

Sexually dimorphic growth in the dioecious tropical shrub, *Siparuna grandiflora*

A. B. NICOTRA*

Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269, USA

Summary

1. To demonstrate evolved sex-based differences in vegetative traits of dioecious plant species, one must consider both pre-reproductive and reproductive individuals, as dimorphic patterns commonly arise secondarily from different effects of reproduction on resource balance.

2. *Siparuna grandiflora*, a neotropical dioecious shrub in which females allocate significantly more biomass to reproduction than males, was studied for 2 years (three reproductive events) to determine whether sex-based differences in stem growth, leaf production and allocation pattern could be detected in pre-reproductive individuals grown from cuttings in field plots or in mature naturally occurring individuals.

3. Among pre-reproductive individuals, females accumulated more stem and leaves than males, but among mature individuals, no sex-based growth differences were apparent. In mature individuals, both growth and leaf longevity were positively correlated with reproductive frequency. With regards allocation, pre-reproductive males had larger leaves than females, and mature females allocated less biomass per unit stem length than males.

4. The capacity of pre-reproductive females to grow faster than males demonstrates innate differences between the sexes. That mature females achieved equivalent growth to males, despite higher reproductive allocation, indicates that the greater growth capacity of young females is sustained in older females and enables them to compensate for greater reproductive allocation.

Key-words: Allocation patterns, leaf longevity, leaf production, reproductive costs, stem growth

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Introduction

Sexual differentiation in vegetative traits of dioecious plants may reflect innate differences between the sexes, or may arise secondarily from different effects of reproduction on resource balance. While theory predicts that innate differentiation can evolve in response to sexual selection or selection for ecological divergence (e.g. Meagher 1984), such differentiation is difficult to detect in naturally occurring plants because it is generally obscured by effects of reproduction (e.g. Gehring 1990). Thus, to demonstrate innate sex-based differences, as distinct from those mediated by reproduction, plants must be followed from the pre-reproductive state through to reproduction, preferably under biologically realistic conditions.

Natural populations of the dioecious tropical shrub *Siparuna grandiflora* Kunth in Humb. & Bonpl., A. DC., syn *S. tonduziana*, Siparunaceae (S. Renner,

personal communication) exhibit a male-biased sex ratio that results from precocious reproduction in males, higher frequency of reproduction in males and, potentially, from greater mortality among mature females (Nicotra 1998). The sexes are found in microenvironments that are not detectably different, although males can flower at lower light availability than females (Nicotra 1998). The difference in frequency of reproduction is most probably related to differences in reproductive allocation and subsequent effects on growth (Nicotra 1999). Females allocate more biomass to reproduction and reproduction has a negative effect on growth in females but not in males. However, when reproductively mature females are prevented from reproducing they grow faster than males of equivalent condition (Nicotra 1999).

Given that *S. grandiflora* females allocate more to reproduction, live in microenvironments equivalent to those of males and grow more when experimentally prevented from reproducing, do naturally occurring females differ in vegetative growth from males? The pattern of equivalent growth despite greater

reproductive allocation has been identified in a wide range of dioecious species (e.g. Putwain & Harper 1972; Grant & Mitton 1979; Onyekwelu & Harper 1979; Gross & Soule 1981; Sakai & Burris 1985; Lovett Doust & Lovett Doust 1987, 1988; Gehring & Linhart 1993; Ramadan *et al.* 1994). Other studies have demonstrated that if there are no mechanisms to compensate for resource allocation to reproduction, males achieve greater growth than females (e.g. Lloyd & Webb 1977 and references therein; Popp & Reinartz 1988; Garcia & Antor 1995). Compensatory mechanisms that enable equivalent growth might derive from greater resource-use efficiency, higher average or maximum carbon assimilation rates or allocational differences that result in greater or more effective leaf display in females. That *S. grandiflora* females have the potential to achieve greater growth when reproduction is suppressed suggests that females have compensatory responses to offset the costs of reproduction. These compensatory responses may cause the average growth rates of females (considering both reproductive and non-reproductive seasons) to exceed or be equivalent to those of males, despite greater reproductive allocation by females during some periods.

Growth and allocation to leaves *vs* stems was examined in *S. grandiflora* plants grown to reproductive maturity in field plots to determine whether there are sexual dimorphisms in vegetative traits prior to reproduction. Naturally occurring mature plants were used to assess the existence and importance of vegetative differentiation under unmanipulated conditions. The objectives of the study were to (1) determine whether innate differentiation between the sexes is apparent in plants grown to reproductive maturity from cuttings in field gardens, (2) determine if greater allocation of biomass to reproduction in mature females is associated with sex-based differences in patterns of stem growth and leaf production under natural field conditions, (3) investigate how frequency of reproduction affects growth under natural conditions and (4) contrast the results from naturally occurring and experimental plants to determine whether any evidence of sexual differentiation found in cuttings grown under modified field conditions is also apparent in naturally occurring mature plants. Based upon my previous work on *S. grandiflora*, I expected that females would compensate to some degree for their greater allocation to reproduction (Nicotra 1999). Under normal field conditions, such compensation might still fall short of making up for resources allocated to reproduction, however, in non-reproductive plants, I expected growth rates of females to exceed those of males.

Materials and methods

STUDY SPECIES AND SITE

The study was conducted in lowland wet tropical forest in the Sarapiquí region of Costa Rica, primarily at

the Organization for Tropical Studies' La Selva Biological Station (for detailed site description see McDade *et al.* 1994). *Siparuna grandiflora* is a common understory shrub in secondary forests of the region. The shrub flowers when *c.* 1 m in stem length and can exceed 5 m in height. At La Selva, flowering occurs primarily between January and May. Staminate flowers (male) do not bear vestigial pistillate structures, nor do pistillate flowers (female) bear any male structures. There are no records of *S. grandiflora* or any other *Siparuna* switching sex, nor did any individual change sex during the course of this study (A. Nicotra, personal observation, S. Renner, personal communication, Antonio 1983; Feil 1992).

STEM GROWTH AND LEAF PRODUCTION OF PLANTS GROWN FROM CUTTINGS

To examine sex-based differences in pre-reproductive plants, eight cuttings were taken from each of 11 female and 13 male genotypes in July 1993. All cuttings were taken from current year's growth on mature branches of the parent plant. In early 1994, during the flowering season, the sex of all plants used for cuttings was verified in the field. Each cutting consisted of two nodes with one leaf (trimmed to half its original size to reduce water loss) and one node below ground. Cuttings were treated with synthetic rooting hormone and propagated in a sand bench in a shadehouse. Not all cuttings survived, so in October 1993 additional cuttings were taken from genotypes that had high initial mortality. Rooted cuttings were transferred to flexible plastic tree tubes with air holes in a sand-soil mix (Forestry Suppliers). Plants were watered as needed and fertilized approximately every other month between October 1993 and May 1994, when they were planted into the field. There were no significant effects of date of cutting initiation on the size of cuttings when planted. Because of mortality in the cutting bench and the sex ratio bias in the cutting stock, there was a male sex ratio bias in the plots (a total of 14 female and 25 male plants \pm 2 per plot). Although the cuttings were taken from mature plants of known sex, they were more like seedlings than adults, in that they began with just two stem nodes and very little leaf area. Their growth pattern in the first year of life may therefore be analogous to that of a seedling.

Four plots were established in the secondary rainforest habitat in which the species naturally occurs. The plants therefore experienced realistic climatic variation and were exposed to natural herbivores, pollinators and seed dispersers. Two existing small light gaps, *c.* 10 m across, were cleared of vegetation and two forest plots were cleared of understory vegetation less than 1.5 m tall, hereafter high and low light treatments, respectively. Plots were 10 m square and plants were in rows with 1 m between all plants and a 1 m border around the plot edge. Plots were weeded throughout the study to minimize interspecific

competition and maintain relatively uniform light levels within plots. Plants were assigned to plots using a structured random approach, so that genotypes were distributed evenly throughout plots.

One hemispheric canopy photograph was taken in the centre of each plot in May 1994 to assess the general light level. Photographs were analysed digitally using the Solarcalc canopy photograph analysis system (Chazdon & Field 1987). The integrated total incident PPF (photosynthetic photon flux density, $\text{mol m}^{-2} \text{day}^{-1}$) for the two high-light plots was 7.15 and 11.51, and for the low light plots was 4.76 and 5.54. In July 1996, nine photographs were taken in each plot to measure light availability and variation. Total incident PPF in 1996 was analysed using ANOVA with the two understorey plots nested within one light level (low), and the small gap plots nested as a second light level (high). The light effect was highly significant ($P = 0.004$) and the plot effect was not significant. In 1996, mean (\pm SE) PPF ($\text{mol m}^{-2} \text{day}^{-1}$) at high light was $9.33(\pm 0.64)$ and at low light was $5.61(\pm 0.24)$. These data indicate that the manipulation successfully altered light availability and yielded a consistent light difference through the duration of the study.

The number of leaves and stem length of each plant were measured prior to planting (April 1994) and 2 weeks after planting, in June 1994. Measurements of stem length and leaf number were repeated every 3 months until June 1996, with the exception of June 1995. Plot differences were investigated using ANOVA with sex, light and plot effects (plot was nested within light) and all possible interactions. There were no significant plot effects, so to simplify subsequent analyses, plot effects were excluded (data not shown). To determine whether sex, light or time since planting affected stem growth and leaf production a repeated measures ANOVA was conducted. Scheffé post-hoc tests were used to identify significant differences among treatment means. All analyses were carried out in Datadesk (Data Description Inc., Ithaca, NY, USA).

To examine relative allocation to stem and leaves in the plants grown from cuttings, leaf area was estimated from the relationship established by regressing leaf width on leaf area. The regression was obtained by tracing the perimeter of leaves on plants in the field in June 1996. For each tracing, leaf width was measured, and area determined using a leaf area meter (Licor, Lincoln, NE, USA). At high light a total of 47 leaves from five age classes were measured, at low light 31 leaves were measured. A regression between leaf width and leaf area was calculated for each light level (high light: $r^2 = 0.91$, low light $r^2 = 0.93$). Sex effects on the width to area relationship examined using ANCOVA but were not significant. The width of all leaves on each plant was measured in June or July 1996, and total plant leaf area calculated based upon the regression equation. Total leaf area and estimated area per leaf were analysed with ANOVA which

included sex and light effects and their interaction term. Finally, sex-based differences in relationships between leaf number, leaf area and stem length were explored using ANCOVA.

STEM GROWTH AND LEAF PRODUCTION IN NATURALLY OCCURRING PLANTS

To determine whether there are sex-based differences in patterns of vegetative growth among mature plants, stem growth and leaf production in naturally occurring plants from three sites (two at La Selva Biological Station and one at the nearby Selva Verde Ecologe) were measured. These plants were also included in a study of sex ratio and spatial distribution in *S. grandiflora* (Nicotra 1998a). *Siparuna grandiflora* is patchily distributed and common only in secondary forests on residual soils. Sites ($c. 0.25$ ha) were therefore selected in areas where at least 50 mature sized individuals were found. Within each site, all reproductively active plants between 100 and 1300 cm stem length were included. The lower boundary is the size at which first reproduction commonly occurs and the upper boundary encompasses nearly 94% of the population (332 of the 355 plants included in Nicotra 1998a) and is roughly the largest plant for which total stem length could be measured without damage. The study began with 109 plants, 10 died during the study, resulting in a total of 35 females and 64 males.

In early 1994, total stem length and leaf number on each plant were measured in the La Selva (January) and Selva Verde (March) sites. The newest meristem was marked with a single strand of coloured wire so that all new stem growth and leaf production could be identified. Measurements were repeated every 3 months until January or March 1996 (eight censuses in each site) and new stem growth and leaf production marked at each census. During each of the three flowering seasons (1994, 1995, 1996), I determined whether or not each individual was flowering and, if flowering, noted the sex of the flowers. In 1994 and 1995 hemispheric canopy photographs were taken above each plant to determine light availability, as light is likely to affect growth rates. Photographs were digitized and analysed using the Solarcalc package (Chazdon & Field 1987).

New stem growth and new leaf production were determined at each census date, and rates of growth and production calculated for each interval. Total stem and leaf production were determined for the full duration of the study (24 months). Mean rates of leaf and stem production were calculated as the average of the single interval production rates. Total stem growth and leaf production and rates thereof were analysed in one-way ANOVAs testing for sex effects. The influence of initial stem length or leaf number and flowering frequency on stem growth and leaf production were examined using ANCOVA because

both size and reproduction were likely to affect growth. Light availability was also investigated as a potentially important covariate. Variables were log transformed to improve normality as needed. The natural male sex ratio bias in populations of *S. grandiflora*, led to uneven cell sample sizes in the dataset.

LEAF LONGEVITY OF NATURALLY OCCURRING PLANTS

The census method used for growth measurements allowed me to determine the number of leaves produced and the number of leaves surviving between censuses in each cohort. With these data leaf longevity at both individual and population levels was analysed. The relationship between leaf age and survivorship was also examined but, because of low sample sizes, the relationship between time of leaf initiation and life span could not be analysed. Therefore, cohorts initiated at different dates were combined to increase the sample size of leaves surviving to 3, 6, 9 (etc.) months. An individual life table for leaves of each plant was calculated, and from this survivorship and leaf life expectancy were determined following standard demographic methods (e.g. Bazzaz & Harper 1977). The individual life table approach was sensitive to initial leaf number, so leaf longevity for each plant was also calculated using the model of Begon & Mortimer (1986):

$$L = \{(N_{i1} + p)/f\} - 1\}T,$$

where L is longevity, N_{i1} is initial leaf number, p is total number of leaves produced, f is the total number of leaves dying and T is the duration of the study. This method is not affected by the initial size of the plant but is sensitive to the duration of the study and does not discern between early and late deaths. The results of the life table and Begon and Mortimer methods will be referred to as life expectancy and longevity, respectively. Values from each of the two methods described above for calculating leaf life-span were analysed for variation between the sexes using ANOVA. Light availability was included as a covariate in preliminary models but did not yield any significant effects and so was not included in final models. When plants were classified by sex and flowering frequency, cell sizes were uneven.

Leaf survivorship was examined at the population level to establish that the individual based analyses were not overly biased by small numbers of leaves on individual plants. Plants were classified by sex and flowering frequency, a single life table was calculated for each classification, and survivorship and life expectancy were calculated using standard demographic methods.

VEGETATIVE ALLOCATION OF NATURALLY OCCURRING PLANTS

To examine vegetative allocation patterns in mature plants, 20 naturally occurring, reproductively active

individuals of each sex were harvested from secondary forest at La Selva Biological Station. Only reproductively active plants greater than 2 m stem length and judged to be less than 20 m total stem length in the field, were harvested. The lower size limit was imposed because females seldom flower in lower size classes, and the upper size limit was set because mean stem length is *c.* 5 m, and because plants larger than 20 m make up approximately 1% of the total population (five of the 355 plants included in Nicotra 1998). Allocation patterns in plants larger than 20 m are therefore not necessarily representative of the population as a whole. Because it is difficult to judge total stem length in the field, some of the harvested plants were larger than 20 m and two of these (one of each sex) were excluded from analyses because they were extreme outliers for a wide range of characters (Nicotra 1999). Plants were harvested from a single population and were selected as they were encountered, up to a total of 20 per sex. Additional data from these plants were also used in a study of reproductive allocation (Nicotra 1999).

For each harvested plant, stems were sawed off at ground level and total stem length of all branches was summed. Total number of leaves was counted and area determined using a leaf area meter (Li-Cor, Lincoln, NE, USA). Stems and leaves were then dried to constant mass at 70 °C, and weighed to the nearest mg. These measurements were analysed using ANCOVA to determine whether the relationships among vegetative traits differ for the sexes. In addition, a segment of stem (*c.* 15 cm) from ground level was taken from each plant to determine if the sexes differed in wood density (mass/volume). Density was analysed using one-way ANOVA. Variables were log transformed to improve normality.

Results

STEM AND LEAF PRODUCTION OF EXPERIMENTALLY GROWN PLANTS

Not only did plants grown from cuttings at high light produce more stem length and leaves than plants grown at low light, but high-light grown females produced both more stem and more leaves than males (Fig. 1). The sexes did not differ in size at the beginning of the study (ANOVA results not shown). However, by the end of the study, repeated measures ANOVA indicated significant light and date effects for both stem and leaf production, a significant sex by date interaction for stem production and a marginally significant sex by date interaction for leaf production (Table 1a, b). Clearly, light was the most important determinant of growth and by the end of the second year, high-light grown females had on average over 4 m of stem and nearly 50 leaves, whereas high-light males had about 3 m of stem and 30 leaves. All but 11 of the high-light grown plants (six females, five males)

flowered during the 1996 season. Despite the significant differences in total leaf number, there were not sex-based differences in total leaf area (Fig. 2a). High-light males actually produced slightly more leaf area on average than females because males had significantly greater area per leaf than females (Fig. 2b).

STEM GROWTH AND LEAF PRODUCTION IN NATURALLY OCCURRING PLANTS

There were no sex-based differences in total stem growth or total leaf production, or in rates thereof among naturally occurring mature plants, analysed on a single census basis, 6 month basis, or as the mean of all censuses. Initial size or leaf number and flowering frequency were tightly correlated with total stem and leaf production (Table 2). Larger plants of both sexes produced more stem and leaves than smaller plants. Plants that flowered in all three seasons produced more stem and leaves than plants that flowered in one or two seasons, regardless of sex (Fig. 3a,b). Although there was not a significant main effect for sex, there was a significant sex by flowering frequency interaction for stem production and a marginally significant interaction for leaf production (Table 2). Both interac-

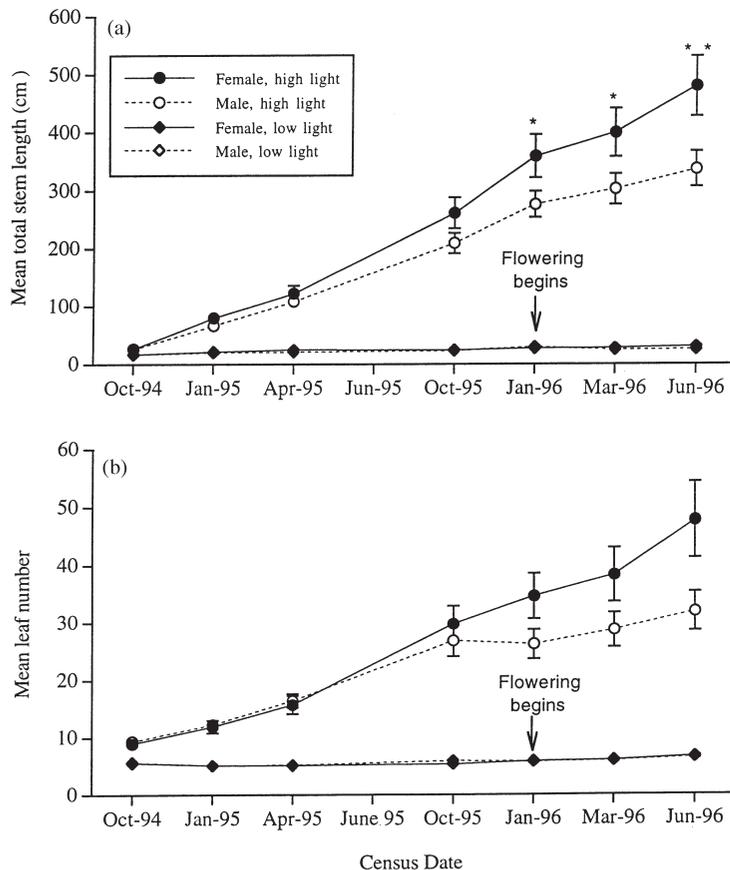


Fig. 1. (a) Mean stem length (\pm SE) and (b) mean leaf production (\pm SE) of cuttings grown in field plots, from October 1994 to June 1996. Asterisks denote significant differences between the sexes at high light according to Scheffé post-hoc tests (* $P \leq 0.05$, ** $P \leq 0.01$).

Table 1. Results of repeated measures analysis of variance for (a) stem and (b) leaf production by *S. grandiflora* cuttings

(a) Log (stem production)			
	df	MS	P
Sex	1	0.591	0.175
Light	1	140.7	≤ 0.001
Subject	122	0.318	≤ 0.001
Date	6	7.103	≤ 0.001
Sex \times light	1	0.246	0.381
Sex \times date	6	0.044	0.006
Light \times date	6	4.045	≤ 0.001
Sex \times light \times date	6	0.014	0.440
Error	724	0.014	
Total	873		
(b) Log (leaf production)			
	df	MS	P
Sex	1	0.140	0.466
Light	1	60.21	≤ 0.001
Subject	122	0.262	≤ 0.001
Date	6	1.704	≤ 0.001
Sex \times light	1	0.052	0.657
Sex \times date	6	0.054	0.067
Light \times date	6	1.096	≤ 0.001
Sex \times light \times date	6	0.015	0.767
Error	723	0.027	
Total	872		

tions were most probably owing to greater stem and leaf production by females that flowered in just one of the 3 years, relative to males of the same status (Fig. 3a,b). The model also indicated significant three-way interactions between sex, flowering frequency, and initial stem length for both stem and leaf production.

Plants at high light levels in 1994 had significantly greater stem production over the course of the study than those at lower light levels in 1994 (light effect $P = 0.02$ and $P = 0.04$ for stem and leaf production, respectively, ANCOVA). However, there was no sex by light interaction, so light effects cannot explain variation in patterns of stem production for the sexes. When initial size was included as a second covariate to light availability, the light effect was no longer significant, suggesting that much of the affect of light on stem growth and leaf production was indirect (data not shown).

LEAF LONGEVITY IN NATURALLY OCCURRING PLANTS

Leaf life span in naturally occurring plants was strongly affected by flowering frequency but not by sex (Table 3). Plants that flowered in all three seasons had significantly longer leaf life spans than plants that flowered in only one of the three seasons. Plants that flowered in two seasons had intermediate leaf life spans (Fig. 4, based on life expectancy calculation).

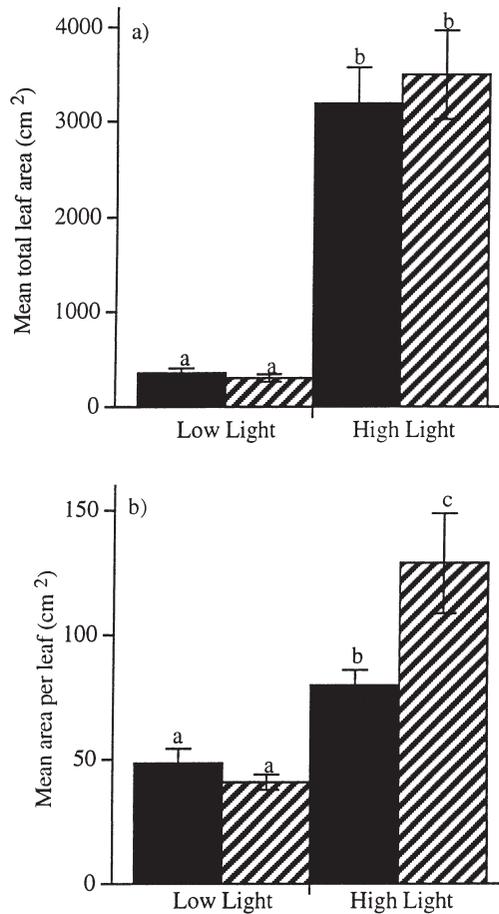


Fig. 2. (a) Mean leaf area (\pm SE) and (b) mean area per leaf (\pm SE) for cuttings growing in field plots in June/July 1996. Females are represented as solid bars, males as hatched bars. Different letters denote significant differences ($P \leq 0.05$) according to Scheffé post-hoc tests.

The life table and Begon and Mortimer estimates of leaf life span were highly correlated ($R = 0.761$, $n = 91$, $P \leq 0.05$), and yielded the same pattern. When the data for all plants in a given flowering frequency classification were pooled, the pattern was qualitatively identical to that of the individual based analyses (data not shown), indicating that the individual based estimates were not unduly influenced by within-plant variation.

VEGETATIVE ALLOCATION OF NATURALLY OCCURRING AND EXPERIMENTALLY GROWN PLANTS

Relationships between leaf area, leaf number and stem length for the plants grown from cuttings were examined to see if sex-based differences were apparent. These parameters were tightly correlated in each case and the sex difference in the relationship between leaf area and leaf number was significant, as would be expected given that males had greater estimated area per leaf (Table 4). The sexes did not differ in leaf area or leaf number as a function of stem length (data not shown).

On the harvested mature individuals, I measured leaf area, leaf number, leaf biomass, stem biomass and stem length, and postulated *a priori* seven relationships of interest in terms of sex-based differences: leaf area vs leaf number, leaf biomass and stem length; leaf biomass vs leaf number, stem biomass and stem length; stem length vs stem biomass. In all cases the traits were highly correlated but only one of the seven relationships (stem length vs stem biomass) showed any sign of sex-based differentiation (sex effect $P = 0.04$, ANCOVA, Fig. 5). Males allocated more

Table 2. Results of ANCOVA for effects of initial size, sex and flowering frequency over the course of the study on (a) stem production and (b) leaf production. Flowering frequency was classified (1,2,3) based upon the number of years that the plant flowered during the study

(a) Log (stem production)	df	SS	MS	F-ratio	P
Sex	1	0.122	0.122	2.150	0.146
Flowering frequency	2	0.396	0.198	3.486	0.035
Log (initial stem length)	1	0.504	0.504	8.867	0.004
Sex \times flowering frequency	2	0.582	0.291	5.124	0.008
Sex \times log(initial stem length)	1	0.128	0.128	2.251	0.137
Flowering frequency \times log (initial stem length)	2	0.494	0.247	4.346	0.016
Sex \times flowering frequency \times log (initial stem length)	2	0.588	0.294	5.177	0.008
Error	87	4.943	0.057		
Total	98	13.454			

(b) Log (leaf production)	df	SS	MS	F-ratio	P
Sex	1	0.001	0.001	0.022	0.883
Flowering frequency	2	0.528	0.264	5.930	0.004
Log (initial leaf number)	1	0.673	0.673	15.131	0.000
Sex \times flowering frequency	2	0.257	0.129	2.892	0.061
Sex \times log(initial leaf number)	1	0.001	0.001	0.013	0.911
Flowering frequency \times log (initial leaf number)	2	0.828	0.414	9.303	0.000
Sex \times flowering frequency \times log (initial leaf number)	2	0.302	0.151	3.394	0.038
Error	85	3.872	0.045		
Total	96	10.098			

biomass per unit stem length than females. The significance of this effect was low, however, and did not withstand correction for multiple comparisons. The one-way ANOVA for stem density did not demonstrate a difference between the sexes ($P = 0.86$).

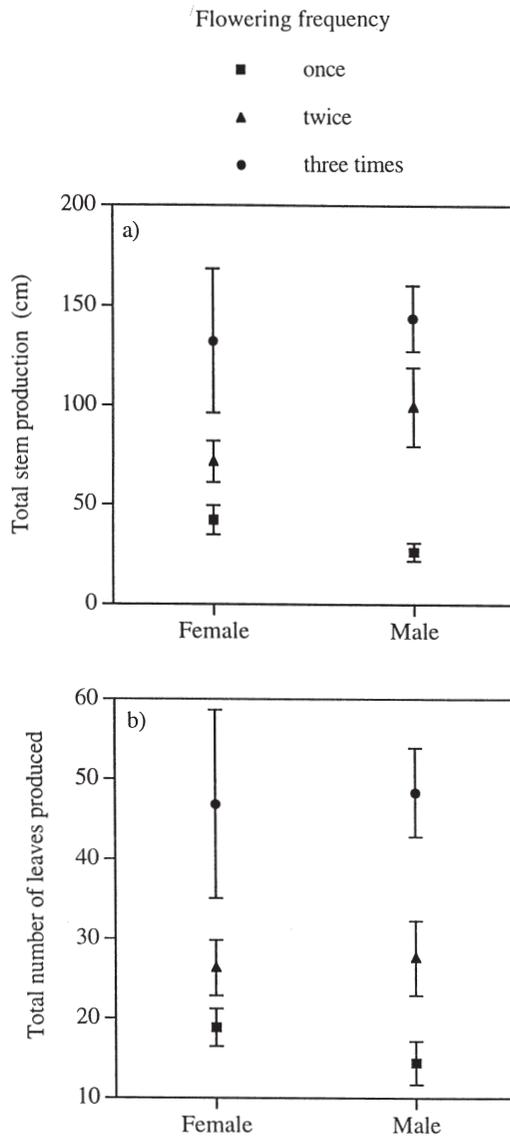


Fig. 3. (a) Mean stem growth (\pm SE) and (b) mean leaf production (\pm SE) for naturally occurring male and female *Siparuna grandiflora* plants classified by flowering frequency in the three seasons of the study.

Table 3. Effects of sex and flowering frequency on leaf longevity. Two methods were used to calculate leaf longevity (see text), both yielded the same pattern of results

	Life expectancy			Longevity		
	df	MS	P	df	MS	P
Sex	1	1.430	0.798	1	102.0	0.288
Flowering frequency	2	118.0	0.006	2	391.2	0.015
Sex \times flowering frequency	2	17.07	0.457	2	0.723	0.992
Error	91	21.60		93	89.23	
Total	96			98		

Discussion

Pre-reproductive female *S. grandiflora* plants have the capacity to grow faster than males of equivalent age and condition, yet this growth difference is not apparent in naturally occurring, reproductively mature individuals. As most field studies of dioecious species have focused on mature individuals (e.g. Gehring 1990 and references therein), the present study is novel in demonstrating sex-based differences in growth that are independent of reproductive effects. The basic capacity of females to grow faster than males may compensate for their greater reproductive allocation and thereby allow them to achieve equal growth to males under natural conditions.

GREATER GROWTH CAPACITY IN FEMALES

How do pre-reproductive females achieve greater growth than males, given equivalent resource conditions? How do mature females achieve equivalent growth rates despite differences in reproductive allocation? The differences could result from differences in allocation patterns, from females maintaining leaves for longer periods and thereby accruing greater life-time assimilation per leaf, or via higher rates of assimilation in females. Sex-based differences in assimilation pattern have been demonstrated in other dioecious species (Dawson & Bliss 1989 1993; Dawson & Ehleringer 1993; Marshall, Dawson & Ehleringer 1993; Gehring & Monson 1994; Laporte & Delph 1996) but are beyond the scope of the present study. There are no sex-based differences in leaf life span in *S. grandiflora* (Fig. 4), thus the growth differences cannot be explained by effects of leaf life span on carbon assimilation. The study does, however, provide evidence that sexual differentiation in allocation patterns may underlie observed growth differences.

Allocational differences in dioecious species have been found in species that are markedly dimorphic, such as the desert shrub *Simmondsia chinensis* (Wallace & Rundel 1979; Kohorn 1994), as well as in species like *S. grandiflora* that are less obviously dimorphic (e.g. Meagher & Antonovics 1982; Oyama & Dirzo 1988). Under natural conditions, *S. grandiflora* females allocate less biomass per unit stem length than males. If females have thinner stems than males on average, they could achieve equal stem length from a lower biomass investment to stems. The experimentally grown males also had larger leaves than females and males therefore achieved greater total leaf area than females, despite having fewer total leaves. To some extent the allocational differences demonstrated in the present study should be viewed with caution, as neither allocational difference could be confirmed in both naturally occurring and experimentally grown plants.

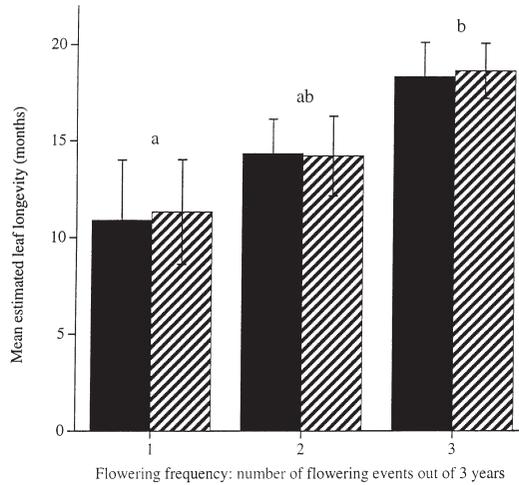


Fig. 4. Mean (\pm SE) leaf longevity classified by sex and flowering frequency in the three seasons of the study. Different letters denote significant differences between flowering frequencies according to Scheffé post-hoc tests. Females are represented as solid bars, males as hatched bars.

Table 4. Results of ANCOVA examining sex differences in relationship between total plant leaf area and leaf number in *S. grandiflora* cuttings

	Log (leaf area)		
	df	MS	P
Sex	1	0.349	0.033
Log(leaf number)	1	42.18	≤ 0.001
Sex \times log(leaf number)	1	0.609	0.005
Error	122	0.075	
Total	125		

None the less, the observed differences in allocation to photosynthetic vs support tissues could have a significant impact on leaf display, and consequently on whole plant carbon gain in *S. grandiflora*.

The demonstration of a differential pattern of growth between the sexes is an indication that that there are costs or constraints for each sex inherent in the other sex's growth strategy. If females display smaller leaves on thinner branches, they might maximize interception of direct light relative to males, and might do so at a lower cost in stem allocation. However, if such a strategy was superior, and without cost, we would expect no difference between the sexes. Perhaps female stems are more vulnerable to damage from disease or falling debris. Elsewhere I argued that males allocate resources to reproduction from current resource supplies, rather than by relying heavily on stored resources (Nicotra 1999). Thus, for males, large leaves supported on more solid stems might fix enough carbon to pay for reproduction from current photosynthate, alleviating dependence on stored reserves, even though these leaves are not displayed optimally for direct light

interception. Ecological differentiation evolves in response to selection to optimize fitness of each reproductive strategy and to reduce long-term costs of reproduction. The costs of and constraints to the evolution of such differentiation may not be immediately apparent. In the case of *S. grandiflora*, the demonstration of differential growth presents some interesting hypotheses about the evolution of sexual dimorphisms in ecological traits.

IS THERE A TRADE-OFF BETWEEN GROWTH AND REPRODUCTION?

At some scale there must be a trade-off between growth and reproduction, as resources allocated to reproduction cannot be simultaneously allocated to growth, however, selection will act to reduce the long-term costs of reproduction. The present study provides no evidence of long-term costs of reproduction in terms of growth or leaf life spans. Rather, there was a significant positive relationship between flowering frequency and growth and leaf life span in both sexes. Indirectly, the present study does demonstrate negative effects of reproduction on growth. If females had both greater growth capacity than males, and showed no negative effects of reproduction, then female growth should actually exceed that of males. In previous work I demonstrated a significant negative impact of reproduction on growth by females during the period of fruit maturation (Nicotra 1999). Given the greater growth capacity of females, it is not surprising that when multiple reproductive events are considered, females compensate for short-term negative effects of reproduction on growth so that there is no general trend toward growth reduction. Notably, the results of the present study, like those of my previous work, found no detectable costs of reproduction on growth in males (Nicotra 1999).

Why then did females that flowered in all 3 years grow more than other females? Although the sexes are not distributed differentially with regard light, in both

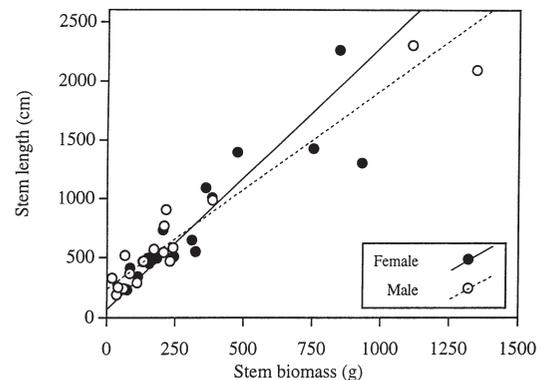


Fig. 5. The relationship between stem biomass and stem length for harvested naturally occurring *S. grandiflora* plants ($n = 19$ per sex).

sexes, flowering frequency is positively correlated with light availability and infrequently flowering females require higher light levels than males of equivalent flowering frequency (Nicotra 1999). Likewise, in both sexes, growth is positively correlated with light availability. Based on these results, I suggest that the positive correlation between growth and reproduction demonstrated in the present study indicates that plants that have favourable resource conditions are vigorous enough to grow quickly and that these plants therefore can also afford to reproduce frequently.

Conclusions

Observed vegetative differentiation in dioecious species may reflect innate differences between the sexes or artefacts of different reproductive history. In *S. grandiflora*, pre-reproductive and newly reproductive females had significantly greater stem growth and leaf production than males. This pre-reproductive growth difference indicates that there are indeed innate differences between the sexes. As mature females of varying size and age achieved on average growth equivalent to that of males, despite greater reproductive allocation and greater associated costs, it is likely that the greater growth capacity observed in young plants is sustained in older females and has evolved in response to selection to reduce long-term costs of reproduction.

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References

- Antonio, T.M. (1983) *Systematics and Reproductive Biology of the Genus Siparuna in Panama*. University of Oklahoma, Norman.
- Bazzaz, F.A. & Harper, J.L. (1977) Demographic analysis of the growth of *Linum usitatissimum*. *New Phytologist* **78**, 193–208.
- Begon, M. & Mortimer, M. (1986) *Population Ecology: a Unified Study of Animals and Plants*. Blackwell Scientific Publications, Oxford.

- Chazdon, R.L. & Field, C.B. (1987) Photographic estimation of photosynthetically active radiation: evaluation of a computerized technique. *Oecologia* **73**, 525–532.
- Dawson, T.E. & Bliss, L.C. (1989) Intraspecific variation in the water relations of *Salix arctica*, an arctic-alpine dwarf willow. *Oecologia* **79**, 322–331.
- Dawson, T.E. & Bliss, L.C. (1993) Plants as mosaics: leaf-, ramet-, and gender-level variation in the physiology of the dwarf willow, *Salix arctica*. *Functional Ecology* **7**, 293–304.
- Dawson, T.E. & Ehleringer, J.R. (1993) Gender specific physiology, carbon isotope discrimination, and habitat distribution in box-elder, *Acer negundo*. *Ecology* **74**, 798–815.
- Feil, J.P. (1992) Reproductive ecology of dioecious *Siparuna* (Monimiaceae) in Ecuador—a case of gall midge pollination. *Botanical Journal of the Linnean Society* **110**, 171–203.
- Garcia, M.B. & Antor, R.J. (1995) Sex ratio and sexual dimorphism in the dioecious *Borderea pyrenaica* (Dioscoreaceae). *Oecologia* **101**, 59–67.
- Gehring, J.L. (1990) *Experimental Analyses of Sexual Dimorphisms in a Dioecious Plant*, Silene Alba. University of Nebraska, Omaha.
- Gehring, J.L. & Linhart, Y.B. (1993) Sexual dimorphisms and response to low resources in the dioecious plant *Silene latifolia* (Caryophyllaceae). *International Journal of Plant Science* **154**, 152–162.
- Gehring, J.L. & Monson, R.K. (1994) Sexual differences in gas exchange and response to environmental stress in dioecious *Silene latifolia* (Caryophyllaceae). *American Journal of Botany* **81**, 166–174.
- Grant, G.C. & Mitton, J.B. (1979) Elevational gradients in adult sex ratio and sexual differentiation in vegetative growth rates of *Populus tremuloides* Mich. *Evolution* **33**, 914–918.
- Gross, L.J. & Soule, J.D. (1981) Differences in biomass allocation to reproductive and vegetative structures of males and female plants of a dioecious perennial herb, *Silene alba*. *American Journal of Botany* **68**, 801–807.
- Kohorn, L.U., Goldstein, G. & Rundel, P.W. (1994) Morphological and isotopic indicators of growth environment: variability in $\delta^{13}\text{C}$ in *Simmondsia chinensis*, a dioecious desert shrub. *Journal of Experimental Botany* **45**, 1817–1822.
- Laporte, M.M. & Delph, L.F. (1996) Sex-specific physiology and source–sink relations in the dioecious plant *Silene latifolia*. *Oecologia* **106**, 63–72.
- Lloyd, D.G. & Webb, C.J. (1977) Secondary sex characteristics in plants. *Botanical Review* **43**, 177–216.
- Lovett Doust, L. & Lovett Doust, J. (1987) Leaf demography and clonal growth in female and male *Rumex acetosella*. *Ecology* **68**, 2056–2058.
- Lovett Doust, J. & Lovett Doust, L. (1988) Modules of production and reproduction in a dioecious clonal shrub, *Rhus typhina*. *Ecology* **69**, 741–750.
- Marshall, J., Dawson, T. & Ehleringer, J. (1993) Gender-related differences in gas exchange are not related to host quality in the xylem-tapping mistletoe, *Phoradendron juniperinum* (viscaceae). *The American Journal of Botany* **80**, 641–645.
- McDade, L.A., Bawa, K.S., Hespenheide, H.A. & Hartshorn, G.S., eds. (1994) *La Selva: Ecology and Natural History of a Neotropical Rain Forest*. University of Chicago Press, Chicago.
- Meagher, T.R. (1984) Sexual dimorphism and ecological differentiation of male and female plants. *Annals of the Missouri Botanical Garden* **71**, 254–264.
- Meagher, T.R. & Antonovics, J. (1982) The population biology of *Chamaelirium luteum*, a dioecious member of the lily family: life history studies. *Ecology* **63**, 1690–1700.

- Nicotra, A.B. (1998) Sex ratio variation and spatial distribution of *Siparuna grandiflora*, a tropical dioecious shrub. *Oecologia* **115**, 102–113.
- Nicotra, A.B. (1999) Reproductive allocation and the long-term costs of reproduction in *Siparuna grandiflora*, a tropical dioecious shrub. *Journal of Ecology* **87**, 138–149.
- Onyekwelu, S.S. & Harper, J.L. (1979) Sex ratio and niche differentiation in spinach (*Spinacia oleracea* L.). *Nature* **282**, 609–611.
- Oyama, K. & Dirzo, R. (1988) Biomass allocation in the dioecious tropical palm *Chamaedorea tepejilote* and its life history consequences. *Plant Species Biology* **3**, 27–33.
- Popp, J.W. & Reinartz, J.A. (1988) Sexual dimorphism in biomass allocation and clonal growth of *Xanthoxylum americanum*. *American Journal of Botany* **75**, 1732–1741.
- Putwain, P.D. & Harper, J.L. (1972) Studies in the dynamics of plant populations. V. Mechanisms governing the sex ratios in *Rumex acetosa* and *R. Acetosella*. *Journal of Ecology* **60**, 113–129.
- Ramadan, A., El-Keblawy, A., Shaltout, K. & Lovett Doust, J. (1994) Sexual polymorphism, growth, and reproductive effort in Egyptian *Thymelaea hirsuta* (Thymelaeaceae). *American Journal of Botany* **81**, 847–857.
- Sakai, A. & Burris, T. (1985) Growth in male and female aspen clones: a twenty-five-year longitudinal study. *Ecology* **66**, 1921–1927.
- Wallace, C.S. & Rundel, P.W. (1979) Sexual dimorphism and resource allocation in male and female shrubs of *Simmondsia chinensis*. *Oecologia* **44**, 34–39.

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