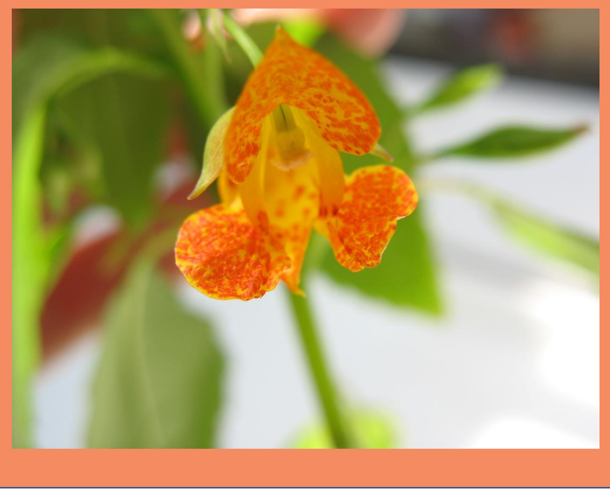

Cotyledon stomatal density differentiation and quantitative genetic analysis of seedling traits in *Impatiens capensis* ecotypes

Alex Shapiro, Lina Farias, Ron Hathaway, & M. Shane Heschel

Colorado College Dept. of Organismal Biology & Ecology, 14 E. Cache La Poudre St. Colorado Springs, CO 80903

Introduction

Impatiens capensis is a weedy annual plant that grows contiguously from the Eastern U.S. to the Rocky Mountains through a range of soil conditions, from the moist habitats of Rhode Island and Pennsylvania to very dry habitats in Colorado. This is possible through physiological and morphological changes in stomatal density, conductance, ABA production, and leaf growth (Bibee et al. 2011; Maruyama et al. 2016). These changes indicate regionally specific ecotypes of *Impatiens capensis* in areas such as Pennsylvania, Rhode Island, and Colorado.

Previous work has examined maternal effects on physiology in the Pennsylvania ecotype, however studies have not yet examined seedling traits across multiple *Impatiens* ecotypes. Using Scanning Electron Microscope (SEM) and physiological data, Colorado and Pennsylvania *Impatiens capensis* seedlings were analyzed for functional traits as well as heritability of photosystem efficiency and stomatal conductance.

In addition to simply measuring and comparing traits, heritability calculations can be used to provide insight into the evolutionary potential of a population. With the changing climate, germination and seedling success could be affected by unpredictable spring weather patterns. Knowing the evolutionary potential of these ecotypes of *Impatiens capensis* seedlings could bolster our understanding of how this species will respond to climate change in regionally specific ways.

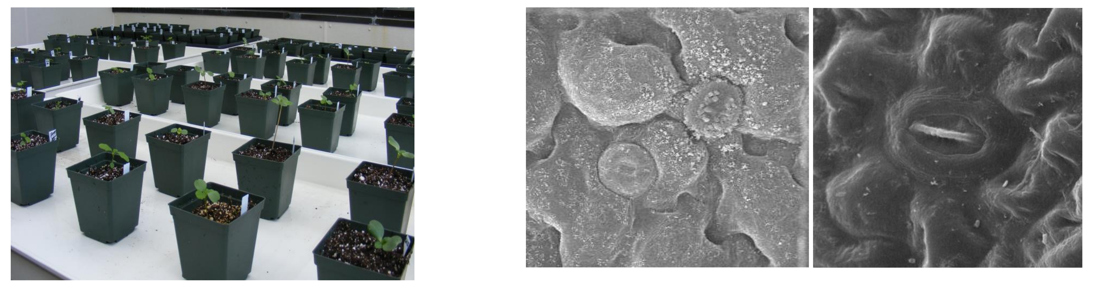

Figure 1. Stomata of *Imaptiens capensis.* Cuticular wax on an adult leaf (left) absence of cuticular wax on a cotyledon (right).

Methods

Scanning Electron Microscope (SEM) Imaging

- Pennsylvania and Colorado seeds were germinated on 1% agar plates in a growth chamber set to 19°C and a photoperiod of 12 hrs. Plates were sprayed once every other day with water, and Gibberellin (10^{-4} M) was added twice during the germination process to accelerate growth.
- 15 cotyledons were fixed with 19% glutaraldehyde for two hours, put through 3 phosphate buffer washes, one two-hour wash of 1% osmium tetroxide, and 3 DI water washes.
- Each cotyledon was observed in four different frames on the SEM. In each frame the stomata were counted and converted to mm^2 density by dividing each plant's average number of stomata per frame by the average area of the frame (.12mm^2).

Maternal Control (Transpiration Rates)

- In late February and early March of 2019, transpiration rates were measured on the 49 maternal *Impatiens* plants; 18 from Pennsylvania and 31 from Colorado.
- All plants had been growing freely in stable conditions at the Colorado College Greenhouse since early December of 2018.
- Stomatal conductance rates were measured on the newest leaf of each plant with a steady-state leaf porometer (Decagon Devices).

Quantitative Genetic Analysis

- Seeds from 44 lines (22 CO, 22 PA) were grown in the Colorado College greenhouse under stable conditions.
- After 13 days of growth, photosystem efficiency (PE) measurements were taken on the largest cotyledon of each seedling with an EARS (NL) fluorometer (Fv/Fm), using a black background paper to shield sunlight; after about 16 days of growth, seedling height was measured (cm) from the soil surface to the apical meristem.
- Quantitative genetic analysis was performed using these PE data and stomatal conductance (g_{ST}) data from seedlings grown in 2012 (CO) and 2015 (PA) in identical greenhouse conditions; these growing periods were effectively grand maternal generations of the lines used in this study and were similarly inbred.

Data Analyses

- All statistical analyses were performed in JMP (vs. 4.0.4, SAS Institute). A one-way ANOVA was done on the stomatal density per region (both Colorado and Pennsylvania). An ANOVA was also performed to compare the transpiration rates of the Colorado and Pennsylvania maternal plants.
- Two-way ANOVAs were performed to estimate genetic and environmental sources of variance in seedling traits; these variance estimates were used to calculate heritability for stomatal conductance and photosystem efficiency to determine the evolutionary potential of these traits between regional ecotypes.
- Jack-knifed genetic variances of PE and g_{ST} were analyzed using a one-way ANOVA to estimate the difference in genetic variance between regions.

Study Questions

1. Do *Impatiens capensis* seedlings from Pennsylvania and Colorado populations show differences in physiological and morphological traits such as stomatal density, stomatal conductance, and photosystem efficiency?
2. Does evolutionary potential differ between Pennsylvania and Colorado varieties of *Impatiens capensis* in seedling traits linked to drought and photosynthesis?

Results

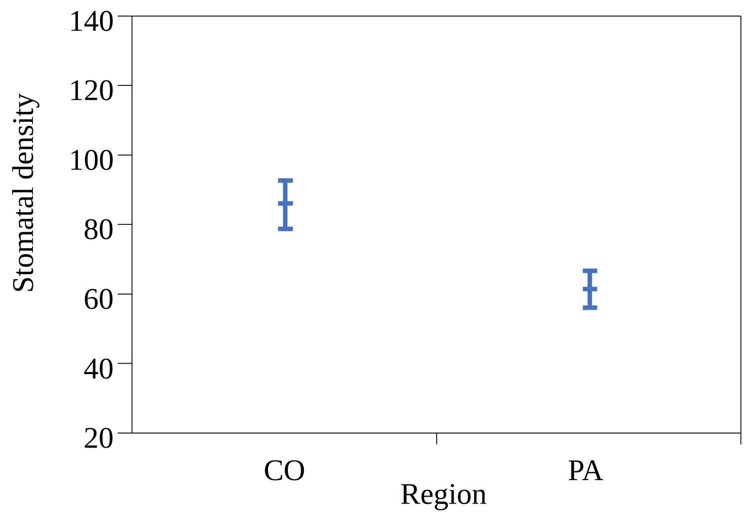

Figure 2. Stomatal densities for CO and PA seedlings. Means ± SE bars shown. [F=8.1, df=1, P=0.008]

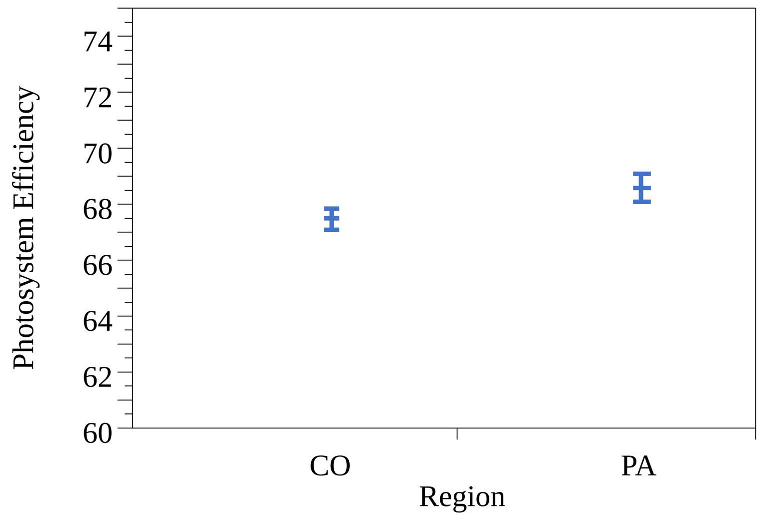

Figure 3. Photosystem efficiencies for CO and PA seedlings. Means ± SE bars shown. [F=3.6, df=1, P=0.07]

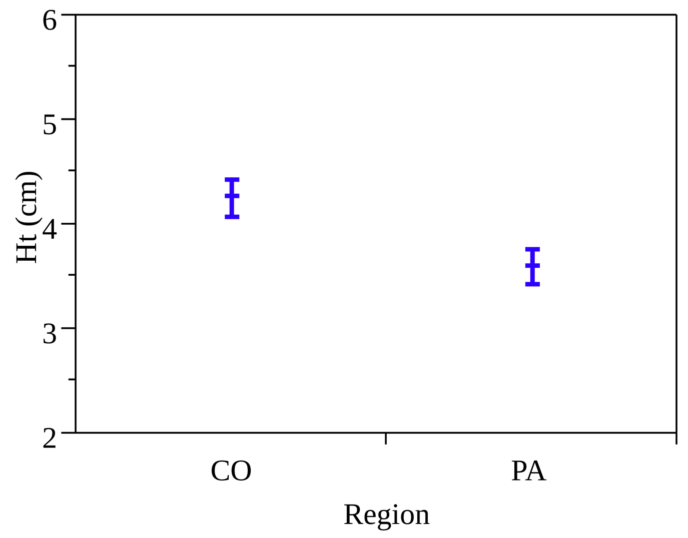

Figure 4. Seedling height after 2weeks of growth. Means ± SE bars shown. [F=7.3, df=1, P=0.008]

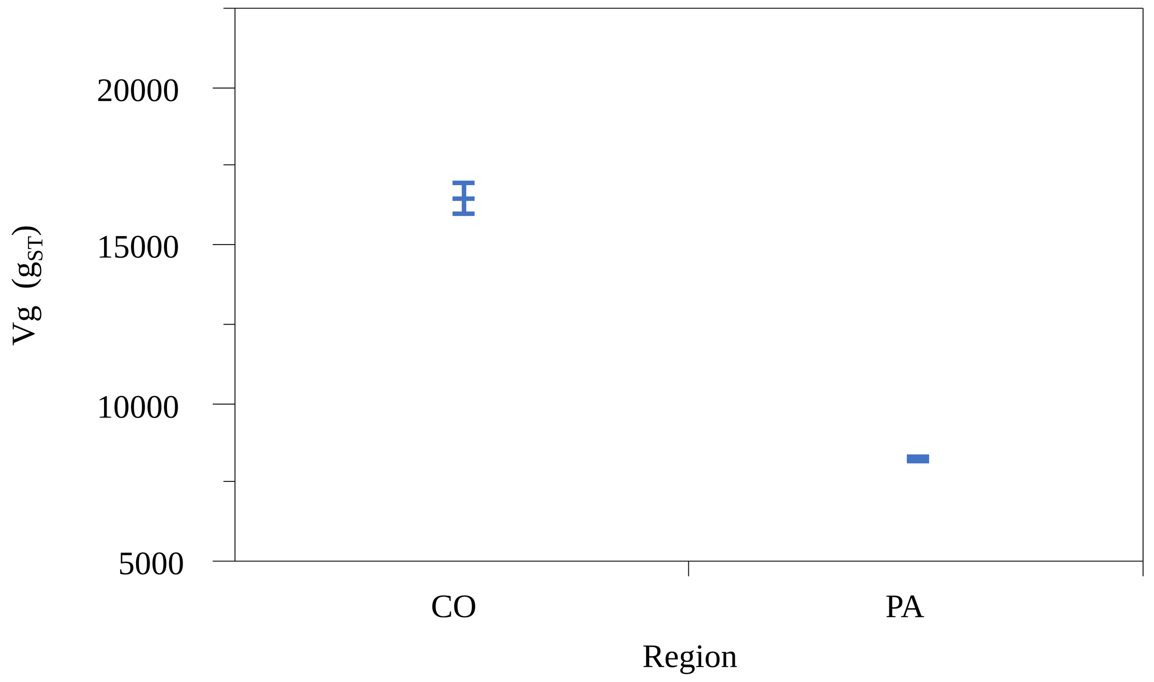

Figure 5. Jack-knifed genetic variance estimates for stomatal conductance (g_{ST}) for CO and PA seedlings. Means ± SE bars shown.

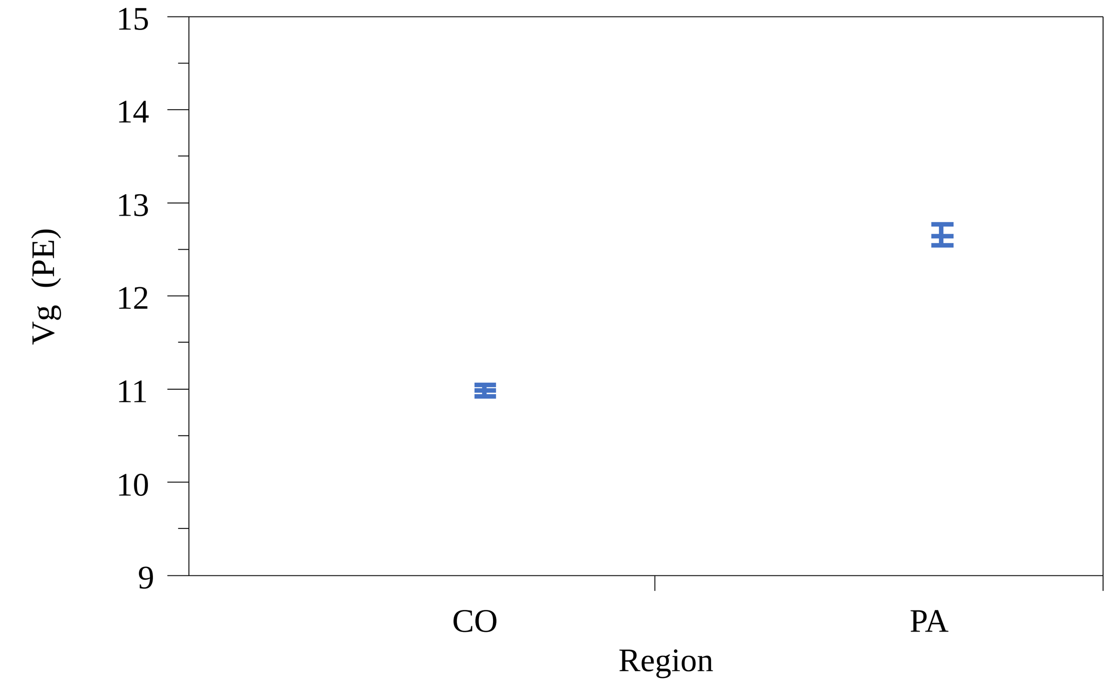

Figure 6. Jack-knifed genetic variance estimates for photosystem efficiency (PE) for CO and PA seedlings. Means ± SE bars shown.

Pop.	Trait	Vg	Ve	Heritability est.	F Ratio	Prob > F
CO	g_{ST}	16452.5	44927.3	0.268	2.3607	0.0093
CO	PE	11.0227	56.763	0.194	1.1348	0.339
CO	Day of emergence	12.0044	24.5489	0.774	3.2034	0.0002
PA	g_{ST}	8255.35	8023.99	0.507	1.0288	0.4471
PA	PE	12.4865	24.3222	0.5134	1.0079	0.4751
PA	Day of emergence	13.1872	9.1838	1.4359	2.3554	0.0191

Table 1. Heritability estimates for stomatal conductance (g_{ST}), photosystem efficiency (PE), and day of cotyledon emergence from both Colorado and Pennsylvania populations. F ratios and P-values are for tests of genetic variance significance. Vg and Ve values were taken from mean squares (MS) of line, block, and error on ANOVA tables.

Literature Cited

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Maruyama, C., Goepfert, Z., Squires, K., Maclay, T., Teal-Sullivan, Q., & Heschel, M. S. (2016). Effects of population site and maternal drought on establishment physiology in *Impatiens capensis* meerb. (Balsaminaceae). *Rhodora*, *118*(973), 32–45.

Discussion

Functional Traits

- Region was a significant predictor of stomatal density in seedlings, but not of cuticular wax. Higher stomatal densities in the Colorado ecotype seedlings (Fig. 2) indicate that strategies related to water use efficiency are likely highly plastic throughout the transition to adulthood. In adult plants from CO, stomatal density is often lower than in other regions (Bibee et al. 2011). The lack of cuticular wax in seedlings (Fig. 1) from both populations contradicts what was found by Bibee et al. (2011) and indicates that wax also changes with developmental age.
- Differences in photosystem efficiency were marginally significant, showing PA seedlings to have slightly higher PE (Fig. 3). Higher stomatal densities in CO populations may be a compensatory mechanism for this lower PE; i.e. CO cotyledons may have greater gas exchange capacity to compensate for less efficient photosystems.
- Day of seedling emergence was not significantly different between populations (Fig. not shown) and was also a highly variable trait (Table 1). This may be beneficial for the evolutionary trajectory of *Impatiens capensis* across all ecotypes. Moreover, equivalent establishment capabilities may be achieved with different combinations of stomatal density and PE.
- Early growth rates were significantly different (Fig. 4); CO lines were significantly taller than PA lines. The combination of increased stomatal density and marginally different PE values made the CO lines capable of faster growth even though plants emerged at the same time; this may translate into drought avoidance strategies in CO populations (Bibee et al. 2011; Maruyama et al. 2016).

Control for Maternal Environment

- Stomatal conductance of Colorado and Pennsylvania adult plants showed no significant differences (maternal plant gst values: CO = 106.8±6.6 & PA = 96.7±9.1 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$). The adult plants were under no stress in the stable greenhouse environment. If differing transpiration rates in maternal plants were shown, this could have influenced the stomatal density of their offspring. Again, this indicates that genetic differences between regions have a greater effect on observed seedling trait values than any maternal effects. This control for maternal environment allows us to examine genetic variances.

Quantitative Genetic Analysis

- Genetic variance was significantly different between populations. Colorado populations had higher Vg for stomatal conductance (Fig. 5), but lower Vg for photosystem efficiency (Fig. 6). These data align with the variation in the respective environments of each ecotype. Colorado environments have variable water availability, but relatively stable light conditions, whereas Pennsylvania environments are opposite. This illustrates that variation within an environment maintains genetic variation in traits linked to those environmental factors.

Conclusions

Differences in physiological and morphological traits in *Impatiens capensis* seedlings from CO and PA are likely due to allelic variation within these populations caused by environmental selection pressures, such as water and light availability. Differences in evolutionary potential between these two regional ecotypes may have implications for the future of these populations as climate change becomes an even more predominant issue in these natural environments.

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