The Spatial Signature of Biotic Interactions of a Clonal and a Non-clonal Palmetto in a Subtropical Plant Community

Mizuki Takahashi  
*Bucknell University*, mt027@bucknell.edu

Toshiro Kubota  
*Susquehanna University*

Liana M. Horner  
*Bucknell University*

Nathan A. Keller  
*Bucknell University*

Warren G. Abrahamson II  
*Bucknell University*

Follow this and additional works at: [https://digitalcommons.bucknell.edu/fac_journ](https://digitalcommons.bucknell.edu/fac_journ)

*Part of the Population Biology Commons*

**Recommended Citation**

Takahashi, Mizuki; Kubota, Toshiro; Horner, Liana M.; Keller, Nathan A.; and Abrahamson, Warren G. II.  
"The Spatial Signature of Biotic Interactions of a Clonal and a Non-clonal Palmetto in a Subtropical Plant Community." *Ecosphere* (2012) : Article 68.
The spatial signature of biotic interactions of a clonal and a non-clonal palmetto in a subtropical plant community

MIZUKI K. TAKAHASHI, TOSHIRO KUBOTA, LIANA M. HORNER, NATHAN A. KELLER, AND WARREN G. ABRAHAMSON

1Department of Biology, Bucknell University, Lewisburg, Pennsylvania 17837 USA
2Mathematical Sciences, Susquehanna University, Selinsgrove, Pennsylvania 17870 USA
3Archbold Biological Station, 123 Main Drive, Venus, Florida 33960 USA

Citation: Takahashi, M. K., T. Kubota, L. M. Horner, N. A. Keller, and W. G. Abrahamson. 2012. The spatial signature of biotic interactions of a clonal and a non-clonal palmetto in a subtropical plant community. Ecosphere 3(7):68. http://dx.doi.org/10.1890/ES12-00101.1

Abstract. Spatial analyses of plant-distribution patterns can provide inferences about intra- and interspecific biotic interactions. Yet, such analyses are rare for clonal plants because effective tools (i.e., molecular markers) needed to map naturally occurring clonal individuals have only become available recently. Clonal plants are unique in that a single genotype has a potential to spatially place new individuals (i.e., ramets) in response to intra- and interspecific biotic interactions. Laboratory and greenhouse studies suggest that some clonal plants can avoid intra-genet, inter-genet, and inter-specific competition via root-placement patterns. An intriguing and yet to be explored question is whether a spatial signature of such multi-level biotic interactions can be detected in natural plant communities. The facultatively clonal Serenoa repens and non-clonal Sabal etonia are ecologically similar and co-dominant palmettos that sympatrically occur in the Florida peninsula. We used amplified fragment length polymorphisms (AFLPs) to identify Serenoa genets and also to assign field-unidentifiable small individuals as Sabal seedlings, Serenoa seedlings, or Serenoa vegetative sprouts. Then, we conducted univariate and bivariate multi-distance spatial analyses to examine the spatial interactions of Serenoa (n = 271) and Sabal (n = 137) within a 20 × 20 m grid at three levels, intragenet, intergenet and interspecific. We found that spatial interactions were not random at all three levels of biotic interactions. Serenoa genets appear to spatially avoid self-competition as well as inter-genet competition. Furthermore, Serenoa and Sabal were spatially negatively associated with each other. However, this negative association pattern was also evident in a spatial comparison between non-clonal Serenoa and Sabal, suggesting that Serenoa genets’ spatial avoidance of Sabal through placement of new ramets is not the explanation of the interspecific-level negative spatial pattern. Our results emphasize the importance of investigating spatial signatures of biotic as well as abiotic interactions at multiple levels in understanding spatial distribution patterns of clonal plants in natural plant communities.

Key words: AFLP; clonal plants; competition; Sabal etonia; Serenoa repens; spatial analysis.

Received 12 April 2012; revised 25 May 2012; accepted 29 May 2012; final version received 22 June 2012; published 30 July 2012. Corresponding Editor: D. P. C. Peters.

Copyright: © 2012 Takahashi et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits restricted use, distribution, and reproduction in any medium, provided the original author and sources are credited.

† E-mail: mizuki.takahashi@bucknell.edu

INTRODUCTION

The signature of biotic interactions both within and among species can be inferred through spatial analyses of plant distribution patterns (Pielou 1977, Silvertown and Wilson 1994, Nanami et al. 1999, Seabloom et al. 2005, Luo et al. 2012). Although such analyses have been...
Conducted on non-clonal plants, few studies have explored naturally occurring clonal-plant populations. This is because the tools (i.e., molecular markers) needed to determine genetic identities of clonal individuals (i.e., ramets) only became available recently (Arnaud-Haond et al. 2007). In particular, small-scale spatial analyses of clonal plants to detect biotic interactions have been rarely done (Honnay and Jacquemyn 2010, Zobel et al. 2010). A unique property of clonal plants is that a single genotype has a potential to spatially place new individuals in response to intra- and interspecific biotic interactions.

Biotic interactions in clonal plants can be examined at three levels: (1) between ramets within the same genet (i.e., clone), (2) between genets within the same species, and (3) between different species. Several laboratory or greenhouse experimental studies tested these interactions by examining root-placement patterns (Huber-Sannwald et al. 1998, Falik et al. 2003, Holzapfel and Alpert 2003, Gruntman and Novoplansky 2004, Semchenko et al. 2007, Semchenko et al. 2010). In general, clonal plants appear to be capable of discriminating between the roots of clonemates and those of other genets and can respond by changing root-placement patterns (de Kroon 2007). Under experimental conditions, clonal plants exhibit stronger avoidance of self-competition for limited belowground resources between clonemates than with other genets within the same species (Falik et al. 2003, Gruntman and Novoplansky 2004). Such avoidance of self-competition through segregation of root systems among ramets can increase efficiency of overall resource acquisition and thus lead to greater genet performance (Holzapfel and Alpert 2003).

Strategies of clonal plants to cope with neighboring roots of other genets can vary among species. For example, when two genets were planted adjacent to one another, the roots of field pea (Pisum sativum) intruded toward the roots of the neighboring genet (Falik et al. 2003). To the contrary, ground ivy (Glechoma hederacea) avoided any belowground competition with their neighbors regardless of their genetic identity (Semchenko et al. 2007). Relatively few studies have tested biotic interactions between clonal plant species. Semchenko and her colleagues (Semchenko et al. 2007, Semchenko et al. 2010) examined biotic interactions between woodland strawberry (Fragaria vesca) and ground ivy. Root behavior in response to interspecific competition varied between the species. When planted adjacent to one another, woodland strawberry did not alter its root-placement pattern; however, ground ivy avoided neighboring woodland strawberries by producing significantly more root mass on the side away from woodland strawberries than on the side nearest its competitor.

Previous studies examined each level of the biotic interactions under controlled environments. In natural environments, however, all three levels of the biotic interactions (i.e., intragenet, intergenet, and interspecific) likely operate simultaneously. Thus, an intriguing and yet to be explored question is whether spatial signatures of such biotic interactions can be detected in natural plant communities. Here, we bring together a DNA-fingerprinting technique, which we use to determine the genetic identities of ramets, with univariate and bivariate multivariate spatial analyses to examine the spatial signatures of biotic interactions at three levels: (1) among clonemates of the same genets, (2) between different genets, and (3) between clonal and non-clonal plants in a natural plant community.

As a model system, we examined spatial distributions of two palmetto (i.e., shrub-size palms) species that occur sympatrically in portions of the Florida peninsula: the clonal Serenoa repens (W.Bartram) Small (saw palmetto; hereafter Serenoa) and non-clonal Sabal etonia Swingle ex Nash (scrub palmetto; hereafter Sabal). Serenoa and Sabal share many life-history characteristics such as habitat preference, dwarf stature, large underground mass, post-fire resilience, and slow recruitment (Abrahamson 1984, Abrahamson 1995, Menges and Kohfeldt 1995, Abrahamson 2007, Abrahamson and Abrahamson 2009). Both species flower during May and mature the resulting fruits during September/October and fruits are consumed and dispersed by numerous vertebrates including white-tailed deer (Odocoileus virginianus), wild turkey (Meleagriscalpalpavo), and gopher tortoise (Gopherus polyphemus) (Abrahamson and Abrahamson 1989, Abrahamson 1999, Layne and Abrahamson 2010). Rhizomes of sister Serenoa ramets can remain connected for
many decades but eventually their connection decays leaving no sign of physical connection, which precludes the detection of true clonal-propagation patterns via observation of clonal connections. By using DNA fingerprinting, our recent study found that 83% of Serenoa’s recruitment was via vegetative means at our study site, indicating that Serenoa is highly clonal (Takahashi et al. 2011).

We hypothesize that spatial signatures of biotic interactions at multiple levels can be detected in the distribution pattern of ramets and genets of Serenoa in a natural plant community. Given the nutrient-poor, drought-prone conditions of our study area (Abrahamson et al. 1984), negative spatial interactions should more equitably distribute ramets and genets to avoid potentially intense resource (e.g., soil nutrients) competition. Thus, belowground intraspecific competition may have led to spatially negative associations among clonemates within Serenoa genets as well as among different Serenoa genets. Finally, based on the shared life-history traits and ecological niches of Serenoa and Sabal, we also predict the negative spatial association between individuals of clonal Serenoa and the sympatric non-clonal Sabal.

Although environmental gradients can also influence spatial patterns of plants, we did not test associations between environmental factors and spatial patterns of Serenoa for two reasons. First, there were no noticeable gradients in environmental factors such as shading pattern, elevation, and soil type within our small study grid (i.e., 20 × 20 m). Second, Serenoa genets are extremely long-lived (i.e., possibly more than 10,000 yr old; Takahashi et al. 2011). It is very likely that the patterns of any environmental gradients in our study grid have changed over such long duration of time; and thus, interference based on the associations between current environmental gradients and spatial patterns of Serenoa and Sabal may be misleading. For example, Serenoa performs better at sites with moist soils, while Sabal tends to perform better at sites with well-drained soils (Abrahamson 1995). Given their overall ecological similarity, it is possible that a few inches of water-table change have altered the distribution of suitable microsites for Serenoa and Sabal within our small grid over the past ten millennia.

**Methods**

**Study site and sampling procedures**

Serenoa and Sabal were sampled from a 20 × 20 m study grid established in scrubby flatwoods at the Archbold Biological Station, which is located near the southern terminus of Florida’s Lake Wales Ridge (LWR) (27°11’ N, 81°21’ W). Because no information was available about the extents of Serenoa clonal spread, we used a grid size that allowed us to collect and genetically analyze every single ramet that occurred within the sampled area. Scrubby flatwoods are a low (1–2 m) shrubby association dominated by evergreen, xeromorphic oaks as well as Serenoa and Sabal (Abrahamson et al. 1984, Abrahamson and Hartnett 1990, Myers 1990, Menges 1999). Pinus elliottii Engelm. (slash pine) occurs as an overstory species at variable but low densities. The density and height of vegetation are dependent on edaphic conditions and time since last fire. Scrubby flatwoods occur on nutrient-poor, moderately well-drained sand soils that have rapid permeability, low available-water capacity, and acidic pH (Abrahamson et al. 1984). The study grid appeared level and was representative of scrubby flatwoods except for the lack of pine overstory.

A leaf fragment was collected from every palmetto that was large enough for its species to be identified within the grid, and its location within the 400 1 × 1 m sub-plots was recorded. Because Serenoa and Sabal typically flower after being burned and do not flower annually, it was not possible to confirm that each ramet was an adult with reproductive capabilities. However, Serenoa and Sabal can flower when their heights are as little as 47 cm (Abrahamson 1999; W. G. Abrahamson, unpublished data). Consequently, these identifiable samples were likely adults and hence, we treated them as adults in our analyses. In total, we collected samples from 218 Serenoa and 55 Sabal adults. We also haphazardly collected leaf fragments from 139 field-unidentifiable small individuals that were scattered across the grid. These individuals could be Sabal seedlings, Serenoa seedlings, or Serenoa vegetative sprouts. These 139 individuals represented roughly one half of the field-unidentifiable small individuals that occurred within the grid. We collected up to ten samples within a 1 × 1 sub-
plot, while no samples were collected from 166 out of 400 sub-plots because of the absence of palmettos. Each leaf fragment was placed into an individually numbered Eppendorf tube with its sub-plot location. Tubes were transferred to Bucknell University in liquid nitrogen and subsequently stored at −20°C.

**AFLP analyses, assignment of field-unidentifiable samples, and detection of Serenoa clones**

We used DNeasy Plant Mini Kit (QIAGEN) to extract DNA from collected leaf samples. For our amplified fragment length polymorphism (AFLP) analyses, we used the three most informative selective-primer pairs of EcoRI and MseI for both Serenoa and Sabal as follows: (1) EcoRI: ACT – MseI: CAA, (2) EcoRI: ACG – MseI: CAA, and (3) EcoRI: ACA – MseI: CAT. We then analyzed the obtained AFLP binary matrix with STRUCTURE (Pritchard et al. 2000) to assign the field-unidentifiable small individuals as Serenoa or Sabal. Details of the procedures used are available in Takahashi et al. (2011).

Using the AFLP binary matrix, we created a frequency distribution of pair-wise genetic distances to detect Serenoa genets. Due to scoring errors or somatic mutations, clonemates can have slightly different multilocus genotypes (MLGs). Thus, the frequency distribution of pair-wise genetic distances of clonal plants is often multi-modal in which the first peak represents clonal individuals with slightly different MLGs while the second peak represents closely related individuals (Douhovnikoff and Dodd 2003, Meirmans and Van Tienderen 2004). Accordingly, a threshold can be assigned to the valley between the first and the second peak to allow individuals with slightly different MLGs to be considered clonemates. Once the threshold was determined, we assigned all Serenoa individuals into genets using GENOTYPE (Meirmans and Van Tienderen 2004). We also generated a frequency distribution of pair-wise genetic distances of Sabal individuals to confirm that our AFLP data were distinguishable between clonal Serenoa and non-clonal Sabal. We duplicated AFLP profiles of randomly selected Serenoa and Sabal samples (N = 10 each) through repetition of the entire AFLP process to estimate reproducibility of AFLP profiles. The power of AFLP fingerprints was estimated by calculating the probability of identity (P.ID) which is the probability of two randomly drawn individuals from a population having the same MLGs (Waits et al. 2001).

**Spatial analyses of Serenoa and Sabal distribution**

We used multi-distance spatial cluster analyses to analyze the spatial pattern of Serenoa and Sabal at multiple different distances by calculating the $L(t)$ function, which is a modified version of Ripley’s $K(t)$ function (Ripley 1977, Nanami et al. 1999, Isagi et al. 2004). $L(t)$ and $K(t)$ indicate the expected number of individuals within distance $t$ of an arbitrary individual and are defined as:

$$L(t) = \frac{K(t)}{\pi t^{1/2}} - t,$$

$$K(t) = n^{-2} A |\Sigma_{i,j} W_{ij}^{-1} I_i(u_{ij})|,$$

where $n$ is the number of individuals in a plot, $|A|$ is the plot area, $u_{ij}$ is the distance between the centers of $1 \times 1$ m sub-plots in which $i$th individual and $j$th individual were collected respectively within $A$, $I_i(u)$ is equal to 1 if $u \leq t$ and 0 otherwise, $W_{ij}$ is the proportion of the circumference of a circle with center at $i$th individual and radius $u_{ij}$ within $A$, and summation is for all pairs of individuals (Ripley 1977, Nanami et al. 1999, Isagi et al. 2004). $L(t) > 0$ indicates a clumped, $L(t) = 0$ indicates a random, and $L(t) < 0$ indicates a uniform distribution pattern.

Following Nanami et al. (1999), we used the bivariate function $L_{12}(t)$ to analyze spatial interactions between two groups ($S$ and $T$). $L_{12}(t)$ is defined as:

$$L_{12}(t) = \frac{|K_{12}(t)|}{n^{1/2}} - t,$$

$$K_{12}(t) = n_S^{-1} n_T^{-1} A |\Sigma_{i \in S} \Sigma_{j \in T} W_{ij}^{-1} I_i(u_{ij})|,$$

where $n_S$ and $n_T$ are the numbers of individuals in $S$ and $T$, respectively.

$L_{12}(t) > 0$ indicates a positive, $L_{12}(t) = 0$ indicates an independent, and $L(t) < 0$ indicates a negative spatial association of two groups within a given space. The significance of $L(t)$ and $L_{12}(t)$ was assessed with Monte Carlo simulations (Nanami et al. 1999, Isagi et al. 2004). Ninety-five percent confidence envelopes were defined as the highest and lowest values of $L(t)$ or $L_{12}(t)$ found in 19 replications of $L(t)$ or $L_{12}(t)$ with random-point distribution (Nanami et al. 1999).
To examine spatial interactions among clone-mates of the same genets, we conducted two types of analyses. First, we used a univariate analysis [i.e., $L(t)$] to examine *Serenoa*’s distribution pattern as a whole. Second, we used a bivariate analysis [i.e., $L_{12}(t)$] to examine the spatial interaction between adult ramets and vegetative sprouts of the same genet. As a comparison, we applied the same analyses to *Sabal* as well with a bivariate analysis testing the spatial interaction between adults and seedlings of *Sabal*.

We used a bivariate analysis to examine spatial interactions between different *Serenoa* genets. Finally, to examine how *Serenoa* clones are distributed in relation to neighboring *Sabal*, we conducted three types of analyses. We examined the spatial interactions (1) between *Serenoa* and *Sabal* as a whole, (2) between *Serenoa* genets and *Sabal*, and (3) between non-clonal individuals of *Serenoa* and *Sabal*. The univariate and bivariate analyses as well as the Monte Carlo simulations were implemented in MATLAB 7 with a statistics toolbox. The source code, the data, and a user guide are available at http://www.susqu.edu/facstaff/k/kubota/PalmSpatialAnalysis.zip.

**RESULTS**

**Demography of Serenoa and Sabal**

Based on the AFLP MLGs (data available via DRYAD entry doi:10.5061/dryad.6th24) of the collected samples, we identified 271 *Serenoa*, of which nine were seedlings and 44 were vegetative sprouts.
tive sprouts, and 137 Sabal, of which 79 were seedlings. Because the frequency distribution of pair-wise genetic distances of Serenoa was multimodal, we set a threshold of 2% dissimilarity and considered pairs with 2% or less dissimilarity to be clonemates. We detected five Serenoa clones: clone 1 with 112 adults and 36 sprouts, clone 2 with 45 adults and three sprouts, clone 3 with six adults and two sprouts, clone 4 with one adult and three sprouts, and clone 5 with three adults and zero sprouts. The AFLP profiles provided no evidence for Sabal clones. The average error rates (i.e., dissimilarity) between duplicated samples were 1.2 ± 0.5% for Serenoa and 2.1 ± 0.7% for Sabal. $P_{ID}$ for Serenoa was 0.0028 and that for Sabal was 0.0002.

### Spatial patterns of Serenoa and Sabal

The $L(t)$ value for the spatial distribution of Serenoa ($n = 271$) was negative at all distances and exceeded the 95% confidence envelope at most distances (Fig. 1A). Thus, Serenoa is non-randomly distributed with a somewhat uniform distribution of individuals. In contrast, Sabal ($n = 137$) was significantly clumped, especially at the scale of ~4–11 m (Fig. 1B).

The spatial interaction between adults ($n = 112$) and sprouts ($n = 36$) of Serenoa clone 1 showed a significant positive association at ~1 m but a negative association was detected at ~6–14 m (Fig. 2A). In contrast, adults ($n = 55$) and seedlings ($n = 79$) of Sabal were spatially positively associated at 6–10 m (Fig. 2B). We excluded Serenoa clones 3, 4 and 5 from all spatial...
analyses because of their small sample size (i.e., <10) and we did not analyze the spatial interaction of adults and sprouts of Serenoa clone 2 because clone 2 had only three sprouts. Within Serenoa, the spatial interaction between clone 1 \((n = 148)\) and clone 2 \((n = 48)\) showed a strong negative association with a peak at 5 m (Fig. 3).

Overall, Serenoa \((n = 271)\) and Sabal \((n = 137)\) were spatially negatively associated with each other at almost all distances (Fig. 4A). Likewise, the spatial interaction between non-clonal individuals of Serenoa \((n = 52)\) and Sabal \((n = 137)\) was negative at almost all distances (Fig. 4B). The spatial interaction between Serenoa clone 1 \((n = 148)\) and Sabal \((n = 137)\) showed negative association at \(\sim 2-9\) m but exhibited an independent distribution pattern (i.e., random) at \(\sim 9-14\) m (Fig. 5A). Serenoa clone 2 \((n = 48)\) was strongly negatively associated with Sabal \((n = 137)\) at almost all distances (Fig. 5B).

**DISCUSSION**

**Spatial interactions between clonemates of the same genet**

Avoidance of self-competition is critical for clonal plants to survive in competition against non-clonal plants in a plant community (Zobel 2008). Serenoa appears to possess a mechanism by which self-competition is mitigated, based on the observed uniform distribution of individuals. This is also supported by the tendency for spatial segregation of sprouts and adults within the same genet, although they were positively associated with adults at a close range because of the nature of Serenoa’s clonal propagation. In contrast, Sabal exhibited the opposite pattern, with a clumped distribution and positive spatial association between adults and seedlings, suggesting that intraspecific competition is not the main driver of Sabal distributions.

Clonal plants can avoid self-competition via directional root growth away from their clonemates in experimental systems (Falik et al. 2003, Gruntman and Novoplansky 2004). Some clonal plants may also be able to establish new ramets by spatially avoiding belowground intra-genet competition, as appears to be the case for Serenoa. Yet, it has not been demonstrated that Serenoa can grow either its rhizomes or adventitious roots differentially toward open spaces in response to below- or aboveground competition with clonemates. It is possible that Serenoa rhizomes or roots that encounter unoccupied soil space tend to grow new ramets more frequently than those that encounter competitive interactions. For example, Foster and Schmalzer (2012) found that Serenoa planted in a former agricultural site on
scrub soil grew faster and developed more ramets when not planted in dense bahia grass (*Paspalum notatum*) presumably because of the lack of the competition from this exotic grass. Alternatively, it is also possible that the detected pattern of the negative association between adults and sprouts of *Serenoa* genet may be the outcome of thinning of new ramets.

**Spatial interactions between different genets**

Isagi et al. (2004) used AFLPs to detect genets of *Phyllostachys pubescens* (Moso bamboo) and found that different genets were intermixed in the field, providing no evidence of spatial avoidance between different genets. In contrast, we found spatial avoidance between two large genets of *Serenoa* (i.e., clone 1 and 2). There was clear separation between *Serenoa* genets with each occupying a different portion within the grid. Our recent work (Takahashi et al. 2011) suggests that these two *Serenoa* clones are thousands of years old, meaning that the observed spatial-avoidance pattern has likely been present for centuries rather than our study simply capturing the initial stages of clonal expansion. However, our grid size allowed us to test spatial interaction of only one genet pair because the extent of *Serenoa* clonal spread was unknown prior to our study. While the current study provides high-resolution spatial data for the relatively small study plot, an ongoing study in our lab is using a much larger study grid with

![Fig. 4. Spatial distribution (left panels) and inter-specific interaction based on bivariate $L_{12}(t)$ values (right panels) shown for (A) *Serenoa repens* vs. *Sabal etonia* and (B) non-clonal *Serenoa* vs. *Sabal*. The left panels illustrate the locations of each sampled palmetto within the 20 × 20 m grid (resolution of 1 × 1 m sub-plot). The black lines in the right panels shows the actual $L_{12}(t)$ values of the focal groups, while the gray lines indicate the 95% confidence envelopes for the pattern expected from an independent distribution of two groups calculated via Monte Carlo simulations.](image)
less intensive sampling regimes in order to include more genets.

The intriguing difference in spatial avoidance of intergenet competition between Moso bamboo and *Serenoa* may result from innate differences in species responses (e.g., Falik et al. 2003, Semchenko et al. 2007). But it is also possible that extremely nutrient-poor and drought-prone sandy soils of our study site (Abrahamson et al. 1984) enhance the intensity of intergenet belowground competition (Schenk et al. 1999). Whether *Serenoa* genets spatially respond to intergenet competition has not been experimentally tested and such a test would be difficult given *Serenoa*'s slow rhizome growth. Alternatively, *Serenoa*'s thick rhizomes (15–40 cm in diameter) may hinder physical crossover of ramets of different genets. Suyama et al. (2000) found that the genets of a dwarf bamboo, *Sasa senanensis*, rarely crossed over and were spatially isolated from one another in a natural stand. The authors suspected that genets did not intermix in part because their rhizomes grow parallel to slope contours. Our study grid was level and thus such parallel pattern of rhizome growth is unlikely to explain the spatial separation of the *Serenoa* genets.

**Spatial interactions between clonal *Serenoa* and non-clonal *Sabal***

It is difficult to examine whether clonal plants spatially avoid sympatric non-clonal plants in a natural community because seedling establishment from non-clonal plants can mask any

---

**Fig. 5.** Spatial distribution (left panels) and inter-specific interaction based on bivariate $L_{12}(t)$ values (right panels) shown for (A) *Serenoa* clone 1 vs. *Sabal* and (B) *Serenoa* clone 2 vs. *Sabal*. The left panels illustrate the locations of each sampled palmetto within the $20 \times 20$ m grid (resolution of $1 \times 1$ m sub-plot). The black lines in the right panels shows the actual $L_{12}(t)$ values of the focal groups, while the gray lines indicate the 95% confidence envelopes for the pattern expected from an independent distribution of two groups calculated via Monte Carlo simulations. Note that the Y axis scale of the right panels differs between (A) and (B).
avoidance attempts of clonal plants. To overcome this difficulty, we compared the spatial interaction of *Serenoa* clonal genets against *Sabal* with that of non-clonal individuals of *Serenoa* against *Sabal*. We also analyzed the spatial interaction between each *Serenoa* clone and *Sabal*. Overall, *Serenoa* and *Sabal* were distributed separately within the grid. However, our analyses provide little evidence in support of *Serenoa* genets spatially avoiding the non-clonal sympatric *Sabal*. First, non-clonal individuals of *Serenoa* were also spatially negatively distributed with *Sabal*. If *Serenoa* genets showed even stronger patterns of negative association with *Sabal*, this would suggest the possibility that *Serenoa* genets avoid *Sabal*. However, the negative association of *Serenoa* clone 1 and *Sabal* was more subtle than those between all *Serenoa* and *Sabal* and between non-clonal *Serenoa* and *Sabal*. Second, *Serenoa* clone 2 showed strong negative spatial association with *Sabal*. This pattern most likely resulted from its intergenet competition against clone 1 preventing *Serenoa* clone 2 from expanding into the area where *Sabal* was more abundant. Therefore, the overall negative spatial association between *Serenoa* and *Sabal* is explained by factors other than interspecific spatial avoidance of *Serenoa* through its clonal placement of new ramets. One possibility is an environmental gradient in abiotic factors such as soil water-holding capacity within our study grid. At a much larger scale, Abrahamson (1995) found that *Serenoa* perform best at sites with poorly and moderately drained soils, while *Sabal* tends to grow best at sites with well-drained soils. Clonal plants can morphologically respond to competition with neighboring plants as well as spatially and temporally variable resources (Oborny 1994, de Kroon and Hutchings 1995, Huber-Sannwald et al. 1998). Slight gradients in soil drainage, soil water-holding capacity, and distance to ground water within even small areas such as our 20 × 20 m grid, may be sufficient to influence the observed pattern of spatial isolation between *Serenoa* and *Sabal*. It is likely that *Serenoa* genets in our study grid took thousands of years to form the present patterns of clonal spread (Takahashi et al. 2011). In such long-lived clonal plants, we need to exercise caution drawing inferences about environmental factors based on the association between current environmental gradients and spatial patterns of plants.

The integration of DNA fingerprinting and multi-distance spatial-cluster analyses offers a valuable tool to analyze spatial patterns of long-lived clonal plants in natural plant communities. In such an analysis, detecting the spatial signature of each of the three levels of biotic interactions: intragenet, intergenet, and interspecific, is critical because each provides a snapshot of the long-term spatial dynamics of clonal plants. Our spatial analyses detected a signature of intragenet and intergenet biotic interactions, while providing no evidence of interspecific competition. These results suggest that biotic as well as abiotic interactions at multiple levels likely shape small-scale distribution patterns of clonal plants. Only with multi-level analyses, does it become possible to attribute the observed spatial variance of the clonal-plant distribution to different levels of biotic interactions.

**Acknowledgments**

We sincerely thank A. Whipple, C. Abrahamson, P. Heinrich, and E. Menges for field assistance and J. Williams and L. Foltz for assistance with molecular work. J. Brown, J. Clarke, S. Jordan, Y. Suyama, and Y. Wester provided valuable help and suggestions for AFLP data analyses. We are also grateful to C. Hawkes and S. Pierce for their valuable comments and suggestions on our earlier draft and P. Schmalzer and an anonymous reviewer for their helpful reviews.

**Literature Cited**

Abrahamson, W. G. 1984. Post-fire recovery of Florida Lake Wales ridge vegetation. American Journal of Botany 71:9–21.

Abrahamson, W. G. 1995. Habitat distribution and competitive neighborhoods of two Florida palmettos. Bulletin of the Torrey Botanical Club 122:1–14.

Abrahamson, W. G. 1999. Episodic reproduction in two fire-prone palms, *Serenoa repens* and *Sabal etonia* (Palmae). Ecology 80:100–115.

Abrahamson, W. G. 2007. Leaf traits and leaf life spans of two xeric-adapted palmettos. American Journal of Botany 94:1297–1308.

Abrahamson, W. G. and C. R. Abrahamson. 1989. Nutritional quality of animal dispersed fruits in Florida sandridge habitats. Bulletin of the Torrey Botanical Club 116:215–228.

Abrahamson, W. G. and C. R. Abrahamson. 2009. Life in the slow lane: palmetto seedlings exhibit remarkable survival but slow growth in Florida’s...
nutrient-poor uplands. Castanea 72:123–132.
Abrahamson, W. G. and D. C. Hartnett. 1990. Pine flatwoods and dry prairies Pages 103–149 in R. L. Myers and J. J. Ewel, editors. Ecosystems of Florida. University of Central Florida Press, Orlando, Florida, USA.
Abrahamson, W. G., A. F. Johnson, J. N. Layne, and P. A. Peroni. 1984. Vegetation of the Archbold Biological Station, Florida: an example of the southern Lake Wales ridge. Florida Scientist 47:209–250.
Arnaud-Haond, S., C. M. Duarte, F. Alberto, and E. A. Serrão. 2007. Standardizing methods to address clonality in population studies. Molecular Ecology 16:5115–5139.
de Kroon, H. 2007. How do roots interact? Science 318:1562–1563.
de Kroon, H. and M. J. Hutchings. 1995. Morphological plasticity in clonal plants: the foraging concept reconsidered. Journal of Ecology 83:143–152.
Douhovnikoff, V. and R. S. Dodd. 2003. Intra-clonal variation and a similarity threshold for identification of clones: application to Salix exigua using AFLP molecular markers. Theoretical and Applied Genetics 106:1307–1315.
Falik, O., P. Reides, M. Gersani, and A. Novoplansky. 2003. Self/non-self discrimination in roots. Journal of Ecology 91:525–531.
Foster, T. E. and P. A. Schmalzer. 2012. Growth of Serenoa repens in a former agricultural site. southeastern Naturalist 11:331–336.
Grunzman, M. and A. Novoplansky. 2004. Physiologically mediated self/non-self discrimination in roots. Proceedings of the National Academy of Sciences USA 101:3863–3867.
Holzapfel, C. and P. Alpert. 2003. Root cooperation in a clonal plant: connected strawberries segregate roots. Oecologia 134:72–77.
Honnay, O. and H. Jacquemyn. 2010. Clonal plants: beyond the patterns—ecological and evolutionary dynamics of asexual reproduction. Evolutionary Ecology 24:1393–1397.
Huber-Sannwald, E., D. A. Pyke, M. M. Caldwell, and S. Durham. 1998. Effects of nutrient patches and root systems on the clonal plasticity of a rhizomatous grass. Ecology 79:2267–2280.
Isagi, Y., K. Shimada, H. Kushima, N. Tanaka, A. Nagao, T. Ishikawa, H. OnoDera, and S. Watanabe. 2004. Clonal structure and flowering traits of a bamboo [Phyllostachys pubescens (Mazel) Ohwi] stand grown from a simultaneous flowering as revealed by AFLP analysis. Molecular Ecology 13:2017–2021.
Layne, J. N. and W. G. Abrahamson. 2010. Spatiotemporal variation of fruit digestible-nutrient production in Florida’s uplands. Acta Oecologica 36:675–683.
Luo, Z. R., M. J. Yu, D. L. Chen, Y. G. Wu, and B. Y. Ding. 2012. Spatial associations of tree species in subtropical evergreen broad-leaved forest. Journal of Plant Ecology doi: 10.1093/jpe/rrt1048
Meirmans, P. G. and P. H. Van Tienderen. 2004. Genotype and genodive: two programs for the analysis of genetic diversity of asexual organisms. Molecular Ecology Notes 4:792–794.
Menges, E. S. 1999. Ecology and conservation of Florida scrub. Pages 7–22 in R. C. Andersen, J. S. Fralish, and J. Baskin, editors. The savanna, barrens, and rock outcrop communities of North America. Cambridge University Press, Cambridge, UK.
Menges, E. S. and N. Kohfeldt. 1995. Life history strategies of Florida scrub plants in relation to fire. Bulletin of the Torrey Botanical Club 122:282–297.
Myers, R. L. 1990. Scrub and high pine. Pages 150–193 in R. L. Myers and J. J. Ewel, editors. Ecosystems of Florida. University of Central Florida Press, Orlando, Florida, USA.
Nanami, S., H. Kawaguchi, and T. Yamakura. 1999. Dioecy-induced spatial patterns of two codominant tree species, Podocarpus nagi and Neolitsea aciculata. Journal of Ecology 87:678–687.
Oborn, B. 1994. Spacer length in clonal plants and the efficiency of resource capture in heterogeneous environments: a monte carlo simulation. Folia Geobotanica & Phytotaxonomica 29:139–158.
Pielou, E. C. 1977. Mathematical ecology. John Wiley & Sons, New York, New York, USA.
Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inferences of population structure using multilocus genotype data. Genetics 155:945–959.
Ripley, B. D. 1977. Modelling spatial patterns. Journal of the Royal Statistical Society, Series B (Methodological) 39:172–212.
Schenk, H. J., R. M. Callaway, and B. E. Mahall. 1999. Spatial root segregation: Are plants territorial? Pages 145–180 in A. Fitter and D. Raffaelli, editors. Advances in ecological research. Volume 28. Academic Press, London, UK.
Seabloom, E. W., O. N. Bjørnstad, B. M. Bolker, and O. J. Reichman. 2005. Spatial signature of environmental heterogeneity, dispersal, and competition in successional grasslands. Ecological Monographs 75:199–214.
Semchenko, M., E. A. John, and M. J. Hutchings. 2007. Effects of physical connection and genetic identity of neighbouring ramets on root-placement patterns in two clonal species. New Phytologist 176:644–654.
Semchenko, M., K. Zobel, and M. Hutchings. 2010. To compete or not to compete: an experimental study of interactions between plant species with contrasting root behaviour. Evolutionary Ecology 24:1433–1445.
Silverstone, J. and J. B. Wilson. 1994. Community
structure in a desert perennial community. Ecology 75:409–417.

Suyama, Y., K. Obayashi, and I. Hayashi. 2000. Clonal structure in a dwarf bamboo Sasa senanensis population inferred from amplified fragment length polymorphism (AFLP) fingerprints. Molecular Ecology 9:901–906.

Takahashi, M. K., L. M. Horner, T. Kubota, N. A. Keller, and W. G. Abrahamson. 2011. Extensive clonal spread and extreme longevity in saw palmetto, a foundation clonal plant. Molecular Ecology 20:3730–3742.

Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10:249–256.

Zobel, K. 2008. On the forces that govern clonality versus sexuality in plant communities. Evolutionary Ecology 22:487–492.

Zobel, M., M. Moora, and T. Herben. 2010. Clonal mobility and its implications for spatio-temporal patterns of plant communities: what do we need to know next? Oikos 119:802–806.