* growth in the summer. A similar pattern of increasing pH from early to mid-summer followed by a decline in the fall was found by Titus and Stephens (1983).

Nutrient availability is related to the solubility of the nutrients, which in turn depends on soil pH (Galston *et al.*, 1980). The optimal pH values for the solubility of the nutrients analysed here are; N: pH 6-8; P: pH 6.5-7.5; K: pH 6-10 (Galston *et al.*, 1980), all of which fall within the ranges measured at the sites over the season (Fig. 3.2.2 d). Water pH and carbon dioxide concentration, in concert with sediment fertility influences the accumulation of minerals in macrophyte shoots (Titus and Andorfer, 1996).

Light quality and quantity can often be limiting to macrophyte growth and distribution in the aquatic environment (Barko *et al.*, 1986; Chambers and Kalff, 1987; Carter *et al.*, 1996; Korschgen *et al.* 1997), often interacting with

temperature (Barko and Smart, 1981; Barko *et al.*, 1982; Duarte, 1991). Titus (1983) found the highest frequency of *V. americana* between 0.5-2.5 m water depth. The results of the current study indicate that there is a significant difference in secchi depth, maintained over time, between the Mitchell's Bay site and Stoney Island with Mitchell's Bay being more turbid (Fig. 3.2.4). This pattern does not appear to be a function of depth sampled, as the Stoney Island and Mitchell's Bay sites were not statistically different in depth. Personal observations of the sites correspond with the pattern of secchi depth. The high water flow through the bay at Stoney Island results in a very short local hydrologic retention time and lower turbidity, whereas the protected channel of Bass Haven off Mitchell's Bay experiences little flow and higher turbidity (C. Lokker, personal observation). Turkey Island, although sampled at a greater range of depths, had similar secchi depth readings to Stoney Island. This site would be considered intermediate in its water flow and turbidity between the other two sites (C. Lokker, personal observation).

Vallisneria americana has been associated with turbid waters in Wisconsin (Nichols, 1992; Nichols and Yandell, 1995) where it maintains its biomass (Korschgen and Green, 1988) and is physiologically able to adapt to low light regimes (Titus and Adams, 1979). One way in which *V. americana* adapts to low light is by changing its canopy morphology; with increasing turbidity resources are diverted from shoot and leaf recruitment to leaf extension (Blanch *et al.*, 1998).

Leach (1991) showed that the distribution of aquatic macrophytes including *V. americana* in Lake St. Clair was related to secchi depth, temperature, plant biomass, number of taxa and mean depth. A dramatic decline in *V. americana* abundance in Put-in-Bay was attributed to increased turbidity, Lake Erie (Stuckey and Moore, 1995) and likewise in Lake Ontario (Crowder and Painter, 1991). Turbidity has been cited as the cause of similar declines of *V. americana* populations in the Detroit River (Schloesser and Manny, 1990), in the Potomac River, MA (Carter and Rybicki, 1985; Carter *et al.*, 1994), and in the Upper Mississippi River (Rogers, 1994; Fischer and Claflin, 1995). The invasion of the Great Lakes of the water column-clearing zebra and quagga mussels (*Dreissena polymorpha* and *D. bugensis*, respectively) has been associated with an increased depth of light penetration and a resurgence of *V. americana* populations in the Great Lakes (Nalepa and Schloesser, 1993; Stuckey and Moore, 1995; Skubinna *et al.*, 1995). Griffiths (1993) reports that *V. americana* now grows in deeper waters.

At sites with higher water flow and water clarity, creeping forms of aquatic macrophytes such as *V. americana* are observed, with more vegetative reproduction present (Korschgen and Green, 1988). In experimental studies by Barko *et al.* (1982) increased light resulted in decreased shoot length. Stoney Island, with the higher observed water flow and secchi depths, experienced lower flowering than did Mitchell's Bay (Tables 3.2.2, 3.2.8). Korschgen *et al.* (1997) suggest that the effect of light on the number and size of turions

produced by *V. americana* limits the distribution and abundance of the species in the Upper Mississippi River.

The pattern of light differences detected with secchi depth measurements was not repeated in the transplant experiment. Water column would reflect such a difference, and was significant, with Turkey Island being different from Mitchell's Bay. Unfortunately, even if differences at Stoney Island existed, they would not be detected with this experiment due to the high mortality of turions and resultant low sample size. The absence of significant results for water, plant and sediment origin, and their interactions, may also reflect the low survivorship of experimental plants.

Sexual reproduction in V. americana

Proportions of flowering ramets were quite high in this study compared to previous reports (Titus and Stephens, 1983; Lovett-Doust and LaPorte, 1991; Laushman, 1993), and ranged from 28-60%, with sites differing in their tertiary sex ratios, and varying from year to year. Comparison of 1993 data (Table 3.2.8) with data obtained in 1991 and 1992 for the same three sites (Table 3.2.1), indicates some year-to-year variation in the proportion of ramets that flower, and the sex ratios of flowering ramets. Stoney and Turkey Islands experienced more flowering in 1993, with Turkey Island becoming slightly more male-biased. At Mitchell's Bay the flowering frequency remained constant, but the sex ratio changed from male-biased to unity over the year. It should be noted there were

differences in sampling strategies between years; in the present study transects were more widely spaced in order to represent the sites more completely.

Seed production at the three sites studied was not significantly different, although Stoney Island produced more seed pods and seeds per m^2 despite a trend towards lower ramet number and plant biomass (Table 3.2.9). Seed pod production regressed significantly with an increasing sex ratio (more female-biased) and increasing ramet density. The results of canonical analysis indicated that lower flowering frequency and increased seed set corresponded with lower K and an increased proportion of sand in the sediment (Table 3.2.11).

Titus and Hoover (1991) have suggested that sexual reproduction in *V. americana* is likely limited by floral induction or pollen transport, and their laboratory experiments suggested that floral induction for female plants of *V. americana* may be a function of plant size (Titus and Hoover, 1991). They interpreted female flowering as a function of resource availability. Other studies have found positive correlations between plant biomass and flowering (Titus *et al.*, 1990; Biernacki *et al.*, 1995 a) or seed set (Rogers *et al.*, 1992). No plants below Titus and Hoover's (1991) size threshold of 0.75 g flowered in a study of the effect of trichloroethylene on *V. americana* growth and reproduction (Biernacki *et al.*,1995 a). Rogers *et al.* (1992) found *V. americana* seed and pod mass to be significantly correlated to each other and also related to above-ground biomass.

Vallisneria americana allocates 1-10% of its biomass to sexual

reproduction (Titus and Stephens, 1983; Donnermeyer and Smart, 1985; Lovett-Doust and LaPorte, 1991; Madsen, 1991). More resources are allocated to the production of overwintering turions (Madsen, 1991), which form at the same time as flower development and seed formation (Donnermeyer and Smart, 1985). Lovett-Doust and LaPorte (1991) found that 12% of *V. americana* plant biomass was allocated to female structures, while 5% was allocated to male structures, and that, overall, females had greater biomass than males.

Environmental influences on turion production in *V. americana* have been extensively studied (e.g., Grisé *et al.*, 1986; Rogers *et al.*, 1992; Titus and Hoover, 1993; Spencer and Ksander, 1995; Korschgen *et al.*, 1997), while those on sexual reproduction remain few. Environmental factors which may affect flowering and seed set include: pH (Titus and Hoover, 1993), nutrient availability (Rogers *et al.*, 1992; vanLent *et al.*, 1995), irradiance (Carter *et al.*, 1996), and organic contamination (Biernacki *et al.*, 1995 a, 1995 b,1996).

Titus and Hoover (1993) found that *V. americana* did not flower, and that shoots grown from seed failed to produce turions at pH < 5. Low pH may therefore restrict seed production. Turion and seed production were not dependent on N levels, but additions of N did promote *V. americana* growth and increased above ground biomass and propagule production (Rogers *et al.*, 1992). Biernacki *et al.* (1995 a) reported a reduction in flowering frequency in *V. americana* plants treated with high, medium and low concentrations of trichloroethylene (TCE), a common water contaminant in the Huron-Erie corridor,

when compared with untreated control plants. Of the few high-TCE plants that survived, however, a greater proportion flowered than did those under lower TCE treatments.

Where light was limiting growth and distribution of the species, Carter *et al.* (1996) found that increased photon irradiance resulted in more plants and greater biomass of *V. americana*. Flowering of *V. americana* only occurred in cages where light was supplemented (Carter *et al.*, 1996), but it is not known whether light per se, or the consequent increase in plant biomass, promoted flowering. Lokker *et al.* (1994) observed a transition from male- to female- biased *V. americana* beds along a depth gradient of <1 m to > 3 m at Turkey Island. Such depth-related differences in flowering frequency may reflect differing flowering cues between the sexes. Such cues could relate to plant size, light availability and composition, nutrient availability etc.

A number of environmental factors affect the growth of *V. americana* (Barko *et al.*, 1986; Titus and Hoover, 1990, 1993; Overath *et al.*, 1991; Rogers *et al.*,1992; Biernacki *et al.*, 1995 a, 1995 b, 1996; Carter *et al.*, 1996), and contribute to its reproductive success. Yet, there was no clear relationship among the environmental variables assayed here and flowering, seed production and growth.

3.3 Pollination and the effects of outcrossing distance

Objective

The objective of this study was to assess the effect on offspring fitness of outcrossing in *V. americana* by carrying out controlled crosses between female *V. americana* clones collected from Turkey Island in the Detroit River with males collected from progressively increasing distances up- and down-stream from the females. Inbreeding and/or outcrossing effects on the formation and vigour of offspring were assessed.

Pollination in *V. americana* occurs at the water surface when pollen enters a small air bubble produced by wave action around the unwettable stigma (Wylie, 1917; Cook, 1982, 1988). Both wind and water currents transport the male flowers (Wylie, 1917; Cook, 1988). Pollen grains often form aggregates (Titus and Hoover, 1991), termed 'search vehicles', that increase the probability that they will encounter a receptive stigma (Cox and Knox, 1989). Despite the pollen's transport on the water, both the sticky pollen and stigma remain dry and pollination is considered epihydrophilous (Cook, 1988). Although *V. americana* is not strictly a hydrophilous species, it shares many characteristics with the hydrophiles in that it is dioecious, highly clonal and has a complex pollination mechanism (Les, 1988). Pollination success has been shown to increase with male plant density and a higher pollen retention time at a site due to lower water flow (Sullivan and Titus, 1996).

The genetic similarity of mates can influence the success of a cross; if

mates are too similar, inbreeding depression may result, but if mates are too dissimilar outbreeding depression may occur (Price and Waser, 1979; Waser and Price, 1983).

Inbreeding depression refers to the reduction in vigour often observed in offspring from matings between close relatives, and is due to the expression of recessive deleterious alleles (Ayala, 1982). Limited seed and pollen dispersal can result in spatial structuring of populations with near neighbours being genetically related (Cleverling, 1995; Byers, 1998). Matings between near neighbours may then result in offspring with lower fitness values due to inbreeding depression (Price and Waser, 1979; Waser and Price, 1983; Byers, 1998).

Outbreeding depression also results in reduced fitness of offspring due to the disruption of gene complexes which are adapted to local selection regimes (Price and Waser, 1979; Waser and Price, 1991). With increasing geographic distance, genetic distance is also expected to increase (Nei, 1972) as a result of drift and selection. Matings between distant individuals can then result in outbreeding depression.

To minimize inbreeding and outbreeding effects, natural selection should favour matings between individuals of intermediate genetic similarity (Price and Waser, 1979). Also, the Fritz Müller Law states that an intermediate degree of outbreeding is optimal if the fitness of a cross is depressed by excessive inbreeding or outbreeding (Price and Waser, 1979; Waser and Price, 1983).

Gene flow and population genetic structure are important determinants of optimal outcrossing distances. For species with localized gene flow, the Fritz Müller Law should apply within populations, and a relatively short outcrossing distance is expected (Waser and Price, 1983). For species with greater gene flow a larger optimal outcrossing distance is expected.

For *V. americana* , the potential for, and the measures of gene flow are high based on the lack of local population subdivision and high migration measures found in the allozyme studies of sites in the Huron-Erie corridor of the Great Lakes (Chapter 2; Lokker *et al.*, 1994; but also see Laushman, 1993). Outbreeding effects among plants from these sites are therefore likely to be negligible, yielding a large optimal outcrossing distance between mates.

The questions addressed here are: does paternal geographic distance affect progeny fitness in terms of seed size, germination or seedling vigour? Do maternal and paternal genetic identities affect progeny fitness?

Methods

Plant collection

Connected ramets of *V. americana* were collected in September 1993. Twenty different female genets (clones) were collected from a particular area (approx. 10 m by 10 m) at the southern end of Turkey Island in the Detroit River. Care was taken to select different multi-ramet female genets from a fairly large area, to maximize the probability that the twenty genets were genetically distinct

(but see Lokker *et al.*, 1994 and Chapter 2 here). A male genet, also composed of a number of attached ramets, was then collected from each of seven sites at 50 m intervals of increasing upstream distance from the maternal plants, starting at 0 m and going to 350 m (two distinct genets were collected at 0 m). Two additional male genets were collected from each of Mitchell's Bay (c. 140 km upstream) and Stoney Island (c. 20 km downstream) to provide distant pollen donors for the crosses.

Each genet was planted in a 68 L plastic tub in the University of Windsor greenhouse. The tubs were half-filled with steam-sterilized sediment collected from Turkey Island, and topped up with tap water.

The plants were allowed to produce turions and senesce during the winter. The pollination experiment began in Spring 1994. Several of the clones did not survive the winter and the males were replaced with connected ramets from the field in the Spring of 1994. A sufficient number of female clones (10) survived.

Parental genetic identities

Genetic analysis of the parents was carried out using stolon samples in order to verify that they represented distinct genotypes (see Chapter 2 for electrophoretic and staining protocols). Most male and female clones had a distinct genotype based on banding patterns for the six allozymes assayed. However, female no. 10 and no. 3 shared composite banding patterns, as did the

males from 100 m and 350 m upstream.

Genetic analysis was also attempted on seedling tissues (using progeny from the crosses), with inconclusive results. Few seedlings produced sufficient stolon material to allow electrophoretic analysis, and the banding obtained from the small stolon samples was not clear enough to test any hypothesis regarding inheritance patterns for the allozymes under study. This could be a result of the small sample size or due to developmental differences in enzyme production at different stages in the life-cycle of *V. americana*.

Pollination

Flowers on each of the females were pollinated as they opened, using the pollen that was available on that particular day. Often, pollen or flowers went unused if both sexes were not at anthesis at the same time. In general, the flowering of the females set the schedule and resultant sample sizes for the experiment. Sample sizes for the various male X female crosses are presented in Table 3.3.1. Care was taken to ensure that the pollen placed on the flowers was not released beyond the immediate area of the test flower. Each flower was tagged with parafilm at the base of the pod. Unfortunately, due to the fragile nature of the peduncle, many pollinated pods were lost when the peduncles dried out on the surface of the water, or were broken by water movement and handling.

Developing pods were left to mature, and removed in mid-November when pod length was measured. Pods were then stored in plastic vials filled with water and placed in a covered box in the cold room (8°C) for 3 months. This

Table 3.3.1. Numbers of crosses performed for the various male X female combinations. Males are represented by code number (1-13) and by the distance from the females. The distance from the source population (Turkey Is.) to Stoney Is. is represented as a negative since Stoney Is. is downstream from Turkey Is. Totals for number of crosses per female are given at the right of the table, totals per male are given along the bottom of the table.

Male code number (1-13) and distance (m or km) from female genets														
	Stoney						Turkey						Mitchell's	
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Female No.	-20km	-20km	0 m	0 m	50m	100m	150m	200m	250m	300m	350m	140km	140km	n
1	3	2	1	1	2	0	1	1	3	2	2	1	2	21
3	2	0	1	1	2	1	1	1	2	2	1	1	1	16
10	2	1	2	2	2	1	1	1	3	3	2	2	2	24
12	2	1	2	2	2	1	1	1	2	2	2	1	2	21
14	3	2	2	3	3	1	3	2	3	3	3	4	3	35
15	1	2	1	1	2	1	0	1	2	3	2	2	1	19
17	3	3	3	2	2	3	1	2	3	2	3	3	3	33
20	2	1	3	1	2	2	1	1	2	2	3	2	2	24
21	2	2	2	2	2	1	2	1	2	2	2	2	1	23
24	1	1	1	1	1	0	0	0	1	1	1	1	1	10
n	21	15	18	16	20	11	11	11	23	22	21	19	18	226

storage time allowed for breakdown of the gelatinous matrix which surrounds the seeds within the pods (see Ferasol *et al.*, 1995). The seeds were then removed from the pods, sorted as mature, immature or aborted and counted. Ten mature seeds per pod were randomly selected for drying and weighing on a Sartorius microbalance. Up to a further 25 seeds were randomly selected for germination and growth studies. Immature and aborted seeds were classified based on their colour, aborted being those structures which were opaque, yet rounded, while immature seeds were light brown-grey in colour (see Ferasol *et al.*, 1995). Seeds classified as immature based on colour were occasionally observed to have germinated.

Germination and seedling growth

On April 24-25, 1995 the seeds to be germinated were planted in peat pots filled with steam-sterilized Turkey Island sediment. A grid of 36 cells were placed in water-filled 4 L dish pans with a height of 13 cm. After initial seedlings were established, the peat pots were moved to deeper 68 L tubs. Germination, classified as the appearance of the hypocotyl, was recorded twice weekly. Seedling growth data were taken at monthly intervals. In June, the number of leaves was recorded; in July, the number of leaves and the maximum leaf length were recorded. In August, during harvest, the number of ramets, number of leaves per ramet and maximum leaf length per ramet were recorded, and the total dry mass of each seedling was measured (including any additional ramets

produced).

Statistical analysis

Kruskal-Wallis one-way ANOVA was used to test the effect of distance on measure of seed production and seedling vigour. Due to small sample sizes within individual male X female cells, parametric MANCOVA or MANOVA could not be performed to test parental genotype effect on progeny fitness . The means model (Milliken and Johnson, 1984) was therefore used to determine the effect of parental genotype. Despite similar banding patterns for some male and female clones, each parent was treated as a unique individual in the analysis. Selected measures of progeny fitness were: number of mature seeds, mean seed weight, percent germination, and seedling dry mass.

Results

No significant effects of paternal distance on seed production measures (Table 3.3.2) or seedling vigour (Table 3.3.3) were detected using Kruskal-Wallis analysis. Mean values for the measures do not show any trend associated with distance (Tables 3.3.2, 3.3.3).

Maternal identity influenced mean seed weight, proportion of seeds that germinated and dry mass (Table 3.3.4). Paternal identity alone proved non-significant, but the specific mother X father cross significantly affected seedling dry mass production (Table 3.3.4). Mean values based on maternal identity are

Table 3.3.2. Measures of seed production and results of Kruskal-Wallis one-way ANOVA for the distance of males (pollen donors) from females. Means (±SE of non-transformed data are given in parenthesis).

Distance	n	Days to mature	Pod length (cm)	No. mature seeds	No. immature seeds	No. aborted seeds	Seed weight (mg)[n]
-20 km downstream	36	63.9 (4.17)	5.58 (0.870)	80.5 (19.4)	1.94 (0.703)	7.03 (3.59)	0.488 (0.042) [18]
0 m	34	62.9 (4.38)	6.68 (0.866)	87.0 (16.4)	4.56 (1.72)	8.85 (2.81)	0.508 (0.048) [20]
50 m	20	66.8 (5.30)	7.85 (1.33)	121 (29.8)	1.60 (0.515)	11.5 (7.02)	0.503 (0.048) [13]
100 m	11	72.2 (1.08)	7.58 (1.37)	76.3 (26.4)	5.91 (3.28)	6.00 (2.70)	0.548 (0.087) [8]
150 m	11	65.0 (5.13)	7.93 (1.49)	106 (31.3)	5.18 (2.91)	9.23 (3.67)	0.454 (0.082) [7]
200 m	11	51.9 (7.23)	6.23 (1.94)	85.7 (35.6)	0.818 (0.325)	3.64 (1.30)	0.606 (0.086) [7]

Distance	n	Days to mature	Pod length (cm)	No. mature seeds	No. immature seeds	No. aborted seeds	Seed weight (mg)[n]
250 m	23	68.4	8.04	116	4.48	9.22	0.514
		(4.01)	(1.14)	(24.0)	(2.15)	(3.11)	(0.041) [16]
300 m	22	70.5	6.31	74.5	2.18	10.1	0.529
		(4.04)	(1.05)	(19.1)	(0.821)	(4.62)	(0.035) [13]
350 m	21	65.9	7.19	114	6.14	7.52	0.492
		(5.76)	(1.30)	(26.4)	(3.20)	(3.00)	(0.059) [14]
140 km upstream	37	68.1	6.07	79.1	3.65	5.44	0.491
		(3.63)	(0.894)	(17.9)	(1.32)	(1.05)	(0.049) [21]
Kruskal-Wallis		10.12	5.653	5.422	5.081	4.892	3.255
p		0.341	0.774	0.796	0.827	0.844	0.953
df		9	9	9	9	9	9

Table 3.3.3. Measures of seedling vigour per pod and results of Kruskal-Wallis one-way ANOVA of distance of males (pollen donors) from females. Means ±(SE) of non-transformed data are given in parenthesis.

Distance	n	Days to germinate	Proportion germination	Seedling dry mass (g)	No. ramets
-20 km	16	22.3 (1.09)	0.792 (0.052)	0.024 (0.002)	1.35 (0.143)
0 m	17	20.4 (2.09)	0.673 (0.076)	0.017 (0.002)	1.03 (0.147)
50 m	10	21.3 (0.734)	0.896 (0.027)	0.022 (0.003)	1.36 (0.185)
100 m	7	22.6 (1.21)	0.768 (0.062)	0.018 (0.004)	1.23 (0.236)
150 m	7	24.4 (1.58)	0.685 (0.117)	0.017 (0.002)	1.22 (0.164)
200 m	5	22.0 (1.93)	0.863 (0.037)	0.019 (0.006)	1.17 (0.305)
250 m	14	22.8 (0.938)	0.834 (0.046)	0.023 (0.002)	1.43 (0.178)
300 m	8	22.4 (1.83)	0.781 (0.072)	0.026 (0.004)	2.22 (0.996)
350 m	11	22.7 (0.875)	0.729 (0.088)	0.023 (0.003)	0.910 (0.197)
140 km	18	22.8 (1.89)	0.618 (0.080)	0.020 (0.004)	1.01 (0.188)
Kruskal-Wallis		4.138	9.272	10.93	9.725
p		0.902	0.413	0.281	0.373
df		9	9	9	9

Table 3.3.4. Results of means model for the effects of maternal and paternal identities and the specific cross performed on seed production and seedling vigour measures. F (p) df; *p<0.05, **p<0.001.

		No. mature seeds	Mean seed weight (mg)	Proportion germination	Seedling dry mass (g)
Female	F	1.22	2.83	2.59	9.78
	p	(0.282)	(0.005)*	(0.013)*	(0.000)**
	df	9	9	8[a]	8[a]
Male	F	0.504	0.749	1.23	0.731
	p	(0.911)	(0.701)	(0.272)	(0.718)
	df	12	12	12	12
Female	F	0.993	1.38	0.742	3.11
X	p	(0.518)	(0.141)	(0.848)	(0.002)*
Male	df	123[a]	101[a]	85[a]	81[a]

[a]missing cells, resulting in lower degrees of freedom

presented in Table 3.3.5. Offspring of females 20, 21, and 24 produced significantly greater dry mass than did others (Table 3.3.5). Mean seed mass for females 17 and 21 was significantly lower than for female 10 (Table 3.3.5). Percentage germination differed significantly between females 1 and 24 (Table 3.3.5). It should be noted that with the exception of mean seed mass and dry mass, and number of mature seeds and dry mass, all of the variables were significantly correlated.

Discussion

Outcrossing effects

The lack of an outcrossing effect on seed formation and seedling vigour is not surprising. Measures of genetic subdivision have already been shown to be low within and among the three sites from which the males originated (Chapter 2). The males are therefore unlikely to be genetically dissimilar enough from the experimental females for outcrossing effects to be observed. Further, inbreeding depression is also not observed with the males sampled closer to the females.

In experimental crosses, an optimal outcrossing distance between 1 - 100 m was reported for *Delphinium nelsonii* (Price and Waser, 1979; Waser and Price 1983; 1991) and *Ipomopsis aggregata* (Waser and Price, 1983), species with localized gene flow. For the rare *Gentianella germanica*, Fisher and Matthies (1997) too found an intermediate outcrossing distance (10 m) to have significantly fitter offspring than those produced by selfing and crosses between

Table 3.3.5. Mean ±(SE) values for seed and seedling variables for the different females. Values, within a column, sharing the same superscript had statistically similar means based on Bonferroni adjusted pairwise comparisons following means model tests.

Female ID No.	No. mature seeds/pod	Mean seed mass (mg)/ pod	Proportion of germinating seeds/pod	Seedling dry mass (g)
1	65.48	0.432^{ab}	0.553^{b}	0.014^{a}
	(17.06)	(0.058)	(0.089)	(0.002)
3	108.3	0.516^{ab}	0.682^{ab}	0.013^{ac}
	(28.97)	(0.058)	(0.060)	(0.002)
10	80.08	0.645^{a}	0.778^{ab}	0.013^{a}
	(17.79)	(0.051)	(0.067)	(0.002)
12	118.0	0.527^{ab}	0.676^{ab}	0.018^{ac}
	(22.97)	(0.040)	(0.055)	(0.004)
14	106.7	0.561^{ab}	0.784^{ab}	0.020^{acd}
	(20.18)	(0.042)	(0.063)	(0.002)
15	106.3	0.492^{ab}	n/a	n/a
	(34.17)	(0.057)		
17	61.03	0.418^{b}	0.833^{ab}	0.020^{acd}
	(15.75)	(0.042)	(0.061)	(0.002)
20	67.75	0.512^{ab}	0.868^{ab}	0.030^{bd}
	(19.84)	(0.056)	(0.057)	(0.002)

Female ID No.	No. mature seeds/pod	Mean seed mass (mg)/ pod	Proportion of germinating seeds/pod	Seedling dry mass (g)
21	106.8	0.387[b]	0.660[ab]	0.027[bcd]
	(24.15)	(0.045)	(0.082)	(0.002)
24	147.7	0.635[ab]	0.960[a]	0.036[b]
	(43.92)	(0.050)	(0.028)	(0.005)

mates 1 m apart and mates from two different sites. Lee and Bazzaz (1982) found no effect of inter-parent distance on the fecundity of *Cassia fasciculata*. Optimal outcrossing distances appear to be less related to pollinator behaviour than by gene flow and population differentiation (Price and Waser, 1979; Waser and Price 1983; 1991).

Many life history traits of *V. americana* are associated with high genetic variability and low genetic subdivision within and among populations. These include a predominately outcrossed breeding system, dioecy, epihydrophilous pollination, seasonal and synchronous phenology of the sexes, widespread geographic range, etc. (Loveless and Hamrick, 1984). Many of these characteristics are associated with a great potential for gene flow, via pollen, seeds or uprooted shoots. In populations of *V. americana*, however, gene flow may be restricted by the predominance of clonal growth (Lovett-Doust and LaPorte, 1991; Laushman, 1993; Lokker *et al.*, 1994); high pollen sterility (Lovett-Doust and LaPorte, 1991); and the low likelihood of reproductive success (Titus and Hoover, 1991; Sullivan and Titus, 1996) and seedling establishment (Lokker *et al.*, 1997).

Clonality in *V. americana* produces 'inbreeding-like effects' since genetically identical individuals are neighbours (see Lokker *et al.*, 1994; Chapter 2). Vegetative spread also makes inter-genet neighbour distance difficult to assess since the origin of ramets are difficult to trace, especially as connections senesce in the fall of each year, new shoots develop from turions and turions

may be dispersed some distance downstream.

Pollination in *V. americana* appears to fall into Cox and Knox's (1989) 'surface pollination syndrome', and as such pollination efficiency is enhanced by limiting it to two dimensions and by the formation of pollen 'search vehicles'. Pollen retention time in a population, contingent upon water and wind currents, and local male density also influence pollination success (Sullivan and Titus, 1996). Live *Ruppia* pollen, stained with rhodamine and injected into a pond, was found to travel rapidly (3.1 m/min), and cover large distances on the water surface (Cox and Knox, 1989). A true measure of realized gene flow within a watershed is not available for *V. americana*, and would need to include measurement of movement of pollen, seeds and vegetative shoots. There are, however, indirect measures of downstream seed and shoot dispersal. McFarland and Rogers (1998) found seeds in the sediments 250 m downstream from beds of *V. americana* in a navigation pool of the Upper Mississippi River. Horvath and Lamberti (1997) studied zebra mussel movement in association with aquatic macrophyte drift, and determined that *V. americana* shoots could drift a distance of 300 m. Movement of shoots, turions, pollen or seeds by animals and humans may contribute to upstream gene flow, although this has not been studied.

Laushman (1993) found greater partitioning of genetic variation among populations of *V. americana* sampled in Ohio rivers than was found in the Great Lakes system (Chapter 2). The greater isolation of Laushman's (1993) populations in comparison to the sites sampled here illustrates the effects of

localized gene flow and extensive clonal spread. In Chapter 2, Nei's genetic distance was significantly correlated with geographic distance between sites extending from upper Lake Huron to the St. Lawrence River. Within the Great Lakes, there is therefore some measure of population differentiation. Given the life history of *V. americana*, the geographic scale of this mating experiment may be too small to detect outcrossing effects. The sample size and the limited range of geographic distances used in the present mating experiment and the high rate of gene flow that has been measured in the Great Lakes system may explain why outcrossing effects were not detected. Pollen from more distant sources may have shown such an effect. However, this study was designed to simulate natural dispersal distances in the system; a study using males from distinct watersheds would address genetic distance, but would have more to do with historic patterns of water flow and isolation events than in assessing optimal outcrossing distance for relevant pollen sources.

Differences in offspring quality often become more apparent at later stages of development (Bertin, 1988; Byers, 1998), and may have escaped detection in this study. Significant effects of pollen donor distance from maternal plant were not detected prior to the second field season of offspring growth in *Eupatorium resinosum and E. perfoliatum*. In this second season, an increase in pollen donor distance (between populations) was correlated with an increase in the size and reproductive success of offspring (Byers, 1998). For *V. americana* in the Huron-Erie corridor, outcrossing effects in terms of any optimal

outcrossing distance appear to be negligible up to the stage of seedling establishment.

Parental genotype effects

Maternal identity proved to be a significant influence on seed formation and seedling vigour in the form of mean seed mass, proportion of seeds germinating and seedling dry mass (Table 3.3.4). The maternal differences may partially be due to genetic effects since environmental conditions (light, temperature and nutrients) were controlled and plants had been cultured under uniform greenhouse conditions for nine months prior to application of the pollination experiments. Random factors or subtle environmental differences could also contribute to the observed maternal differences.

Examples of maternal effects on fitness measures have commonly been observed (Roach and Wulff, 1987; Haig and Westoby, 1988; Richardson and Stephenson, 1991). With respect to seed size, the relative ability of the female to provision the seed has a stronger influence than does the genotype of the embryo (Roach and Wulff, 1987). The maternal plant determines the number of ovules and their longevity, supplies resources for fruit and seed production, produces the seed coat, contributes nearly all cytoplasm, two-thirds of the endosperm genome and half of the embryo genome (Richardson and Stephenson, 1991). There is also mounting evidence that in some plant species female behaviour may influence mate selection (see Lovett-Doust and Lovett-

Doust, 1988a, Chapters 1, 2, 3; Waser and Price, 1993; Stanton, 1994; Marshall, 1998; Mitchell and Marshall, 1998).

Maternal effects do diminish over time, with significant influence on seed size and early seedling development (Roach and Wulff, 1987). As the seedling grows, maternal effects are reduced as the offspring genotype effect takes hold (Roach and Wulff, 1987; Byers, 1998). The significant maternal effect on germination and seedling dry mass of *V. americana* may stem from original differences in seed size.

Bateman (1948) found that variance in reproductive success for male *Drosophila* was greater than that for females; some males proved to be highly successful, while others made no contribution to the following generation. Differences in male competitive abilities or the preference of a majority of females for particular males is the likely reason for this variation (Bateman, 1948; Cruzan, 1993). Most females, on the other hand, were assured some reproductive success. A fully factorial design, with suitable sample sizes in each cell, is a necessity for the variance component of fitness to be analyzed.

Paternal effects on seed sizes and offspring performance have also been observed for a number of species (Bertin, 1988). Mean seed weight of *Campanula americana* was affected by both maternal and paternal plant genotypes, with the paternal effect attributed to the nuclear contribution of the male to the embryo/endosperm (Richardson and Stephenson, 1991). Paternal effects on seed size, partly attributed to the ability to initiate seed formation, was

observed in *Crepis tectorum* (Andersson, 1990). Seedling growth measures were significantly different among pollen donors in *Raphanus sativa* (Marshall and Whittaker, 1989). However, Lee and Bazzaz (1982) found no paternal effect on seed maturation rate in *Cassia fasciculata*.

In the current study, the lack of paternal effects on seed formation and production may be a result of subtle differences not detected with single donors. The lack of pollen competition, since pollen from single specific donors was applied by hand, may mean subtle differences in pollination success were not exposed. Certainly, in natural populations it is possible that a number of males fertilize any single female flower. Paternal effects are often more discernible and reflective of the field situation when multiple pollen donors are compared with the effects of single pollen donors, as in the studies by Schemske and Paulter (1984) and Marshall and Ellstrand (1986).

Nevertheless, in the present study, maternal identity appears to be a more important influence on seed production and seedling vigour than does paternity, and the effects of outcrossing appear to be negligible up to the stage of seedling establishment.

3.4 Seed set and fate: Seed bank and deposition [1]

Objective

Although many aquatic plants are capable of both sexual reproduction and vegetative increase, it is generally believed that populations are maintained by the latter (Sculthorpe, 1967; Hutchinson, 1975; Les, 1988; Barrett *et al.*, 1993). However, recent studies of seed production and seed banks in aquatic plant populations suggest that seeds may be important for colonization (McMillan, 1988; Terrados, 1993), maintenance (McMillan, 1988; Smits *et al.*, 1990; Terrados, 1993), and re-establishment of populations following adverse environmental conditions (Leck and Simpson, 1993; Grillas *et al.*, 1993; Bonis and Lepart, 1994; Kimber *et al.*, 1995). Most studies of seed banks to date have concentrated on annual emergent species growing in temporary marshes, for which seed production is clearly vital to species persistence.

The importance of seed production and seed fate in highly clonal aquatic macrophyte populations has not been fully assessed. Despite the emphasis on vegetative propagation, many clonal species have been observed to flower and produce significant numbers of seeds. Studies of the seed bank in the seagrasses *Cymodocea nodosa* (Terrados, 1993) and *Halophila decipiens* (McMillan, 1988) and for *V. americana* (McFarland and Rogers, 1998) suggest

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[1] Major results from this section (3.4) have been published in American Journal of Botany (1997) 81:1420-1428.

that the seeds produced by these species play important roles in colonization at the edges of populations (as a result of sediment movement), as well as a primary role in population maintenance. Kimber *et al.* (1995) and McFarland and Rogers (1998) reported that seeds of *V. americana* may be an important source for recolonization of sites and dispersal to new sites in the Upper Mississippi River following adverse conditions such as a drought. Studies of seed dispersal and patterns of seed deposition, again mostly for wetland marsh communities, indicate the importance of hydrological regime, life history characteristics, and seed buoyancy to dispersal distances and patterns (Orth *et al.*, 1994; Rea and Ganf, 1994 b; Leck and Simpson, 1995).

Vallisneria americana is a dominant species in the Huron-Erie corridor of the Great Lakes (Schloesser and Manny, 1986; Catling *et al.*, 1994). Vegetative propagation results from shoot production along stolons during the growing season, resulting in numerous interconnected ramets. In the fall, each shoot in turn produces a number of tubers that can overwinter buried in the sediments, and germinate the following spring.

Flowering and the production of viable seed has been observed for many populations of the species (Lovett-Doust and LaPorte, 1991; Titus and Hoover, 1991; Sullivan and Titus 1996; C. Lokker, personal observations). In all cases flowering frequency, in terms of the proportion of ramets or shoots that flowered, was generally low, ranging from a minimum of 5% (Lokker *et al.*, 1994) to a maximum of 42% (Lovett-Doust and LaPorte, 1991). Seed production in *V.*

americana along the Huron-Erie corridor has been reported by Lovett-Doust and LaPorte (1991), with seed pods containing on average 167-288 seeds, with 93-98% viability (based on tetrazolium chloride tests). Seed pods are potential dispersal vehicles for the species (Korschgen and Green, 1988; Titus and Hoover, 1991).

Given the evidence that significant resources are being directed to sexual reproduction, the present study aims to quantify flower and seed production at three sites of *V. americana* in the Huron-Erie corridor of the Great Lakes, to establish whether a seed bank is present in the following spring, and to describe patterns of seed deposition. The questions addressed are: what is the extent of flowering and seed output at the three *V. americana* sites? What is the magnitude and species composition of the seed bank at these sites? How does it relate to seed production in the previous season? And what is the distribution pattern and species composition of seed deposition at these sites compared with the seed bank?

Methods

Flowering and seed production

During September 1993, the three 100 m long transects, which had been established previously at Mitchell's Bay and Turkey and Stoney Islands, were sampled (see Chapter 3.2 *study sites* for descriptions) to assess flowering and sexual reproduction. Quarter-metre quadrats (0.25 m^2)were excavated by hand

at five random distances along each transect (see Chapter 3.2 *sampling procedures*). All ramets within these quadrats were sampled and taken to the laboratory where they were inspected and the flowering status of each ramet was assessed. Seed pods were collected and placed in jars filled with water in a cold room (5° C) for 3 months. The seeds were then assessed as either mature (firm, brown) or immature (white but filled; see Ferasol *et al.*, 1995).

Seed bank

At each site, from 1-10 June 1994, 1-m^2 quadrats were placed at 25 m intervals along the established 100 m transects. These were placed laterally, approximately 1 m from the transects where plants had been removed late in the previous summer. There were five quadrats along each of three transects per site, for a total N=45. Each quadrat was subdivided into 16 subquadrats, each 0.0625 m^2 . Five random subquadrats served as sources for replicate seed bank samples.

Sediment samples were collected during the first week of June 1994, using a post hole digger (diameter=12.5 cm; depth=25 cm). One or two "post hole" cores were taken for each sample, depending on the type of substrate, e.g., samples at Stoney Is. included many rocks, and sediment tended to flow out of the sampler, so more than one core was taken. Care was taken to ensure that all of the sediment from the samples was placed in plastic bags. As a result, the values for seed densities presented are minimum estimates. The volume of

each sediment sample was measured by volume displacement and the sediment was then placed in 4 L dishpans, covered with water, and left to settle. Clear plastic sheets covered the dishpans to diffuse light and reduce evaporation. Sediment depth ranged from < 1 cm to ≈ 4 cm. Samples were kept at room temperature with constant light for the first 8 weeks, and then were moved to a greenhouse where they experienced natural light conditions. Each pan was monitored for seedlings monthly for eight months, by which time (January 1995) germination had become negligible. Following each census, all identified seedlings were removed and the sediment was stirred to allow any of the remaining seeds to move to the top of the sediment layer. In January 1995, three of the five replicates were removed, and the other two were maintained and monitored until September 1995.

Germination of seeds, rather than actual seed counts, was monitored in the present study as this method more effectively detects the presence of the small seeds and oospores characteristic of the species encountered in the freshwater aquatic environment. This method also emphasizes the germinable portion of the seeds present in sediment (Gross, 1990).

Seed deposition

At the time of seed bank sample collection in the spring, exclosure mats (25 cm X 25 cm) were placed into three randomly chosen subquadrats within each quadrat, giving a total of 15 per transect, 45 per site. These mats consisted

of one layer of weed-control cloth covered on the top and bottom with a layer of housewrap (Typar™, Old Hickory, TN). Holes were cut into the four corners, through which 10" or 12" spikes were used to anchor the mats flush with the sediment surface. These mats were designed to retard plant growth, to maintain integrity during 5 months of submergence, and to provide easily retrievable landing pads for any sediment and seeds deposited (see Leck and Simpson, 1993). The mats were retrieved in late October 1994.

The accumulated material underwent cold treatment in a 5°C cold room until March 1995, at which time it was placed under water in dish pans in the greenhouse, similar to the seed bank sediment samples. Germination was recorded monthly until October 1995. Sediment volumes were determined after the germination observations were completed.

Seedling identification

Germinated seedlings were left to grow until reliable taxonomic identifications could be made. Seedlings of all species were identified according to Schloesser (1986), Hotchkiss (1972), Muenscher (1944), and Fassett (1940), except for *Potamogeton* spp., which were identified only to genus due to difficulty in distinguishing species at such an early stage. *Chara* spp. and *Nitella* spp. were grouped in the family Characeae.

Statistical analysis

Plant production in the previous fall was based on totals per quadrat (N=15) and was previously presented in Chapter 3.2, Tables 3.2.8 and 3.2.9. For the seed bank and seed deposition data, samples were standardized to seed densities per square metre (converted from a 0.0625-m^2 sampling area). The means of subquadrat samples were used as the per quadrat measure. Bonis and Lepart (1994) reported that, for wetland communities, the majority of seeds were within the top 2 cm of sediment, and were rarely below 4 cm. Also, Kimber *et al.*, (1995) collected only the top 5 cm of sediment cores in their study of the seed bank in *V. americana*. Given that all of the present samples sampled at least the top 10 cm of sediment, and that regression analysis of total seed number in the seed bank samples vs. sediment volume was nonsignificant (R^2=0.010, F=2.340, p=0.128, df=1, 223), measures of seed density are given as they are considered more meaningful.

Due to violations of normality that could not be corrected by transformation, the data were analysed using Kruskal-Wallis one-way analysis of variance (ANOVA; Zar, 1984) to test for differences among sites in measures of flowering frequency, seed production, seed bank size and seed deposition. A posteriori multiple comparisons of significant Kruskal- Wallis tests were performed using a nonparametric Tukey-like test (Zar, 1984). Means and standard errors given here are based on non-transformed data. (See Chapter 3.2, *data analysis*).

In order to derive any association between seed production and various flowering measures, stepdown multiple regression was carried out, with site initially included as an independent factor (N=45). To determine relationships between (a) flowering frequency and the sex ratio, and (b) the seed bank and seed production in the previous year, regression analysis was employed. Analysis of covariance (ANCOVA) was first performed to test for homogeneity of slopes between sites (Zar, 1984). Regression analysis was performed for individual sites when site interacted with the main factors (N=15). In the absence of significant interactions among factors of interest and site, regression analysis was performed on pooled site measures (N=45). All parametric analyses were carried out using both increasing rank-transformed data and raw data. The results were interpreted in light of both data sets, following Conover (1980). Non-transformed data and results are presented here since nonparametric and parametric analysis produced the same general outcomes.

Results

Flowering and seed production

(results previously presented in Chapter 3.2)

Totals of 536, 740, and 934 ramets were excavated from the fifteen 0.25-m^2 quadrats at Mitchell's Bay, Stoney Is. and Turkey Is., respectively. Flowering frequency was higher at Mitchell's Bay (0.603) than either Turkey Is. (0.497) or Stoney Is. (0.275), and was represented by a sex ratio near 1 (1.03). Stoney Is.

was significantly female biased with a sex ratio of 1.21, while Turkey Is. was significantly male biased with a sex ratio of 0.813.

The sites did not differ significantly in number of seed pods, mature or immature seeds produced per 0.25 m^2. Density and fresh mass of *V. americana* also did not differ significantly among sites (see Table 3.2.9)

Seed bank and deposition

Species germinating in the seed bank samples from the three sites included *V. americana* Michx., *Heteranthera dubia* (Jacq.) MacM., *Lythrum salicaria* L., *Potamogeton* spp., Characeae, *Zannichellia palustris* L., *Eriocaulon septangulare* With., *Isoetes* spp. L., and *Najas flexilis* (Willd.) Rostk. and Schmidt. All nine taxa were present in the seed bank samples at each of the sites. Seed deposition samples also included *Myriophyllum spicatum* L. and *Elodea canadensis* Michx.

For most species observed, seed density measures were much greater in the deposition samples than in the seed bank samples (Table 3.4.1). Densities of *E. septangulare* and *Isoetes* spp. at Stoney Is. were exceptions, being greater in the seed bank than in deposition samples. Marked increases between seed banks and depositions occurred for *Isoetes* spp. at Turkey Is., *H. dubia* at Stoney Is., and *V. americana* at Mitchell's Bay. The number of turions at Turkey Island was 2-fold that of Mitchell's Bay, and almost 3-fold that of Stoney Island (Table 3.4.1).

Table 3.4.1. Seed densities/m^2 ± 1SE for species observed in the germinable seed bank, and seed deposition at three sites in the Huron-Erie corridor. Densities of turions of *Vallisneria americana* in the sediment are also given. Values are based upon means of 15 quadrats per site, within which five 0.0625m^2 areas were sampled for the seed bank and three 0.0625 m^2 areas were sampled for patterns of seed deposition.

Species	Seed Bank			Seed Deposition		
	Mitchell's	Stoney	Turkey	Mitchell's	Stoney	Turkey
Vallisneria americana seeds	113±26.4	52.3±21.2	31.4±12.1	878±268	373±197	73.6±22.9
Vallisneria americana turions	6.60±2.08	4.30±1.94	12.2±2.93	---	---	---
Characeae	101±15.9	134±43.7	365±82.1	159±24.4	193±55.4	308±42.8
Heteranthera dubia	22.8±3.24	10.9±3.95	2.30±1.48	73.6±13.3	160±64.3	33.1±13.3
Lythrum salicaria	1.30±0.680	17.5±8.05	40.5±14.1	10.0±3.45	21.3±6.65	169±47.1
Najas flexilis	20.5±4.29	24.3±10.4	31.4±10.4	47.6±12.5	6.40±2.71	72.2±29.9
Eriocaulon septangulare	10.2±2.12	374±107.2	18.1±5.27	16.7±3.12	28.4±6.94	4.50±1.60
Isoetes spp.	6.00±1.36	427±113	19.0±5.55	20.6±3.37	252±55.4	156±26.1
Potamogeton spp.	22.6±4.86	20.5±7.78	3.2±10.4	41.2±10.7	48.0±22.9	7.80±2.80
Zannichellia palustris	35.2±8.16	3.20±1.29	19.2±5.80	43.4±11.8	12.4±7.28	3.60±1.34
Myriophyllum spicatum	---	---	---	---	---	1.40±1.10
Elodea canadensis	---	---	---	1.10±0.770	7.10±4.62	3.60±2.19
All species totals	333 ±47.5	1070 ±253	530 ±102	1290 ±309	1100 ±224	833 ±84.6

The Shannon-Weaver Index calculated for each site (Table 3.4.2) indicated that at Mitchell's Bay there was slightly greater diversity in terms of species in the seed bank than at Stoney Is., which was followed by Turkey Is. Since each site had the same nine species in the seed bank, the differences in species diversity were attributed to the proportions in which species were present, the equatability measure. The trend showed Turkey Is. as the least equitable compared to Stoney Is. or Mitchell's Bay (Table 3.4.2). Two taxa were dominant at Mitchell's Bay, *V. americana* (33.4%) and Characeae (29.7%). At Stoney Is., *Isoetes* spp. (40.0%) and *Eriocaulon septangulare* (35.0%) dominated the seed bank. Characeae dominated at Turkey Is. (67.3%), producing its low equatability value (Figure 3.4.1 a-c).

For seed deposition, *V. americana* (68.0%) and Characeae (12.3%) again dominated at Mitchell's Bay. At Stoney Is., *Isoetes* spp.(22.9%) were still a dominant group, and *V. americana* and *H. dubia* increased greatly (Figure 3.4.2 d-e). Deposition at Turkey Is. was more equitable than was the pattern in the seed bank, with Characeae (37.0%) and *L. salicaria* (20.2%) dominating (Figure 3.4.2 f). Percentage similarity between seed bank and seed deposition samples was 48.4% at Stoney Is., 65.6% at Turkey Is., and 62.1% at Mitchell's Bay.

With respect to *V. americana*, seed densities differed between sites for both seed bank ($H=10.296$, $p<0.01$) and seed deposition ($H=7.919$, $p<0.01$). Mitchell's Bay sediment contained significantly more germinable seeds than either Stoney Is. or Turkey Is. for both measures (seed bank: $q_{m-s}=3.843$, $p<0.05$;

Table 3.4.2. Indices of species diversity and evenness for three sites based on the number of species and abundance of seedlings germinating from sediment samples for seed bank and seed deposition.

Site	Shannon-Weaver index (H')	No. of species (S)	H'_{max} ($\log_e S$)	Equatability $J'=H'/H'_{max}$
Seed bank				
Mitchell's Bay	1.705	9	2.197	0.776
Stoney Island	1.437	9	2.197	0.654
Turkey Island	1.197	9	2.197	0.545
Seed deposition				
Mitchell's Bay	1.195	10	2.303	0.519
Stoney Island	1.710	10	2.303	0.743
Turkey Island	1.691	11	2.398	0.705

Figure 3.4.1. Species composition of the germinable seed bank and seed deposition at three sites. Seed bank: (a) Mitchell's Bay; (b) Stoney Is.; and (c) Turkey Is. Seed deposition: (d) Mitchell's Bay; (e) Stoney Is.; and (f) Turkey Is. Species represented are: *Heteranthera dubia* , *Lythrum salicaria*., *Potamogeton* spp., Characeae, *Zannichellia palustris*, *Eriocaulon septangulare*, *Isoetes* spp., *Najas flexilis*, *Myriophyllum spicatum* and *Elodea canadensis* .

Figure 3.4.1.

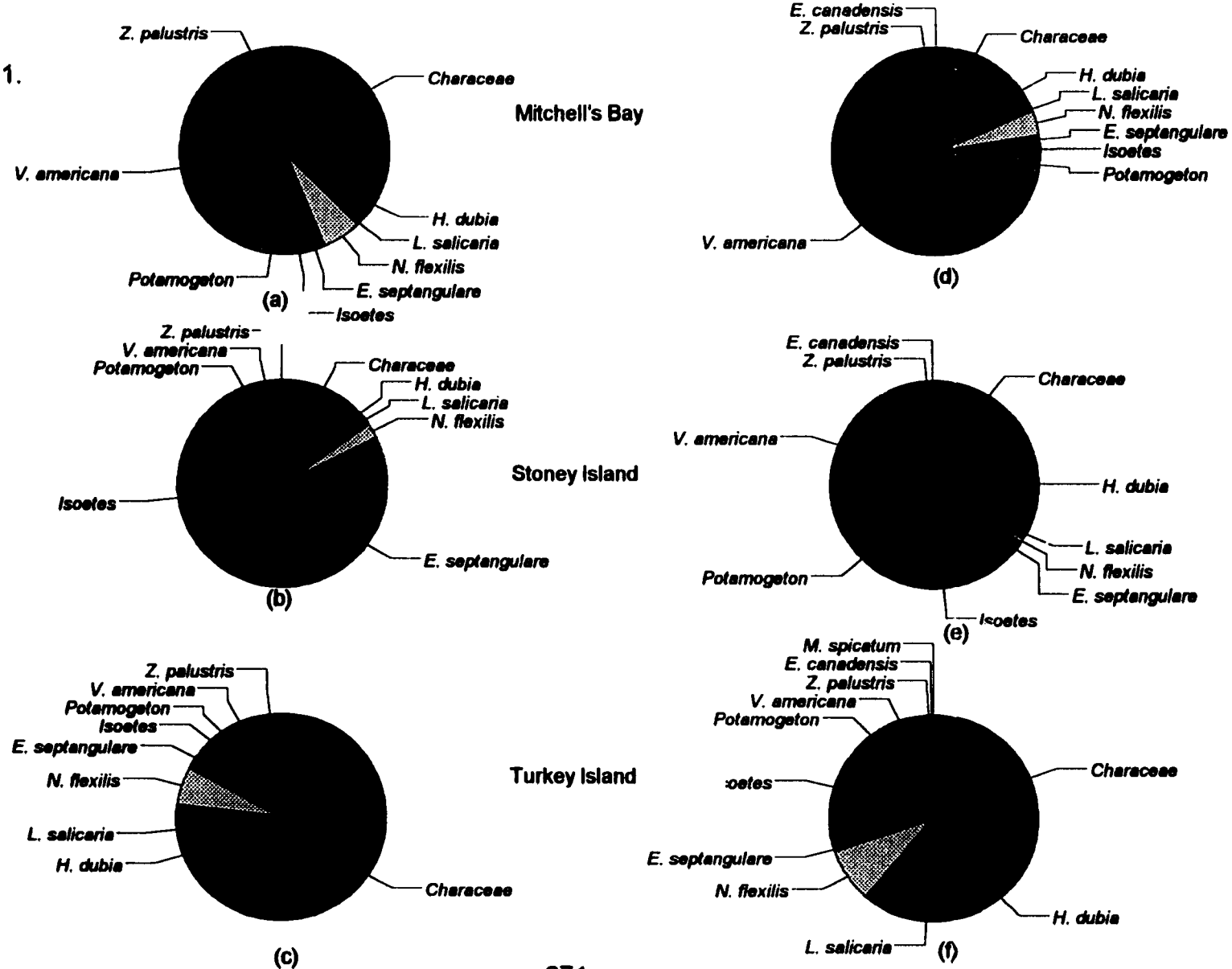
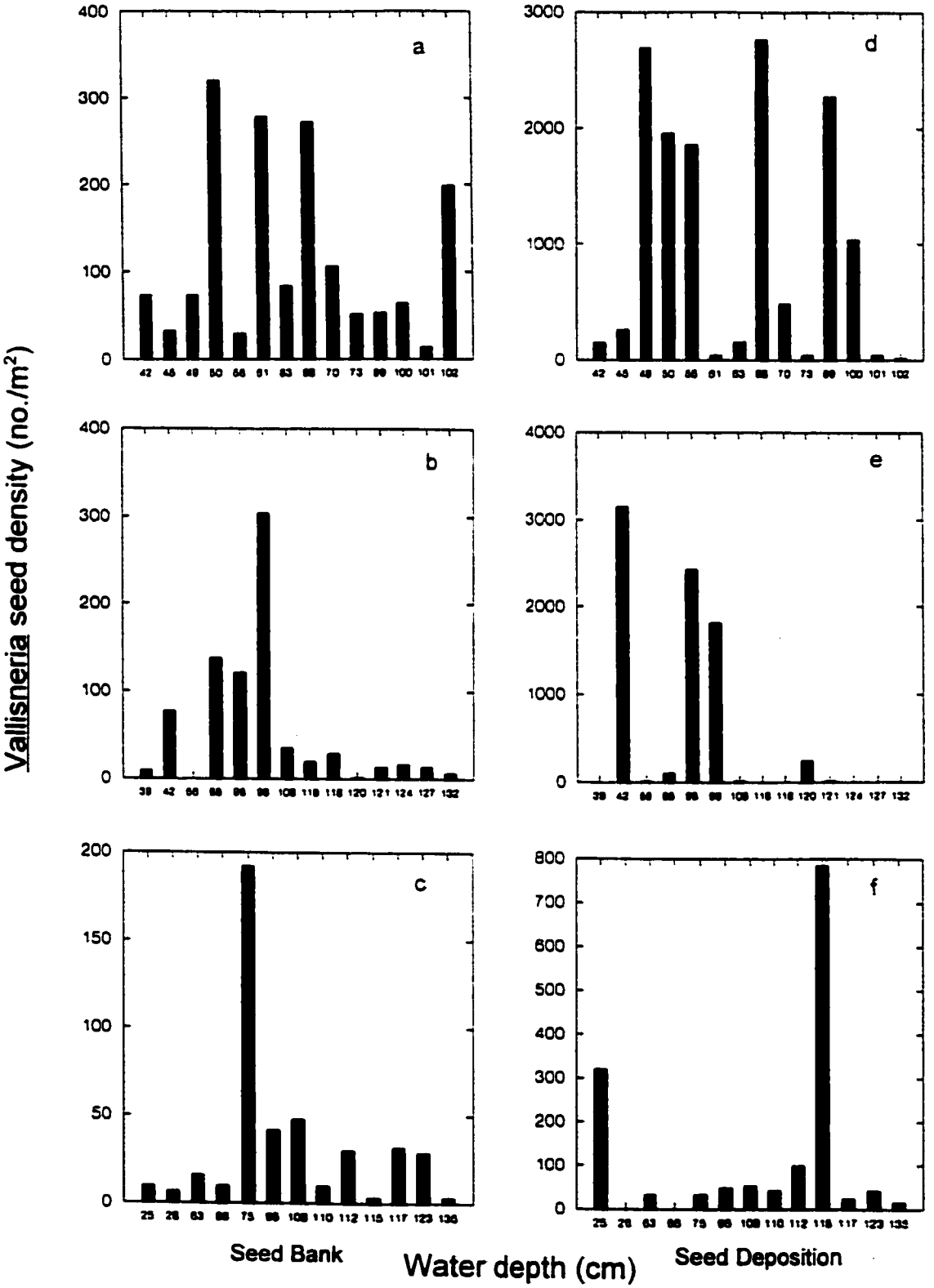

Mitchell's Bay
Z. palustris
Characeae
V. americana
H. dubia
L. salicaria
N. flexilis
Potamogeton
E. septangulare
Isoetes
(a)
E. canadensis
Z. palustris
Characeae
H. dubia
L. salicaria
N. flexilis
E. septangulare
Isoetes
Potamogeton
V. americana
(d)
Stoney Island
Z. palustris
V. americana
Potamogeton
Characeae
H. dubia
L. salicaria
N. flexilis
Isoetes
E. septangulare
(b)
E. canadensis
Z. palustris
Characeae
V. americana
H. dubia
L. salicaria
N. flexilis
E. septangulare
Potamogeton
Isoetes
(e)
Turkey Island
Z. palustris
V. americana
Potamogeton
Isoetes
E. septangulare
N. flexilis
L. salicaria
H. dubia
Characeae
(c)
M. spicatum
E. canadensis
Z. palustris
V. americana
Potamogeton
Characeae
E. septangulare
N. flexilis
H. dubia
L. salicaria
(f)

Figure 3.4.2. Distribution of *V. americana* by water depth for seed bank and seed deposition samples from three sites. Seed bank: (a) Mitchell's Bay; (b) Stoney Is.; and (c) Turkey Is. Seed deposition: (d) Mitchell's Bay; (e) Stoney Is.; and (f) Turkey Is.

Figure 3.4.2.


Vallisneria seed density (no./m²)
a
b
c
d
e
f
Seed Bank
Water depth (cm)
Seed Deposition

q_{m-t}=4.374, p<0.05; seed deposition: q_{m-s}=4.236, p<0.05; q_{m-t}=3.489, p<0.05). The volume of material deposited differed significantly among sites (H=47.384, p<0.001; q_{m-s}=9.705, p<0.05; q_{m-t}=4.255, p<0.05; q_{s-t}=5.450, p<0.05), with Mitchell's Bay (9.705 ± 0.071 L) having greater deposition than Turkey Is. (0.956 ± 0.075 L), which was greater than Stoney Is. (0.543 ± 0.045 L).

Seeds of *V. americana* were present in both the seed bank and seed deposition samples from all depths at Mitchell's Bay and Turkey Is., but were missing from some deposition samples from Stoney Is. Comparisons of standard-error-to-mean ratios for the seed density values indicated that Stoney Is. was more patchy in its distribution of *V. americana* seeds, for both the seed bank and deposition, and it also experienced greater variation in seed pod formation and seed production (Chapter 3.2, Table 3.2.9). There was up to a tenfold increase in seed number in the deposition samples compared to the seed bank samples collected from the same sites (Figure 3.4.2), although deposition values represent direct seed input for the 1994 season, whereas seed bank values represent accumulated seed deposition and seed survival prior to 1994.

Seed densities in the seed bank were not significantly related to seed output in the previous year, based on regression analysis of seed bank densities and pod production (F=0.739, df=1,43, p=0.395, N=45).

The size of the seed bank was not correlated with seed production, measured in terms of pods (F=0.154, P=0.697) or the number of mature seeds produced (F=0.263, P=0.611) in the previous season. Nor was it correlated with

ramet density in the previous season (F=0.503, P=0.482). An ANCOVA was performed to test for a correlation between the seed bank and the (ranked) proportion of ramets flowering. The initial test for an interaction between site and flowering frequency was non-significant (F=0.691, P>0.05), verifying the homogeneity of the slopes for the relationship between seed bank and flowering frequency at each site. Both site (F= 5.338, P<0.01) and flowering frequency (F=5.943, P<0.05) were significant factors in a multiple regression analysis. The size of the seed bank was greater where a greater proportion of the ramets flowered in the previous season, and although this relationship was the same at each site (equal slopes), there were differences among sites in their y-intercepts. These differences were tested using a SAS General Linear Model (SAS, 1986), with Turkey Is. as the control site. This analysis indicated that the intercepts for Turkey Is. and Stoney Is. were not significantly different (P=0.0831), but the intercepts for Turkey Is. and Mitchell's Bay were different (P<0.05).

Discussion

Flowering and seed production in V. americana

Proportions of flowering ramets were quite high at the three sites, compared to previous reports, ranging from 28 to 60%, with sites varying in their tertiary sex ratios. These values represent ramet sex ratios, and may underestimate genet flowering frequencies, as ramets determined to be vegetative may have belonged to a flowering genet. The issue of physiological

independence and genetic identity is a difficult one to address in a clonal macrophyte such as *V. americana*.

Biased sex ratios are common in natural populations of aquatic plants, and greater female bias tends to be associated with an increase in the proportion of ramets flowering (Les, 1988). For *V. americana* this trend was reported by Lovett-Doust and LaPorte (1991) for sites along the Huron-Erie corridor, including Turkey Is. In the present study, Stoney Is. followed the expected pattern, but Turkey Is. and Mitchell's Bay showed no significant relationship (see Chapter 3.2, Fig. 3.2.5). At Turkey Is. this may be greatly influenced by the fact that males predominate and females were present in very low frequency in shallow water. In the present study sampling was carried out in water < 2 m deep. In contrast, flowering females were predominant in water > 2 m deep at the Turkey Is. site (Lokker *et al.*, 1994). At Stoney Is., the low frequency of flowering (27%, see Chapter 3.2, Table 3.2.8) may have skewed the sex ratios disproportionately, producing the strong significant relationship between flowering frequency and sex ratio. Overall, however, the variation among sites suggests that there is no consistent relationship between flowering frequency and sex ratio.

Pollen transport and pollination success in *V. americana* depends on such factors as population density, sex ratio, flowering synchrony, wind, and water currents (Titus and Hoover, 1991; Sullivan and Titus, 1996). The production of seeds of *V. americana* in the present study was consistently

related to both sex ratio and ramet density at each of the three sites, with an increase in seed production corresponding to increases in ramet densities and increasing female bias among flowering ramets.

Aggregates of floating male flowers in *V. americana* have been described as "search vehicles" (Titus and Hoover, 1991), which significantly increase pollination efficiency (Cox and Knox, 1989). This delivery mechanism should be particularly useful in *V. americana* given the finding of Lovett-Doust and LaPorte (1991) that about half of the male flowers they examined were sterile. Dispersal of clusters of male flowers would therefore also increase the likelihood of a fertile male flower encountering a fertile female flower.

In the present study, as in that of Lovett-Doust and LaPorte (1991), we conclude that pollen limitation was not likely to be an important factor regulating seed production. Lovett-Doust and LaPorte (1991) observed high seed production and viability, despite frequent pollen infertility. In the present study, seed production did not increase with increasing male bias (as might be expected under pollen limitation). In contrast, we note that seed production increased with increasing female bias (see Chapter 3.2, Tables 3.2.8 and 3.2.9). Floral induction in female ramets seems more likely to be a significant factor limiting seed production in *V. americana*.

Seed bank and deposition

The existence of a seed bank suggests that successful seed production, dispersal, and overwintering occurred at each site, while the lack of association between seed production and the seed bank, and the marked differences in seed bank and seed deposition densities, although based on seed production in different years, indicate that the stages between seed production and "potential" seed germination are dynamic.

These differences between seed bank and seed deposition densities point out the vulnerable nature of the seed stage in the plant life cycle (Harper, 1977; Fenner, 1985). It is likely that many seeds die or are eaten (Fenner, 1985; Korschgen and Green, 1988). Alternatively, they may be further dispersed by water and sediment movement. Studies of seed deposition (involving, for example, leaving a subset of the exclosure mats over the winter; or covering some with predator exclosures), would give a better comparison between input within a season and the proportion of seeds surviving or being dispersed through the winter.

It is possible for entire seed pods to be transported when a parent ramet detaches from sediment and drifts downstream. Alternatively, movement by waterfowl may occur (Korschgen and Green, 1988), or following seed pod rupture, the gelatinous matrix within which seeds are contained may drop to the sediment (negatively buoyant; Kaul, 1978). More frequently seed pods are observed to detach from the parent plant after decay of the peduncle (C. Lokker,

personal observation). These positively buoyant pods may then drift downstream. Light, wind carried diaspores are more likely to be dispersed over long distances (Cook, 1985). Water dispersed diaspores rely on water currents as well as wind, the latter likely having a more significant role in dispersal over large water bodies (Cook, 1987 a). McFarland and Rogers (1998) found *V. americana* seeds in to be most prevalent in sediment samples within *V. americana* beds and up to 250 m downstream.

Dispersal may also be extended over time, as opposed to space. Measures of germination from the seed bank in this study may include seeds produced over more than one year. As yet, however, the longevity of seeds of *V. americana* has not been determined. Although no germination studies have indicated dormancy in seeds of *V. americana*, the present study recorded germination in sediment, rather than in sterile petri dishes. In the natural sediments used here, germination occurred over a 15-month period, and it was necessary to disturb the sediment regularly, to distribute ungerminated seeds closer to the sediment surface. In contrast, studies of germination in *V. americana* under controlled conditions in petri plates were completed after 30 d (Ferasol *et al.*, 1995). Germination studies in experimental ponds by Kimber *et al.*, (1995) indicate that light is not a requirement for germination, but is an important factor in seedling survival. Further multi-factorial, controlled, *in situ* germination experiments would greatly enhance our understanding of the natural germination and seedling survival requirements of this species (Titus and

Hoover, 1991).

The relative decrease in seed densities of some species between the seed deposition and seed bank stages represented by our field samples, was likely due to lack of establishment of seed-bank-derived seedlings during the growing season. For instance, *E. septangulare* and *Isoetes* spp., although present in great numbers in the Stoney Is. seed bank, were rarely observed growing in that area between 1992 and 1995 (C. Lokker, personal observation). The initial seed bank of these species may have been the result of dispersal into the site from upstream. Similarly, for Isoetes spp. at Turkey Is., large densities are observed in the deposition samples, but the species was present in low densities in the seed bank for that season, and plants have rarely been observed *in situ* (C. Lokker, personal observation).

The potential role of seeds

Although few established seedlings have been found in the field for *V. americana*, short-lived flushes of seedlings have been observed on sand banks around Turkey Is. (C. Lokker, personal observation). Seedling establishment events within established adult populations occur at low frequency but are not uncommon in terrestrial clonal species, and have been reported in 40% of 68 species (Eriksson, 1989). They may be particularly important when populations first become established (Lovett-Doust, 1981). A small-scale experiment where 24 *V. americana* seedlings were planted in Ostego Lake, New York resulted in

67% of the seedlings producing an average of 6.3 rosettes and 1.9 g dry mass within 4 month, supporting the contention that seedling establishment can occur (Titus and Hoover, 1991). Kimber *et al.*, (1995) report the presence of a seed bank, and the production of tubers by seedlings after one season's growth, in addition to observations of seedling establishment in areas previously lacking *V. americana*. They suggest that recolonization by this species (and other clonal aquatics), may be more common than previously thought. The findings of *V. americana* seeds 250 m downstream from established beds suggest that seeds may be important in dispersal to new sites (McFarland and Rogers, 1998). The failure to observe establishment of *V. americana* seedlings in the field may be a consequence of their high mortality and the challenge of demographic studies during the early summer when the water is still cold and quite turbid in the Huron-Erie corridor.

Cleverling (1995) described sexual reproduction as a means of long-distance dispersal and a mechanism to allow dormancy in emergent aquatic macrophytes (although tubers in *V. americana* are also winter dormant). For *V. americana*, seed production may serve as a mechanism for dispersal, with seeds being transported to colonize new suitable sites (Kimber *et al.*, 1995), or it may serve to establish new genotypes within the source population. A true measure of sexual reproductive success in the Huron-Erie corridor, however, cannot be made without further studies on the probability of seedling establishment within a population, and the subsequent production of successfully overwintering tubers.

We have, however, shown that a seed bank does exist within meadows of *V. americana*, and that seed production and deposition are significant processes occurring at *V. americana* sites in the Huron-Erie corridor.

Chapter 4

General Discussion:

Evolutionary Processes in Populations of *Vallisneria americana*

Evolutionary processes in aquatic plants

Ayala (1982) describes evolution at the genetic level as a simplified two-step process of the generation of genetic variation (mutation and recombination), followed by the differential transmitting of genetic variants from generation to generation (genetic drift, natural selection, gene flow). Assessing the reproductive system of a species allows for the biological potential for genetic variability, gene flow and population differentiation detected in genetic studies to be evaluated (Barrett *et al.*, 1993). An understanding of evolutionary processes in aquatic macrophytes therefore requires information on reproductive system (i.e., the potential for the generation of genetic variability) in concert with population genetic parameters over time and space.

Studies on evolutionary processes in aquatic macrophytes are becoming more common (Les, 1988; Barrett and Husband, 1990; Waycott and Sampson, 1997; Procaccini and Mazzella, 1998; Waycott, 1998; Pellegrin and Hauber, 1999); slowly our understanding of these processes is expanding. Recent studies, including those contained in Chapters 2 and 3, pertain to population

genetic structure, clonal diversity, colonization, sexual reproduction and dispersal and gene flow in aquatic macrophytes (Les, 1988; Harrison and Durance, 1991; Eckert and Barrett, 1993; Laushman, 1993; Lokker *et al.*, 1994; Procaccini and Mazzella, 1998; Waycott, 1998; Pellegrin and Hauber, 1999). These studies indicate that populations are often clonally diverse. Other measures of evolutionary significance, such as effective population size (N_e), outcrossing and mating system parameters (Barrett *et al.*, 1993; Waycott and Sampson, 1997) and patterns of genetic differentiation (Barrett *et al.*, 1993) have seldom been estimated, as they require extensive studies which are often difficult to undertake. Estimates of the aforementioned measures would allow for more precise models of evolutionary processes in clonal macrophytes to be developed. Some of these estimates require tracking temporal genetic changes in populations, measures of sex ratio, quantifying mating systems and determining variation in reproductive output among individuals (Barrett *et al.*, 1993). A number of biological factors such as the prevalence of clonal propagation, the low reporting of establishment of plants from seeds, and the vagaries of the aquatic habitat makes the study of the population biology and genetic diversity of these plants an interesting, but often difficult, pursuit.

The potential for genetic recombination

Vallisneria americana shares a number of the biological traits described by Barrett *et al.* (1993) as characteristic of aquatics plants. These include high

phenotypic plasticity, which can buffer genotypes against environmental heterogeneity and thereby reduce selection intensities; prolific clonal growth which can lead to genetically uniform populations and reduces the mortality risk of certain genotypes; potentially limited sexual reproduction which leads to reduced genetic diversity; a pollination mechanism similar to hydrophily whereby gene flow is limited to water body boundaries; and water-dispersed diaspores, which results in local and long-distance dispersal (Barrett *et al.*, 1993).

Reproductive systems are complex and vary greatly among aquatic plant species (Sculthorpe, 1967; Hutchinson, 1975; Barrett *et al.*, 1993), often comprising both sexual, asexual and vegetative modes of reproduction (Briggs and Walters, 1984). These modes can take a number of forms including dicliny, self-incompatibility, autogamy and agamospermy, often in combination with clonal propagation via rhizomes, runners, stolons, turions or tubers (Barrett *et al.*, 1993), and various levels of outcrossing. The reproductive system that a species has and the balance between sexual and vegetative reproduction are known to be significant determinants of population genetic structure (Loveless and Hamrick, 1984; Hamrick and Godt, 1989).

Each mode of reproduction has its associated costs, benefits and consequences. Sexual reproduction requires more specialized structures, and is generally more costly in terms of resource utilization than is vegetative reproduction (Harper, 1977). The success of sexual reproduction is contingent upon a number of environmental and biological factors being present; flowering

cues, pollinators and acceptable mates, as well as sufficient resources (Harper, 1977). However, successful sexual reproduction has the benefit of producing genetic variation through recombination, which is important for coping with environmental fluctuations and other selection pressures. Vegetative propagules, especially overwintering structures, have the benefit of greater initial growth than do seeds, allowing them to establish more quickly in the face of inter- and intra-specific competition (Madsen, 1991). Further, through vegetative increase, well adapted genotypes proliferate and become established, especially in homogenous or stable environments. However, vegetative propagation has the effect of reducing genetic variability and increasing the likelihood of inbreeding in a population. Alternately, sexual reproduction increases genetic variability, and contributes to the potential for gene flow and migration among populations. Most aquatic species have mixed mating systems, undergoing reproduction through both sexual and vegetative means (Sculthorpe, 1967; Hutchinson, 1975; Barrett *et al.*, 1993). The relative proportions of these modes of reproduction are likely to vary from season-to-season, even within a population, as a result of environmental changes (Briggs and Walters, 1984). The relative levels of these modes of reproduction also influence population genetic structure (Holderegger *et al.*, 1998).

Clonal propagation can result in a population being spatially or temporally structured, rather than a random assemblage of genetic individuals (Harper, 1977). A departure from random mating due to clumping of clones may occur,

and could result in reduced genetic variability due to higher levels of inbreeding (Levin, 1988). However, a review of clonal terrestrial plant species by Ellstrand and Roose (1987) suggests that genetic uniformity and extreme substructuring are not necessarily characteristic of all clonal plant species.

Arber (1920), Sculthorpe (1967) and Les (1988) all belabour the suggestion that the propensity for clonal growth in aquatic macrophytes acts as a deterrent to sexual reproduction. Yet, this widely-held view has not been adequately tested by these authors. Certainly the trend of limited successful seed production in clonal species exists (Sculthorpe, 1967; Hutchinson, 1975; Bartley and Spence, 1987; Les, 1988). However, many authors have reported successful sexual reproduction in highly clonal aquatic species (McMillan, 1988; Harrison and Durance, 1991; Terrados, 1993; Laushman, 1993), most notably for *V. americana* (Titus and Hoover, 1991; Laushman, 1993; Lokker *et al.*, 1994, Sullivan and Titus, 1996; Lokker *et al.*, 1997). A more important question to address may be the relative success of sexual reproduction and the conditions under which it is favoured.

Rea and Ganf (1994 b) suggest that sexual reproduction in clonal aquatic macrophytes is likely considered to be rare due to the slim possibilities of seed presence coinciding with suitable germination or establishment conditions. However, seedlings are often small and overlooked and establishment events are irregular and patchy so investigators may simply have missed germination flushes in nature. The constraints for seedling establishment in *V. americana*

could well be competition from larger ramets that are sprouting from turions, and the small size of turions produced by new genets in their first year (ramets originating from seeds, C. Lokker, personal observation; Titus and Hoover, 1991). Other factors which often influence the success of seedling establishment in many plant species, such as interspecific competition, the presence of suitable substrate, water and light regimes (Fenner, 1985), may also act to limit seedling establishment in *V. americana.*

Although detailed observations of germination and establishment have not been made as a part of the present study of *V. americana,* field observations of a number of shoots with seed coats attached to the roots have been made (C. Lokker, personal observation), and are clear indications that germination requirements are being met. Furthermore, sites were multiclonal (see Chapter 2), which is suggestive of successful germination and establishment events in the past.

Rea and Ganf (1994 b) studied flowering in the highly clonal emergent *Triglochin procerum* and found seed production and seedling establishment to be associated with water regime and elevation gradient. The authors suggested that sexual reproduction may be adaptive and selected for under unfavourable conditions. For *V. americana*, Kimber *et al.* (1995) similarly found seed banks to play a vital role in colonization after drought in Chesapeake Bay. In the Huron-Erie corridor, it is possible that sexual reproduction may play a similar role given records of population declines due to pollution and turbidity in parts of the Great

Lakes where *V. americana* is currently a dominant species (Schloesser and Manny, 1990; Stuckey and Moore, 1995). The rebounding of these populations must partly be a result of successful sexual reproduction as these populations show high levels of genetic and clonal diversity (see Chapter 2).

Role of sexual reproduction in aquatic macrophytes

Sexually produced progeny may play important roles in colonization and contribute to the genetic variation observed in the species, and the seed bank may reflect the cumulative reproduction of more than the preceding year. Based on these findings, it is now important to examine the influences on subsequent stages of recruitment, and their resulting effects on the population dynamics and structure of populations of *V. americana*. Different sites experience different environmental conditions, and these in turn likely influence local flowering, fruit and seed production and the fate of sexually produced progeny.

Bartley and Spence (1987), in assessing dormancy and propagation in hydrophytes, concluded that turions provide dispersal and establishment near the parent plant. Cleverling (1995) sees sexual reproduction as a means of long-distance dispersal and a mechanism providing dormancy for emergent aquatic macrophytes (although turions also allow winter dormancy in *V. americana*). For *V. americana*, sexual reproduction allows for dispersal, with seeds being transported to colonize new suitable sites (Kimber *et al.*, 1995; McFarland and Rogers, 1998), and it establishes under appropriate conditions new genotypes in

an existing population. Sexual reproduction may also contribute genetic variation to downstream sites though the dispersal of both pollen and seeds.

Genetic diversity in other clonal species

It is widely maintained that highly clonal species will have genetically depauperate populations (Hutchinson, 1975; Briggs and Walters, 1984; Les, 1988; Barrett *et al.*, 1993), although demographic evidence for this contention is widely lacking. Yet a recurrent theme in population genetic studies of highly clonal species, both of animals and plants, is the finding of high clonal diversity. These findings are suggestive of a significant role for sexual reproduction in populations characterized by infrequently observed sexual recruitment.

Species which follow this pattern of high clonal diversity despite a prevalence for vegetative reproduction include the sedge *Carex bigelowii* (Jonsson *et al.*,1996), aphids (Sunnucks *et al.*, 1997), oaks *Quercus havardii* (Mayes *et al.*, 1998) and *Q. chrysolepis* (Montalvo *et al.*,1997) , and *V. americana* (Lokker *et al.*,1994; Chapter 2). Some sexual reproduction must have occurred in these species, the variation observed cannot be explained by somatic mutation alone. It seems clear that sexual reproduction is maintained despite its associated costs. Mayes *et al.* (1998) suggest that for *Q. havardii* vegetative propagules act as a means of dispersing sexually reproduced individuals, maintaining genetic diversity despite a reduction in effective population size due to clonality.

The evolutionary significance of the observed variation in these species warrants further study. It is possible that rare sexual reproduction coupled with reduced genetic diversity describes a subgroup of species which have successful all-purpose genotypes, and are able to survive environmental heterogeneity. On the other hand, other species, such as *V. americana*, maintain sufficient variation, through sexual reproduction, to survive in their potentially heterogenous environments.

Future studies

The studies described in this thesis have brought to light some interesting information regarding populations of *V. americana,* but many questions remain. What are the conditions needed for successful recruitment of seeds? What are the dynamics of the seedling stage? How is the detected gene flow realized? What is the rate of outcrossing and effective population size in *V. americana*?

Given that *V. americana* does not follow the predicted pattern of low genetic diversity in clonal species (Hutchinson, 1975; Briggs and Walters, 1984; Les, 1988; Barrett *et al.*, 1993), it could serve as a system to model clonal population structure and dynamics.

Waycott (1998) suggested that allozyme studies are better for detecting broader scale processes such as gene flow and population establishment, while DNA methodologies appear to be better for detecting finer-scale processes such as local recruitment events and shorter-term population processes. The use of

DNA techniques could provide many more genetic markers in *V. americana* studies and could therefore be used to distinguish between ramets of the same genet and individuals of possible sexual origin. It could provide more precise estimates of rates of seedling recruitment. These studies should be augmented with careful field demography observations during the early spring.

Mating system parameters such as outcrossing rates need to be ascertained using temporal and spatial genetic data (Barrett *et al.*, 1993; Waycott and Sampson, 1998), in combination with demographic studies assessing sex ratios, reproductive success of individuals, and the longevity and mobility of pollen .

Spatial autocorrelational analysis of clones is becoming common (Geburak and Tripp-Knowles, 1994; Maki and Masuda, 1994; Widén *et al.*, 1994). Based on these studies, growth patterns and their effect on population genetics can be elucidated. Such studies can further be used to estimate relative contributions of sexual and asexual reproduction based on allelomorph locations, as done by Holderegger *et al.* (1998) applying the methods of Harada and Iwasa (1994) and Harada *et al.* (1997). Before such studies are undertaken, a well defined sampling strategy needs to be devised, preferably one where a single population is sampled at different scales.

Conclusions

Population genetics

* *Vallisneria americana* sites were genetically variable and clonally diverse, suggestive of a significant role for sexual reproduction in generating variation.

* Genetic structure of *V. americana* populations was complex, with a mosaic pattern of genet distribution.

* Great Lakes *V. americana* sites did not show evidence of marked genetic differentiation, rather they appeared to be panmictic, implicating gene flow as a significant process in the system.

* Great Lakes sites were strongly genetically differentiated from Florida sites, supporting the role of gene flow in populations sharing a water system.

* Florida sites were genetically differentiated from one another, corresponding to their geographic isolation and separate water systems.

Sexual reproduction

* Despite having an effective method of vegetative propagation and an obligate outcrossing breeding system, *V. americana* flowered regularly, many female flowers were successfully fertilized and numerous viable seeds were produced, suggesting a significant role for sexual reproduction in population maintenance, growth and gene flow.

* Male and female shoots of *V. americana* did not differentially compete, nor did

they appear to be allelopathic. Observed spatial segregation of the sexes in the field was either a sampling effect or a result of different flowering cues for male and female ramets.

* Nutrient regimes, sediment composition and light differences were not correlated with flowering, seed production or biomass.

* Outcrossing and inbreeding effects were not detected in seed production or seedling vigour in crosses between nearby mates, nor between distant mates; rather differences in seedling performance were associated with maternal identity.

* *Vallisneria americana* in the Huron-Erie corridor produced significant numbers of seeds, and maintained a significant seed bank.

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Appendix A

Cellulose Acetate Electrophoresis

Protocol and Recipes

Cellulose acetate gel electrophoresis protocol development for *V. americana* was carried out by Dr. Lesley Lovett-Doust and David Susko in 1991, with 21 enzyme systems involved in the preliminary testing. Of these, six enzymes showed consistently good resolution and intensity. These were phosphoglucomutase (PGM), malic enzyme (ME), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6PGDH), malate dehydrogenase (MDH), and phosphoglucose isomerase (PGI) (Table 3.2.1). Stolon material, the underground structures connecting ramets, provided the best results.

Tanks

Figure A1 Shows the electrophoresis tank ("rig") used for cellulose acetate gel electrophoresis. Electrode buffer levels were maintained at 15-17 mm. Moistening the wicks in the rigs before each gel run improved the outcome of the runs.

Gel and Electrophoresis Buffers

The three buffers used in this study ranged in pH from 6.0 to 8.5. The buffers were: tris glycine (TG; pH 8.5), tris citrate (TC; pH 7.0), and citric acid aminopropyl morpholine (CAAPM; pH 6.0). The recipes for these buffers are given in Table A1. Prior to gel runs all cellulose acetate plates were soaked, for at least 30 minutes, in the buffer corresponding to that used in the rigs.

Sample Preparation

The tissues of most plants must be homogenized in a grinding buffer that maintains the intensity and resolution of the banding patterns (Hebert and Beaton, 1989). Thus a tris hydrogen chloride grinding buffer, developed by Hebert and Beaton (1989), was used in this study (Table A2). At the time of grinding, approximately 0.2- 0.3 g of stolon was removed from the ultracold freezer. This material was homogenized in approximately 0.7 ml of the grinding buffer along with 30-50 mg of polyvinylpolypyrrolidone (PVPP) and a few grains of instant coffee. The addition of the PVPP and coffee grains served to stabilize the enzymes by removing phenolic compounds (Hebert and Beaton, 1989). The grinding of stolons was performed in individual depressions of a plexiglass grinding block with a teflon pestle using fine sand as an abrasive.

Sample Loading

Once homogenized, 10 μL aliquots of stolen extracts were transferred to

Helena sample wells (containing 12 wells each) using a micro-pipette. Eight samples were added to the sample wells in lanes 3-10. The first and last samples were replicated in their respective adjacent wells occupying lanes 2 and 11. Stolon extracts were then loaded onto 76 x 76 mm cellulose acetate plates using a Super Z-12 applicator (Helena Laboratories, Beaumont, TX) following the procedure of Hebert and Beaton (1989). Each set of stolon samples was applied twice to every plate.

Gel Runs

Gel plates were placed onto the wicks of the electrophoresis rig as described by Hebert and Beaton (1989). Electrophoresis was then performed at room temperature using 100 volts for 45 minutes with TG and TC buffers. CAAPM buffers were run at 100 volts for 90 minutes.

Gel Staining

After completion of the gel run, plates were stained according to the procedures for cellulose acetate gel electrophoresis employed by Hebert and Beaton (1989). The plates run on TG rigs were stained for the enzymes PGM and ME. The three enzymes MDH, IDH, and 6PGDH were stained for on the three plates run on TC rigs. The plates of CAAPM rigs were stained for PGI. The staining recipes for these enzymes were based on recipes developed by Hebert and Beaton (1989) with modifications made to improve the resolution and

staining intensity of the banding patterns (Table A3). Agar noble was added to each stain solution just prior to staining, allowing the solution to remain on the plate while the enzymatic reaction occurred. The plates were then placed in a dark incubator at 35 ^{0}C for 10-45 minutes, with the time varying according to the enzyme being assayed. Plates were removed from the incubator to prevent over-staining, or when there was no further noticeable change in staining intensity. The agar was washed off under cold running water, and plates were temporarily stored in water in the dark until they were scored.

Gel Scoring

Following gel staining and incubation, dark blue bands were observed at the sites of enzyme activity. The distances the bands moved from the origin were measured and recorded on gel scoring sheets which had twelve columns corresponding to the twelve sample lanes on each plate. The position, intensity and thickness of the individual bands as they appeared on the gels were also recorded by drawing the bands in pencil on the scoring sheets. In addition, the name of the enzyme that was stained for, the duration of the gel run, the voltage and current at which the run was performed, the electrode buffer used, and the site from which the stolons were collected were all noted. The sample number of the stolon was included above the lane on which an extract was.

Table A1. Recipes for gel and electrode buffers. Recipes for TG adapted from Hebert and Beaton, 1989; TC from Soltis and Soltis, 1989; CAAPM from Paul Hebert, Pers. Comm.

Buffer name	**Ingredients**	**Recipe**
Tris Glycine (TG) pH=8.5	30 g/L Trizma base 144 g/L glycine	Dilute the buffer 1 part TG : 9 parts distilled water for general use
Tris Citrate (TC) pH=7.0	16.35 g/L Trizma base 9.04 g/L citric acid monohydrate	Adjust pH with HCl or NaOH
Citric Acid Aminopropyl Morpholine (CAAPM) pH=6.0	42 g/L citric acid monohydrate 50 mL/L – (3-aminopropyl) - morpholine	Dilute the buffer 1 part CAAPM : 4 parts distilled water for general use. Adjust pH with – (3-aminopropyl) - morpholine

Table A2. Recipe for Grinding Buffer adapted from Hebert and Beaton (1989).

Grinding Buffer:	
0.1 M tris HCL, pH=8.0	1 L
Beta-mercaptoethanol	50 mL
Additions during grinding:	
polyvinylpolyprrolidone (PVPP)	30-50 mg
instant coffee grains	5-6 mg

Table A3. Enzyme Stain Recipes, adapted from Hebert and Beaton (1989). Abbreviations for chemicals used include: NAD (nicotinamide adenine dinucleotide); NADP (nicotinamide adenine dinucleotide phophate); MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide); PMS, phenazine methosulfate; MgCl (magnesium chloride).

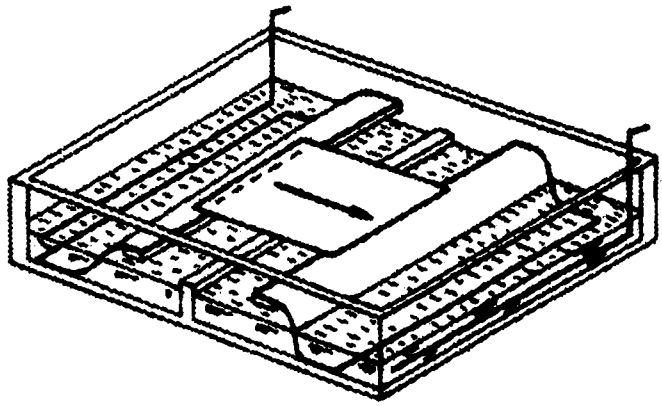
Buffer System	Enzyme	Ingredients	Amounts
TG	ME	tris HCl pH=8.0	0.6 mL
		NADP	1.5 mL
		malic substrate	12 drops
		MgCl	2 drops
		MTT	5 drops
		PMS	5 drops
		agar noble	2.0 mL
	PGM	tris HCL pH=8.0	1.0 mL
		NAD	1.5 mL
		glucose-1-phosphate	5 drops
		MTT	5 drops
		PMS	5 drops
		glucose-6-phosphate dehydrogenase	20 μmL
		agar noble	2.0 mL

TC	IDH	tris HCl pH=7.0	1.0 mL
		NADP	1.5 mL
		DL-isocitric acid	15 drops
		MgCl	8 drops
		MTT	5 drops
		PMS	5 drops
		agar noble	2.0 mL
	6PGDH	tris HCl pH=8.0	0.6 mL
		NADP	1.5 mL
		6-phosphogluconic acid	6 drops
		MgCl	6 drops
		MTT	5 drops
		PMS	5 drops
		agar noble	2.0 mL
	MDH	tris HCl pH=8.0	1.0 mL
		NAD	1.5 mL
		malic substrate	18 drops
		MTT	5 drops
		PMS	5 drops
		agar noble	2.0 mL

CAAPM	PGI	tris HCL pH=8.0	1.0 mL
		NADP	1.5 mL
		fructose-6-phosphate	5 drops
		MTT	5 drops
		PMS	5 drops
		glucose-6-phosphate dehydrogenase	20 μL
		agar noble	2.0 mL

Figure A1. Schematic drawing of cellulose acetate gel electrophoresis apparatus. Courtesy of Ruben Boles. Arrow indicates direction of enzyme movement.

Figure A1.



Appendix B

Genetic Data

Table B1. Equations used to calculate population genetic statistics. [a]Li and Horvitz, 1953, [b]Workman and Niswander 1970; [c]Nei, 1972; [d]Nei, 1973; [e]Hartl and Clark, 1989; [f]Weir, 1990; [g]Eckert and Barrett, 1993. n= no. of samples; k= no. of sites.

Statistic	Equation	Description
P [e]	no. of polymorphic loci / no. of loci	Percent polymorphic loci
A_e [e]	1 / ($\sum p_i^2$ for each allele)	Effective number of alleles. Means across loci and survey areas were used in the study.
H_{obs} [e]	no. of heterozygotes / n	Observed heterozygosity
H_{exp} [e]	2pq	Expected heterozygosity under HWE. p=frequency of A allele; q=frequency of B allele.
p [e]	(2(no. of AA) + no. of AB) / 2n	Frequency of A allele
q [e]	(2(no. of BB) + no. of AB) / 2n	Frequency of B allele
H_I [e]	($\sum H_{obs}$) / k	Mean observed heterozygosity
H_S [d]	1 - ($\sum p^2$ for each allele)	Mean expected heterozygosity if population in HWE (random mating). $\bar{H}_s$ is H_S averaged over sites.

Statistic	Equation	Description
H_T [e]	$1 - (\sum \overline{p^2}$ for each site)	Total gene diversity. Mean expected heterozygosity under random mating. $H_T = H_S + D_{ST}$
D_{ST} [d]	$H_T - H_S$	
G_{ST} [d]	D_{ST} / H_T	Co-efficient of gene differentiation (Nei, 1975). Equivalent to Wright's F_{ST} in a 2 allele system.
Wright's F-statistics [f]		
F_{IS}	$(F_{IT} - F_{ST}) / (F_{ST} - 1)$	Estimated inbreeding due to non-random mating within sites.
F_{ST}	see Weir (1990)	Fixation index. Amount of inbreeding due to population subdivision. $F_{ST} = 0$ if population in HWE.
F_{IT}	see Weir (1990)	Total inbreeding co-efficient. Estimated extent of inbreeding due to non-random mating within sites and random genetic drift among sites.
Nm [e]	$Nm = ((1/F_{ST}) - 1)/4$	Number of migrants per generation.

Statistic	Equation	Description
Nei's genetic distance [c]		
D	-ln (I)	Nei's genetic distance
I	$J_{xy}/(\sqrt{J_{xx}\ J_{yy}})$	Nei's genetic identity
J_{xx}	$\sum p_i^2$	*i* = *i*th locus
J_{yy}	$\sum q_i^2$	
Jxy	$\sum p_i\ q_i$	
Clonal diversity[g]		
G		Number of alllelomorphs (genotypes)
Nr		Number of ramets sampled
G_{max}	$(^{m}{}_{i=1}\prod a_i(a_i+1)/2)M$ a_i: no. of alleles at *i*th locus M: no. ofgenders	Maximum number of possible allelomorphs. Equal to Nr if G_{max}>Nr.
G/Nr	G/Nr	Proportion of alllelomorphs which were distinguishable
D	$1 - ((\sum n_i*(n_i-1)) / (Nr*(Nr-1)))$	Diversity (n is the number of ramets representing each allelomorph)
E	$(D - D_{min}) / (D_{max} - D_{min})$	Evenness
D_{min}	$(G-1) *((2*Nr - G)/((Nr*(Nr-1)))$	
D_{max}	$(Nr*(G-1)) / (G*(Nr-1))$	
χ2 tests		

Statistic	Equation	Description
$\chi 2$ HWE [a]	$N*F^{2}*(k-1)$	Test of HWE. N=sample size,
	df = (k(k-1)) / 2	k=number of alleles,
		$F=(H_{EXP}-H_{OBS})/H_{EXP}$
$\chi 2$ [b]	$(\sum(2N_i)p_i^2 - \overline{p} \sum((2N_i)p_i)) / \overline{pq}$	Test of heterogeneity of allele
	df = (no. loci -1)(no. alleles -1)	and genotype frequencies. i
		represents different sites

Local populations

Table B2. Ramet genotype values for the transects sampled at Turkey Is., Mitchell's Bay and Stoney Is. In 1991-1993.

Locus	Site	Year	AA	AB	BB	n
PGM-2	Turkey Is.-shal	1991	241	0	0	241
	Turkey Is.-mid	1991	273	0	0	273
	Turkey Is.-shal	1993	150	0	0	150
	Turkey Is.-mid	1993	150	0	0	150
	Stoney Is.-shal	1992	405	0	0	405
	Stoney Is.-mid	1992	405	0	0	405
	Stoney Is.-shal	1993	181	0	0	181
	Mitchell's Bay-shal	1992	405	0	0	405
	Mitchell's Bay-mid	1992	405	0	0	405
	Mitchell's Bay- shal	1993	150	0	0	150
ME-1	Turkey Is.-shal	1991	118	87	19	224
	Turkey Is.-mid	1991	176	51	19	246
	Turkey Is.-shal	1993	106	33	7	146
	Turkey Is.-mid	1993	82	42	3	127
	Stoney Is.-shal	1992	170	67	69	306
	Stoney Is.-mid	1992	263	37	8	308
	Stoney Is.-shal	1993	62	63	9	134
	Mitchell's Bay-shal	1992	159	53	9	221
	Mitchell's Bay-mid	1992	93	46	8	147
	Mitchell's Bay- shal	1993	53	59	4	116
IDH-1	Turkey Is.-shal	1991	145	74	9	228
	Turkey Is.-mid	1991	223	28	8	259

Locus	Site	Year	AA	AB	BB	n
	Turkey Is.-shal	1993	82	65	1	148
	Turkey Is.-mid	1993	82	66	0	148
	Stoney Is.-shal	1992	210	139	1	350
	Stoney Is.-mid	1992	342	48	1	391
	Stoney Is.-shal	1993	55	64	2	121
	Mitchell's Bay-shal	1992	134	199	12	345
	Mitchell's Bay-mid	1992	133	249	12	394
	Mitchell's Bay- shal	1993	38	97	2	137
6PGDH-1	Turkey Is.-shal	1991	130	62	36	228
	Turkey Is.-mid	1991	204	33	13	250
	Turkey Is.-shal	1993	95	42	10	147
	Turkey Is.-mid	1993	86	60	3	149
	Stoney Is.-shal	1992	205	103	42	350
	Stoney Is.-mid	1992	324	68	3	395
	Stoney Is.-shal	1993	78	67	6	151
	Mitchell's Bay-shal	1992	135	163	55	353
	Mitchell's Bay-mid	1992	134	214	40	388
	Mitchell's Bay- shal	1993	64	67	11	142
MDH-2	Turkey Is.-shal	1991	222	0	0	222
	Turkey Is.-mid	1991	229	0	0	229
	Turkey Is.-shal	1993	150	0	0	150
	Turkey Is.-mid	1993	150	0	0	150
	Stoney Is.-shal	1992	405	0	0	405
	Stoney Is.-mid	1992	405	0	0	405
	Stoney Is.-shal	1993	181	0	0	181

Locus	Site	Year	AA	AB	BB	n
	Mitchell's Bay-shal	1992	405	0	0	405
	Mitchell's Bay-mid	1992	405	0	0	405
	Mitchell's Bay- shal	1993	150	0	0	150
PGI-1	Turkey Is.-shal	1991	195	0	0	195
	Turkey Is.-mid	1991	191	0	0	191
	Turkey Is.-shal	1993	131	0	0	131
	Turkey Is.-mid	1993	124	0	0	124
	Stoney Is.-shal	1992	268	0	0	268
	Stoney Is.-mid	1992	346	0	0	346
	Stoney Is.-shal	1993	113	0	0	113
	Mitchell's Bay-shal	1992	255	0	0	255
	Mitchell's Bay-mid	1992	321	4	0	325
	Mitchell's Bay- shal	1993	89	0	0	89

Table B3. Ramet allele frequencies, observed and expected heterozygosities for the transects sampled at Turkey Is., Stoney Is., and Mitchell's Bay. Deviations from HWE were detected using Wright's inbreeding coefficient (Li and Horvitz, 1953). Transects sampled in 1993 were used for further genetic data analysis, and are presented in bold. *p<0.05, **p<0.01, ***p<0.001.

Locus	Site	Year	p(A)	q(B)	n	Hobs	Hexp	F	χ^2	df
PGM-2	Turkey Is.-shal	1991	1.000	0.000	241	0.000	0.000			0
	Turkey Is.-mid	1991	1.000	0.000	273	0.000	0.000			0
	Turkey Is.-shal	**1993**	1.000	0.000	150	0.000	0.000			0
	Turkey Is.-mid	1993	1.000	0.000	150	0.000	0.000			0
	Stoney Is.-shal	1992	1.000	0.000	405	0.000	0.000			0
	Stoney Is.-mid	1992	1.000	0.000	405	0.000	0.000			0
	Stoney Is.-shal	**1993**	1.000	0.000	181	0.000	0.000			0
	Mitchell's Bay-shal	1992	1.000	0.000	405	0.000	0.000			0
	Mitchell's Bay-mid	1992	1.000	0.000	405	0.000	0.000			0
	Mitchell's Bay- shal	**1993**	1.000	0.000	150	0.000	0.000			0
ME-1	Turkey Is.-shal	1991	0.721	0.279	224	0.388	0.402	0.036	0.284	1
	Turkey Is.-mid	1991	0.819	0.181	246	0.207	0.296	0.302	22.4***	1
	Turkey Is.-shal	**1993**	0.839	0.161	146	0.226	0.270	0.163	3.90 *	1

Locus	Site	Year	p(A)	q(B)	n	Hobs	Hexp	F	χ^2	df
	Turkey Is.-mid	1993	0.811	0.189	127	0.331	0.307	-0.080	0.807	1
	Stoney Is.-shal	1992	0.665	0.335	306	0.219	0.446	0.508	79.1 ***	1
	Stoney Is.-mid	1992	0.914	0.086	308	0.120	0.157	0.237	17.3 ***	1
	Stoney Is.-shal	**1993**	0.698	0.302	134	0.470	0.422	-0.115	1.77	1
	Mitchell's Bay-shal	1992	0.839	0.161	221	0.240	0.270	0.112	2.75	1
	Mitchell's Bay-mid	1992	0.789	0.211	147	0.313	0.333	0.060	0.528	1
	Mitchell's Bay- shal	**1993**	0.711	0.289	116	0.509	0.411	-0.239	6.60 *	1
IDH-1	Turkey Is.-shal	1991	0.798	0.202	228	0.325	0.322	-0.008	0.015	1
	Turkey Is.-mid	1991	0.915	0.085	259	0.108	0.156	0.306	24.2 ***	1
	Turkey Is.-shal	**1993**	0.774	0.226	148	0.446	0.350	-0.275	11.2 ***	1
	Turkey Is.-mid	1993	0.777	0.223	148	0.439	0.347	-0.267	10.5 **	1
	Stoney Is.-shal	1992	0.799	0.201	350	0.397	0.321	-0.236	19.5 ***	1
	Stoney Is.-mid	1992	0.936	0.064	391	0.123	0.120	-0.027	0.2775	1
	Stoney Is.-shal	**1993**	0.719	0.281	121	0.530	0.404	-0.312	11.8 ***	1
	Mitchell's Bay-shal	1992	0.677	0.323	345	0.577	0.437	-0.319	35.2 ***	1
	Mitchell's Bay-mid	1992	0.654	0.346	394	0.632	0.453	-0.396	61.9 ***	1

Locus	Site	Year	p(A)	q(B)	n	Hobs	Hexp	F	χ^2	df
	Mitchell's Bay- shal	**1993**	0.631	0.369	137	0.708	0.466	-0.520	37.1 ***	1
6PGDH-1	Turkey Is.-shal	1991	0.706	0.294	228	0.272	0.415	0.345	27.1 ***	1
	Turkey Is.-mid	1991	0.882	0.118	250	0.132	0.208	0.366	33.5 ***	1
	Turkey Is.-shal	**1993**	0.789	0.211	147	0.286	0.333	0.141	2.92	1
	Turkey Is.-mid	1993	0.779	0.221	149	0.400	0.344	-0.162	3.90 *	1
	Stoney Is.-shal	1992	0.733	0.267	350	0.294	0.391	0.249	21.7 ***	1
	Stoney Is.-mid	1992	0.906	0.094	395	0.172	0.170	-0.010	0.038	1
	Stoney Is.-shal	**1993**	0.738	0.262	151	0.444	0.387	-0.148	3.31	1
	Mitchell's Bay-shal	1992	0.613	0.387	353	0.462	0.474	0.026	0.2435	1
	Mitchell's Bay-mid	1992	0.621	0.379	388	0.552	0.471	-0.173	11.6 ***	1
	Mitchell's Bay- shal	**1993**	0.687	0.313	142	0.472	0.430	-0.098	1.35	1
MDH-2	Turkey Is.-shal	1991	1.000	0.000	222	0.000	0.000			0
	Turkey Is.-mid	1991	1.000	0.000	229	0.000	0.000			0
	Turkey Is.-shal	**1993**	1.000	0.000	150	0.000	0.000			0
	Turkey Is.-mid	1993	1.000	0.000	150	0.000	0.000			0
	Stoney Is.-shal	1992	1.000	0.000	405	0.000	0.000			0

Locus	Site	Year	p(A)	q(B)	n	Hobs	Hexp	F	χ^2	df
	Stoney Is.-mid	1992	1.000	0.000	405	0.000	0.000			0
	Stoney Is.-shal	**1993**	1.000	0.000	181	0.000	0.000			0
	Mitchell's Bay-shal	1992	1.000	0.000	405	0.000	0.000			0
	Mitchell's Bay-mid	1992	1.000	0.000	405	0.000	0.000			0
	Mitchell's Bay- shal	**1993**	1.000	0.000	150	0.000	0.000			0
PGI-1	Turkey Is.-shal	1991	1.000	0.000	195	0.000	0.000			0
	Turkey Is.-mid	1991	1.000	0.000	191	0.000	0.000			0
	Turkey Is.-shal	**1993**	1.000	0.000	131	0.000	0.000			0
	Turkey Is.-mid	1993	1.000	0.000	124	0.000	0.000			0
	Stoney Is.-shal	1992	1.000	0.000	268	0.000	0.000			0
	Stoney Is.-mid	1992	1.000	0.000	346	0.000	0.000			0
	Stoney Is.-shal	**1993**	1.000	0.000	113	0.000	0.000			0
	Mitchell's Bay-shal	1992	1.000	0.000	255	0.000	0.000			0
	Mitchell's Bay-mid	1992	0.994	0.006	325	0.010	0.012	0.162		0
	Mitchell's Bay- shal	**1993**	1.000	0.000	89	0.000	0.000			0

Table B4. Genotype and allele frequencies for putative genets based on 6 loci and gender, sampled in 1993 only. Sites are coded as TI (Turkey Island), SI (Stoney Island) and MB (Mitchell's Bay). All X^2 values testing for HWE were non-significant at $p<0.05$.

Locus	Site	AA	AB	BB	p(A)	q(B)	n	Hobs	Hexp	F	X^2	df
PGM-2	TI	23			1.000	0.000	23	0.000	0.000			0
	SI	21			1.000	0.000	21	0.000	0.000			0
	MB	27			1.000	0.000	27	0.000	0.000			0
ME-1	TI	10	10	3	0.652	0.348	23	0.435	0.454	0.042	0.041	1
	SI	7	10	4	0.571	0.429	21	0.476	0.490	0.028	0.016	1
	MB	11	12	4	0.630	0.370	27	0.444	0.466	0.047	0.060	1
IDH-1	TI	9	13	1	0.674	0.326	23	0.565	0.440	-0.286	1.88	1
	SI	8	11	2	0.643	0.357	21	0.524	0.459	-0.141	0.416	1
	MB	8	17	2	0.611	0.389	27	0.630	0.475	-0.325	2.85	1
6PGDH-1	TI	10	9	4	0.630	0.370	23	0.391	0.466	0.160	0.591	1
	SI	9	9	3	0.643	0.357	21	0.429	0.459	0.067	0.090	1
	MB	9	10	8	0.519	0.481	27	0.370	0.499	0.258	1.80	1
MDH-2	TI	23			1.000	0.000	23	0.000	0.000			0
	SI	21			1.000	0.000	21	0.000	0.000			0

Locus	Site	AA	AB	BB	p(A)	q(B)	n	Hobs	Hexp	F	X^2	df
	MB	27			1.000	0.000	27	0.000	0.000			0
PGI-1	TI	23			1.000	0.000	23	0.000	0.000			0
	SI	21			1.000	0.000	21	0.000	0.000			0
	MB	27			1.000	0.000	27	0.000	0.000			0

Table B5. Confidence intervals (95%) for Wright's F-statistics: F_{IS}, F_{ST}, and F_{IT}. Intervals were generated by bootstrapping values over loci using the Genetic Data Analysis program (Lewis, 2000), 5000 replicates were performed.

		F_{IS}	F_{ST}	F_{IT}
Ramet	Upper	-0.0439	0.0331	-0.0288
	Lower	-0.3728	0.0145	-0.3424
Genet	Upper	-0.0032	0.1549	0.0939
	Lower	-0.4037	-0.0102	-0.4035

Geographic Survey

Table B6. Ramet genotype numbers for the Great Lake and Florida sites.

Locus	Area	Site	AA	AB	BB	AC	BC	AD	CC/DD	n
PGM-2	Huron	Thess	160							160
		Blind	144							144
		Serp	152							152
		Span	160							160
		Bayf	160							160
		Key	144							144
		Big C	160							160
	Huron-	Clay	150							150
	Erie	Walpole	300							300
		Mitchell	810							810
		Peche	300							300
		Stoney	810							810
	Erie	Rondeau	310							310
		Long Point	299							299
		Put	83							83
	Ontario	Niag	159							159
		Brigh	160							160
		Adol	160							160
		Howe	160							160

Locus	Area	Site	AA	AB	BB	AC	BC	AD	CC/DD	n
		SLan	160							160
	Florida	Blue	95							95
		Chas	50							50
		Hom	90							90
		StM	137							137
		Wak	76							76
ME-1	Huron	Thess	46	94	3					143
		Blind	26	84	7					117
		Serp	43	65	14					122
		Span	36	101	4	1				142
		Bayf	45	84	5					134
		Key	34	70	9					113
		Big C	6	58	35					99
	Huron-	Clay	60	73	7					140
	Erie	Walpole	181	97	5					283
		Mitchell	252	99	17					368
		Peche	160	38	20					218
		Stoney	433	104	77					614
	Erie	Rondeau	188	61	6					255
		Long Point	145	80	17					242
		Put	30	65	1					96
	Ontario	Niag	42	58	40					140

Locus	Area	Site	AA	AB	BB	AC	BC	AD	CC/DD	n
		Brigh	48	77		8	2			135
		Adol	47	74	10	1				132
		Howe	61	76		3	1			141
		SLan	67	43	9					119
	Florida	Blue	44	4				7	9	64
		Chas	72	22	3					97
		Hom	54	46	4					109
		StM	98	14					12	124
		Wak	48	13	5					66
IDH-1	Huron	Thess	36	119						155
		Blind	35	95	3					133
		Serp	24	119	4					147
		Span	20	132	3					155
		Bayf	63	83	4					150
		Key	25	101	2					128
		Big C	84	37	4					125
	Huron-	Clay	53	95						148
	Erie	Walpole		192	5					197
		Mitchell	267	448	24					739
		Peche	172	97	9					278
		Stoney	101	187	2					290
	Erie	Rondeau	552	124	6					682

Locus	Area	Site	AA	AB	BB	AC	BC	AD	CC/DD	n
		Long Point	176	96	5					277
		Put	78	58						136
	Ontario	Niag	29	86	23					138
		Brigh	29	127	3					159
		Adol	32	112	10					154
		Howe	48	94						142
		SLan	39	87	23					149
	Florida	Blue	125	11	2					138
		Chas	11	78	25					114
		Hom	87	36	1					124
		StM	132	25						157
		Wak	122	4						126
6PGDH-1	Huron	Thess	26	119	12					157
		Blind	24	88	18					130
		Serp	31	106	10					147
		Span	20	129	8					157
		Bayf	35	113	8					156
		Key	21	86	19					126
		Big C	34	68	20					122
	Huron-	Clay	35	104	6					145
	Erie	Walpole	103	177	19					299
		Mitchell	269	377	95					741

Locus	Area	Site	AA	AB	BB	AC	BC	AD	CC/DD	n
		Peche	135	97	36					268
		Stoney	529	171	45					745
	Erie	Rondeau	148	115	39					302
		Long Point	159	91	33					283
		Put	51	88	1					140
	Ontario	Niag	33	68	53					154
		Brigh	27	125	8					160
		Adol	42	91	21					154
		Howe	44	99						143
		SLan	38	80	28					146
	Florida	Blue	135		2					137
		Chas	116							116
		Hom	119	7						126
		StM	150	8						158
		Wak	93	1						94
MDH-2	Huron	Thess	159							159
		Blind	141							141
		Serp	142	2	1					145
		Span	155		1					156
		Bayf	153		2					155
		Key	131	4						135

Locus	Area	Site	AA	AB	BB	AC	BC	AD	CC/DD	n
		Big C	115							115
	Huron-	Clay	150							150
	Erie	Walpole	300							300
		Mitchell	810							810
		Peche	300							300
		Stoney	810							810
	Erie	Rondeau	310							310
		Long Point	299							299
		Put	154							154
	Ontario	Niag	130	3						133
		Brigh	151							151
		Adol	134							134
		Howe	146							146
		SLan	134	7						141
	Florida	Blue	96	55						151
		Chas	72	55						127
		Hom	1	118	15					134
		StM	153	5						158
		Wak	123							123
PGI-1	Huron	Thess	120	36	1					157

Locus	Area	Site	AA	AB	BB	AC	BC	AD	CC/DD	n
		Blind	118	4						122
		Serp	142							142
		Span	155			1				156
		Bayf	137	9	1	1				148
		Key	127	1		1				129
		Big C	88							88
	Huron-	Clay	115			1				116
	Erie	Walpole	267	7						274
		Mitchell	576	4						580
		Peche	196			5			2	203
		Stoney	614							614
	Erie	Rondeau	256						2	258
		Long Point	208		1	6				219
		Put	143	2		2				147
	Ontario	Niag	104			1				105
		Brigh	149							149
		Adol	125							125
		Howe	120	18	1				1	140
		SLan	123	11						134
	Florida	Blue	83	16			28			127
		Chas	86	26		4				116
		Hom	94	27						121

Locus	Area	Site	AA	AB	BB	AC	BC	AD	CC/DD	n
		StM	70	87						157
		Wak	116	11						127

Table B7. Ramet allele frequencies, observed and expected heterozygosities for the sites surveyed in the Great Lakes and Florida. Testing for HWE using χ^2 based on Wright's inbreeding coefficient (Li and Horvitz, 1953).*p<0.05, **p<0.01, ***p<0.001. Alleles are only included in the χ^2 analysis when their frequency is greater than 0.05.

Locus	Area	Site	p(A)	q(B)	r(C)	s(D)	n	Hobs	Hexp	F	χ^2	df
PGM-2	Huron	Thess	1.000	0.000			160	0.000	0.000			0
		Blind	1.000	0.000			144	0.000	0.000			0
		Serp	1.000	0.000			152	0.000	0.000			0
		Span	1.000	0.000			160	0.000	0.000			0
		Bayf	1.000	0.000			160	0.000	0.000			0
		Key	1.000	0.000			144	0.000	0.000			0
		Big C	1.000	0.000			160	0.000	0.000			0
	Huron	Clay	1.000	0.000			150	0.000	0.000			0
	-Erie	Walp	1.000	0.000			300	0.000	0.000			0
		Mitch	1.000	0.000			810	0.000	0.000			0
		Pech	1.000	0.000			300	0.000	0.000			0
		Ston	1.000	0.000			810	0.000	0.000			0
	Erie	Rond	1.000	0.000			310	0.000	0.000			0
		Long	1.000	0.000			299	0.000	0.000			0
		Put	1.000	0.000			83	0.000	0.000			0
	Ontario	Niag	1.000	0.000			159	0.000	0.000			0
		Brigh	1.000	0.000			160	0.000	0.000			0
		Adol	1.000	0.000			160	0.000	0.000			0
		Howe	1.000	0.000			160	0.000	0.000			0
		SLan	1.000	0.000			160	0.000	0.000			0
	Florida	Blue	1.000	0.000			95	0.000	0.000			0
		Chas	1.000	0.000			50	0.000	0.000			0

Locus	Area	Site	p(A)	q(B)	r(C)	s(D)	n	Hobs	Hexp	F	χ^2	df
		Hom	1.000	0.000			90	0.000	0.000			0
		StM	1.000	0.000			137	0.000	0.000			0
		Wak	1.000	0.000			76	0.000	0.000			0
ME-1	Huron	Thess	0.650	0.350			143	0.657	0.455	-0.444	28.2***	1
		Blind	0.581	0.419			117	0.718	0.487	-0.475	26.4***	1
		Serp	0.619	0.381			122	0.533	0.472	-0.130	2.06	1
		Span	0.613	0.384	0.004		142	0.718	0.479	-0.500	35.5***	1
		Bayf	0.649	0.351			134	0.627	0.456	-0.376	19.0***	1
		Key	0.611	0.389			113	0.619	0.475	-0.302	10.3**	1
		Big C	0.354	0.646			99	0.586	0.457	-0.281	7.83**	1
	Huron-	Clay	0.689	0.311			140	0.520	0.429	-0.213	6.37*	1
	Erie	Walp	0.811	0.189			283	0.340	0.307	-0.109	3.37	1
		Mitch	0.819	0.181			368	0.270	0.296	0.089	2.94	1
		Pech	0.821	0.179			218	0.170	0.294	0.422	38.8***	1
		Ston	0.790	0.210			614	0.170	0.332	0.488	146***	1
	Erie	Rond	0.857	0.143			255	0.240	0.245	0.021	0.110	1
		Longt	0.764	0.236			242	0.330	0.361	0.085	1.74	1
		Put	0.651	0.349			96	0.677	0.454	-0.490	23.0***	1
	Ontario	Niag	0.507	0.493			140	0.410	0.500	0.180	4.53*	1
		Brigh	0.670	0.293	0.037		135	0.644	0.464	-0.388	20.35***	1
		Adol	0.640	0.356	0.004		132	0.568	0.464	-0.225	6.69**	1
		Howe	0.713	0.273	0.014		141	0.567	0.417	-0.360	18.3***	1
		SLan	0.744	0.256			119	0.361	0.381	0.052	0.326	1
	Florida	Blue	0.773	0.031		0.195	64	0.172	0.361	0.524	17.6***	1
		Chas	0.856	0.144			97	0.227	0.247	0.079	0.609	1
		Hom	0.706	0.248		0.046	109	0.422	0.438	0.036	0.289	3

Locus	Area	Site	p(A)	q(B)	r(C)	s(D)	n	Hobs	Hexp	F	χ^2	df
		StM	0.847	0.056		0.097	124	0.113	0.270	0.582	83.9***	3
		Wak	0.826	0.174			66	0.197	0.287	0.315	6.54*	1
IDH-1	Huron	Thess	0.616	0.384			155	0.768	0.473	-0.623	60.2***	1
		Blind	0.620	0.380			133	0.714	0.471	-0.515	35.3***	1
		Serp	0.568	0.432			147	0.810	0.491	-0.651	62.2***	1
		Span	0.555	0.445			155	0.852	0.494	-0.725	81.4***	1
		Bayf	0.697	0.303			150	0.553	0.422	-0.309	14.3***	1
		Key	0.590	0.410			128	0.789	0.484	-0.631	50.9***	1
		Big C	0.820	0.180			125	0.296	0.295	-0.003	0.001	1
	Huron-Erie	Clay	0.679	0.321			148	0.640	0.436	-0.468	32.4***	1
		Walp	0.487	0.513			197	0.970	0.500	-0.941	175***	1
		Mitch	0.664	0.336			739	0.610	0.446	-0.367	99.6***	1
		Pech	0.793	0.207			278	0.350	0.328	-0.066	1.21	1
		Ston	0.671	0.329			290	0.640	0.442	-0.450	58.6***	1
	Erie	Rond	0.900	0.100			682	0.180	0.180	0.000	0	1
		Long	0.809	0.191			277	0.350	0.309	-0.133	4.87*	1
		Put	0.787	0.213			136	0.426	0.335	-0.271	9.96**	1
	Ontario	Niag	0.522	0.478			138	0.623	0.499	-0.248	8.52**	1
		Brigh	0.582	0.418			159	0.799	0.487	-0.642	65.6***	1
		Adol	0.571	0.429			154	0.727	0.490	-0.484	36.1***	1
		Howe	0.669	0.331			142	0.662	0.443	-0.495	34.8***	1
		SLan	0.554	0.446			149	0.584	0.494	-0.182	4.92*	1
	Florida	Blue	0.946	0.054			138	0.080	0.102	0.217	6.50*	1
		Chas	0.439	0.561			114	0.684	0.493	-0.389	17.2***	1
		Hom	0.847	0.153			124	0.290	0.259	-0.119	1.75	1
		StM	0.920	0.080			157	0.159	0.147	-0.080	1.01	1

Locus	Area	Site	p(A)	q(B)	r(C)	s(D)	n	Hobs	Hexp	F	χ^2	df
		Wak	0.984	0.016			126	0.032	0.031			0
6PGDH-1	Huron	Thess	0.545	0.465			157	0.758	0.496	-0.528	43.8***	1
		Blind	0.523	0.477			130	0.677	0.499	-0.357	16.6***	1
		Serp	0.571	0.429			147	0.721	0.490	-0.472	32.7***	1
		Span	0.538	0.462			157	0.822	0.497	-0.654	67.1***	1
		Bayf	0.587	0.413			156	0.724	0.485	-0.493	37.9***	1
		Key	0.508	0.492			126	0.683	0.500	-0.366	16.9***	1
		Big C	0.557	0.443			122	0.557	0.494	-0.129	2.02	1
	Huron-	Clay	0.600	0.400			145	0.720	0.480	-0.500	36.3***	1
	Erie	Walp	0.640	0.360			299	0.590	0.461	-0.280	23.5***	1
		Mitch	0.617	0.383			741	0.510	0.473	-0.079	4.64*	1
		Pech	0.685	0.315			268	0.360	0.432	0.166	7.37**	1
		Ston	0.825	0.175			745	0.230	0.289	0.203	30.8***	1
	Erie	Rond	0.680	0.320			302	0.380	0.435	0.127	4.86*	1
		Long	0.723	0.277			283	0.320	0.401	0.201	11.4***	1
		Put	0.679	0.321			140	0.629	0.436	-0.443	27.5***	1
	Ontario	Niag	0.435	0.565			154	0.442	0.492	0.101	1.57	1
		Brigh	0.559	0.441			160	0.781	0.493	-0.584	54.6***	1
		Adol	0.568	0.432			154	0.591	0.491	-0.204	6.43*	1
		Howe	0.654	0.346			143	0.692	0.453	-0.529	40.0***	1
		SLan	0.534	0.466			146	0.548	0.498	-0.101	1.49	1
	Florida	Blue	0.985	0.015			137	0.000	0.030			0
		Chas	1.000	0.000			116	0.000	0.000			0
		Hom	0.972	0.028			126	0.056	0.054			0
		StM	0.975	0.025			158	0.051	0.049			0
		Wak	0.995	0.005			94	0.011	0.010			0

Locus	Area	Site	p(A)	q(B)	r(C)	s(D)	n	Hobs	Hexp	F	χ^2	df
MDH-2	Huron	Thess	1.000	0.000			159	0.000	0.000			0
		Blind	1.000	0.000			141	0.000	0.000			0
		Serp	0.986	0.014			145	0.014	0.028			0
		Span	0.994	0.006			156	0.000	0.012			0
		Bayf	0.987	0.013			155	0.000	0.026			0
		Key	0.985	0.015			135	0.030	0.030			0
		Big C	1.000	0.000			115	0.000	0.000			0
	Huron-	Clay	1.000	0.000			150	0.000	0.000			0
	Erie	Walp	1.000	0.000			300	0.000	0.000			0
		Mitch	1.000	0.000			810	0.000	0.000			0
		Pech	1.000	0.000			300	0.000	0.000			0
		Ston	1.000	0.000			810	0.000	0.000			0
	Erie	Rond	1.000	0.000			310	0.000	0.000			0
		Long	1.000	0.000			299	0.000	0.000			0
		Put	1.000	0.000			154	0.000	0.000			0
	Ontario	Niag	0.989	0.011			133	0.023	0.022			0
		Brigh	1.000	0.000			151	0.000	0.000			0
		Adol	1.000	0.000			134	0.000	0.000			0
		Howe	1.000	0.000			146	0.000	0.000			0
		SLan	0.975	0.025			141	0.050	0.049			0
	Florida	Blue	0.818	0.182			151	0.364	0.298	-0.222	7.48**	1
		Chas	0.783	0.217			127	0.433	0.340	-0.274	9.55**	1
		Hom	0.448	0.552			134	0.881	0.495	-0.781	81.8***	1
		StM	0.984	0.016			158	0.032	0.031			0
		Wak	1.000	0.000			123	0.000	0.000			0
PGI-1	Huron	Thess	0.879	0.121			157	0.229	0.213	-0.077	0.920	1

Locus	Area	Site	p(A)	q(B)	r(C)	s(D)	n	Hobs	Hexp	F	χ^2	df
		Blind	0.984	0.016			122	0.033	0.031			0
		Serp	1.000	0.000			142	0.000	0.000			0
		Span	0.997	0.000	0.003		156	0.010	0.006			0
		Bayf	0.959	0.037	0.003		148	0.068	0.077			0
		Key	0.992	0.004	0.004		129	0.016	0.016			0
		Big C	1.000	0.000			88	0.000	0.000			0
	Huron-	Clay	0.996	0.000	0.004		116	0.010	0.008			0
	Erie	Walp	0.987	0.013			274	0.030	0.026			0
		Mitch	0.997	0.003			580	0.010	0.006			0
		Pech	0.978	0.000	0.022		203	0.020	0.043			0
		Ston	1.000	0.000			614	0.000	0.000			0
	Erie	Rond	0.992	0.000	0.008		258	0.000	0.016			0
		Long	0.963	0.005	0.032		219	0.030	0.072			0
		Put	0.986	0.007	0.007		147	0.027	0.028			0
	Ontario	Niag	0.995	0.000	0.005		105	0.010	0.010			0
		Brigh	1.000	0.000			149	0.000	0.000			0
		Adol	1.000	0.000			125	0.000	0.000			0
		Howe	0.921	0.071	0.007		140	0.129	0.145	0.108	1.64	1
		SLan	0.959	0.041			134	0.082	0.079			0
	Florida	Blue	0.717	0.173	0.110		127	0.346	0.444	0.221	12.4**	3
		Chas	0.871	0.112	0.017		116	0.259	0.229	-0.133	2.06	1
		Hom	0.888	0.112			121	0.223	0.199	-0.121	1.77	1
		StM	0.723	0.277			157	0.554	0.401	-0.383	23.0***	1
		Wak	0.957	0.043			127	0.087	0.000			0

Table B8. Allele frequencies based on putative genets for sites survey in the Great Lakes and Florida.

Locus	Area	Site	p(1)	q(2)	r(3)	s(4)	n	H-obs	H-exp	F	chi	df
PGM-2	Huron	Thess	1.00	0.00			25					0
		Blind	1.00	0.00			30					0
		Serp	1.00	0.00			24					0
		Span	1.00	0.00			25					0
		Bayf	1.00	0.00			26					0
		Key	1.00	0.00			28					0
		Big C	1.00	0.00			32					0
	Huron-Erie	Clay	1.00	0.00			28					0
		Walp	1.00	0.00			33					0
		Mitch	1.00	0.00			48					0
		Pech	1.00	0.00			41					0
		Ston	1.00	0.00			42					0
	Erie	Rond	1.00	0.00			36					0
		Long	1.00	0.00			43					0
		Put	1.00	0.00			25					0
	Ontario	Niag	1.00	0.00			44					0
		Brigh	1.00	0.00			24					0

Locus	Area	Site	p(1)	q(2)	r(3)	s(4)	n	H-obs	H-exp	F	chi	df
		Adol	1.00				23					0
		Howe	1.00				28					0
		SLan	1.00				28					0
	Florida	Blue	1.00				21					0
		Chass	1.00				17					0
		Hom	1.00				24					0
		StM	1.00				15					0
		Wak	1.00				10					0
ME-1	Huron	Thess	0.60	0.40			25	0.560	0.480	-0.167	0.694	1
		Blind	0.60	0.40			30	0.533	0.480	-0.111	0.370	1
		Serp	0.50	0.50			24	0.500	0.500	0.000	0	1
		Span	0.64	0.34	0.02		25	0.560	0.474	-0.180	0.814	1
		Bayf	0.63	0.37			26	0.654	0.466	-0.403	4.21*	1
		Key	0.55	0.45			28	0.607	0.495	-0.227	1.44	1
		Big C	0.42	0.58			32	0.531	0.487	-0.090	0.262	1
	Huron-Erie	Clay	0.52	0.48			28	0.464	0.499	0.070	0.137	1
		Walp	0.65	0.35			33	0.394	0.455	0.134	0.594	1
		Mitch	0.63	0.38			48	0.375	0.479	0.217	2.26	1

Locus	Area	Site	p(1)	q(2)	r(3)	s(4)	n	H-obs	H-exp	F	chi	df
		Pech	0.54	0.46			41	0.341	0.497	0.313	4.01*	1
		Ston	0.50	0.50			42	0.381	0.500	0.238	2.38	1
	Erie	Rond	0.68	0.32			36	0.472	0.435	-0.085	0.261	1
		Long	0.58	0.42			43	0.512	0.487	-0.050	0.108	1
		Put	0.66	0.34			25	0.600	0.449	-0.337	2.84	1
	Ontario	Niag	0.48	0.52			44	0.455	0.499	0.089	0.352	1
		Brigh	0.63	0.25	0.13		24	0.667	0.544	-0.226	2.45	3
		Adol	0.57	0.41	0.02		23	0.522	0.507	-0.030	0.021	1
		Howe	0.70	0.23	0.07		28	0.571	0.452	-0.264	3.89	3
		SLan	0.66	0.34			28	0.393	0.449	0.125	0.435	1
	Florida	Blue	0.45	0.07		0.48	21	0.333	0.562	0.407	6.96	3
		Chass	0.74	0.26			17	0.294	0.385	0.236	0.944	1
		Hom	0.60	0.31		0.08	24	0.458	0.518	0.115	0.629	3
		StM	0.60	0.13		0.27	15	0.267	0.550	0.515	7.97**	3
		Wak	0.50	0.50			10	0.400	0.500	0.200	0.400	1
IDH-1	Huron	Thess	0.68	0.32			25	0.640	0.435	-0.471	5.54*	1
		Blind	0.62	0.38			30	0.700	0.471	-0.486	7.07*	1
		Serp	0.60	0.40			24	0.708	0.480	-0.476	5.43*	1

Locus	Area	Site	p(1)	q(2)	r(3)	s(4)	n	H-obs	H-exp	F	chi	df
		Span	0.64	0.36			25	0.720	0.461	-0.563	7.91**	1
		Bayf	0.71	0.29			26	0.500	0.412	-0.214	1.19	1
		Key	0.61	0.39			28	0.714	0.476	-0.501	7.04**	1
		Big C	0.81	0.19			32	0.313	0.308	-0.015	0.007	1
	Huron-Erie	Clay	0.73	0.27			28	0.536	0.394	-0.359	3.61	1
		Walp	0.62	0.38			33	0.576	0.471	-0.222	1.63	1
		Mitch	0.63	0.38			48	0.583	0.479	-0.218	2.29	1
		Pech	0.67	0.33			41	0.366	0.442	0.173	1.22	1
		Ston	0.74	0.26			42	0.524	0.385	-0.361	5.48*	1
	Erie	Rond	0.64	0.36			36	0.611	0.461	-0.326	3.83	1
		Long	0.74	0.26			43	0.512	0.385	-0.330	4.67*	1
		Put	0.76	0.24			25	0.480	0.365	-0.316	2.49	1
	Ontario	Niag	0.51	0.49			44	0.659	0.500	-0.319	4.47*	1
		Brigh	0.65	0.35			24	0.708	0.455	-0.557	7.44**	1
		Adol	0.63	0.37			23	0.652	0.466	-0.399	3.66	1
		Howe	0.73	0.27			28	0.536	0.394	-0.359	3.61	1
		SLan	0.52	0.48			28	0.464	0.499	0.070	0.137	1
	Florida	Blue	0.86	0.14			21	0.190	0.241	0.209	0.917	1

Locus	Area	Site	p(1)	q(2)	r(3)	s(4)	n	H-obs	H-exp	F	chi	df
		Chass	0.44	0.56			17	0.529	0.493	-0.074	0.094	1
		Hom	0.79	0.21			24	0.417	0.332	-0.256	1.57	1
		StM	0.80	0.20			15	0.400	0.320	-0.250	0.937	1
		Wak	0.90	0.10			10	0.200	0.180	-0.111	0.123	1
6PGDH-1	Huron	Thess	0.50	0.50			25	0.600	0.500	-0.200	1	1
		Blind	0.58	0.42			30	0.500	0.487	-0.026	0.021	1
		Serp	0.60	0.40			24	0.542	0.480	-0.129	0.400	1
		Span	0.52	0.48			25	0.720	0.499	-0.442	4.89*	1
		Bayf	0.60	0.40			26	0.577	0.480	-0.202	1.06	1
		Key	0.48	0.52			28	0.607	0.499	-0.216	1.31	1
		Big C	0.56	0.44			32	0.563	0.493	-0.142	0.649	1
	Huron-Erie	Clay	0.55	0.45			28	0.607	0.495	-0.226	1.43	1
		Walp	0.52	0.48			33	0.424	0.499	0.151	0.749	1
		Mitch	0.55	0.45			48	0.396	0.495	0.200	1.92	1
		Pech	0.49	0.51			41	0.341	0.500	0.318	4.14*	1
		Ston	0.61	0.39			42	0.357	0.476	0.250	2.62	1
	Erie	Rond	0.46	0.54			36	0.417	0.497	0.161	0.929	1
		Long	0.56	0.44			43	0.372	0.493	0.245	2.58	1

Locus	Area	Site	p(1)	q(2)	r(3)	s(4)	n	H-obs	H-exp	F	chi	df
		Put	0.68	0.32			25	0.560	0.435	-0.287	2.06	1
	Ontario	Niag	0.43	0.57			44	0.409	0.490	0.165	1.21	1
		Brigh	0.65	0.35			24	0.708	0.455	-0.557	7.44**	1
		Adol	0.67	0.33			23	0.478	0.442	-0.082	0.153	1
		Howe	0.75	0.25			28	0.500	0.375	-0.333	3.11	1
		SLan	0.54	0.46			28	0.429	0.497	0.137	0.528	1
	Florida	Blue	0.95	0.05			21	0.000	0.095	1.000	21.0***	1
		Chass	1.00				17	0.000	0.000			0
		Hom	0.98	0.02			24	0.042	0.039			0
		StM	0.93	0.07			15	0.133	0.130	-0.024	0.009	1
		Wak	1.00				10	0.000	0.000			0
MDH-2	Huron	Thess	1.00				25	0.000	0.000			0
		Blind	1.00				30	0.000	0.000			0
		Serp	0.92	0.08			24	0.083	0.147	0.436	4.57*	1
		Span	0.96	0.04			25	0.120	0.077			0
		Bayf	0.96	0.04			26	0.000	0.077			0
		Key	0.95	0.05			28	0.107	0.095	-0.126	0.447	1
		Big C	1.00				32	0.000	0.000			0

Locus	Area	Site	p(1)	q(2)	r(3)	s(4)	n	H-obs	H-exp	F	chi	df
	Huron-Erie	Clay	1.00				28	0.000	0.000			0
		Walp	1.00				33	0.000	0.000			0
		Mitch	1.00				48	0.000	0.000			0
		Pech	1.00				41	0.000	0.000			0
		Ston	1.00				42	0.000	0.000			0
	Erie	Rond	1.00				36	0.000	0.000			0
		Long	1.00				43	0.000	0.000			0
		Put	1.00				25	0.000	0.000			0
	Ontario	Niag	0.98	0.02			44	0.045	0.039			0
		Brigh	1.00				24	0.000	0.000			0
		Adol	1.00				23	0.000	0.000			0
		Howe	1.00				28	0.000	0.000			0
		SLan	0.95	0.05			28	0.107	0.095	-0.126	0.447	1
	Florida	Blue	0.76	0.24			21	0.476	0.365	-0.305	1.95	1
		Chass	0.71	0.29			17	0.588	0.412	-0.428	3.11	1
		Hom	0.38	0.63			24	0.667	0.479	-0.393	3.71	1
		StM	0.97	0.03			15	0.067	0.058			0
		Wak	1.00				10	0.000	0.000			0

Locus	Area	Site	p(1)	q(2)	r(3)	s(4)	n	H-obs	H-exp	F	chi	df
PGI-1	Huron	Thess	0.88	0.08	0.04		25	0.200	0.218	0.081	0.164	1
		Blind	0.83	0.10	0.07		30	0.233	0.296	0.213	2.73	3
		Serp	0.85	0.13	0.02		24	0.250	0.260	0.039	0.037	1
		Span	0.92	0.08			25	0.160	0.147	-0.087	0.189	1
		Bayf	0.88	0.08	0.04		26	0.192	0.218	0.116	0.351	1
		Key	0.88	0.07	0.05		28	0.179	0.218	0.180	1.81	3
		Big C	0.91	0.08	0.02		32	0.188	0.185	-0.015	0.007	1
	Huron-Erie	Clay	0.79	0.16	0.05		28	0.321	0.348	0.077	0.333	3
		Walp	0.89	0.06	0.05		33	0.091	0.202	0.549	9.95*	3
		Mitch	0.81	0.14	0.05		48	0.292	0.322	0.093	0.823	3
		Pech	0.91	0.09			41	0.171	0.164	-0.044	0.079	1
		Ston	0.79	0.14	0.07		42	0.333	0.351	0.052	0.230	3
	Erie	Rond	0.85	0.08	0.07		36	0.222	0.266	0.166	1.99	3
		Long	0.86	0.10	0.03		43	0.233	0.230	-0.015	0.009	1
		Put	0.92	0.08			25	0.160	0.147	-0.087	0.189	1
	Ontario	Niag	0.84	0.11	0.05		44	0.227	0.280	0.189	1.57	1
		Brigh	0.83	0.10	0.06		24	0.167	0.278	0.398	7.62	3
		Adol	0.89	0.07	0.04		23	0.130	0.201	0.355	2.89	1

Locus	Area	Site	p(1)	q(2)	r(3)	s(4)	n	H-obs	H-exp	F	chi	df
		Howe	0.95	0.05			28	0.107	0.095	-0.126	0.447	1
		SLan	0.89	0.07	0.04		28	0.179	0.201	0.113	0.36	1
	Florida	Blue	0.95	0.05			20	0.100	0.095	-0.053	0.055	1
		Chass	0.94	0.06			17	0.118	0.113	-0.046	0.036	1
		Hom	0.88	0.10	0.02		24	0.208	0.215	0.033	0.027	1
		StM	0.97	0.03			15	0.067	0.058			0
		Wak	0.90	0.10			10	0.200	0.180	-0.111	0.123	1

Table B9. Confidence intervals (95%) for Wright's F-statistics: F_{IS}, F_{ST}, and F_{IT} for the different survey areas studied. Intervals were generated by bootstrapping values over loci using the Genetic Data Analysis program (Lewis, 2000), 5000 replicates were performed. If upper and lower values do not overlap with zero, than the F value is said to be significantly different from zero. Those ranges different from zero are indicated in bold.

	Survey area		F_{IS}	F_{ST}	F_{IT}
Ramets	Huron	Upper	**-0.2586**	**0.0417**	**-0.2083**
		Lower	**-0.4994**	**0.0047**	**-0.4577**
	Huron-Erie	Upper	0.2158	**0.0626**	0.2223
		Lower	-0.2898	**0.0083**	-0.2090
	Erie	Upper	0.0763	**0.0455**	0.0771
		Lower	-0.1522	**0.0002**	-0.1518
	Ontario	Upper	**-0.1028**	**0.0366**	**-0.0636**
		Lower	**-0.3735**	**0.0116**	**-0.3569**
	Great Lakes	Upper	**-0.0347**	**0.0615**	0.0269
		Lower	**-0.3350**	**0.0461**	-0.2558
	Florida	Upper	0.1705	**0.2961**	0.2524
		Lower	-0.3526	**0.0525**	-0.0205
	All sites	Upper	**-0.0454**	**0.1639**	0.0656
		Lower	**-0.3248**	**0.0770**	-0.1797

	Survey area		F_{IS}	F_{ST}	F_{IT}
Genets	Huron	Upper	0.0437	0.0153	0.0447
		Lower	-0.3014	-0.0080	-0.2857
	Huron-Erie	Upper	0.0808	**0.1096**	0.1411
		Lower	-0.1371	**0.0046**	-0.1228
	Erie	Upper	0.1111	0.0224	0.1295
		Lower	-0.2499	-0.0025	-0.2394
	Ontario	Upper	0.1169	**0.0514**	0.1330
		Lower	-0.2217	**0.0112**	-0.1795
	Great Lakes	Upper	0.0714	**0.0664**	0.1016
		Lower	-0.2216	**0.0053**	-0.1943
	Florida	Upper	0.3044	**0.2424**	**0.3596**
		Lower	-0.2384	**0.0478**	**0.0526**
	All sites	Upper	0.0563	**0.1669**	0.1604
		Lower	-0.2065	**0.0227**	-0.1407

Table B10. Allelomorph codes and frequencies at the Great Lake sites. All observed allelomorphs are included.

Allelo.	thes	blin	serp	span	bay	key	big	clay	walp	mitc	pech	ston	rond	long	put	niag	brig	adol	howe	slan
fbaaaaa	1	3	0	3	6	0	1	5	21	10	4	53	9	1	6	4	2	3	11	4
fbaaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
fbaaaca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fbaabaa	2	2	0	1	1	0	1	5	4	1	0	5	3	0	3	0	0	0	1	1
fbaacaa	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
fbabaaa	0	1	0	0	0	0	0	2	10	1	0	2	0	0	0	1	0	4	1	1
fbababa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fbabaca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fbabbaa	3	0	0	1	5	0	1	6	34	5	0	6	5	2	1	2	5	6	4	10
fbabbab	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
fbabbae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
fbabcaa	0	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0
fbabcac	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
fbacbaa	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
fbaccaa	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
fbbaaaa	4	0	0	0	2	0	3	1	4	0	1	7	0	1	3	0	2	2	3	0
fbbaaab	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
fbbaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fbbaaca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fbbabaa	4	4	0	0	5	0	7	1	2	0	1	1	0	0	4	0	1	0	5	0
fbbabab	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
fbbabac	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
fbbacaa	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
fbbbaaa	0	0	0	0	1	0	1	0	0	0	0	2	0	0	0	0	0	2	1	0
fbbbaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Allelo.	thes	blin	serp	span	bay	key	big	clay	walp	mitc	pech	ston	rond	long	put	niag	brig	adol	howe	slan
fbbbbaa	13	7	0	16	13	6	3	15	14	4	0	5	2	3	14	7	14	15	16	7
fbbbbab	0	0	0	1	0	0	0	3	0	0	0	0	0	0	1	1	0	0	1	0
fbbbbac	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
fbbbbae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
fbbbbaf	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
fbbbbba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
fbbbbbf	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
fbbbcaa	2	0	0	1	1	1	0	0	0	0	0	1	3	1	0	1	0	2	0	3
fbbbcab	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
fbbcbba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
fbbccaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
fbcbbaa	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
fbdaaaa	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0
fbdaaab	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
fbdaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fbdabaa	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0
fbdacaa	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
fbdacab	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
fbdbaaa	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
fbdbbaa	0	0	0	1	0	0	3	0	0	0	0	1	0	0	0	1	0	0	0	0
fbdbbab	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
fbdbcaa	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	3	0	0	0	0
fbdbcab	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
fbdccaa	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	4
fbdccab	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
fbdccaf	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
fbeaaaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Allelo.	thes	blin	serp	span	bay	key	big	clay	walp	mitc	pech	ston	rond	long	put	niag	brig	adol	howe	sian
fbebbaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
fbgaaab	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fbgaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbaaaaa	9	3	6	0	1	5	0	0	3	44	29	0	48	32	5	6	5	0	1	11
mbaaaab	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
mbaaaac	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
mbaaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbaabaa	2	0	0	1	0	1	0	0	0	3	7	0	7	3	1	0	0	0	1	4
mbaacaa	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0
mbabaaa	0	1	2	0	0	0	0	0	0	11	3	0	3	1	1	1	2	0	4	1
mbababa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbabbaa	12	3	12	6	1	6	0	0	4	46	13	0	12	11	0	2	13	0	7	9
mbabbab	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
mbabbac	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
mbabcaa	0	0	0	0	0	1	0	0	0	10	3	0	2	2	0	0	0	0	0	0
mbabcab	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
mbacaaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbacaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbacbaa	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
mbaccaa	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
mbaccab	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
mbbaaaa	2	1	1	0	1	2	1	0	0	9	6	0	5	10	5	3	0	0	1	1
mbbaaab	0	0	1	0	0	0	0	0	0	2	1	0	0	0	1	0	0	0	0	0
mbbaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbbabaa	1	7	2	1	1	2	3	0	0	3	2	0	3	3	2	2	3	0	0	0
mbbabab	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
mbbabca	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Allelo.	thes	blin	serp	span	bay	key	big	clay	walp	mitc	pech	ston	rond	long	put	niag	brig	adol	howe	slan
mbbacaa	0	0	0	0	0	0	4	0	0	0	0	0	0	2	0	0	0	0	0	0
mbbbaaa	0	1	1	0	0	1	0	0	0	1	0	0	2	0	1	0	0	0	0	0
mbbbaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbbbbaa	16	8	27	26	1	18	3	0	8	32	3	0	11	5	7	3	28	1	13	7
mbbbbab	1	1	2	1	0	1	0	0	0	11	0	0	5	1	1	1	2	0	0	0
mbbbbac	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1
mbbbbae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbbbbaf	1	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0
mbbbbba	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbbbbbb	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbbbbcb	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbbbcaa	3	0	0	1	0	3	1	0	2	4	0	0	5	3	0	2	0	0	0	0
mbbbcab	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
mbbccab	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
mbcbbaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0
mbcbbab	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
mbdaaaa	0	0	0	0	0	0	1	0	0	4	2	0	0	2	0	0	0	0	0	0
mbdaaab	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
mbdaaaf	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
mbdabaa	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
mbdabab	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
mbdbaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbdbbaa	1	0	4	1	0	1	0	0	0	5	1	0	0	1	1	1	0	0	0	0
mbdbbab	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbdbbaf	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbdbbba	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbdbcaa	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	2	0	0	0	0

Allelo.	thes	blin	serp	span	bay	key	big	clay	walp	mitc	pech	ston	rond	long	put	niag	brig	adol	howe	slan
mbdbcab	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
mbdcaab	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbdcbab	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
mbdccaa	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0
mbdccab	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
mbebbab	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
mbfaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbgaaaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mbgaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbaaaaa	4	3	7	6	7	3	1	13	27	22	31	205	35	43	2	5	6	11	11	5
vbaaaab	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
vbaaaac	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
vbaaaae	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0
vbaaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbaaaca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbaabaa	0	0	0	2	6	0	0	6	8	4	9	23	5	6	1	3	0	3	3	1
vbabaaa	0	1	2	2	0	0	0	3	5	1	3	1	0	1	0	4	1	4	2	1
vbababa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbabaca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbabbaa	2	1	6	8	11	3	0	11	29	16	12	33	13	7	3	6	1	7	5	6
vbabcaa	0	2	0	0	0	0	0	0	2	6	5	5	3	3	0	1	0	0	0	0
vbabcab	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
vbacaaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbacaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbaccaa	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
vbbaaaa	0	0	0	0	5	0	2	1	6	5	7	22	1	13	0	1	0	0	0	0
vbbaaab	0	0	0	0	1	0	0	0	0	0	1	5	0	2	0	0	0	0	1	0

Allelo.	thes	blin	serp	span	bay	key	big	clay	walp	mitc	pech	ston	rond	long	put	niag	brig	adol	howe	slan
vbbaaac	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
vbbaaaf	0	0	0	0	0	0	0	1	0	0	0	2	0	1	0	0	0	0	0	0
vbbaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbbaabb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbbaabf	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbbaacb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbbabaa	0	4	0	1	7	0	8	4	1	0	5	4	1	2	2	0	0	2	3	0
vbbabab	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0
vbbabad	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
vbbabaf	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
vbbabba	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbbacaa	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
vbbbaaa	0	1	1	4	0	1	1	0	1	1	1	3	0	1	1	2	0	3	2	1
vbbbaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbbbabb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbbbacb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbbbbaa	22	22	7	29	27	11	1	28	36	9	4	17	7	10	8	12	7	32	16	4
vbbbbab	1	6	1	1	1	0	1	7	1	3	1	7	3	0	0	1	0	1	0	0
vbbbbac	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
vbbbbad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
vbbbbae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
vbbbbaf	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
vbbbbba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
vbbbbca	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbbbcaa	1	5	0	1	1	3	0	3	2	1	1	7	6	5	1	3	0	0	0	0
vbbbcab	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
vbbbcaf	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0

Allelo.	thes	blin	serp	span	bay	key	big	clay	walp	mitc	pech	ston	rond	long	put	niag	brig	adol	howe	slan
vbbcbaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
vbbccaa	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0
vbcaaaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0
vbcbaaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
vbcbbaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
vbdaaaa	0	0	0	0	0	0	4	0	1	1	3	26	0	5	0	0	0	1	0	0
vbdaaab	0	0	0	0	0	0	0	0	0	0	0	6	0	1	0	0	0	0	0	0
vbdaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbdabaa	0	0	0	0	0	0	2	1	0	0	0	9	0	0	0	0	0	0	0	0
vbdacaa	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
vbdacab	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
vbdbaaa	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0
vbdbaab	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
vbdbbaa	0	1	0	0	0	1	0	1	0	1	3	10	0	4	0	0	0	3	0	0
vbdbbab	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0
vbdbcaa	0	0	0	0	0	0	0	1	0	0	0	8	3	1	0	3	0	0	0	0
vbdbcab	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0
vbdbcaf	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
vbdccaa	0	2	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0
vbdccab	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
vbfaaaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbfaaab	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbfaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbgaaaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbgaaaf	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbgaaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbgaabb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Allelo.	thes	blin	serp	span	bay	key	big	clay	walp	mitc	pech	ston	rond	long	put	niag	brig	adol	howe	slan
vbgbaaa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vbgbaba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table B11. Allelomorph code and frequencies for the five Florida sites. All observed allelomorphs are included.

Allelo	blue	chas	hom	stm	wak
fbaaaaa	0	0	0	8	13
fbaaaba	0	0	7	0	0
fbaaaca	0	0	2	0	0
fbaabaa	0	0	0	0	0
fbaacaa	0	0	0	0	0
fbabaaa	0	0	0	2	1
fbababa	0	0	2	0	0
fbabaca	0	0	1	0	0
fbabbaa	0	0	0	0	0
fbabbab	0	0	0	0	0
fbabbae	0	0	0	0	0
fbabcaa	0	0	0	0	0
fbabcac	0	0	0	0	0
fbacbaa	0	0	0	0	0
fbaccaa	0	0	0	0	0
fbbaaaa	0	0	0	0	1
fbbaaab	0	0	0	0	1
fbbaaba	0	0	9	0	0
fbbaaca	0	0	1	0	0
fbbabaa	0	0	0	0	0
fbbabab	0	0	0	0	0
fbbabac	0	0	0	0	0
fbbacaa	0	0	0	0	0
fbbbaaa	0	0	0	0	0
fbbbaba	0	0	4	0	0
fbbbbaa	0	0	0	0	0
fbbbbab	0	0	0	0	0
fbbbbac	0	0	0	0	0
fbbbbae	0	0	0	0	0
fbbbbaf	0	0	0	0	0
fbbbbba	0	0	1	0	0
fbbbbbf	0	0	0	0	0
fbbbcaa	0	0	0	0	0
fbbbcab	0	0	0	0	0
fbbcbba	0	0	0	0	0
fbbccaa	0	0	0	0	0
fbcbbaa	0	0	0	0	0
fbdaaaa	0	0	0	0	1
fbdaaab	0	0	0	0	1
fbdaaba	0	0	1	0	0
fbdabaa	0	0	0	0	0
fbdacaa	0	0	0	0	0
fbdacab	0	0	0	0	0
fbdbaaa	0	0	0	0	0
fbdbbaa	0	0	0	0	0
fbdbbab	0	0	0	0	0

Allele	blue	chas	hom	stm	wak
fbdbcaa	0	0	0	0	0
fbdbcab	0	0	0	0	0
fbdccaa	0	0	0	0	0
fbdccab	0	0	0	0	0
fbdccaf	0	0	0	0	0
fbeaaaa	0	0	0	0	0
fbebbaa	0	0	0	0	0
fbgaaab	0	0	0	1	0
fbgaaba	0	0	0	1	0
mbaaaaa	3	0	0	21	0
mbaaaab	0	0	0	0	0
mbaaaac	0	0	0	0	0
mbaaaba	11	0	0	0	0
mbaabaa	0	0	0	0	0
mbaacaa	0	0	0	0	0
mbabaaa	0	9	0	3	0
mbababa	0	7	0	0	0
mbabbaa	0	0	0	2	0
mbabbab	0	0	0	0	0
mbabbac	0	0	0	0	0
mbabcaa	0	0	0	0	0
mbabcab	0	0	0	0	0
mbacaaa	0	3	0	0	0
mbacaba	0	1	0	0	0
mbacbaa	0	0	0	0	0
mbaccaa	0	0	0	0	0
mbaccab	0	0	0	0	0
mbbaaaa	2	0	0	3	0
mbbaaab	0	0	0	0	0
mbbaaba	0	1	0	0	0
mbbabaa	0	0	0	0	0
mbbabab	0	0	0	0	0
mbbabca	0	0	0	0	0
mbbacaa	0	0	0	0	0
mbbbaaa	0	0	0	1	0
mbbbaba	1	3	1	0	0
mbbbbaa	0	0	0	0	0
mbbbbab	0	0	0	0	0
mbbbbac	0	0	0	0	0
mbbbbae	0	0	0	0	0
mbbbbaf	0	0	0	0	0
mbbbbba	0	0	0	0	0
mbbbbbb	0	0	0	0	0
mbbbbcb	0	0	0	0	0
mbbbcaa	0	0	0	0	0
mbbbcab	0	0	0	0	0
mbbccab	0	0	0	0	0
mbcbbaa	0	0	0	0	0
mbcbbab	0	0	0	0	0
mbdaaaa	0	0	0	0	0

Allelo	blue	chas	hom	stm	wak
mbdaaab	0	0	0	0	0
mbdaaaf	0	0	0	0	0
mbdabaa	0	0	0	0	0
mbdabab	0	0	0	0	0
mbdbaba	0	2	0	0	0
mbdbbaa	0	0	0	0	0
mbdbbab	0	0	0	0	0
mbdbbaf	0	0	0	0	0
mbdbbba	0	0	0	0	0
mbdbcaa	0	0	0	0	0
mbdbcab	0	0	0	0	0
mbdcaab	0	1	0	0	0
mbdcbab	0	0	0	0	0
mbdccaa	0	0	0	0	0
mbdccab	0	0	0	0	0
mbebbab	0	0	0	0	0
mbfaaba	3	0	0	0	0
mbgaaaa	2	0	0	2	0
mbgaaba	1	0	0	0	0
vbaaaaa	8	1	0	49	19
vbaaaab	0	0	0	0	0
vbaaaac	0	0	0	0	0
vbaaaae	0	0	0	0	0
vbaaaba	11	2	15	0	0
vbaaaca	0	0	3	0	0
vbaabaa	0	0	0	0	0
vbabaaa	0	12	1	2	0
vbababa	1	9	8	0	0
vbabaca	0	0	1	0	0
vbabbaa	0	0	0	0	0
vbabcaa	0	0	0	0	0
vbabcab	0	0	0	0	0
vbacaaa	0	5	0	0	0
vbacaba	0	4	0	0	0
vbaccaa	1	0	0	0	0
vbbaaaa	1	0	0	0	6
vbbaaab	0	0	0	0	0
vbbaaac	0	0	0	0	0
vbbaaaf	0	0	0	0	0
vbbaaba	0	0	15	0	0
vbbaabb	0	0	2	0	0
vbbaabf	0	0	1	0	0
vbbaacb	0	0	1	0	0
vbbabaa	0	0	0	1	0
vbbabab	0	0	0	0	0
vbbabad	0	0	0	0	0
vbbabaf	0	0	0	0	0
vbbabba	0	0	0	0	0
vbbacaa	0	0	0	0	0
vbbbaaa	0	7	0	3	1

Allele	blue	chas	hom	stm	wak
vbbbaba	0	2	4	0	0
vbbbabb	0	1	0	0	0
vbbbacb	0	0	1	0	0
vbbbbaa	0	0	0	0	0
vbbbbab	0	0	0	0	0
vbbbbac	0	0	0	0	0
vbbbbad	0	0	0	0	0
vbbbbae	0	0	0	0	0
vbbbbaf	0	0	0	0	0
vbbbbba	0	0	0	0	0
vbbbbca	0	0	0	0	0
vbbbcaa	0	0	0	0	0
vbbbcab	0	0	0	0	0
vbbbcaf	0	0	0	0	0
vbbcbaa	0	0	0	0	0
vbbccaa	0	0	0	0	0
vbcaaaa	0	0	0	0	0
vbcbaaa	0	0	0	0	0
vbcbbaa	0	0	0	0	0
vbdaaaa	0	0	0	0	2
vbdaaab	0	0	0	0	0
vbdaaba	0	0	1	0	0
vbdabaa	0	0	0	0	0
vbdacaa	0	0	0	0	0
vbdacab	0	0	0	0	0
vbdbaaa	0	0	0	0	0
vbdbaab	0	0	0	0	0
vbdbbaa	0	0	0	0	0
vbdbbab	0	0	0	0	0
vbdbcaa	0	0	0	0	0
vbdbcab	0	0	0	0	0
vbdbcaf	0	0	0	0	0
vbdccaa	0	0	0	0	0
vbdccab	0	0	0	0	0
vbfaaaa	2	0	0	0	0
vbfaaab	1	0	0	0	0
vbfaaba	1	0	0	0	0
vbgaaaa	1	0	0	8	0
vbgaaaf	1	0	0	0	0
vbgaaba	1	0	4	0	0
vbgaabb	1	0	1	0	0
vbgbaaa	1	0	0	0	0
vbgbaba	1	0	0	0	0

Appendix C

Site Information

General site data

Table C1. Sites sampled in the Great Lakes and Florida, with frequency of flowering data presented. Sex ratio relates to flowering shoots.

Site	Survey	Frequency males	Frequency females	Flowering frequency	Sex Ratio
Thessalon	Lake Huron	0.228	0.165	0.393	0.949
Blind River	Lake Huron	0.173	0.202	0.375	1.03
Serpent River	Lake Huron	0.735	0.000	0.735	0.576
Spanish Harbour	Lake Huron	0.226	0.112	0.338	0.907
Bayfield Inlet	Lake Huron	0.025	0.129	0.153	1.10
Key River	Lake Huron	0.320	0.085	0.405	0.822
Big Chute	Lake Huron	0.149	0.163	0.312	1.01
Clay Creek	Huron-Erie	0.002	0.255	0.257	1.25
Walpole Island	Huron-Erie	0.047	0.415	0.462	1.35
Mitchell's Bay	Huron-Erie	0.649	0.064	0.714	0.645
Peche Isle	Huron-Erie	0.499	0.043	0.543	0.696
Stoney Island	Huron-Erie	0	0.154	0.154	1.15
Rondeau Bay	Lake Erie	0.414	0.099	0.513	0.778
Long Point Bay	Lake Erie	0.342	0.036	0.378	0.772
Put-in-Bay	Lake Erie	0.368	0.263	0.632	0.923
Niagara River	Lake Ontario	0.203	0.145	0.349	0.952
Brighton	Lake Ontario	0.410	0.156	0.566	0.819
Adolphustown	Lake Ontario	0.031	0.117	0.148	1.08

Site	Survey	Frequency males	Frequency females	Flowering frequency	Sex Ratio
Howe Island	Lake Ontario	0.158	0.191	0.349	1.03
South Lancaster	Lake Ontario	0.286	0.170	0.456	0.910
Blue Springs	Florida	0.238	0.000	0.238	0.808
Chassahowitza	Florida	0.193	0.000	0.193	0.838
Homosassa	Florida	0.008	0.278	0.286	1.27
St. Marks River	Florida	0.240	0.111	0.351	0.896
Wakulla River	Florida	0.000	0.249	0.249	1.25

Table C2. Sampling information for the sites, including the number of 1-m^2-quadrats sampled, the number of ramets removed from each site and the estimated density of ramets (shoots) per m^2.

Site	No. of quadrats	No. of ramets	Density /m^2
Thessalon	10	728	291.2
Blind River	10	341	136.4
Serpent River	10	381	152.4
Spanish Harbour	10	770	308.0
Bayfield Inlet	10	1259	503.6
Key River	10	375	150.0
Big Chute	8	1100	550.0
Clay Creek	n/a	480	761.9
Walpole Island	n/a	1046	536.4
Mitchell's Bay	n/a	1062	351.1
Peche Isle	n/a	1403	746.3
Stoney Island	n/a	1164	1012
Rondeau Bay	n/a	1339	847.5
Long Point Bay	n/a	1730	660.3
Put-in-Bay	10	437	174.8
Niagara River	10	978	391.2
Brighton	10	424	169.6
Adolphustown	10	1124	449.6
Howe Island	8	1121	560.5
South Lancaster	8	1028	514.0
Blue Springs	8	538	269.0
Chassahowitza	n/a	482	n/a
Homosassa	n/a	378	n/a
St. Marks River	n/a	388	n/a
Wakulla River	n/a	325	n/a

Site descriptions: population genetics study

Site name:	Thessalon, ON
Abbreviation:	Thess
Associated Water body	North Channel, Lake Huron
Longitude/latitude:	83°26' / 46°16'
Geographic Description:	The west shore of river was sampled from Hwy 17B bridge south to the mouth. Quadrats were spaced 20 m apart along one transect.
Limnological characteristics:	Water was murky, with clarity to 40-50 cm. The bottom was steeply sloped. Water flow was strong with considerable boat disturbance. The river was c. 30-40 m wide.
Biological characteristics:	*V. americana* plants were long-leaved, and grew in scattered patches along a narrow band, and comprised >80% of the vegetation.

Site name:	Blind River, ON
Abbreviation:	Blind
Associated Water body:	North Channel, Lake Huron
Longitude/latitude:	82°57' / 46°11'
Geographic Description:	The east shore of river, behind the secondary school was sampled. Quadrats were spaced 20 m apart along one transect
Limnological characteristics:	Water was clear to bottom throughout sampling area (up to 1 m). Water flow strong, with little to no disturbance. The river was c. 100 m wide.
Biological characteristics:	Plants grew in scattered patches, often only scattered shoots. Sediments were sandy and rocky. *V. americana* accounted for approximately 40% of vegetation. *V. americana* shoots were small, 20-50 cm tall at all depths and the leaves were red and curled

Site name:	Serpent River, ON
Abbreviation:	Serp
Associated Water body:	North Channel, Lake Huron
Longitude/latitude:	82°33' / 46°13'
Geographic Description:	The site was sampled 1-2 km from the mouth of river. One long transact was taken parallel to the shore, with quadrats spaced 5 m apart.
Limnological characteristics:	Water was clear to c. 60 cm. Water flows were strong, with some boat traffic. The river was 40-50 m wide.
Biological characteristics:	*V. americana* (c. 70%)and other macrophytes grew in a narrow band along the shore which dropped rapidly. *V. americana* plants were large and grew densely.

Site name:	Spanish Harbour, ON
Abbreviation:	Span
Associated Water body:	North Chanel, Lake Huron
Longitude/latitude:	82°21' / 46°11'
Geographic Description:	The site was sampled at the south end of harbour. Two perpendicular transects were taken with five quadrats in each spaced at 20 m.
Limnological characteristics:	Many old logs and other debris were strewn across the bottom of the bay throughout the area. Water clear to all depths sampled (c. 1.5 m). Strong waves with very little other disturbance.
Biological characteristics:	Small bay was heavily vegetated with a number of species, c. 80% of which was *V. americana*. *V. americana* grew densely. Shoots were large with many leaves.

Site name:	Bayfield Inlet, ON
Abbreviation:	Bayf
Associated Water body:	Georgian Bay, Lake Huron
Longitude/latitude:	80°30' / 45°37'
Geographic Description:	The site was a long, narrow inlet off Georgian Bay, 20-30 m wide at the sampling point. Quadrats were sampled 10m apart along one long transect roughly parallel to shore.
Limnological characteristics:	Water was fairly clear to 60-80 cm. Boats responsible for majority of disturbance and water flow. The substrate was rocky.
Biological characteristics:	Vegetation grew in low density with a patchy distribution. *V. americana* comprised c. 80% of the vegetation. Shoots were generally small.

Site name:	Key River, ON
Abbreviation:	Key
Associated Water body:	Lake Huron
Longitude/latitude:	80°33' / 45°54'
Geographic Description:	The site was a long, narrow, navigable inlet extending east past hwy 69. Two transects were taken, one on either side of the disused boat ramp with quadrats spaced 5m apart.
Limnological characteristics:	Bottom was muddy further out; sandy near the shore. Water flow was fairly strong flow. Water clarity extended to 50-60cm.
Biological characteristics:	*V. americana* grew in medium density in a small meadow. Plants were of a good size. *V. americana* accounted for >90% of the vegetation.

Site name:	Big Chute, ON
Abbreviation:	Big C
Associated Water body:	Trent/ Severn waterway
Longitude/latitude:	79°40' / 44°53'
Geographic Description:	Located along the Trent/ Severn waterway, the site was part of a river, with a hydro dam at the former waterfall which has created an extensive lake above the dam. Three transects were taken along the south shore of the lake, the first was parallel to the shore with two quadrats 10m apart, two further transects parallel to the road were taken with two quadrats spaced at 15m.
Limnological characteristics:	Flow was fairly perceptible due to the drop in elevation. The sediment was muddy. There was some disturbance due to the the docking of boats. Water was clear to 60-70cm
Biological characteristics:	Plants grew in high density with leaves reaching the water surface. *V. americana* accounted for >80% of the vegetation.

Site name:	Clay Creek, ON
Abbreviation:	Clay
Associated Water body:	St. Clair River
Longitude/latitude:	82°28' / 42°47'
Geographic Description:	The site was a small creek off the St. Clair River, south of Sarnia, ON. Contiguous plants were sampled along two 20 cm wide transects.
Limnological characteristics:	Substrate was clay and rock. Water flow was slow. Water clarity was to the sediment, up to 1 m. The creek was narrow, c. 30 m wide.
Biological characteristics:	Patchy distribution of plants, mostly *V. americana* (>90%). High water clarity resulting in short statured plants with reddish leaves.

Site name:	Walpole Island, ON
Abbreviation:	Walpole
Associated Water body:	Lake St. Clair
Longitude/latitude:	82°27' / 42°30'
Geographic Description:	Site was located at south end of Island, close to hunting lodge, in Chanel Escarte. Contiguous plants were sampled along two 20 cm wide transects.
Limnological characteristics:	Substrate was loose and sandy. Water clarity to c. 60 cm. Water flow was moderate.
Biological characteristics:	Lush, dense meadows of *V. americana* (>80%) grew with some other species present.

Site name:	Mitchell's Bay, ON
Abbreviation:	Mitchell
Associated Water body:	Lake St. Clair
Longitude/latitude:	82°24' / 42°29'
Geographic Description:	The site was situated within a boat channel leading to Bass Haven. The channel was narrow (c. 30 m wide) with vegetation growing along steep banks. Contiguous plants were sampled along two 20 cm wide transects.
Limnological characteristics:	High turbidity and water flow was experienced due to boat traffic. Ovehanging vegetation on north side where sampling was done provided of accumulated decaying leaf matter.
Biological characteristics:	*V. americana* accounted for >90% of the vegetation. Plants grew tall and vigorously in dense meadows.

Site name:	Peche Island, ON
Abbreviation:	Peche
Associated Water body:	Detroit River
Longitude/latitude:	82°59' / 42°21'
Geographic Description:	Peche Island is situated where Lake St. Clair meets the Detroit River. The study site was within the waterway which meanders through the island. Contiguous plants were sampled along two 20 cm wide transects.
Limnological characteristics:	The site with shady with moderate to low water flow. The substrate soft and sandy, and the water relatively murky, with clarity o c. 30 cm..
Biological characteristics:	Dense plant growth, with large *V. americana* shoots, which accounted for >90% of the vegetation.

Site name:	Stoney Island, MI
Abbreviation:	Stoney
Associated Water body:	Detroit River
Longitude/latitude:	83°8 / 42°10'
Geographic Description:	The site was the Bay of Stoney Island, facing Grosse Isle, MI. Contiguous plants were sampled along two 20 cm wide transects.
Limnological characteristics:	Water flow was high, and clarity was to c. 2 m. The site was shallow throughout (less than 3 m) and the substrate was stoney substrate.
Biological characteristics:	*V. americana* grew in patches in along the shore, with increasing density as water depth increased. *V. americana* accounted for >80% of the vegetation, and was of moderate size.

Site name:	Rondeau Bay, ON
Abbreviation:	Rondeau
Associated Water body:	Lake Erie
Longitude/latitude:	81°55' / 42°18'
Geographic Description:	The Bay was sampled along bank by the 'Somewhere Else' cafe. Contiguous plants were sampled along two 20 cm wide transects.
Limnological characteristics:	Sbstrate was stoney. Water flow was rough due to weather conditions.
Biological characteristics:	Few other species grew at the site. Growth of *V. americana* was patchy, and plants were of moderate size.

Site name:	Long Point Bay, ON
Abbreviation:	Long Point
Associated Water body:	Lake Erie
Longitude/latitude:	80°26' / 42°35'
Geographic Description:	The site was situated along an enclosed area of the bay, leading to the Provincial Park. Contiguous plants were sampled along two 20 cm wide transects.
Limnological characteristics:	Substrate was sandy. Water flow was moderate and clarity was to c. 80 cm.
Biological characteristics:	The site was a large duck staging area, especially Canvasbacks. *V. americana* growth was dense and lush, with shoots accounting for >90% of the vegetation.

Site name:	Put-in-Bay, OH
Abbreviation:	Put
Associated Water body:	Lake Erie
Longitude/latitude:	
Geographic Description:	The site was on South Bass Island in Put-in-Bay, Lake Erie. A small bay of the island was sampled. Quadrats were sampled along two transects parallel to shore, spaced c. 15 m apart.
Limnological characteristics:	Water clarity was to the bottom at all depths (up to 1.5 m). There was little water movement. The substrate was a cobble bottom.
Biological characteristics:	Many zebra mussels and ducks were observed at the area. Algae growth around *V. americana* shoots was abundant. *V. americana* shoots had very long leaves, grew lushly and many formed seed pods were present.

Site name:	Niagara River, ON
Abbreviation:	Niag
Associated Water body:	Lake Ontario
Longitude/latitude:	79°1' / 43°2'
Geographic Description:	The west side of the river was sampled near Fort Erie, ON. Two transects were taken parallel to shore, with quadrats spaced 20m apart. The river was fairly wide (c. 250 m) between the mainland and Grand Island, NY.
Limnological characteristics:	Water was murky with lots of suspended sediment. Water flow was very strong, somewhat dampened in weed beds. The sediment was sandy and firm.
Biological characteristics:	There was a wide shallow area heavily vegetated all along the shore; more than 95% *V. americana* growing in high density. Shoots were of average size.

Site name:	Brighton, ON
Abbreviation:	Brigh
Associated Water body:	Lake Ontario
Longitude/latitude:	77°43' / 44°1'
Geographic Description:	A small inlet on the north shore of Presqu'ile Bay was sampled. The inlet was quite shallow and about 50 m wide. Quadrats were sampled along two transects.
Limnological characteristics:	There was a very thick layer of black muck over the sediment, at least 30cm deep. Water clarity was good to over 1m, while the water flow was moderate.
Biological characteristics:	All plants were larger than normal, stolons were up to 43.5 cm long and the inter-ramet stolon length was routinely 20 cm or more. *Vallisneria* grew denselt, partly due to size of plants and accounted for >90% of the vegetation.

Site name:	Adolphustown, ON
Abbreviation:	Adol
Associated Water body:	Lake Ontario
Longitude/latitude:	77°3' / 44°3'
Geographic Description:	The site was in the Bay of Quinte at the Glenora Ferry Dock on Hwy 33. One long transect was taken parallel to shore with quadrats 20 m apart. The bay was c. 500 m wide.
Limnological characteristics:	Good water clarity up to 1 m. There was lots of wave action and strong water flow. The substrate was cobble/ sand, with lots of rocks.
Biological characteristics:	*V. americana* grew well but sparsely, and accounted for 90% of the vegetation.

Site name:	Howe Island, ON
Abbreviation:	Howe
Associated Water body:	Lake Ontario
Longitude/latitude:	76°19' / 44°16'
Geographic Description:	This site lies in the Bateau Channel between Howe Island and the mainland. The area around the Ferry Dock at the end of Frontenac County Rd 16 was sampled. Two parallel transects were taken, four quadrats were sampled per transect. Quadrats were taken10m apart.
Limnological characteristics:	Water clarity was good up to 1m. Wave motion was entense, up to 2.5 m without much wind. Sediment was sandy and compacted.
Biological characteristics:	*V. americana* was dominant, with greater than 90% frequency in meadows. Shoot density was high, plants were of average size. An abundance of zebra mussels was observed.

Site name:	South Lancaster, ON
Abbreviation:	SLan
Associated Water body:	Lake St. Louis, St. Lawrence Seaway
Longitude/latitude:	74°30' / 45°8'
Geographic Description:	The site was at a boat ramp into Lac St. Louis. Two perpendicular transects were sampled, with quadrats spaced 15 m apart.
Limnological characteristics:	Water clarity was excellent up to c. 2 m. There was little perceptible water flow. The substrate was sandy with scattered "cobble-sized" rocks. There was some disturbance near shore where vegetation has been removed for swimming.
Biological characteristics:	Overall vegetation consisted of 80-90% of *V. americana* in meadows, although there were patches where *Najas* dominated. All parts of *V. americana* plants were big; leaves grew lushly to the water surface.

Site name:	Blue Springs, FL
Abbreviation:	Blue
Associated Water body:	Santa Fe River, flows to Suwanee and Gulf of Mexico
Longitude/latitude:	
Geographic Description:	Spring run on south side of the Santa Fe River west of High Springs. The site was within 50 m of the spring boil hole. Eight quadrats were sampled along a transect at the the edge of a cleared area.
Limnological characteristics:	Water was crystal clear to at least 5 m. Water flow was quite brisk down the spring run. The sediment was pure sand.
Biological characteristics:	*V. americana* covered the middle of the spring except where cleared for swimmers. Shoots were huge with leaves 2-3 cm wide and stolons up to 7-8mm thick, and plants had woody "root stalks". Plant size made density deceptive, ramet density was low although shoot growth was thick. In the spring head *V. americana* accounted for 75% of the vegetation, while further down the run, its frequency was reduced to c. 10%.

Site name:	Chassahowitzka, FL
Abbreviation:	Chass
Associated Water body:	Gulf of Mexico
Longitude/latitude:	
Geographic Description:	Chassahowitzka spring flows out to the Gulf of Mexico along the eastern shore of Northen Florida. The sample site was c. 5 km in from the gulf, and c. 200-300 m downstream from the boil hole. The channel was c. 20 m wide at the sampling site. Large 'grabs' of plants, at least 5 m apart, were taken scattered throughout the *V. americana* meadow,.
Limnological characteristics:	Water clarity was good to c. 1.5 m. Water flow was strong. There was a layer of flotsam over the sandy substrates. Boats caused some disturbance.
Biological characteristics:	*V. americana* comprised approximately 90% of vegetation. Shoots were big and grew lushly, leaves were thick and wide.

Site name:	Homosassa, FL
Abbreviation:	Hom
Associated Water body:	Gulf of Mexico
Longitude/latitude:	
Geographic Description:	The Homossasa River was sampled c. 2 km from its mouth at the Gulf of Mexico. Non-transect/ non-quadrat, widely scattered grab samples were taken from the hazardous alligator infested river.
Limnological characteristics:	Tides influence this river. The water was clear with a strong flow. The sediment was firm and sandy.
Biological characteristics:	Where *V. americana* occurred it was a solid patch but other vegetation predominated, *V. americana* accounting for <50% of the vegetation. The shoots were large.

Site name:	St. Marks River, FL
Abbreviation:	StM
Associated Water body:	northern shore of Gulf of Mexico
Longitude/latitude:	
Geographic Description:	The spring run river was sampled on the south side. 'Grabs' of plants were taken every 5-10 m along one transect.
Limnological characteristics:	Water flow was moderate. The substrate was firm and sandy. Water clarity to c. 1 m.
Biological characteristics:	Plants were intermediate in size, and grew in patches. *V. americana* comprised c. 70% of the vegetation.

Site name:	Wakulla, FL
Abbreviation:	Wak
Associated Water body:	St. Marks River; Gulf of Mexico
Longitude/latitude:	
Geographic Description:	Another spring run river sampled on the east side of a south flowing river, c. 2 km from the biol hole.
Limnological characteristics:	The site had very strong water flow and high water clarity. The substrate was soft and silty.
Biological characteristics:	*V. americana* shoots were large, and grew lushly. Leaves were often 2 and 3m in length. *V. americana* accounted for >80% of the vegetation.

Appendix D

Glossary

allele Alternative form of a gene.

allelomorph Multilocus allozyme phenotype.

allozyme Form of an enzyme, controlled by alleles of the same locus, that differ in electrophoretic mobility.

effective number of alleles (A_e) The number of equally frequent alleles in an ideal population that would be required to produce the same homozygosity in an actual population.

effective population size (N_e) The number of reproducing individuals in a population. The number of individuals in an ideal population with the same variance in allele frequencies or level of inbreeding as observed in the actual population.

gene flow The exchange of genes (in one or both directions) at low rate between populations, due to the dispersal of gametes or of individuals from one population to another; also called migration.

genetic drift Variation in gene frequency from one generation to another due to chance fluctuations.

genet A genetic individual, often comprising a number of clonal shoots or ramets.

HWE Hardy-Weinberg Equilibrium. A principle by which genotypic frequencies can be predicted on the basis of gene frequencies, under the assumption of random mating.

isozyme Different electrophoretic forms of the same enzyme, with differences attributed to differing subunit configurations rather than allelic differences (see allozyme).

ISSR Inter-simple sequence repeat. A technique used to detect genetic differences between samples using segments of amplified DNA.

locus The position of a gene on a chromosome.

ramet An individual belonging to a clone.

RAPD Randomly amplified polymorphic DNA. A technique used to detect genetic differences between samples using segments of amplified DNA.

selection The differential reproduction of alternative genotypes due to variable fitness.

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