# Partitioning adaptive differentiation across a patchy landscape: shade avoidance traits in *Impatiens capensis*

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## Abstract:

Adaptation to different habitat types across a patchy landscape may either arise independently in each patch or occur due to repeated colonization of each patch by the same specialized genotype. We tested whether open- and closed-canopy forms of *Impatiens capensis*, an herbaceous annual plant of eastern North America, have evolved repeatedly by comparing hierarchical measures of $F_{ST}$ estimated from AFLPs to morphological differentiation measured by $Q_{ST}$ for five pairs of populations found in open and closed habitats in five New England regions. Morphological differentiation between habitats ($Q_{HT}$) in elongation traits was greater than marker divergence ($F_{HT}$), suggesting adaptive differentiation. Genotypes from open- and closed-canopy habitats differed in shade avoidance traits in several population pairs, whereas patterns of AFLP differentiation suggest this differentiation does not have a single origin. These results suggest that open- and closed-canopy habitats present different selective pressures, but that the outcome of diversifying selection may differ depending on specific closed- and open-canopy habitats and on starting genetic variation. Hierarchical partitioning of $F_{ST}$ and $Q_{ST}$ makes it possible to distinguish global stabilizing selection on traits across a landscape from diversifying selection between habitat types within regions.

**Keywords:** $F_{ST}$-$Q_{ST}$ | parallel evolution | phenotypic plasticity | population differentiation | shade avoidance

## Article:

Evolutionary diversification is the outcome of three interacting processes occurring within and among populations: selection, gene flow, and genetic drift. Classical population genetic theory

predicts that even small amounts of gene flow between populations can effectively homogenize gene pools in the absence of very strong selection (Wright 1969; Crow and Kimura 1970). In this context, gene flow inhibits local adaptation in response to heterogeneous environments (Lenormand 2002), although local adaptation can occur in the face of gene flow (e.g., Kruckeberg 1951; Antonovics 1968; Linhart and Grant 1996; Stanton et al. 1997; Sambatti and Rice 2006). To understand the interplay of local selection and gene flow it is necessary to examine adaptation over habitat mosaics in which it is possible for variation in gene flow, selection, and drift to produce varying evolutionary outcomes (Coyne and Orr 2004). However, most reciprocal transplant and common garden experiments have used only a small number of populations to test for local adaptation (reviewed in Schluter 2000).

Even in the few species in which local adaptation has been rigorously demonstrated at a landscape scale, it is usually not known whether local adaptation is achieved by a single specialist "ecotype" colonizing all the available patches of available habitat, by locally adapted forms arising independently in situ, or some combination of both (but see Nyberg-Berglund et al. 2004). Distinguishing these possibilities requires a careful examination of heritable quantitative variation, of gene flow, and of the forces that may limit local adaptation. A number of forces and constraints can limit local adaptation in the face of divergent natural selection, including gene flow, lack of genetic variation, genetic drift, counteracting temporal fluctuations in local environments, and genetic correlations between traits (Van Tienderen 1991;Kirkpatrick and Barton 1997; Stanton and Galen 1997; Sultan and Spencer 2002).

Gene flow, potentially the most important constraint on local adaptation, can be estimated by using neutral molecular markers to calculate$F_{ST}$, an index of how variation is portioned within and between populations, and related statistics (Wright 1951). If quantitative traits have a similar genetic basis as molecular markers (Lande 1992; Whitlock 1999), variation in quantitative traits can be partitioned between populations and lines using $Q_{ST}$, an analog to $F_{ST}$ for quantitative traits (Spitze 1993). When $Q_{ST}$ exceeds the range of $F_{ST}$ values derived from neutral or nearly neutral markers, then morphological variation is greater than random genetic variation and differentiating selection has therefore acted on the traits (reviewed by Merila and Crnokrak 2001; McKay and Latta 2002). If $Q_{ST}$ is less than $F_{ST}$, then morphological variation is low compared to neutral genetic variation, implying homogenizing selection across populations or diversifying selection within them (Merila and Crnokrak 2001; McKay and Latta 2002). If $Q_{ST}$ and $F_{ST}$ are equal, genetic drift cannot be rejected as the cause of population differentiation. Studies with mice of known artificial selection regimes have shown that $F_{ST}$-$Q_{ST}$ comparisons can successfully distinguish artificially imposed directional selection on morphological traits from neutral marker differentiation by comparing confidence intervals around $Q_{ST}$ and $F_{ST}$ estimates (Morgan et al. 2005). This method has been useful for identifying the signature of differentiating selection in natural populations in a range of organisms (e.g., Cano et al. 2004; Le Corre 2005; Gravuer et al. 2005;Stenoien et al. 2005; Volis et al. 2005; Waldmann et al. 2005).

Stabilizing selection will result in similar trait values in all populations, and may occur due to similar selection between populations or strong constraints on a trait, such as selection and constraints on seed size in wind-dispersed plants (Gravuer et al. 2003, 2005). However, diversifying selection on a trait due to habitat heterogeneity across a patchy landscape (e.g., between adjacent open and closed habitats) will also result in low $Q_{ST}$ relative to $F_{ST}$ at a regional or population level, because variation in the trait within populations or regions will be relatively high relative to variation among populations or regions. Comparison of $F_{ST}$ and $Q_{ST}$ at the population level cannot distinguish local diversifying selection from stabilizing selection across populations. One way to distinguish these possibilities is to further parse $F_{ST}$ and $Q_{ST}$ hierarchically into regional and subpopulation measures. With stabilizing selection we expect little differentiation between local subpopulations, whereas with diversifying selection due to variable habitats we would expect substantial differentiation between subpopulations from different patch types.

Differences between patch types in the within-patch heterogeneity of selective environments may result in adaptive differentiation between patches in plasticity to those selective environments. However, in some cases genetic constraints may prevent rapid population differentiation in plasticity and constitutive differences may instead evolve. If so, similar selective pressures may result in different adaptive outcomes depending upon the genetic variation present in the ancestral populations. Constitutive differences may also evolve if the selective environment within patches is relatively uniform or the frequency of one environment is very high (Van Tienderen 1991; de Jong 1995, 1999; Sultan and Spencer 2002). It is therefore of interest to test whether adaptation to similar patch types has the same evolutionary outcome in different populations across the landscape.

Phytochrome-mediated “shade avoidance” responses are an ideal system for investigating adaptive differentiation of plastic traits across a landscape. Numerous studies on phylogenetically diverse plant species suggest that differentiation between species pairs or populations of herbaceous plants frequently occurs between forest understories and open-canopy habitats (e.g., Morgan and Smith 1979;Dudley and Schmitt 1995; Gilbert et al. 1995; Linhart and Grant 1996; Van Hinsberg 1997; Van Hinsberg and Van Tienderen 1997;Donohue et al. 2000a,b; Gilbert et al. 2001; Weinig 2000a,b; Schmitt et al. 2003). Differentiation between open- and closed-canopy lineages can be manifested either as differences in phenotypically plastic responses to shading, such as stem elongation, or as constitutive differences in traits related to alternate light environments, such as height or branching. Evidence consistent with differences in plastic elongation responses between populations or species has been found in several plant lineages (e.g., Morgan and Smith 1979;Dudley and Schmitt 1995; Gilbert et al. 1995; Linhart and Grant 1996; Van Hinsberg 1997; Van Hinsberg and Van Tienderen 1997;Donohue et al. 2000a,b; Weinig 2000a,b; Gilbert et al. 2001; Schmitt et al. 2003).

Theory predicts that herbaceous plants in open-canopy habitats can benefit from plastic elongation more than plants from forest understories (Morgan and Smith 1979; Smith

1982; Schmitt and Wulff 1993; Schmitt et al. 1995). In open habitats, elongating only in response to shading from other plants can allow shaded plants to grow into higher light areas (Ballaré, et al. 1987, 1990; Novoplansky 1990), but avoid the costs of elongating in the absence of competitors for light (Schmitt et al. 1995; Dudley and Schmitt 1995). Herbaceous plants in forest understories should benefit less from elongating in response to foliage shade than plants from open-canopy habitats because they cannot elongate over the forest canopy above them. However, if plants in open habitats predictably experience high levels of competition, it is also possible that in some cases constitutively elongated "shade avoidance" phenotypes may evolve.

The basis of phenotypically plastic responses to foliage shade is perception of a light cue indicative of shade and a subsequent physiological response. A primary light cue for shading is reduced ratios of red (680 nm) to far red (720 nm) light (or R:FR) (Smith 1982). This ratio is a good indication of shading by other plants because absorption of red light by the canopy causes a large disparity between R:FR in full sunlight, approximately 1.2:1, as compared to under a plant canopy where it can be as low as 1:18. Plants can even perceive future shade before full shading develops because of the refraction of light from plants of a similar height (Ballaré et al. 1987, 1990;Novoplansky et al. 1990). R:FR ratios can be perceived by plants through phytochrome photoreceptors, key controllers of a number of plant development processes including stem elongation. Either plastic or constitutive differentiation may occur in a population with changes to any of a range of processes downstream of perception of light ratios. Because the targets of phytochrome activity may include nearly 80% of the transcriptome in *A. thaliana* (Tepperman et al. 2001), such a wide variety of mechanisms are possible that different mechanisms may be selected in different lineages. However, in any lineage, distinguishing population differentiation in plasticity from constitutive differences requires experimental approaches that quantify plasticity, such as artificial manipulation of wavelength ratios with filters.

## PREDICTIONS

To distinguish independent evolution of traits from repeated colonization of different patches by a single specialist genotype, and to test for diversifying selection on plastic traits between habitat types across a patchy landscape, we used *Impatiens capensis*, a widespread herbaceous annual in eastern and central North America with documented occurrences of "sun" and "shade" populations (e.g., Schmitt 1993; Dudley and Schmitt 1995; Donohue and Schmitt 1999; Donohue et al. 2000a,b, 2001; von Wettberg and Schmitt 2005). Greenhouse common garden studies have shown heritable differences in sensitivity to low R:FR (Dudley and Schmitt 1995) between two closed-canopy stands and an open-canopy stand from Rhode Island, USA. Reciprocal transplant experiments have shown that these differences give each form an advantage in the habitat from which they came, suggesting an adaptive significance to this differentiation (Donohue et al. 2000a,b, 2001; Schmitt et al. 2003).

To examine the pattern of differentiation across a landscape of open- and closed-canopy populations, we examined morphological and molecular genetic variation within and among

neighboring pairs of open-canopy “sun” and closed-canopy “shade” populations of *I. capensis* in five regions in southern New England. Open-canopy and closed-canopy populations inferred to be about 20–80 years of age were found within a kilometer of each other on in five different areas, or regions, of Rhode Island and Massachusetts. By comparing morphological differentiation between populations with differentiation in neutral molecular markers, we were able to test whether differentiation in shade avoidance traits exceeded differentiation expected by gene flow between populations as predicted by local adaptation. By growing plants from these populations under low and equal R:FR conditions, we tested whether population differentiation in shade avoidance traits resulted from differences in phenotypic plasticity or constitutive expression of those traits.

We test four alternative hypotheses of patterns of differentiation between populations: **H1:** Differentiation is primarily due to drift and isolation by distance. This hypothesis will be supported if the degree of differentiation increases similarly with geographic distance in both markers and morphological traits. **H2:** Local adaptation to regional environments has occurred, irrespective of canopy cover. This hypothesis will be supported if morphological differentiation among pairs of populations from different regions exceeds neutral marker differentiation among regions. **H3:** Local adaptation to canopy cover occurred once in *Impatiens* in southeast New England, such that all open- or closed-canopy sites are populated by the same ancestral ecotypes from previously existing open- or closed-canopy sites. This hypothesis will be supported if there is morphological differentiation between open- and closed-canopy populations and all open-canopy forms are less differentiated from one another in molecular markers than adjacent closed-canopy forms. **H4:** Local adaptation to canopy cover has occurred independently and repeatedly, such that populations in one habitat type can develop quickly from populations of the other. This hypothesis will be supported if there is morphological differentiation between open- and closed-canopy populations, if adjacent open- and closed-canopy populations are less differentiated from one another in molecular markers than in morphological traits, and if molecular differentiation among pairs of open- and closed-canopy populations from different regions is greater than molecular differentiation within pairs.

## Methods

### STUDY SPECIES

*I. capensis* Meerb. (Balsaminaceae) is an annual, diploid, self-compatible herb of North American deciduous forests and wetlands (Gleason and Cronquist 1963; Leck 1979, 1996). *I. capensis* has a mixed mating system, commonly producing self-fertilized cleistogamous flowers as well as outcrossed chasmogamous flowers on the same plants (Waller 1979), allowing the production and maintenance of inbred lines. Self-fertilized seeds are the only progeny produced in many populations, and are regularly a large proportion of all seeds produced in all populations. Inbreeding depression is generally weak in *Impatiens* (Schemske 1978; Waller 1979; Schmitt and Ehrhardt 1987) and strongest under stressful drought conditions (Heschel et

al. 2005). *I. capensis* occurs across a range of canopy habitats, and differentiated open- and closed-canopy forms have been observed (e.g., Schmitt 1993; Dudley and Schmitt 1995; Donohue and Schmitt 1999; Donohue et al. 2000a,b; Donohue et al. 2001). Seeds disperse ballistically, traveling up to two meters (Schmitt et al. 1987), but can be dispersed secondarily by rodents or by flowing water (Parker and Leck 1985). In New England, where the current study was based, most forests were cleared with European settlement. Most contemporary forests, where closed-canopy *Impatiens*populations would be found, arose relatively recently after agricultural abandonment in the 19th and 20th century (Foster 1992).

## GENOTYPE COLLECTION AND HABITAT CHARACTERIZATION

In the spring of 2003 we collected seedlings from five pairs of populations in southern New England (Fig. 1, Table 1). Collections were made from conservation lands in the area to ensure that sites could be revisited and would remain protected, and to facilitate the gathering of historical data. At each site both a sun and a shade stand approximately 0.5–1 km separate were selected in mid-April of 2003 based on the presence of surrounding trees. Canopy cover was verified during the summer by taking hemispheric overhead canopy images with a Nikon Coolpix 950 Digital camera (Nikon Cameras, Tokyo, Japan) to which a Nikon FC-E* Fisheye Converter was attached. Images were taken in late afternoon or early morning to avoid interference by direct sunlight. Canopy cover was quantified as the percent of total light transmitted through the canopy (calculated as the sum of direct and indirect light) using Gap Light Analyzer 2.0 (Frazer et al. 1999). A few shade sites were chosen to overlap with an unpublished long-term *Impatiens* density and soil moisture level dataset collected by M. S. Heschel. Sun and shade populations were collected at Caratunk Audubon Preserve (CP), Lincoln Woods State Park (LW), Norman Bird Sanctuary (NB), Touissett Marsh Audubon Sanctuary (TM), and Weetomo Woods Tiverton town park (WW). Historical aerial photographs available from the RIGIS consortium and UMASS Amherst GIS library were scored on a 1–3 scale of forest cover to determine the length of time a site has had an open or closed canopy.

**Figure 1.** Location of sites from which genotypes were collected in Southeastern New England. Each point represents both open- and closed-canopy populations, which are too geographically proximate to chart separately.

**Table 1.** Characteristics of populations. Current canopy cover was calculated from hemispheric photographs. Historical canopy cover was scored from publicly available aerial photographs.

| **Region** | **Canopy type** | **Latitude** | **Longitude** | **Current canopy openness** | **Historical canopy cover** |
|---|---|---|---|---|---|
| Caratunk Preserve (CP) | Open canopy | 41.87 | −71.314 | 83.6%±14.3 | Open since 1952 |
| Caratunk Preserve (CP) | Closed canopy | 41.875 | −71.318 | 12.5%±6.6 | Open in 1952, closed after 1972 |
| Lincoln Woods S.P. (LW) | Open canopy | 41.886 | −71.436 | 38.5%±13.9 | Partially open since 1939 |
| Lincoln Woods S.P. (LW) | Closed canopy | 41.886 | −71.437 | 6.1%±4.4 | Intermediate in 1939, closed after 1972 |
| Norman Bird Sanctuary (NB) | Open canopy | 41.502 | −71.253 | 73.3%±25.3 | Open since 1939 |
| Norman Bird Sanctuary (NB) | Closed canopy | 41.503 | −71.254 | 17.2%±6.8 | Partially closed in 1939, fully closed after 1952 |
| Touisset Marsh (TM) | Open canopy | 41.708 | −71.237 | 58.5%±40.5 | Open since 1939 |
| Touisset Marsh (TM) | Closed canopy | 41.705 | −71.235 | 0.8%±32.3 | Open in 1939, partially closed by 1962, fully closed by 1981 |
| Weetamo Woods (WW) | Open canopy | 41.575 | −71.177 | 81.3%±29.6 | Open since 1939 |
| Weetamo | Closed | 41.574 | −71.179 | 6.1%±3.2 | Closed canopy since |

| | | | | | |
|---|---|---|---|---|---|
| Woods (WW) | canopy | | | | 1939 |

At each population a roughly 20 m square permanent grid was established to determine the location of seedlings that would be collected to found inbred lines. Within the permanent grids at approximately 2-m intervals, 48 seedlings were collected at known x,y coordinates in each site. Distances between collection points varied from 2 to 4 m due to differences in population density. Seedlings were returned to the Brown University greenhouse, and grown under natural light in 4-inch pots with Metromix 360 coir soil (Scotts-Sierra Horticultural Products Co., Marysville, OH). Plant positions were randomized on the benches to limit micro-environmental effects. Self-fertilized, cleistogamous seeds were collected from these plants in July 2003 to begin inbred lines. Seeds were cold-stratified for 4 months at 4°C, following Schmitt (1993). Seedlings were grown for a second generation, and cleistogamous seeds again collected in February 2004, to remove maternal-environmental effects (Roff 1997).

## AFLP SCORING

We used Amplified Fragment Length Polymorphism (AFLP) markers (Vos et al. 1995, LI-COR Biosciences, Lincoln, NE) to detect polymorphism within and between populations. AFLPs are dominant markers, such that an individual either has or does not have at least one allele yielding a specific amplified fragment due to modification of restriction sites or changes in neighboring selective nucleotides. The selective amplification allows one to separate the amplified DNA fragments by size.

Leaves of the initial field-collected plants were collected and stored at −80°C. DNA was extracted from the leaves with Qiagen Plant DNeasy kits (Qiagen, Valencia, CA). We used Li-Cor AFLP kits to digest genomic DNA with *EcoR*1 and *Mse*1. Ligations were performed with T4 ligase. We used four selective primer combinations: E+AAC-M+CTC, E+AGC-M+CAA, E+AAC-M+CTT, and E+AGG-M+CTC. PCR products from the selective PCR were run on a Li-Cor model 4200 sequencer, and scored with Li-Cor's SAGA-MX AFLP analysis software. Following Bonin et al. (2004) and Pompanon et al. (2005), one quarter (27.5%) of the samples were replicated to remove loci with poor reproducibility. Before we began scoring we examined images for evidence of artifacts from poor-quality template, such as random variation in sample-to-sample intensity of individual fragments and absence of monomorphic fragments due to allelic dropout. In addition, we took care to avoid scoring fragments that showed evidence of being amplification artifacts or comigrating fragments from different loci (e.g., slight size variation among samples or intensity variation beyond that expected homozygote–heterozygote differences). In total we identified 175 AFLP loci.

## MORPHOLOGICAL DIFFERENTIATION—GREENHOUSE EXPERIMENT

To determine patterns of differentiation, plants from all five pairs of sun and shade populations were grown under simulated foliage shade and a control neutral shade treatment in the Brown University greenhouse. Simulated foliage shade selectively filtered red light and decreased the R:FR ratio, whereas the neutral shade treatment reduced the overall light intensity but did not alter R:FR. The experiment had a randomized split plot design, with two blocks and two treatments per block. A total of 20–30 replicate genotypes were used from each sun or shade population, with eight replicate seeds per genotype-treatment interaction. Cleistogamous seeds collected from the second generation of greenhouse grown parents were planted onto the surface of 10 cm plastic pots with Metro-mix 360 coir (Scotts-Sierra Horticultural Products Co., Marysville, OH) between June 14 and 16, 2004. Germination was censused every other day until June 30. In total 6256 seeds were planted and 1513 germinated. Germination was not equal among all maternal families; sample sizes of genotypes were equivalent within open- and closed-canopy pairs, but not among regions. Initial height, internode length, node number, branch number, and early flower number were measured nondestructively for emerged seedlings on July 8.

Overhead foliage shade (low R:FR) or neutral shade were imposed on July 10 when most seedlings were at the four-leaf stage, at the time that the first and second internodes were elongating. In the field, this developmental stage occurs at a time when overhead canopies close at closed-canopy sites in Southeastern New England, so imposing a shade treatment at this developmental stage most closely simulates natural conditions. To reduce R:FR, a red-absorbing plastic filter (SRX-4, Mitsui Chemical Corp, Kyoto, Japan) was affixed to PVC cages above the plants. Clear plastic sheeting affixed to 1-m high PVC caging provided a control treatment with an R:FR similar to full sunlight and an amount of photosynthetically active radiation (PAR) equal to the low R:FR treatment. The PAR for both treatments, as measured with a decagon ceptometer, was 35.7% of ambient. R:FR, the ratio of flux at 680 and 730 nm as measured with a LiCor spectroradiometer, was 0.6 below the low R:Fr treatment, and 1.1 in the control treatment. Plants were bottom watered daily and fertilized weekly to ensure that growth was not limited by other factors.

Plants were harvested by block between July 29 and August 7. We measured hypocotyl length, first, second, and third internode lengths, total height, number of branches, and flower and fruit production at harvest. All aboveground biomass was harvested and dried to obtain dry weight.

Because length traits are highly correlated, we used principal components to decompose them into two primary axes of variation. We calculated principal components with PROC PRINCOMP in SAS 9.13 to summarize variation in several traits. Previous work with*Impatiens* has shown are involved in shade avoidance. We calculated principal components from values in both treatments at the final harvest. We also calculated principal components from absolute and relative growth rates from initial census to harvest. Absolute growth rates were calculated by subtracting trait values before the imposition of the shade treatment from final values. The traits used were hypocotyl length, first, second, and third internode lengths, final height, biomass, and branch

number. Those traits that could not be measured at the initial census (third internode length and biomass) were excluded from the growth rate principal components (PCs). Nondestructive relative growth rates are not used because zero values at the initial harvest for many traits in some individuals make the RGR estimates uninterpretable.

## DATA ANALYSIS

Polymorphism within and between populations was assessed using a Bayesian algorithm implemented in the Hickory 1.03 freeware package (Holsinger 1999; Holsinger et al. 2002). We used the default settings with the full model, with a calculation of f (an equivalent of Fis), because it gave a better fit as calculated by Deviance Information Criterion (DIC, a metric similar to the more common Akaike's information criterion that takes into account both fit of a model and the number of parameters used) than the f-free model. As suggested by Holsinger et al. (2002), we used uniform priors, a burn-in of 50,000 and runs of 1,000,000, and a thinning interval of 10.

To compare differentiation at presumably neutral AFLP loci to our quantitative characters, we took a hierarchical approach. We calculated several *Q* and *F*-statistics according to three models: (1) a nonhierarchical model in which we estimated global $F_{LT}$ and $Q_{LT}$ (where l is for local) by separating all regions and habitat pairs into 10 populations; (2) a hierarchical model to test for local adaptation, in which we estimated $F_{RT}$ and $Q_{RT}$ representing differentiation among the five regions, and $F_{HR}$ and $Q_{HR}$ for differentiation between open- and closed-canopy habitats within regions; and (3) an ecotype model in which we estimated $F_{HT}$ and $Q_{HT}$ by combining all open-canopy habitats and all closed-canopy habitats to test for differentiation between canopy types. To estimate $F_{HR}$ and $Q_{HR}$ we first calculated pairwise $F_{HR(i)}$ and $Q_{HR(i)}$ between open- and closed-canopy habitats within each region *i* and then averaged these pairwise values (Weir and Cockerham 1984). For all measurements of *F*-statistics, we calculated $\theta^B$, an estimate of *F*-statistics, with Hickory. To estimate ranges around $\theta^B$ estimates we used the standard errors generated by Hickory. To estimate credible intervals around $F_{HR}$ and $Q_{HR,}$ we calculated the difference between estimated mean and estimated CI (from Hickory or Winbugs) for each pair, squared the difference, divided the square by 25 ($n^2$), took the square root, and averaged over all five pairs.

Due to imbalance in our data, we used a Bayesian approach to calculate credible intervals around our estimates of *Q*-statistics (O'Hara and Merila 2005). To accomplish the Bayesian estimates, we used a script implemented by Waldmann et al. (2005) in WinBUGS14 (Spiegelhalter et al. 2003). In this approach, we calculated *Q*-statistics as $\sigma^2_{(pop)}/\sigma^2_{(pop)}+c(\sigma^2_{(family)})$ where *c* is a function of the kinship coefficient and (pop) represents all 10 populations separated nonhierarchically for $Q_{LT}$, the combined open- and closed-canopy genotypes for each region combined for $Q_{RT}$, the open- versus closed-canopy populations within region *i* for $Q_{HR(i)}$, and the combined open-canopy versus the combined closed-canopy populations for $Q_{HT}$. We used a Gamma (1.0, 0.10) distribution as priors for the inverse of the variances ($1/\sigma^2_{(pop)}$, $1/\sigma^2_{(family)}$, and

$1/\sigma^2_{(individual)}$). To calculate confidence intervals we ran two chains for 550,000 iterations, with a 50,000 burn-in, and thinned by 10. All calculations were done separately for the simulated foliage shade and neutral shade treatments.

Because *I. capensis* has a mixed mating system that is environment dependent (i.e., Schemske 1978; Waller 1979; Schmitt and Ehrhardt 1987), and we performed two generations of inbreeding to reduce maternal environmental effects, the actual mating design for the scored plants varied between being selfed progeny of partially inbred parents (with inbreeding coefficients of 0.75, assuming they have undergone two generations of selfing from outbred field-collected plants) and selfed progeny of completely inbred parents (assuming the field-collected plants were themselves inbred, with inbreeding coefficients of 1). To account for this variation, we calculated *Q*-statistics for both mating models, as the true relatedness must fall between the two. Thus, the standard formula (Spitze 1993) for $Q_{ST}$ becomes: $Q_{ST}=\sigma^2_{(pop)}/(\sigma^2_{(pop)})+(8/7 *\sigma^2_{(family(pop))})$ for partially inbred parents; and $Q_{ST}=\sigma^2_{(pop)}/(\sigma^2_{(pop)})+(1 *\sigma^2_{(family(pop))})$ for completely inbred parents. The two approaches gave very similar results, with the clonal model invariably giving a *Q* estimate 0.02–0.03 higher than the partial outbreeding case, and using one or the other never affected our conclusions. As a consequence, we only report the partial outbreeding estimates.

Spearman correlations were calculated between the difference in current canopy cover between a paired closed- and open-canopy site, the amount of time they have differed in canopy cover, and between $F_{HR}$ and $Q_{HR}$ for the open–closed canopy pair. We also calculated coefficient of genetic variation ($CV_g$) as the square root of half the family variance component divided by the mean (Houle 1992) and heritability (broad sense) as $\frac{1}{2}\sigma^2_{(family)}/(\frac{1}{2}\sigma^2_{(family)}+\sigma^2_{(error)})$.

We distinguished four differentiation scenarios by comparing *F*- and *Q*-statistics. If differentiation is due to drift (**H1**), $F_{ST}$ and $Q_{ST}$ are expected to be equal at all hierarchical levels. If local adaptation occurs among regions (**H2**) in response to conditions other than canopy cover (or correlated factors), $Q_{RT}$ will be greater than $F_{RT}$. Repeated differentiation of open- and closed-canopy forms can be distinguished from a single origin of canopy forms (**H3**) by the comparison of neutral $F_{HT}$ and $F_{RT}$: if $F_{HT}$ is larger than $F_{RT}$, one origin of ecotypes is more likely than in situ origins. If there is local adaptation to canopy within regions (**H4**), $Q_{HR}$ should be larger than $F_{HR}$.

DNA from laboratory maintained inbred lines from the previously studied Haffenreffer Estate (Bristol RI) (Schmitt 1993; Dudley and Schmitt 1995) open–closed canopy populations were included in the AFLP survey. We excluded these inbred lines from the greenhouse study because their germination was several months out of cycle with the other populations, and because new collections could not be made in the field due to potential contamination from previous reciprocal transplant experiments (Donohue et al. 2000a,b). As a consequence, we did not include these populations in the $F_{ST}$-$Q_{ST}$ comparisons, but report their pairwise AFLP $F_{HR}$ ($\theta^B$) differences as a comparison to differences between the five open–closed canopy comparisons.

To examine patterns of trait variation more closely, we measured how principal components differ across shade treatments, regions, and habitats with ANOVA using PROC Mixed in SAS 9.13 (SAS Institute, Cary, NC). To test whether open-canopy populations are generally more responsive to low R:FR than closed-canopy populations, we used a mixed model analysis of variance with canopy habitat, region, and treatment as fixed effects and family nested within region and habitat as a random effect to test for population type × shade treatment interactions for the first two traits and growth principal components. As none of the three-way interactions were statistically significant ($P > 0.2$ for all), they were removed. We retained second-order interactions in the model regardless of statistical significance, as these interactions were in many cases the tests of our hypotheses. Denominator degrees of freedom for *F*-tests were determined by Satterthwaite approximation (the "DDFM = SATTERTH" statement in Proc Mixed). Means contrasts for interaction terms were performed using the "slice" statement of the "lsmeans" function of Proc Mixed (Schabenberger et al. 2000; Herrera et al. 2002). Interactions between habitat and treatment were further examined separately within regions.

## Results

### HABITAT CHARACTERIZATION

Measures of current canopy cover and historical aerial photographs showed that open- and closed-canopy sites differ in the extent and age of their canopy cover (Table 1). Among these sites open-canopy sites were older than closed-canopy sites, consistent with the recent reforestation of Southern New England, and suggesting that colonization in contemporary times was from open-canopy to closed-canopy habitats. All but one of the open-canopy habitats were open for the full period for which aerial photographs were available, whereas all but one of the closed-canopy populations shifted from open to closed during the same period.

### PATTERNS OF MARKER DIFFERENTIATION

Global $\theta^B$, a proxy for $F_{ST}$ for dominant markers like AFLPs, was 0.3697 when estimated nonhierarchically across all 10 populations ($\theta_{lt}$), with a 95% credible interval from 0.3518 to 0.3873 (Table 2). There was significant isolation by distance across all pairwise $\theta^B$comparisons (Fig. 2); however, pairwise comparisons between open-canopy sites, or between closed-canopy sites did not display significant isolation by distance. Genotypes from different habitats within a region were generally more closely related to each other than to genotypes from other regions (Fig. 2). Differentiation between the five regions in the hierarchical model ($\theta_{rt}$) was 0.32, with a 95% credible interval from 0.3005 to 0.3450 (Table 2). In contrast, differentiation between all open and closed populations in the ecotype model ($\theta_{ht}$) was only 0.02, with a 95% credible interval of 0.0155 to 0.0269 (Table 2). Differentiation between habitats within regions ($\theta_{hr}$) in the hierarchical model was 0.14 (Table 4). Pairwise $F_{LT}$ between all 45 possible combinations of two populations varied from 0.06 to 0.42, and increased with increasing geographical distance (Fig. 2). Migration rate, or gene flow, calculated from pairwise $F_{LT}$ by the relationship$F_{ST} = 1/4N_eM$,

varied from 0.59 to 4.17 $N_eM$ per generation. Although $F_{ST}$= 1/4$N_eM$ only holds in situations of migration–drift equilibrium, an assumption that is almost always violated, it still gives an estimate of the extent of gene flow under the assumption that migration and drift are the main factors shaping population structure rather than sequential episodes of complete reproductive isolation from panmictic populations.

**Table 2.** FST–QST comparisons. F- and Q-statistics are broken up hierarchically into habitat, region, and total forms. F-statistics are calculated as θB from Hickory, whereas Q-statistics were calculated from variance components with a Bayesian algorithm. We calculated FHR and QHR by taking the average pairwise FHR(i) and QHR(i) from open–closed canopy habitat pairs. Credible intervals are shown below means, in italics.% polymorphic loci, estimated genetic diversity (Hs), and estimated expected heterozygosity (ht) are given above the θB estimates for each hierarchical level. For these descriptive values ranges rather than standard errors are given. *Indicate Q-statistics that fall outside the 95% credible intervals for the F-statistic.

| **Trait** | **$F_{LT}$ and $Q_{LT}$ Total between population variation (nonhierarchical model)** | **$F_{RT}$ and $Q_{RT}$ Among regions (hierarchical model)** | **$F_{HR}$ and $Q_{HR}$ Between habitats within regions (hierarchical model)** | **$F_{HT}$ and $Q_{HT}$ Between habitats, pooling regions (ecotype model)** |
|---|---|---|---|---|
| % polymorphic loci | 100 | 100 | 100 | 89 |
| | | | | *81–95* |
| $H_s$ | 0.26 | 0.28 | 0.39 | 0.30 |
| | | | | *0.27–0.35* |
| $H_t$ | 0.40 | 0.40 | 0.40 | 0.32 |
| | | | | *0.28–0.35* |
| $\theta^B$ | 0.37 | 0.32 | 0.12 | 0.02 |
| | *0.35–0.39* | *0.30–0.35* | *0.10–0.16* | *0.01–0.03* |
| PC1, foliage shade | 0.22 | 0.14 | 0.24* | 0.07 |
| | *0.08–0.47* | *0.03–0.41* | *0.20–0.34* | *0.01–0.30* |
| PC2, foliage shade | 0.21 | 0.18 | 0.39* | 0.23* |
| | *0.07–0.43* | *0.05–0.48* | *0.34–0.49* | *0.05–0.72* |
| PC1, neutral shade | 0.36 | 0.19 | 0.32* | 0.12 |
| | *0.16–0.64* | *0.04–0.50* | *0.27–0.42* | *0.02–0.51* |
| PC2, | 0.23 | 0.16 | 0.29* | 0.55* |

| | | | | |
|---|---|---|---|---|
| neutral shade | | | | |
| | *0.08–0.48* | *0.04–0.43* | *0.25–0.39* | *0.19–0.94* |
| PC 1 absolute growth, foliage shade | 0.22 | 0.15 | 0.22* | 0.08 |
| | *0.08–0.46* | *0.04–0.42* | *0.18–0.32* | *0.01–0.35* |
| PC 2 absolute growth, foliage shade | 0.35 | 0.26 | 0.38* | 0.31* |
| | *0.16–0.61* | *0.08–0.59* | *0.32–0.47* | *0.07–0.80* |
| PC 1 absolute growth, neutral shade | 0.37 | 0.19 | 0.30* | 0.15 |
| | *0.16–0.65* | *0.05–0.50* | *0.25–0.10* | *0.02–0.57* |
| PC 2 absolute growth, neutral shade | 0.35 | 0.20 | 0.40* | 0.58* |
| | *0.15–0.62* | *0.06–0.50* | *0.33–0.49* | *0.21–0.94* |

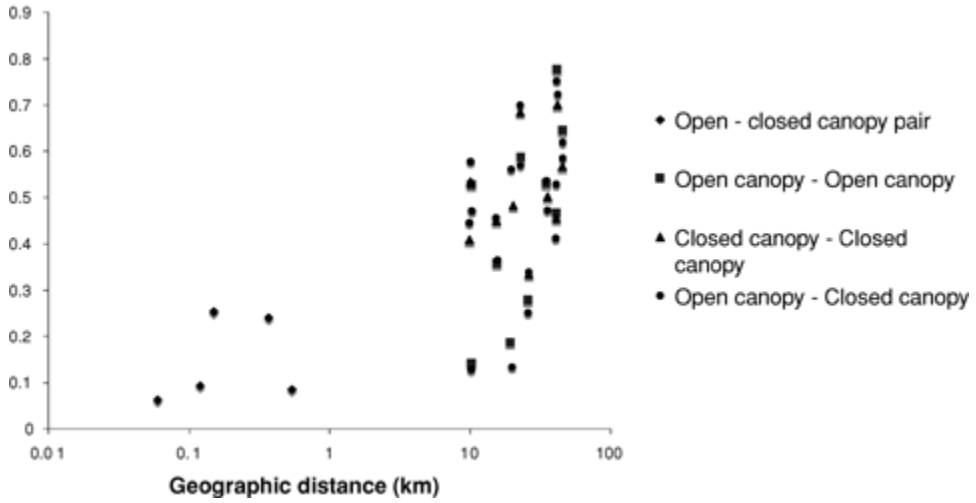


**Figure 2.** Isolation by distance between all populations from 175 dominant AFLP loci. Pairwise $\theta_{st}/(1-\theta_{st})$ by log distance is shown. Pairwise comparisons are separated by canopy types involved (between open–closed canopy pairs, open-canopy populations from different regions,

closed-canopy populations from different regions, and open-versus closed-canopy populations from different regions. The linear regression on log distance has a slope of 0.17133, with an adjusted $R^2$ 0.3915, and $P < 0.0001$. The geographic distance and genetic distance matrices were correlated (Mantel's*r* 0.64, $P = 0.0004$).

**Table 4.** Principal components differing significantly in a mixed model ANOVA between regions and habitats in our common garden greenhouse experiment. Plants were exposed to a low R:FR treatment simulating overhead foliage shade, and a control, equal R:FR treatment, simulating low PAR in an open canopy habitat. Nonsignificant three-way interactions were removed (P>0.14). F-values for Region, Habitat, Treatment, and their interactions from a mixed model are reported with * for P<0.05, ** for P<0.01, ***for P<0.001, and **** for P<0.0001, with degrees of freedom in parentheses. Log-likelihood ratios are reported for family and family by treatment, with * for P<0.025, ** for P<0.0025, with significance assessed with a $\chi 2$ distribution and one degree of freedom.

| **Trait** | **Trait PC 1** | **Trait PC 2** | **Abs PC 1** | **Abs PC2** |
|---|---|---|---|---|
| R:FR Light Treatment | 1.82 | 70.0 **** | 0.53 | 52.49 **** |
| | (1, 1042) | (1, 1035) | (1, 1087) | (1, 345) |
| Region | 14.28 **** | 4.98*** | 13.69 **** | 7.19 **** |
| | (4, 203) | (4, 212) | (4, 201) | (4, 217) |
| Habitat | 2.12 | 15.32 **** | 4.02 * | 27.59 **** |
| | (1, 222) | (1, 227) | (1, 219) | (1, 238) |
| R:FR×Region | 0.83 | 0.4 | 0.8 | 0.26 |
| | (4, 997) | (4, 992) | (4, 1040) | (4, 175) |
| R:FR×Habitat | 3.19 | 2.59 | 3.96 * | 0.38 |
| | (1, 969) | (1, 964) | (1,1012) | (1, 130) |
| Region×Habitat | 8.04 **** | 1.9 | 7.39 **** | 5.67*** |
| | (4, 201) | (4, 209) | (4, 198) | (4, 215) |
| Family (Habitat (Region)) | 228 ** | 338.2 ** | 226.7 ** | 256.5** |

Pairwise $F_{HR(i)}$ between open- and closed-canopy populations ranged from 0.06 to 0.20 for the five pairs of open- and closed-canopy populations. Differentiation between the previously studied (e.g., Dudley and Schmitt 1995; Donohue et al. 2000a,b) Haffenreffer open- and closed-canopy populations was 0.1567.

There was a correlation between $F_{HR(i)}$ between paired open-canopy and closed-canopy populations in individual regions and the age of open-canopy sites (Spearman rank coefficient = 0.89, $P = 0.04$), with the older open-canopy sites being more diverged from paired closed-canopy sites. However, there was no significant correlation between $F_{HR(i)}$ and difference in current canopy cover or age of differentiation in canopy cover. Furthermore, there was no significant relationship between pairwise $Q_{HR(i)}$ for any PC and difference in current canopy cover or age of difference in canopy cover, although the site with the most stable canopy cover through the

period examined (Weetamo Woods, WW) did have the largest pairwise $Q_{HR(i)}$ estimate for the second PC (see below).

## COMPARISON OF MORPHOLOGICAL AND MOLECULAR DIFFERENTIATION

All traits considered had significant genetic variation and heritability (see on-line Supplementary Table S1). To look at differentiation across a large number of traits, traits were summarized with principal components (Table 3). We interpret PC 1 of trait values and absolute growth rate as representing general size and vigor, as indicated by large loadings from node number, height, and branch number, as well as biomass and the lengths of some internodes. PC 2 for traits and growth rates loads heavily on early elongation traits, such as internode lengths and hypocotyl length.

**Table 3.** Cumulative variation explained by principal components and loading of traits onto principal components. Principal components were calculated in Proc PRINCOMP in SAS 9.13. Principal components for growth rates were calculated from growth rates of individual traits before the imposition of shade treatment and at final harvest. Days to emergence, third internode length, and biomass were dropped from the growth rate principal components because growth rates for these traits cannot be calculated (NC).

| | **Trait PC 1** | **Trait PC 2** | **Absolute growth PC 1** | **Absolute growth PC 2** |
|---|---|---|---|---|
| Cumulative variation explained | 0.59 | 0.74 | 0.56 | 0.75 |
| Loadings | | | | |
| Days to emergence | −0.33 | 0.17 | NC | NC |
| Hypocotyl length | 0.01 | 0.59 | −0.26 | 0.39 |
| First internode length | 0.4 | 0.12 | 0.01 | 0.82 |
| Second internode length | 0.13 | 0.67 | 0.42 | 0.33 |
| Third internode length | 0.35 | 0.26 | NC | NC |
| Upper internode length | 0.38 | −0.01 | 0.46 | −0.2 |
| Height | 0.34 | −0.26 | 0.48 | 0.14 |
| Nodes | 0.36 | −0.12 | 0.4 | −0.08 |
| Branches | 0.34 | −0.1 | 0.38 | 0.02 |
| Biomass | 0.27 | −0.04 | NC | NC |

Across all 10 populations, global $Q_{ST}$ for traits and growth rates summarized by principal components measured in our glasshouse study fell within the range of $\theta^B$, although many of the point estimates were below $\theta^B$ estimated from AFLPs (Table 2). Both assumptions of mating system and relatedness gave similar estimates of differentiation (data not shown). Differentiation among the five regions, $Q_{RT}$, fell within the range of $\theta^B$ for traits and growth rates as well (Table 2). The first principal components for traits and absolute growth rates, indices of size and vigor,

were not significantly more differentiated between habitats ($Q_{HT}$) than the AFLP markers ($F_{HT}$) in either the foliage and neutral shade treatments. However, the second principal components of traits and growth rates, indices of early elongation, were significantly and substantially more differentiated between habitats ($Q_{HT}$) than the AFLP markers ($F_{HT}$) in both the foliage and neutral shade treatments (Table 2), as predicted if there were differentiation between habitats in shade avoidance traits. $Q_{HR}$ estimates were all higher than the $F_{HR}$ estimates, consistent with differentiation of traits between canopy habitats within regions.

## RESPONSES TO SHADE—DIFFERENCES BETWEEN CLOSED- AND OPEN-CANOPY POPULATIONS

To examine patterns of morphological differentiation, we measured how the principal components differ across R:FR treatments, regions, and habitats (Table 4, Fig. 3A–D). The first principal components of both traits and growth rates were not significantly affected by the R:FR treatment, consistent with our interpretation of this axis of variation as primarily representing size or vigor rather than elongation (Table 3,Fig. 3A, C). The second principal component of traits and growth rates was significantly higher in the R:FR treatment (Table 4, Fig. 3B, D), consistent with our interpretation of this axis of variation as related primarily to elongation.

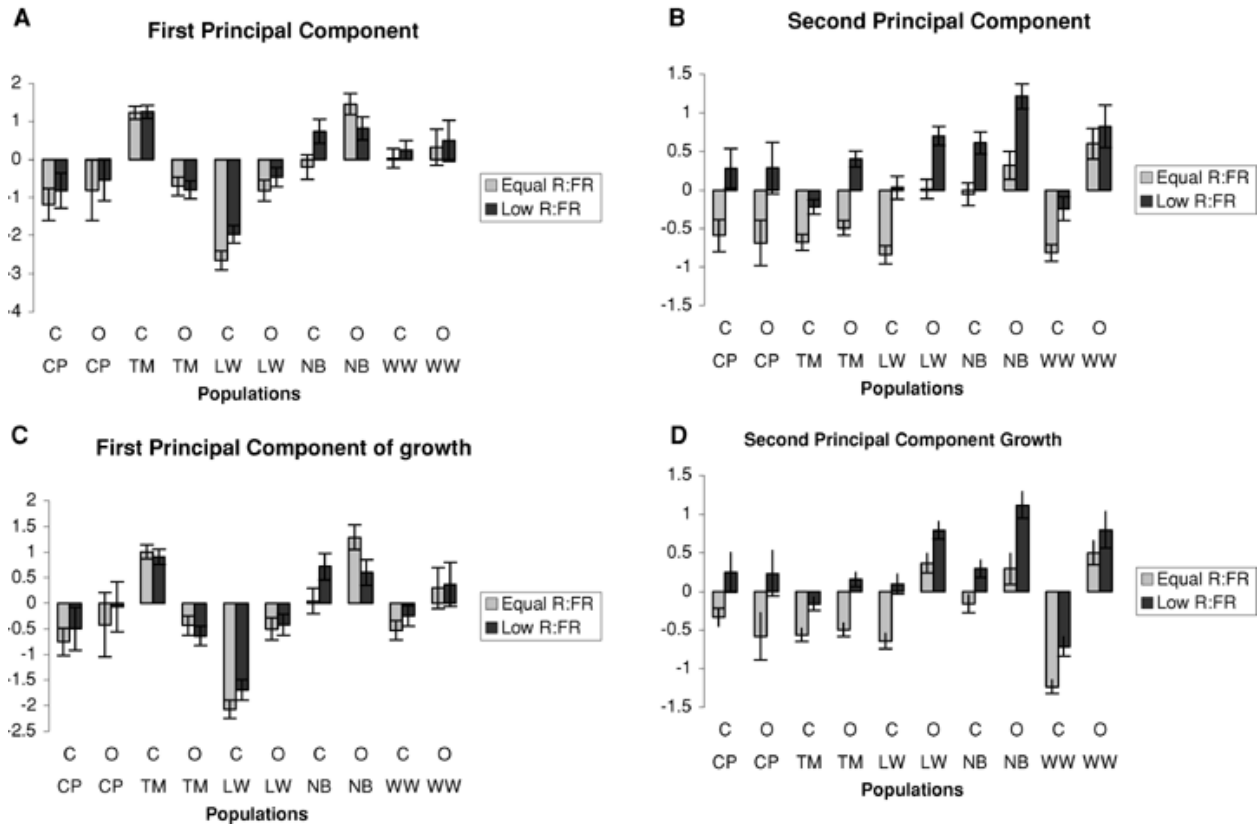


**Figure 3.** A–D. Variation in principal component values across regions, habitats, and treatments. PC values with standard errors are shown. Habitats are distinguished as C for closed canopy, and

O for open canopy. The five regions (ordered north to south) are Caratunk (CP), Touissett (TM), Lincoln Woods (LW), Norman Bird (NB), and Weetamoo Woods (WW). A. PC 1 calculated from final trait values. B. PC 2 for final trait values. C. PC 1 of absolute growth (before and after shade treatments) of traits. D. PC 2 of absolute growth.

There was differentiation between habitats in the second principal component of traits and growth rate, but not the first principal component. Overall, populations from open-canopy habitats displayed higher values of PC2, that is, more elongated phenotypes than populations from closed-canopy habitats (Table 4). This trend is driven by three of the five populations (Fig. 3B, D). This observation supports the results of the $Q_{ST}$ analysis, suggesting that open- and closed-canopy populations do differ consistently in elongation traits. However, there were no significant R:FR treatment × habitat interactions (Table 4); open- and closed-canopy populations did not differ significantly in response to R:FR, as predicted by Morgan and Smith's hypotheses (1979) and observed by Dudley and Schmitt (1995). Although plants from open- and closed-canopy habitats differed in elongation traits in the present study, they did not differ overall in plasticity. Even if we look at differences between habitats within regions, only one pair of populations (TM) displayed a significant R:FR treatment × habitat interaction caused by the open-canopy genotypes having greater elongation than the closed-canopy genotypes only in the foliage shade treatment (Fig. 3B, D; T, R:FR × habitat interaction, $F$ (1,390) = 5.9, $P$= 0.015). In all other regions there was no significant difference between foliage and neutral shade treatments in the amount of difference between the paired open- and closed-canopy populations (Fig. 3B, D) (data not shown). This result suggests that open-canopy sites do not always have greater plastic responses to R:FR than nearby closed-canopy populations.

Across all principal components, there was significant differentiation among regions, suggesting multivariate variation across regions (Table 4). Variation between regions did not depend on R:FR treatment, as indicated by the lack of significant interactions between region and R:FR treatment. However, there were strong interactions between region and habitat, suggesting that the amount of differentiation between habitats differs between regions. Furthermore, there was substantial variation among families, indicating substantial variation within habitats within regions.

## Discussion

### WHAT PATTERN OF POPULATION DIFFERENTIATION DO THE DATA SUPPORT?

We tested four hypotheses that could explain patterns of differentiation in open- and closed-canopy habitats in *I. capensis* by comparing different measures of $F_{ST}$ and $Q_{ST}$. Our data allow us to reject three of these hypotheses: drift, adaptive regional differentiation, and a single origin of canopy ecotypes. Although we found isolation by distance in neutral markers, differentiation between open- and closed-canopy habitats ($Q_{HT}$ and $Q_{HR}$) exceeded neutral expectation

($F_{HT}$ and $F_{HR}$, respectively), suggesting adaptive differentiation to canopy type across the landscape and allowing us to reject the hypothesis of differentiation in morphological traits solely through drift (**H1**). However, morphological differentiation among regions ($Q_{RT}$) did not exceed the expectation from neutral markers ($F_{RT}$), and thus could not be attributed to adaptive differentiation (**H2**). The observed differentiation between habitat types cannot be attributed to colonization of specific canopy habitats in multiple sites by ancestral specialist genotypes (**H3**); if so, $F_{RT}$ would have been lower than $F_{HT}$, and we observed the opposite.

However, the data are consistent with the hypothesis of repeated independent adaptive differentiation between open- and closed-canopy forms in different regions (**H4**). The estimates of $Q_{HT}$ and $Q_{HR}$ for trait and growth rate principal components that fall above $F_{HT}$ and $F_{HR}$suggest frequent independent adaptive differentiation between open- and closed-canopy habitats within regions. Although local adaptation obviously may occur due to factors other than canopy cover, such as soil moisture (Bennington and McGraw 1995; Heschel et al. 2002), and correlated selection pressures may have a strong role in shaping shade avoidance responses (Maliakal et al. 1999; Huber et al. 2004; von Wettberg et al. 2005), our results indicate that overhead canopy cover is a significant driver of adaptive population differentiation.

## PLASTIC VERSUS CONSTITUTIVE DIFFERENCES: WHY DOES DIFFERENTIATION HAVE DIFFERENT END POINTS?

Although we found evidence for adaptive differentiation between open- and closed-canopy populations within regions, the pattern of differentiation differed among regions. Only one of the five population pairs displayed a pattern of differentiation consistent with expectations from Dudley and Schmitt (1995) and Donohue et al. (2000b). Dudley and Schmitt (1995) found that field-collected seedlings from an open-canopy site elongated more in response to simulated foliage shade than those from two closed-canopy sites at the Haffenreffer Estate, in Bristol, RI, USA. In reciprocal transplantation experiments with two of these populations at the Haffenreffer Estate,Donohue et al. (2000b) found selection for increased plasticity of shade avoidance traits at high density in the open-canopy site, but not in the closed-canopy site, suggesting that selection can drive differentiation between open- and closed-canopy sites. However, the present study, with five different pairs of populations from other New England sites, detected significantly greater elongation of open-canopy genotypes in response to low R:FR in only one of the population pairs (TM). In contrast, open-canopy genotypes were constitutively more elongated than closed-canopy genotypes in three regions. Thus, adaptive differentiation in response to canopy cover had different end points in different regions across the landscape.

Population differentiation may produce different results for several reasons, either alone or acting in concert. For example, different starting genetic variation, and different genes with polymorphisms available to selection, could result in different end points. Selection could be spatially or temporally variable, causing differences in the amount of differentiation. There could

be selection from other factors that vary spatially, such as drought or herbivores and pathogens, that drive local populations to different end points. Alternatively, sites may be of different ages and/or gene flow might differ between pairs, altering the amount of differentiation. All of these are possible in the case of*Impatiens.*

From this study and others (e.g., Schmitt 1993; Paoletti and Holsinger 1999; Donohue et al. 2000a) we know there is substantial quantitative genetic variation in *I. capensis*, and that due to epistasis and other factors selection might drive phenotypes in different directions from different starting materials. There could be differences in which genes have polymorphisms available to differentiating selection in different regions. Field transplant studies of the frequency of selection on elongated and unelongated *Impatiens* plants suggest that selection on elongation can be spatially variable within sites (Huber et al. 2004; von Wettberg et al. 2005). Long-term microsite monitoring (Schmitt et al. 2003; M. S. Heschel, unpubl. data) suggests a strong possibility for temporally variable selection as well.

Drought stress (Maliakal et al. 1999; Huber et al. 2004; von Wettberg et al. 2005) and leaf litter (Stinchcombe and Schmitt 2006; E. J. von Wettberg and J. Schmitt unpubl. data) have been found to alter shade avoidance responses in *Impatiens*. Soil moisture availability and leaf litter composition do not covary completely with overhead canopy cover, suggesting that selection from other factors may drive correlated responses differently in different sites, even if closed and open canopies present consistently different selective pressures. However, there are only limited genetic correlations between responses to leaf litter and foliage shade (E. J. von Wettberg and J. Schmitt, unpubl. data). The extent of genetic correlation between responses to drought and shade has not been explored in *Impatiens*, although greenhouse and field experiments provide suggestive evidence that there is some genetic correlation (Maliakal, et al. 1999; Heschel et al. 2002, 2005; Huber et al. 2004; von Wettberg et al. 2005).

Using historical aerial photographs we have characterized canopy cover over the past 70 years. Although sites have been relatively stable over that time, succession from open to closed sites has occurred in several closed-canopy sites. Differences in the age of habitats, particularly closed-canopy habitats, contribute to differences in the amount of differentiation observed between open- and closed-canopy populations. Selective forces may be more important in open-canopy habitats where populations are frequently larger and plants are more fecund. However, this explanation may not be complete; although the open-canopy habitats in this study have been more stable than closed-canopy habitats for the period of time covered by aerial photographs, this stability may not predate the past century (Foster 1992).

We observed low pairwise $\theta^B$ estimates between open- and closed-canopy populations, suggesting gene flow greater than one migrant per generation between open and closed stands. This is a sufficiently large number of migrants to disrupt local adaptation in the absence of strong selection. The previously examined Haffenreffer populations, with their documented local adaptation and differences in plasticity (Schmitt 1993; Dudley and Schmitt 1995; Donohue et al.

2000a,b, 2001), has a pairwise $\theta^B$ of 0.1567 (E. von Wettberg unpubl. data); this value was within the range of pairwise $\theta^B$s we observed, and corresponds to 1.5 migrants per generation, which should be sufficient to disrupt local adaptation. In addition, small patches of individuals occur between every open- and closed-canopy site used in this study, including those at the Haffenreffer Estate. Given that *Impatiens* is an extremely widespread and abundant colonizing species, with ballistically dispersed floating seeds that can spread through any wetland, it is impossible to find sites that are geographically proximate, inhabit different canopy environments, and do not have individuals or patches occurring between them. Any differentiation that occurs in spite of this gene flow, suggesting that canopy features pose a consistently strong selective pressure on *Impatiens*, even if the result is not always identical.

## DIVERSIFYING SELECTION OR STABILIZING SELECTION?

$Q_{ST}$ values that are less than $F_{ST}$ values are often accepted as evidence for stabilizing selection between populations (Merila and Crnokrak 2001; McKay and Latta 2002), in which homogenous selective pressures and/or constraints on traits cause more similarity in traits between populations than expected based on gene flow as inferred from molecular markers. However, a low $Q_{ST}$ relative to $F_{ST}$could also indicate excess variation within populations due to diversifying selection across subpopulations within a region, in which variability is maintained between patches in a population. Global $F_{ST}$-$Q_{ST}$ comparisons cannot distinguish these two possibilities, but the hierarchical partitioning of $F_{ST}$ and $Q_{ST}$ allows us to distinguish between stabilizing and diversifying selection. If we had examined $F_{RT}$and $Q_{RT}$ in this study by only pooling between habitats, we would have only been able to reject stabilizing selection, as has commonly been done. Yet, the high values of $Q_{HR}$ demonstrate significant differences between habitats within regions, suggesting that diversifying selection shapes quantitative genetic variation within regions. We suggest that this hierarchical approach is a useful way to parse selective forces on a landscape scale, and $F_{ST}$-$Q_{ST}$ comparisons would benefit from being performed at multiple levels.

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