

**LATITUDINAL VARIATION IN PALATABILITY OF SALT-MARSH
PLANTS: CONSTITUTIVE OR INDUCED?**

A Thesis

Presented to

The Faculty of the Department of Biology and Biochemistry
University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Cristiano Salgado

May 2004

**LATITUDINAL VARIATION IN PALATABILITY OF SALT-MARSH
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ABSTRACT

A central biogeographic theory argues that consumer-prey interactions are more intense, and prey defenses better developed, at lower latitudes. Along the Atlantic Coast of the United States, southern salt marsh plants are less palatable than northern conspecifics. To test the hypothesis that latitudinal variation in palatability would occur in the absence of geographically-different environmental cues (i.e., that differences in palatability are constitutive rather than induced by climate or herbivore damage), I grew high- and low-latitude individuals of three species of salt marsh plants from seeds (*Solidago sempervirens*) or rhizome cuttings (*Distichlis spicata* and *Spartina alterniflora*) in a common-garden greenhouse environment, and compared their palatability to herbivores over time. I also quantified leaf toughness and nitrogen content of those plants in order to help explain results of feeding assays. My results document a pattern for northeastern salt marsh plants to be more palatable than southeastern conspecifics after being germinated in a greenhouse or kept under common-garden conditions for several clonal generations, suggesting that the latitudinal variation of salt marsh plants observed in the field is constitutive rather than induced by environmental cues. Latitudinal variation in plant traits depended on the plant species. Toughness varied as a function of latitude for *Spartina* and *Distichlis*, with southern plants being tougher than northern conspecifics across clonal generations. For all generations of *Spartina* and for seed-propagated *Solidago*, northern plants had higher nitrogen content than southern plants. Results are consistent with the theory that herbivory is a strong selective pressure that might be shaping geographical variation in plant palatability. However, many other

factors, such as differences in growth season length and external disturbances could be of crucial importance in mediating this latitudinal pattern of palatability. Understanding the genetic and environmental bases of intraspecific variation and how they covary on broad geographic scales can provide important clues to how organisms adapt to different and changing environments.

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INTRODUCTION

A fundamental paradigm of biogeography is that consumer-prey interactions vary across latitude, with predation and herbivory being more intense in the tropics than in the temperate zones. Theory predicts that increased consumer pressure at low latitudes should select for increased defenses of prey relative to high latitudes (McArthur 1972, Bakus 1974, 1981, Bakus and Green 1974, Green 1977, Vermeij 1978, Jeanne 1979, Bertness *et al.* 1981, Gaines and Lubchenco 1982, Louda 1982, Fawcett 1984, Heck and Wilson 1987, Coley and Aide 1991, Stiven and Gardner 1992, Jablonski 1993, Cronin *et al.* 1997). Greater herbivore pressure will increase the risk of damage and the potential benefit of defenses, favoring selection for enhanced defense against herbivory (Feeny 1976, Levin 1976, Coley *et al.* 1985). Why herbivore pressure should show a latitudinal trend is uncertain. Possible explanations for high herbivore pressure at low latitudes include less severe abiotic control of herbivore populations and greater predictability and productivity of plants (Coley and Aide 1991).

One important element of plant-herbivore interactions over both evolutionary and ecological time scales is plant defense. Defenses are thought to influence the population dynamics and distribution of plants and herbivores, and, in turn, this interaction might affect the evolution of defenses (Coley and Aide 1991). Despite considerable interest in biogeographical patterns in plant-herbivore interactions (Brower *et al.* 1972, Gaines and Lubchenco 1982, Krischik and Denno 1983, Hay and Fenical 1988, Horn 1989, Duffy and Hay 1990, Coley and Aide 1991, Steinberg 1992, Coley and Barone 1996, Hay 1996), the evidence to date for latitudinal gradients in plant palatability is largely

circumstantial or laden with caveats (Pennings *et al.* 2001). Because of the logistical constraints inherent in working across geographical scales, experimental studies of latitudinal differences in plant-herbivore interactions are almost entirely lacking, and most studies have been forced into indirect measurements of plant palatability or compromises of design. Few studies have directly compared the palatability of fresh plants (but see Steinberg *et al.* 1991, Swihart *et al.* 1994, Pennings *et al.* 2001), working instead with freeze-dried plants or plant extracts, or measuring plant traits that might correlate with palatability (e.g., Levin 1976, Rodriguez 1977, Coley and Aide 1991, Bolser and Hay 1996). Moreover, some studies have utilized only one study site in each geographic region (e.g., Cronin *et al.* 1997), raising the concern that variation within a region might be as large as that observed between regions (Coley and Aide 1991, Bolser and Hay 1996). Finally, because many plant species are not widely distributed, many latitudinal studies compare distantly-related groups of plants (e.g., Van Alstyne and Paul 1990, Coley and Aide 1991), raising the concern that phylogenetic constraints unrelated to plant-herbivore interactions might influence the comparisons (Pennings *et al.* 2001).

Despite these caveats, most of the limited number of studies available to date are consistent with the general hypothesis that tropical plants are better defended than temperate ones (Hay and Fenical 1988, Coley and Aide 1991, Bolser and Hay 1996, Pennings *et al.* 2001), although brown algae, boreal trees, and sponges show no trend or the opposite trend (Van Alstyne and Paul 1990, Steinberg 1992, 1995, Swihart *et al.* 1994, Becerro *et al.* 2003), and several studies suggest important regional differences in plant palatability not related to latitude (Steinberg 1989, 1992, Coley and Aide 1991,

Targett *et al.* 1992, 1995, Bryant *et al.* 1994, Steinberg *et al.* 1995, Bolser and Hay 1996, Coley and Barone 1996).

The most comprehensive data set addressing latitudinal variation in plant defense comes from work in Atlantic Coast salt marshes. Results of palatability trials with fresh plants demonstrated strong preferences for northern vs. southern individuals of 10 salt marsh plant species, regardless of season of plant collection or geographic origin of herbivore used (Pennings *et al.* 2001), documenting a striking pattern for high-latitude plants to be more palatable than low-latitude conspecifics. Moreover, recent research shows that different traits, including toughness, chemical defenses, and N content, have the potential to produce this striking geographic pattern (Pennings and Paul 1992, Pennings *et al.* 1998, Siska *et al.* 2002). A variety of factors, including herbivore densities, climate, length of growing season, salinity, and anthropogenic eutrophication may differ between low and high-latitude salt marshes, and could play a role in mediating plant traits and palatability (Siska *et al.* 2002). Although it seems clear that latitudinal variation in palatability exists amongst salt marsh plants and that multiple plant traits may affect palatability, it is still unknown whether this geographical pattern is dictated by differences in the environmental conditions experienced by the plants or if it is genetically controlled.

Whatever the ultimate factors responsible for this latitudinal variation are, they could create differences in plant traits either through selection for fixed traits or by altering expression of plastic traits. Phenotypic plasticity is of particular importance to plants, whose sessile condition requires them to deal with enormous variation in the

environment. Plants are highly plastic for a variety of traits, and individuals within a species may largely vary in growth rate, size, reproduction, allocation to different organs, and chemical composition (Callaway *et al.* 2003). Furthermore, phenotypic plasticity in chemical defense is thought to play a major role in plant-herbivore interactions, with cues associated to herbivores causing an increase or induction of the defensive phenotype of the plant (Agrawal *et al.* 2002).

Herbivores can reduce seed production and other correlates of plant fitness, and this decrease can result in natural selection for either constitutively expressed or inducible defenses (Karban and Baldwin 1997, Agrawal 1998). Thus, latitudinal differences in plant palatability might be constitutive (always expressed) or might be induced by latitudinal differences in environmental cues such as physical stress, day-light length, or herbivore damage. We know almost nothing about the importance of these two mechanisms in producing geographic patterns in plant palatability. Whether differences are constitutive or induced has important implications for the kind of temporal and spatial variability in palatability that we might see in the field, for our ability to perform experiments in order to explore these patterns, and for our understanding of the selective forces that shape plant defenses. My goal was to test the hypothesis that latitudinal variation in palatability would occur in the absence of geographically-different environmental cues (i.e., that differences in palatability are constitutive rather than induced by climate or herbivore damage). To test this hypothesis, I grew high- and low-latitude individuals of three species of salt marsh plants from seeds or rhizome cuttings in

a common-garden greenhouse environment, and compared their palatability to herbivores over time.

Environmental conditions experienced by the mother may affect the offspring's performance (Lacey 1998) and environmental variation may be important in the expression of parental effects (Rossiter 1998). Parental effects are defined as 'any parental influence on offspring phenotype that cannot be attributed solely to offspring genotype, to the direct action of the non-parental components of the offspring's environment, or to their combination' (Lacey 1998). In plants, because the mother provides the seed, there is ample potential for seed quality to result in maternal effects on offspring performance (Agrawal *et al.* 1999). Therefore, when looking at population differentiation, differences that seem to be the results of genetic differences may in fact relate to differential maternal contributions (Berven *et al.* 1979). Laugen *et al.* (2002) argue that one way to overcome this problem is to raise offspring under common environment conditions for a few generations in order to eliminate environmentally induced effects.

Over 100 years ago, Bonnier (1895; as cited by Linhart and Grant 1996) and other botanists acknowledged the experimental advantages resulting from the facts that plants can be easily moved, subdivided by experimenters, and then grown under highly controlled conditions (Linhart and Grant 1996). Common garden experiments have often been used to demonstrate the genetic basis of intraspecific differentiation in a variety of plant characteristics, and Karban and Baldwin (1997) pointed out that using a common garden to keep environmental conditions as constant as possible provides a good way to

try to ensure that much of the observed variation is heritable. The most rigorous way to examine the genetic basis of traits is through experimental crosses. Nevertheless, because common-garden experiments remove the majority of environmentally-induced effects on phenotype (Laugen *et al.* 2002), results of common-garden experiments are typically regarded as strong, although not incontrovertible, evidence for the genetic basis of traits (Karban and Baldwin 1997).

Here I will present strong evidence that latitudinal variation in palatability of salt marsh plants has a genetic basis (i.e., differences are constitutive rather than induced by environmental conditions), and discuss possible explanations for this pattern.

MATERIALS AND METHODS

Collection sites

Salt marshes are the dominant intertidal habitat along the Atlantic Coast of the United States (Pennings and Bertness 2001), and offer an attractive system for examining latitudinal variation in plant palatability. Similar plant and animal communities occur across a wide latitudinal range, making it possible to make intraspecific comparisons across latitude (Duncan and Duncan 1987, Pennings and Bertness 1999). In addition, salt marshes host a diverse assemblage of herbivores that at times can strongly reduce plant biomass and/or mediate plant distributions (Pennings and Bertness 2001).

To look at latitudinal variation in plant palatability, I worked at salt marshes located within high latitudes (northeast coast of the United States) and low latitudes (southeast coast of the United States) (hereafter, high latitude = north and low latitude = south). In

order to ensure that working at any one site did not bias the results, I collected plants and seeds from ten northern and ten southern sites (Table 1). Logistical constraints precluded sampling of mid-Atlantic or Canadian sites.

■ **Table 1**

Collection sites and geographic coordinates.

Site	State*	Latitude	Longitude
Northern Sites			
Drake Island	ME	43°15' N	69°50' W
Little River	ME	43°15' N	69°50' W
Great Neck	MA	42°70' N	70°79' W
Nelson Island	MA	42°74' N	70°82' W
Providence Point	RI	41°37' N	71°19' W
100 Acres	RI	41°45' N	71°19' W
Rumstick Cove	RI	41°43' N	71°17' W
Nag Creek	RI	41°39' N	71°20' W
Bluff Point	CT	41°19' N	72°02' W
Cottrell Marsh	CT	41°20' N	71°56' W
Southern Sites			
Goat Island	SC	33°19' N	79°11' W
3 rd Boundary	SC	33°21' N	79°10' W
Ace Basin	SC	32°33' N	80°28' W
Broad River	SC	32°23' N	80°45' W
Dean Creek	GA	31°23' N	81°16' W
North Sapelo	GA	31°31' N	81°13' W
Hunt Camp	GA	31°23' N	81°16' W
Visitor Center	GA	31°25' N	81°15' W
Eulonia	GA	31°25' N	81°15' W
Amelia Island	FL	30°40' N	81°30' W

* ME = Maine, MA = Massachusetts, RI = Rhode Island, CT = Connecticut, SC = South Carolina, GA = Georgia, FL = Florida

Plants and herbivores

I worked with three plant species common at both northern and southern sites (Table 2). *Solidago sempervirens* was propagated by seed. I collected multiple seeds from at least six individual plants from each of the 20 sites. Seeds from within a site were pooled, brought to the laboratory, and kept refrigerated for about five months. In January of 2003 the seeds were germinated and four to ten seedlings were raised per site. *Distichlis spicata* and *Spartina alterniflora* were propagated clonally. Four individual ramets of *Spartina alterniflora* and four rhizome cuttings, each with several shoots, of *Distichlis spicata* were collected from each site in June of 2002. To minimize the possibility of collecting identical plants (same genotype), I collected plants that were spaced widely from one another. Recent studies of genetic diversity in *Spartina alterniflora* have indicated that populations are highly diverse, and that there is little likelihood of repeatedly sampling the same clone with collections taken >1 m apart (Richards *et al.* in preparation). Individual plants were potted and kept under common-garden conditions and free of herbivory in a greenhouse at the University of Houston. For all three plant species, I used a mixture of 60% potting soil (Fafard's Professional Formula 52 Mix) and 40% sand. Individual plants of *S. sempervirens*, *D. spicata* and *S. alterniflora* were planted in 200, 400, and 1000 ml plastic pots, respectively. Plants were maintained in fresh water and saturated soil. This experiment was not designed to mimic the environmental conditions of any parental field population but rather to eliminate or minimize confounding environment effects. The individual ramets of *Spartina* and *Distichlis* collected in the field (here referred to as "generation 1") were allowed to

acclimate in the greenhouse for approximately 45 days before being used in feeding trials. Successive clonal generations of *D. spicata* and *S. alterniflora* were separated from the mother plant and repotted periodically as growth rates permitted (~ 2-3 times/growth season). I followed *D. spicata* over 4 generations and *S. alterniflora* over 5 generations (counting the plants collected from the field as generation 1).

The relative palatability of plants from northern and southern sites was assessed with appropriate herbivores. The herbivore species that were used in feeding trials depended on their availability in the field. I used adult individuals of the grasshoppers *Orchelimum fidicinum* Rehn and Hebard 1907 (Tettigoniidae), *Orphulella pelidna* Burmeister 1838 (Acrididae), *Paroxya clavuliger* Serville 1838 (Acrididae), and *Dicromorpha elegans* Morse 1896 (Acrididae) to assess the palatability of different plant species (Table 3). Whenever possible, two herbivores were used per plant species to ensure that results were general across herbivore species. All the consumers used are normally present in salt marshes and would naturally feed on the plant species used in the feeding assays. The animals used in the feeding trials were collected from salt marshes around Sapelo Island, Georgia, except the *O. pelidna* that were used for the feeding trials with the fourth clonal generation of *D. spicata* and the fifth clonal generation of *S. alterniflora*, which were collected in salt marshes near Surfside, Texas.

■ **Table 2**

Plant species studied and typical habitats in which each occurs.

Species	Common name	Habitat ¹
Asteraceae		
<i>Solidago sempervirens</i> L.	Seaside goldenrod	High marsh, terrestrial border
Poaceae		
<i>Distichlis spicata</i> (L.) Greene	Salt or spike grass	High marsh, high salinity areas
<i>Spartina alterniflora</i> Loisel	Smooth cordgrass	Low marsh dominant

1. For the purposes of this table, the marsh is divided into three elevation zones: low marsh, high marsh and terrestrial border. Taxonomy is based on Radford *et al.* (1964).

■ **Table 3**

Herbivores used as consumers and plant species evaluated in palatability assays by each herbivore.

Species	Plants tested	References ¹
Orthoptera		
<i>Dicromorpha elegans</i>	<i>Distichlis spicata</i>	Otte (1981)
<i>Orchelimum fidicinum</i>	<i>Spartina alterniflora</i> , <i>Distichlis spicata</i>	Smalley (1960)
<i>Orphulella pelidna</i>	<i>Spartina alterniflora</i> , <i>Distichlis spicata</i>	Blatchley (1920), Davis and Gray (1966), Otte (1981)
<i>Paroxya clavuliger</i>	<i>Solidago sempervirens</i>	Blatchley (1920), Helfer (1953), Davis and Gray (1966)

1. References point to selected papers on the taxonomy and/or ecology of each consumer.

Feeding trials

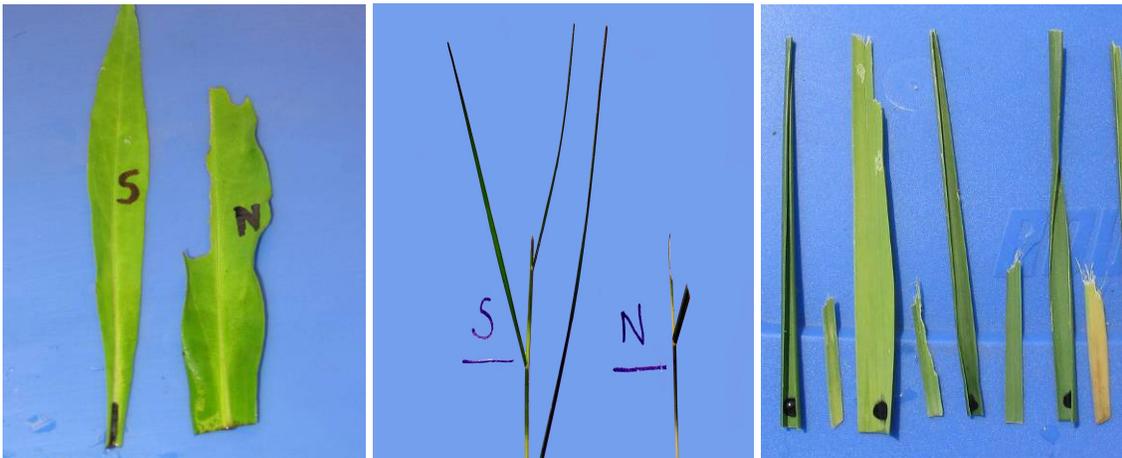
I measured relative plant palatability using paired-choice feeding assays. Feeding assays were conducted following Pennings *et al.* (2001). Consumers were collected in the field, brought to the laboratory, and experiments were set up approximately one hour after plant material was obtained. Grasshoppers were housed individually within 1000 ml glass jars that contained a vial of water (to keep leaves hydrated), and were offered a choice between an undamaged leaf from a northern plant and one from a conspecific southern plant (Figure 1). To reduce the effect of leaf age on herbivore preference, I collected leaves in standard positions (e.g., second fully expanded leaf). Northern and southern sites were randomly paired, and each feeding trial began with up to 23 replicates, depending on the availability of herbivores. As much as possible, I avoided using the same clone in more than one replicate, but in some cases a limited number of healthy clones forced me to do so (18 of 225 replicates). In these cases, leaves from the same clone in one geographic region were paired with different clones from the other geographic region, making each choice test a unique comparison between northern and southern clones. Individual replicates were checked twice a day and were terminated when substantial feeding (~30%) on at least one leaf had occurred or at the end of the third day (Figure 2). Typically, more than half of the replicates were terminated within a single day. Individual animals were used only once. Replicates in which both leaves were completely consumed or in which neither leaf was eaten after a period of 72 hours provided no information on latitudinal variation of plant palatability and were omitted (total of 9 replicates).

Repeating the feeding assays over time/generations allowed me to determine whether the differences in palatability persisted or disappeared when plants were kept under identical conditions. Feeding trials were conducted for all the clonal generations of *D. spicata* and *S. alterniflora* (four and five clonal generations, respectively) and for seed-germinated *S. sempervirens*. Feeding was measured as square millimeters of leaf area consumed. This estimate of consumption might have been biased if leaves from different geographic locations consistently differed in thickness or density (Pennings *et al.* 2001). Given that differences in amount consumed were often large and were consistent across all 3 species, I did not attempt to correct for differences in leaf mass per unit area. Consumption was compared between geographic regions with paired t-tests. To determine whether the strength of the preference (magnitude of the differences) shown by consumers changed within generations, I subtracted the amount consumed of southern plants from the amount consumed of northern plants (north – south) in each replicate. Differences in feeding preferences were nested within plant generations and compared using ANOVA.



■ Figure 1

Consumers housed individually with a choice of an undamaged leaf from a northern plant and one from a conspecific southern plant.



■ Figure 2

Southern and northern leaves of *Solidago sempervirens*, *Distichlis spicata* and *Spartina alterniflora* after being offered to herbivores in paired-choice feeding assays.

Field experiment

Northern and southern individuals of *S. sempervirens* were germinated from seeds and grown in the greenhouse under controlled conditions, as described above. Four healthy plants per site (eight northern and eight southern sites, for a total of 64 plants) were chosen and two undamaged leaves from each plant were measured and tagged. For easy identification and retrieval, plants were retained within pots. Potted plants were placed in the field at the Airport Marsh, on Sapelo Island, Georgia. Airport Marsh is a typical southern salt marsh, housing a variety of grasshopper species (including *Orchelimum fidicinum*, *Paroxya clavuliger*, and *Orphulella pelidna*) (personal observation). Plants were placed in the high marsh, a vegetation zone that *Solidago* would naturally occupy, adjacent to naturally-occurring *S. sempervirens*. A fence (10 cm mesh) was placed around the plants to exclude deer and any other large herbivores. The fence allowed easy access to invertebrate herbivores. Plants were collected after 15 days and the amount of accumulated damage on all tagged leaves was measured as square millimeters of leaf area. Values for individual leaves and plants were averaged to yield a single value per site. I examined variation in the amount of leaf tissue consumed between northern and southern source populations with a two-sample t-test, using sites as replicates.

This experiment allowed me to investigate latitudinal variation on plant palatability using a more realistic scenario, with a broad set of herbivores (a natural assemblage) and intact plants. Similar field assays were not conducted for *D. spicata* or *S. alterniflora* for logistical reasons.

Toughness and Nitrogen content measurements

Toughness, N content, and secondary metabolites are plant traits that play an important role in determining palatability (Siska *et al.* 2002). Although this was not the primary focus of my study, I quantified leaf toughness and nitrogen content of all clonal generations of *S. alterniflora* and *D. spicata*, and of seed-germinated *S. sempervirens* in order to help explain results of feeding assays. Because the secondary metabolites that might affect palatability of my study plants have not been unambiguously identified (Siska *et al.* 2002, but see Valiela *et al.* 1979 and Rietsma *et al.* 1988), I did not work with secondary metabolites. Identifying the compounds in each plant species that mediate palatability and quantifying biogeographic variation in these compounds would be a large task well beyond the scope of this thesis.

To compare the toughness of northern and southern plants, I measured leaf toughness using a penetrometer test, as described in Pennings *et al.* (1998). Briefly, penetrometer measurements determined the force necessary to drive a steel rod through the leaf tissue. For each test, four individual leaves per site were collected from each plant species and tested within 30 minutes. Values of individual leaves were averaged to yield a single value per species per site. I examined variation in latitudinal differences in toughness using a two-way ANOVA, with sites as replicates and latitude and generation as main effects. Toughness data for *S. sempervirens* (one generation only) were analyzed with a two-sample t-test, using sites as replicates.

I measured total N content of northern and southern plants as an indicator of nutritional quality. Plant material was freeze-dried, pulverized using an amalgamator and

sent to a laboratory for N content measurements. CHN analysis was performed in the laboratory of Dr. Samantha Joye, at the University of Georgia, using a ThermoFinnigan Flash Elemental Analyzer. Nitrogen content (percentage of dry mass) was arcsine (square-root) transformed and compared for each species using a two-way ANOVA, with sites as replicates (two leaves nested within a site) and latitude and generation as main effects. Nitrogen data for *S. sempervirens* were analyzed with a two-sample t-test (two plants per site and sites as units of replication). I also used a chlorophyll meter (OPTI-Sciences CCM-200) to obtain chlorophyll measurements. The chlorophyll meter provides a quick and nondestructive method for estimating leaf chlorophyll content (Watanabe *et al.*, 1980), and since chlorophyll content in a leaf is often closely correlated with leaf N concentration (Evans 1983, Blackmer and Schepers 1994), the measurement of chlorophyll provides an indirect assessment of leaf N status (Yang *et al.* 2003). Because the leaves of *S. alterniflora* and *D. spicata* were not broad enough to be read by the instrument, I only measured chlorophyll content on *S. sempervirens* leaves. I examined latitudinal variation in chlorophyll content using a two-sample t-test (two to six plants per site and sites as units of replication).

RESULTS

Feeding trials

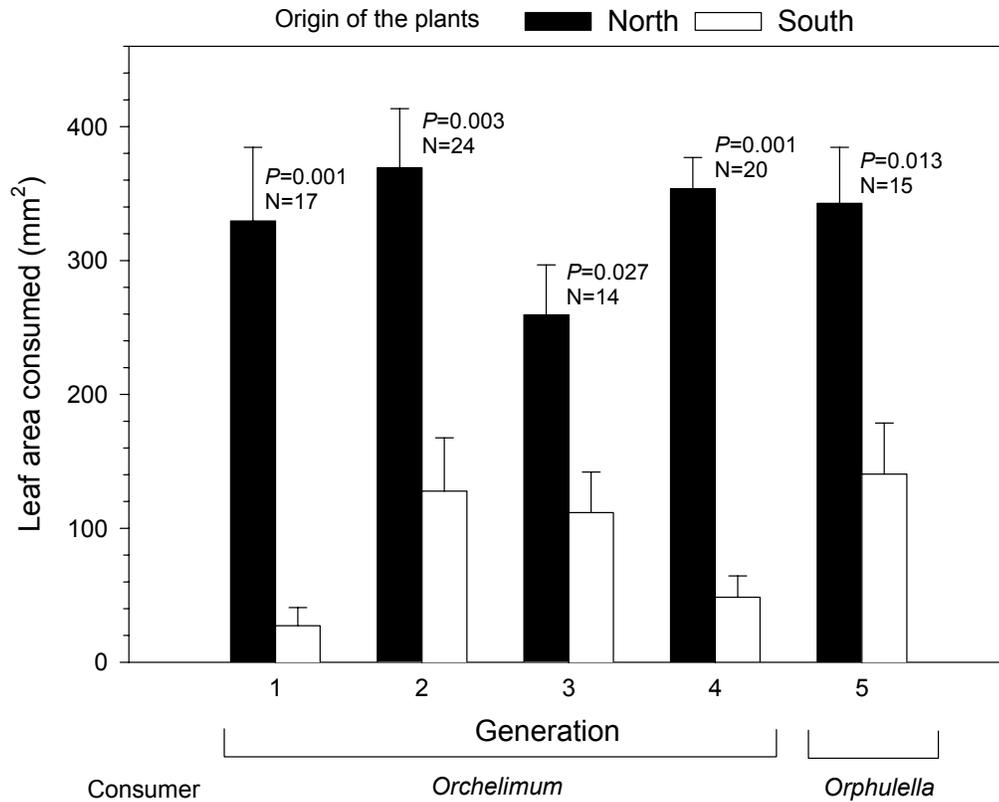
Overall, 13 of 13 trials resulted in a significant preference for high versus low-latitude plants. In most cases, preferences were very strong, with herbivores eating several times more of the northern plant than of the southern plant when given a choice between the two. This pattern held true across all plant species and clonal generations, independent of the herbivore used. Results for specific plants and herbivores are discussed in more detail below.

Spartina alterniflora. Herbivores (*Orchelimum fidicinum*) preferred high latitude plants over their low latitude conspecifics throughout the experiment. Results showed a significantly greater consumption of northern plants for the “wild plants” (generation 1), as well as for all the subsequent clonal generations produced in the common garden (Figure 3). The strength of the preference did not vary within generations ($P = 0.45$), as shown by the differences in amount consumed in each generation (Figure 4).

Distichlis spicata. Herbivores (*Orchelimum fidicinum* and *Dicromorpha elegans*) preferred high latitude plants over their low latitude conspecifics throughout the experiment (Figures 5, 6). The strength of preference did not change directionally over time (Figure 7, 8). However, the assay conducted with third clonal generation plants and *D. elegans* resulted in an unusually strong preference for northern plants in this particular clonal generation.

Solidago sempervirens. Herbivores (*Paroxya clavuliger*) showed a significant ($P = 0.002$) preference for plants originating from northern versus southern seeds (Figure 9). Although I did not conduct assays with “wild” *Solidago* collected directly from the field, previous work by Pennings *et al.* (2001) with field-collected plants showed strong preferences for northern over southern plants (significant in 11 of 17 assays). Similarly, when plants from the common-garden were exposed in the field to a natural herbivore assemblage, northern plants accumulated significantly ($P = 0.002$) greater herbivore damage than did their southern conspecifics (Figure 9).

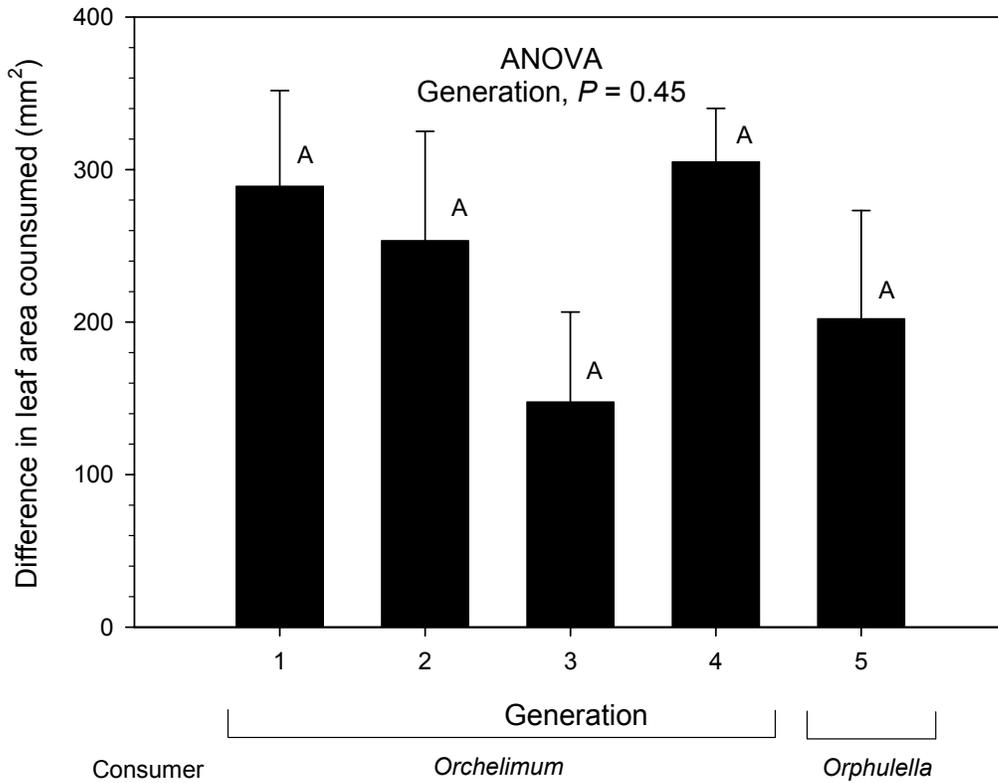
Spartina alterniflora



■ Figure 3

Spartina alterniflora. Consumption of northern vs. southern leaves in paired feeding trials. Experiments were conducted for 5 clonal generations. Bars represent means + 1 SE. Sample sizes and P-values are given above bars. Generation 1 refers to ramets collected in the field. Herbivores species used are indicated below paired bars.

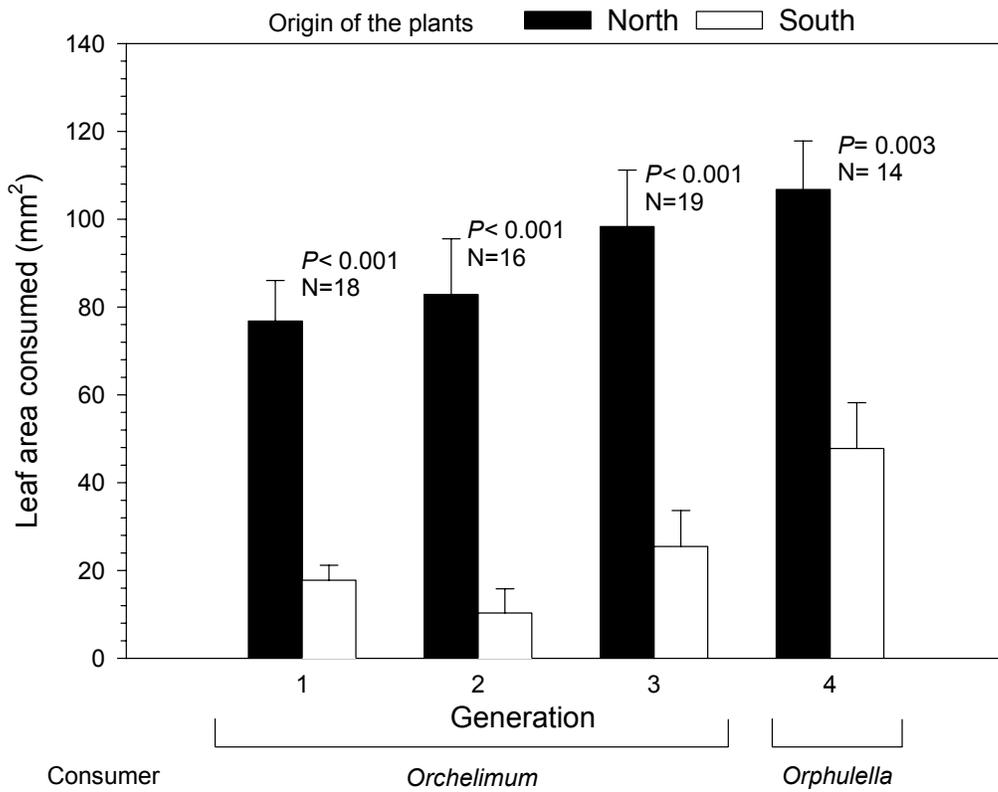
Spartina alterniflora



■ **Figure 4**

Spartina alterniflora. Difference in consumption of northern vs. southern leaves (amount consumed of northern leaves minus the amount consumed of southern leaves) as an estimate of strength of preference. Bars represent means + 1 SE. Generation means marked with the same letter do not differ significantly at $\alpha = 0.05$ (ANOVA with Tukey means test).

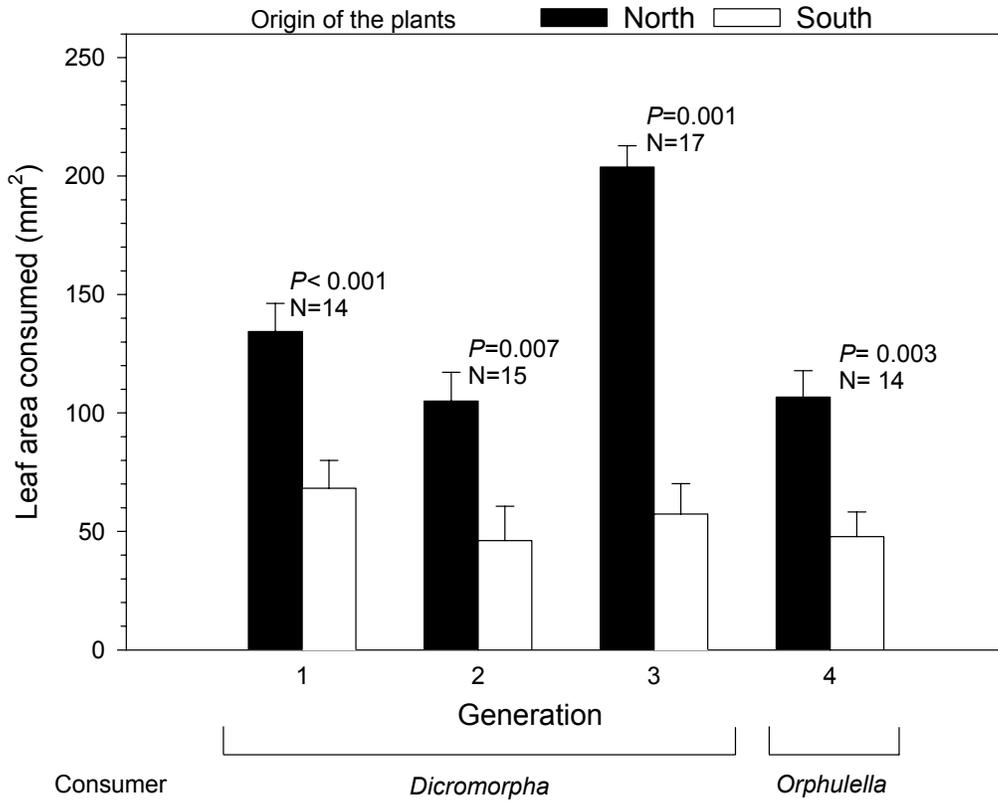
Distichlis spicata



■ **Figure 5**

Distichlis spicata. Consumption of northern vs. southern leaves in paired feeding trials. Experiments were conducted for several clonal generations of *D. spicata*. Bars represent means + 1 SE; sample sizes and P-values are given above bars. Generation 1 refers to ramets collected in the field. Herbivores species used are indicated below paired bars.

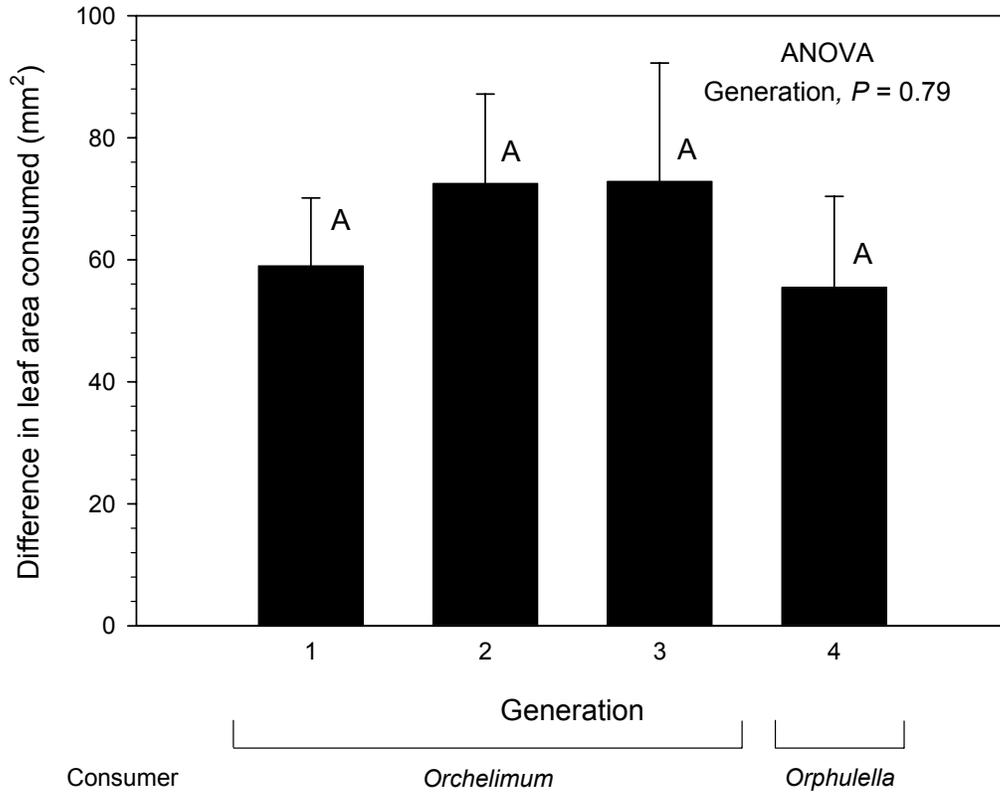
Distichlis spicata



■ **Figure 6**

Distichlis spicata. Consumption of northern vs. southern leaves in paired feeding trials. Experiments were conducted for several clonal generations of *D. spicata*. Bars represent means + 1 SE; sample sizes and P-values are given above bars. Generation 1 refers to individual ramets collected in the field. Herbivores species used are indicated below paired bars. Note that data for generation 4 are the same data shown in Figure 5 (repeated here for comparison purposes).

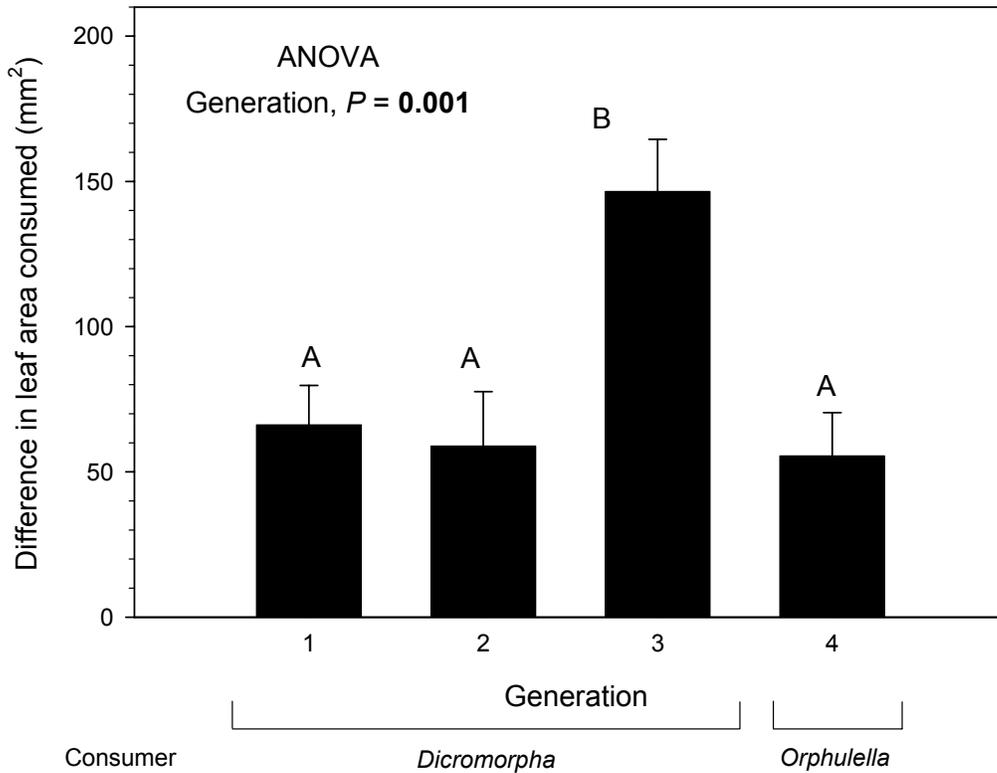
Distichlis spicata



■ **Figure 7**

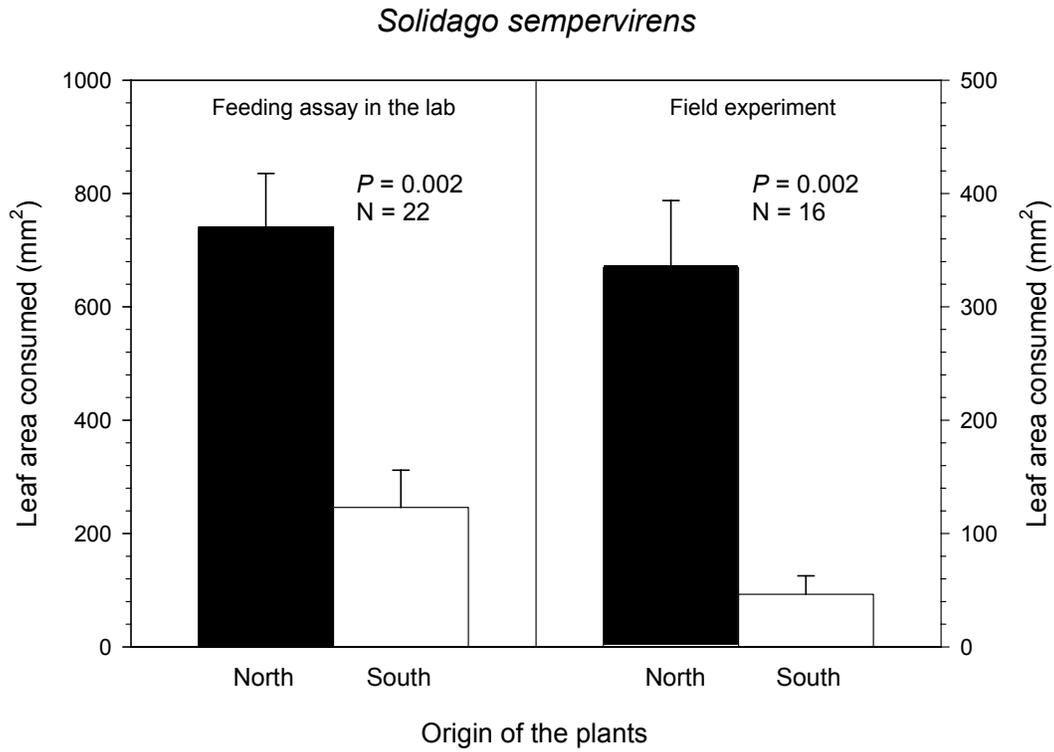
Distichlis spicata. Difference in consumption of northern vs. southern leaves (amount consumed of northern leaves minus the amount consumed of southern leaves) as an estimate of strength of preference. Bars represent means + 1 SE. Generation means marked with the same letter do not differ significantly at $\alpha = 0.05$ (ANOVA with Tukey means test).

Distichlis spicata



■ **Figure 8**

Distichlis spicata. Difference in consumption of northern vs. southern leaves (amount consumed of northern leaves minus the amount consumed of southern leaves) as an estimate of strength of preference. Bars represent means + 1 SE. Note that data for generation 4 are the same data shown in Figure 7 (repeated here for comparison purposes). Generation means marked with the same letter do not differ significantly at $\alpha = 0.05$ (ANOVA with Tukey means test).



■ **Figure 9**

Solidago sempervirens. Consumption of northern vs. southern leaves. *Left*: results of paired feeding trials using the grasshopper *Paroxya clavuliger*. *Right*: results of field experiment. Bars represent means + 1 SE; sample sizes and P-values are given above bars.

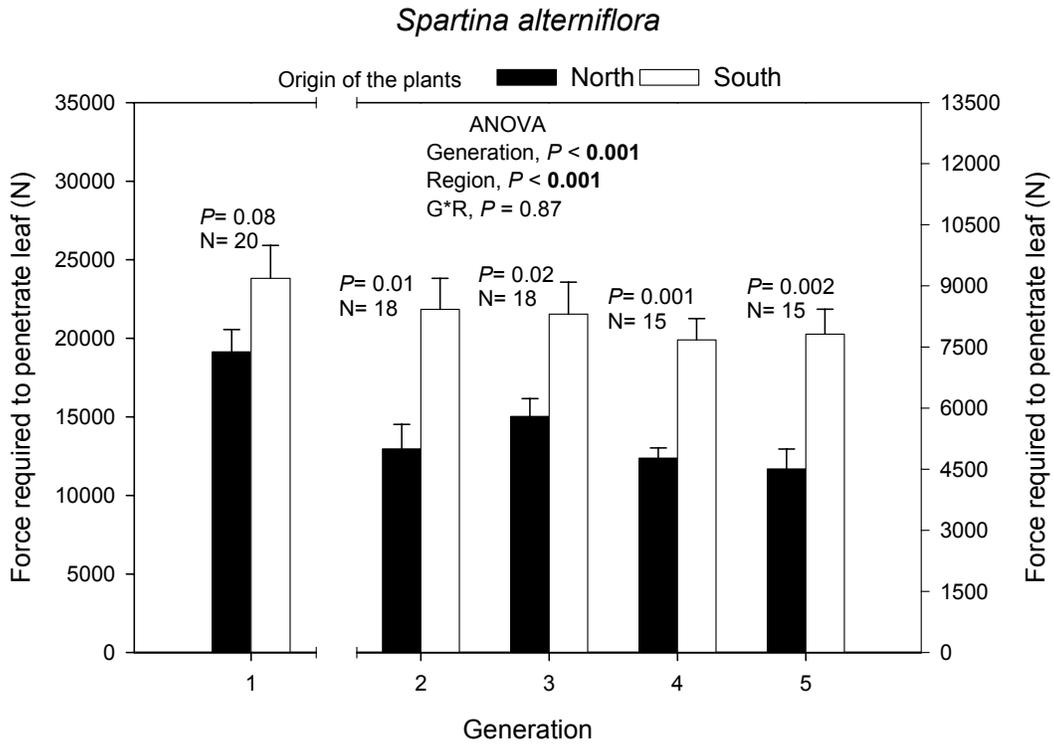
Toughness measurements

Overall, low latitude plants tended to be tougher than their high latitude conspecifics. In no case did the data suggest that northern plants were tougher than southern plants. Results for specific plants are discussed below.

Spartina alterniflora. Comparisons within individual generations indicated that southern plants were tougher than northern plants in all generations except the first, which was marginally ($P = 0.08$) significant (Figure 10). Across all generations a two-way ANOVA showed southern plants to be significantly ($P < 0.001$) tougher than northern plants (Figure 10).

Distichlis spicata. Individual two-sample t-tests for each generation showed no significant difference in toughness between northern and southern plants, although the 3rd and 4th generations showed strong trends towards greater toughness in southern plants (Figure 11). Across all generations, a two-way ANOVA (with sites as replicates and latitude and generation as main effects), indicated that southern plants were significantly ($P = 0.01$) tougher than northern conspecifics (Figure 11).

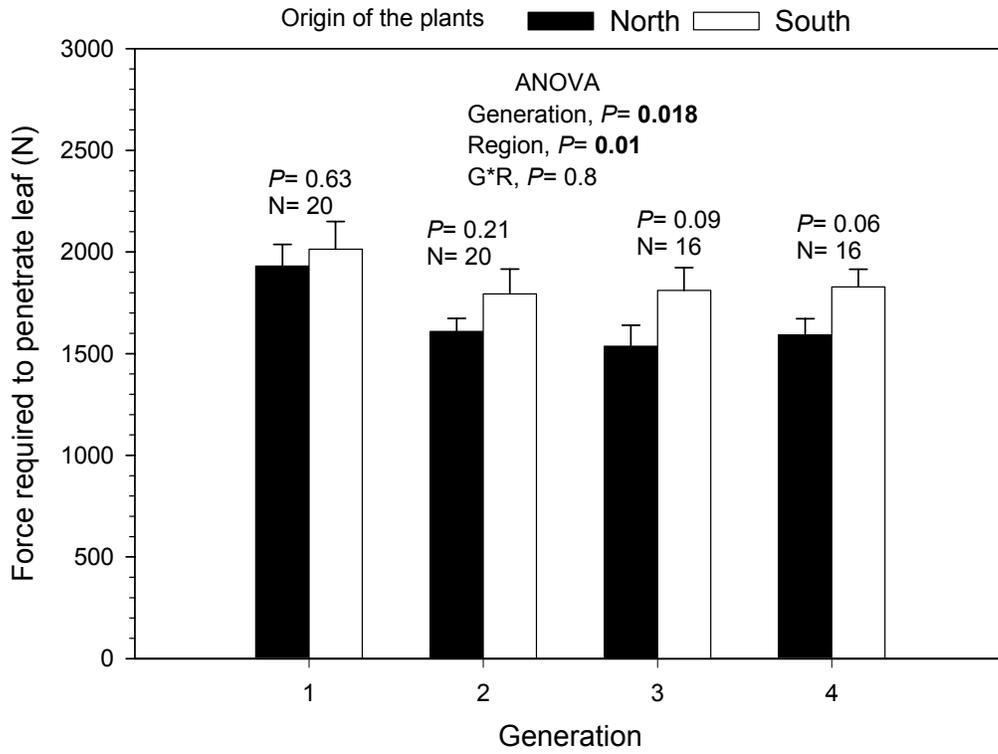
Solidago sempervirens. Although southern plants tended to be slightly tougher than northern conspecifics, this trend was not significant ($P = 0.3$) (Figure 12).



■ **Figure 10**

Spartina alterniflora. Toughness of northern and southern plants measured for 5 clonal generations. Bars represent means + 1 SE; sample sizes and P-values are given above bars. Toughness measurements for generation 1 plants were obtained with the use of a different instrument than the one used for the consecutive generations, and therefore are presented here with a different scale.

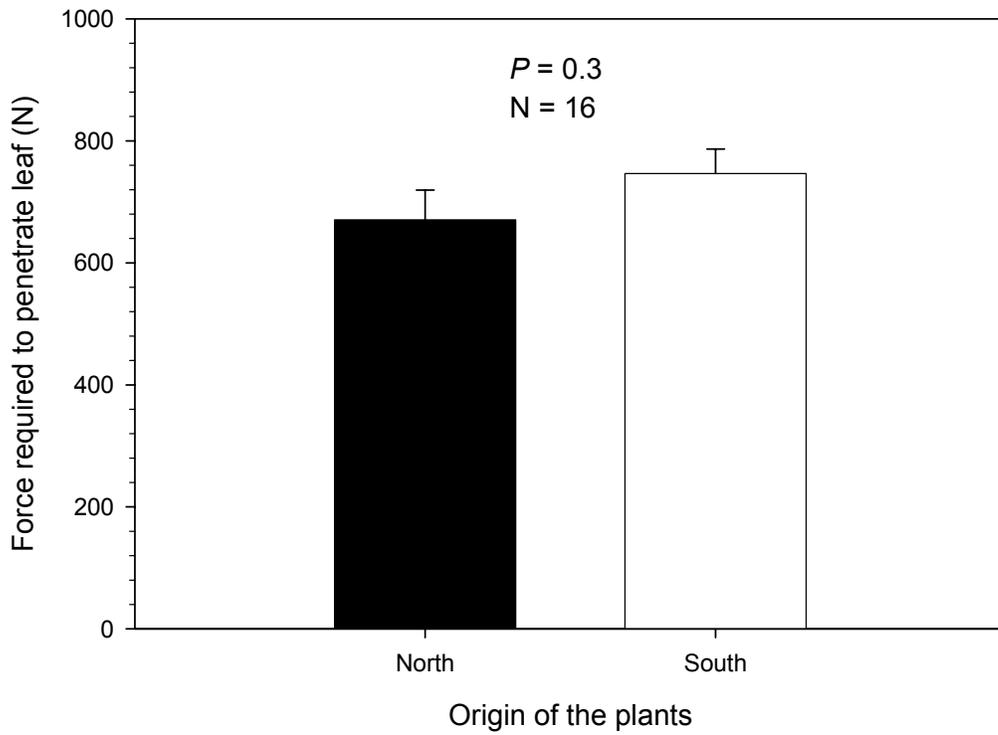
Distichlis spicata



■ Figure 11

Distichlis spicata. Toughness of northern and southern plants measured for several clonal generations. Bars represent means + 1 SE; sample sizes and P-values are given above bars.

Solidago sempervirens



■ **Figure 12**

Solidago sempervirens. Toughness of northern and southern plants. Bars represent means + 1 SE; sample size and P-value are given above bars.

Nitrogen content

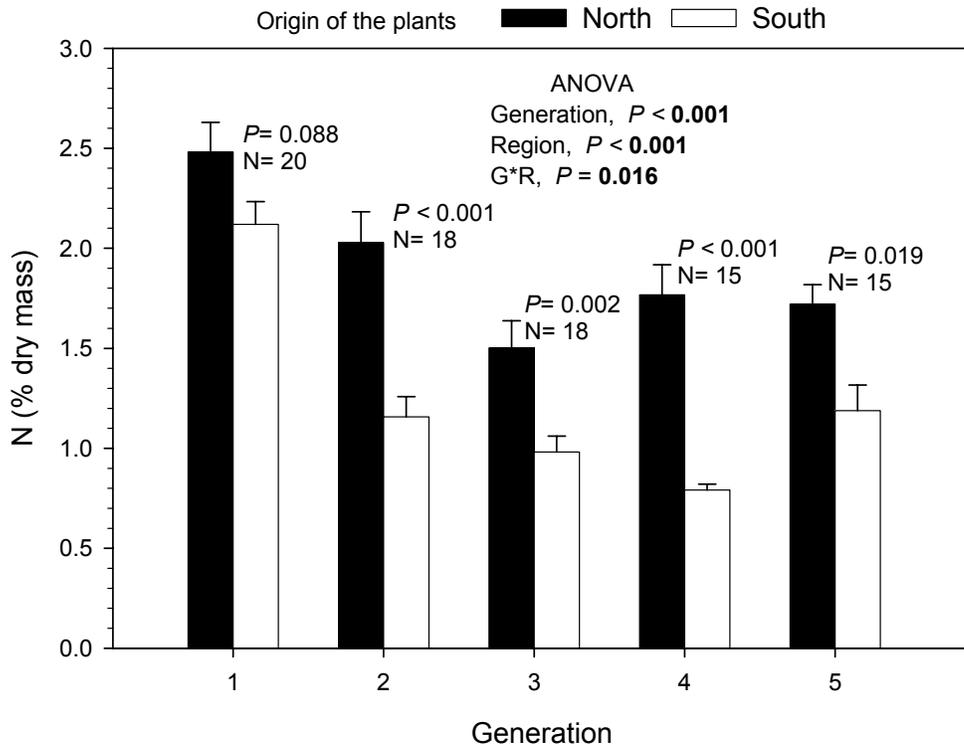
Patterns of variation in nitrogen content across latitude were mixed. Overall, 5 of 10 comparisons (50%) showed significantly higher N content in northern versus southern plants. No comparison found significantly higher N content in southern plants. Results for specific plants are discussed below.

Spartina alterniflora. Comparisons within individual generations indicated that northern plants had a higher nitrogen content than southern plants in all generations except the first, which was marginally ($P = 0.088$) significant (Figure 13). Across generations, northern plants had a significantly higher total N content than southern plants (ANOVA, $P < 0.001$) although a significant generation X region interaction reflected the weaker difference in the first generation (Figure 13).

Distichlis spicata. Nitrogen content of northern and southern plants did not differ significantly, either in any one generation or across the data set as a whole (Figure 14).

Solidago sempervirens. Northern plants had a significantly higher N and chlorophyll content than did southern conspecifics (Figure 15). Chlorophyll and nitrogen were significantly and positively related (Figure 16). Both nitrogen and chlorophyll content, but not leaf toughness, were significant predictors of damage to plants in the field experiment (Figure 17). A composite variable (PC1) of all three traits was a slightly better predictor of damage than was any single trait.

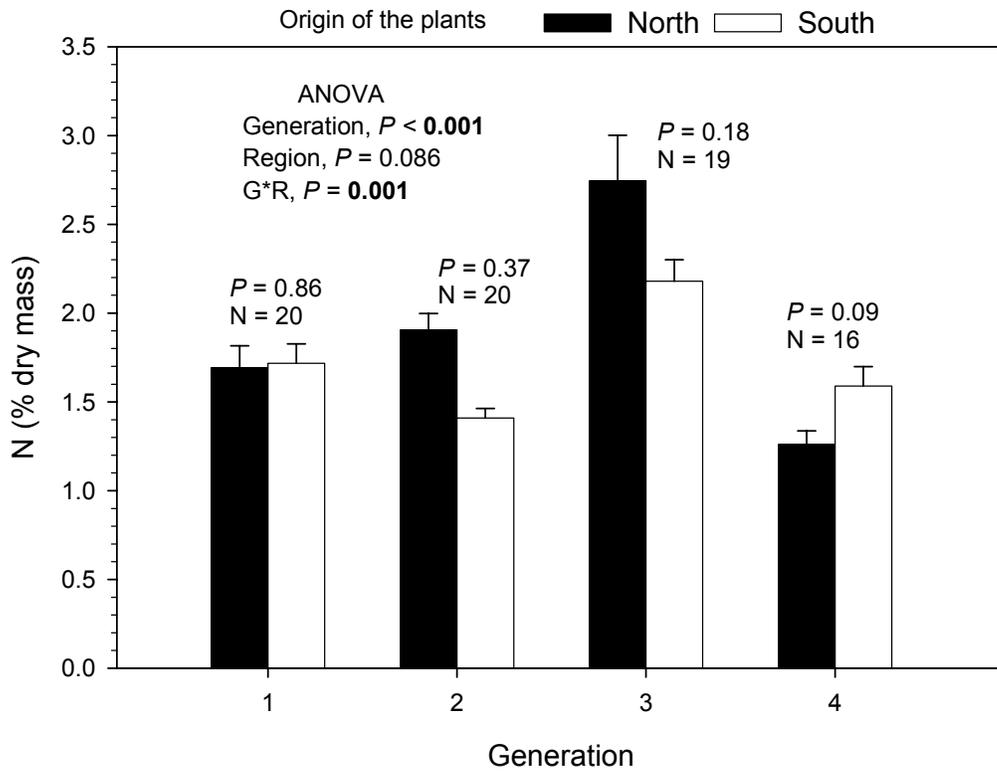
Spartina alterniflora



■ Figure 13

Spartina alterniflora. Nitrogen content of northern and southern plants measured for 5 clonal generations. Bars represent means + 1 SE; sample sizes and P-values are given above bars.

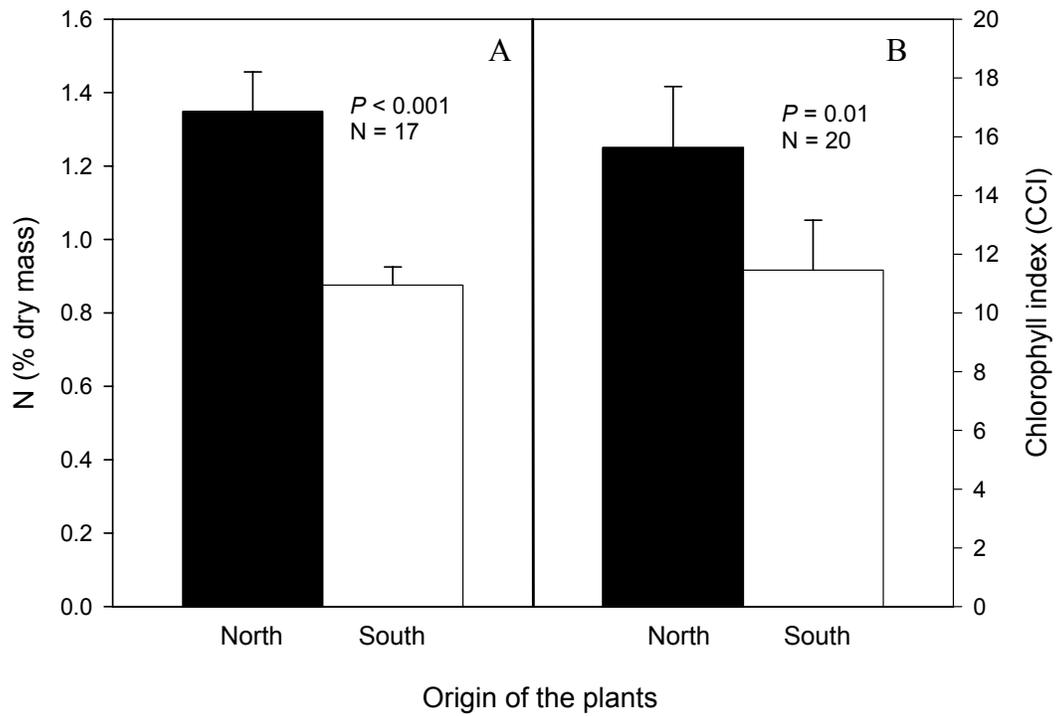
Distichlis spicata



■ **Figure 14**

Distichlis spicata. Nitrogen content of northern and southern plants measured for 4 clonal generations. Bars represent means + 1 SE; sample sizes and P-values are given above bars.

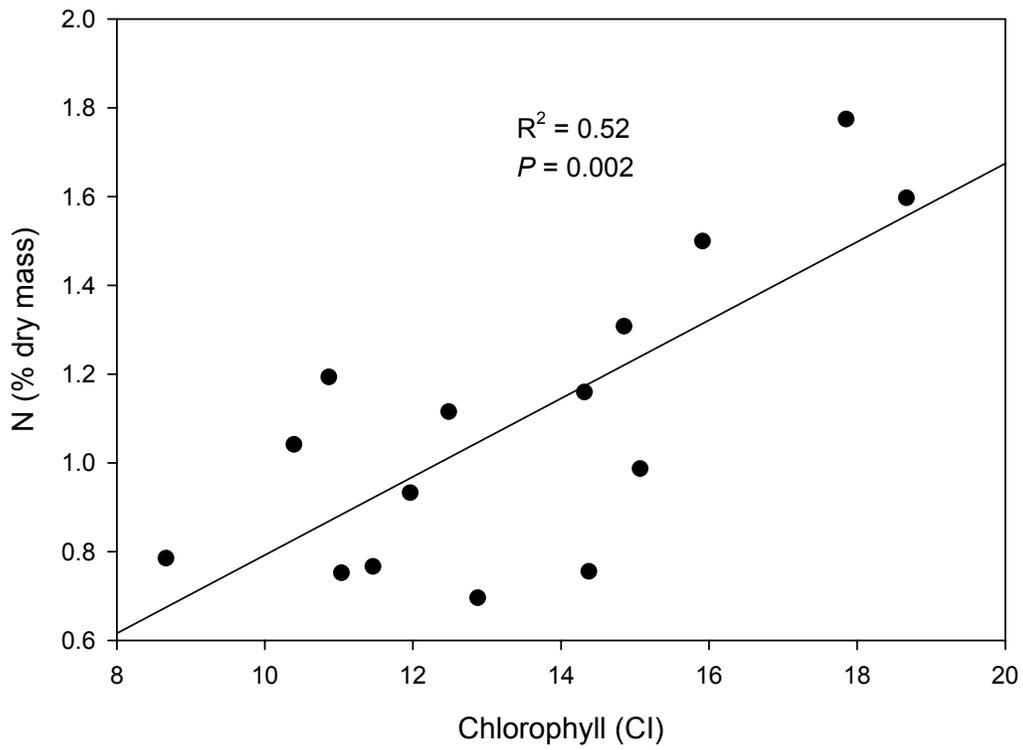
Solidago sempervirens



■ Figure 15

Solidago sempervirens. Nitrogen (A) and chlorophyll (B) content of northern and southern plants. Bars represent means + 1 SE; sample sizes and P-values are given above bars.

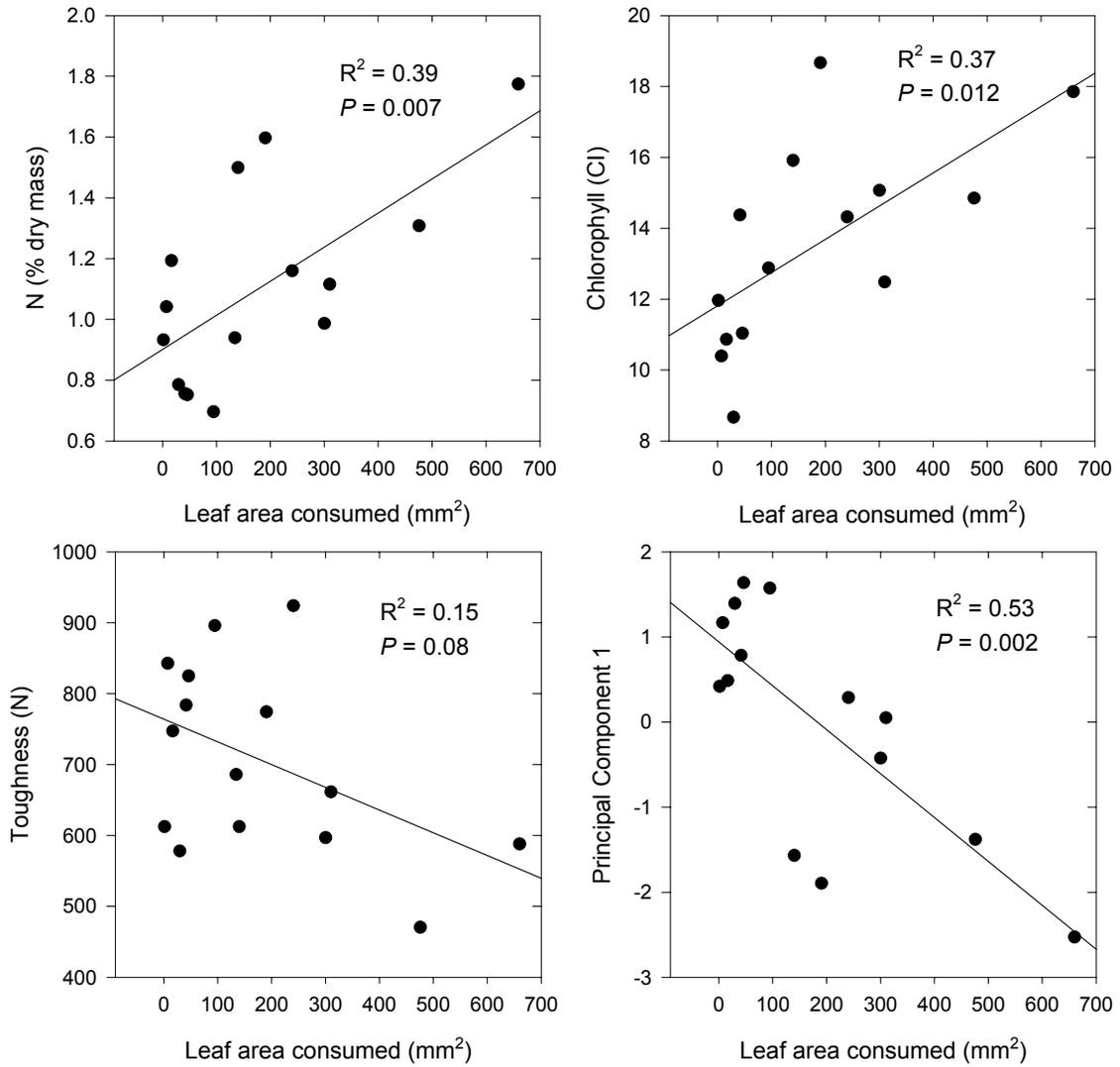
Solidago sempervirens



■ **Figure 16**

Solidago sempervirens. Leaf nitrogen content vs. leaf chlorophyll index. Points represent mean values for each site.

Solidago sempervirens



■ Figure 17

Solidago sempervirens. Leaf area consumed by herbivores during field experiment vs. leaf traits. Points represent mean values for each site. Principal component one was a composite of the other three traits.

DISCUSSION

Intraspecific phenotypic differentiation among geographically distinct populations is frequent (Mayr 1963, Endler 1977), but the ultimate determinants of this differentiation continue to puzzle biologists (Laugen *et al.* 2002). Along the Atlantic Coast of the United States, southern salt marsh plants are less palatable than northern conspecifics (Pennings *et al.* 2001). Different plant traits, including toughness, chemical defenses, and nitrogen content, have the potential to produce this pattern (Pennings and Paul 1992, Pennings *et al.* 1998, Siska *et al.* 2002). A variety of factors, including herbivore densities, climate, length of growing season, salinity, and anthropogenic eutrophication may differ between low and high-latitude salt marshes, and could play a role in mediating plant traits and palatability (Siska *et al.* 2002). Nevertheless, it is still unknown if the observed differences in palatability are due to differences in the environmental conditions experienced by those plants (e.g., climate, length of growing season, salinity, anthropogenic eutrophication), and to what extent they are genetically controlled. Here I have begun to identify the importance of constitutive versus induced mechanisms in producing geographic patterns in plant palatability, and also to obtain additional information on which plant traits might contribute to the observed latitudinal variation in palatability.

My results document a pattern for northeastern salt marsh plants to be more palatable than southeastern conspecifics after being germinated in a greenhouse (*S. sempervirens*) or kept under common-garden conditions for several clonal generations (during a period

of approximately 16 months) (*S. alterniflora* and *D. spicata*). Overall, 13 of 13 trials resulted in a significant preference for feeding on high versus low-latitude plants. In most cases, preference was very strong, with herbivores eating several times more of the northern plants than of the southern plant when given a choice between the two. This pattern held true across all plant species, generations, plant propagation methods, and herbivores tested. My results were more consistent than the tests with field plants in Pennings *et al.* (2001), which always included some non-significant trials. Results for seed-germinated *Solidago sempervirens* followed the same pattern in both laboratory and field experiments. For *Spartina alterniflora* and *Distichlis spicata* (when using *Orchelimum* as the herbivore), the direction and strength of the preference shown by consumers did not change over successive clonal generations. When using the grasshopper *Dicromorpha elegans* for feeding trials with *D. spicata*, the preference for northern plants shown in “generation 3” was even stronger than the previous generations. This is unlikely to reflect any important change in plant characteristics, but rather to reflect some variation in the assay process (e.g., animals might have been starved for a longer period of time before the assay, or replicates might not have been checked as frequently). The fact that this striking variation in palatability was retained for several generations when plants were grown under similar environmental conditions suggests that latitudinal differences in palatability of the three plant species tested are not driven by latitudinal variation in environmental conditions. In other words, latitudinal differences in palatability of *Distichlis spicata*, *Spartina alterniflora*, and *Solidago sempervirens* seem to be constitutive (always expressed) rather than induced by

latitudinal differences in environmental cues. One could argue that, because I only assessed the palatability of one generation of *S. sempervirens*, the respective results still reflect the conditions experienced by the parental generation. However, there is evidence that maternally induced resistance is a short lived response in progeny plants, relaxing in the face of low herbivore pressure (Agrawal 2002). Most ecologists would accept this type of result—phenotypic differences persisting without diminution in a common garden—as strong evidence for genotypic differentiation among populations. This assertion would have to be tempered with the caveat that it is at least conceivable that environmental effects could be highly persistent in a common garden, either through genes that are irreversibly switched on or off, or through maternal effects. Rigorously excluding these possibilities would require crossing experiments, which were not done here.

Nearly all of the models looking at constitutive vs. induced responses assume that the reliability of cues as predictors of future environments plays a major role in determining when and where induced defenses would be favored over constitutive ones. Constitutive strategies would be favored when the environment is relatively predictable, whereas induced strategies would be favored when the environment is unpredictable (Karban and Baldwin 1997). Vince *et al.* (1981) suggested that salt marsh plants are predictably abundant and do not escape herbivory in space or time, although herbivory pressure is generally most intense in the warm summer months. If that is the case, my results are in agreement with the notion that induced defenses are not favored over constitutive strategies when the environment (probability/intensity of attack) is predictable, and as the

risk of herbivory becomes more stochastic, induced resistance becomes relatively more favored compared to constitutive strategies (Clark and Harvell 1992, Åström and Lundberg 1994).

My results come with two potential caveats. First, all my feeding assays were conducted with consumers from only one geographic region (southern herbivores). It is possible that consumers might prefer or avoid foods based on novelty rather than on intrinsic palatability. This matter is probably more of a concern when comparing distantly related plants, which are likely to differ qualitatively, than when comparing congeners or conspecifics that are likely to differ quantitatively (e.g., in the concentration rather than the types of secondary metabolites, or in leaf toughness rather than in the presence or absence of mineral defenses). Although high- and low-latitude herbivores may differ in sensitivity to particular chemical defenses (Cronin *et al.* 1997), the results available to date suggest that they display similar rank preferences for high- vs. low-latitude plants (Bolser and Hay 1996, Pennings *et al.* 2001). Thus, although the use of herbivores from different locations could have altered some of the details of my results, the overall patterns and conclusions likely would not have changed.

Second, the plant propagation process and palatability assays were not replicated in both northern and southern greenhouses. Greenhouse experiments addressed the degree to which differences seen in the field would persist in a common environment. Nonetheless, it is possible that the results of my common garden experiments are themselves a function of day-length or other environmental cues. A different common garden, with different environmental cues, might have led to different patterns of

phenotypic expression in plants from one or both geographic regions. My greenhouse experiment was not a match to either native environment, and it would be very hard to assess the seriousness of this caveat without doing the reciprocal experiment. Nevertheless, in a previous study with fresh plants (Pennings *et al.* 2001), a suite of consumers exhibited the same patterns of preference for northern vs. southern salt marsh plants, regardless of the geographic location in which the experiments were conducted. Moreover, a reciprocal transplant experiment using *Spartina alterniflora* and *Solidago sempervirens* resulted in northern plants accumulating more herbivore damage in both geographic regions (S. C. Pennings, *unpublished data*). Thus, although it is possible that different environmental cues might have led to different patterns of phenotypic expression in plants from one or both geographic regions, there is some circumstantial evidence that the differences in palatability are maintained in both low- and high-latitude conditions.

Proximate and ultimate causes of latitudinal variation in plant palatability in Atlantic Coast salt marshes

The observation that the amount of nutrients (e.g., nitrogen) that a plant possesses affects the way it repels herbivores helps to explain possible physiological mechanisms of defense. This is a proximate explanation and addresses “how” questions such as “how do plant defenses work?” A complete understanding of the proximate mechanisms of plant-herbivore interactions is critical and can lead to a formulation of better ultimate explanations. The argument that plant defenses are adaptations to herbivory tries to offer

an ultimate explanation for the characteristics observed in nature. It tackles “why” questions such as, “why are differences in defense level maintained?” Yet, proximate explanations cannot substitute ultimate explanations, and the two should not be seen as opposing hypotheses (Karban and Baldwin 1997).

Given the evidence that latitudinal differences in plant palatability in coastal salt marshes appear to be constitutive, I can raise two further lines of inquiry. First, how do plant traits thought to be important in generating latitudinal differences in palatability vary under a common environment? Second, why are latitudinal differences in plant traits mediating palatability maintained?

A variety of plant traits including toughness, nitrogen and mineral content, and secondary metabolites may affect palatability to consumers (Pennings and Paul 1992, Pennings *et al.* 1998), and any of these might differ across latitude. Experiments using fresh and reconstituted plant material, as well as plant extracts (Siska *et al.* 2002), suggest that no single factor explains geographic variation in palatability for all salt marsh plants species, and multiple traits have the potential to contribute to the variation in palatability of some species. Because it is logistically difficult to measure every single trait that might matter to herbivores, I centered my attention on toughness and nitrogen content, both of which are relatively easy to measure. Leaf toughness and high fiber content provide structural support for the leaf, but have also been shown to be very efficient anti-herbivore defenses (Feeny 1970, Coley 1983, 1987, Raupp 1985). Nitrogen content is an important component of leaf nutritional quality (White 1978, 1993), and several studies report it to affect herbivore choices within a plant species (Vince *et al.* 1981, Denno *et al.*

1986, Bowdish and Stiling 1998, Gratton and Denno 2003), with N rich plants appearing to be more attractive to herbivores than N poor plants. Although this was not the primary focus of my study, I quantified leaf toughness and nitrogen content of all my three target plant species in order to determine whether variation in those traits might help explain latitudinal variation in feeding preferences of herbivores.

Latitudinal variation in plant traits depended on the plant species. With the exception of nitrogen content for *D. spicata*, my results were very similar to the trends found by Siska *et al.* (2002). Toughness varied as a function of latitude for *Spartina* and *Distichlis*, with southern plants being tougher than northern conspecifics. This result did not change across clonal generations. For all generations of *Spartina* and for seed-propagated *Solidago*, northern plants had higher nitrogen content than southern plants. Those outcomes correlate well with the idea that toughness is a good deterrent to herbivory and that high N content equals better food. Furthermore, the amount of damage that *Solidago* accumulated during the field experiment correlated with both N and chlorophyll content (strong positive correlation). Chlorophyll content correlated well with total nitrogen, and it is likely just a proxy for N content. Thus, it seems reasonable to argue that latitudinal differences in leaf toughness and nitrogen content are not strongly influenced by variation in environmental conditions, and have the potential to contribute to latitudinal differences in palatability of some plant species. My results suggest that different plant species have different mechanisms to reduce herbivory. For example, leaf toughness seems to be important for *S. alterniflora* and *D. spicata*, but not for *S. sempervirens*. Nitrogen content, on the other hand, appears to influence *S. sempervirens* and *S. alterniflora*, but

not *D. spicata*. I did not perform experiments in which I manipulated different traits to address consumer responses, and it is important to be aware that correlation does not equal causation. Many other factors, such as secondary metabolites, water content, silica, salt, or nutrients other than nitrogen, likely varied and could have contributed to the observed pattern in palatability.

The second question addresses ultimate causation: Why are latitudinal differences in plant traits mediating palatability maintained? It is tremendously difficult to determine the specific selective factors that shaped a trait (Endler 1986). It seems, then, unrealistic to assume that traits that defend against herbivory necessarily evolved to “accomplish this function”. They may or may not have been maintained in the recent past because they aid to execute this supposed function. A variety of traits that provide defense against consumers have multiple functions. For example, tannins, resins, and toughened leaves may contribute to drought tolerance and leaf durability; secondary chemicals that are a deterrent or an antibiotic to herbivores may provide benefits in reducing germination or growth to other plant competitors. At some level almost all precursors to existing traits evolved to serve tasks other than their current uses (Karban and Baldwin 1997). Viewing traits as adaptations molded exclusively by herbivory is naive, and arguing that herbivory had no role merely because the traits have multiple functions is equally naive. Because it is so difficult to determine the evolutionary origin of a defensive trait (Endler 1986), the best we can do is to understand something about the evolutionary factors that maintain it (Berenbaum *et al.* 1986).

The evidence presented here argues that the variation in palatability of high- and low-latitude salt marsh plants is maintained when the differences in the environment (such as water and light regime, nutrient availability, and temperature) are eliminated or minimized. This only says that the observed differences in palatability are constitutive, but does not tell us anything about what selected for them. A variety of factors including herbivore pressure, climate, shading, desiccation, and nutrient availability may affect plant traits that mediate palatability (Clausen *et al.* 1948, Coley *et al.* 1985, Bryant *et al.* 1989, Karban and Myers 1989, Cronin and Hay 1996), and any of these might cause variation across latitude (Pennings *et al.* 2001). Intraspecific genetic variation can cause huge differences in plant resistance to herbivores (Berenbaum *et al.* 1986, Simms and Rausher 1989, Agrawal *et al.* 1999). Plant populations show an extraordinary degree of spatial genetic differentiation that includes a diverse collection of traits, and occurs primarily as a result of different selection regimes (Linhart and Grant 1996).

My work has been presented in the perspective of standard biogeographic theories arguing that consumer-prey interactions are more intense at lower latitudes, and hence that prey defenses should be better developed at lower latitudes (Feeny 1976, Levin 1976, Coley *et al.* 1985). Herbivores can reduce seed production and other correlates of plant fitness, and this reduction can result in natural selection for either constitutively expressed or inducible defenses (Karban and Baldwin 1997, Agrawal 1998). Herbivores can have both direct and indirect impacts that negatively affect aspects of plant growth, reproduction, and status in the community. Herbivory can have strong and presumably selective effects on salt marsh plants. For example, Foster (1984), examining the impact

of the aphid *Sticobium sticis* on the sea lavender *Limonium vulgare* in salt marshes, noticed that most of the plants failed to produce seed due to aphid infestation. Experimental removals with insecticides showed that with the elimination of aphids, 100% of the inflorescences produced fruits, versus only 23% of the controls.

Comparative tropical-temperate observations in a number of systems support the idea of an increase in the intensity of predation (Cody 1966, Bakus 1974, Vermeij 1978, Jeanne 1979, Palmer 1979, Bertness *et al.* 1981) and herbivory (Doutt 1960, Baker 1970, Levin 1976) with a decrease in latitude. Some observational evidence (Pennings *et al.* 2001, S. C. Pennings, *unpublished data*) suggests that in Atlantic Coast salt marshes, a higher herbivore density as well as a greater amount of herbivore damage to plants can be found at low vs. high latitudes, supporting the hypothesis that herbivore pressure is stronger in low- than high-latitude salt marshes. Such evidence, of course, does not exclude other factors that might be selecting for geographic variation in plant palatability.

Variability in traits that affect plant defense may also be affected by other selective forces that are difficult to appreciate when considering only specific, limited defensive functions (Karban and Baldwin 1997). It is possible, for example, that the ultimate factors behind the observed latitudinal variation in palatability lie not in the intensity of a specific selective pressure, but rather in distinct selective pressures. It might be that high herbivore pressure shapes plant defenses in the south, whereas a different selective force (rather than just different intensity of a same pressure, in this case low herbivore pressure) could be of greater relative importance in the north. High-latitude marshes are target to great levels of natural disturbances by ice and “wrack” (rafts of floating dead

plant material), which can kill large areas of vegetation (Pennings and Bertness 2001). In some cases, ice damage is so severe that it limits the growth of the “low marsh” (vegetation adjacent to the creeks), which is continually in a state of recovery. The burial of plants by wrack represents a chronic disturbance in high-latitude marshes, with wrack mats that can frequently be 100-1000 m² in area and 10-30 cm thick, and can completely destroy underlying vegetation if they remain stationary for most of a growing season (Pennings and Bertness 2001). Such disturbances do not have great importance at low-latitude marshes. Plants could use different ways other than chemical and/or physical defenses to deal with external disturbances. van der Meijden and coworkers (1988) proposed that regrowth represents an inexpensive option to constant deployment of costly and unnecessary defenses. They argued that defense and regrowth are redundant strategies and proposed that plants should invest heavily in either regrowth or defense, but not both. It seems plausible to suggest that such a strategy would be beneficial when repeated disturbance occurs by an external cause against which plants cannot defend. Thus, ice and wrack disturbances could represent strong selective forces shaping plant strategies in high-latitude salt marshes towards a regrowth-based strategy, while different forces (e.g., herbivore pressure) might be molding the way in which low-latitude plants deal with herbivory (i.e., chemical/physical defense strategy), which could explain the differences in palatability observed. I have not tested the idea that northern marsh plants favor regrowth over defense, and therefore this hypothesis is highly speculative.

In sum, my results are consistent with the theory that herbivory is a strong selective pressure that might be shaping geographical variation in plant palatability. I cannot

discard, however, the possibility that many other things could be of crucial importance in mediating this latitudinal pattern of palatability. For example, differences in growth season length may be affecting plant palatability to herbivores (a short growing season at high-latitude marshes would favor rapid growth as opposed to long-lived, and hence tough, leaves). It would be possible to construct a list with many other hypothetical scenarios, but such a list would get increasingly speculative.

Understanding the genetic and environmental bases of intraspecific variation and how they covary on broad geographic scales is an important goal of evolutionary ecology, dating back to seminal work by Clausen *et al.* (1940). Such information can provide important clues to how organisms adapt to different and changing environments. My results suggest that latitudinal variation in the palatability of Atlantic Coast salt marsh plants is constitutive, and thus likely under genetic control, and that the traits responsible for the differences in palatability are not strongly influenced by variation in environmental conditions. Since phenotypic plasticity is itself under genetic control, high genetic variation and high plasticity need not to be mutually exclusive (Schlichting and Levin 1986), and hence it is likely that the studied plants possess many other traits possibly involved in dealing with herbivory that are highly plastic. My experiments addressed variation in palatability under common environmental conditions; by no means had they tested for the presence or absence of induced defenses. Further studies involving the use of common garden plants grown under different environmental conditions (high- and low-latitude greenhouses) combined with studies of population genetics would be

beneficial to our understanding of how natural selection is working upon the palatability of these populations of salt marsh plants.

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