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# Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species

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To understand herbivore selection in natural plant populations, it is important to understand the landscape of plant chemical phenotypes that herbivores face and the sources of variation that will define this landscape. We studied the spatial patterns of variation in leaf secondary chemistry of the tropical tree *Quararibea asterolepis*, Pitt. (Bombacaceae) in a natural population on Barro Colorado Island, Panama, and used this background to discuss hypotheses of natural selection by herbivores. *Quararibea* plants collected from different sites had consistent differences in their chemical phenotypes. Some of these differences were explained by developmental and environmental sources of variation. Canopy trees had 13% lower yield of leaf extracts than gap seedlings, explained by 41% lower concentrations of the more abundant metabolites in the secondary compound profile. Also, plants growing in gaps had 25% higher yield than those in the understory, explained by two-fold increases in the concentration of some of the less polar secondary compounds in the profile. Differences in soil type did not affect the secondary chemistry of leaves, but sites with different topography had differences in the secondary compound profile that were not explained by any of the measured environmental sources of variation. Neighboring parent-offspring pairs and sibling/half sibling clusters displayed equal or higher variance among themselves than unrelated individuals at farther distances. Assuming that related plants should be more similar in their phenotypes, this pattern is consistent with local selection by herbivores overriding the similarity of related plants in a frequency- or distance-dependent manner.

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Plants display an enormous diversity of secondary metabolites generally believed to be the outcome of their coevolutionary interactions with herbivores (Ehrlich and Raven 1964). Within species, plants express complex mixtures of secondary chemicals and, among individuals of the same species, these mixtures frequently differ in the total or relative concentration of each compound. As a result, natural plant populations exhibit a large amount of phenotypic variation in secondary metabolites. The levels and spatial structure of this variation may influence the capacity of herbivores and pathogens to adapt and exert selection on plant chemicals (Zangerl and Berenbaum 1993, Thrall et al. 2001) and for that reason, the patterns of phenotypic variation in plant secondary chemistry have strong ecological significance that is often overlooked and are an important factor in understanding the coevolutionary interactions in natural populations.

Population variation of plant chemical phenotypes can be explained by a combination of genetic (Berenbaum and Zangerl 1992), developmental (Bowers and Stamp 1993) and environmental (Agrell et al. 2000) sources of variation. Thus, the age structure, environmental heterogeneity and limits in gene flow in a natural

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population, will determine the variability and the spatial structure of the secondary chemistry landscape of plants. Trees provide a potentially rich system in which to study secondary chemistry variation in a spatial context. Due to their long life span and large developmental changes, trees may benefit from high levels of developmental and environmental variation in their chemical phenotype. Furthermore, the spatial variation in tree secondary chemistry may provide a horizontal and vertical structure to the secondary metabolite landscape presented to the herbivores; and due to the difference in life spans, this structure may remain constant over many generations of herbivores, favoring their adaptation to particular phenotypes.

Specialist invertebrate herbivores can potentially adapt to the phenotypes of particular localities (Berenbaum and Zangerl 1998, Zangerl and Berenbaum 2003), forest strata (Kearsley and Whitham 1989) and perhaps even individual trees (Van Zandt and Mopper 1998). Herbivores can potentially influence the spatial structure of tree populations and their phenotype distribution by exerting selection pressure on tree recruitment. For example, Janzen (1970) and Connell (1971) suggested that tropical specialist herbivores caused increased mortality of seedlings growing under the canopy of their mother tree and that this increased spacing between recruits. Langenheim and Stubblebine (1983) extended this hypothesis and suggested that if herbivores adapt to the particular phenotype of an adult tree, they can cause the selective mortality of seedlings whose chemistry is more similar to that of the mother, in the same distance-dependent fashion. An analogous way in which herbivores may influence the spatial distribution of phenotypes is by preferentially attacking the locally common phenotypes in a frequency-dependent fashion. In plants, frequency-dependent selection is best known from studies in plant–pathogen gene-for-gene interactions (Chaboudez and Burdon 1995), but it can potentially be applied to quantitative traits like secondary chemistry (Berenbaum and Zangerl 1998, Zangerl and Berenbaum 2003). Either of these two spatial patterns of selection, frequency- or distance-dependent, could leave a signature in the variability of phenotypes of neighboring conspecifics. Neighboring plants would benefit from being as different as possible, and the variability of the population should be maximized at small, as well as large, scales.

In this paper we describe the spatial patterns of variation in the leaf secondary chemistry of one population of the tropical tree *Quararibea asterolepis*, Pitt. (Bombacaceae). We tested the effects of different environmental and developmental sources of variation on the chemical phenotype of plants and evaluated their impact on the secondary compound landscape of the population. We addressed the following questions: were there spatial differences in the chemical phenotype of this species? Did plant size, soil type or light levels influence this variability? What was the phenotypic variability of the population at different spatial scales? And are the observed patterns of variability consistent with hypotheses of local selection by herbivores?

## Study system and methods

### Study site

The survey was conducted on Barro Colorado Island (BCI; 9°09′N, 79°51′W), a field station run by the Smithsonian Tropical Research Institute in Panama. BCI is a 1500 ha artificial island in the Gatun Lake, created when the Panama Canal was flooded in 1914. The climate is typical of a lowland tropical moist forest. Average daily temperature is 27°C and the average total yearly rainfall is 2600 mm, 90% of which falls during the rainy season from May through December (Leigh et al. 1996). The maximum elevation on BCI is a plateau 137 m above the lake level. The bed rock of the plateau is an Andesite flow that has weathered into a clay-rich, yellow-brown oxisol $<50$ cm deep. The flanks of the island have two types of formations: the Caimito and the Bohio. These formations are sedimentary and have weathered into a silty-clay, yellow-brown alfisol $<30$ cm deep (Yavitt 2000).

### Study species

*Quararibea asterolepis* is a Neotropical, shade-tolerant, canopy species found in Costa Rica and Panama (Croat 1978). On BCI, this species is very abundant, and the only other species in the genus is very rare. *Quararibea* is self-incompatible (Hamrick and Murawski 1990) and pollinated by bats and moths (Murawski et al. 1990). Its large seeds ($>1$ cm long) are dispersed by bats, monkeys and parrots (Murawski et al. 1990, De Steven 1994) but most recruitment of seedlings and saplings occurs close to the mother tree (Condit et al. 1992, De Steven 1994). *Quararibea* adult trees exchange their leaves twice a year, at the beginning and the end of the wet season, with peaks of leaf flush in May and December. Subcanopy trees keep their leaves for at least a year and saplings keep them even longer (Wright and van Schaik 1994, Wong et al. 1990, Barone 2000). There are few data on pathogens that attack this species, but some herbivores that feed on *Quararibea* have been described (Wong et al. 1990, Barone 2000).

### Plant sampling

All leaf collections were done in December 2000 from plants found along the Armour trail, a roughly 2 km

long transect from the hilltop down to the SW slope of the island (Fig. 1). Preliminary studies showed that expanding leaves had 55% higher concentration of extracts ($p = 0.02$) and different secondary compound profiles ($p < 0.0001$) than mature leaves. Since these changes in chemical phenotype during leaf expansion can complicate the comparison among plants, we collected only mature leaves (one or two) from each *Quararibea* plant. For seedlings and smaller saplings we randomly collected any mature leaf. In the case of larger juveniles we collected a leaf from a lower branch that could be reached by hand. For larger trees we shot a small branch, usually in the lower canopy, and selected an intact leave from it.

Whenever we found a *Quararibea* tree or juvenile, we looked for nearby conspecifics that could be related and therefore possibly subject to distance- or frequency-dependent selection. Juveniles, especially seedlings, were usually found in groups of plants of equal size growing nearby (within a 5 m radius). We called these groups "clusters" and interpreted them to be the outcome of the germination of cohorts of seeds from the same mother tree and, thus, possibly siblings or half siblings.

We recorded the spatial coordinates of each plant sampled and categorized them into the groups described in Table 1. The samples were transported from the field into sealed plastic bags, and once in the laboratory, they were vacuum dried the same day and stored in a dry cool place until analysis.

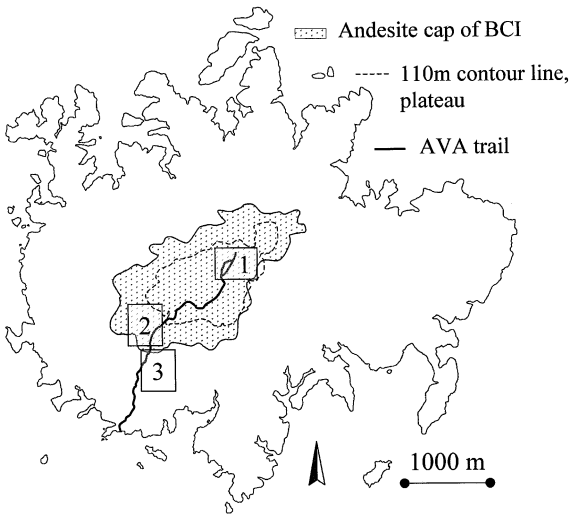


Fig. 1. Map of BCI with the soil types, topography and sites along the AVA trail where *Quararibea* plants were more abundant. 1 = top-oxisol site, 2 = slope-oxisol site and 3 = slope-alfisol site.

## Analyses of leaf secondary chemistry

Preliminary studies showed that the polar fraction of the leaf extracts contained most of the secondary chemicals that differentiate *Quararibea* from other species. When using the same extraction method on three other unrelated species, *Protium tenuifolium* (Burseraceae), *Heisteria concinna* (Olacaceae) and *Pouteria unilocularis* (Sapotaceae), the HPLC peaks analyzed in this paper were found only in *Quararibea*. Spot color tests of the *Quararibea* polar extracts, showed some positives with the Folin–Denis test for phenolics and the o-toluidine test for nitrogen-containing compounds but negatives for the Dragendorf's test for alkaloids.

The polar fraction was extracted in the following way. Dry leaf material (leaf weight $= 20 - 30 \pm 0.1$ mg) was ground in an eppendorf tube and extracted overnight at 5°C in 1.5 ml of 50% MeOH. The extract was filtered, loaded onto C18 solid phase extraction cartridges (Burdick & Jackson, capacity 1 ml) and washed with 1.5 ml of 50% MeOH to eliminate chlorophyll, lipids and other non-polar metabolites. The resultant yellowish solution was then vacuum dried and weighed (extract weight $\pm 0.1$ mg). The yield of the polar extracts (extract weight (mg)/leaf weight (mg)) was quantified for each sample and was used as one of the variables to describe the chemical phenotype.

To characterize the secondary compound profile, the extracts were re-dissolved in 1 ml of 50% MeOH. A 15 µl sample was injected in the HPLC [Pump: Hitachi L-6200; Detector: Hitachi L-4500 Diode Array Detector; scan $\lambda = 320$ nm; column: Varian Omnisorb C18, 5 µm, $250 \times 4.6$ mm ID; Eluents: MeOH and 20 mM phosphate buffer (pH 2.1); flow rate 1 ml $min^{-1}$ with a linear gradient from 10% to 20% MeOH in 20 min and constant 20% MeOH for 5 min]. All peaks observed at 320 nm were integrated and named by their retention time. The area under each peak was divided by the weight of the leaf sample to correct for differences in the size of the samples analyzed. These values (peak areas/leaf weight (mg)) are proportional to the concentration of the metabolites in the leaf and, while they can not be used to compare two peaks in the same chromatogram, they give information about the changes in concentration of each metabolite between samples.

## Data analyses

All analyses were done using SAS software (SAS Institute, v8.1). We analyzed the yield separately from the secondary compound profile of the leaves. Yield (in units of mg/mg) was treated as a single dependent variable to describe changes in the mass concentration of polar extracts in a leaf. On the other hand, the secondary compound profile (in units of area/mg) comprised dozens of HPLC peaks (metabolites) and

Table 1. Sample size within each category for the analysis of the sources of variation.

| Topography-Soil[a] | Seedlings ( <0.5 m) | | Saplings (0.5–5 m) | | Adults ( >5 m) | |
|---|---|---|---|---|---|---|
| | Gap | Understory | Gap | Understory | Canopy | Total |
| Slope-Alfisol | 23 | 11 | | 1 | 16 | 51 |
| Slope-Oxisol | 17 | 8 | 9 | 7 | 4 | 45 |
| Hilltop-Oxisol | 3 | 9 | 2 | 25 | 8 | 47 |
| Total | 43 | 28 | 11 | 33 | 28 | 143 |

[a]Soil type was categorized based on the maps provided in Leigh (1996).

some of them may be biosynthetically related. Thus, to aid in the interpretation of the results, the secondary compound profile was summarized before the analyses using principal components analysis.

### Summary of the secondary compound profile

Of the 59 peaks detected across all HPLC profiles (Fig. 2), 15 peaks were discarded because they were present in only a few of the samples and had areas close to the detection limit of the instrument. Among the remaining 44 peaks, 26 had poor resolution and overlapped in many of the chromatograms. The areas of these overlapping peaks were summed, collapsing the 26 peaks into nine distinct groups. The resultant 27 peaks, consisting of the nine groups of overlapping peaks and 18 individual peaks, were factorized using principal component analyses (PCA, SAS proc factor, covariance matrix, varimax rotation). These conditions facilitate the identification of the covarying peaks that explain the variation of each principal component (PC). The group of peaks that explain the variation of one PC represent metabolites whose concentrations covary throughout the population, probably indicating that they are biosynthetically related. The robustness of this grouping of peaks was investigated changing the PCA parameters and using subsamples of the data. Most of the peaks were grouped in a similar way under different conditions, suggesting that these PCs are good descriptors of the relationship among the peaks.

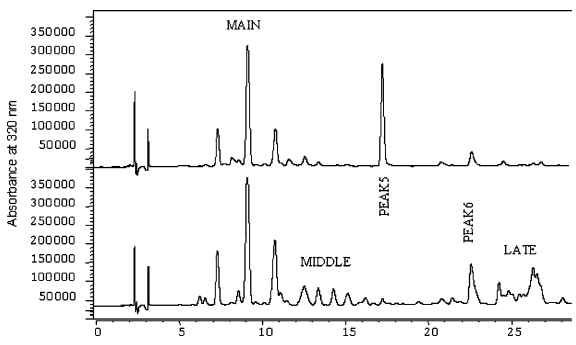


Fig. 2. Two examples of HPLC traces obtained from mature leaves of *Quararibea*. The labels indicate the location of the covarying peaks that belong to five of the groups. GROUP1 and GROUP3 comprise several odd peaks throughout the chromatogram and are not labeled.

For all the analyses described in the results, we used the first seven PCs, which we renamed according to the general position and characteristics of the peaks they grouped (Table 2). These PCs had eigenvalues higher than one, explained the covariance of 23 of the 27 peaks analyzed and accounted for 97% of the variance in the secondary compound profile. The remaining four peaks did not contribute much to the variability of the system. Since the PCs are non-dimensional variables that can not be used to describe the magnitude of the difference in concentrations between samples, we calculated another set of summary variables. These new variables were the sum of the areas of the peaks or metabolite groups that explained each PC. Because we are summing the areas of covarying peaks, these values are roughly proportional to the concentrations of that group of metabolites in leaves.

## Results

There were significant levels of variation in the yield and secondary compound profile of mature leaves throughout the population. The yield varied more than threefold, ranging from 60 mg to 210 mg per gram of dry leaf. The summed areas of the metabolite groups also presented substantial variation. The least variable of the metabolite groups (MIDDLE, Table 2) exhibited more than a seven-fold variation in concentration, while other metabolite groups varied up to a 32-fold (GROUP1) or were altogether absent in some plants (Table 2).

### Spatial differences in the chemical phenotype of the population

To look for large scale spatial differences in the chemical phenotypes of *Quararibea* we compared two different sites where this species was especially abundant. Those two sites were 1000 m away from each other and were named by their topography as: hilltop and slope (Fig. 1). There were significant differences between sites in the yield of leaf extracts ($F_{1,140} = 31.77$ $p < 0.0001$) and in

Table 2. Name and description of the seven principal components used to summarize the secondary compound profile of *Quararibea* mature leaves. The retention time lists the position of the covarying peaks that explain each principal component (coefficient >0.5); and the last two columns give the summed areas of those peaks, population's maximum and minimum.

| Principal component | Eigenvalue | Retention time (minutes) | Sum of areas | |
|---|---|---|---|---|
| | | | min | max |
| GROUP1 | 5.0 | 6.2, 10.1, 19.7 | 260 | 8528 |
| MAIN | 4.0 | 6.8, 7.6, 8.9, 9.5, 11.2, 13.9 | 7886 | 59202 |
| GROUP3 | 3.5 | 10.5, 13.6 | 0 | 6063 |
| MIDDLE | 2.1 | 13.0, 14.8, 15.7, 16.8, 20.0 | 2218 | 16837 |
| PEAK5 | 1.5 | 17.8 | 0 | 11679 |
| PEAK6 | 1.1 | 22.9 | 0 | 9020 |
| LATE | 1.1 | 21.0, 22.0, 24.8, 26.0, 26.8 | 1497 | 33282 |

the principal components (PCs) of the multivariate secondary compound profile (Wilk's $\lambda$ $F_{7,136}=2.58$ $p=0.0159$). Plants growing on the slope had higher yield ($0.14\pm0.02$) than those on the hilltop ($0.12\pm0.02$) and the difference was significant even after controlling for light level and size class (3-way ANOVA, effect of site $F_{1,123}=4.34$ $p=0.0391$). To describe the multivariate differences in the chemical profile, we used canonical discriminant analysis (SAS proc Candisc), a test that evaluates multivariate differences between categorical groups of samples. Candisc creates linear functions of the metabolite PCs, and each coefficient in the function (canonical coefficient) is proportional to the importance of that PC in the difference between the groups. When comparing the two sites using Candisc, we determined that the difference between the slope and hilltop sites was largely driven by variation in the MIDDLE PC (canonical coefficient $=0.70$). However, this difference was significant only for the older established size classes. Adults and saplings growing on the slope respectively had 37% and 51% higher sum of areas of MIDDLE metabolites than those on the hilltop, but seedlings could not be differentiated between sites.

## Sources of variation in the chemical landscape

We tested how plant size, light gaps and soil type affected the chemical phenotypes of mature leaves. To identify significant effects and interactions among the three sources of variation, we used 3-way ANOVA on the yield and MANOVA on the PCs that summarize the secondary compound profile. Since we suspected that clusters of juveniles (plants of equal size and growing close together) were composed of siblings or half siblings, and since those relationships would break the assumption of independence in the ANOVA, we averaged the phenotypes of these clusters. Both the yield and the secondary compound profile had significant differences for the effects of light gaps and size class of the plant, while soil type had no effect on the phenotype (Table 3). Due to small sample size in some categories, not all interactions could be estimated, but those that were showed no significant interactions between the sources of variation. Most important, since there were no interactions between light and size, they can be described independently of each other.

## Effect of light gaps

The yield and the secondary compound profile of *Quararibea* leaves were significantly different between plants growing at different light levels (Table 3). Since adults have only one level of light availability (canopy), we used only juvenile trees (gap and understory) to describe the effect of light. Seedlings and saplings growing in gaps had on average 28% higher yields than in the understory (Fig. 3). This difference in yield was driven by changes in the PCs LATE, PEAK5 and MIDDLE (canonical discriminant analysis $R^2=0.55$, $p<0.0001$; canonical coefficients, 1.24, 0.77 and 0.65, respectively). These metabolite groups had up to 120% higher sum of areas in gaps than in the understory (Fig. 4).

## Effect of plant size

The chemical phenotype of *Quararibea* changed significantly between plants in different size classes (Table 3). Bigger plants had lower yields within each light level (Fig. 3) and the PCs of the secondary compound profile were also good descriptors of the effect of size. Canonical discriminant analysis (canonical $R^2=0.55$, $p<0.0001$) indicated that the differences between size classes were explained mostly by changes in the concentration of MAIN metabolites (canonical coefficient $=1.27$). MAIN metabolites had 40% lower sum of areas for bigger plants than for smaller ones (Fig. 5). Three other metabolite groups (MIDDLE, PEAK5 and PEAK6) also had significantly different concentrations for the different size classes, but their effect was not as dramatic as that of MAIN metabolites.

Table 3. Effects and interactions of the environmental sources of variation in the yield and in the multivariate secondary compound profile of *Quararibea* leaves (SAS, proc glm).

| Source | Yield | | | Profile | | |
|---|---|---|---|---|---|---|
| | DF | F value | Pr > F | DF | F value | Pr > F |
| Size | 2 | 3.14 | 0.0498 | 14/124 | 3.09 | 0.0004 |
| Light | 1 | 20.97 | <0.0001 | 7/62 | 3.62 | 0.0025 |
| Size × light | 1 | 0.23 | 0.6349 | 7/62 | 0.22 | 0.9788 |
| Soil | 1 | 0.03 | 0.8636 | 7/62 | 0.23 | 0.9771 |
| Size × soil | 2 | 2.97 | 0.0893 | 7/62 | 0.29 | 0.9574 |
| Light × soil | 0 | – | – | – | – | – |
| Size × light × soil | 0 | – | – | – | – | – |

## Local patterns of phenotypic variability

To look for patterns of variation consistent with local selection by herbivores, we compared phenotypes of plants at different spatial scales. The phenotypes were compared calculating Euclidean distances. The Euclidian distance is the squared difference of all the PCs that summarize the secondary compound profile, in other words, the phenotypic difference in chemical profile between two plants. These phenotypic differences are non-dimensional and can be compared directly or used to calculate the variance of a group of plants. The variance is the average of the squared differences (Euclidean distances) between each individual and the mean (divided by degrees of freedom). Using the data on the spatial coordinates of the plants and their phenotypes, we looked for the distribution of phenotypic differences expected under distance- and frequency-dependent selection by herbivores.

## Distance-dependent variability

Distance-dependent selection by herbivores (sensu Langenheim and Stubblebine 1983) predicts that successful recruits that become established nearby differ in chemical phenotype from the parent plant. Due to developmental differences, seedling phenotypes are very different from adults regardless of their location, so we limited the comparisons to saplings. To test this hypothesis we compared the average adult-sapling phenotypic difference between near saplings ( <10 m) and saplings growing further away (10–500 m). In the presence of distance-dependent selection, we expected no difference between the two groups. Alternatively, in the absence of selection, near saplings should be more similar to the adult tree because they are likely to be its offspring. There were 18 saplings growing under the canopy of seven adults, though only one adult had more than two saplings under its canopy. Three of the seven adults had nearby saplings that were more similar in phenotype as

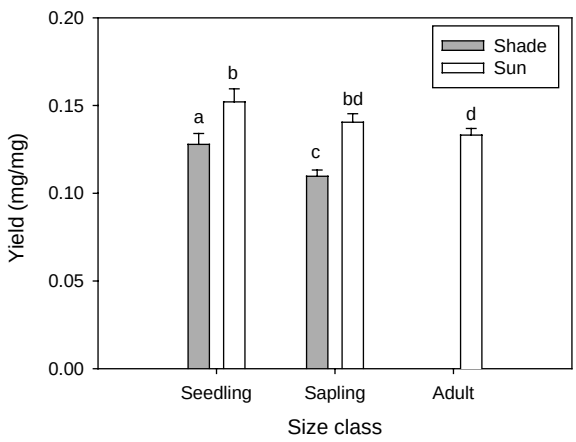


Fig. 3. Average yield of leaf extracts (±se) in plants classified by size and light level. Means with the same letter are not different at $\alpha = 0.05$ in planned comparisons of the effect of light within each size class or the effect of size within each light level. Adults have only one light level (sun) because they are at or close to canopy level.

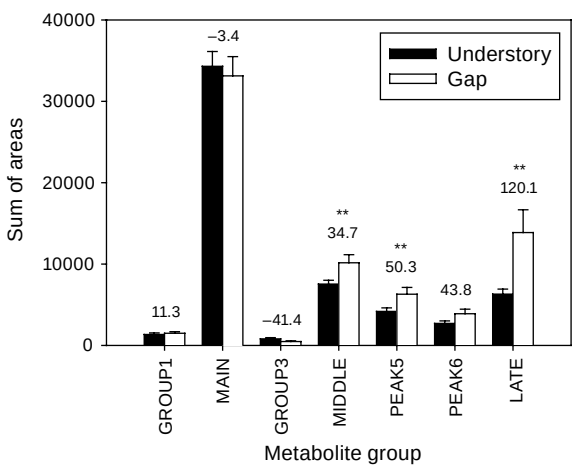


Fig. 4. Summed areas of the covarying peaks (±se) for juvenile samples grouped by their light level. The number above each set of bars is the percentage increase in area from understory to gap and ** indicates differences significantly different in a two sample t-test at $\alpha = 0.01$ level.

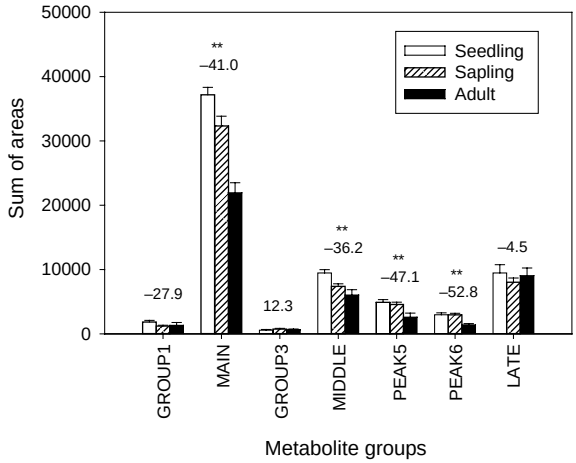


Fig. 5. Summed areas of the covarying peaks (±se) for samples grouped by their size class. The number above each set of bars is the percentage reduction in area from seedlings to adult, and ** represents reductions significant in a two sample t-test at $\alpha = 0.01$ level.

we would expect if they were related (Fig. 6). Yet, when we averaged all adult-sapling phenotypic differences by distance group, a one-tailed t-test revealed no significant difference ($x_1 - x_2 = 0.02$, $p = 0.51$). Thus, nearby saplings were as different from the adults as were distant saplings, meaning that we can not reject selection by herbivores or pathogens.

### Frequency-dependent variability

The expectation under frequency-dependent selection is similar to that of distance-dependent selection: neighboring plants under selection would be as different from each other as possible. We tested this by comparing the phenotypic variance of clusters. Unless herbivores were selecting against common phenotypes, clusters should have lower phenotypic variance for two reasons: First juveniles growing in clusters were expected to be siblings or half-siblings and have more similar genotypes. Second, due to their proximity and similar size, plants within a cluster might also be expected to have less environmental and developmental differences. We predicted that, if frequency-dependent selection was important, cluster variances should be equal to or higher than the variance of unrelated plants (in the same size class and light level) widespread throughout the population. Nine clusters comprising more than four juveniles each, could be used for this analysis (Fig. 7). Four of them had lower variance than unrelated plants within the same size class and light level, but only two cases were at least marginally significant (Fig. 7). One of them (QA00, $p = 0.02$; Fig. 7) was collected inside a mammal seed predation exclosure where seedling survival was significantly increased compared to the other clusters. In total, in 7 out of nine comparisons, the surviving seedlings were as or more variable than unrelated plants. Consistent with selection by pathogens or herbivores.

## Discussion

Heterogeneity in tropical forests provides a great number of variables that contribute to the spatial structure of phenotype expression and possibly to the spatial structure of the selection pressure exerted by herbivores.

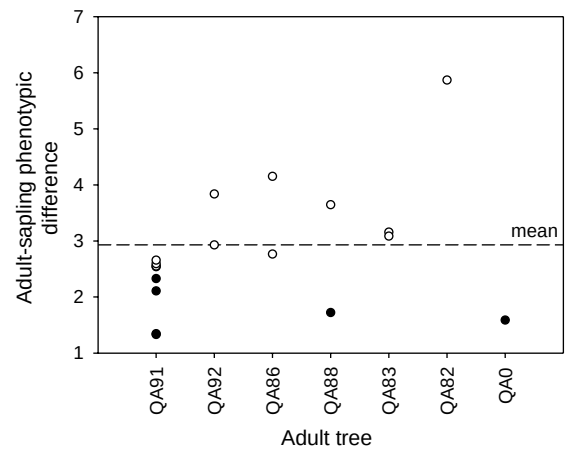


Fig. 6. Phenotypic difference between seven adults and their near understory saplings. Each circle is one sapling growing within 10 m of the adult tree. Black symbols represent saplings that were significantly more similar to the adult than the average ($\alpha = 0.05$). The reference line (dashed line) is the mean phenotypic difference for all near and far adult-sapling comparisons.

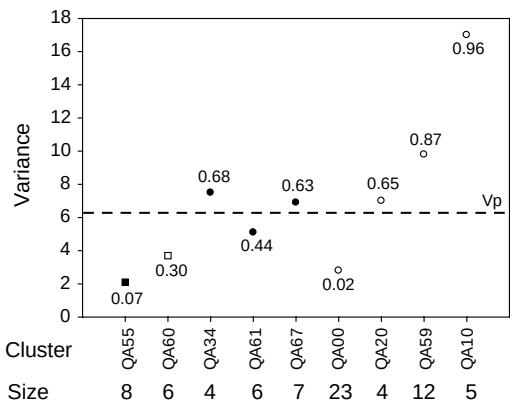


Fig. 7. Phenotypic variance of juvenile clusters. Each cluster is a group of saplings (squares) or seedlings (circles) growing in gaps (open) or in the understory (solid). The number next to the symbol is the p value supporting the null hypothesis that the cluster variance is equal or higher than the phenotypic variance of widespread plants in the same size class and light level. The reference line, Vp (dashed line) is the total variance of the population.

## Phenotypic variation and the spatial structure of secondary chemicals

Our results described significant levels of variability in the chemical phenotype of mature leaves of *Quararibea* at different spatial scales and due to environmental (Fig. 3, 4) and developmental (Fig. 3, 5) sources of variation.

At the larger spatial scale, we found that two different sites of collection within the population (slope and hill top) had differences in the average chemical phenotypes of *Quararibea.* The hilltop site was 1000 m away from the slope site, but it is unlikely that the observed differences were caused by limitations in gene flow for three reasons. First, studies in allozyme variation have demonstrated that *Quararibea* has high levels of gene flow throughout the BCI population (Hamrick and Murawski 1990, Murawski et al. 1990). Second, most of the pollinators and seed dispersers known for *Quararibea* (monkeys, parrots and bats) are likely to move distances larger than 1000 m. And third, our results indicated that seedlings could not be differentiated between sites suggesting that they are randomly distributed with respect to their phenotype. Topography has been found to influence species recruitment in some cases (Clark et al. 1999), but only to the extent that it represents differences in soil types. This is not the case in our comparison, because the slope site spans an Alfisol-Oxisol boundary and, when tested directly, soil type did not affect plant phenotype. Another untested possibility is that the observed chemical difference is the result of greater water availability on slopes during the dry season (Becker et al. 1988).

The main metabolite group responsible for the difference between sites (MIDDLE) responded also to changes in light availability and plant size. However, we were unable to link this source of variation to the differences between sites. Thus, with the data collected for this study, we can not establish whether this large scale difference between sites was caused by some form of local adaptation or by the effect of another source of variation that we did not measure.

## Effect of light

We found differences in the chemical phenotypes of *Quararibea* leaf extracts between plants growing in gaps and plants growing in the understory. Such light effects have been reported often for carbon-based secondary compounds in a wide variety of temperate (Agrell et al. 2000), tropical (Coley 1986) and aquatic (Gross 2003) study systems. In a meta-analysis of literature data, Koricheva et al. (1998) showed that only one particular kind of carbon-based secondary compounds, the phenylpropanoid-derivatives (phenolics), changed concentration in response to environmental manipulations. Accordingly, in our results, the yield was significantly higher in gaps, but not all compounds in the chemical profile had higher concentrations (Fig. 4). While we did not identify the peaks in the HPLC profile, our spot color tests, using Folin-Denis reagent, indicated that our polar extracts included some phenolics, which could be the ones responding to differences in light availability.

There are two possible explanations for an increase in phenolic concentration under high light conditions. Plants with greater access to light have higher rates of photosynthesis and thus have higher carbon availability. The carbon-nutrient balance hypothesis (CNB) suggests that the availability of resources limits biosynthesis of secondary compounds (Bryant et al. 1983; but see Hamilton et al. 2001). An alternative explanation is that those compounds that respond to light levels can be involved in UV screening or antioxidant functions in the plant (Close and McArthur 2002).

## Effect of plant size

Changes in leaf chemical phenotype with plant development have been addressed less frequently in the literature and earlier studies have reported diverse results. For example, while aspen, birch and poplar had higher resin content in the sapling stage, spruce and green alder showed no ontogenetic differences (Bryant and Kuropat 1980), and the California bay tree had lower concentrations (Goralka and Langenheim 1996).

We observed a vertical structure in the secondary compound landscape of the population that resulted from an interaction between light availability and plant size. Because of the higher light availability due to their position in the canopy, adults had significantly higher yields than juveniles in the understory (Fig. 3). They also had higher concentrations of the same compounds that responded to light availability. Yet, for a given light level, there was a clear decrease in the yield of secondary metabolites with increasing size class (Fig. 3). Furthermore, the metabolites responsible for size differences were different from those that responded to light (Fig. 4, 5) suggesting that the difference between size classes is developmental and independent of the difference in light availability.

These developmental changes can be the cause of vertical stratification of herbivores observed both in temperate (Kearsley and Whitham 1989, Waltz and Whitham 1997) and tropical (Basset et al. 2003) forests. Whether this is also true in the case of *Quararibea* we cannot tell. Barone (2000) found no difference in the species of herbivores that visited saplings and adults. However, during our leaf collections we found that seedlings were easy to recognize because of a particular kind of leaf damage caused by a leaf miner that was not seen in saplings and adults (Brenes-Arguedas, pers. obs.).

Higher investment in secondary chemicals by young plants is consistent with the hypothesis that defenses should be allocated preferentially to stages or structures that are more vulnerable and valuable (McKey 1979, Rhoades 1979). In *Quararibea*, the observed developmental variation in chemical phenotype could be caused by adaptive ontogenic changes for three main reasons. First, younger plants are smaller, with fewer leaves and roots, and thus are less tolerant of defoliation. Second, juveniles often grow in the shady and moist understory where they may more frequently be the targets of pathogen attack. And finally, *Quararibea* may invest more in defending the leaves of juveniles because they are long-lived, while adults drop their leaves at least once a year.

### Frequency- and distance-dependent variability

Limits in gene flow in the form of pollen and seed dispersal should create small-scale spatial patterns in the variability of secondary chemistry. In the case of *Quararibea*, seed rain results in higher seedling recruitment under a parent tree (Condit et al. 1992), and neighboring plants would most likely be related. Thus, assuming that the traits under study are heritable, limited seed dispersal should lead to greater similarity of neighboring plants in their secondary chemistry and the variability at small spatial scales should be lower than at larger spatial scales. Local environmental conditions that affect seedling recruitment may neutralize or even reverse this pattern. For example, herbivore pressure on seedling recruitment may increase the local variability of plant chemistry.

Our results are consistent with the expected result if phenotypic similarity between neighboring related plants was dampened by distance- or frequency- dependent selection. First, the majority of juveniles (12 out of 18, Fig. 6) do have different phenotypes from their closest adult; and second, cluster variance was higher than expected for seven out of the nine comparisons (Fig. 7). It is interesting to note that the one cluster whose variance was significantly lower than the population variance (QA00, Fig. 7) was found inside a mammal exclosure plot. Seedlings inside this plot had much higher recruitment than any other cluster, and it is possible that it is indeed mortality at early stages that increases the variability of the cohorts.

Since *Quararibea* is strictly out-crossing (Bawa 1974), seed cohorts should be very variable and if mechanisms such as herbivore pressure weed out the more common phenotypes in a cluster, the variability of the survivors may seem equal to or even exceed that of unrelated plants. However, an alternative explanation is that the observed patterns of variation are the outcome of extremely efficient seed and pollen dispersion or of very low genetic variability in the population. The main assumptions in which we based our conclusions were that neighboring plants were more likely to be related, that related plants were more likely to have similar phenotypes and that the measured phenotypes were under natural selection. In support of these assumptions are the following arguments: Condit et al. (1992) found that *Quararibea* saplings were more likely to recruit close to an adult tree, suggesting that seeds actually land close to the mother tree more often than not and neighboring plants are likely related. Second, secondary chemical production is commonly heritable (Berenbaum and Zangerl 1992) supporting the assumption that the phenotype of related plants should be more similar. Finally, we do not know if the measured phenotypes are under selection by herbivores, but *Quararibea* saplings have been found to share the herbivores of the adults (Barone 2000) suggesting that, from the point of view of the herbivores, saplings are comparable to the adult.

## Conclusions

Geographic variation in secondary chemistry has been studied in many other study systems. Some recent examples have discussed between population differences in tundra (Graglia et al. 2001), temperate (Talley et al. 2002), subtropical (Cantonwine and Downum 2001) and tropical (Ronning et al. 2000) plant species. However, all these studies focus on large-scale geographic patterns where there are also limits in gene flow between populations. In this study we described the small-scale patchiness of the secondary compound landscape within one *Quararibea* population. The observed patterns of variation underscore the high levels of variability in the secondary chemistry of *Quararibea*. This variation presumably presents herbivores with a food source whose quality varies widely even at small spatial scales.

In natural systems it is difficult to detect signals of selection by herbivores due to the enormous phenotypic variation caused by environmental influences. For that reason some of the most conclusive studies in selection by herbivores have been done in controlled environments (Mauricio and Rausher 1997). Yet, for the study of coevolution in a natural context it is necessary to understand the actual conditions under which plants and herbivores interact. In tropical ecosystems, given their high diversity and heterogeneity, it is impossible to do this without understanding the spatial patterns of phenotypic variation and their potential ecological role. Our study suggests, that it is possible to detect evidence for selection against this backdrop of developmental and environmental variation.

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## References

Agrell, J., McDonald, E. P. and Lindroth, R. L. 2000. Effects of $CO_2$ and light on tree phytochemistry and insect performance. – Oikos 88: 259–272.

Barone, J. A. 2000. Comparison of herbivores and herbivory in the canopy and understory for two tropical tree species. – Biotropica 32: 307–317.

Basset, Y., Hammond, P. M., Barrios, H. et al. 2003. Vertical stratification of arthropod assemblages. – In: Basset, Y., Novotny, V., Miller, S. E. et al. (eds), Arthropods of tropical forests: spatio-temporal dynamics and resource use in the canopy. Cambridge Univ. Press, pp. 17–27.

Bawa, K. S. 1974. Breeding systems of tree species in a lowland tropical community. – Evolution 28: 85–92.

Becker, P., Rabenold, P. E., Idol, J. R. et al. 1988. Water potential gradients for gaps and slopes in a Panamenian tropical moist forest's dry season. – J. Trop. Ecol. 4: 173–184.

Berenbaum, M. R. and Zangerl, A. R. 1992. Genetics of secondary metabolism and herbivore resistance in plants. – In: Rosenthal, G. A. and Berenbaum, M. R. (eds), Herbivores: their interactions with secondary plant metabolites. Academic Press, pp. 415–439.

Berenbaum, M. R. and Zangerl, A. R. 1998. Chemical phenotype matching between a plant and its insect herbivore. – Proc. Natl Acad. Sci. USA 95: 13743–13748.

Bowers, M. D. and Stamp, N. E. 1993. Effects of plant age, genotype, and herbivory on *Plantago* performance and chemistry. – Ecology 74: 1778–1791.

Bryant, J. P. and Kuropat, P. J. 1980. Selection of winter forage by subarctic browsing vertebrates: the role of plant chemistry. – Annu. Rev. Ecol. Syst. 11: 261–285.

Bryant, J. P., Chapin III, F. S. and Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. – Oikos 40: 358–368.

Cantonwine, E. G. and Downum, K. R. 2001. Phenylheptatriyne variation in *Bidens alba* var. radiata leaves. – J. Chem. Ecol. 27: 313–326.

Chaboudez, P. and Burdon, J. J. 1995. Frequency-dependent selection in a wild plant-pathogen system. – Oecologia 102: 490–493.

Clark, D. B., Palmer, M. W. and Clark, D. A. 1999. Edaphic factors and the landscape-scale distributions of tropical rain forest trees. – Ecology 80: 2662–2675.

Close, D.-C. and McArthur, C. 2002. Rethinking the role of many plant phenolics: protection from photodamage not herbivores? – Oikos 99: 166–172.

Coley, P. D. 1986. Costs and benefits of defense by tannins in a neotropical tree. – Oecologia 70: 238–241.

Condit, R., Hubbell, S. P. and Foster, R. B. 1992. Recruitment near conspecific adults and the maintenance of tree and shrub diversity in a neotropical forest. – Am. Nat. 140: 261–286.

Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. – In: de Boer, P. J. and Gradwell, G. R. (eds), Dynamics of populations, Proceedings on the Advanced Study Institute on Dynamics of Numbers in Populations. Center for agricultural Publishing and Documentation, pp. 298–312.

Croat, T. B. 1978. Flora of Barro Colorado Island. – Stanford Univ. Press.

De Steven, D. 1994. Tropical tree seedling dynamics: recruitment patterns and their population consequences for three canopy species in Panama. – J. Trop. Ecol. 10: 369–383.

Ehrlich, P. R. and Raven, P. H. 1964. Butterflies and plants: a study in coevolution. – Evolution 18: 586–608.

Goralka, R. J. L. and Langenheim, J. H. 1996. Implications of foliar monoterpenoid variation among ontogenetic stages of the California bay tree (*Umbellularia californica*) for deer herbivory. – Biochem. Syst. Ecol. 24: 13–23.

Graglia, E., Julkunen-Tiitto, R., Shaver, G. R. et al. 2001. Environmental control and intersite variations of phenolics in *Betula nana* in tundra ecosystems. – New Phytol. 151: 227–236.

Gross, E. M. 2003. Differential response of tellimagrandin II and total bioactive hydrolysable tannins in an aquatic angiosperm to changes in light and nitrogen. – Oikos 103: 497–504.

Hamilton, J. G., Zangerl, A. R., DeLucia, E. H. et al. 2001. The carbon-nutrient balance hypothesis: its rise and fall. – Ecology Lett. 4: 86–95.

Hamrick, J. L. and Murawski, D. A. 1990. The breeding structure of tropical tree populations. – Plant Species Biol. 5: 157–166.

Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. – Am. Nat. 104: 501–528.

Kearsley, M. J. C. and Whitham, T. G. 1989. Developmental changes in resistance to herbivory: implications for individuals and populations. – Ecology 70: 424–434.

Koricheva, J., Larsson, S., Haukioja, E. et al. 1998. Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. – Oikos 83: 212–226.

Langenheim, J. H. and Stubblebine, W. H. 1983. Variation in leaf resin composition between parent tree and progeny in *Hymenaea*: implications for herbivory in the humid tropics. – Biochem. Syst. Ecol. 11: 97–106.

Leigh, E. G. J., Rand, A. S. and Windsor, D. M. 1996. The ecology of a tropical forest: seasonal rhythms and long-term changes. – Smithsonian Institution Press.

Mauricio, R. and Rausher, M. D. 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. – Evolution 51: 1435–1444.

McKey, D. 1979. The distribution of secondary compounds within plants. – In: Rosenthal, G. A. and Janzen, D. H. (eds), Herbivores: their interaction with secondary plant metabolites. Academic Press, pp. 56–133.

Murawski, D. A., Hamrick, J. L., Hubbell, S. P. et al. 1990. Mating systems of two Bombacaceous trees of a neotropical moist forest. – Oecologia 82: 501–506.

Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. – In: Rosenthal, G. A. and Janzen, D. H. (eds), Herbivores: their interaction with secondary plant metabolites. Academic Press, pp. 1–54.

Ronning, C. M., Kowalski, S. P., Sanford, L. L. et al. 2000. Geographical variation of solanidane aglycone glycoalkaloids in the wild potato species *Solanum chacoense* Bitter. – Genet. Resour. Crop Evol. 47: 359–369.

Talley, S. M., Coley, P. D. and Kursar, T. A. 2002. Antifungal leaf-surface metabolites correlate with fungal abundance in sagebrush populations. – J. Chem. Ecol. 28: 2141–2168.

Thrall, P. H., Burdon, J. J. and Young, A. 2001. Variation in resistance and virulence among demes of a plant host-pathogen metapopulation. – J. Ecol. 89: 736–748.

Van Zandt, P. A. and Mopper, S. 1998. A meta-analysis of adaptive deme foundation in phytophagous insect populations. – Am. Nat. 152: 595–604.

Waltz, A. M. and Whitham, T. G. 1997. Plant development affects arthropod communities: opposing impacts of species removal. – Ecology 78: 2133–2144.
Wong, M., Wright, S. J., Hubbell, S. P. et al. 1990. The spatial pattern and reproductive consequences of outbreak defoliation in *Quararibea asterolepis*, a tropical tree. – J. Ecol. 78: 579–588.
Wright, S. J. and van Schaik, C. P. 1994. Light and the phenology of tropical trees. – Am. Nat. 143: 192–199.
Yavitt, J. B. 2000. Nutrient dynamics of soil derived from different parent material of Barro Colorado Island, Panama. – Biotropica 32: 198–207.
Zangerl, A. R. and Berenbaum, M. R. 1993. Plant chemistry, insect adaptations to plant chemistry, and host plant utilization patterns. – Ecology 74: 47–54.
Zangerl, A. R. and Berenbaum, M. R. 2003. Phenotype matching in wild parsnip and parsnip webworms: causes and consequences. – Evolution 57: 806–815.