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**A tradeoff between sexual and asexual reproduction in the
dioecious clonal macrophyte *Vallisneria americana*: Environmental
and genetic influences**

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State University of New York at Binghamton, 1994

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A TRADEOFF BETWEEN SEXUAL AND ASEXUAL REPRODUCTION
IN THE DIOECIOUS CLONAL MACROPHYTE VALLISNERIA AMERICANA:
ENVIRONMENTAL AND GENETIC INFLUENCES

BY

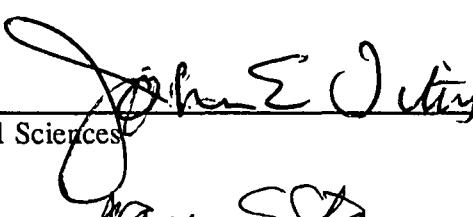
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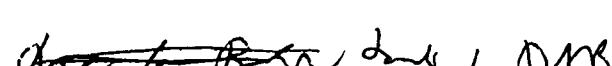
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ABSTRACT

Life history theory has long predicted that tradeoffs should exist between sexual and asexual reproduction in clonal organisms. Preliminary research on the submersed aquatic macrophyte *Vallisneria americana* suggested that in females from a single population grown in the greenhouse, the production of fruit and tubers (the asexual propagule and sole perennating organ) was inversely related. To investigate the nature of the relationship between sexual and asexual reproductive allocation further, I (1) examined the relationship between sexual reproductive effort (SRE) and asexual reproductive effort (ARE) in males and females; (2) manipulated male and female SRE by removing immature inflorescences (males) or flowers (females) in the early stages of development to examine the effect on ARE; (3) examined the effect of dissolved inorganic carbon or sediment availability on male and female reproductive allocation patterns; (4) tested for a genetic basis to the tradeoff observed between sexual and asexual reproduction in females; and (5) conducted a field survey of sites with different physical characteristics to examine what effect site characteristics play in limiting pollination success among natural populations.

A tradeoff in the form of a negative correlation between SRE and ARE was consistently found among females grown under different greenhouse conditions, but this tradeoff was not observed in males. When SRE was manipulated in females by removing floral buds, biomass that might have gone into developing fruits was allocated to tuber production, so that there was no difference in total reproductive effort (TRE: sexual plus asexual) between manipulated and unmanipulated plants. No differences were observed in ARE between males with buds removed and buds intact. This tradeoff appears to be related to the timing of reproductive investment, where fruit development overlaps with tuber development in females, but there is little overlap between inflorescence production and tuber production among males.

When males and females were grown under different resource conditions, I found that in response to increased carbon or mineral nutrient availability, males tended to decrease the mass specific number of tubers produced and increase the mean size of tubers. In contrast, females increased the mean size of fruits, but did not change the mean size or mass specific number of tubers. Females did increase TRE in response to increased carbon or mineral nutrient availability, but the balance between sexual and asexual reproduction was not affected.

The genetic basis of several traits related to the tradeoff between sexual and asexual reproduction among females was investigated within 13 clonal and 12 sexually produced families. The intrafamily correlations for SRE, ARE, and the balance between them showed that there was significant genetic variation in these traits. In addition, a significant negative genetic correlation between SRE and ARE was estimated from the family means of these traits. These results suggested that the tradeoff observed in females between sexual and asexual reproduction had a significant genetic component.

An examination of pollination success at different field sites showed that fruit-set in natural populations ranged from zero to nearly 100%. Pollen limitation at different field sites was influenced by wind and waves, currents, and water depth. Wind and waves in open sites reduced pollination success by moving male flowers away from the tethered female flowers before they could be pollinated. Many females growing in a river current had male flowers swept away before they could be pollinated, and pollination was inhibited entirely when females grew in current strong enough to sweep female flowers beneath the surface. All females observed growing in water depths in excess of their maximum peduncle length were also unpollinated.

These results suggest that a range in life history strategies may be found among females within a single population. A similar range in strategies was not observed among male plants. Variation among females plants in the balance between sexual and asexual reproductive allocation provided direct evidence of a physiological tradeoff between these

two modes of reproduction in a clonal macrophyte. These results further indicate that there was cost of (sexual) reproduction paid in clonal reproduction, and that this cost has a significant genetic basis.

The timing and magnitude of reproductive allocation may account for differences between males and females in reproductive strategies. The nature of this relationship may be further influenced in natural populations by variation in fruit-set due to the influence of site characteristics on the magnitude of female sexual reproductive allocation.

DEDICATION

To Marilyn, who has been unbelievably patient, understanding, and supportive. To Kellen and Ryhan, who have dealt incredibly well with their absentee father. To all the friends and family who have supported me and mine.

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PREFACE

My initial interest in *Vallisneria* was in how sexual reproduction was affected by depth and/or light intensity. Depth might have been limiting because the female flowers are pollinated at the surface, while male flowers float unattached and independent of the male plant. I hypothesized that female flower production would be reduced or eliminated if depth was too great for flowers to reach the surface, while male sexual reproduction would not be directly affected by depth. A second question was how a potential reduction in female sexual reproduction with depth might subsequently affect asexual reproduction. Experimental field and greenhouse studies in 1989 indicated that flower production was not directly affected by light or depth.

However, I did find that the number of fruits produced among individual females within a depth or light treatment was highly variable, unrelated to plant size, and negatively correlated with the number of clonal propagules produced. In contrast, inflorescence production among males was positively correlated with plant size, as was the number of clonal propagules. This suggested that the allocation of biomass to sexual and asexual reproductive structures or functions may be coupled among females, which subsequently became the focus of my research in 1990. I hypothesized that allocation to fruit production might reduce the availability of resources for clonal reproduction, and that nutrient availability would influence this relationship. This hypothesis was strongly influenced by the predictions of resource allocation theory (Cody, 1966; Harper, 1977) and clonal life history theory (Caswell, 1985; Loehle, 1987; Sackville-Hamilton, Schmid, and Harper, 1987).

This hypothesis was tested by examining the relationship between sexual and asexual reproductive effort in males and females after removing flower buds from some individuals early in development (Chapter II). Flower removal manipulates the biomass allocated to

sexual reproduction, and may simulate the effects of herbivory or mechanical damage. These experimental manipulations confirmed that there was a tradeoff between sexual and asexual reproduction expressed among females from a single population, but not among the males. This tradeoff was characterized by variation in the proportion of total reproductive biomass allocated to sexual reproduction (SR/TR). SR/TR was strongly and positively correlated with the number of flowers produced by an individual. However, the proportion SR/TR among females was not strongly influenced by resource availability (Chapter III), suggesting that plasticity in the balance between sexual and asexual reproduction may be limited (unless externally imposed). Females did respond to carbon and mineral nutrient availability by increasing the proportion of plant biomass allocated to reproduction, chiefly by producing larger fruits. Males responded to increased carbon and mineral nutrient availability by producing larger clonal propagules.

Because of limited plasticity among females in the balance between sexual and asexual reproduction and since there was wide variation in the number of flowers produced that could not be related to treatment conditions or plant size, I hypothesized that variation in SR/TR might have a genetic basis (Chapter IV). A subsequent analysis indicated that there was significant genetic variation in flower production, sexual reproductive effort, and SR/TR. Few other studies have examined the genetic basis behind the tradeoff between sexual and asexual reproduction within clonal populations. This result was in agreement with the predictions of clonal life history theory. This was also most intriguing as it indicated that a range of clonal life history strategies may be found within a single population.

The nature of the relationship between the number of flowers produced and the biomass allocated to sexual reproduction directly affects the proportion of a female's reproductive biomass allocated to sexual reproduction (SR/TR) and hence the shape of the tradeoff function. This relationship may also be strongly influenced by pollination success. The biomass allocated to fruit development in pollen limited populations may be much reduced,

resulting in a corresponding increase in asexual reproductive allocation. My experience with greenhouse grown plants was that fruit-set was very high (approaching 100%). I examined several natural populations of *V. americana* known to have flowering females and males to assess the likelihood of pollen limitation in the field (Chapter V). I found that pollination was highly dependent upon specific site characteristics such as water depth, exposure to wind and waves, and current velocity, and that male density also exerts an important influence. This result indicated that although little plasticity was found in SR/TR as a function of resource availability, this balance could be, and was in natural populations, externally influenced by pollen limitation in addition to flower loss. Therefore, the tradeoff and balance between sexual and asexual reproduction within individual females will be strongly shaped in natural populations by both genetic and environmental influences.

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CHAPTER I

INTRODUCTION

Life history theory, costs of reproduction, and tradeoffs

Life history theory was developed in part to account for the great variety of reproductive tactics utilized by different organisms (Stearns, 1976). An explicit assumption of the theory is that there is a cost of reproduction (Williams, 1966; Gadgil and Bossert, 1970; Partridge and Harvey, 1988). Two types of reproductive costs are generally recognized: (1) costs related to the risks associated with producing offspring, and (2) costs related to the resources spent in the production of offspring (Cody, 1966; Callow, 1979; Bell and Koufopanou, 1986; Reznick, 1992). Risk-based costs may be manifested as increased exposure to predators or reduced ability to compete for resources as a result of either producing or nurturing offspring. Resource-based costs may be manifested as physiological tradeoffs within the organism between reproduction and other functions or structures, such as growth, maintenance, and/or defense (Bazzaz et al., 1987; Reznick, 1992). An understanding of these tradeoffs is critical when examining how investment in current reproduction affects survivorship or the ability to reproduce in the future (Stearns, 1989).

A physiological tradeoff occurs when allocation from a finite pool of resources into one structure or function precludes allocation to others (Bell and Koufopanou, 1986). It is assumed that different structures or activities represent alternatives, so that a given resource can not be simultaneously invested in more than one activity (Harper, 1977). However, this assumption has been questioned more recently. Bazzaz, Carlson, and Harper (1979) found that flowers and fruits can make a significant photosynthetic contribution to a plant's

reproductive effort. Thompson and Stewart (1981) point out that some structures can support more than one function, such as the "tall, leafy flowering spikes" of *Digitalis* supporting photosynthetic and reproductive structures. Reekie and Bazzaz (1987) report that the reproductive structures in some genotypes of *Agropyron repens* can support in excess of their reproductive carbon needs through photosynthesis in reproductive structures. Despite such examples, the existence of tradeoffs between reproduction and other activities are partly self-evident. The allocation of resources to reproduction eventually must have some impact on the organism (Caswell, 1985). If reproductive investment did not involve a cost, then selection would drive all fitness-related traits towards the limits imposed by design (Stearns, 1989). This runaway selection could result in the evolution of "Darwinian demons", organisms that simultaneously maximize all aspects of their reproductive performance (Law, 1979; Partridge and Harvey, 1988). However, since all organisms are ultimately resource limited, it is the balance struck between reproduction and other activities that determines an organism's fitness.

A physiological or demographic cost of reproduction is usually demonstrated as a negative phenotypic correlation between reproduction and some measure of an organism's current or future performance. However, life history theory predicts an evolutionary response to selection, and thus assumes that there is a genetic basis to the associated costs of reproduction (Caswell, 1985; Reznick, 1985). Negative phenotypic correlations do not by themselves indicate that such costs have a genetic basis (Horvitz and Schemske, 1988). An evolutionary cost of reproduction principally results from antagonistic pleiotropy (Futuyma, 1986), and as such, may be demonstrated by measuring negative genetic correlations between life history characters, or by measuring the correlated response in one trait to selection on another (Reznick, 1985, 1992). The genetic basis of reproductive costs have also been demonstrated by assessing the resemblance among relatives within groups relative to all individuals across groups (Geber, 1991). Determining the genetic basis of the relationship between fitness related traits is important because genetic correlations may

differ from phenotypic correlations in both sign and magnitude due to environmental effects on expression of the genotype (Primack and Antonovics, 1982; Falconer, 1989). Many studies among plants have found that differences in the relationship between fitness related traits disappear when they are reared in a common garden (e.g. Holler and Abrahamson, 1977). Thus, it is imperative to demonstrate the genetic basis of negatively correlated traits through quantitative genetic studies or artificial selection experiments when evaluating the predictions of life history theory. Reznick (1992) points out that though phenotypic correlations or manipulations of the phenotype can provide invaluable information about how organisms allocate their resources, or how characters are physiologically related to each other, they may be demonstrating what are essentially environmental effects rather than the pleiotropic effects of genes.

*Clonal life histories and the tradeoff between
sexual and asexual reproduction*

The life histories of clonal organisms are fundamentally different from non-clonal or unitary organisms. Many clonal species do not appear to have a natural life span, and are in effect potentially immortal (Harper, Rosen, and White, 1986). Indeed, there are many examples of very long-lived clones in the literature (reviewed in Cook, 1985), such as the 11,000 plus year-old clone of *Larrea tridentata* (Vasek, 1980). Because clonal individuals often leave no record of their existence through time, it is nearly impossible to determine the age of most clones, suggesting that other long-lived clones may exist undetected. A critical distinction thus arises between clonal and unitary organisms in that the reproductive value of clonal organisms may increase indefinitely with age, in contrast to unitary organisms whose reproductive value increases up until reproductive maturity and then declines. A second distinction is that the interaction of life history traits may occur at different levels of organization: the entire genet, individual ramets, or among

physiologically integrated units comprised of several ramets. Consequently, different types of life history tradeoffs arise within clonal organisms.

Fitness gains for clonal organisms may come directly from sexually produced offspring, or by investing in clonal growth (Abrahamson, 1980). Harper (1967) has suggested that sexual and asexual reproduction are competing processes within a plant. Thus one of the most interesting tradeoffs predicted by clonal life history theory is that between sexual and asexual reproduction (Caswell, 1985; Sackville-Hamilton, Schmid, and Harper, 1987). The cost of reproduction (sexual) may be paid as a reduction in clonal reproduction, which may directly affect the growth and the probability of survival of the clone, and indirectly affect the probability of producing more sexual offspring in the future. On the other hand, producing clonal offspring at the expense of sexual reproduction may be counter-productive in terms of fitness towards the center of well established clones since there may be little or no room for fitness gains through clonal expansion (Williams, 1975). Since both sexually and asexually produced offspring make contributions to fitness, the optimal clonal life history balances seed production with clonal reproduction to maximize the long term production of offspring.

Several hypotheses have been advanced to predict how clonal organisms should allocate their resources to sexual and clonal functions. The balance between sexual and asexual reproduction may be affected by population growth rates, the probability of seedling establishment, juvenile development rates, and the probability of adult mortality (Caswell, 1985). Extending these predictions, others have suggested that the balance between clonal growth and sexual reproduction will also be affected by the physiological cost of sexual reproduction, resource availability, clonal morphology and geometry (guerrilla vs. phalanx growth form), and the predictability of mortality risks (Watkinson and White, 1985; Sackville-Hamilton, Schmid, and Harper, 1987). It has also been suggested that sexual reproductive effort should respond to changes in density (Holler and Abrahamson, 1977; Abrahamson, 1979; Eriksson, 1985; Jurik, 1985). Loehle (1987)

took a more mechanistic view, suggesting that the relative costs and benefits of sexual vs. asexual reproduction in a given environment will determine the quantity of resources allocated to each mode of reproduction, assuming that plants can detect and respond to environmental conditions. Interconnected ramets have been shown to both detect and respond to local environmental conditions independently of other ramets (Salzman, 1985; Harper, Rosen, and White, 1986; Eriksson, 1988; Solangaarachchi and Harper, 1989; de Kroon and Schieving, 1991; Evans, 1992). Assuming that the balance between sexual and asexual reproduction is a plastic characteristic, it may vary with the availability of resources, such as carbon, mineral nutrients, or light.

A note on pollen limitation

Those making these predictions assume that plant allocation patterns are internally controlled. However, sexual reproductive allocation and allocation to other functions (e.g. clonal growth or defense) may be strongly related to such external factors as herbivory (Bazzaz et al., 1987) or pollen limitation (Bierzychudek, 1981). It is partly self-evident that pollination success or failure affects reproductive allocation by influencing the number of flowers setting fruit. Several studies have shown that the magnitude of reproductive effort is directly related to fruit-set within a population (e.g. Armstrong and Irvine, 1989; Johnston, 1991). Conversely, populations that appear to be pollen limited (evidenced by low fruit-set) may in fact have adequate pollen loads to fertilize more ovules, but fruit development is strained by the lack of sufficient resources to mature more than a fraction of the fruits (e.g. Lee and Bazzaz, 1982). It remains largely unknown to what extent the allocation patterns of natural populations are influenced by pollination success or lack thereof. Bierzychudek (1981) convincingly argues that pollination limitation should be considered when interpreting how plants allocate their resources in field or greenhouse studies.

RESEARCH ORGANISM:
VALLISNERIA AMERICANA MICHX. (HYDROCHARITACEAE)

Submersed aquatic macrophytes have adopted a wide array of reproductive strategies, many of which are unique to the aquatic environment (Arbor, 1920; Sculthorpe, 1967). Perennial macrophyte communities may be further characterized by various means of asexual reproduction, including shoot fragmentation, the production of tubers and turions, and/or the propagation of clonal offshoots from stolons or rhizomes. Indeed, the proportion of asexually reproducing species in many aquatic communities is relatively high when compared to terrestrial communities (Sculthorpe 1967, Hutchinson 1975). At the same time, aquatic macrophytes may allocate a large portion of their resources to the production of seeds, often producing them in large numbers (Arber 1920). The impact of seed production on the population dynamics of many aquatic perennial species is largely unknown.

I have chosen *Vallisneria americana* as the organism in which to study the relationship between sexual and asexual reproduction in aquatic environments. *V. americana* is a dioecious submersed aquatic macrophyte widely distributed in lakes and streams throughout much of eastern North America, with varieties found in several parts of eastern Asia and Australia (Lowden, 1982). *V. americana* is found in a wide range of environments, from temperate to subtropical, fresh water to estuarine, and oligotrophic to eutrophic.

V. americana may be an ideal subject for studying questions related to reproduction among clonal species. Since *V. americana* is a dioecious species, allocation to male vs. female function is unambiguously separated. Male inflorescences, female flowers and fruits, and perennating tubers (asexual reproductive propagules) occur as discrete, easily measured units. Since the entire plant, with the exception of seeds and tubers, senesces at the end of the growing season, resources acquired in prior seasons can not be confounded

with resources allocated to the current season's growth or reproductive output. This is critical when relating reproductive output to conditions found within the current growing season.

V. americana germinates in the spring from seeds or perennating tubers, and produces a rosette of long ribbon-like leaves. Rosettes may initiate a stolon within the axil of every third leaf (Sculthorpe, 1967). The stolons may then grow laterally above or below the sediment surface. New rosettes arise sequentially at the nodes of stolons, so that a chain is formed of interconnected rosettes. Occasional branching may occur when a 2nd or (rarely) a 3rd rosette bearing stolon is initiated (personal observation).

Sexual reproduction begins in upstate NY in late June or July. Females produce buoyant flowers that extend to the surface on an elongated peduncle. Males produce inflorescences on short peduncles at the base of the rosette. When the inflorescence is mature, the spathe ruptures, releasing as many as 2000 individual buoyant flowers (Wylie, 1917). Pollination takes place at the surface when free floating male flowers collide with and tip into the surface tension dimple surrounding a female flower. Pollen from the projecting anthers brushes against the stigma (Wylie, 1917; Svedelius, 1932; Kausik, 1939). Buoyant cigar shaped fruits, 10 to 18 cm long, develop over a period of several weeks and may remain attached until either the peduncle, the fruit itself, or the parent rosette begins to senesce (personal observation). Flower initiation ends late in August or early in September. Fruit maturation may continue through the end of the growing season. Seeds (as many as 300 to 400 per fruit) are dispersed locally if the fruit senesces while still attached, or they are dispersed away from the parent if the peduncle senesces prior to the fruit (Sullivan, personal observation).

V. americana reproduces asexually when tubers bearing a single bud are produced at the nodes of stolons growing down into the sediment (Wylie, 1917). Two to three tubers (rarely more than three) will develop sequentially on a single stolon. These tubers are the sole perennating organ. The initiation of stolons that will bear tubers begins late in July or

early in August. Tubers are developing by late in August and development continues until the plant senesces at the end of the growing season.

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CHAPTER II

A tradeoff between sexual and asexual reproduction in the dioecious clonal macrophyte *Vallisneria americana* (Michx.)

INTRODUCTION

A key assumption of life history theory is that tradeoffs occur between reproduction and other functions (Bazzaz et al., 1987; Reznick, 1992). Among clonal organisms, one of the most important of these tradeoffs may be between sexual and asexual reproduction (Harper, 1967; Abrahamson, 1980). The allocation of resources to sexual offspring may reduce those resources available for clonal growth (Williams, 1975). Allocating resources to clonal growth may occur at the expense of sexual reproduction (Sarukhan, 1976), but may also increase the probability of survival and future sexual reproduction (Caswell, 1985). Thus the tradeoff and eventual balance struck between sexual and asexual reproduction will have a critical impact on an organism's lifetime fitness (Abrahamson, 1980; Loehle, 1987; Sackville-Hamilton, et al., 1987).

Negative correlations between measures of sexual and asexual reproduction have been offered as evidence of tradeoffs between them. Such correlations have been reported among closely related species (Sarukhan, 1976; Sutherland and Vickery, 1988) and among populations within a species (Werner, 1979; Jurik, 1980; Swamy and Ramakrishnan, 1988). Others have reported that the relationship between sexual and asexual reproduction is relatively weak (e.g. Lovett-Doust, 1980). Negative correlations between sexual and asexual allocation have been found within dioecious species, where fruiting females may allocate more to sexual reproduction and less to asexual reproduction than do pollen-producing males (Putwain and Harper, 1972; Hancock and Bringhurst, 1980).

Differences in sexual reproductive allocation between males and females have been related to the different costs associated with pollen production vs. fruit maturation (Korpelainen, 1992).

However, interspecific or interpopulational correlations may not convincingly demonstrate tradeoffs between sexual and asexual reproduction for at least two reasons. (1) The reproductive allocation patterns of one population are determined independently of other populations, such differences resulting from different genetic backgrounds and environmental pressures (Antonovics, 1980; Thompson and Stewart, 1981). Correlations across populations may be confounded with different genetic and environmental factors (Reekie and Bazzaz, 1992). (2) Tradeoffs assume that the magnitude of one trait is dependent upon another. Within a given population, the resources allocated to sexual reproduction may be unrelated to those allocated to asexual reproduction. For example, Holler and Abrahamson (1977) found that sexual reproductive effort in *Fragaria virginiana* from two populations remained constant across densities, but asexual reproductive effort was significantly increased in the lower density plots. Tradeoffs observed under one set of circumstances may disappear under others. Conversely, reproductive allocation may simply be a linear function of plant size. Eriksson (1985) found that the proportion of total biomass in *Potentilla anserina* allocated to either sexual reproduction or clonal growth did not differ significantly across three densities, despite a 3.5-fold range in mean plant size. Under such circumstances, the underlying relationship between sexual and asexual allocation remains unknown. Thus, comparisons across populations may lead to spurious correlations, or find no relationship when one may in fact exist.

Consequently, investigating of the effects of reproduction on other functions may best be carried out among members of a single population to eliminate evolutionary history and environmental differences as potential confounding factors. A comparison of reproductive and (manipulated) non-reproductive individuals has been advocated as a powerful means of determining the effects of reproduction on other characters (Antonovics, 1980; Bazzaz and

Ackerly, 1992). Such an approach has often been utilized in examining the effects of reproduction on vegetative growth (Antonovics, 1980; Reekie and Bazzaz, 1987; Horvitz and Schemske, 1988; Snow and Whigham, 1989; Reekie and Bazzaz, 1992). However, there has been little experimental work examining tradeoffs between sexual and asexual reproduction within clonal populations (Lovett Doust, 1989; but see Westley, 1993).

Toward this end, I have investigated the relationship between sexual and asexual reproduction in the dioecious clonal macrophyte *Vallisneria americana* Michx. (Hydrocharitaceae). A preliminary study in 1989 indicated that the number of fruits among females was negatively correlated with the number of clonal propagules, while the number of inflorescences produced among males was positively correlated with clonal propagule number. This suggested that males and females may utilize different reproductive "strategies". I hypothesized that female sexual reproductive effort (Table 1) would be negatively correlated with asexual reproductive effort, so that fruit production will have a direct negative impact on clonal reproductive output. Secondly, I hypothesized that male sexual reproductive effort is a linear function of plant size. These hypotheses were evaluated using plants from a single source population by (1) relating sexual reproduction to asexual reproduction and plant size, and (2) manipulating sexual reproductive investment through the removal of flower or inflorescence buds, and comparing subsequent clonal reproduction between manipulated and unmanipulated plants. The following questions were addressed in two greenhouse experiments:

- (1) What is the relationship between reproductive allocation and plant size?
- (2) Is there a tradeoff in the allocation of biomass to sexual reproduction with the allocation of biomass to asexual reproduction?
- (3) What effect does a manipulated reduction in allocation to sexual reproduction have on growth and/or asexual reproduction?
- (4) Do reproductive strategies differ between males and females?

In the first experiment, I examined the relationship between reproductive allocation and plant size. I tested for tradeoffs in the relative allocation of biomass between sexual and asexual reproduction, and I examined the effects of manipulating female sexual reproductive allocation on asexual reproductive allocation. Male reproductive allocation was not manipulated to ensure an adequate pollen supply. In the second experiment, tradeoffs in the relative allocation of biomass between sexual and asexual reproduction were examined in males and females, and male sexual reproductive allocation was manipulated to determine what effect this might have on asexual reproduction. The manipulation of male plants precluded the pollination of females in this second experiment.

MATERIALS AND METHODS

Study Species

Vallisneria americana (wild celery) is a submersed dioecious clonal macrophyte widely distributed throughout eastern North America (Lowden, 1982). *V. americana* reproduces in the northeast U.S. and Canada from both perennating tubers and seeds. A rosette of ribbon-like leaves germinates from each tuber, with additional rosettes developing at the nodes of stolons arising from axillary meristems (Sculthorpe, 1967). Flowers or inflorescences develop from two separate meristems flanking each of the axillary meristems that (potentially) develop into stolons. Buoyant female flowers are tethered on an elongated peduncle (Wylie, 1917). Anthesis occurs at the surface, but will occur below the surface in deeper water (Sullivan, personal observation). Up to 2000 male flowers (approximately 0.6 mm in diameter) may develop on a short peduncled inflorescence (Wylie, 1917). Buoyant male flowers abscise after the spathe opens over a period of usually less than 36 hours (Sullivan, personal observation), and are supported on the surface film. Pollination occurs at the surface when male flowers are caught in the surface tension depression surrounding the female perianth, causing stamens to brush the stigma (Wylie, 1917;

Svedelius, 1932; and Cox, 1988). Buoyant fruits develop quickly and remain attached for several weeks, until the fruit either senesces or is dispersed when the peduncle senesces (Sullivan, personal observation). One to several perennating tubers (usually two or three) develop on stolons growing down into the sediment from mature or terminal rosettes. The entire plant, except for tubers and seeds, senesces at the end of the growing season.

Plant collection and growth

In 1990, plants germinating from tubers of unknown sex were collected by divers from Chenango Lake, NY ($42^{\circ}15'N$; $75^{\circ}50'W$). On May 29, the plants were collected from the south end of the lake from a population loosely spread over several hectares. They were stored for seven days at $5^{\circ} C$ in lake water until transplanted into the Binghamton University greenhouse, where they were grown in seven replicate, 85 cm deep, 1200 liter, fiberglass tanks. All plants were first weighed and assigned to one of five weight classes. One hundred forty plants were used in this experiment. Four plants from each weight class were randomly assigned to each of the seven tanks to ensure an even distribution of initial plant sizes per tank. The tanks were filled with deionized water to which was added the following to facilitate growth and osmotic balance (see Smart and Barko, 1985): $CaCl_2 \cdot 2H_2O$ (91.7 mg l⁻¹), $MgSO_4 \cdot 7H_2O$ (69.0 mg l⁻¹), and $KHCO_3$ (15.4 mg l⁻¹). To compensate for evaporative loss, the tanks were topped off weekly with deionized water. Each plant was grown in a plastic pot (20 cm wide x 18 cm deep) in three liters of sediment from Big Moose Lake, NY ($43^{\circ}49'N$; $74^{\circ}51'W$). pH was maintained at 7.25 ± 0.25 units with Horizon Model 5997 pH control units through the addition of a 0.4N mixture of 30% HNO_3 and 70% H_2SO_4 , or 0.4N NaOH. Temperature was maintained at $23^{\circ} \pm 1^{\circ} C$ with Remcor CFF500 water circulating units. Light level was unmanipulated, with approximately 95% of ambient intensity naturally found within the greenhouse. The plants were monitored daily until harvested on September 21, 1990.

In 1993, plants were collected from Chenango Lake and grown in the Binghamton University greenhouse as in 1990, with the following exceptions. Plants of unknown sex were collected on May 27 and transplanted into the greenhouse on June 3. An artificial sediment comprised of a 20:1 mixture of local "topsoil" (a mixture of clay, silt, and sand) and cow manure was used in place of natural lake sediment. Fifty six plants were randomly assigned to two replicate tanks, with 28 plants per tank. The plants were monitored daily until harvested on October 5.

Manipulation of sexual reproduction

In 1990, female reproductive investment was manipulated in half of the female plants in each tank by removing their immature flower buds as they began to develop. Every other female was assigned to the flower removal treatment as it was identified when flower buds were first observed. Flowers were removed by clipping the peduncle at the base. Peduncle length at flower removal was between 5 and 20 cm. Twenty clipped flower buds were saved to provide a biomass estimate of the flower buds that were removed. Unmanipulated females were pollinated by the males in each tank. Fruits were harvested at maturity (after turning from green to brown) or at peduncle senescence. Male plants were not manipulated so that an adequate supply of male flowers was available. However, inflorescence biomass was estimated by multiplying the number of inflorescences by the mean biomass of 20 mature inflorescences harvested prior to releasing their flowers.

In 1993, male reproductive investment was manipulated in half of the male plants by removing immature inflorescences as they began to develop. Every other male in each tank was assigned to the inflorescence removal treatment as it was identified by the first appearance of an immature inflorescence. Inflorescences were allowed to mature in the remaining male plants, but were harvested at maturity (just prior to releasing flowers). Maturity was determined by the change in inflorescence color and transparency that

precedes the opening of the spathe. All plants were inspected daily, and inflorescences were removed by clipping the peduncle at the base. Since male inflorescences were removed prior to releasing flowers, female flowers were not pollinated. The female flowers were harvested when peduncles had elongated, and after the perianth changed from white to brown.

Measured and derived variables

In 1990, counts were made of the number of rosettes, stolons, tubers, female flowers/fruits, and male inflorescences. Inflorescence number was determined by counting the number of inflorescence peduncle "stubs" that remained after inflorescence senescence. Rosettes (plus their interconnecting stolons), tubers (plus their tuber bearing stolons), female fruits (plus their peduncles), and the sampled flower buds and inflorescences were dried at 105° C and then weighed to determine their biomass (= dry mass). Root biomass was not measured as the fine roots could not be separated from the coarse particles of Big Moose sediment. Several derived variables were then calculated (Table 1).

Reproductive effort was calculated as the proportion of total plant biomass allocated to reproduction (for a discussion of methods in calculating reproductive effort, see Hickman, 1975; Thompson and Stuart, 1981; Bazzaz and Reekie, 1985). Asexual reproductive effort (ARE) was calculated as asexual reproductive biomass / total plant biomass. Sexual reproductive effort (SRE) was calculated as sexual reproductive biomass / total plant biomass. Flower biomass of females in the flower removal treatment was estimated by multiplying flower number by the mean biomass of the 20 immature flowers sampled. The inflorescence biomass of males was estimated by multiplying inflorescence number by the mean biomass of the 20 mature inflorescences sampled. Total reproductive effort (TRE) was determined as the biomass of all reproductive structures (sexual plus asexual) / total plant biomass (TRE = SRE + ARE). The proportion of total reproductive biomass (TR:

sexual plus asexual) allocated to sexual reproduction (SR/TR) was calculated as sexual biomass / (sexual plus asexual biomass).

In 1993, plant parts were collected and dried as in 1990, except that roots were also collected, dried, and weighed (to be included in total plant biomass). All inflorescence buds and mature inflorescences that were removed were also dried and weighed, so that male sexual reproductive effort was directly measured rather than estimated. Unpollinated female flowers were also dried and weighed. Derived variables were calculated as in 1990.

Statistical analysis

Data were analyzed with the SYSTAT statistical analysis package (SYSTAT, 1992). A mixed model two factor analysis of variance was used to examine differences between males, females, and non-flowering plants, with tank as a random factor. Residuals were graphically examined for violations of statistical assumptions. Some measures were subsequently log transformed ($Y' = \log [Y + 1]$) as noted to improve normality or achieve homoscedasticity. In post-hoc comparisons between plant groups, alpha was corrected to control family-wise error using Keppel's (1982) modification of the Bonferroni test. The Friedman's test was used to examine differences between males and females in measures of sexual reproductive allocation (Zar, 1984).

To examine how sexual and asexual reproductive output were related to plant size, reproductive biomass (sexual, asexual, and sexual plus asexual) was correlated with total biomass. Reproductive biomass was also correlated with an alternative measure of plant size: vegetative biomass [total biomass - reproductive biomass (sexual plus asexual)] to avoid the statistical problem of non-independence if total biomass is used. However, when considering the relationship between reproductive biomass and plant size, vegetative biomass may not be the most relevant measure. Reproductive and vegetative functions are not physiologically independent of one another (reviewed in Bazzaz and Ackerly, 1992).

The fruits, peduncles, and above sediment stolons and tubers of *V. americana* appear to be photosynthetic (they are green) and may contribute to the plant's resource pool. This potentially confounds the relationship between vegetative and reproductive functions. In addition, if there is a dependent relationship between sexual and asexual reproduction, then reproductive allocation is partially independent of vegetative biomass. Moreover, total biomass may be the best integrative measure of the plant's total resource pool, and may thus yield the best predictive relationship.

RESULTS

Not all of the plants flowered in each experiment, nor were there equal sex ratios found among the plants used in each of the experiments. In the first experiment (1990), 47 male and 76 female plants flowered. Seventeen plants were non-flowering. All immature flower buds from 36 of the 76 female plants were removed. Flowering plants were significantly larger (total biomass) than non-flowering plants, while differences in total biomass between males, females, and females with buds removed were not significant. Fruit set among flowering females was high at 97%. Pollination failure occurred only when male flowers were unavailable for pollen donation. All plants flowered in the second experiment (1993). There were 15 females and 38 males. All immature inflorescence buds from 19 of the 38 males were removed. Differences in plant size between males and females were not significant. Female flowers were not pollinated.

Reproductive allocation and plant size

In 1990, sexual and asexual measures of reproduction were generally positively correlated with plant size (Table 2). Among males and females, the biomass of tubers was positively correlated with both total biomass and vegetative biomass. The number of

inflorescences among males was positively correlated with both total biomass and vegetative biomass. Interestingly, the number of flowers and the biomass of fruits and flowers among unmanipulated females was correlated with total biomass, but was not significantly correlated with vegetative biomass. There appears to be a predictable relationship between total reproductive biomass (sexual plus asexual) and plant size. Among both males and females, the allocation of total reproductive biomass was more strongly correlated with plant size than either asexual or sexual reproductive biomass alone. Over 81% of the variation in reproductive biomass was accounted for by total biomass in manipulated ($r = 0.904$) and unmanipulated ($r = 0.932$) females, while 72% of the variation in reproductive biomass was accounted for in males ($r = 0.851$). Differences in the slope of total reproductive biomass regressed on either total biomass or vegetative biomass among all plant groups (males, females, manipulated females, and non-flowering plants) were not significant. Further, an analysis of covariance indicated that the effect of plant group on the intercept of these regressions was not significant. This indicated that the relationship between reproductive biomass and plant size among all of these plants could be described by a single regression (Fig. 1a), suggesting that plants exposed to the same conditions all allocate the same proportion of their biomass to reproduction regardless of how it is divided between sexual and asexual activities.

The correlations between reproductive allocation and plant size in 1993 (Table 3) were very similar to those in 1990. Total reproductive biomass was more strongly related to plant size than either sexual or asexual reproductive biomass alone. As in 1990, differences in the slope or intercept of total reproductive biomass regressed on total biomass or vegetative biomass were not significant, indicating that both groups of males and the unpollinated females could be described by the same relationship (Fig. 1b).

Reproductive tradeoffs

Negative correlations in 1990 between relative measures (mass specific) of asexual and sexual reproductive allocation among unmanipulated females indicate that a tradeoff occurred between fruit and tuber production (Table 4). Relative measures were used because reproductive allocation was strongly influenced by plant size, which ranged four-fold between 1.46 and 5.79 g biomass. The number of tubers per gram total biomass among females was negatively correlated with the number of fruits per gram total biomass ($r = -0.544$, $p = 0.0003$). Fifty percent of the variation in tuber number was accounted for by the multiple regression of tuber number on fruit number and total biomass ($R^2 = 0.504$, $p < 0.0001$). There was a stronger relationship between sexual and asexual reproduction in terms of biomass and reproductive effort (reproductive biomass/total biomass). Sexual reproductive effort (SRE) was negatively correlated with asexual reproductive effort (ARE; Fig. 2a). Seventy eight percent of the variation in tuber biomass was accounted for by the multiple regression of tuber biomass on fruit biomass and total biomass ($R^2 = 0.781$, $p < 0.0001$). There was also a strong negative correlation among the unpollinated females in 1993 between ARE and SRE (Fig. 2c).

The expression of this tradeoff among these females was reflected in variation in the proportion of reproductive biomass (TR: sexual plus asexual) allocated to sexual reproduction (SR/TR). SR/TR ranged tenfold from 0.096 to 0.994. This variation in SR/TR was related to mass specific flower production. SR/TR was positively correlated with flowers per gram (Table 5), which ranged from 0.257 to 2.115. The number of flowers per gram and SR/TR appear to be independent of plant size as they were not correlated with either total biomass or vegetative biomass. This relationship did not appear to be influenced by pollination intensity as flower number was strongly correlated with sexual reproductive biomass (Table 6).

Among the males in 1990, there was no evidence of a tradeoff between allocation to sexual and asexual reproduction (Fig. 2b). The number of tubers per gram was not significantly correlated with the number of inflorescences per gram (Table 4). The relationship between male SRE and ARE was not examined in 1990 since inflorescence biomass was not directly measured. There was no evidence of this tradeoff among the unmanipulated males in 1993 (Fig. 2d). The number of tubers per gram was not significantly correlated with the number of inflorescences per gram, nor was ARE significantly correlated with SRE (Table 4).

Effects of manipulating sexual reproduction

The biomass allocated to sexual reproduction might have been a fixed proportion of total biomass. If so, then inflorescence or flower bud removal might have resulted in the production of more floral buds (assuming meristem limitation was not a factor). However, neither females nor males appear to respond in this way (Table 7). In 1990, a two way ANOVA among females showed that differences in either the number of flowers per plant or flowers per gram in the two treatments were not significant. A two way ANOVA among males in 1993 showed that differences in either the number of inflorescences per plant or inflorescences per gram were not significantly increased when inflorescence buds were removed. Although meristem limitation was not specifically examined in either experiment, the range in flowers per gram among females (eightfold in 1990 and fourfold in 1993) and inflorescences per gram among males (sevenfold in 1990 and fourfold in 1993) suggested that meristem availability was not limiting since flower or inflorescence production ranges widely among plants of the same size.

In 1990, the removal of female flower buds resulted in an estimated 94% decrease in biomass allocated to sexual reproduction (0.565 vs. 0.033 grams). This decrease did not appear to be associated with an increase in plant growth. In post-hoc comparisons of

females and females with buds removed, differences in both total biomass and reproductive biomass were not significant (Fig. 3a, b). However, there was a significant effect of bud removal on asexual reproduction. The biomass of tubers produced was increased by 93% (Fig. 3c) and the number of tubers produced was increased by 79% (Fig. 3d) when buds were removed. Consequently, bud removal significantly increased female ARE by 91% (Fig. 3g). There was, however, no effect of bud removal on the mean size of tubers produced (mean tuber biomass; Fig. 3e).

The biomass allocation pattern of females with buds removed was similar to that of the males, which also allocated less biomass to sexual reproduction than the unmanipulated females (an estimated 68% less). Differences between females with buds removed and males in tuber biomass, tuber number, and ARE were not significant (Fig. 3c, d, g). Interestingly, differences in the proportion of total biomass allocated to total reproduction (TRE: SRE + ARE) among females, females with buds removed, males, and non-flowering plants were not significant (Fig. 3f).

In 1993, the removal of inflorescence buds from males resulted in a 59% decrease in biomass allocated to sexual reproduction (0.061 vs. 0.150 grams). However, inflorescence bud removal did not have a significant effect on any measure of growth or asexual reproductive allocation (Fig. 4a, b, c, d, e). There also were no differences between males and females in any measure of growth. However, females allocated 3.5 x more biomass to unpollinated flowers than males allocated to inflorescences (Fig. 4h). ARE in females was significantly smaller than in males (Fig. 4g), but there were no differences in TRE among males, males with buds removed, and unpollinated females (Fig. 4f).

DISCUSSION

Allocation theory assumes that resources are finite, and predicts that tradeoffs occur in the allocation of finite resources between competing functions. Results from these experiments suggest that *Vallisneria* allocates a given proportion of total biomass to reproduction, and that different females partition this reproductive allotment differently between sexual and asexual reproduction. Three lines of evidence support this conclusion: (1) naturally occurring variation in the proportion of reproductive biomass allocated to sexual reproduction, (2) the effect of manipulating sexual reproductive allocation on asexual reproductive allocation, and (3) differences in reproductive allocation between males and females.

Sexual and asexual reproductive tradeoffs

Reproductive allocation among males, females, and non-flowering plants in 1990 (Fig. 1a), and among males and females in 1993 (Fig. 1b) was a linear function of plant size. Consequently, differences in TRE among plant groups within each experiment were not significant (Fig. 3f, 4f). This suggested that these plants all had a similar reproductive "budget", which was sub-divided between sexual and asexual reproductive structures.

Negative correlations among *Vallisneria* females between SRE and ARE in 1990 and in 1993 provide strong evidence of a tradeoff between sexual and asexual reproduction (Fig. 2a, c). This tradeoff was observed because of naturally occurring variation in the proportion of the reproductive budget allocated to sexual reproduction (SR/TR). Variation in SR/TR was directly related to variation in both SRE and ARE (Table 5). The relatively broad range in SR/TR (ten-fold in 1990 and five-fold among unpollinated females in 1993) indicates that some females invested a much greater proportion of their reproductive capital in seed production than others, and at the expense of asexual reproduction. Accounts of

similar variation among members of a single population have been rare (but see McCrea and Abrahamson, 1987), and seldom investigated.

The balance between sexual and asexual allocation expressed among individual females will be strongly influenced by the relative number of flowers initiated and matured, and subsequent pollination intensity. Since the number of flowers was strongly correlated with the biomass of fruits and flowers (Table 5), pollination intensity probably had little impact on the allocation of biomass to sexual reproduction here. However, the number of flowers per gram ranged eight-fold and was strongly correlated with SR/TR (Table 5). This strongly suggests that SR/TR was a function of the relative number of flowers produced.

One of the more interesting questions raised by this finding regards the basis of variation in flower production, and hence SR/TR. Neither bud removal treatment nor tank had a significant effect on female flower number or flowers per gram (Table 6). This suggests that variation in the relative number of flowers produced has a genetic basis. Thus, variation in the balance between sexual and asexual reproduction among *Vallisneria* females may also have a genetic component. This would be of interest as it runs counter to the predictions of clonal life history theory. Current models predict that the balance between sexual and asexual reproduction is a life history characteristic selected for by the circumstances under which a given population evolves. However, the variation in SR/TR among these females suggests that the balance between sexual and asexual reproduction has not been optimized. Selection among potentially immortal clones may be relatively slow (Caswell, 1985) compared to the rate at which new genotypes are introduced. Even with intense interclonal competition taking place among interdigitating clones, genet diversity may be maintained within environments that are sufficiently complex. There is abundant evidence that clonal polymorphisms in other traits are relatively common (Silander, 1985), but there is currently little known about life history polymorphisms within clonal populations.

A similar tradeoff between inflorescence production and tuber production was not found among males (Fig. 2b, d). Only 4.6 % of the variation in tubers per gram was explained by inflorescences per gram in 1990, and only 4.8 % of the variation in ARE was explained by SRE in 1993. This indicates that variation in biomass allocation to inflorescences had little influence on the allocation of biomass to tubers, which suggests a much looser coupling between sexual and asexual reproductive functions. Male inflorescence and tuber production both appear to be a function of plant size.

Differences between males and females in the degree of coupling between sexual and asexual reproductive functions may be related to differences in the relative magnitude and timing of reproductive allocation. Inflorescence biomass in 1993 represented a relatively small portion of reproductive biomass (6.9 %), while on average fruit biomass in 1990 (48.2 %), and unpollinated flower biomass in 1993 (20.9 %) represented a much larger portion of reproductive biomass. Moreover, the development of tubers overlaps little with inflorescence development, but may overlap much longer with flower development and the subsequent development of fruits. In field and greenhouse grown plants, tuber development may begin late in July or early in August and continue through the end of the growing season. Inflorescence development begins early in July and continues through mid to late August. Female flowers have a similar phenology, but then fruit maturation can continue late into September or early October. Mature fruits often remain attached to the plant until senescence. Consequently, female sexual and asexual functions compete simultaneously for the same resource pool for a longer period of time.

Effects of bud removal

The removal of flower buds in 1990 demonstrated that the tradeoff between sexual and asexual reproductive allocation among females is a result of the close physiological coupling of these two functions. When flower buds were removed, total reproductive

allocation remained relatively constant (Fig. 3b) with biomass that might have gone into flowers and fruits allocated to the production of tubers (Fig. 3d). The decrease in SRE was balanced by an increase in ARE, so that differences in TRE between manipulated and unmanipulated females were not significant. This represents an extremely flexible reproductive strategy that compensates for sexual reproductive failure with increased clonal reproduction. Partial or complete sexual reproductive failure can result from herbivory, low resource availability (Titus and Hoover, 1991), depth limitation, or pollination limitation (Sullivan and Titus, in preparation). Paige and Whitham (1987) found that artificially limiting pollination or removing flower buds in *Ipomopsis aggregata* resulted in increases in clonal reproduction. Westley (1993) found that artificially reducing allocation to *Helianthus tuberosus* inflorescences resulted in increases in tuber number and biomass. Similar reports of intrapopulational flexibility in sexual/asexual reproductive allocation have been rare.

The increase in biomass allocated to tubers after female flower bud removal (Fig. 3c) was a function of an increase in tuber number, though not in tuber size (Fig. 3d, e). Females investing a greater proportion of resources into sexual offspring incurred a reproductive "cost" paid in fewer clonal offspring. Thus seed production limits the number of perennating tubers that can compete the following season. This demographic cost of reproduction may be dramatic in populations primarily or exclusively maintained through clonal reproduction. Many clonal populations may be characterized in this way (Cook, 1985, Erickson, 1988), especially among the aquatic macrophytes (Sculthorpe, 1967; Titus and Hoover, 1991). In Chenango Lake, very few *V. americana* seedlings have been observed, and of these, none have exceeded 6 cm in height as late as the first week in September (personal observation). In a July 1, 1993 sampling of *V. americana* seedlings in Chenango Lake, only one seedling was observed in five 50 m by 1 m transects (Sullivan and Andorfer, unpublished data). Twelve *V. americana* seedlings were observed in a follow up survey on August 9, 1993 along a 100 m by 1 m transect cutting through an area

densely populated with female plants (Sullivan and Titus, unpublished data). The scarcity of seedlings coupled with their small stature late in the growing season make it unlikely that sexual reproduction plays a significant role in the population dynamics of the Chenango Lake plants. Thus, over a period of time, there is a demographic penalty for those females that allocate a greater proportion of their reproductive budget to seed production rather than to tubers.

The removal of inflorescence buds among males in 1993 did not have a significant effect on the biomass or number of tubers produced. This experiment was unable to demonstrate a physiological coupling of male sexual and asexual reproductive allocation. There wasn't a demographic cost of sexual reproduction as in females, as ARE was not significantly increased by bud removal nor were differences in TRE significant (Fig. 4). Perhaps more importantly, differences in ARE between males and non-flowering plants in 1990 were not significant. There may be little selective pressure for reproductive flexibility when differences in ARE between flowering and non-flowering plants are not significant.

Males vs. females

Evidence for the tradeoff between sexual and asexual reproduction can be found in the different reproductive allocation patterns of males and females. Males and females both allocated the same proportion of total biomass to reproduction in 1990, and in 1993: differences in TRE between them were not significant (Fig. 3f, 4f). However, males allocated more reproductive biomass towards producing tubers, and less to sexual reproductive structures, than did fruiting females in 1990 (Fig. 3h), or unpollinated females in 1993 (Fig. 4h). This suggests that females allocating more to sexual reproductive structures than males have less reproductive biomass available to allocate to asexual reproductive structures, the basis of a tradeoff between these functions. Although the literature is replete with examples among dioecious species of females allocating more

to reproduction and less to growth than males, indicating a tradeoff between reproduction and growth (Gross and Soule, 1981; Agren, 1988; Allen and Antos, 1988; Armstrong and Irvine, 1989; and Korpelainen, 1992), reports of females allocating more to sexual reproduction and less to asexual reproduction than males are rare (but see Putwain and Harper, 1972). Males allocating more to asexual reproduction may be better colonizers or competitors than females. Hancock and Bringhurst (1980) suggested this may be the basis of sex-ratios biased in favor of staminate plants among 12 populations of *Fragaria chiloensis*. The demographic cost of reproduction paid by *V. americana* females investing heavily in seed production may also be paid by females relative to males, so that males should produce on average more clonal offspring than do females, resulting in male-biased sex-ratios. Lovett Doust and Laporte (1991) offer evidence in support of this prediction, reporting that sex-ratios among three populations of *V. americana* in the Detroit-St. Clair River systems were in fact significantly male biased.

Summary

Males and females of the dioecious, clonal, macrophyte *V. americana* appear to use different reproductive "strategies". Three lines of evidence support the hypothesis that a tradeoff takes place in the allocation of biomass between sexual and asexual reproductive functions in female, but not in male plants. First, male, female, and non-flowering plants all allocated the same proportion of total biomass towards reproduction (sexual plus asexual). Female asexual reproductive effort (ARE) was negatively correlated with sexual reproductive effort (SRE), while male ARE was uncorrelated with SRE. Second, there was a close physiological coupling of sexual and asexual reproductive allocation among female, but not male plants. When flower buds were removed from females, resources that might have gone into sexual reproduction were allocated to asexual reproductive structures, so that total reproductive allocation was maintained. When inflorescence buds were

removed from males, asexual reproductive allocation was not affected. Third, although total reproductive effort is the same between males and females, females allocate on average more to sexual reproduction, and less to asexual reproduction than do males. The results suggest that female allocation to sexual reproductive structures is variable, and that female allocation to asexual reproductive structures is dependent upon allocation to sexual reproductive structures. Consequently, a tradeoff occurs between them. Male allocation to sexual reproductive structures is variable, but has little impact on asexual reproductive allocation.

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Table 1. The calculation of derived variables, including reproductive effort and the ratio SR/TR. Biomass was measured as the dry mass of tissues dried at 105° C.

V =	Vegetative biomass: biomass of rosettes and their interconnecting stolons
AR =	Asexual reproductive biomass: biomass of perennating tubers and the stolons giving rise to them
SR =	Sexual reproductive biomass: biomass of flowers or inflorescences and the peduncles supporting them
T =	Total biomass = V + AR + SR
TR =	Reproductive biomass = AR + SR
ARE =	Asexual reproductive effort = the proportion of total biomass allocated to asexual reproduction = AR/T
SRE =	Sexual reproductive effort = the proportion of total biomass allocated to sexual reproduction = SR/T
TRE =	Total reproductive effort = TR/T = ARE + SRE
SR/TR =	The proportion of reproductive biomass allocated to sexual reproduction

Table 2. 1990 correlation coefficients and levels of significance between measures of reproductive allocation and total biomass, and vegetative biomass. All measures of reproductive allocation were log transformed [$Y' = \log(Y + 1)$].

Plant Group	n	Variable	Total Biomass		Vegetative Biomass	
			r	p	r	p
All plants	140	Reproductive biomass	0.890	<0.0001	0.690	<0.0001
Males	47	Reproductive biomass	0.851	<0.0001	0.577	<0.0001
		Tuber biomass	0.825	<0.0001	0.537	0.0001
		Inflorescence number	0.466	0.001	0.451	0.002
Females (buds intact)	40	Reproductive biomass	0.904	<0.0001	0.669	<0.0001
		Tuber biomass	0.723	<0.0001	0.560	0.0002
		Flower + fruit biomass	0.381	0.015	0.225	0.16
		Flower number	0.425	0.006	0.261	0.10
Females (buds removed)	36	Reproductive biomass	0.932	<0.0001	0.816	<0.0001
		Tuber biomass	0.931	<0.0001	0.817	<0.0001
		Flower number	0.028	0.87	0.067	0.70
Non-flowering	17	Reproductive biomass	0.828	<0.0001	0.570	0.017

Table 3. 1993 correlation coefficients and levels of significance between measures of reproductive allocation and total biomass, and vegetative biomass.

Plant Group	n	Variable	Total Biomass		Vegetative Biomass	
			r	p	r	p
All plants	53	Reproductive biomass	0.859	<0.0001	0.530	<0.0001
Males (mature buds removed)	19	Reproductive biomass	0.844	<0.0001	0.473	0.041
		Tuber biomass	0.823	<0.0001	0.444	0.057
		Inflorescence biomass	0.484	0.036	0.411	0.080
		Inflorescence number	0.389	0.10	0.424	0.071
Males (immature buds removed)	19	Reproductive biomass	0.818	<0.0001	0.482	0.037
		Tuber biomass	0.799	<0.0001	0.454	0.051
		Inflorescence biomass	0.552	0.014	0.575	0.010
		Inflorescence number	0.659	0.002	0.675	0.002
Females (unpollinated)	15	Reproductive biomass	0.912	<0.0001	0.671	0.006
		Tuber biomass	0.659	0.007	0.454	0.089
		Flower biomass	0.611	0.016	0.485	0.067
		Flower number	0.560	0.030	0.423	0.12

Table 4. Correlation coefficients and levels of significance between mass specific measures of sexual and asexual reproductive allocation. Negative correlations indicate a tradeoff between these measures.

Year	Sex	n	First variable	Second variable	r	p
1990	F	40	# fruit • g ⁻¹	# tubers • g ⁻¹	-0.544	0.0003
	F	40	SRE	ARE	-0.710	<0.0001
	M	47	# infl. • g ⁻¹	# tubers • g ⁻¹	-0.176	0.24
1993	F	15	# fls. • g ⁻¹	# tubers • g ⁻¹	-0.577	0.024
	F	15	SRE	ARE	-0.787	0.0005
	M	19	# infl. • g ⁻¹	# tubers • g ⁻¹	-0.210	0.39
	M	19	SRE	ARE	-0.219	0.37

Table 5. Correlations in 1990 between the proportion of reproductive biomass allocated to sexual reproduction (SR/TR) and total biomass, vegetative biomass, flowers per gram, SRE, or ARE among unmanipulated females. n = 40.

SR/TR	Variables	r	p
SR/TR	Total biomass	0.255	0.11
SR/TR	Vegetative biomass	0.264	0.10
SR/TR	Flowers • g ⁻¹	0.835	<0.0001
SR/TR	SRE	0.923	<0.0001
SR/TR	ARE	-0.889	<0.0001

Table 6. Correlations between flower number and sexual reproductive biomass, and between tuber number and asexual reproductive biomass for unmanipulated females in 1990. n = 40.

First variable	Second variable	r	p
Flowers	Sexual reproductive biomass	0.925	<0.0001
Tubers	Asexual reproductive biomass	0.901	<0.0001

Table 7. The effect of bud removal on the number of flowers and flowers per gram among females (1990), and on the number of inflorescences and inflorescences per gram among males (1993). Data were analyzed with a mixed model analysis of variance.

	Source of variation	df	MS	F	p
a)	1990: # flowers				
	treatment	1	13.721	1.623	0.25
	tank	6	4.964	1.207	0.32
	treatment x tank	6	8.453	2.055	0.072
	error	62	4.114		
b)	1990: # fls. • g ⁻¹				
	treatment	1	3.029	1.775	0.23
	tank	6	1.030	1.844	0.11
	treatment x tank	6	1.706	3.056	0.011
	error	62	0.558		
c)	1993: # inflorescences				
	treatment	1	14.935	2.154	0.38
	tank	1	50.601	2.320	0.14
	treatment x tank	1	6.935	0.318	0.58
	error	34	21.812		
d)	1993: # infl. • g ⁻¹				
	treatment	1	0.572	0.325	0.67
	tank	1	0.023	0.016	0.90
	treatment x tank	1	1.762	1.245	0.27
	error	34	1.415		

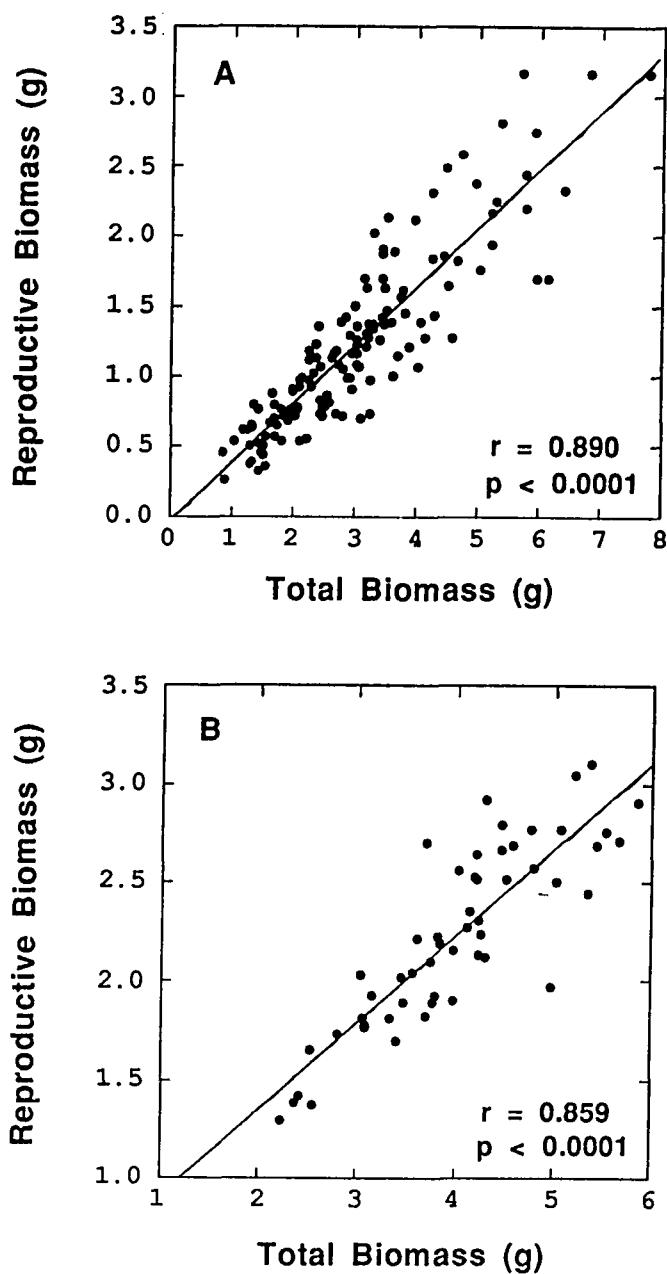


Figure 1. Relation between reproductive biomass and total biomass in *V. americana* for all plant groups combined in 1990 (A) and 1993 (B).

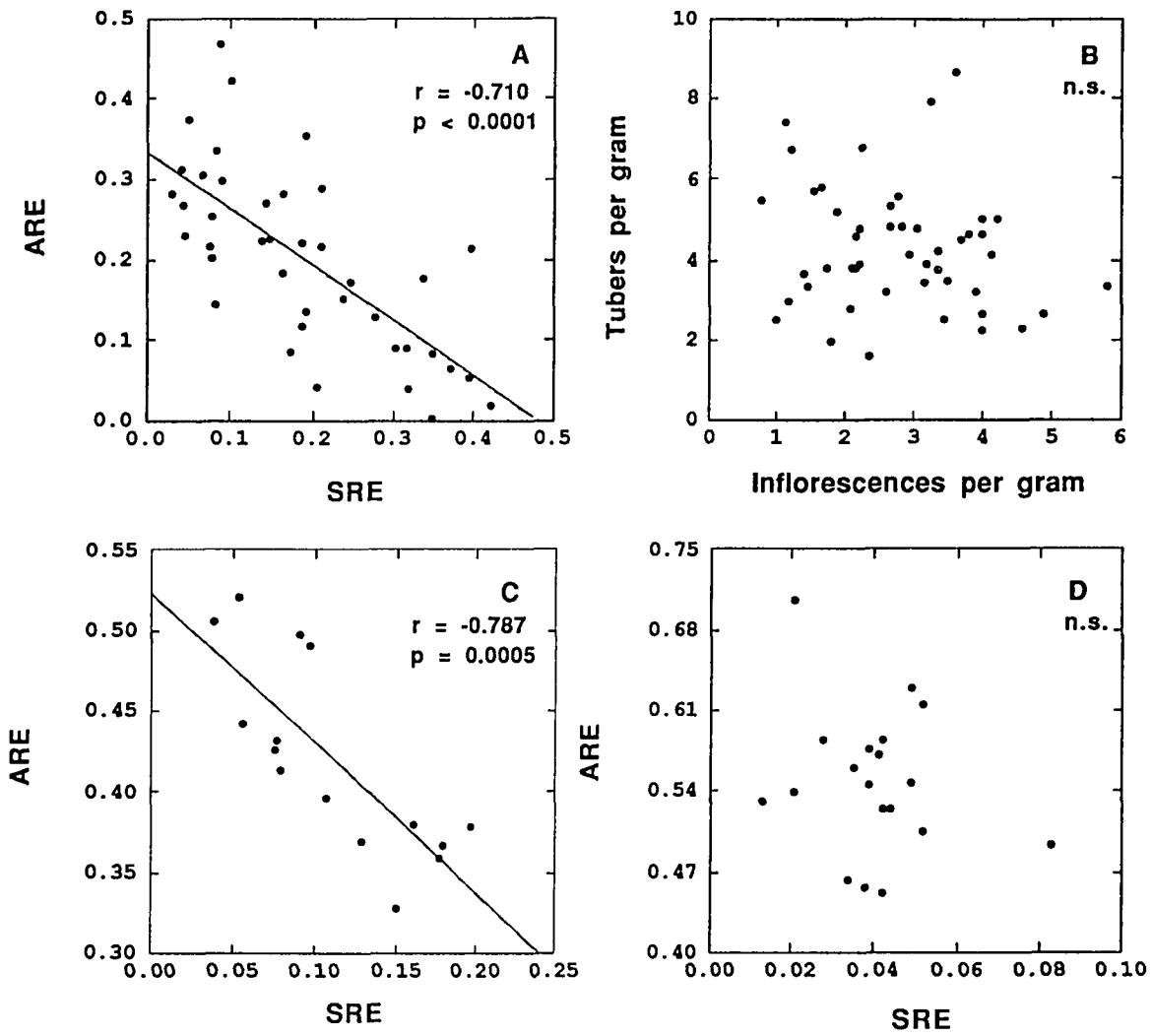


Figure 2. Relation between ARE and SRE among unmanipulated females (A), and between tubers per gram and inflorescences per gram among males (B) in *V. americana* in 1990. Relation between ARE and SRE among females (C) and unmanipulated males (D) in *V. americana* in 1993.

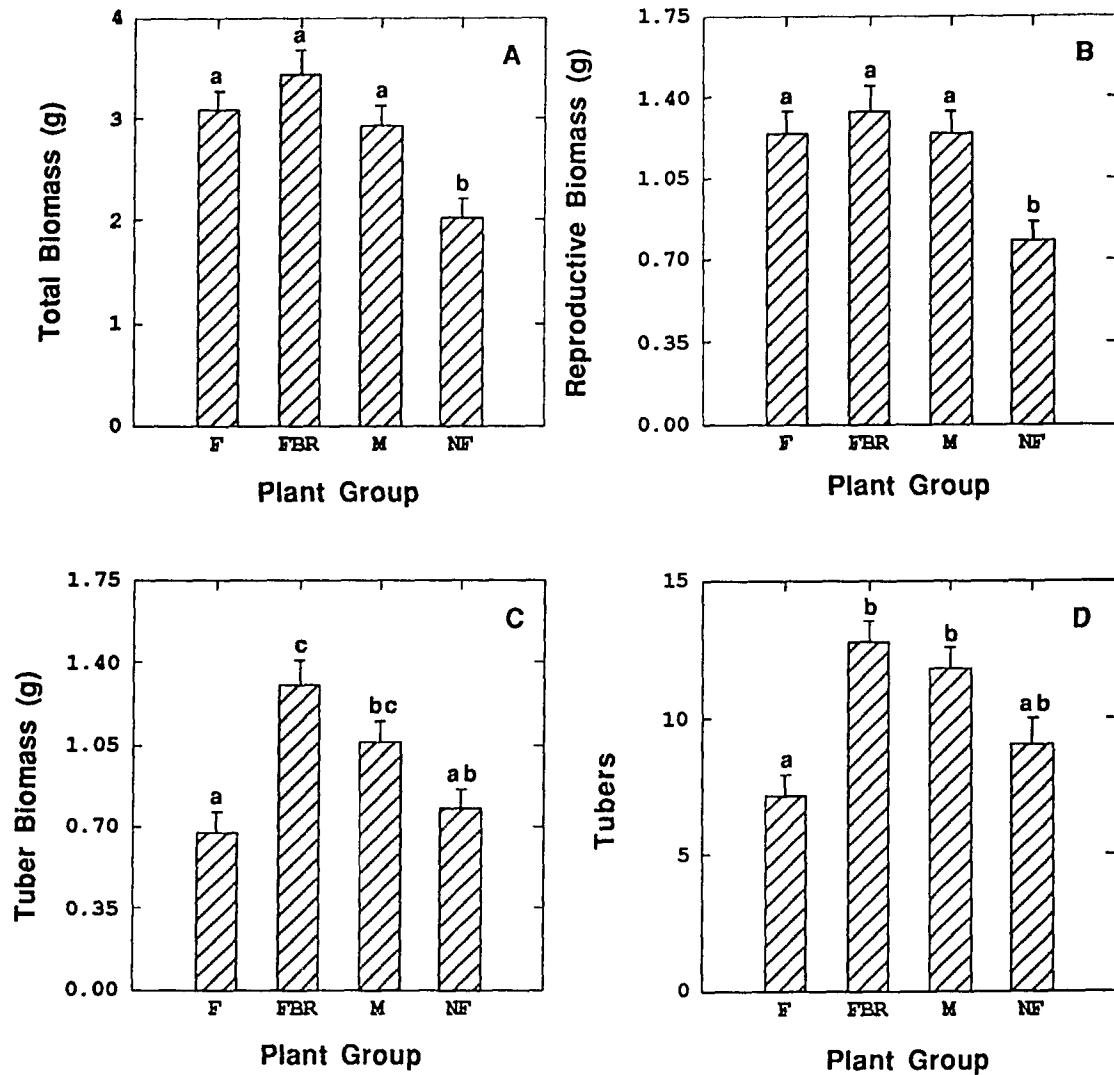
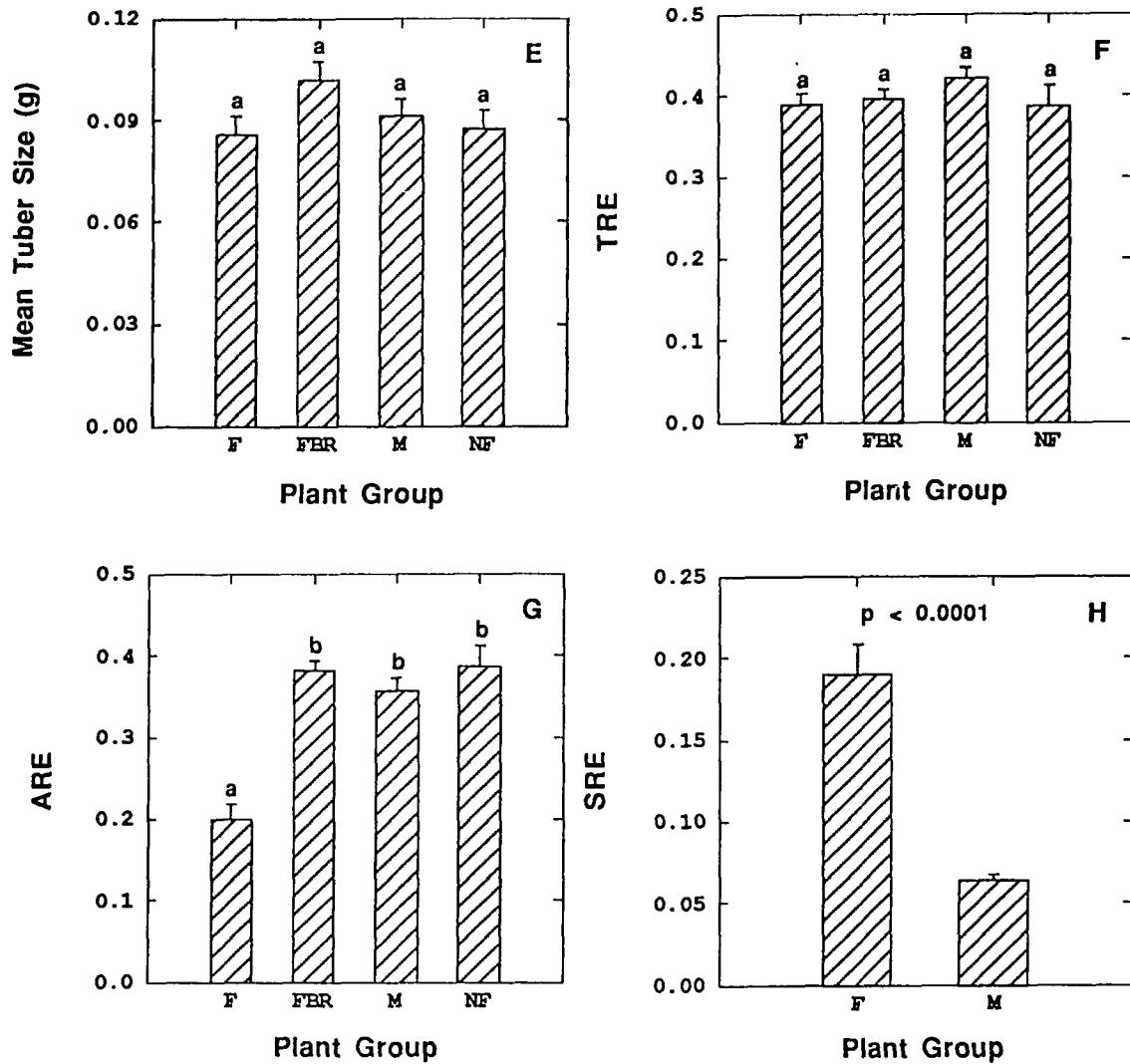
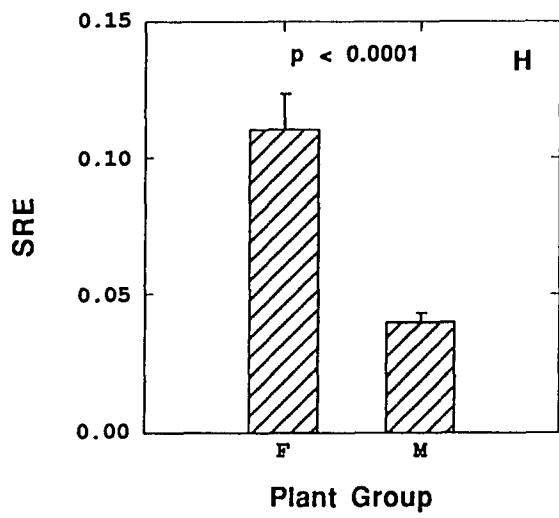
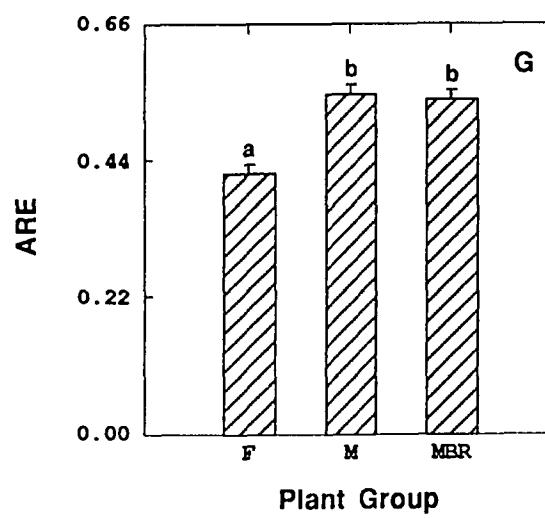
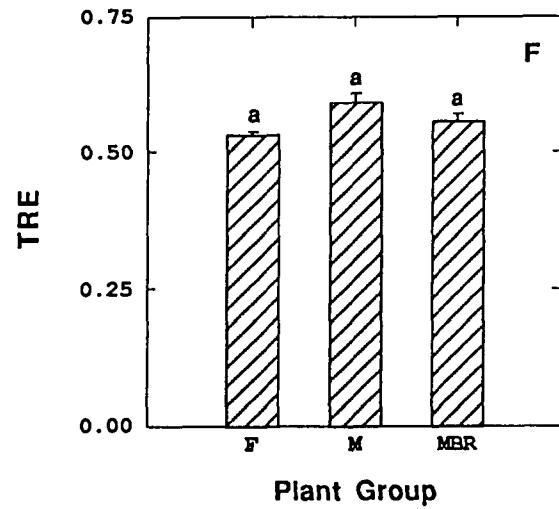
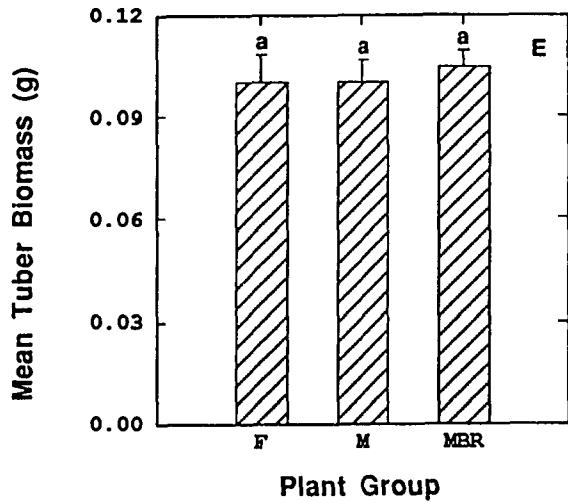


Figure 3. Comparisons of relative and absolute measures of growth and reproduction among F (females), FBR (females with flower buds removed), M (males), and NF (non-flowering plants) in *V. americana* in 1990. Error bars represent standard errors. Means sharing common letters do not differ significantly, based on post-hoc comparisons (Keppel, 1984).





CHAPTER III

The effect of resource availability on
sexual versus asexual reproductive allocation
in the dioecious clonal macrophyte *Vallisneria americana* (Michx.)

INTRODUCTION

A great deal of attention has been given to how resources are divided between seed production and asexual reproduction in clonal organisms (Holler and Abrahamson, 1977; Armstrong, 1982, 1984; McCrea and Abrahamson, 1987; Reekie, 1991). Several theories have been advanced to account for differences in the relative allocation to sexual versus asexual reproduction (Abrahamson, 1975; Williams, 1975; Caswell, 1985; Loehle, 1987; Sackville-Hamilton, Schmid, and Harper, 1987). An assumption of these theories is that tradeoffs occur between these two modes of reproduction, and that the balance between them is shaped by the relative success of sexual and asexual offspring. For some species, this balance may be characterized by considerable plasticity, with the ratio of sexual to asexual reproductive allocation influenced by environmental conditions (Sarukhan and Harper, 1973; Douglas, 1981; Turkington and Maze, 1981; Jurik, 1983; Jurik, 1985; Swamy and Ramakrishnan, 1988; Reekie, 1991). Plasticity in reproductive allocation may be influenced by the relative costs and benefits associated with investing in one mode of reproduction versus the other (Loehle, 1987), assuming that the plant can detect conditions within its environment.

Several studies among various species have examined plasticity in reproductive allocation associated with density (Holler and Abrahamson, 1977; Pitelka, Stanton, and Peckenham, 1980; Waite and Hutchings, 1982; Bishop and Davy, 1985), environmental

gradients (Hickman, 1975; Bostock, 1980; Soule and Werner, 1981; Turkington and Maze, 1981 Jurik, 1985; Chapin, et al., 1987), or resource availability (Ashmun, Brown, and Pitelka, 1985; Reekie and Bazzaz, 1987; Stafford, 1989; Evans, 1992). Many of these studies have yielded inconsistent results. Variation has been detected in relative allocation to both sexual and asexual reproduction, to one mode but not the other, or to neither mode of reproduction. Plasticity in a life history strategy may be an important factor influencing reproductive success in unpredictable environments, or in environments that change predictably, such as during successional change or the aging of a clone (Williams, 1975). For example, Swamy and Ramakrishnan (1988) found that sexual reproductive effort decreased while asexual reproductive effort increased in *Makania micantha* across successional environments in abandoned fields. Such changes were related to increasing competition for limiting nutrients within more mature environments. Hartnett (1990) has shown that among some clonal composites, sexual and asexual reproductive effort may vary across habitats, but that they vary in the same direction and magnitude, so that the balance between them is unaffected.

In a preliminary study relating sexual reproductive output to asexual reproductive output in the submersed aquatic macrophyte *Vallisneria americana* Hydrocharitaceae, a tradeoff between the number of fruits produced and the number of clonal propagules was observed. Subsequent work has shown that there is a significant genetic component to variation in sexual reproductive allocation (Sullivan, in preparation). My interest in this study was to examine plasticity in sexual and asexual reproductive allocation, and in the balance between them, as function of resource availability. I specifically wished to relate plasticity in reproductive performance to mineral nutrient, carbon, and light availability. I had three major objectives: (1) to examine potential change in both the numbers and biomass of clonal propagules per gram plant mass (relative allocation) as a function of resource availability, (2) to examine potential change in both the numbers and biomass of fruit per gram plant mass (females), or in the number of inflorescences per gram plant mass

(males), as a function of resource availability, (3) to examine potential change in the balance between sexual and asexual reproductive allocation.

The availability of carbon was of particular interest in this regard. Unlike the availability of CO₂ to terrestrial plants, the availability of dissolved inorganic carbon (DIC) may vary a great deal among aquatic systems. In a survey of 16 Adirondack lakes, DIC was found to range in concentration from levels below saturation up to 9 times the concentration expected if CO₂ was in equilibrium with the atmosphere (Titus, Feldman, and Grisé, 1990). Cole and Caraco (1992) found that a great many North American lakes are supersaturated with CO₂, many with 4 to 5 times the amount that would be found if CO₂ was in equilibrium with the atmosphere.

Sediment mineral nutrient levels were of interest because sediment quality can vary a great deal between and within aquatic systems, and growing plants in different sediments can have a dramatic effect on their growth (Barko and Smart, 1986; Titus, 1992). Moreover, one of the specific predictions of Loehle's model (1987) that I wished to evaluate was that an increase in mineral nutrient availability will result in an increase in sexual reproduction at the expense of asexual reproduction due to the greater cost of seed production relative to asexual reproduction.

Light was also of interest because it is the ultimate source of plant energy, and it varies a great deal among systems and seasonally within systems. This variation may be related to interactions taking place among the various biotic and abiotic components of an aquatic system, including: (1) phytoplankton density, (2) phytoplanktivore activity, (3) nutrient availability, (4) suspended material, and (5) physical characters (temperature, O₂ concentration, etc.). Light quality also varies with depth as light wavelengths are differentially attenuated by the water column and the substances dissolved in it.

METHODS AND MATERIALS

Study Species

Vallisneria americana (wild celery) is a submersed dioecious clonal macrophyte widely distributed throughout eastern North America (Lowden, 1982). *V. americana* reproduces in the northeast as an asexual annual (Hutchinson, 1975) from perennating tubers in addition to seeds. A rosette of ribbon-like leaves germinates from each tuber, with additional rosettes developing at the nodes of stolons arising from axillary meristems (Sculthorpe, 1967). Flowers and inflorescences develop from the two separate meristems flanking each axillary meristem giving rise to a stolon. Buoyant female flowers are tethered on an elongated peduncle (Wylie, 1917). Anthesis occurs at the surface, but will occur below the surface in deeper water (Sullivan, personal observation). Up to 2000 male flowers (approximately 0.6 mm in diameter) may develop on a short peduncled inflorescence (Wylie, 1917). Buoyant male flowers abscise over a period of hours after the spathe opens (usually less than 36: Sullivan, personal observation), and are supported on the surface film. Pollination occurs at the surface when male flowers are caught in the surface tension depression surrounding the female perianth, causing stamens to brush the stigma (Wylie, 1917; Svedelius, 1932; and Cox, 1988). Buoyant fruits develop quickly and remain attached for several weeks, until the fruit either senesces or disperses when the peduncle senesces (Sullivan, personal observation). One to several perennating tubers (usually two or three) develop on stolons growing down into the sediment from mature or terminal rosettes. The entire plant senesces at the end of the growing season, except for tubers and seeds.

Plant collection, growth, and harvest

In 1990, plants germinating from tubers were collected by divers from Chenango Lake, NY (42°15'N; 75°50'W) on May 29. Two hundred twenty four plants of unknown sex

were collected from a population covering from two to three hectares at the south end of the lake. They were stored for seven days at 5° C in lake water until transplanting into the Binghamton University greenhouse, where they were grown in seven replicate 1200 liter fiberglass tanks.

A randomized block design was used with tank as the blocking factor. All plants were first weighed and assigned to one of five weight classes. Six plants from each weight class were randomly assigned to each of the seven tanks to ensure an even distribution of initial plant sizes per tank. Plants were also randomly assigned to one of two sediment treatments, four from each weight class to the high quality sediment treatment and two from each weight class to the low quality sediment treatment, so that 20 plants per tank were grown in the high quality sediment and 10 plants per tank were grown in the low quality sediment.

Each plant was grown in a plastic pot (20 cm wide x 18 cm deep) in three liters of sediment. Sediment from Big Moose Lake, NY (43°49'N; 74°51'W) was used in the high quality sediment treatment. Big Moose sediment diluted with #2 quartz sand (1 part sediment to 3 parts sand) was used in the low quality sediment treatment. The tanks were filled with deionized water to which was added the following to facilitate growth and osmotic balance (see Smart and Barko, 1985): $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (91.7 mg l⁻¹), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (69.0 mg l⁻¹), and KHCO_3 (15.4 mg l⁻¹). To compensate for evaporative loss, the tanks were topped off weekly with deionized water. pH was maintained at 7.2 ± 0.25 units with Horizon Model 5997 pH control units through the addition of a 0.4N mixture of 30% HNO_3 and 70% H_2SO_4 , or 0.4N NaOH. Temperature was maintained at $23^\circ \pm 1^\circ \text{C}$ with Remcor CFF500 water circulating units.

The plants were monitored daily. Thirty six of the plants that flowered as females had their flowers removed to study the effects of removal on reproductive allocation, and these plants were not considered in the analysis. The plants were harvested on Sept. 21, 1990. Counts were made of the number of rosettes, stolons, tubers, female flowers/fruits, and

male inflorescences. Inflorescence number was determined by counting the number of inflorescence peduncle "stubs" that remained after inflorescence senescence. Inflorescence biomass was not measured because inflorescences senesce at maturity. However, 20 inflorescences from each treatment were harvested at maturity to provide an estimate of inflorescence biomass. Rosettes (plus their interconnecting stolons), tubers (plus their tuber bearing stolons) and fruits (plus their peduncles) were dried at 105° C and then weighed to determine their biomass (= dry mass). Root biomass was not measured as the fine roots could not be separated from the course particles of Big Moose sediment.

Several derived variables were then calculated (Table 1). Reproductive effort was calculated as the proportion of total plant biomass allocated to reproduction (for a discussion of methods in calculating reproductive effort, see Hickman, 1975; Thompson and Stuart, 1981; Bazzaz and Reekie, 1985). Asexual reproductive effort (ARE) was calculated as asexual reproductive biomass / total plant biomass. Sexual reproductive effort (SRE) was calculated for females as sexual reproductive biomass / total plant biomass. Total reproductive effort (TRE) was determined to be the sum of SRE and ARE. The proportion of total reproductive biomass (TR: sexual plus asexual) allocated to sexual reproduction (SR/TR) was calculated as sexual biomass / (sexual plus asexual biomass).

On January 3, 1991, 90 *V. americana* tubers of unknown sex were planted in the Binghamton University greenhouse to examine the effects of carbon availability and inflorescence removal on reproductive allocation. The tubers had been collected in September of 1990 from Chenango Lake and stored in sediment at 4° C for three months. The tubers were planted in pots (16 cm wide x 18 cm deep) in sediment collected from Montezuma Bay on Cayuga Lake, NY (42°55'N; 76°45'W). The plants were grown in two of the fiberglass tanks detailed above. Two dissolved inorganic carbon (DIC) levels were imposed, one on each tank. DIC was enhanced to approximately 4.5 times ambient levels by adding pure CO₂ to air bubbling through the water column (see Titus, Feldman, and

Grisé, 1990). Half of the plants had their immature inflorescence buds removed to examine the effect of bud removal on asexual reproduction. Plants were induced to grow and flower by manipulating photoperiod with timer-controlled General Electric GEH 2877 multi-vapor lamps. The photoperiod between January and April was altered to promote flowering and tuber formation. The plants were otherwise grown as detailed above. Plants were harvested on April 25, 1991, and processed as outlined in the 1990 greenhouse experiment. The harvested plants had flowered and produced tubers, but not all of the tubers were fully developed at the time of harvest. Consequently, estimates of ARE may have been low.

In the summer of 1991, *Vallisneria* plants germinating from tubers were collected by divers from Seneca Lake, NY (42°46'N; 76°58'W) on June 15. One hundred sixty plants of unknown sex were collected from a population covering several hectares at the northwest end of the lake. They were stored for two days at 5° C in lake water until transplanting into the Binghamton University greenhouse, where they were grown in four 1200 liter fiberglass tanks. All plants were weighed and randomly assigned to one of the tanks. Each plant was grown in a plastic pot (16 cm wide x 18 cm deep) in three liters of sediment.

A split plot design was used where dissolved inorganic carbon (DIC) was crossed with light, and sediment quality levels were split within tanks. DIC levels were ambient (the concentration found if DIC was in equilibrium with the atmosphere) and 2 x ambient. DIC was enhanced by adding pure CO₂ to air bubbling through the water column (see Titus, Feldman, and Grisé, 1990). The levels of light were unfiltered light (filtered only by the glasshouse structure, a loss of approximately 5%) and light filtered by four layers of neutral density shade cloth (approximately 85% of light was attenuated). A high quality sediment collected from Otsego Lake, NY (42°47'N; 74°52'W) was used. The sediment nutrient levels used were 100% and 25% (sediment diluted with #2 quartz sand: three parts

sand to one part sediment by volume). All coarse material was removed from the sediment by filtering with 0.13 cm hardware cloth. The plants were otherwise maintained as in 1990. Plants were harvested on September 20. Data were collected as in 1990. In addition, biomass data on roots were collected by spraying the fine sediment away from the roots with a hose at harvest.

In the summer of 1992, plants germinating from tubers were collected by divers from Chenango Lake, NY on May 26. Eighty four plants of unknown sex were collected from a population covering several hectares at the south end of the lake. They were stored for five days at 5° C in lake water until transplanting into the Binghamton University greenhouse, where they were grown in four replicate 1200 liter fiberglass tanks. All plants were weighed and randomly assigned to a treatment, and to one of the tanks. Each plant was grown in a plastic pot (20 cm mouth diameter x 18 cm deep) in three liters of sediment collected from Chenango Lake.

A nested design was used where tanks were nested in two levels each of dissolved inorganic carbon (DIC). DIC levels were ambient and 4.5 x ambient (similar DIC levels have been measured in Chenango Lake). The plants were otherwise maintained as in 1990. The experiment ran from June 3 to harvest on September 24. The biomass of male inflorescences used in calculating SRE and TRE was estimated from the biomass of mature inflorescences harvested during the experiment.

Statistical analysis

Data were analyzed with the SYSTAT statistical analysis package (SYSTAT, 1992). In 1990, a randomized block design was used with tank as the blocking factor. A mixed model two factor analysis of variance was used to examine differences in growth and relative allocation between sediment levels in males and in females, with tank as the random

factor. Because only eight females flowered in the low quality sediment treatment, the means of females in each tank were used in the analysis, so that the design became randomized block without replication. The Friedman's test was used to examine differences in male total reproductive effort between sediment treatments.

In the spring 1991 experiment, a two-factor analysis of variance was used to examine differences in growth and relative allocation between carbon levels in males plants with inflorescences intact or removed. There were only eleven female plants in the experiment, so they were eliminated from the design. A Mann-Whitney U test was used to examine differences in mean tuber biomass between high and low carbon treatments.

In the summer 1991 experiment, a three-way analysis of variance was used to examine differences in growth and relative allocation between carbon, sediment, and light treatments among males.

In the 1992 experiment, a mixed model, nested, two-factor analysis of variance was used, with tanks nested in levels of DIC, to examine differences in growth and relative allocation between DIC levels in males and females.

The calculation of derived variables is detailed in Table 1. Residuals were graphically examined in all analyses for violations of statistical assumptions. Some characters were subsequently log transformed ($Y' = \log [Y + 1]$), natural log transformed ($Y' = \ln Y$), or arcsin transformed ($Y' = \arcsin \sqrt{Y}$) as noted to improve normality or meet assumptions of homoscedasticity.

RESULTS

Not all of the plants in each experiment flowered, nor where there equal sex ratios found in each of the groups. In 1990, 47 of the 104 plants in the high quality sediment flowered as males, 40 as females, while 17 failed to flower. Of the 70 plants in the low quality sediment treatment, 35 flowered as males, eight as females, and 27 failed to flower.

In the spring 1991 experiment, 79 of the 90 plants were males and 11 were females. Twenty of the 41 males whose inflorescences were left intact were in the high DIC treatment and 21 were in the low DIC treatment. Twenty of the 38 males whose inflorescences were removed were in the high DIC treatment and 18 were in the low DIC treatment. The eleven females were not considered in this experiment. In the summer 1991 experiment, all 160 plants flowered as males. In the 1992 experiment, 25 of the 54 plants in the high DIC treatment flowered as males, with 29 flowering as females. Eleven of the 28 plants in the low DIC treatment flowered as males, with 17 flowering as females.

Carbon availability

Increasing the availability of dissolved inorganic carbon (DIC) had a strong effect on the growth of *V. americana*. Males in the spring 1991, summer 1991, and 1992 experiments responded to an increase in resource levels with a significant increase in total plant biomass (Fig. 1a, 2a, 3a). There were also a response in total biomass to multiple resource level combinations in the summer 1991 experiment (Table 3). Total biomass in all the low carbon treatments was a relatively consistent proportion (39 to 49%) of biomass in the high carbon treatments. This consistency in proportion reflected a multiplicative (rather than additive) response to light and sediment at different levels of DIC, so that significant DIC*light and DIC*sediment interactions were detected in the analysis (Fig. 2a). Despite the dramatic effect resource availability had on plant size, DIC had little impact on the relative production of sexual reproductive structures by males. Differences in the number of inflorescences per gram between high and low DIC levels in each experiment were not significant (Tables 2, 3, and 4). SRE and SR/TR were not evaluated in the spring 1991, summer 1991, and 1992 experiments because biomass data on inflorescences were not collected.

There was a consistent response in asexual reproductive allocation to increased carbon availability among males. Males grown in the higher carbon environments produced significantly larger tubers (mean biomass per tuber) than those in the lower carbon environments (Fig. 1b, 2b, 3c). ARE was on average higher for males in the high DIC environment, but this increase was only significant in the summer 1991 experiment (Fig. 2c). Interestingly, although the production of larger tubers by males in the summer 1991 experiment resulted in a significant increase in ARE, these plants produced fewer tubers per gram plant mass (Fig. 2d). Fewer tubers per gram were also observed for males in the high DIC treatment in the spring 1991 experiment (Fig. 1c). This suggested a possible tradeoff between the size and number of tubers for the plants grown in different DIC environments.

The effect of carbon availability on sexual reproductive allocation in females was different than in males. In the 1992 experiment, the size of fruits (mean biomass per fruit) for females grown in the high DIC treatment was significantly larger than for females in the lower DIC treatment (Fig. 3d). Because of this increase in fruit size, female SRE was on average greater in the higher carbon environment, although this increase was not significant (Table 4). Average levels of ARE were also greater in the high DIC treatment in females in the 1992 experiment, but this increase was not significant either. However, TRE (SRE + ARE) was significantly higher in the higher carbon environment (Table 4). Despite this increase in TRE among these females, the proportion of reproductive biomass (sexual plus asexual biomass) allocated to sexual reproduction was not affected by carbon availability (Table 4), indicating that the balance between sexual and asexual reproduction was not affected by the disproportionate increase in reproductive biomass.

Mineral nutrient effects

Growing *V. americana* in different quality sediments had significant effects on plant size among both males and females. Male and female plants in the 1990 experiment were significantly larger when grown in full strength Big Moose sediment than those grown in the diluted sediment (Fig. 4a, b). The same effect was observed in the summer 1991 experiment where males grown in full strength Otsego Lake sediment were significantly larger than those grown in the diluted sediment (Fig. 2a). There was a significant sediment*light interaction on plant growth in the summer 1991 experiment where the effect of sediment quality on plant growth was nearly twice as great in the high light environment as in the low light environment (Fig. 2a, Table 3). Despite the differences in plant size between sediment quality treatments, differences in the relative allocation to sexual reproduction were not significant. The number of inflorescences per gram did not differ between treatment levels in males in either experiment (Tables 3, 5). Differences in the estimated values of male SRE in the 1990 experiment were not significant either. However, there was a significant decrease in the proportion of reproductive biomass allocated to sexual reproduction (SR/TR) in the higher sediment quality treatment, suggesting a shift in reproductive allocation towards asexual reproduction in higher sediment quality environments (Table 5).

There was an effect of sediment quality on the relative allocation to asexual reproductive structures in males in both experiments, but this effect appeared to differ between plants grown on Big Moose vs. Otsego sediments. On average, males tended to produce larger tubers in both higher quality sediment treatments, although this response was not significant for the males grown in Big Moose sediment in 1990 (Fig. 2b, 4c). However, ARE was significantly higher among the males grown in higher quality Big Moose sediment in 1990 (Fig. 4e). Despite the larger tubers produced by males in the summer 1991 experiment, there was virtually no difference in ARE as a function of sediment

quality, probably because these plants were producing significantly fewer tubers per gram plant mass (Table 3). There also was a significant sediment*light interaction among these males where even more tubers per gram were produced in the low quality sediment environment when the plants were grown in low light (Fig. 2d). Sediment quality did not have an effect on the number of tubers produced per gram among males in 1990 (Table 5).

The effect of sediment quality on reproductive allocation among females in the 1990 experiment was qualitatively similar to the response to DIC. There was a significant effect of sediment quality on plant size (Fig. 4b). Plants grown on higher quality Big Moose sediment also produced larger fruits (Fig. 4d). There was no difference in ARE between sediment quality levels, and differences in SRE were not significant (Table 5). As in the response to elevated DIC, TRE was greater in females grown in the high quality sediment as well. Of particular interest, differences in the balance between sexual and asexual reproduction (SR/TR) between sediment treatments were not significant (Table 5).

Light effects

The availability of light was only examined in the summer 1991 experiment on male plants, and predictably was found to be a limiting factor for plant growth (Table 3). Plant biomass was significantly reduced among plants grown under low light conditions (Fig. 2a). Despite the effect of light intensity on plant size, light levels had no effect on the relative allocation to male sexual reproduction (inflorescences per gram) or ARE. Light availability did have a significant effect on mean tuber size, so that plants grown in higher light conditions produced significantly larger tubers than those grown in low light conditions (Fig. 2b). There was also a significant reduction in the number of tubers produced per gram for males grown under low light conditions.

DISCUSSION

These results suggest that *V. americana* exhibits a certain amount of plasticity in its reproductive allocation patterns in response to changes in resource availability. Specifically, they suggest that (1) males and females may utilize different response strategies, and (2) the effects of either DIC availability or sediment quality on male or female reproductive allocation were not qualitatively different from one another.

The response among females to an increase in sediment quality or carbon availability was to produce larger fruits, while there was no change in the size of tubers. Consequently, there was little impact on asexual reproductive effort (ARE). However, in both experiments, females significantly increased the proportion of plant biomass allocated to sexual plus asexual reproduction: total reproductive effort (TRE). Interestingly, although females in both experiments increased TRE, the balance between sexual and asexual reproductive allocation was unaffected. This result is in agreement with Sullivan (unpublished manuscript) who found that there was a significant genetic component to the proportion of reproductive biomass allocated to sexual reproduction among females from this same population. This result also qualitatively differs from the predictions of Loehle (1987) who suggested that sexual reproduction would increase in response to greater mineral nutrient availability at the expense of asexual reproduction. Neither SRE , ARE, nor the balance between them were significantly affected by sediment quality in these experiments despite the dramatic impact of sediment quality on plant size.

Males appeared to have a different response to changes in resource availability. In response to higher levels of DIC or light, the males in these experiments increased the size of their tubers, though the increase in tuber size among males grown in higher quality sediment was not significant. The production of larger tubers is an important response in that it can confer a competitive advantage upon the clonal offspring if larger tubers produce faster growing or larger plants. Initial tuber size in *V. americana* males has been positively

correlated with final plant biomass in other studies (Titus and Hoover, 1991). Although mean tuber biomass was increased in the higher DIC or light environments, an increase in ARE was not always found. This may have resulted because of the general, although not always significant, decrease in the relative number of tubers produced among males in the higher resource environments, suggesting that the plants may be producing proportionately fewer, though larger, tubers.

There was no effect of resource availability on the mass specific production of inflorescences (inflorescences per gram). When inflorescence biomass was estimated in the 1990 experiment, the effect of sediment quality on SRE was not significant either. Since this response was coupled with a significant increase in ARE, there was a significant decrease in the proportion of reproductive biomass allocated to inflorescence production (SR/TR). This contrasts sharply with the results found among the females, where differences in SR/TR were not significant. It has long been recognized that males and females may differ in their response to resource availability (Bazzaz, et al, 1987). Here, the differences in the response of SR/TR to these resource environments between males and females may be related to the timing and magnitude of reproductive investment. Inflorescence development is tailing off at the time males begin developing tubers. Consequently, there is little internal competition for reproductive resources. The plant is able to allocate all of its reproductive biomass to tubers late in the season, and any additional reproductive biomass resulting from greater resource availability can result in either more or larger tubers. Among females however, developing fruits may compete for reproductive resources with developing tubers well into the later part of the growing season, and potentially through plant senescence (Sullivan and Titus, unpublished manuscript). These data suggest that any additional reproductive biomass resulting from greater resource availability may be allocated to fruits at the expense of tubers. Thus, differences between males and females in the magnitude of clonal reproduction (number of

tubers produced) may become exacerbated in better environments, conferring a demographic advantage to male plants.

Several other studies have shown that the magnitude or timing of fruit development plays a critical role in defining the differences between males and females in reproductive allocation. Female reproductive effort (RE) may be greater than male RE as a function of fruit production (Allen and Antos, 1988; Antos and Allen, 1990), although male RE may be greater than female RE in pollen limited individuals (Armstrong and Irvine, 1989).

Gross and Soule (1981) found that RE among *Silene alba* females with less than 20% fruit set was lower than in males due to the lower cost of male flowers. They also report that the lower early season cost to female flowers (relative to males) accounts for the larger size of female plants in that and other populations. Agren (1988) reported that RE in *Rubus chamaemorus* females was greater than in males, and that fruit producing ramets delayed the onset of rhizomatous branching beyond that of males or non-fruiting females.

Korpelainen (1992) found that the magnitude of male and female RE in *Rumex acetosa* and *R. acetosella* changes with the end of male flowering and the onset of fruit development, so that by late in the season, fruiting females allocate both a greater total and proportionate amount towards reproduction than do males. Delph, Lu, and Jayne (1993) showed that similar differences in the timing and magnitude of reproductive allocation among males and females led to differences in the rate of reproductive tillering in *Carex picta*.

The results of this study indicate that *V. americana* exhibits plasticity in reproductive allocation related to carbon, mineral nutrient, or light levels. Males may react to better resource environments by producing more and larger clonal offspring, while fruiting females may react by producing larger fruits. All else being equal, males appear to gain a demographic advantage in better resource environments if we assume that seedling establishment is not particularly high.

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Table 1. The calculation of derived variables. Biomass was measured as the dry mass of tissues dried at 105° C.

ARE = Asexual reproductive effort = the proportion of total biomass allocated to asexual reproduction = asexual reproductive biomass / total biomass

SRE = Sexual reproductive effort = the proportion of total biomass allocated to sexual reproduction = sexual reproductive biomass / total biomass

TRE = Total reproductive effort = ARE + SRE

SR/TR = The proportion of reproductive biomass allocated to sexual reproduction

Mean tuber biomass = Biomass allocated to tubers and tuber bearing stolons / tuber number

Mean fruit biomass = Biomass allocated to fruits and peduncles / flower number

Tubers per gram = Tuber number / total biomass

Inflorescences per gram = Inflorescence number / total biomass

Table 2. Effect of dissolved inorganic carbon (DIC) level on growth and reproductive allocation in *V. americana* males in the spring 1991 experiment. Means \pm one standard error, with significance level. The low DIC level is ambient, the high DIC level is 4.5 x ambient.

	High DIC Mean \pm SE n = 40	Low DIC Mean \pm SE n = 39	p value
Total biomass (g) [†]	6.595 \pm 0.394	4.361 \pm 0.248	<0.0001
ARE	0.167 \pm 0.012	0.155 \pm 0.011	n.s.
Inflorescences per gram	2.339 \pm 0.126	2.360 \pm 0.154	n.s.
Tubers per gram	1.757 \pm 0.087	2.070 \pm 0.121	0.033
Mean tuber biomass (g)	0.098 \pm 0.007	0.075 \pm 0.003	0.0085

[†] values were log transformed to meet assumption of homoscedasticity: $Y' = \log(Y+1)$

Table 3. P values from a split-plot analysis of variance (main effects and interactions) on growth and reproductive allocation in *V. americana* males in the summer 1991 experiment. DIC (dissolved inorganic carbon) levels were ambient and 2 x ambient, light levels were 15% and 100% light intensity, and sediment nutrient levels were 25% and 100% Otsego lake sediment.

	Total Biomass [†]	ARE	Inflorescences per gram	Tubers per gram ^{††}	Mean Tuber Biomass
DIC	<0.0001	0.0001	n.s.	0.0009	<0.0001
Light	<0.0001	n.s.	n.s.	0.0033	0.0001
Sediment	<0.0001	n.s.	n.s.	<0.0001	<0.0001
D x L	0.0096	n.s.	n.s.	n.s.	n.s.
D x S	0.0097	n.s.	n.s.	n.s.	n.s.
S x L	<0.0001	n.s.	n.s.	0.0093	n.s.
D x L x S	n.s.	n.s.	n.s.	n.s.	n.s.

[†]values were log transformed to meet assumption of homoscedasticity: $Y' = \log(Y+1)$

^{††}values were natural log transformed to meet assumption of homoscedasticity: $Y' = \ln(Y)$

Table 4. Effect of dissolved inorganic carbon (DIC) levels on growth and reproductive allocation in *V. americana* males and females in the 1992 experiment. Means \pm one standard error, with significance level. The low DIC level is ambient, the high DIC level is 4.5 x ambient.

	High DIC Mean \pm SE	Low DIC Mean \pm SE	p value
Males			
Total biomass (g)	n = 11 8.725 \pm 0.411	n = 25 5.237 \pm 0.218	0.0031
ARE	0.438 \pm 0.018	0.402 \pm 0.012	n.s.
Inflorescences per gram	3.789 \pm 0.367	3.848 \pm 0.182	n.s.
Tubers per gram	3.872 \pm 0.313	4.838 \pm 0.290	n.s.
Mean tuber biomass (g)	0.117 \pm 0.006	0.089 \pm 0.005	0.019
Females			
Total biomass (g)	n = 17 9.106 \pm 0.413	n = 29 5.356 \pm 0.192	0.0020
ARE	0.117 \pm 0.014	0.095 \pm 0.013	n.s.
SRE ^{†††}	0.413 \pm 0.020	0.341 \pm 0.019	n.s.
TRE ^{†††}	0.529 \pm 0.015	0.436 \pm 0.013	0.033
SR/TR	0.776 \pm 0.027	0.775 \pm 0.031	n.s.
Tubers per gram	1.045 \pm 0.146	0.908 \pm 0.133	n.s.
Flowers per gram	1.704 \pm 0.081	2.283 \pm 0.112	n.s.
Mean tuber biomass (g)	0.116 \pm 0.008	0.116 \pm 0.012	n.s.
Mean fruit biomass (g)	0.245 \pm 0.010	0.155 \pm 0.082	0.0057

^{†††}values were arcsin transformed to meet assumption of homoscedasticity: $Y' = \arcsin \sqrt{Y}$

Table 5. Effect of sediment mineral nutrient levels on growth and reproductive allocation in *V. americana* males and females in the 1990 experiment. Means \pm one standard error, with P value less than 0.10 given. The low sediment mineral nutrient level is 25% Big Moose Lake sediment, the high sediment mineral nutrient level is 100% Big Moose Lake sediment. Analysis of variance in females done on treatment means per tank due to low number of flowering females in the low sediment treatment.

	High nutrient sediment Mean \pm SE	Low nutrient sediment Mean \pm SE	p value
Males	n = 47	n = 35	
Total biomass (g) [†]	2.937 \pm 0.189	1.876 \pm 0.129	0.0019
ARE	0.358 \pm 0.015	0.282 \pm 0.014	0.0015
SRE	0.064 \pm 0.004	0.072 \pm 0.005	n.s.
TRE	0.423 \pm 0.014	0.354 \pm 0.015	0.0009
SR/TR	0.161 \pm 0.011	0.209 \pm 0.013	0.025
Inflorescences per gram	2.801 \pm 0.166	3.437 \pm 0.231	n.s.
Tubers per gram	4.271 \pm 0.225	4.385 \pm 0.388	n.s.
Mean tuber biomass (g)	0.091 \pm 0.005	0.077 \pm 0.006	0.071
Females	n = 40	n = 8	
Total biomass (g)	2.981 \pm 0.359	2.305 \pm 0.308	0.044
ARE	0.204 \pm 0.032	0.202 \pm 0.044	n.s.
SRE	0.184 \pm 0.029	0.126 \pm 0.034	n.s.
TRE	0.388 \pm 0.020	0.328 \pm 0.018	0.037
SR/TR	0.469 \pm 0.073	0.409 \pm 0.118	n.s.
Tubers per gram	2.433 \pm 0.354	3.066 \pm 0.552	n.s.
Flowers per gram	0.838 \pm 0.083	0.785 \pm 0.202	n.s.
Mean tuber biomass (g)	0.086 \pm 0.007	0.069 \pm 0.011	n.s.
Mean fruit biomass (g)	0.203 \pm 0.016	0.152 \pm 0.030	0.041

[†] values were log transformed to meet assumption of homoscedasticity: $Y' = \log(Y+1)$

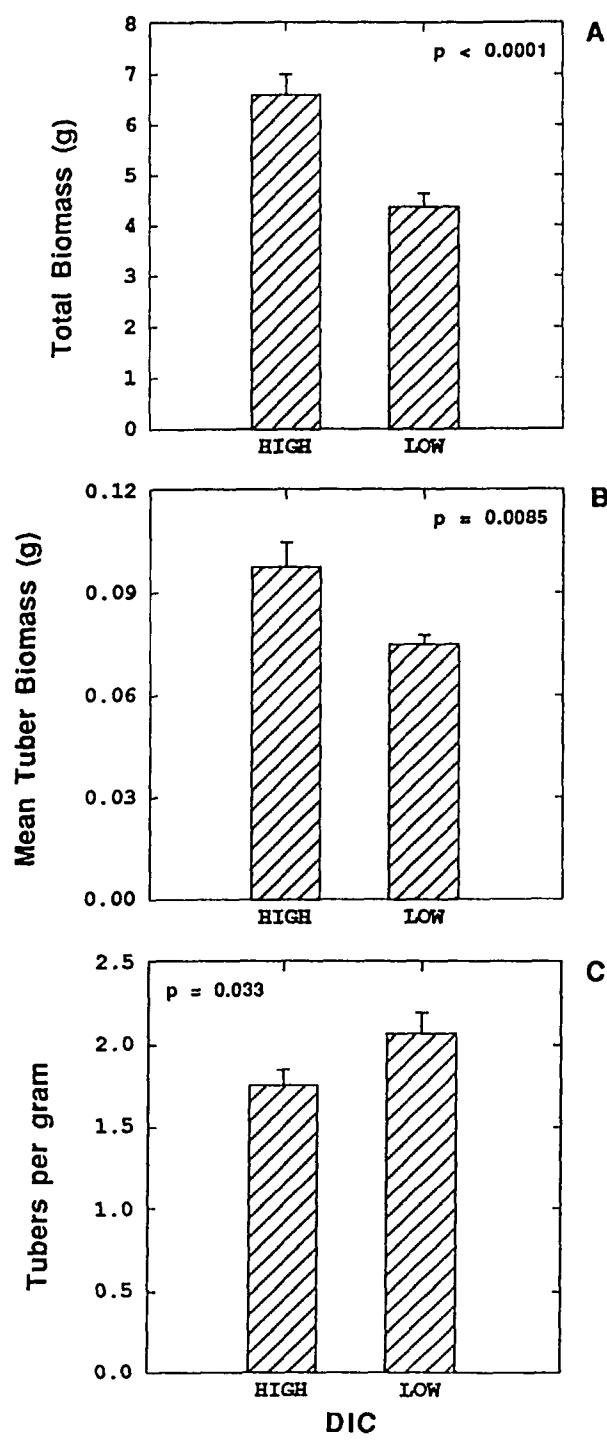


Figure 1. Effects of DIC availability on (A) total biomass, (B) mean tuber biomass, and (C) the number of tubers per gram in the spring 1991 experiment. Values are untransformed. Error bars represent standard errors. P values from single factor analysis of variance.

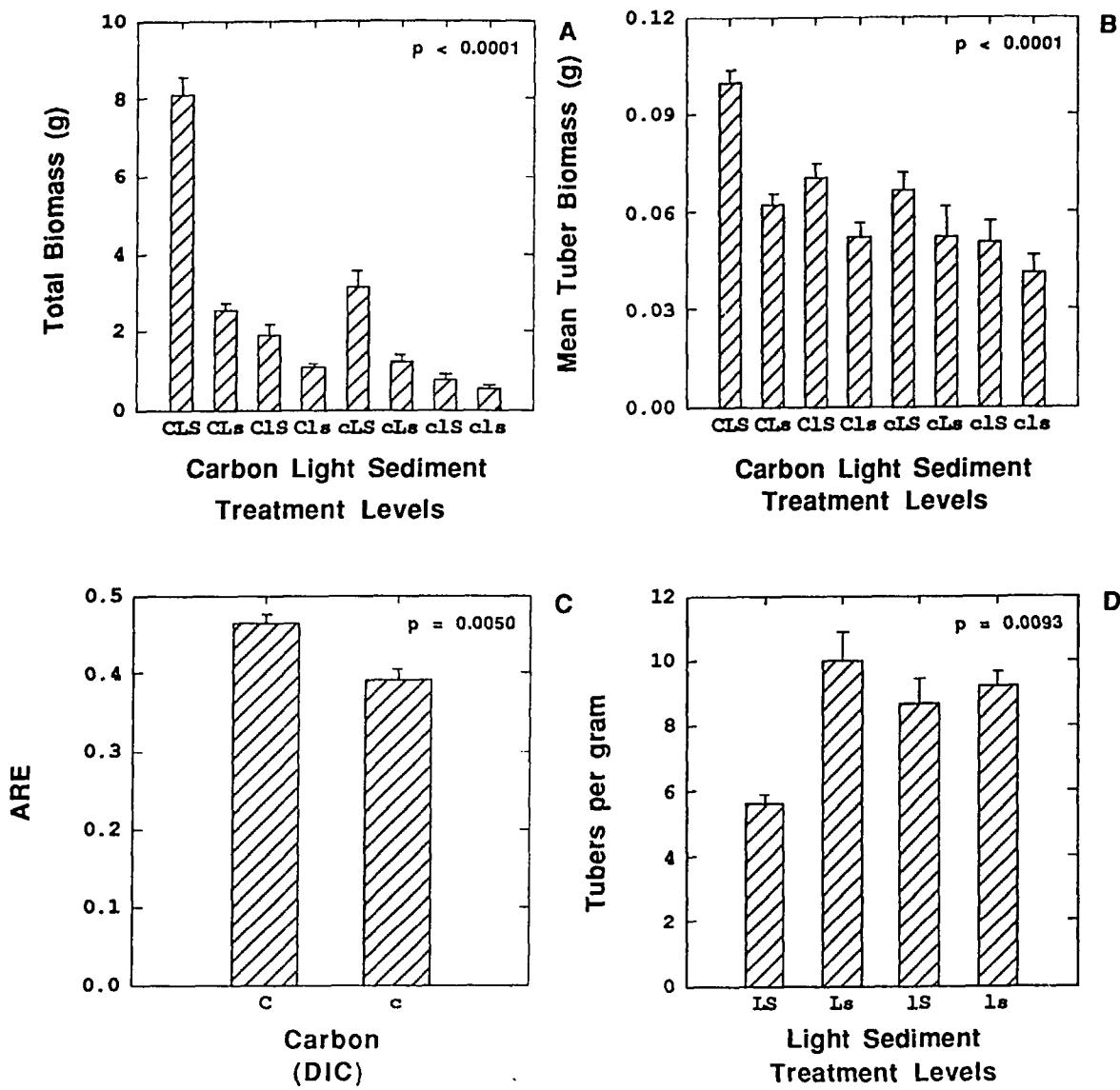


Figure 2. Effects of dissolved inorganic carbon (C, c), light (L, l), and sediment quality (S, s) on (A) total biomass, (B) mean tuber biomass, (C) ARE, and (D) the number of tubers per gram in the summer 1991 experiment. Upper case letters represent high resource levels, lower case letter represent low resource levels. Values are untransformed. Error bars represent standard errors. P values from analysis of variance

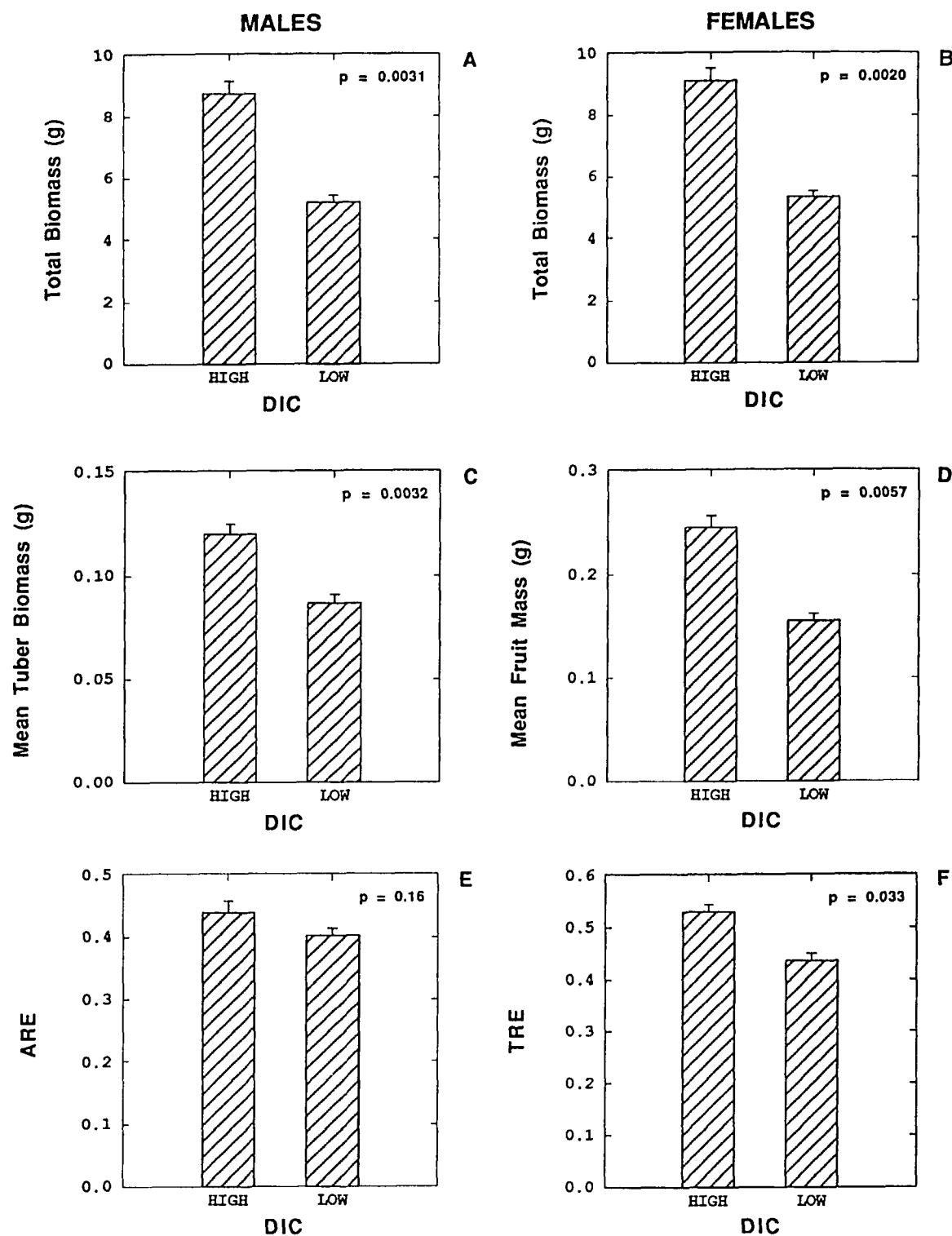


Figure 3. Effects of carbon availability (DIC: dissolved inorganic carbon) on (A) total biomass, (C) mean tuber biomass, and (E) ARE among males, and on (B) total biomass, (D) mean fruit biomass, and (F) TRE among females in the 1992 experiment. Values are untransformed. Error bars represent standard errors. P values from analysis of variance.

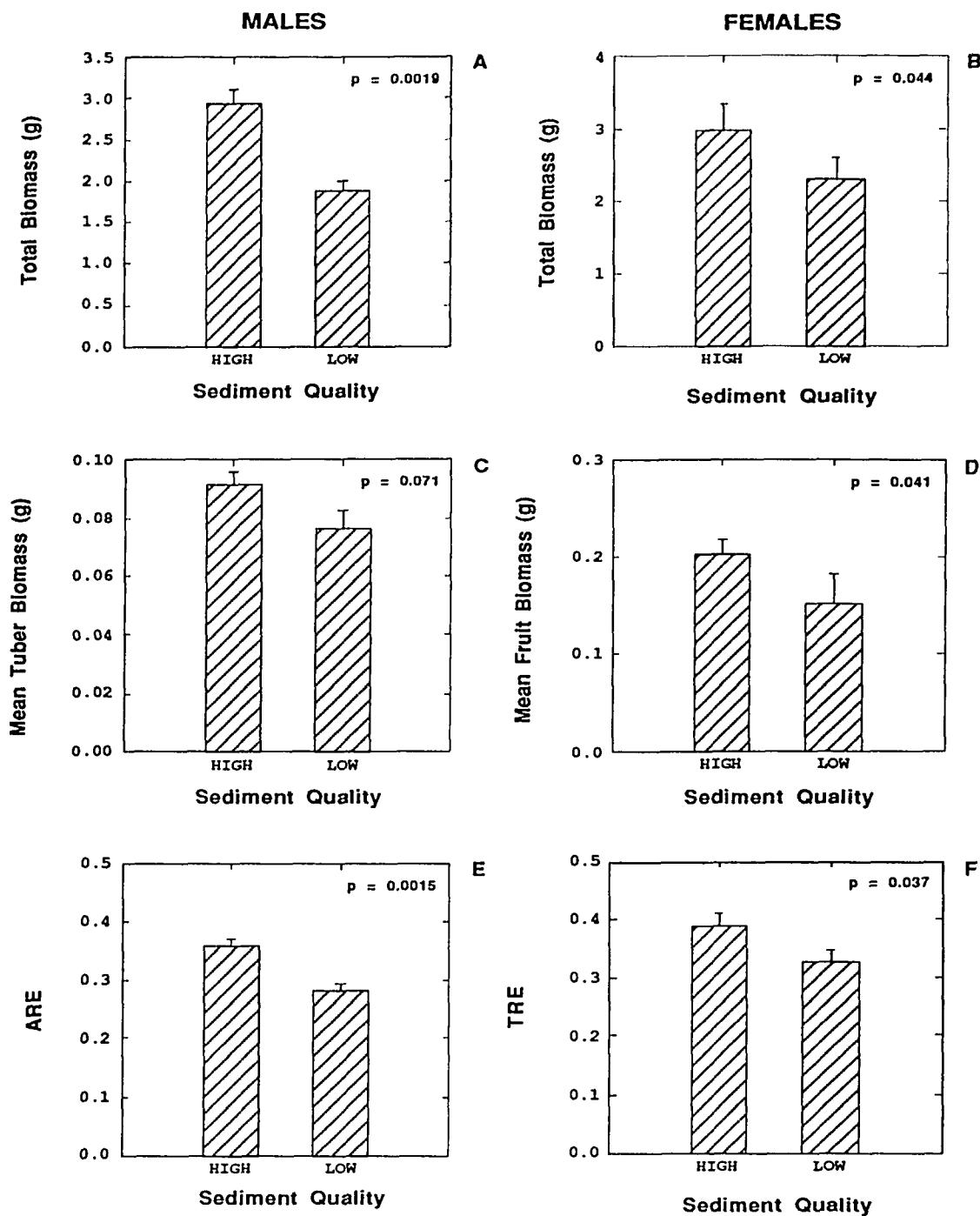


Figure 4. Effects of sediment quality on (A) total biomass, (C) mean tuber biomass, and (E) ARE among males, and on (B) total biomass, (D) mean fruit biomass, and (F) TRE among females in the 1990 experiment. Values are untransformed. Error bars represent standard errors. P values from single factor analysis of variance.

CHAPTER IV
A cost of reproduction
in the dioecious clonal macrophyte *Vallisneria americana*

INTRODUCTION

Much of life history theory is concerned with understanding the relationships among various fitness related traits. A key assumption is that tradeoffs occur among traits: increased allocation to one involves a decrement to one or more others (Gadgil and Bossert, 1970; Bell, 1980; Stearns, 1989). One of the most interesting of these tradeoffs involves the cost of reproduction, in which an increase in current reproduction may be coupled with a decrease in growth, survival, or future reproduction (Stearns, 1992). Consequently, the timing and magnitude of reproductive investment may have a critical impact on lifetime fitness. Different life history "strategies" have evolved to maximize offspring production within the context of these costs.

The life histories of clonal organisms are fundamentally different from those of unitary organisms. Many species do not appear to have a natural life span, and in effect are potentially immortal (Harper, Rosen, and White, 1986). A critical distinction thus arises in that reproductive value may indefinitely increase with age. A further distinction is that the interaction of life history traits may occur at different levels of organization: the entire genet, individual ramets, or among physiologically integrated units comprised of several ramets. Consequently, different types of tradeoffs can evolve, one of the most important being the tradeoff predicted between sexual and asexual reproduction. The cost of reproduction (sexual) may be paid as a reduction in clonal reproduction, which may then indirectly affect the growth, survival, or future sexual reproduction of the clone. Since

both sexually and asexually produced offspring make contributions to fitness (Abrahamson, 1980), the optimal clonal life history balances seed production with clonal reproduction to maximize the long term production of offspring. In modeling the life histories of clonal organisms, Caswell (1985) predicted that the balance between sexual and asexual reproduction will favor asexual reproduction when the population is growing rapidly, where early development is slow, when seedling establishment is low, and/or when adult mortality is low. Sackville-Hamilton, Schmid, and Harper (1987) predicted that the balance between clonal growth and sexual reproduction is affected by the cost of sexual reproduction, growth form (guerrilla vs. phalanx), the risk of mortality, and the predictability of such risks.

Many of the studies investigating the life histories of clonal organisms have examined various phenotypic correlations between measures of sexual and asexual reproduction. However, life history theory predicts an evolutionary response to selection, and thus assumes that there is a genetic basis to any associated cost of reproduction (Caswell, 1985; Reznick, 1985). Negative correlations between sexual and asexual reproduction do not by themselves indicate a genetically based cost of reproduction (Horvitz and Schemske, 1988). Reznick (1992) points out that though phenotypic correlations or manipulations of the phenotype may provide invaluable information about how organisms allocate their resources, or how characters are related to each other, they may be demonstrating what are essentially environmental effects rather than the pleiotropic effects of genes. In evaluating the predictions of life history theory, it remains imperative to demonstrate the genetic basis of negatively correlated traits through quantitative genetic studies or artificial selection experiments.

In previous experiments on the dioecious, clonal, macrophyte *Vallisneria americana* Michx. (Hydrocharitaceae), a phenotypic tradeoff between sexual and asexual reproduction was observed among female (but not male) plants grown under greenhouse conditions (Sullivan and Titus, unpublished manuscript). This tradeoff was measured as negative

correlations between the allocation of biomass to sexual and asexual reproductive structures. This indicated that a demographic cost of sexual reproduction among *V. americana* females was being paid in lost clonal growth. These correlations were observed because of naturally occurring variation in the proportion of reproductive biomass allocated to sexual reproduction among females sampled from a single population and grown under the same treatment conditions. This suggested that the tradeoff between sexual and asexual reproduction may have an underlying genetic basis. In this study, I examined variation in the allocation of biomass to sexual and asexual reproductive structures. The ultimate goal was simply to determine if there was significant genetic variation in reproductive characters, and if this variation results in significant negative genetic correlations between sexual and asexual reproductive characters. A sib analysis was performed on (1) female clonal "sibs" and on (2) the female sibs among their sexual offspring. Intraclass correlation coefficients measuring the proportion of total variance among offspring attributable to being a member of a sib group (family or clone; Zar, 1984; Falconer, 1989) were calculated, and the genetic correlation between sexual and asexual reproductive allocation was estimated.

METHODS

Study Species

Vallisneria americana (wild celery) is a submersed dioecious clonal macrophyte widely distributed throughout eastern North America (Lowden, 1982). *V. americana* reproduces in the northeast primarily from perennating tubers, as well as seeds. A rosette of ribbon-like leaves germinates from each tuber, with additional rosettes developing at the nodes of stolons arising from axillary meristems (Sculthorpe, 1967). Flowers or inflorescences develop from the two separate meristems flanking each of the axillary meristems that may develop a stolon. Buoyant female flowers are tethered on an elongated peduncle (Wylie, 1917). Anthesis occurs at the surface, but will occur below the surface in water too deep

for the peduncle to elongate to the surface (Sullivan, personal observation). Up to 2000 male flowers (approximately 0.6 mm in diameter) may develop on a short peduncled inflorescence (Wylie, 1917). Buoyant male flowers abscise over a period of usually less than 36 hours after the spathe opens (Sullivan, personal observation), and are supported on the surface film. Pollination occurs at the surface when male flowers are caught in the surface tension depression surrounding the female perianth, causing stamens to brush the stigma (Wylie, 1917; Svedelius, 1932; and Cox, 1988). Buoyant fruits develop quickly and remain attached for several weeks, until the fruit either senesces or disperses when the peduncle senesces (Sullivan, personal observation). One to several perennating tubers (usually two or three) develop on stolons growing down into the sediment from mature or terminal rosettes. The entire plant senesces at the end of the growing season, except for tubers and seeds.

Experimental approach

Twenty clone lines were established to study the resemblance among relatives in traits related to the tradeoff between sexual and asexual reproduction among female clonal and sexual offspring. Plants were collected from the field and grown in the greenhouse in the summer of 1992. Each "plant" consisted of the rosette germinating from a perennating tuber plus the interconnected rosettes it gives rise to during the current growing season. Clonal offspring were collected from these plants to be grown during the following winter. A "sib" analysis was performed on these clonal offspring on variables related to the tradeoff between sexual and asexual reproduction. One fruit from each female was then randomly selected as a source of seeds for the production of sexual offspring in the last part of this study. Seeds were germinated and seedlings from each family were selected, replanted, and grown during the summer of 1993. A sib analysis was performed on these sexually produced offspring on the same variables examined in the first analysis.

Collection, growth, and harvest of plants

Plants germinating from tubers were collected by divers from Chenango Lake, NY (42°15'N; 75°50'W) on May 29, 1992. One hundred twelve plants of unknown sex were collected from a population covering two to three hectares at the south end of the lake. They were stored for seven days at 5° C in lake water until they were transplanted into the Binghamton University greenhouse, where they were grown in replicate 1200 liter fiberglass tanks. Fifty six of the plants were randomly chosen, weighed, and assigned to one of the two tanks.

The tanks were filled with deionized water to which was added the following to facilitate growth and osmotic balance (see Smart and Barko, 1985): $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (91.7 mg l⁻¹), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (69.0 mg l⁻¹), and KHCO_3 (15.4 mg l⁻¹). To compensate for evaporative loss, the tanks were topped off weekly with deionized water. Each plant was grown in a plastic pot (20 cm opening diameter x 18 cm deep) in three liters of sediment from Otsego Lake, NY (42°47'N; 74°52'W). pH was maintained at 7.0 ± 0.25 units with a Horizon Model 5997 pH control unit through the addition of 0.4N HCl or NaOH. Temperature was maintained at $23^\circ \pm 1^\circ \text{C}$ with Remcor CFF500 water circulating units. Dissolved inorganic carbon (DIC) levels were maintained in equilibrium with the atmosphere by bubbling air through the water column. Water was circulated between the tanks so that the plants in each tank experienced the same water quality.

The plants were monitored daily until harvested on September 24, 1992. Counts were taken at harvest on the number of rosettes, tubers, stolons, and sexual reproductive structures (fruits in females, inflorescences in males). Some fruits were collected before the harvest after maturing and prior to senescence. The number of male inflorescences was determined by counting the peduncle stubs remaining after inflorescence senescence. Plant parts were separated into shoots (rosettes plus the stolons interconnecting them), roots, asexual reproductive tissue (tubers plus the stolons giving rise to them), and sexual

reproductive tissue (fruits and peduncles). (See Thompson and Stewart (1981) for a discussion of what comprises reproductive tissue.) Twenty females and eight males were randomly selected to donate tubers (clonal offspring) for subsequent germination and growth over the following winter. All other plant parts were dried at 105° C and weighed to determine dry mass (= biomass). Fresh weight to dry weight ratios were calculated for estimating the biomass of the selected tubers. The biomass of inflorescences could not be directly measured as they senesce prior to harvest.

Growth and harvest of clonal offspring

The tubers set aside in the first part of the study were germinated in cups of sediment prior to transplanting into the tanks. Germination was asynchronous, so that only 18 of the 20 female clone lines had germinated tubers within the time frame for beginning the experiment. Forty female tubers and 16 male tubers were planted in pots in Otsego Lake sediment on November 3, 1992. Thirteen of the female clone lines and all eight of the male lines were represented by at least two clonal offspring (five female clone lines had germinated only one tuber each). Each tuber was randomly assigned to one of two tanks and grown under the conditions discussed above. In addition, the winter photoperiod was adjusted every four to five days to mimic that found naturally between June 1 and October 1. Natural light was supplemented with artificial light from timer-controlled General Electric GEH 2877 multi-vapor lamps to induce flowering and tuber production at the appropriate time. All plants were harvested on March 26, 1993 when natural photoperiods began to exceed the artificially maintained photoperiod needed to induce tuber formation. Plant parts were separated, dried, and weighed as detailed above. The middle third of one fruit randomly selected from each of the forty females was removed to provide seeds for use in the final part of this study. Fresh weight to dry weight ratios were calculated for estimating the biomass of the selected fruits.

Growth and harvest of sexual offspring

The sections of fruit were subjected to a three week cold period at 5° C. Seeds from each fruit were then planted on April 16 in separate cups in an artificial sediment. The sediment was composed of a 20:1 mixture of local "topsoil" (a mixture of clay, silt, and sand) and cow manure. The cups were kept in an aquarium while seeds germinated over a period of six weeks. Seed germination was asynchronous, so that by May 25, seedling size ranged from newly germinated to 3.5 cm maximum leaf length. Five seedlings between two and three cm maximum leaf length from each clone line were chosen for transplanting.

Seedlings were transplanted on May 25 into plastic pots (as above) in three liters of the artificial sediment. Each seedling was randomly assigned to one of three tanks and grown under the conditions discussed above. In addition, DIC was increased to promote seedling growth and increase the probability of flowering. DIC was enhanced to 5 x ambient levels by adding pure CO₂ to the air bubbling through the water column (see Titus, Feldman, and Grise', 1990 for details on CO₂ control and monitoring). (5 x the ambient level at pH 7.0, 0.325 mM C • l⁻¹, was measured in Chenango Lake.) The plants were monitored daily until harvested on October 1. Plant parts were separated, dried, and weighed as detailed above.

Analysis

As a branch of evolutionary theory, life history theory assumes that the costs of reproduction have a genetic basis (Reznick, Perry, and Travis, 1986). The goal here was to determine if there was a genetic basis to the predicted tradeoff between sexual and asexual reproduction among *V. americana* females, measured as significant genetic variation in characters thought to determine or be related to this tradeoff. First, a sib

analysis was performed where genetic variation in relevant characters was estimated from the intraclass correlation of clone and family members. Total phenotypic variance (s^2_T) was partitioned into between or among group (s^2_B) and within group (s^2_W) components through a Model II analysis of variance. The intraclass correlation (t) was estimated from these components as the proportion of total phenotypic variance among groups:

$t = s^2_B / [s^2_B + s^2_W]$ (Sokal and Rohlf, 1981; Falconer, 1989). Second, genetic correlations were estimated as the Pearson product-moment correlations of clone or family means between relevant characters showing significant genetic variation (Roach, 1986; Weis, Hollenbach, and Abrahamson, 1987).

The among group portion of variance (s^2_B) is a measure of the covariance of the members of the groups. The covariance of clonal sibs is composed of the total genetic variance (s^2_G) plus environmental variance resulting from maternal effects (s^2_{Ec}). The covariance of family sibs is composed of a portion of the additive genetic variance (s^2_A), environmental variance resulting from maternal effects (s^2_{Ec}), and potentially, a portion of any variance resulting from dominance deviations (s^2_D) or epistatic interactions (s^2_I ; Falconer, 1989). Since the paternal identity of family sibs was unknown in tanks where mating occurred at random, the relative contributions of each type of variance to the between group covariance remains unknown.

Maternal effects on seedling and adult plant performance have been demonstrated in a number of studies (reviewed in Roach and Wulff, 1987). I attempted to minimize maternal effects here as they potentially contribute to the between group covariance. For the clonal offspring analysis, I first raised the parental generation in a common greenhouse environment to eliminate differences in maternal environments as a source of variation. Secondly, each of the clonal offspring (tubers) that were to be used in the second part of the study came from different rosettes, so that no two clonal offspring shared the same provisioning rosette. Last, to account for variation due to differences in the maternal provisioning of tubers (tuber size), I removed the effects of initial tuber biomass in an

analysis of covariance procedure. The components of variance were calculated from the mean squares resulting from a Model II ANOVA (SYSTAT, 1992) performed on the residuals of the dependent variable regressed on the covariate, with initial tuber fresh weight as the covariate (see Geber, 1991). I then recalculated the intraclass correlations from these components of variance.

The clonal parents of the sexual offspring were the second generation to be raised in the common greenhouse environment, further minimizing any residual maternal effects due to different maternal environments. I did not use initial seed size as a covariate in an analysis of covariance. Although seed size has often been used as a means to estimate or control for maternal effects (Schaal, 1984; Geber, 1991), maternal effects primarily influence plants at the seed and seedling stages, so that seed size is not a good indicator of maternal effects on later life traits (Roach and Wulff, 1987; Weis, Hollenbach, and Abrahamson, 1987). The seedlings that were used were approximately the same size (maximum leaf length between two and three cm) so that potential differences in seed provisioning and the timing of germination were minimized.

Phenotypic correlations were calculated for all traits related to sexual and asexual reproductive allocation showing significant genetic variation as pairwise Pearson's product-moment correlations. Genetic correlations were estimated as pairwise Pearson's product-moment correlations on the clonal or family mean values of these same traits. This estimate is an approximation because the genetic correlation results from the additive genetic covariance of traits, while this correlation may include non-additive components of variance (Reznick, Perry, and Travis, 1986).

Dependent variables

The number of flowers per plant was a measure of sexual reproductive allocation, while the number of tubers per plant was a measure of asexual reproductive allocation. Mass

specific numbers of flowers and tubers (numbers per gram) take variation in plant size into account. Flower and fruit biomass and tuber biomass were a measure of the resources allocated to sexual and asexual reproductive structures. The proportion of total biomass allocated to sexual reproduction (SRE: sexual reproductive effort) and the proportion of total biomass allocated to asexual reproduction (ARE: asexual reproductive effort) were relative measures of biomass allocation to reproductive structures. The tradeoff between sexual and asexual reproduction may be expressed by variation in the proportion of reproductive biomass allocated to sexual reproduction, calculated as sexual/total reproductive biomass (SR/TR).

The number of flowers and the number of flowers per gram were log transformed ($Y' = \log[Y + 1]$) to meet the assumptions of homoscedasticity in the clonal offspring analysis. The number of flowers and the number of tubers were log transformed ($Y' = \log[Y + 1]$), SRE and SR/TR were arcsin transformed ($Y' = \arcsin \sqrt{Y}$), and the number of flowers per gram were square root transformed ($Y' = \sqrt{[Y + 0.5]}$) to meet the assumptions of homoscedasticity or normality in the sexual offspring analysis.

RESULTS

Physiological tradeoffs

Negative phenotypic correlations were calculated between different measures of sexual and asexual reproductive allocation among females in both the clonal and sexual sibships (Table 1). These correlations suggested that from a physiological perspective, there was a tradeoff in biomass allocation between sexual and asexual reproductive structures. In the clonal offspring generation, the relationship between the allocation of biomass to sexual and asexual reproductive structures tends to be stronger than between the number of flowers and tubers produced, in both absolute and relative terms (tubers per gram). The number of tubers produced may not have been a good reflection of asexual reproductive

allocation in this group of plants because many of the tuber bearing stolons were not yet fully developed at harvest. Despite this, ARE was negatively correlated with SRE, a strong indication of the tradeoff between sexual and asexual reproduction (Fig. 1a). Another indication of this tradeoff was the wide variation among all females in the proportion of reproductive biomass allocated to sexual reproduction (SR/TR), which ranged from 0.37 to 0.99. Variation in SR/TR was strongly related to the number of flowers produced per plant ($r = 0.776$, $p < 0.0001$).

Negative phenotypic correlations were also calculated among the female sexual offspring in sexual and asexual reproductive characters (Table 1). Both the number and biomass of flowers were negatively correlated with the number and biomass of tubers. The number of flowers per gram was also negatively correlated with the number of tubers per gram. ARE was negatively correlated with SRE here as well, strongly indicating a tradeoff between sexual and asexual reproduction among female sexual offspring (Fig. 1b). SR/TR ranged from 0.01 to 0.81 and was also positively correlated with the number of flowers per plant ($r = 0.785$, $p < 0.0001$).

Intraclass correlations

The overall means and the range in clonal and family means for the nine characters are reported in Table 2. The intraclass correlations (t) calculated among clonal and sexual offspring groups indicated that there was significant genetic variation in nearly all measures of reproductive allocation (Table 3). Removing variation due to initial tuber size among the clonal offspring sibs indicated that a portion of the among group variation in the number of flowers per gram could be accounted for by maternal effects. The correlations (t) on other characters were either affected little or were increased after removing variation due to initial tuber size. An increase in the correlation indicated that initial tuber size was contributing to the within group or error variance. The decrease in error variance on ARE

showed that there was significant genetic variation in that trait that would otherwise have gone undetected. The intraclass correlations for the mass specific number of flowers (flowers per gram) and biomass of flowers and fruits (SRE), and the proportion of reproductive biomass allocated to sexual reproduction (SR/TR) were all significant (Fig. 2). Genetic variation was not detected in the number of tubers produced per gram, though it may have been underestimated because the plants were harvested prior to full development of the perennating tuber-bearing stolons.

There was significant genetic variation found among the females in the sexual offspring sibships in all measures of sexual and asexual reproductive allocation (Table 4). Significant family differences were detected in the number of flowers produced per gram, SRE, and SR/TR (Fig. 3), as well as for the overall number of flowers produced, sexual reproductive biomass, the number and biomass of tubers, the number of tubers per gram, and ARE.

Genetic correlations

Genetic correlations were calculated for all reproductive characters showing significant genetic variation in both the clonal and sexual offspring generations (Table 5). The genetic correlations were of the same sign and were generally stronger than the phenotypic correlations. In the clonal sibships, the number of flowers was negatively correlated with the number of tubers, and the biomass of flowers and fruits was negatively correlated with the biomass of tubers. This indicated that the tradeoff between fruit and tuber production has a genetic basis. Since significant genetic variation in the number of tubers per gram was not detected, the genetic correlation between mass specific flower and tuber production was not calculated. The effect of allocating resources to sexual reproduction on asexual reproductive allocation may best be seen in the relative allocation of biomass to fruit and

tuber production (Fig. 4a). SRE was strongly and negatively correlated with ARE in the clonal offspring generation.

In the sexual generation, the number of flowers was negatively correlated with the number of tubers, and the number of flowers per gram was negatively correlated with the number of tubers per gram. This indicates that there was a tradeoff in both the absolute and relative number of flowers and tubers produced, and that this tradeoff had a genetic basis. The genetic correlation between fruit plus flower biomass and tuber biomass was not significant, though this correlation was strongly affected by an outlier (from the family mean of the largest plants). Removing this outlier resulted in a strongly significant relationship ($r = -0.779$, $p = 0.0047$). The relative allocation of biomass to flowers and fruits (SRE) was strongly and negatively correlated with the relative allocation of biomass to tubers (ARE; Fig. 4b). Strong negative genetic correlations between SRE and ARE in the clonal and sexual offspring generations indicated that there was a genetically based tradeoff between the allocation of biomass to sexual and asexual reproduction among *V. americana* females.

DISCUSSION

Phenotypic cost of reproduction

Flower production and fruit development exact a significant cost in clonal reproductive potential. Reproductive biomass allocated to flowers and fruits appears to be a constraint on biomass allocation to tubers. This indicated a demographic cost of reproduction in that fruiting females leave fewer tubers to compete for space and resources during the next growing season. A similar cost was observed in *Helianthus tuberosus* (Westley, 1993), where the number and biomass of tubers was inversely related to the biomass allocation to inflorescences. Since a change in sexual reproductive allocation may be offset by an opposite change in asexual reproduction, it may be argued that both forms of reproduction

lead to fitness gains, so that this demographic cost is diminished. However, seeds and clonal propagules may not contribute equally to the production of offspring. The phenotypic cost of reproduction may be severe in populations where seedling establishment is low, or where competition takes place between adjacent clones.

Genetic variation

Significant genetic variation was found among clones and families of *V. americana* females in several characters related to the tradeoff between sexual and asexual reproductive allocation. Although the numbers and biomass of most reproductive structures showed high levels of genetic variation, the traits were not independent of one another as they were all highly correlated. Four of these traits are key in evaluating the genetic basis of the phenotypic tradeoffs observed. (1) First is the mass specific number of flowers produced (flowers per gram). Asexual reproductive allocation has been shown to be strongly and inversely related to sexual reproductive allocation (Sullivan and Titus, unpublished manuscript), so that biomass allocation to fruits influences biomass allocation to tubers. The biomass allocated to fruits is ultimately limited by the number of flowers produced, although differences among families in fruit size or in pollination success could confound the relationship between flower number and sexual reproductive biomass. Differences in the mean biomass of flowers or fruits (flower or fruit size) among clones or families were not significant, suggesting that flower number is a good predictor of sexual reproductive biomass. (2) Next is the biomass of flowers and fruits per gram of plant (SRE). SRE reflects the proportion of resources that the plant allocates to flower and fruit development, which can differ among individuals producing the same number of flowers per gram. Phenotypically, there was a much stronger relationship between SRE and ARE than there was between the number of flowers per gram and the number of tubers per gram, suggesting that tradeoffs in biomass allocation are more intimate than between numbers of

reproductive structures. (3) Third is the biomass allocated to tubers per gram plant mass (ARE). ARE represents the proportion of plant resources allocated to asexual reproduction, and is strongly correlated with the number of clonal propagules produced. The negative correlation between ARE and SRE forms the basis of the tradeoff between sexual and asexual reproduction. (4) Last is the proportion of reproductive biomass allocated to sexual reproduction (SR/TR). SR/TR reflects the balance between sexual and asexual reproduction. Although SR/TR varied a great deal among both clonal and sexual sibships, a significant proportion of this variation was accounted for by group affiliation. Despite a relatively small sample of clones and families with few members in each group, significant genetic variation was measured in each of these variables.

Evolutionary cost of reproduction

The clone or family mean correlations showed that clones or families that produced more flowers produced fewer clonal offspring. This reflects the genetic basis for the demographic cost of reproduction, and is consistent with the predictions of clonal life history theory. These correlations are of particular interest because these characters are intimately associated with fitness. Although phenotypic tradeoffs between sexual and asexual reproduction have been reported (e.g. Sarukhan, 1976; Sutherland and Vickery, 1988; Swamy and Ramakrishnan, 1988), few studies of wild clonal plants have demonstrated the genetic basis behind this tradeoff. Weis, Hollenbach, and Abrahamson (1987) report that such a tradeoff likely takes place in *Solidago altissima*, but they were unable to demonstrate it with their experimental design. Geber, Watson, and Furnish (1992) report that inflorescence production in *Eichhornia crassipes* was negatively correlated with ramet production, and that this tradeoff has a genetic basis. The basis of this tradeoff was meristem limitation, which is not a factor in *V. americana* as stolons and flowers utilize different axillary meristems.

The genetic correlations calculated in this study may not result entirely from the additive effects of genes. Not all of the environmental variation due to common environment could have been removed in the experimental design, so that some maternal effects may have influenced the correlations. However, maternal effects have been shown to have little effect on later stage adult characteristics, so that these effects may have been minimal (Roach, 1986; Roach and Wulff, 1987; Weis, Hollenbach, and Abrahamson). A potentially greater influence on the genetic correlations occurs among the clonal sibships, where the genetic variation includes all of the non-additive effects of genes in addition to the additive genetic effects (Falconer, 1989). The offspring within each sibship in the sexual offspring generation may be either full or half sibs, as mating was random within the tanks. It is possible that all of the pollen fertilizing a given flower came from one donor, resulting in full sibs. However, some sibs were chosen from different clonal mothers, so that the probability of half sibships is increased. Variation among half sibs is not influenced by non-additive effects, but may have influenced covariation among any full sib groups (Falconer, 1989). Consequently, the genetic correlations for the clonal and sexual offspring sibships should be considered an upper bound.

The course of future investigations will be to investigate the tradeoff between sexual and clonal reproduction in natural populations of *V. americana*, and to examine how high levels of genetic variation can be maintained. The level of genetic variation observed in SR/TR among these females is greater than might be predicted for a trait having such a large impact on both seed and clonal offspring production. Many clonal populations have been characterized as dominated by one or a few clones (Cook, 1985; Silvertown, 1987), and one of six local populations of *V. americana* may be characterized as monoclonal (Grise', personal communication), and another as unisexual (personal observation). In competition among long-lived individuals, better adapted genotypes should tend to eliminate less fit genotypes, even as new genotypes are introduced (Abrahamson, 1980; Harper, Rosen, and White, 1986). Several long term studies have shown that the number

of genotypes present in experimental plots steadily declines over a period of time (Langer, Ryle, and Jewiss, 1964; Kays and Harper, 1974; Hartnett and Bazzaz, 1985), and that the establishment of new genotypes may be rare in mature habitats (Hartnett and Bazzaz, 1985; Tamm, 1972). However, Burdon (1980) points out that diversity is maintained when ecotypes evolve due to microhabitat variation resulting from local differences in the physical or biotic environments. Calvo (1993) has suggested that selection pressure on the level of fruit or seed production will be weak or nonexistent if there is little correlation between seed production and fitness. This may well be the case for *V. americana* as seedling establishment may be exceedingly low (Sullivan, unpublished data).

SUMMARY

The relationship between sexual and asexual reproductive allocation among female clonal and sexual sibships was examined in *V. americana*. Negative phenotypic correlations between sexual and asexual reproductive characters indicated that females investing more in the production of flowers and fruits invested less in the production of perennating tubers. This tradeoff indicates a demographic cost of reproduction. An examination of intraclass correlations showed that there was significant genetic variation in characters related to this tradeoff. Negative genetic correlations estimated from the family means of sexual and asexual reproductive characters indicate that there also was an evolutionary cost to reproduction. This result is consistent with the predictions of clonal life history theory: tradeoffs exist between sexual and asexual reproduction and these tradeoffs have a genetic basis.

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Table 1. Phenotypic correlations calculated as pairwise Pearson's product-moment correlations in *Vallisneria americana* females between absolute and relative measures of sexual and asexual reproduction in the clonal and sexual offspring sibships.

Sexual Reproductive Variables	Asexual Reproductive Variables	Clonal Offspring n = 33		Sexual Offspring n = 35	
		r	p	r	p
Flower number	Tuber number	-0.651	<0.0001	-0.553	0.0006
Flowers per gram	Tubers per gram	-0.407	0.019	-0.458	0.0057
Fruit + flower biomass (g)	Tuber biomass (g)	-0.692	<0.0001	-0.592	0.0002
SRE	ARE	-0.701	<0.0001	-0.835	<0.0001

Table 2. Overall character means \pm SD, and the range in clone and family means in untransformed characters of *V. americana* females.

Character	Clonal Offspring		Sexual Offspring	
	Mean \pm SD	Range in means	Mean \pm SD	Range in means
Number of flowers	6.212 \pm 2.355	3.0 - 9.0	5.457 \pm 2.650	0.5 - 10.0
Number of flowers per gram	1.376 \pm 0.678	0.502 - 1.906	1.812 \pm 0.951	0.162 - 3.836
Flower + fruit biomass (g)	1.388 \pm 0.508	0.822 - 2.002	0.627 \pm 0.305	0.055 - 1.030
SRE	0.301 \pm 0.133	0.130 - 0.424	0.205 \pm 0.097	0.018 - 0.293
SR/TR	0.753 \pm 0.188	0.440 - 0.950	0.376 \pm 0.175	0.029 - 0.556
Number of tubers	4.788 \pm 2.924	1.5 - 9.5	24.114 \pm 8.804	14.0 - 37.0
Number of tubers per gram	0.974 \pm 0.587	0.370 - 1.593	7.835 \pm 2.684	4.666 - 11.514
Tuber biomass (g)	0.423 \pm 0.330	0.088 - 1.827	1.082 \pm 0.410	0.589 - 1.865
ARE	0.083 \pm 0.056	0.022 - 0.176	0.345 \pm 0.114	0.229 - 0.578

Table 3. By-clone intraclass correlation coefficients (*t*) for absolute and relative measures of sexual and asexual reproductive allocation among 13 female clones in the clonal offspring generation. Coefficients and levels of significance on the right are for variables after the effects of initial tuber fresh weight had been removed in an analysis of covariance (residual values). Biomass = g dry mass.

dependent variable	Actual values		Residual values	
	<i>t</i>	p	<i>t</i>	p
Number of flowers [†]	0.424	0.019	0.421	0.020
Number of flowers per gram [†]	0.606	0.0009	0.484	0.0082
Fruit, flower biomass (g)	0.448	0.014	0.513	0.0052
SRE	0.602	0.0010	0.588	0.0014
SR / TR	0.561	0.0024	0.618	0.0007
Number of tubers	0.432	0.017	0.399	0.025
Number of tubers per gram [†]	0.037	0.41	0.104	0.30
Tuber biomass (g)	0.628	0.0006	0.680	0.0002
ARE	0.312	0.069	0.450	0.015
Mean biomass per flower (g)	0.051	0.39	0.00	0.60
Mean biomass per tuber (g)	0.247	0.11	0.262	0.099

[†] values were log transformed to meet assumptions of homoscedasticity: $Y' = \log(Y + 1)$.

Table 4. By-family intraclass correlation coefficients (*t*) and levels of significance for absolute and relative measures of sexual and asexual reproductive allocation among the females from 12 families in the sexual offspring sibships. Biomass = g dry mass.

dependent variable	<i>t</i>	p value
Number of flowers [†]	0.667	<0.0001
Number of flowers per gram ^{††}	0.550	0.0008
Fruit, flower biomass (g) [†]	0.426	0.0076
SRE ^{†††}	0.463	0.0040
SR / TR ^{†††}	0.437	0.0064
Number of tubers [†]	0.534	0.0011
Number of tubers per gram	0.414	0.0093
Tuber biomass (g)	0.440	0.0061
ARE	0.328	0.030
Mean biomass per flower (g)	0.046	0.23
Mean biomass per tuber (g)	0.00	0.65

[†] values were log transformed to meet assumptions of homoscedasticity : $Y' = \log(Y + 1)$

^{††} values square root transformed to meet assumptions of homoscedasticity or normality: $Y' = \sqrt{Y + 0.5}$

^{†††}values were arcsin transformed to meet assumptions of homoscedasticity: $Y' = \arcsin(\sqrt{Y})$

Table 5 Genetic correlations calculated as pairwise Pearson's product moment correlations among clonal or family character means of *Vallisneria americana* females between absolute and relative measures of sexual and asexual reproduction in the clonal and sexual offspring generations.

Sexual Reproductive Characters	Asexual Reproductive Characters	Clonal Offspring n = 13		Sexual Offspring n = 12	
		r	p	r	p
Flower number	Tuber number	-0.744	0.0035	-0.744	0.0055
Flowers per gram	Tubers per gram	-	-	-0.780	0.0028
Fruit, flower biomass (g)	Tuber biomass (g)	-0.707	0.0069	-0.512	0.089†
SRE	ARE	-0.755	0.0028	-0.875	0.0002

† after removing one outlier from the family with the plants, the correlation was much stronger:
 $r = -0.779$, $p = 0.0047$.

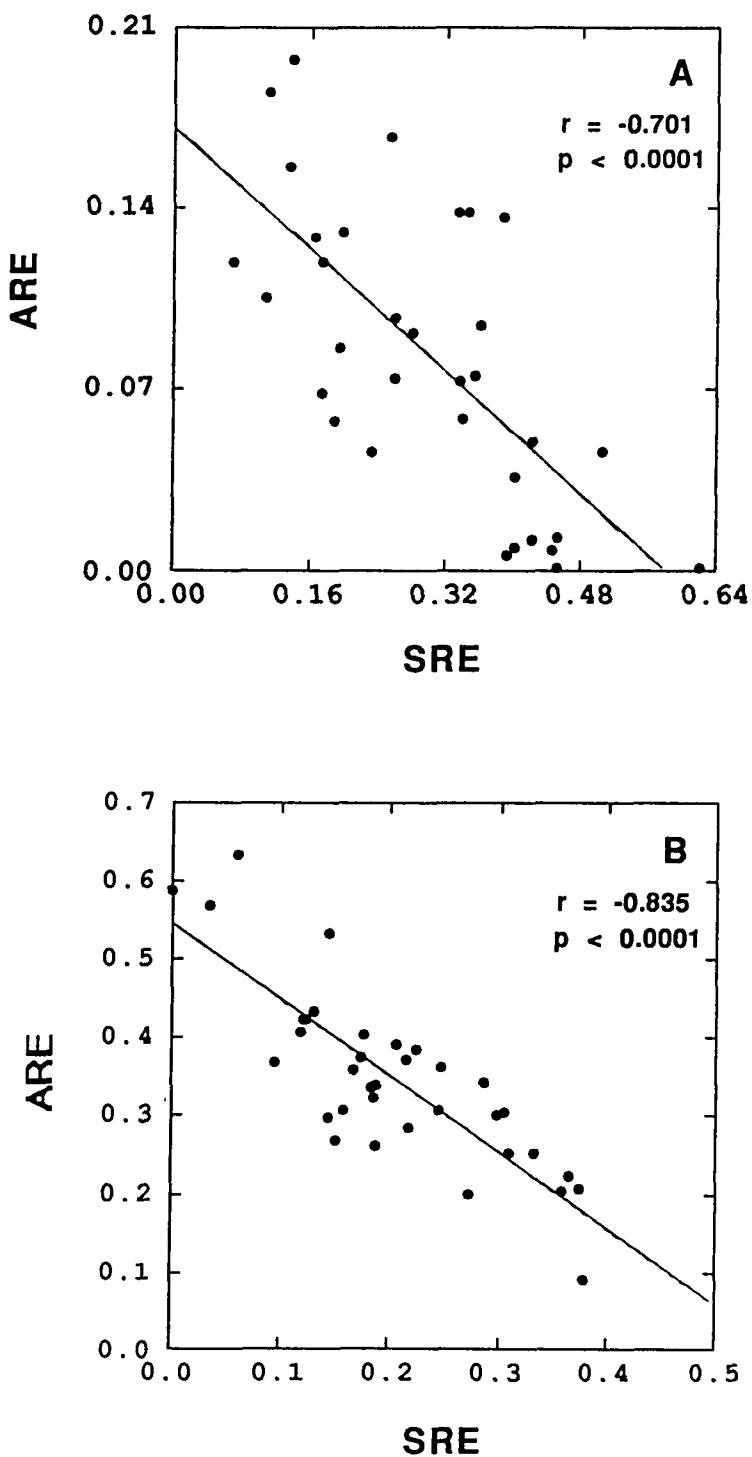


Figure 1. Overall relationship between sexual reproductive effort (SRE) and asexual reproductive effort (ARE) in (A) the clonal sibships, and (B) the sexual family sibships. Values are untransformed.

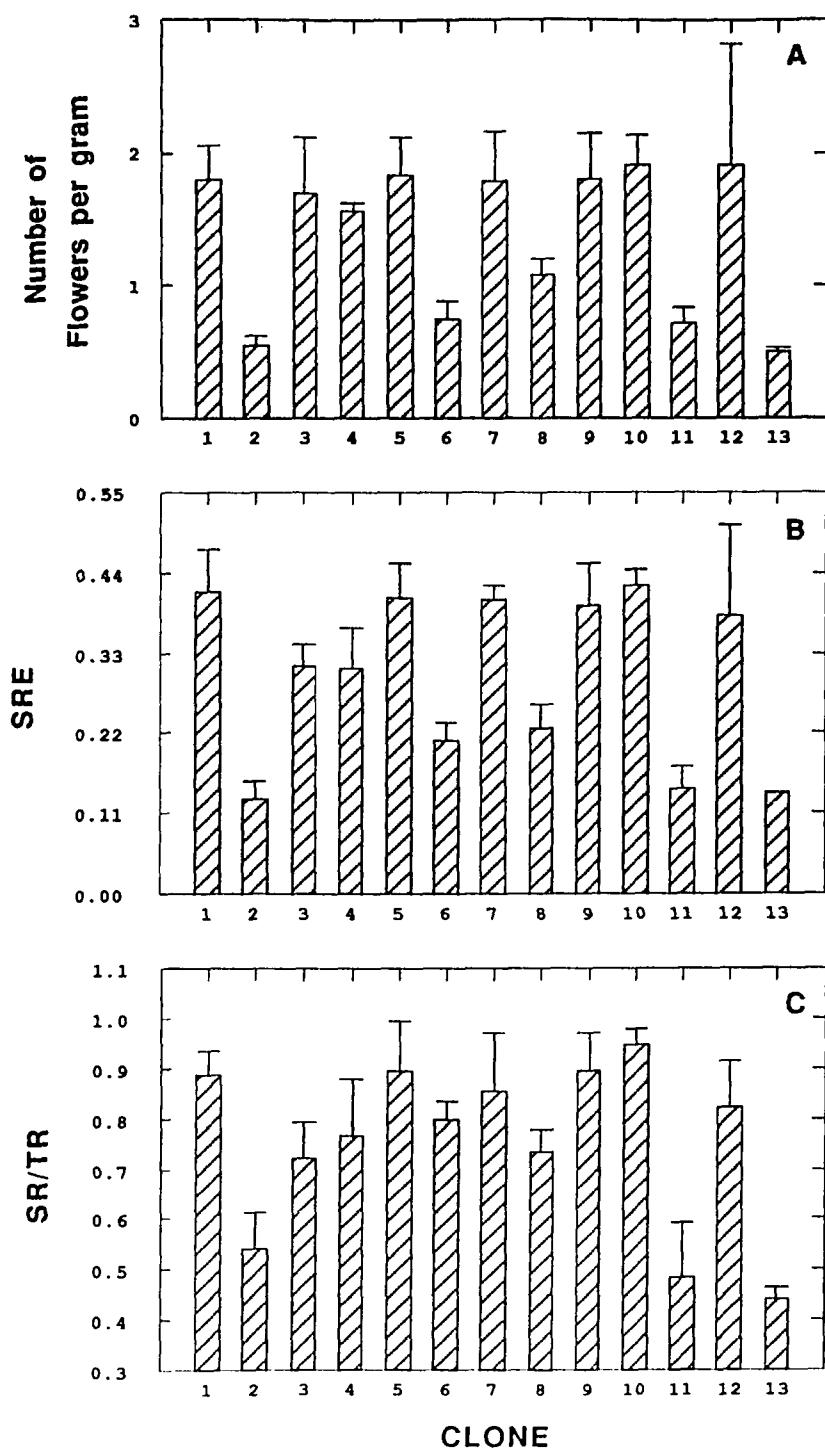


Figure 2. Clonal sibship means \pm SE of (A) number of flowers per gram, (B) sexual reproductive effort (SRE), and (C) the proportion of reproductive biomass allocated to sexual reproduction (SR/TR). Values are untransformed.

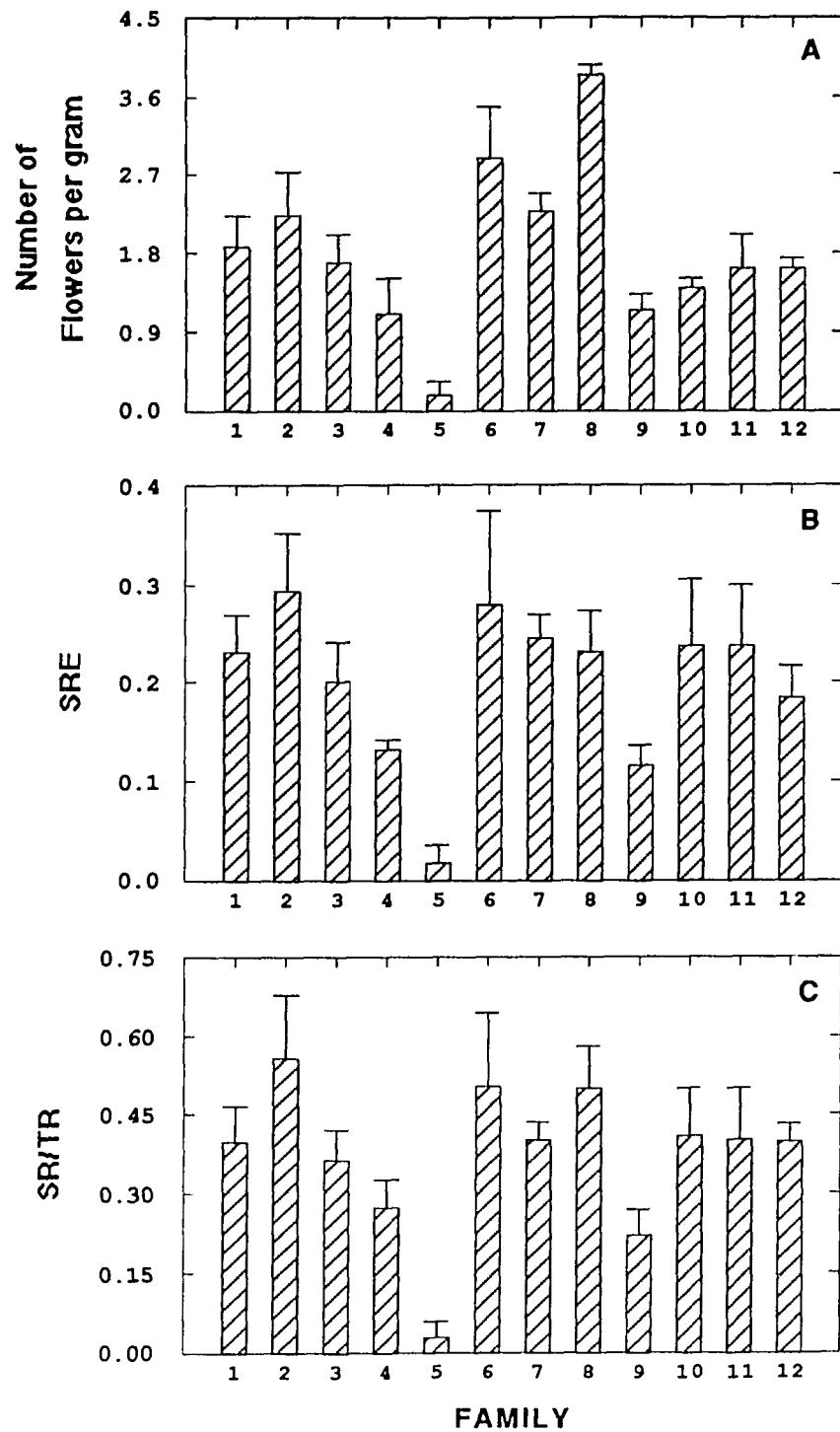


Figure 3. Sexual family sibship means \pm SE of (A) number of flowers per gram, (B) sexual reproductive effort (SRE), and (C) the proportion of reproductive biomass allocated to sexual reproduction (SR/TR). Values are untransformed.

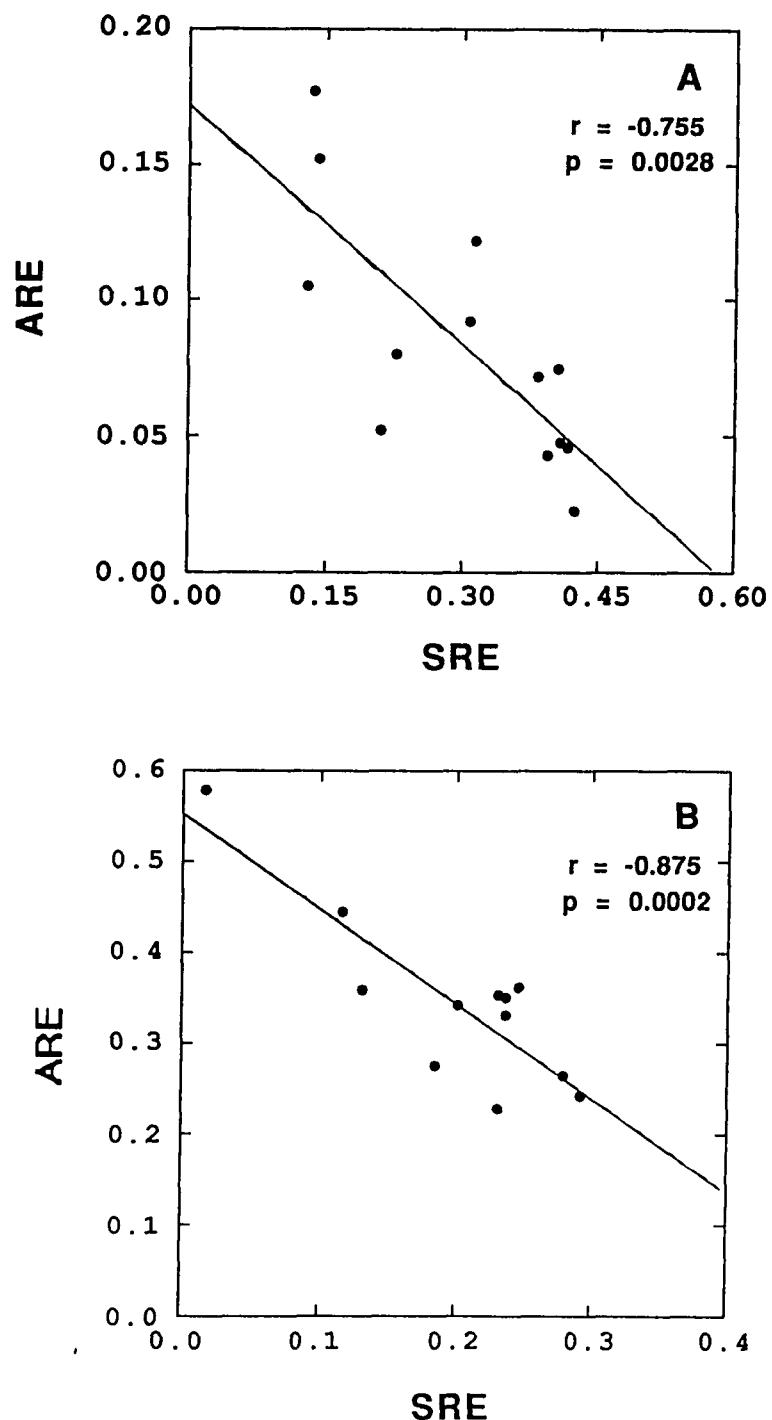


Figure 4. Relationship between the group mean values of sexual reproductive effort (SRE) and asexual reproductive effort (ARE) in (A) the clonal sibships, and (B) the sexual family sibships. Values are untransformed.

CHAPTER V

Site-specific pollen limitation

in a dioecious, hydrophilous species

INTRODUCTION

The relative importance of pollen supply in limiting fruit-set among natural populations of flowering plants remains unresolved for many species. Sexual selection theory suggests that female reproductive function should be limited by resources (other than pollen; Bateman, 1948; Willson, 1979). Several non-mutually exclusive hypotheses consistent with resource limitation have been advanced to account for non-fruiting flowers: male function, whereby non-fruiting flowers increase fitness through pollen donation; selective abortion, whereby some fruits are aborted to favor the development of higher quality fruits; and bet hedging, whereby extra flowers develop as a hedge against variation in resource availability (Darwin, 1876; Janzen et al., 1980; Bawa and Webb, 1984; Sutherland and Delph, 1984; Sutherland, 1986). At odds with this theoretical and intuitive view of what limits female reproduction are the many examples of pollen limitation reported in the literature, although Zimmerman (1988) has argued that more conclusive studies are needed. Recently, some have concluded that pollen and resources may both be important factors limiting reproductive success among natural populations (McCall and Primack, 1985; Campbell and Halama, 1993).

Evidence of pollen limitation is of critical interest for several reasons. Studies of how plants allocate their resources assume that seed production is resource limited. Thus, conclusions regarding the reproductive allocation patterns of pollen limited populations may be in error (Bierzychudek, 1981). Secondly, all hypotheses based on resource limitation of

female reproduction (e.g. the male function or selective abortion hypotheses) assume adequate pollination. These hypotheses can not be properly evaluated if the population being considered is pollen limited (Whelan and Goldingay, 1989). Thirdly, theory suggests that pollen limited populations are not evolutionarily stable (Haig and Westoby, 1988). Within populations consistently growing under pollen limited conditions, there should be strong selection pressure favoring individuals that increase pollen attraction, until the population is limited by resources. It therefore remains important to assess when reproduction is limited by pollen supply in natural populations.

Prior investigations of pollen limitation have examined populations of hermaphroditic species characterized by low fruit to flower ratios. However, all obligate outcrossers are potentially pollen limited, including the dioecious species which are generally thought to have high fruit to flower ratios (Sutherland and Delph, 1984). Prior investigations have also primarily focused on taxa with zoophilous pollination systems. Here we extend examination of pollen limitation to the dioecious, hydrophilous aquatic macrophyte *Vallisneria americana* (Hydrocharitaceae). An advantage in considering pollen limitation in a dioecious species is that there is no potential confound with the allocation of resources to male function. The unique hydrophilous pollination system of *V. americana* (see below) also adds another dimension to the study of pollen limitation in that prior studies have not considered pollen limitation in a two-dimensional environment.

The purpose of this study was to investigate the influence of different site characteristics in limiting pollination in *V. americana*. Many of the studies examining the role played by pollination intensity in limiting reproductive success have failed to consider spatial variation (but see Hagerup, 1951; Galen, 1985; McCall and Primack, 1985; Campbell, 1987; and Johnston, 1991). I found evidence in a field experiment relating reproductive effort to depth among *V. americana* females suggesting that populations may be pollen limited if they are found where wind or surface currents carry male flowers away before females can be effectively pollinated. Such areas might be found along the shores of

lakes with relatively narrow littoral zones that are exposed to sufficient wind and/or wave action, or in streams and rivers with sufficient current velocity. Populations may also be entirely pollen limited if they occur and flower at depths beyond the maximum peduncle length, assuming that pollination can not take place beneath the surface. *V. americana* pollination has been described by several authors (see Wylie, 1917; Svedelius, 1932; and Cox, 1988) as a remarkable process occurring at the surface. However, since flowers have been observed maturing below the surface in deeper water, it may be possible that subsurface pollination takes place.

I hypothesized that some populations of *V. americana* may commonly be found at sites whose characteristics limit or preclude successful pollination. Prior experience suggested that fruit-set in natural populations is very high. A series of field observations were made at sites where flowering populations of *V. americana* were known to occur to test this hypothesis. The influences of wave and wind exposure, current velocity, or depth on fruit-set was examined.

METHODS

The approach I took in evaluating pollen limitation was to consider natural variation in fruit-set between treatments or sites, rather than to compare natural levels of fruit-set with fruit-set augmented by hand-pollination. The latter approach looks for an increase in fruit-set, but such increases may be confounded with declining fruit-set in subsequent seasons if populations are in fact resource limited instead of pollen limited (see Janzen et al., 1980). An advantage in considering natural pollination levels is that fruit-set in subsequent seasons need not be considered as there is no unnatural and potentially draining expenditure of resources on the fruit development of hand-pollinated flowers.

Study Species

Vallisneria americana is a dioecious, clonal, submersed macrophyte distributed in lakes and streams throughout much of eastern North America, with subspecies found in eastern Asia, Oceania, and Australia (Lowden, 1982). *V. americana* produces a rosette of ribbon-like leaves from a perennating tuber in the spring. Additional rosettes are produced at the nodes of rhizomes arising in the axils of leaves (Sculthorpe, 1967). In upstate New York, flowering begins from late in June to late in July, and may continue into early September. Buoyant female flowers are borne on elongated peduncles, and anthesis occurs at the surface (Wylie, 1917). Female flowers in deeper water may not grow to the surface, but anthesis still occurs after the peduncle has elongated (Sullivan, personal observation). Up to 2000 tiny round male flowers (approximately 0.6 mm in diameter) may be borne on a short stemmed inflorescence (Wylie, 1917). The mature male flowers are abscised over a period of usually less than 36 hours after the spathe opens. They immediately float to the surface where they are supported on the surface film. The sepals soon open with the stamens oriented in an upright position. The male flowers are then propelled about by wind or currents (Svedelius, 1932; Cox, 1988). Pollination occurs when male flowers collide with a female flower and tip into the surface tension depression surrounding the perianth, causing anthers to brush the stigma surface (Wylie, 1917; Svedelius, 1932; and Cox, 1988). The buoyant cigar shaped fruits develop over several weeks and many remain attached to the female until the end of the growing season. Seeds are dispersed locally if the fruit senesces before the peduncle, and they are dispersed away from the parent when the peduncle senesces before the fruit (Sullivan, personal observation).

A factor to consider among dioecious clonal species such as *V. americana* is the distribution of male and female clones, which may need to be in close proximity for effective pollination. Although unisexual populations of *V. americana* may be common

(Lowdon, 1982), abundant males were naturally present within close proximity of females in all of the study sites except Silver Lake, where only transplanted males flowered.

Field experimentation: Silver Lake

The effects of depth and light availability on flower and fruit production were investigated experimentally at Silver Lake, PA, (41°56' N; 75°57' W) during the summer of 1989. The native population of *V. americana* at Silver Lake has been non-flowering in the recent past. On June 6, 240 germinating *V. americana* tubers of unknown sex from Chenango Lake, NY (42°15'N; 75°50'W) were transplanted into pots containing lake sediment and placed at the south end of Silver lake in a relatively open area with little surface vegetation. Sixty plants were grown at 0.9 m and at 1.7 m depth, with 20 plants at 1.3 m, 2.1 m, and 2.5 m depth. An additional 60 plants were grown at 0.9 m depth (adjacent to the other plants at 0.9 m) inside a 3 x 3 m enclosure. The enclosure at 0.9 m was made of neutral density shadecloth suspended 0.2 m above the lake surface and extending down to the sediment surface. The enclosure inhibited wave action, and the drifting away of male flowers. The shadecloth passed approximately 70% of incident radiation, so that the light level at 0.9 m depth within the enclosure approximated that at 1.7 m depth. Plants were harvested on September 3, after fruits had matured. Fruit-set and peduncle length as well as the number of rosettes, flowers, and tubers were recorded.

Field observations: unmanipulated populations

Observations were made in late summer toward the end of the fruiting season at Seneca Lake, NY (Sept. 7, 1993), Nuthatch Hollow Pond, NY (Aug. 25, 1991), Cayuga Lake, NY (Aug. 31, 1993), and the Hudson River, NY (Aug. 25, 1993). At all sites, fruit-set was calculated as:

(fruit number) / (fruit + mature unpollinated flower number).

Flowers were classified as either pollinated (including fruits), unpollinated, or recently opened. Because recently opened flowers may have been pollinated, but had not yet begun developing a fruit, they were not considered in the calculation. Recently opened flowers could be identified because the perianth remains white and unwettable. Unpollinated flowers were distinguished from recently opened flowers because the perianth of older flowers is wettable, and the color turns grey or brown as they senesce. Pollinated flowers were distinguished from unpollinated flowers as the ovary elongates as the fruit develops (ovary elongation begins shortly after pollination, personal observation). Fruit-set was calculated per patch rather than per individual because individuals could not be readily distinguished. The individual female patches sampled were generally distinct with recognizable borders defined by their fruits or flowers at the water surface or in the water column. Some of these patches may have represented a single clone as the patch was a single color morph.

Seneca Lake ($42^{\circ}34' N$, $76^{\circ}53' W$) was sampled to examine how peduncle length and the probability of being pollinated varies with depth for a population found primarily in deeper water. We sampled offshore of Lamoreaux Landing on the eastern shore of Seneca Lake (approximately 21 km north of Watkins Glen) where *V. americana* may be found in abundance between 2.25 m and 5.25 m depth. Flowers were sampled across patches along 50 m transects with SCUBA gear along depth contours at 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 m depth. The status of flowers (pollinated or unpollinated) and peduncle length were recorded for every flower encountered along a 1.0 m wide belt transect.

Nuthatch Hollow Pond ($42^{\circ} 5' N$, $5^{\circ} 59' W$) was sampled to determine how fruit-set was related to flower position relative to the surface, to assess whether female flowers

could be pollinated below the surface of the water. Nuthatch Hollow Pond was chosen because *V. americana* is the dominant macrophyte there, many flowers in deeper water do not elongate to the water surface, and the surface is often densely covered with male flowers while in bloom. *V. americana* occurs and may flower from 0.1 m to 2.25 m depth. All fruits and flowers from one randomly chosen patch of female plants growing between 1.4 m to 2.2 m depth were examined. The position of the perianth relative to the surface was determined for flowers as the distance from the surface to the perianth (above or below the surface), or for fruits as a point 20 mm above the fruit base (the approximate position of the perianth prior to fruit development).

Cayuga Lake ($42^{\circ}55' \text{ N}$, $76^{\circ}45' \text{ W}$) was sampled to relate different site characteristics to the probability of being pollinated. Fruit-set was measured at three site in the north end of Cayuga Lake differing primarily in exposure to surface wind and waves, and incidentally in depth. Site I was located within a relatively sheltered, shallow area in a bay just north of the railroad line crossing the lake at the north end. The water was approximately 1.0 m deep and protected from wave action by a railroad causeway and by the dense stand of macrophytes extending to the water surface. *V. americana* was the dominant species in this area. All fruits and flowers were sampled in four 0.5 m wide belt transects, each one bisecting a distinct patch of females encountered along an objectively chosen heading.

Site II was in the northwest corner of the lake just south of the rail line. The site chosen was approximately 0.9 m deep and was protected from wave action by land to the north, west, and southwest, and by the dense macrophytes growing throughout the water column. The water in this area remained calm despite a brisk south wind, while in deeper water ($> 1.25 \text{ m}$) it was relatively rough (wave amplitude approximately 40 cm at the time of sampling). As in site I, *V. americana* was the dominant species. All fruits or flowers

were sampled in five 0.5 m wide belt transects, each one bisecting a distinct female patch encountered along an objectively chosen heading.

Site III was in the middle of the lake approximately 2.75 km south of the rail line.

Plants were sampled along a northerly drift line originating in the middle of the lake directly east of Cayuga Lake State Park. Depth varied between 2.45 and 2.55 m. The lake bottom in this area was densely covered with *V. americana* and other macrophytes. Site III differed from sites I and II in that very few of the macrophytes grew within a meter of the surface, with the exception of the flowers of female *V. americana*. Four patches were sampled as each was encountered by our drifting boat after objectively selected time intervals. All fruits and flowers that had reached the surface in a given patch were harvested and later counted for calculating fruit-set.

The Hudson River ($43^{\circ}10' N$, $73^{\circ}35' W$) was sampled to examine how pollination might be affected in a flowing water environment. We sampled the river between Northumberland Bridge and Fort Edward to seek evidence of pollen limitation as a function of surface water velocity. *V. americana* grows in the river throughout this area in a range of flow regimes from quiet backwaters to stretches with rapidly moving water. Still and slow moving waters were sampled around the two small islands near the mouth of Black House Creek, while faster flow regimes were sampled downstream on the east side of Thompson Island. Flowers and fruits were sampled along 0.5 m wide belt transects cutting through randomly chosen patches in areas with different current velocities. Surface water velocity was calculated from the mean time of three trials for a 15 mm diameter plastic bobber to travel 6.0 m downstream.

Statistical analysis

Data from Seneca Lake were analyzed in a single factor analysis of variance. Pairwise multiple comparisons were performed with a Tukey test (SYSTAT, 1992). Cayuga Lake data were analyzed in a nested design with patch nested in Site. Peduncle data from Silver Lake failed to meet the homogeneity of variance assumption of ANOVA despite transformations, and were therefore analyzed with a Kruskal-Wallis one-way ANOVA. Nonparametric multiple contrasts similar to the Tukey test were used to compare means across depths (Zar, 1984). Fruit-set was correlated with water velocity in the Hudson River data after an arcsin transformation of fruit to flower ratio (Zar, 1984). A chi-square analysis was performed on the Nuthatch Hollow Pond data to distinguish differences in the distribution of fruits between surface and subsurface flowers.

RESULTS

Field experiment

Two hundred fourteen of the 240 transplants in the Silver Lake experiment flowered, including 67 females and 145 males. Non-flowering plants were scattered among all treatment groups. Contrary to expectations, we found that *V. americana* females were surprisingly insensitive to depth. Female plants growing in deeper water flowered, even though they could not elongate their flowers to the surface (Fig. 1a). Peduncle length increased significantly ($p < 0.001$) with depth, but the response was highly variable within and among individuals and could not compensate for depth beyond 1.3 m. All mature female flowers found at 0.9 m depth at both open and enclosed sites were able to reach the surface. One of the 8 female flowers growing at 1.3 m failed to reach the surface, though others on the same plant were successful. Only 2 of the 33 flowers on females growing at 1.7 m did reach the surface. These were on different individuals, each with

other flowers that failed to reach the surface. None of the female flowers at 2.1 m or 2.5 m depth reached the surface.

All mature female flowers that failed to reach the surface still opened, and none of these were pollinated. Unexpectedly, none of the female flowers that did reach the surface were pollinated either, except for those growing within the enclosure at 0.9 m (Table 1).

All female flowers in the enclosure were pollinated and developed into fruits. The proportion of plants that were male in each treatment group was similar (nearly 2 of every 3 plants). Male plants produced on average 4.06 ± 1.76 (s.d.) inflorescences. Differences between treatments were not significant. Since there was nearly complete overlap in the timing of male and female flower maturation, male flowers were available for donating pollen in each treatment group.

Field observations

Somewhat surprisingly, we found that *V. americana* females readily flowered in water well beyond the depth at which peduncles could reach the surface. In Seneca Lake, a large population of *V. americana* was observed flowering across its depth range (from 2.25 m out to 5.25 m) with 100% of the flowers failing to reach the surface. There was a significant effect of depth on peduncle length (Fig. 1b). Pairwise comparisons indicated that differences in peduncle length beyond 3.5 m depth were not significant until they decreased at 5.0 m. Regardless of depth, lengthening peduncles could not compensate for growing in deeper water.

Despite the wide occurrence of females flowering below the water surface, subsurface pollination did not appear to take place. At the Seneca Lake site, none of the female flowers sampled (and none of the many others observed) had developed into fruit by the end of the flowering season. At Nuthatch Hollow Pond, where male flowers often densely cover the water's surface, the probability of being pollinated was significantly higher

among surfacing flowers than among subsurface flowers ($p < 0.0001$, chi-square analysis; $\chi^2 = 125.23$, $df = 1$). Fruit-set was 0.0% among subsurface flowers and 92.2% among surfacing flowers.

Deep water may not be the only constraint to successful pollination among *V. americana* females. Wind or surface currents may be an impediment to pollen donation. When fruit-set was contrasted at three sites on Cayuga Lake (Fig. 3), it was significantly lower in the deeper open water site exposed to wind and waves (79%) than in the exposed shallow water site (97%) or in the sheltered shallow water site (97%). The dense canopy of *V. americana* leaves extending to and along the water surface in both shallow water sites appears to facilitate pollination by reducing wave action, and by hindering the dispersal of male flowers that are blown or drift into patches of females.

Fruit-set of *V. americana* females growing in the Hudson River decreased as surface velocity increased (Table 2). Fruit-set ranged from 93% in a still backwater to 0% in the fastest moving current. Fruit-set in the first five sites was significantly and negatively correlated with current velocity, decreasing from 93% at $0.09 \text{ m}\cdot\text{s}^{-1}$ to 2.3% at $0.19 \text{ m}\cdot\text{s}^{-1}$ ($r = -0.934$, $p = 0.020$). At site four (current velocity $0.19 \text{ m}\cdot\text{s}^{-1}$) fruit-set was slightly lower than at site five, despite a slower current velocity ($0.21 \text{ m}\cdot\text{s}^{-1}$). I suspect the lower fruit-set was partially due to greater depth at site five (1.15 m vs. 0.8 m at site four), where some flowers were being intermittently driven beneath the surface by the river's current, and thus unavailable to male flowers. Fruit-set among female flowers at site six in the fastest current ($> 0.22 \text{ m}\cdot\text{s}^{-1}$; we were unable to get an accurate measurement) was 0% due to the female flowers being swept beneath the surface by the current. These results suggested that *V. americana* may be pollen limited while flowering in a current due to males being swept away prior to capture by a female flower and/or to female flowers being held below the surface by the current.

DISCUSSION

Populations of the hydrophilous species *Vallisneria americana* may be found on sites where they are entirely or partially pollen limited. Depth at a given site may impose an insurmountable barrier to successful pollination. Females at the Seneca Lake site readily produced flowers throughout their range of depth (to 5.25 m), and none of these flowers elongated to the water surface. Subsurface flowers did not have access to floating male flowers and thus were not pollinated. Subsurface pollination may not be possible even under what may be the most favorable of conditions. Subsurface female flowers at Nuthatch Hollow Pond were not pollinated even when found just a few centimeters beneath a blanket of floating male flowers. Peduncle elongation responded to depth, but could not always compensate for it. Females at Silver Lake could not reach the surface beyond 1.7 m depth, and did not reach the surface anywhere in the Seneca Lake population (Fig. 1). *V. americana* at the Seneca Lake site may not have successfully reproduced (sexually) in many years, perhaps centuries. There may be relatively few other examples of complete long-term pollen limitation among zoophilous or anemophilous species where both males and females are found in close proximity.

V. americana growing in shallow water may also be pollen limited if wind, waves, or current carry male flowers away before females are pollinated. When surface currents in the Hudson River increased from 0.09 to 0.17 $\text{m}\cdot\text{s}^{-1}$, fruit-set was reduced from 83% to 9% (Table 1). Females growing in faster currents were hindered further as their flowers were swept below the surface, precluding any chance of successful pollination. Fruit-set on Cayuga Lake was very high (97%) in areas protected from waves, and where leaves and female flowers trailing along the surface hindered the dispersal of male flowers. In contrast, fruit-set in open water exposed to wind and waves was significantly lower at 79%. This difference is more compelling given that these plants were located in the middle of an extensive littoral zone comprising several square kilometers and dominated by

V. americana. Fruit-set was lower in this area despite legions of male flowers continuously drifting by. This situation contrasts sharply with the experimental plants on Silver Lake, where fruit-set was 0% outside the enclosure, and where the population of males was relatively small. This suggests that male densities may play an important role in determining pollination success. The 100% fruit-set within the enclosure at Silver Lake was due to maintaining a high local density of male flowers. Thus, pollination may be severely limited where the local residence time of male flowers is relatively short, unless there is a continuous infusion of new male flowers. Conditions of low male flower density may be commonly found on large lakes with relatively narrow littoral zones.

Site-specific pollen limitation among other species

Pollen limitation of fruit or seed set in zoophilous or anemophilous species may also differ among sites, though few studies have considered that pollen limitation may be a function of the physical characteristics of the site on which a population is found. Hagerup (1951) found that pollination in a number of species on the Faeroe Islands are limited by the weather and general paucity of insect pollinators. Factors limiting fruit-set in *Lysimachia quadrifolia* (pollen vs. other resources) were investigated at an open and a scrubby site just 30 m apart over a three year period. Pollen did limit fruit-set in some years, but fruit-set varied more between the two sites than among years, suggesting that the difference in limiting factors was related to differences between the sites (McCall and Primack, 1985). Seed set for two ecotypes of *Polemonium viscosum* was examined at three elevations differing in the relative abundance of the pollinators attending each ecotype. Seed set was pollen limited at all sites, but differed significantly between the two ecotypes according to the abundance of their pollinators (Galen, 1985). Johnston (1991) found that *Lobelia cardinalis* was pollen limited at two different sites, but that the degree of limitation was greater at one of the sites because of lower pollinator attendance. These studies

indicate that pollen limitation is a site-specific phenomena, and suggest that many species may be pollen limited in different parts of their range. The results of this study are in strong agreement with this conclusion.

Implications of pollen limitation among clonal species

Theory suggests pollen limitation is not an evolutionarily stable condition, and that natural selection will favor those individuals in pollen limited populations that increase allocation to pollen attraction (Haig and Westoby, 1988). However, Calvo (1993) has suggested that pollen limitation may be an evolutionarily stable condition if there is little or no relationship between fruit and seed production and seedling establishment. Under these circumstances, there may be no correlation between reproductive effort and fitness. Thus, an evolutionary response to pollen limitation may not be likely among clonal populations with poor recruitment. Seedling establishment has been found to be rare in many clonal populations on more mature sites (e.g. Hartnett and Bazzaz, 1985), and seedling recruitment may be a relatively rare event among some *V. americana* populations. In a recent survey of Chenango Lake, NY where there is an extensive population of flowering and fruiting *V. americana*, only 1 seedling was recorded in the 250 m² sampled (Sullivan and Andorfer, unpublished data). It may be difficult for such populations to adapt to pollen limited conditions on sites such as these. Even if conditions for seedling recruitment are potentially high, some deep water populations may spend long periods of time between the successful production of seeds.

I conclude that pollination in the dioecious and hydrophilous *Vallisneria americana* is highly dependent upon specific site characteristics such as water depth, exposure to wind and waves, and current velocity. I strongly suspect that male density also exerts an

important influence. Overall, pollen limitation in natural populations varies from complete to remarkably low.

SUMMARY

Experimental and observational studies of the submersed, freshwater macrophyte *Vallisneria americana* Michx. revealed that water depth, site exposure, and current velocity may all influence fruit-set. In this dioecious species, long-pedunculate female flowers are pollinated by free-floating male flowers at the water surface. Among sites studied, fruit-set in natural populations varied from zero to 97%. With increasing water depth at field sites in New York and Pennsylvania, 100% of female plants continued to flower though female flowers were unable to reach the surface, however these flowers set no fruit. Fruit-set was also low in relatively exposed sites, presumably because male flowers do not remain long in the vicinity of female flowers. This was particularly true at a site with low male flower densities, but was experimentally increased to 100% at that site by confining the floating male flowers within a field enclosure. Finally, fruit-set was negatively correlated with current velocity in a river.

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Table 1. Effect of depth and site on pollination success and fruit-set for females, and on the availability of inflorescences at each site for pollen donation at Silver Lake during the summer of 1989. Each site was within 5 m of the next deeper site.

Depth	Site	M/F	Fruits	Unpollinated Flowers	Fruit-set (%)	Male Inflorescences
0.9	Open	39/17	0	47	0	170
0.9	Enclosed	37/19	29	0	100	160
1.3	Open	13/2	0	8	0	47
1.7	Open	29/20	0	27	0	124
2.2	Open	13/5	0	9	0	45
2.6	Open	14/4	0	11	0	43

Table 2. Effect of depth and current velocity on the number of fruits, number of unpollinated flowers, and fruit-set at six sites on the Hudson River on Aug. 25, 1993. Fruit-set in the first five sites was negatively correlated with current velocity ($r = 0.934$, $p < 0.05$). We were unable to get an accurate measurement of the fastest current velocity (at site six).

Site	Depth (m)	Current Velocity ($m \cdot s^{-1}$)	Fruits	Unpollinated Flowers	Fruit-set (%)
1	0.7	0.000	162	12	93.0
2	0.5	0.090	124	25	83.2
3	0.9	0.167	19	205	9.3
4	1.2	0.188	7	304	2.3
5	0.8	0.214	22	217	9.2
6	0.6	> 0.22	0	176	0.0

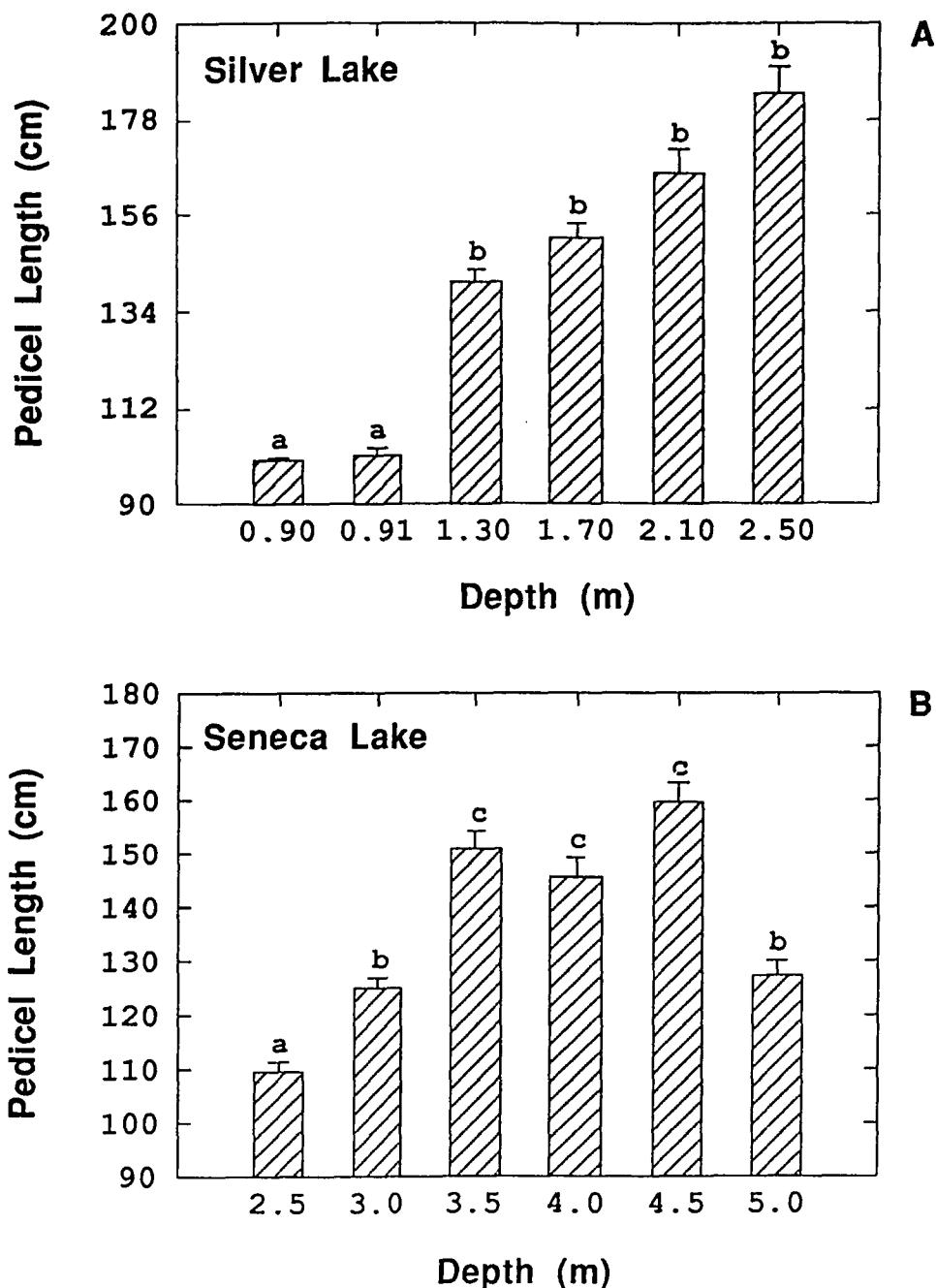


Figure 1. Peduncle length of female flowers as a function of depth: (a) Silver Lake experimental transplants (0.91 m depth refers to plants within the enclosure at 0.90 m); (b) the unmanipulated population at Seneca Lake. Error bars represent 1 SE. Means with different lowercase letters differ significantly ($p < 0.05$; multiple contrast of nonparametric mean ranks in (a), Tukey's multiple comparison test in (b)).

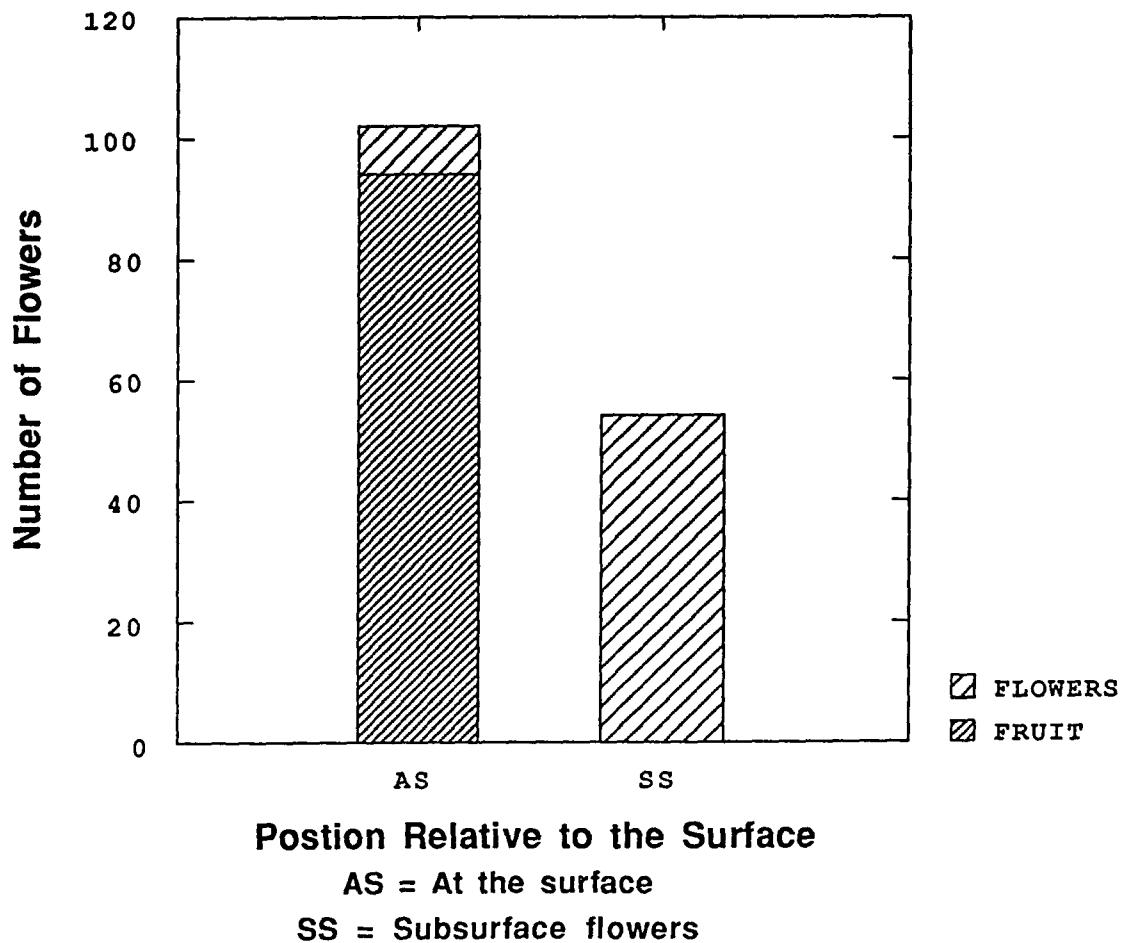


Figure 2. Number of unpollinated and pollinated flowers sampled just below and at the surface in Nuthatch Hollow Pond.

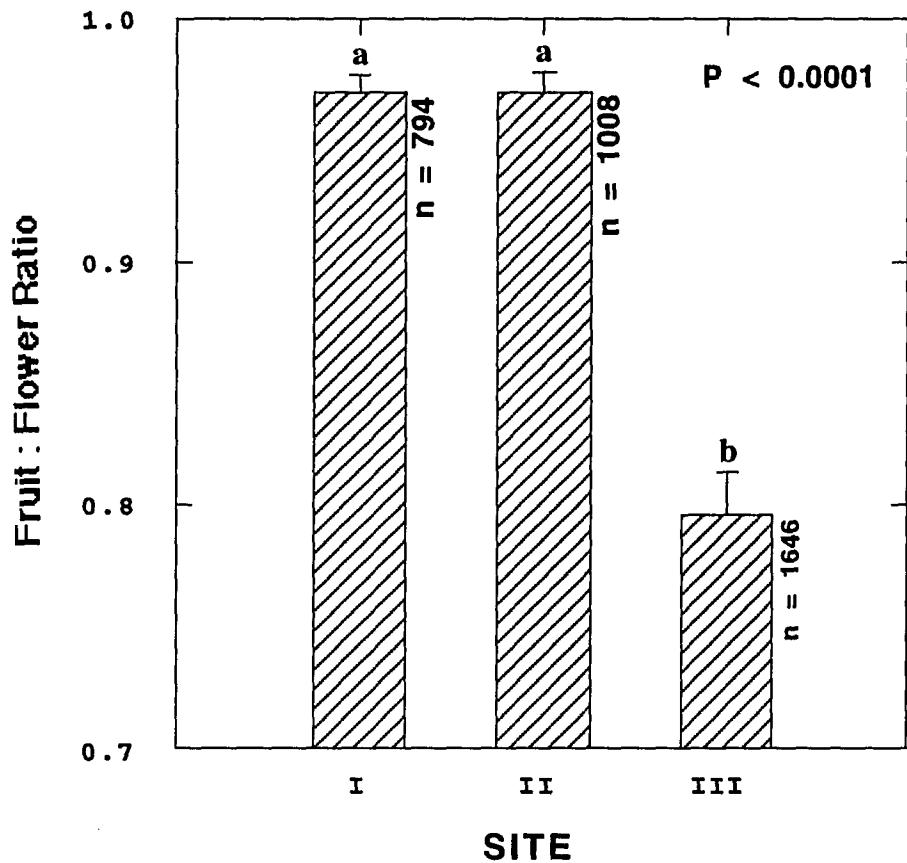


Figure 3. Fruit-set in *V. americana* at three sites in Cayuga Lake. Sites I and II are relatively sheltered while site III was exposed to wind and waves. Means are of four patches at sites I and III, or five patches at site II. Means with different lowercase letters differ significantly ($p < 0.001$; Tukey's test). Error bars represent 1 SE. n = the total number of pollinated and unpollinated flowers contributing to each mean.

