

Sex ratio and spatial pattern of males and females in the dioecious sandhill shrub, *Ceratiola ericoides ericoides* (Empetraceae) Michx

John Paul Schmidt

Received: 6 December 2006 / Accepted: 17 August 2007 / Published online: 6 September 2007
© Springer Science+Business Media B.V. 2007

Abstract Sex ratio, size, age, and spatial pattern were investigated for males and females of the dioecious shrub *Ceratiola ericoides ericoides* (Empetraceae) Michx. within seven mapped populations in Georgia and South Carolina, USA. Among the sites studied, two are regularly burned and one site long-unburned. Age was estimated from node counts of individual shrubs. Only one (fire-suppressed) population showed a female-biased sex ratio, while all others did not differ significantly from 1:1. Mean age estimates did not differ between sexes at any site nor did mean shrub canopy diameter. Bivariate Ripley's *K* analysis with a null hypothesis of random labeling was used to investigate whether any of the mapped populations exhibited spatial segregation of the sexes (SSS). No population showed strong evidence of SSS. Rather all sites but one showed males and females to be associated (though not significantly) at a scale of 1–10 m. At a scale of 10–35 m male and female shrubs were located randomly with respect to one another at all sites.

Keywords Dioecy · *C. ericoides* · Spatial segregation of the sexes · Sandhill · Ripley's *K* · Sex ratio

Introduction

Research comparing allocation patterns between genders in dioecious species indicates that female plants usually have higher resource requirements and/or reproduction imposes a greater drain on resources (Crawley 1997). Perhaps for this reason, a recurring pattern observed among dioecious plant species is an increasing proportion of males within populations along an axis of decreasing site productivity. This can occur over a range of scales, and with respect to broad environmental gradients or to microsite preferences. Male frequency has been shown to increase as environments become more stressful with increasing altitude (Pickering and Hill 2002), lower moisture availability (Dawson and Ehleringer 1993), increasing salinity (Eppley 2001; Vitale et al. 1987), more anoxic conditions (Shea et al. 1993), decreasing soil fertility (Lawton and Cothran 2000), and decreasing light levels (Nicotra 1998; Thomas and LaFrankie 1993). As related phenomena, higher levels of parasitism, herbivory, and disease have been detected in females (Gehring and Whitham 1992; Krischik and Denno 1990). Perhaps as a result of these patterns, sex ratios in dioecious plant populations are typically male-biased—often strongly so. Only rarely have biased primary sex ratios (sex ratio among seeds or seedlings) been documented (Eppley 2001). Rather, differential survival (Krischik and Denno 1990), differential competitive ability (Cox 1981; Agren 1988) or divergent flowering phenology (Bram and

J. P. Schmidt (✉)
Institute of Ecology, University of Georgia, Athens, GA
30602, USA
e-mail: jps@uga.edu

Quinn 2000; Purrington 1993) between males and females are the specific mechanisms invoked most often to explain apparently skewed sex ratios.

A phenomenon related to sex ratio bias is spatial segregation of the sexes (SSS) within dioecious plant species. In environments that are heterogeneous and/or where resources are patchily distributed, populations taken as a whole may have equal proportions of males and females. However, sex ratio bias at a localized scale—between microsites or landscape features—results in SSS. SSS may alleviate, at least in part, the higher reproductive cost of females if they preferentially occupy resource-rich microsites. Whereas dioecy is associated with production of fleshy fruits (Renner and Ricklefs 1995; Muenchow 1987), among island floras (Sakai et al. 1995), and with abiotic pollination (Vamosi et al. 2003; Renner and Ricklefs 1995), SSS has been reported most often among wind-pollinated dioecious species. SSS has been shown for *Acer negundo* (box elder) (Freeman et al. 1997; Dawson and Ehleringer 1993), *Nyssa aquatica* (water tupelo) (Shea et al. 1993) and *Juniperus virginiana* (red cedar) (Lawton and Cothran 2000), and spinach (*Spinacia oleracea*) (Vitale et al. 1987) among other species.

My objective in this study was to investigate whether *Ceratiola ericoides* (Empetraceae) Michx., a dioecious shrub which grows to 2 m in height and is found on xeric, sandy sites on both coastal and inland sites in the southeastern United States: (1) evidenced any sex ratio bias within surveyed populations, (2) determine whether SSS at any scale was apparent, and (3) investigate possible intersexual differences in mean size and age.

Materials and methods

Study species

Ceratiola ericoides (Empetraceae) Michx. is an aromatic, needle-leaved, evergreen, wind-pollinated, which grows to 2 m in height and is found on dry, sandy soils on both coastal and inland sites in the southeastern United States. In central Florida where it occurs most abundantly, *C. ericoides* is typically dominant only on xeric, deep sands on the uplands of old dune ridges. However, on many sites north of the Lake Wales Ridge in Florida, *C. ericoides* grows in

close association with *Quercus laevis* and *Pinus palustris*. Many populations of *C. ericoides* occurring in Georgia or South Carolina appear to be suppressed by *Q. laevis*, which has encroached probably as a result of fire exclusion.

Ceratiola ericoides is the sole member of its genus within the Empetraceae (crowberry family), which is comprised of three genera and six species, all but two of which are dioecious (Heywood 1993). In mature plants, a flower is borne in each leaf axil of the previous year's growth during a September–October bloom period. Pollinated female flowers develop into 3-mm diameter fleshy fruits which ripen during the month of January–April. Since remnant flowers and fruits persist in all seasons, reproductive shrubs are easily sexed. *C. ericoides* has a whorled branching pattern with the main unshaded branches producing new shoots once a year, therefore shrubs can be aged nondestructively by counting the nodes on the main branches (Johnson 1982). The fruits of *C. ericoides* are apparently dispersed by vertebrates and birds such as towhees are known to consume them. Harvester ants have also been observed to collect and consume the fruits of *C. ericoides* (Johnson 1982).

Study sites

The study was carried out in Georgia and South Carolina (USA). Individual study sites were located in the Coastal Plain physiographic province of both states, sandhill habitats occurring in distinct sub-provinces (Fig. 1). Populations mapped at Fort Gordon and in Burke County, Georgia and Aiken County, South Carolina are located within the Fall Line sandhills, a formation of remnant dunes which marked the Atlantic shoreline during the Miocene epoch (Ivester et al. 2001). Three other populations (at the state-owned Ochoopee Dunes Natural Area and at two sites near Cobbtown, Georgia) were mapped on riverine sandhills located along the Ochoopee River in southeastern Georgia. Sites at Fort Gordon are burned annually whereas most other sites have not burned within the last 10 years, though detailed fire histories prior to 1990 are not known for any sites other than Fort Gordon. Soil substrates also differ between sites: some sites at Fort Gordon and in Aiken County occur on kaolin (highly leached clay) rather

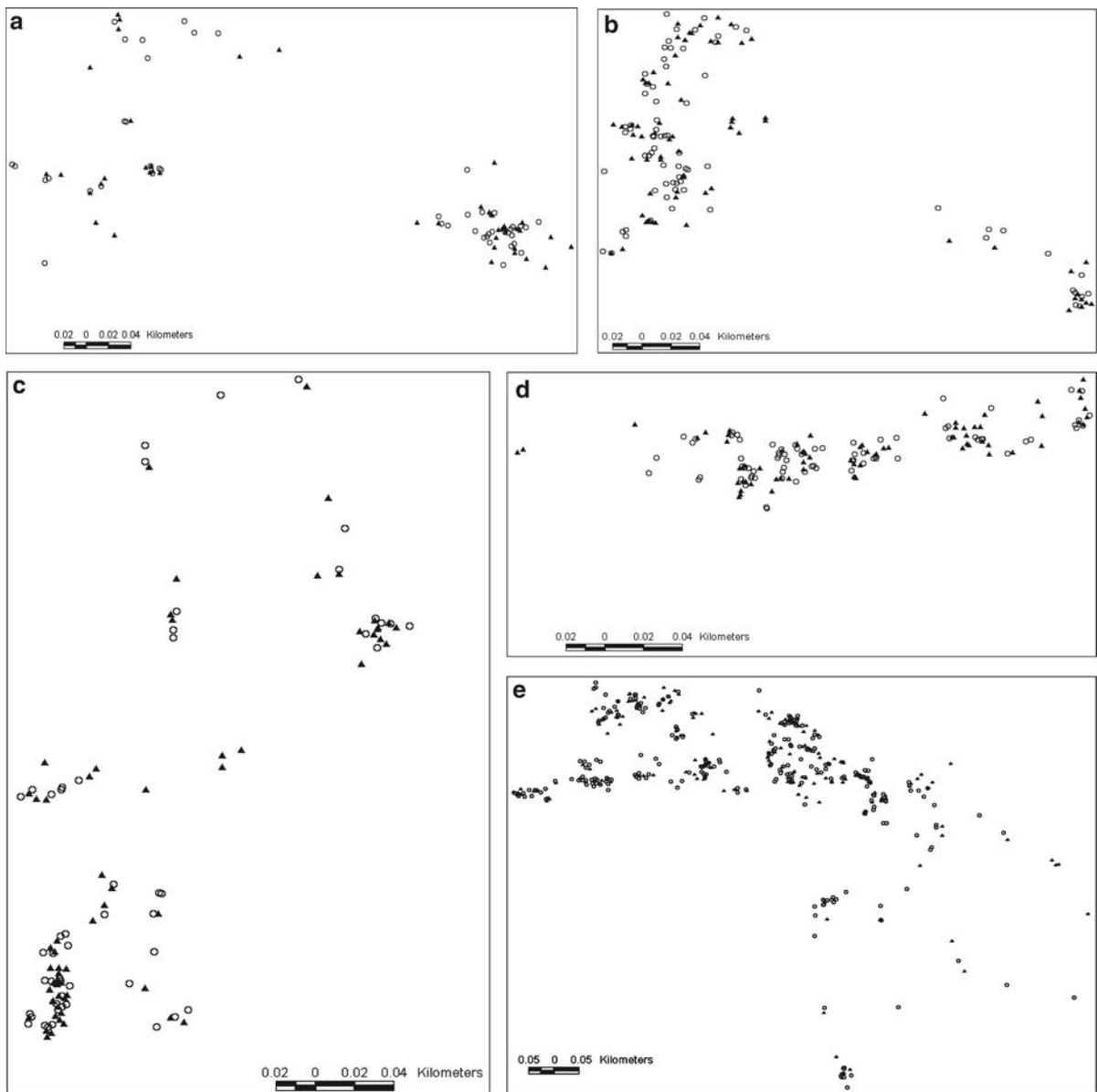


Fig. 1 Mapped adult shrubs, males (▲) and females (○) by site: (a) Burke County, (b) Cobbtown 1, (c) Cobbtown 2, (d) Fort Gordon 1, and (e) Oohoopee Dunes Natural Area

than on thick sandy deposits (Table 1). Of the populations mapped, Oohoopee Dunes site was the largest population completely mapped and the only population available for study which spanned a topographic gradient from floodplain to upper dune.

Field mapping

A total of 1,583 shrubs from seven populations from three habitat variants were mapped (Table 1) using a

Trimble GeoExplorer 3 GPS unit (and associated Trimble Pathfinder software to perform differential correction from base-station files). Trimble advertises 1–5 m accuracy. Comparing deviation of coordinates of GPS-mapped landmarks (roads, large trees) with their locations on rectified aerial photos (DOQQs), I estimate an accuracy of 1–2 m. GPS mapping was facilitated by the open terrain and the low, open canopy of sandhill vegetation. For all populations except Cobbtown 2, the entire population was

Table 1 Site summary showing ecoregion, fire regime, soil type and tallies of males, females, and juveniles for each mapped population

Population	Region	Fire regime	Soil	Latitude (dd)	Longitude (dd)	Elev. (m)	Male	Females	Juv.	Total
Fort Gordon 1	Fall Line	Annually burned	Sandy	33.32160	-82.33966	130	18	17	22	57
Fort Gordon 2	Fall Line	Annually burned	Clay	33.39107	-82.17445	130	73	77	340	490
Burke County	Fall Line	<10 years	Sandy	33.19952	-82.17660	85	45	46	32	124
Aiken County	Fall Line	>10 years	Clay	33.60645	-81.74906	160	14	14	38	66
Ohoopsee Dunes ^a	Coastal Plain	>15 years	Sandy	32.54257	-82.45319	102	193	258	123	583
Cobbtown 1	Coastal Plain	? >15 years	Sandy	32.27847	-82.16640	76	62	53	0	115
Cobbtown 2	Coastal Plain	? >15 years	Sandy	32.31347	-82.21843	76	68	73	78	219
							473	538	633	1654

^a Sex ratio significantly female-biased ($\chi^2 = 9.39$, $df = 1$, $P = 0.002$)

mapped with transects established to find all plants. For Cobbtown 2, plots were arbitrarily established to include a sample of 300–400 plants. For each shrub, sex (male, female, juvenile), canopy diameter, and maximum number of nodes counted from the tallest central stem were recorded.

Spatial pattern and comparison of size and age between sexes

At each site, sex ratio was tallied and a χ^2 test was used to determine whether ratios differed significantly from equality. In addition, size (canopy by diameter of shrub in meters), and age (estimated from node counts) were tallied by sex and analyzed statistically for significant differences between sexes using the Proc GLM in SAS (SAS Institute, Inc. 2000–2004) to perform an analysis of variance.

The occurrence of spatial segregation between males and females was investigated for the five populations larger than 100 individuals using Ripley's K -function (1981) analysis of spatial pattern. Ripley's K is a second-order statistic based on the distribution of distances of pairs of points. Ripley's K and other second-order statistics describe the spatial correlation of the point pattern. Bivariate Ripley's K -function was used to assess patterns of male and female *C. ericoides* shrubs in relation to one another. In order to remove scale-dependence and stabilize the variance, a square root transformation of K , called the L -function, is used instead:

$$L_{12}(r) = \left(\sqrt{\frac{K_{12}(r)}{\pi}} - r \right)$$

where r = distance. $L_{12}(r)$ are determined from the number of males within a distance r of an arbitrary female, and $L_{21}(r)$ is determined by the number of females within distance r of an arbitrary male (Diggle 1979).

In bivariate point pattern analyses, the choice of null hypotheses is between independence and random labeling. To test the first case, simulations are performed that involve random shifts of the whole of one component pattern relative to the other. To test against a null hypothesis of random labeling, labels (1 or 2) are assigned to points whose locations are fixed. The null hypothesis of random labeling is applied not to questions about the interaction between two processes, but rather about the process that assigns labels to points (Wiegand and Moloney 2004). Random labeling is appropriate when the "locations of type 1 and 2 points result from a completely random process affecting *a posteriori* a single spatial pattern (Goreaud and Pelissier 2003)." Therefore, random labeling is the appropriate null model in testing for spatial segregation of male and female shrubs. The specific test for bivariate random labeling used here corresponds to that proposed by Goreaud and Pelissier (2003): $L_{12}(r) = L(r)$. Values of $L_{12}(r) > 0$ indicate that there are on average more points of pattern 2 within distance r of pattern 1 than expected under the null hypothesis. Conversely, $L_{12} < 0$ indicates repulsion between the two patterns up to distance r . When $L_{12} = 0$, the

relationship between the two patterns is interpreted as random. Analyses were performed using Programita (http://www.oesa.ufz.de/towi/towi_programita.html), a software program written by Wiegand and Moloney (2004). $L_{12}(r)$ was calculated over an area corresponding to the minimum bounding box of each population. Results are given as $L_{12}(r) - L_{11}(r)$, which represents the dispersion pattern of females to males relative to the dispersion of all shrubs within the joint population.

Results

Sex ratio, age, and size

Only at the Ohoopsee Dunes site did sex ratios differ significantly from 1:1 for any age class (Table 1). However, this difference was not statistically significant. No significant differences between age or size of males and females occurs at any site (Table 2).

Spatial segregation of sexes

Maps (Fig. 2) of the five largest populations depict a random to clumped spatial pattern of individuals within each population. With the exception of Cobbtown 2 and Burke County, bivariate Ripley's K comparisons of the spatial distribution of males and females revealed them to be patterned either randomly or associated (though not significantly) with respect to one another at a scale of 1–10 m, and randomly with the exception of the Fort Gordon population. Only the Fort Gordon populations showed a significant—though barely so—pattern of association between males and females at a scale of

1–5 m. Cobbtown 2 showed a negative, though barely significant, association between males and females at the scale 1–10 m. Burke County exhibits an alternating, though non-significant, positive and negative association between males and females. At all sites, males and females are patterned randomly with respect to each other at the scale 10–35 m.

Discussion

As a strictly dioecious, fleshy-fruited, wind-pollinated species growing in a stressful environment where resources appear to be both patchy and distributed along a gradient, *C. ericoides* was expected to demonstrate some form of differentiation between the sexes in growth or survivorship and/or sex ratio bias or male/female spatial segregation. Based on the results of this study, there appears to be little differentiation between sexes in *C. ericoides* in either mean age or size within populations suggesting differences in growth or survivorship between males and females are unlikely. Nor do there appear to be skewed sex ratios or evidence for SSS within the *C. ericoides* populations mapped. [At Fort Gordon a marginally significant clumped pattern of association (the opposite of SSS) probably results from the more frequent burning which has occurred there. Relatively few safe sites for shrub establishment and persistence result in a more clumped pattern of occurrence for all shrubs and therefore for males and females as well.] Therefore this study concurs with that of Gibson and Menges (1994) who investigated spatial pattern in seaside and central Florida populations of *C. ericoides*, habitats which are more homogeneous than the sandhill sites included in this study. A similar study

Table 2 Mean age (nodes), and mean size (shrub canopy diameter in meters) by sex and site, standard deviations in parentheses

Site	Nodes		Diameter	
	Males	Females	Males	Females
Aiken County	9.36 (3.41)	8.14 (3.01)	0.52 (0.36)	0.57 (0.46)
Burke County	13.00 (2.71)	12.56 (2.03)	1.71 (0.65)	1.78 (0.51)
Cobbtown 1	15.45 (5.89)	13.46 (4.66)	2.42 (0.94)	2.23 (1.18)
Cobbtown 2	12.08 (5.86)	12.00 (7.11)	1.49 (1.07)	1.61 (1.11)
Gordon 1	8.94 (3.84)	7.47 (3.32)	0.74 (0.65)	0.46 (0.27)
Gordon 2	9.49 (3.33)	11.40 (7.33)	0.74 (0.46)	0.90 (0.69)
Ohoopsee Dunes Natural Area	17.41 (6.80)	18.76 (6.45)	2.80 (1.50)	3.01 (1.44)

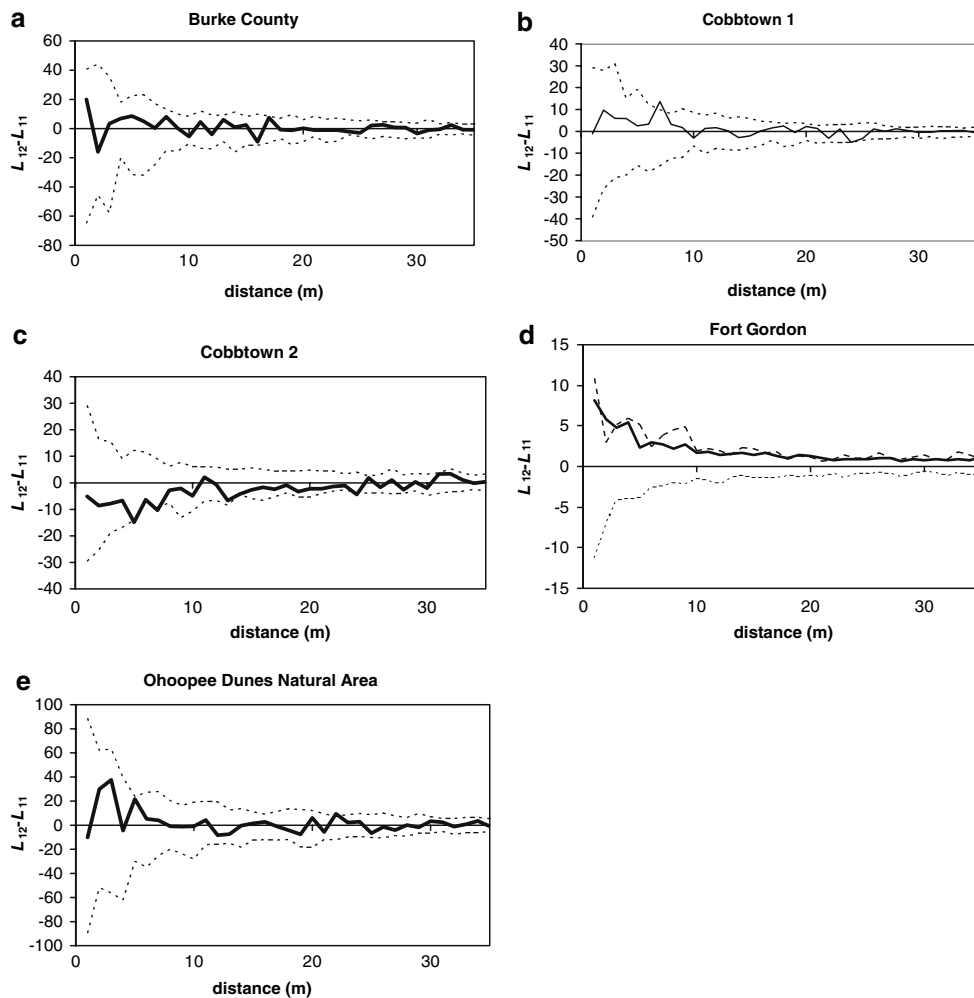


Fig. 2 Results of Ripley bivariate $L(t)$ analysis of male and female *C. ericoides* shrubs. In (a) Burke County, (b) Cobbtown 1, (c) Cobbtown 2, (d) Fort Gordon 1, and (e) Ochoopee Dunes Natural Area. $L_{12}-L_{11}$ values are represented by solid lines. Dashed lines represent 95% confidence intervals generated from 1000 Monte Carlo simulations of a randomly distributed

population. $L_{12}-L_{11}$ values within the confidence envelop indicate a random association between males and females, above the envelop indicate significant association between males and females, and below the envelop indicate significant segregation between males and females

of *Corema conradii* (Rocheleau and Houle 2001), an Empetraceous shrub of the coastal dune heathlands of northeastern North America, also found no spatial segregation between males and females although males were significantly larger.

From an evolutionary standpoint there are also many reasons to predict a finding of little segregation. Selection can be expected to minimize differences in reproductive costs between the sexes. Differences between the sexes in reproductive costs, if they exist at all, may be very slight given the large volume of pollen produced by males in the case of *C. ericoides*

(Pedro Quintana-Ascencio pers comm.) and the variability in fruit production by females. Female reproductive investment may exceed the average outlay of resources by males in some years, but fall well short in other years, averaging to a similar or lesser value.

However, reproductive costs have been inferred from lower growth, survival, and/or frequency of flowering often reported for females, and/or by a more variable reproductive effort through time (Popp and Reinartz 1988; Obeso et al. 1998). Allen and Antos (1988), Cipollini and Stiles (1991), Guitian

et al. (1997), Nicotra (1999), and Rocheleau and Houle (2001) demonstrate larger investments in reproduction by females. Where reproductive costs have been quantified in terms of differential allocation patterns to flowers and fruits, males frequently invest more in reproduction at time of flowering (Rocheleau and Houle 2001; Guitian et al. 1997; Cipollini and Whigham 1994; Thompson and Edwards 2001). However, total reproductive investments in biomass (Thompson and Edwards 2001; Cipollini and Stiles 1991), N (Cipollini and Whigham 1994) and Mg and Ca (Rocheleau and Houle 2001) were higher for females than males—as a result of fruit production.

Determining real reproductive costs in *C. ericoides*, a highly aromatic (terpene-releasing) shrub which grows in an environment where light is generally not limiting, is difficult since carbon compounds probably do not represent a significant expense. Carbon invested to fruit biomass, for example, may not always represent a limiting resource. Some reproductive structures may yield a significant photosynthetic return and nutrients expended in reproductive structures may be reabsorbed (Ashman 1994). Yet for many plants of arid or low-productivity environments, growth and reproduction may be limited by the availability of critical nutrients, yet C-based compounds produced through photosynthesis may be in surplus (Crawley 1997).

If reproductive costs for *C. ericoides* in either nutrients or carbon are higher for females as has often been reported for dioecious species of similarly stressful habitats, then females may be compensating via greater allocation of photosynthate to roots or mycorrhizae. Such a difference in allocation might not be apparent in intersexual growth differences in most environments if males are volatilizing more aromatic compounds and/or expending more photosynthate during flowering through pollen and flower production. However, where a combination of stressors exists, compensation by females may not be possible. Gehring and Whitham (1992), for example, found that mistletoe-infested females *Juniperus monosperma* trees showed lower rates of root mycorrhizal infection. In the case of *C. ericoides* populations investigated in this study, contrasts in soils and fire histories did not result in differences in sex ratios or SSS, nor in mean size or age of males versus females.

Acknowledgments The author would like to thank Jon Ambrose and the staff of the Georgia Natural Heritage Program (Georgia Department of Natural Resources) who provided funding and support for this study as well as the Georgia Botanical Society which supported this research through a Marie Mellinger Grant.

References

- Agren AB (1988) Sexual differences in biomass and nutrient allocation in the dioecious *Rubus-chamaemorus*. *Ecol* 69(4):962–973
- Allen GA, Antos JA (1988) Relative reproductive effort in males and females of the dioecious shrub *Oemleria cerasiformis*. *Oecologia* 76:111–118
- Ashman TL (1994) A dynamic perspective on the physiological cost of reproduction in plants. *Am Nat* 144:300–316
- Bram MR, Quinn JA (2000) Sex expression, sex-specific traits, and the effects of salinity on growth and reproduction of *Amaranthus cannabinus* (Amarathaceae), a dioecious annual. *Am J Bot* 87:1609–1618
- Cipollini ML, Stiles EW (1991) Costs of reproduction in *Nyssa sylvatica*—sexual dimorphism in reproductive frequency and nutrient flux. *Oecologia* 86:585–593
- Cipollini ML, Whigham DF (1994) Sexual dimorphism and cost of reproduction in the dioecious shrub *Lindera benzoin* (Lauraceae). *Am J Bot* 81:65–75
- Cox PA (1981) Niche partitioning between sexes of dioecious plants. *Am Nat* 117:295–307
- Crawley MJ (1997) Dioecy. In: Crawley MJ (ed). *Plant ecology*. Oxford, Malden, MA
- Dawson TE, Ehleringer JR (1993) Gender-specific physiology, carbon isotope discrimination, and habitat distribution in boxelder, *Acer negundo*. *Ecol* 74:798–815
- Diggle PJ (1979) On parameter estimation and goodness-of-fit testing for spatial point patterns. *Biometrics* 35:87–101
- Eppley SM (2001) Gender-specific selection during early life history stages in the dioecious grass *Distichlis spicata*. *Ecol* 82:2022–2031
- Freeman DC, Doust JL, et al (1997) Sexual specialization and inbreeding avoidance in the evolution of dioecy. *Bot Rev* 63:65–92
- Gehring CA, Whitham TG (1992) Reduced mycorrhizae on *Juniperus monosperma* with mistletoe—the influence of environmental stress and tree gender on a plant parasite and a plant–fungal mutualism. *Oecologia* 89:298–303
- Gibson DJ, Menges ES (1994) Population structure and spatial pattern in the dioecious shrub *C. ericoides*. *J Veg Sci* 5:337–346
- Goreaud F, Pelissier R (2003) Avoiding misinterpretation of biotic interactions with the intertype K12-function: population independence vs. random labeling hypotheses. *J Veg Sci* 14:681–692
- Guitian P, Medrano M, Guitian P et al (1997) Reproductive biology of *Corema album* (L.) D. Don (Empetraceae) in the northwest Iberian Peninsula. *Act Bot Gall* 144:119–128
- Heywood VH (1993) *Flowering plants of the world*. Oxford University Press, New York

- Ivester AH, Leigh DS, Godfrey-Smith DI (2001) Chronology of inland eolian dunes on the coastal plain of Georgia, USA. *Quater Res* 55:293–302
- Johnson AF (1982) Some demographic characteristics of the Florida rosemary *C. ericoides* Michx. *Am Mid Nat* 108:170–174
- Krischik VA, Denno RF (1990) Difference in environmental response between the sexes of the dioecious shrub, *Baccharis-Halmifolia* (Compositae). *Oecol* 83:176–181
- Lawton RO, Cothran P (2000) Factors influencing reproductive activity of *Juniperus virginiana* in the Tennessee Valley. *J Torr Bot Soc* 127:271–279
- Muenchow GE (1987) Is dioecy associated with fleshy fruit? *Am J Bot* 74(2):287–293
- Nicotra AB (1998) Sex ratio variation and spatial distribution of *Siparuna grandiflora*, a tropical dioecious shrub. *Oecol* 115:102–113
- Nicotra AB (1999) Reproductive allocation and the long-term costs of reproduction in *Siparuna grandiflora*, a dioecious neo-tropical shrub. *J Ecol* 87:138–149
- Obeso JR, Alvarez-Santullano M, et al (1998) Sex ratios, size distributions, and sexual dimorphism in the dioecious tree *Ilex aquifolium* (Aquifoliaceae). *Am J Bot* 85:1602–1608
- Pickering CM, Hill W (2002) Reproductive ecology and the effect of altitude on sex ratios in the dioecious herb *Aciophylla simplicifolia* (Apiaceae). *Aus J Bot* 50:289–300
- Popp JW, Reinartz JA (1988) Sexual dimorphism in biomass allocation and clonal growth of *Xanthoxylum americanum*. *Am J Bot* 75:1732–1741
- Purrlington CB (1993) Parental effects on progeny sex ratio, emergence, and flowering in *Silene latifolia* (Caryophyllaceae). *J Ecol* 81:807–811
- Renner SS, Riklefs RE (1995) Dioecy and its correlates in flowering plants. *Am J Bot* 82(5):596–606
- Ripley BD (1981) *Spatial statistics*. Wiley, New York
- Rocheleau AF, Houle G (2001) Different cost of reproduction for the males and females of the rare dioecious shrub *Corema conradii* (Empetraceae). *Am J Bot* 88:659–666
- Sakai AK, Wagner WL, Ferguson DM, Herbst DR (1995) Biogeographical and ecological correlates of dioecy in the Hawaiian flora. *Ecol* 76(8):2530–2543
- SAS Institute Inc. (2000–2004) *SAS 9.1.3 Help and documentation*. SAS Institute Inc., Cary, NC
- Shea MM, Dixon PM et al (1993) Size differences, sex-ratio, and spatial distribution of male and female water tupelo, *Nyssa aquatica* (Nyssaceae). *Am J Bot* 80:26–30
- Thomas SC, LaFrankie JW (1993) Sex, size, and inter-year variation in flowering among dioecious trees of the Malayan rain-forest. *Ecol* 74:1529–1537
- Thompson DI, Edwards TJ (2001) Breeding biology, resource partitioning and reproductive effort of a dioecious shrub, *Clusia pulchella* L. (Euphorbiaceae). *Plant Syst Evol* 226:13–22
- Vamosi JC, Otto SP, Barrett SCH (2003) Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. *J Evol Biol* 16(5):1006–1018
- Vitale JJ, Freeman DC et al (1987) Patterns of biomass allocation in *Spinacia Oleracea* (Chenopodiaceae) across a salinity gradient—evidence for a niche separation. *Am J Bot* 74:1049–1054
- Wiegand T, Moloney KA (2004) Rings, circles, and null-models for point pattern analysis in ecology. *Oikos* 104:209–229