2015

Intraspecific Variation in Leaf Traits Across an Environmental Gradient in the Cape Floristic Provence of South Africa

Bruce H. Ramos
bramos_8321@email.ric.edu

INTRASPECIFIC VARIATION IN LEAF TRAITS ACROSS AN ENVIRONMENTAL GRADIENT IN THE CAPE FLORISTIC PROVINCE OF SOUTH AFRICA

By

Bruno H. Ramos

An Honors Project Submitted in Partial Fulfillment of

The Requirements for Honors

in

The Department of Biology

The School of Arts and Sciences

Rhode Island College

2015

Abstract

Intraspecific variability has been an overlooked and under-investigated driving force behind biodiversity. Both interspecific and intraspecific variability contribute to species community assembly, and the South African Cape Floristic Region (CFR), as an area of remarkable biodiversity, provides the perfect opportunity to the latter. The objective of this research is to study intraspecific variability in leaf traits across three biomes that span rainfall and temperature gradients, and to assess the partitioning of that variability across biomes, across shrubs within biomes, and within shrubs of four focal species - *Rushia intricata*, *Aridaria noctiflorum*, *Diospyros austro-africana* and *Chrysocoma ciliata*. All collections were made in the towns of Calvinia, Nieuwoudtville, and Sutherland from July 28-August 27, 2014. ANOVAs were performed using the statistical package R. The results suggest that for all species except *Diospyros austro-africana*, most of the variability occurs at the within-shrub level. This study also suggests that the focal species, with the exception of *Rushia intricata*, are in fact responding to environmental conditions.

Introduction

The evolution of biodiversity has always been a topic of great interest in biology. Accounting for the adaptive radiation of species within a community and understanding their interactions has been an enormous challenge taken by evolutionary biologists and ecologists to understand the reasons and implications behind species diversity and their coexistence in any given biome (Messier, *et al.*, 2010). It is no surprise that rainforests, where biodiversity is high, can be very attractive locations for research. Even more interesting, however, are locations where the environmental factors are, theoretically, not favorable to the adaptive radiation of many species, and yet high levels of diversity are present. One of these locations is the Cape Floristic Region (CFR) of South Africa, where species diversity is so high that some have called it one of the world's floral kingdoms (Golblatt, 2002).

The landscape of the CFR ranges from mountains of different elevations to arid plains and fertile fields, each influenced by different amounts of rain, temperature, types of soil, and degree of grazing. These ecosystems are made of their own unique species assemblages,

particularly their flora (Holsinger, 2011). The climatic differences, available resources and biotic factors, such as competition, are the ecological filters that determine which species can or cannot inhabit a specific community. While natural selection and evolution are observable at the species level, the actual response to these filters occurs at the individual level (Clark *et al.*, 2011). The ecological features of an ecosystem and the pressures they exert determine community assemblies because species must have a particular set of traits to thrive there (Kamiyama *et al.*, 2014) (Jung *et al.*, 2014). Plants with different characteristics may be able to coexist in the same environment because they each exhibit traits suitable for fitness and survival. It is the interspecific genetic variation among species and the resulting range in phenotypes (observable traits) that permit some species to succeed when faced with unfavorable conditions. However, intraspecific variations can also be observed among individuals of the same species as they respond to environmental gradients thanks to their phenotypic plasticity (Kamiyama *et al.* 2014, Violle et al. 2012). Thus, plants can display interspecific genetic variations or phenotypical plasticity or both at the intraspecific level to cope with gradients in biotic and abiotic factors (Jung, 2010).

Interspecific variation has received more attention and has been studied to a greater extent than intraspecific variability perhaps because it is a more obviously observed and measurable phenomenon. In fact, traditional community assembly theories and the phylogenetics approaches to community ecology all have been based on interspecific variation alone, and, one could argue, have overlooked intraspecific variation (Weiher *et al*. 2011, Cavender-Bares *et al*. 2009, Violle *et al.* 2012). Intraspecific variability has only recently been researched as a promoter of phenotypic variation, even though both intraspecific and interspecific variation serve as important modes by which diversity can arise (Violle *et al.* 2012, Messier *et al.* 2010).

Messier *et al.* (2010) demonstrate that intraspecific variation can account for biodiversity just as much as interspecific variation, and that in some cases it can be even greater. In separate studies, Jung *et al.* (2010) and Kamiyama *et al.* (2014) revealed that intraspecific variability in leaf measurements across a gradient was lower than interspecific variability; the former accounted for 44% and 34% of all the variability in Jung's (2010) and 22.9-57.9% in Kamiyama's (2014) data. In both studies intraspecific variability accounted for a big portion of the variability, demonstrating its importance in community assembly and biodiversity, but also its contribution to community-level changes (Kamiyama, 2014). Because biodiversity is the result of biotic and abiotic factors that affect species at the individual level, studies of community species diversity should focus on intraspecific variation as well (Violle *et al.,* 2012).

The objective of this research was to determine if intraspecific variation in leaf morphology occurs within four species of perennial shrubs broadly distributed across three biomes, located near the towns of Calvinia, Nieuwoudtvile and Sutherland in the CFR of South Africa, that span an environmental gradient for rainfall and temperature. First, I examined the intraspecific variability in leaf traits of each species across the three different biomes, but, since biodiversity ultimately stems from natural selection at the individual level and not at the species level, I secondly evaluated intraspecific variability in leaf traits amongst individuals within each biome. Thirdly, intraspecific variation in leaf traits at the scale of the individual shrub was examined for all sampled individuals. In summary, intraspecific variation was measured (a) among the three different biomes, (b) among individuals within each biome, and (c) within individuals in each sampled biome. My question was: is intraspecific variability in leaf traits greater within individual shrubs, among shrubs of a given biome, or across all three biomes? Clark *et al.* (2011) explains that when variability is looked at solely at the interspecific level, we

may fail to observe important ecological processes that only produce patterns at the intraspecific level. I suspect that when looking at intraspecific variation, a similar problem may be happening in that most studies look for variation between individuals whereas the majority of variation will be within an individual shrub. Based on observations made when collecting leaves and based on the fact that comparing all the leaves collected from one biome against another's might mask some of the variability (Clark *et al.* 2011, Messier *et al.* 2010), I predicted that intraspecific leaf traits variability will be greatest within individuals, followed by variability between shrubs at each biome, and finally across all three biomes.

Material and Methods

Intraspecific variation in leaf traits was measured on four perennial shrub species: *Chrysocoma ciliata* (henceforth "CHCI"), *Rushia intricata* ("RUIN"), *Diospyros austro-africana* ("DIAU"), and *Aridaria noctiflora* (also known as *Mesenbryanthemum noctiflorum*) ("ARNO"). Leaf collections were made in three different locations across an environmental gradient of rainfall and temperature: nearby the town of Calvinia (mean annual temp. 16.2 ^{o}C, mean annual precip. 209 mm) (lon. 19.77549, lat. -31.45082) in the transition from Nama Karoo to Succulent Karoo ecosystems (henceforth "biome 1"); nearby Nieuwoudtville (mean annual temp. 17.6 ^{o}C, mean annual precip. 332 mm) (lon. 19.11265, lat. -31.32524) in the Fynbos and Renosterveld ecosystems (henceforth " biome 2"); and in Sutherland (mean annual temp. 14.3 ^{o}C, mean annual precip. 230 mm) (lon. 20.38178, lat. -32.11337) in the Nama Karoo and Tanqua Karoo ecosystems (henceforth "biome 3").

A total of 1200 leaves were collected, 100 leaves per species in each of three biomes. At each biome, five randomly selected shrubs per species were sampled as population representatives. Each shrub was measured (crown height and diameter) and divided into quadrants (North, South, East and West). Stratified random sampling was used to collect one branch at mid-height from each quadrant, for a total of 4 branches representative of the individual shrub regardless of the plant's sun-exposed versus shaded side, or grazing bias, etc. All the remaining branches for that species were collected at the same height across the three biomes. All the branches for *Rushia intricata, Diospyros austro-africana, Aridaria noctiflorum,* and *Chrysocoma ciliata* were collected at 17 cm, 80 cm, 15 cm and 25 cm respectively, placed in 1-gallon plastic bags with wet paper towels and brought back to the field station where they were refrigerated at 9 ^{0}C until processed. Processing occurred within 1-2 days after collection. In the lab, all the leaves from one branch were plucked, 5 leaves were selected haphazardly, and (a) their length and width were measured with a Mitutoyo caliper, (b) their thickness was measured with a Mitutoyo micrometer and (c) their area was measured with a LICOR portable area meter (model Li-3000A). The *Chrysocoma ciliata* leaves were too small to measure area with the portable area meter, so leaf area was estimated by approximating the small leaves as rectangles using width and length measurements.

All measurements were normalized by taking their natural logs (Fig. 1-A and B) and data were analyzed using ANOVA models in R (R Core Team 2014). Following Messier's *et al.* (2010) protocol, nlme R package, a mixed effects model used to fit and compare linear and nonlinear data, was used (Appendix 1). The nlme R package includes functions to perform nested ANOVA capable of handling the nested design of the data: variability of leaves within individual shrubs, variability of shrubs within a given biome and across the three biomes. The

null hypothesis tested with these analyses was that there were no differences in traits (a) across leaves within each shrub, (b) across shrubs within a biome, and (c) across biomes. To address comparisons across the 3 biomes, mean trait values were calculated for each plant and subject to a Tukey Honest Significant Differences (HSD) test of standard one-way ANOVAs. This analysis accounted for the nested design and tested the null hypothesis that there was no difference among shrubs across the three biomes.

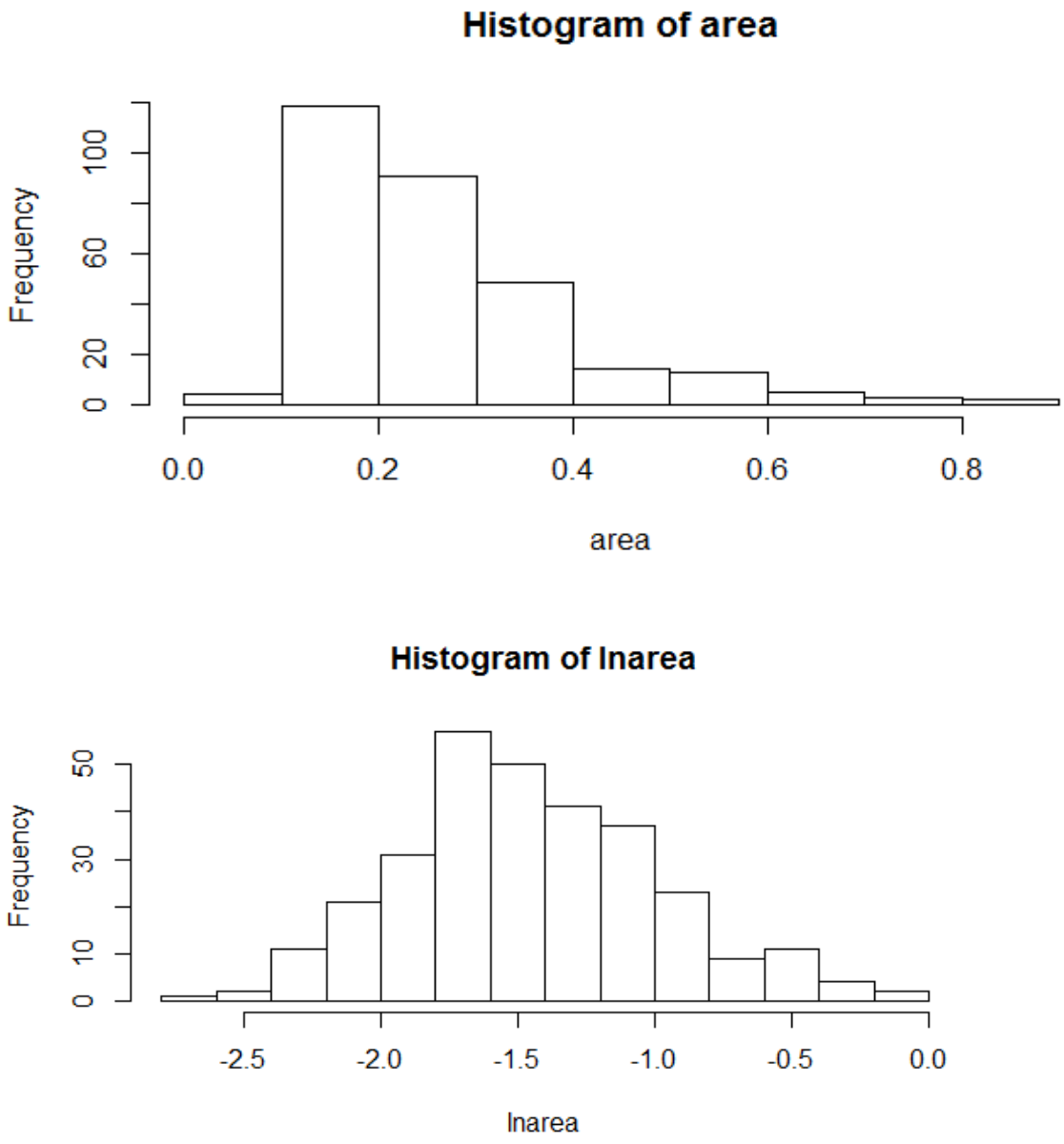

Fig. 1: Histograms of (a) leaf area and (b) ln(leaf area) for RUIN.

Results

Of the produced density plots, the ln(leaf area) and ln (leaf length) produced graphs that showed matching patterns for each species unlike those of both ln(leaf width) and ln(leaf thickness). This fact was ruled as a possible indicator that errors might have occurred during measurements (i.e. slightly crushing leaves with micrometer when measuring thickness). ln(leaf area) was deemed most reliable and was selected for boxplot graphs and for quantitative ANOVA analysis. For the other three remaining variables, only graphical non-quantitative results are presented in this thesis.

Leaf area **-** *Rushia intricata*'s leaf area was the same across the three biomes; *Chrysocoma ciliata* and *Diospyros austro-africana* had the same leaf area for biomes 1 and 3 but had bigger leaf areas in biome 2; leaves of *Aridaria noctiflorum* had about the same area for biomes 1 and 2 but they were bigger in biome 3 (Fig. 2; Table 2).

The five individuals measured for each of the focal species exhibited different medians and distribution of ln(leaf area) at any given biome (Figs. 3, 4, 5 and 6). Variance, in all but one of the focal species, was greatest within shrubs than across shrubs at each biome or across biomes. RUIN, ARNO and CHCI had 84.6%, 60.3% and 73.6% within-shrub variance respectively. DIAU's variability was greatest when comparing across biomes with 53.1%, while 42.6% of variability was within shrubs (Table 1). In both ARNO and CHCI, the variability by location and by bush within one biome was roughly the same with 19.2%-20.5% and 12.7%-13.7% respectively. RUIN showed the least amount of variability across biomes with 2.8%, while DIAU was least variable among shrubs with 4.3%. All *p*-values for this analysis were >0.05 (Table 1).

The Tukey HSD test results showed that there were no significant differences for the RUIN species ln (leaf area) when doing pair-wise comparisons between biomes; the ARNO species ln (leaf area) was only significantly different when comparing sut-cal (biomes 3 and 1); DIAU was significantly different in all but sut-cal; and CHCI was only significantly different in sut-neu (biomes 3 and 2) (Table 2).

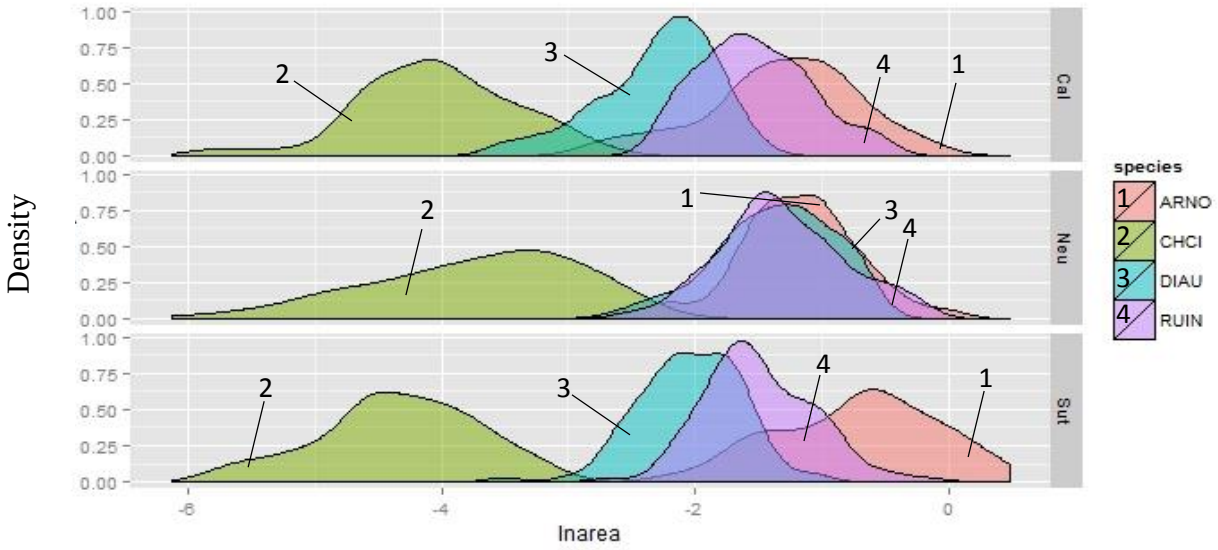

Fig. 2: Density functions of ln(leaf area) for each species at each of the three biomes ("Cal" = biome 1, "Neu" = biome 2, "Sut" = biome 3). The y-axis ("density") represents the abundance of leaves with a given ln(leaf area) for each species at each biome.

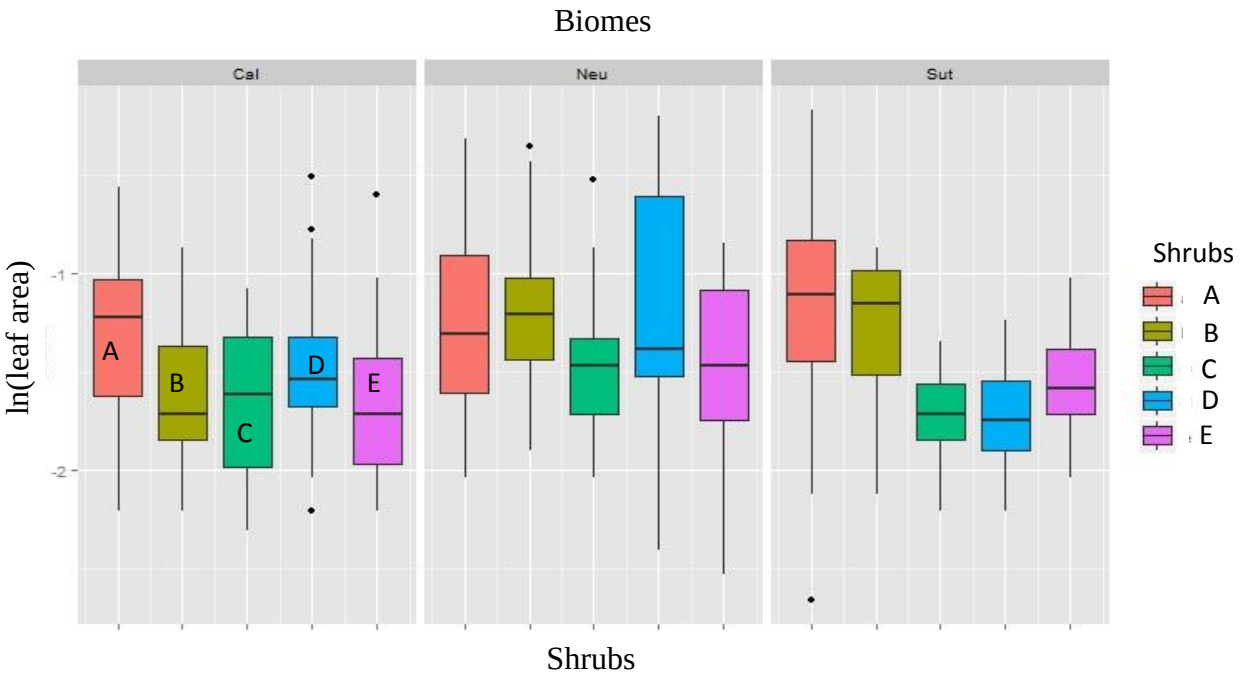

Fig. 3: Boxplots of ln(leaf area) for the 5 individuals of *Rushia intricata (*A-E) at each of the three biomes ("Cal" = biome 1, "Neu" = biome 2, "Sut" = biome 3). The y-axis represents variability in ln(leaf area) and the x-axis represents each shrub at each biome.

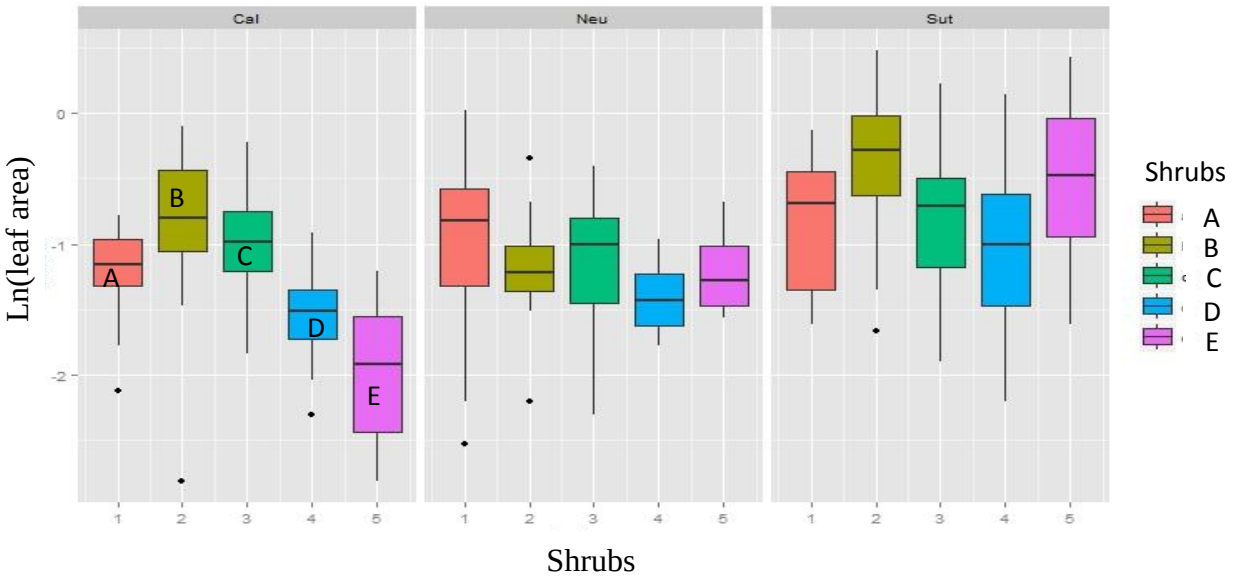

Fig. 4: Boxplots of ln(leaf area) for the 5 individuals of *Aridaria noctiflorum* (A-E) at each of the three biomes ("Cal" = biome 1, "Neu" = biome 2, "Sut" = biome 3). The y-axis represents variability in ln(leaf area) and the x-axis represents each shrub at each biome.

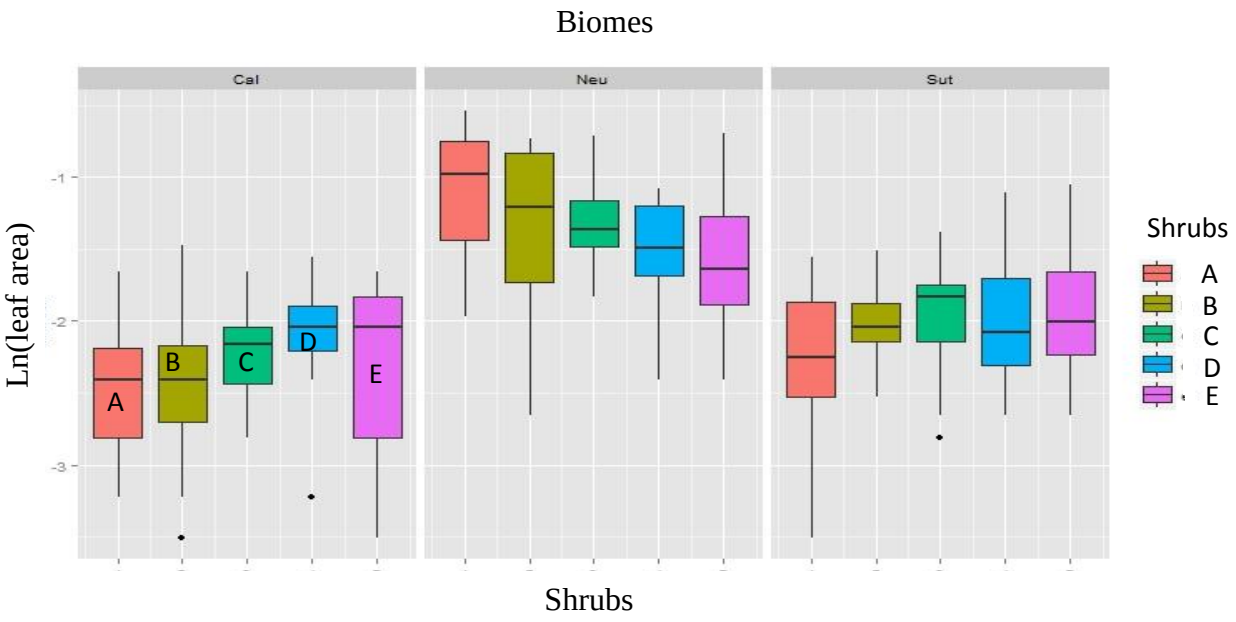

Fig. 5: Boxplots of ln(leaf area) for the 5 individuals of *Diospyros austro-africana* (A-E) at each of the three biomes ("Cal" = biome 1, "Neu" = biome 2, "Sut" = biome 3). The y-axis represents variability in ln(leaf area) and the x-axis represents each shrub at each biome.

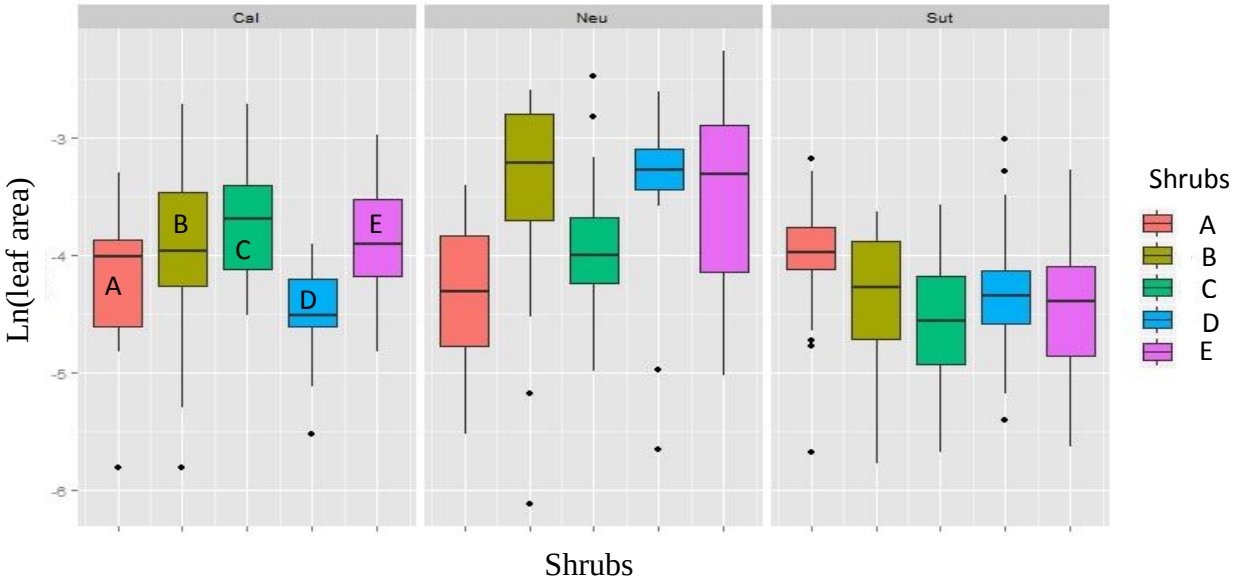

Fig. 6: Boxplots of ln(leaf area) 5 individuals of *Chrysocoma ciliata* (A-E) at each of the three biomes ("Cal" = biome 1, "Neu" = biome 2, "Sut" = biome 3). The y-axis represents variability in ln (leaf area) and the x-axis represents each shrub at each biome.

Table 1. Partitioning of variance based on results of nested ANOVA for ln(leaf area) across the three biomes across shrubs within a given biome and within a single shrub of a given biome for all 4 species. *** Variances are significantly different (null hypothesis is rejected).

Species	Variance across biomes	Variance across shrubs within a biome	Variance within a shrub	*p*-values
RUIN	2.8%	12.6%	84.6%	1.07×10^{-5}***
ARNO	19.2%	20.5%	60.3%	1.575×10^{-12}***
DIAU	53.1%	4.3%	42.6%	0.0005508***
CHCI	12.7%	13.7%	73.6%	5.830×10^{-7} ***

Table 2. Probability of observed differences in leaf area between biome pairwise comparison being the result of chance, as assessed by Tukey HSD test for ln(leaf area) of all four species. * Significant difference in ln(leaf area) for a given species and a given biome pair (null hypothesis is rejected).

	Biomes		
Species	2 vs. 1	1 vs 3	2 vs 3
RUIN	0.2210881	0.9965309	0.2487178
ARNO	0.7920348	0.0181828*	0.0585132
DIAU	0.0000016*	0.0655120	0.0000372*
CHCI	0.2022862	0.4272388	0.0224580*

Leaf length - *Rushia intricata*'s leaf length appeared to be the same across the three biomes; *Chrysocoma ciliata* and *Diospyros austro-africana* appeared to have the same leaf length for biomes 1 and 3 but longer leaf length in biome 2; leaves of *Aridaria noctiflorum* appeared to be the same length for biomes 1 and 2 but longer in biome 3 (Fig. 7).

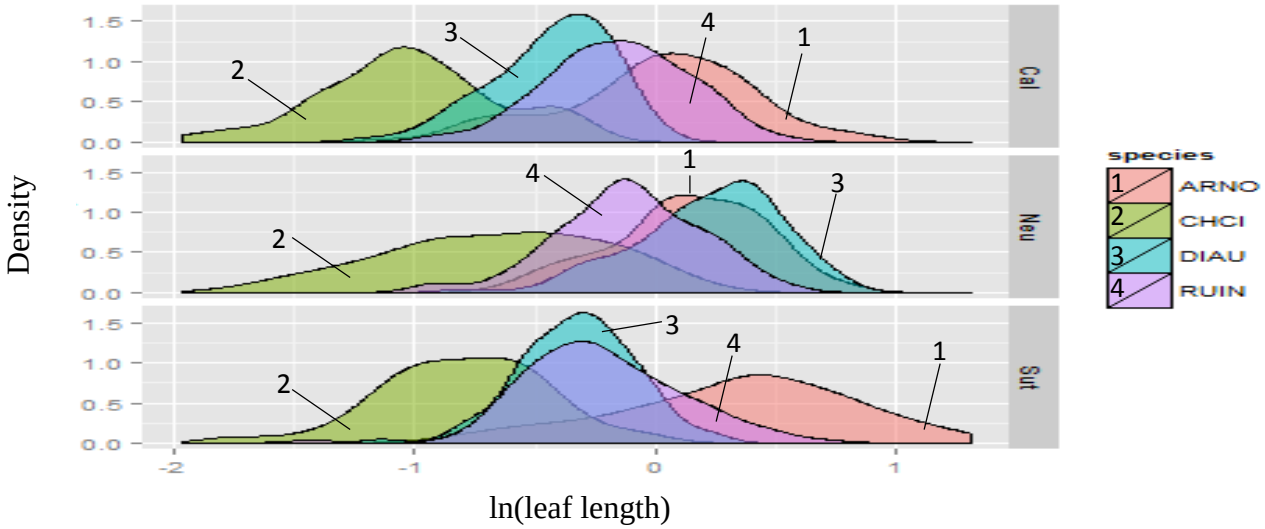

Fig. 7: Density functions of ln(leaf length) for each species at each of the three biomes ("Cal" = biome 1, "Neu" = biome 2, "Sut" = biome 3). The y-axis ("density") represents the abundance of leaves with a given ln(leaf length) for each species at each biome.

Leaf width - *Rushia intricata*'s leaf width appeared to bevthe same across the three biomes; *Diospyros austro-africana* appeared to have the same leaf width for biomes 2 and 3 with smaller leaf width in biome 1; leaves of *Aridaria noctiflorum* appeared to have the same width for biomes 1 and 2 but wider in biome 3; *Chrysocoma ciliata* appeared to have the same leaf width for biomes 1 and 2, with smaller leaf width in biome 3 (Fig. 8).

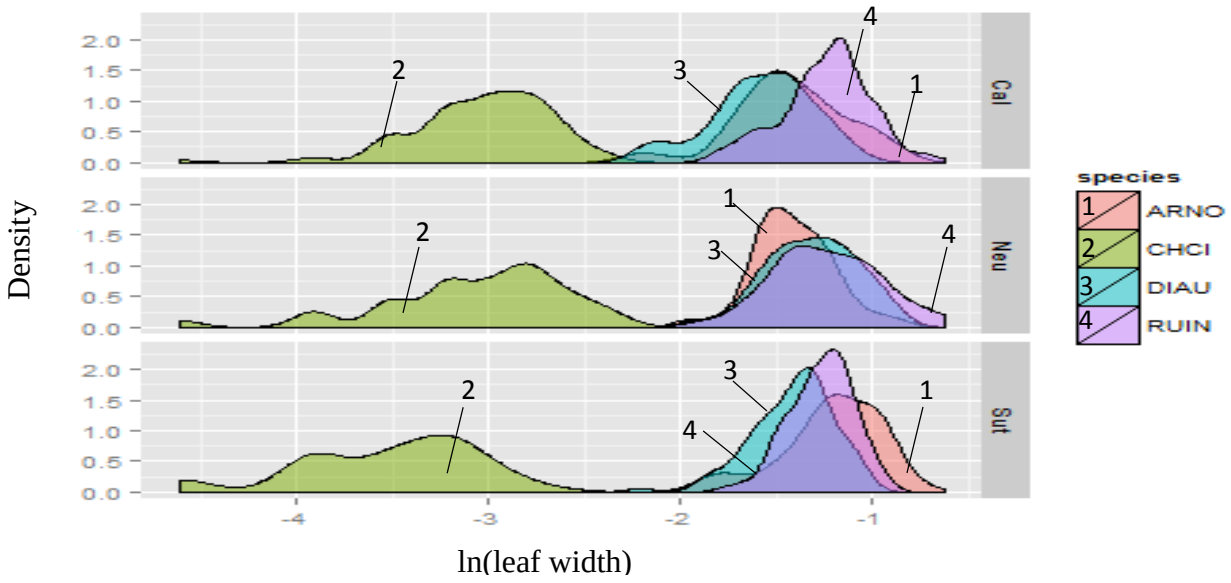

Fig. 8: Density functions of ln(leaf width) for each species at each of the three biomes ("Cal" = biome 1, "Neu" = biome 2, "Sut" = biome 3). The y-axis ("density") represents the abundance of leaves with a given ln(leaf width) for each species at each biome.

Leaf thickness - *Rushia intricata* and *Diospyros austro-africana* appeared to have the same leaf thickness across the three biomes; *Chrysocoma ciliata* appeared to have the same leaf thickness in biomes 2 and 3, with thinner leaves in biome 1; leaves of *Aridaria noctiflorum* appeared to have the same thickness in biomes 1 and 2, but thicker in biome 3; *Chrysocoma ciliata* appeared to have the same leaf width in biomes 1 and 2, with smaller leaf width in biome 3 (Fig. 9).

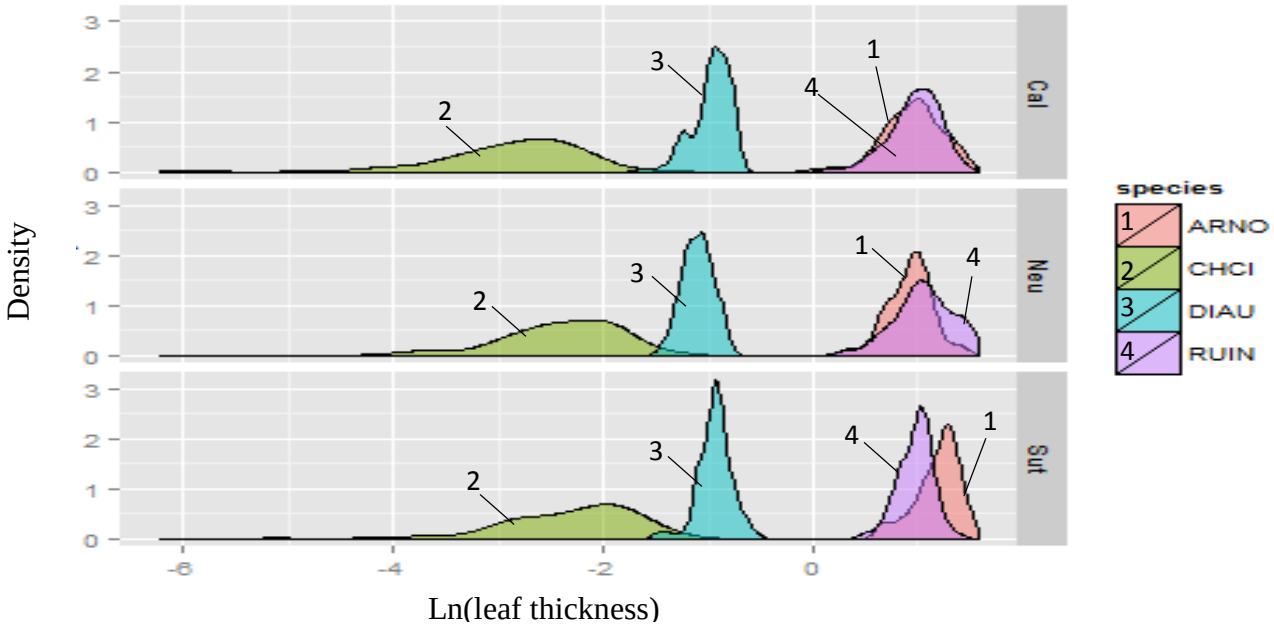

Fig. 9: Density functions of ln(leaf thickness) for each species at each of the three biomes ("Cal" = biome 1, "Neu" = biome 2, "Sut" = biome 3). The y-axis ("density") represents the abundance of leaves with a given ln(leaf thickness) for each species at each biome.

Fig. 10: *Diospyros austro-africana* leaves prior to haphazard selection (top). *Chrysocoma ciliata* leaves prior to haphazard selection (bottom). Photos: R. de Gouvenain.

Discussion

I observed a very high amount of variation in ln(leaf area) values within shrubs, compared to among shrubs of a biome and across biomes. In RUIN, the density graphs show that leaf area is approximately the same in all 3 biomes and that is supported by the Tukey HSD test where there are no significant differences between any pair-wise comparisons of the 3 biomes. However, when taking a look at the boxplot of the individual RUIN shrubs collected per each of the 3 biomes, they all have different measurements both amongst each other within 1 biome, and when comparing the 3. In fact, according the nested ANOVAS performed there is great variance (84.6%) within shrubs. The same concept is applicable to the other focal species when comparing any of their boxplots against their density graphs -a lot more detail becomes apparent in the boxplots. This is a perfect example of how aggregation can mask details, because when looking only at interspecific variation you lose all of the details that are apparent if one accounts for intraspecific variation, as mentioned by Clark *et al* (2010). In the case of this research, the aggregation is not as critical because the overall distribution indicated by the shape of the boxplots is similar to the distribution of the density graphs, minus individual shrub details. But in reality, aggregation of results can often result in complete misrepresentations of data where the data with all the details leads to one conclusion and the aggregated data lead to a different or even opposing explanation (Clark *et al*, 2010). The Tukey HSD test used in this research is based on calculated mean trait values. In this situation it is acceptable because by calculating mean values, we are not over-stating the number of replicates of plants that we have for each species, at each site. Looking at the group-level information, which can hide details at the smaller scale, may lead to false conclusions and is referred to as the ecological fallacy. To avoid this pitfall, not only should interspecific variability be accounted for when studying community assemblies and

biodiversity, but so should intraspecific variability. This research has demonstrated that the majority of the variability for leaf area occurs at the within individual level and, as so, to not account for it could also be seen as an example of aggregation.

Both CHCI and DIAU exhibited bigger leaf area in biome 2, where climate is the warmest and rainiest, while leaf area was roughly the same in the other 2 biomes. Interestingly enough, morphologically speaking these two species are almost opposite with respect to leaf area and size of the shrub. At these three studied biomes, CHCI shrubs ranged from approximately 1-2 feet with leaves between 0.009-0.07 cm^2 while DIAU shrubs ranged from about 4-8 feet with leaves between 0.04-0.5 cm^2 (Figure 10). However, these two species both demonstrated bigger leaves where both water and sunlight was most available to them. This is unusual as plants tend to make bigger leaves in places where it's hardest to obtain sunlight. Also, despite the fact that biome 2 had the highest annual precipitation out of the three biomes, one wouldn't expect bigger leaves because the climate is still fairly dry and bigger leaves tend to result in greater water loss.

It would be interesting, in future research, to track which quadrant of the sampled shrubs sampled leaves came from. This extra level in the structure of the data could then be used to compare leaves collected on the side of a shrub that is mostly exposed to the sun against leaves that are mostly on the shaded side. Also, noteworthy is the fact that DIAU grew much taller and CHCI much smaller than the other shrub species, because in the case of DIAU different sized shrubs may have been a confounding factor for my results and analysis; and with CHCI the leaves were so small that measurements may have been taken with some degree of error. In this study, while others were helping with the collecting and processing tasks, the measurer was always the same, in an attempt to minimize error and to remain consistent; but this study might also have benefited from more time in the field, collecting a bigger sample of leaves, which

could then possibly expose what are considered outliers (i.e., DIAU seems to be an exception to the observed greatest variability found at the within-shrub level).

Biomes 1, 3, and 2, in that order, are located along an increasing gradient of mean annual rainfall, while mean annual temperature increased from biome 3, to 1 and then 2, in that order. There was no apparent connection between the natural log of leaf area and these ecological gradients four the RUIN species. The natural log of leaf area for RUIN was about the same in the 3 biomes, with roughly the same means and variability. One of the possible explanations as to why the leaves would be about the same area despite variable rain or temperatures, is that RUIN is a succulent species and is better able to retain water than other plants. However, ARNO, which is also a succulent species, had bigger leaf area in biome 3 with leaf area being significantly different when comparing biome 3 and 2. Now, being a succulent, one would expect the same speculation applied to RUIN to be true for ARNO unless for some reason the latter would be less efficient at retaining water in warmer temperatures. Future research efforts should, perhaps, take these hypotheses into consideration.

DIAU had significant differences in 2 pair-wise comparisons (biomes 2-1 and 3-2) and nearly so in the third (biomes 3-1, p-value = 0.0655120). DIAU seems to be different everywhere, suggesting that it may vary its trait values in response to local environmental conditions. ARNO was only significantly different when comparing biomes 3 and 1, although it was nearly so for biomes 3 and 2 as well (p-value = 0.0585132). CHCI was only significantly different when comparing biomes 3 and 2. It appears that CHCI and ARNO are intermediate species when varying its trait values as a response to environmental gradients. However, based on the fact that I see more differences in traits between biomes 3 and 2 and I know they have

different environmental conditions, one could interpret this as suggesting that these plants are in fact responding to environmental conditions.

Conclusion

Overall, this research suggests that a significant amount of variability in nature is overlooked when only taking into account interspecific variation. It is at the individual level that species respond to ecological filters and it is intraspecific variability that accounts for how they respond to those filters. This study suggests that intraspecific variation accounts for much of the variability in nature, specifically in the CFR of South Africa, and therefore that intraspecific variability should be taken into account when investigating biodiversity and community assembly processes, as well as those that highlight intraspecific variability as a major component of ecological and evolutionary processes (Jung *et al* 2010, Violle *et al* 20112, and Kamiyama *et al* 2014).

Acknowledgements

Votes of gratitude go out to Dr. Roland de Gouvenain of Rhode Island College and Dr. John Silander of the University of Connecticut for making the trip to South Africa a reality, thus creating the opportunity for this research. The two plus Hayley Kilroy Mollmann are gratefully acknowledged for assisting in the protocol set up and data collection. A big thanks to Matthew Aiello-Lammens as well for aiding with the analysis and familiarization with the R program. Finally, a special thanks to Douglas Euston-Brown, a South African Botanist, who helped choose the focal species for this research and helped identify them in the field. Funding for the research, travel, living expenses and student stipend was provided by the National Science Foundation through a Supplemental RUI grant attached to the University of Connecticut's project *Dimensions of Biodiversity: Parallel Evolutionary radiations in Protea and Pelargonium in the Greater Cape Floristic Region* under grant No. DEB-1046328.

Work Cited

Cavender-Bares, Jeannine *et al*. The merging of community ecology and phylogenetic ecology. Ecology Letters 12 (2009): 693-715. Print.

Carlson, Jane E., Kent E. Holsinger and Rachel Prunier. Plant Responses to Climate in the Cape Floristic Region of South Africa: Evidence for Adaptive Differentiation in the Proteaceae. Evolution 65 (2011): 108-124. Print.

Clark, James S. *et al*. Individual-scale variation, species-scale differences: inference needed to understand diversity. Ecology Letters 14 (2011): 1273-1287. Print.

Goldblatt, Peter, John C. Manning. Plant Diversity of the Cape Region of South Africa. Annals of the Missouri Botanical Garden 89 (2002): 281-302. Print.

Jung, Vincent, *et al.* Intraspecific variability and trait-based community assembly. Journal of Ecology 98 (2010): 1134-1140. Print.

Kamiyama, Chiho, *et al.* Leaf-trait responses to environmental gradients in moorland communities: contribution of intraspecific variation, species replacement and functional group replacement. Ecological Reseach 29 (2014): 607-617. Print.

Messier, Julie, Brian McGill and Martin J. Lechowicz. How do traits vary across ecological scales? A case for trait-based ecology. Ecology Letters 13 (2010): 838-848. Print.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Violle, Cyrille *et al.* The return of the variance: intraspecific variability in community ecology. Trends in Ecology and Evolution 27 (2012): 244- 252. Print.

Weiher, E. *et al.* Advances, challenges and a developing synthesis pf ecological community assembly theory. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 366 (2011): 2403-2313. Print.

Appendix 1 - R Script

```
#Attach the data
attach(BrunoData05)
#creating data subsets per locations
RUIN<-subset(BrunoData05, specod==1)
ARNO<-subset(BrunoData05, specod==2)
DIAU<-subset(BrunoData05, specod==3)
CHCI<-subset(BrunoData05, specod==4)
#Creating data subsets per species per locations
attach(RUIN)
RUINCal<-subset(RUIN, loccode==1)
RUINNeu<-subset(RUIN, loccode==2)
RUINSut<-subset(RUIN, loccode==3)
attach(ARNO)
ARNOCal<-subset(ARNO, loccode==1)
ARNONeu<-subset(ARNO, loccode==2)
ARNOSut<-subset(ARNO, loccode==3)
attach(DIAU)
DIAUCal<-subset(DIAU, loccode==1)
DIAUNeu<-subset(DIAU, loccode==2)
DIAUSut<-subset(DIAU, loccode==3)
attach(CHCI)
CHCICal<-subset(CHCI, loccode==1)
CHCINeu<-subset(CHCI, loccode==2)
CHCISut<-subset(CHCI, loccode==3)
#Creating histograms to show that natural log normalized data
attach(RUIN)
hist(area)
hist(lnarea)
#looking at density plots for the measured traits (all species) by location
```

```
library("ggplot2", lib.loc="~/R/win-library/3.1")
ggplot( data = BrunoData05, aes( x = lnlength, fill = species ) ) +
  geom_density( alpha = 0.5 ) +
  facet_grid( location ~ . )
ggplot( data = BrunoData05, aes( x = lnwidth, fill = species ) ) +
  geom_density( alpha = 0.5 ) +
  facet_grid( location ~ . )
ggplot( data = BrunoData05, aes( x = lnthickness, fill = species ) ) +
  geom_density( alpha = 0.5 ) + facet_grid( location ~ . )
#histograms for the measured traits by location
ggplot( data = BrunoData05, aes( x = lnlength, fill = species ) ) +
  geom_histogram( position = "dodge" )+ facet_grid (location ~ . )
ggplot( data = BrunoData05, aes( x = lnwidth, fill = species ) ) +
  geom_histogram( position = "dodge" )+ facet_grid (location ~ . )
ggplot( data = BrunoData05, aes( x = lnthickness, fill = species ) ) +
  geom_histogram( position = "dodge" )+ facet_grid (location ~ . )
ggplot( data = BrunoData05, aes( x = lnarea, fill = species ) ) + geom_histogram( position = "dodge" )+ facet_grid
(location ~ . )
#Boxeplots by species that show individual bushes
attach(RUIN)
ggplot(data=RUIN, aes(x=bushnum,y=lnlength, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=RUIN, aes(x=bushnum,y=lnwidth, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=RUIN, aes(x=bushnum,y=lnthickness, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=RUIN, aes(x=bushnum,y=lnarea, fill=bush))+geom_boxplot()+ facet_wrap(~location)
attach(ARNO)
ggplot(data=ARNO, aes(x=bushnum,y=lnlength, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=ARNO, aes(x=bushnum,y=lnwidth, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=ARNO, aes(x=bushnum,y=lnthickness, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=ARNO, aes(x=bushnum,y=lnarea, fill=bush))+geom_boxplot()+ facet_wrap(~location)
attach(DIAU)
ggplot(data=DIAU, aes(x=bushnum,y=lnlength, fill=bush))+geom_boxplot()+ facet_wrap(~location)
```

```
ggplot(data=DIAU, aes(x=bushnum,y=lnwidth, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=DIAU, aes(x=bushnum,y=lnthickness, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=DIAU, aes(x=bushnum,y=lnarea, fill=bush))+geom_boxplot()+ facet_wrap(~location)
attach(CHCI)
ggplot(data=CHCI, aes(x=bushnum,y=lnlength, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=CHCI, aes(x=bushnum,y=lnwidth, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=CHCI, aes(x=bushnum,y=lnthickness, fill=bush))+geom_boxplot()+ facet_wrap(~location)
ggplot(data=CHCI, aes(x=bushnum,y=lnarea, fill=bush))+geom_boxplot()+ facet_wrap(~location)
#attaching packages for nested ANOVA
library(nlme)
library(ape)
library(dplyr)
#perform lme fit (CHCI)
chci.lme<- lme(data= filter(BrunoData05, species== "CHCI"), lnarea~1, random= ~1| location/bush)
summary (chci.lme)
chci.varcomp<-varcomp(chci.lme)
chci.varcomp
#%variance in each level(CHCI)
chci.varcomp/sum(chci.varcomp)
#perform lme fit (ARNO)
arno.lme<- lme(data= filter(BrunoData05, species== "ARNO"), lnarea~1, random= ~1| location/bush)
summary (arno.lme)
arno.varcomp<-varcomp(arno.lme)
arno.varcomp
#%variance in each level(ARNO)
arno.varcomp/sum(arno.varcomp)
#perform lme fit (RUIN)
ruin.lme<- lme(data= filter(BrunoData05, species== "RUIN"), lnarea~1, random= ~1| location/bush)
summary (ruin.lme)
ruin.varcomp<-varcomp(ruin.lme)
ruin.varcomp
```

```
#%variance in each level(RUIN)
ruin.varcomp/sum(ruin.varcomp)
#perform lme fit (DIAU)
diau.lme<- lme(data= filter(BrunoData05, species== "DIAU"), lnarea~1, random= ~1| location/bush)
summary (diau.lme)
diau.varcomp<-varcomp(diau.lme)
diau.varcomp
#%variance in each level(DIAU)
diau.varcomp/sum(diau.varcomp)
#p-values % variance
area.chci.lm<-lm(data=filter(BrunoData05, species == "CHCI"),
lnarea~location * location/bush)
anova(area.chci.lm)
area.ruin.lm<-lm(data=filter(BrunoData05, species == "RUIN"),
lnarea~location * location/bush)
anova(area.ruin.lm)
area.arno.lm<-lm(data=filter(BrunoData05, species == "ARNO"),
lnarea~location * location/bush)
anova(area.arno.lm)
area.diau.lm<-lm(data=filter(BrunoData05, species == "DIAU"),
lnarea~location * location/bush)
anova(area.diau.lm)
#Test Across Biomes- TukeyHSD (probability of differences)
library(dplyr)
BrunoData05summary<- BrunoData05%>%
group_by(species, location, bush, add =FALSE)%>%
summarize(lnlength=log(mean(length)),
        lnwidth=log(mean(width)),
        lnthickness=log(mean(thickness)),
        lnarea= log(mean(area)))
TukeyHSD(aov(data=filter(BrunoData05summary, species=="CHCI"),
```

```
        lnarea~location))
TukeyHSD(aov(data=filter(BrunoData05summary, species=="RUIN"),
        lnarea~location))
TukeyHSD(aov(data=filter(BrunoData05summary, species=="ARNO"),
        lnarea~location))
TukeyHSD(aov(data=filter(BrunoData05summary, species=="DIAU"),
        lnarea~location))
```