Reproductive allocation and the long-term costs of reproduction in *Siparuna grandiflora*, a dioecious neo-tropical shrub

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Summary

1 Using a combination of observational and experimental approaches, both allocation of resources to reproduction (often called the direct cost of reproduction) and the subsequent long-term costs (the indirect, delayed or demographic cost) associated with reproductive allocation to male and female function in *Siparuna grandiflora* (Siparunaceae), a tropical dioecious shrub, were examined.

2 The objectives were to determine whether females allocate more biomass or nitrogen per reproductive episode than males, and whether there is a long-term cost of reproduction in terms of subsequent growth or reproduction for either sex. If there is no long-term cost of reproduction, then reproduction may be viewed as free in an evolutionary sense.

3 As is generally the case in dioecious species, females allocated more biomass and nitrogen to reproduction than males. Females also showed delayed costs of reproduction in terms of decreased growth and subsequent reproduction, whereas males did not.

4 The lack of measurable delayed costs in males suggests that with the evolution of dioecy, selection has reduced delayed costs of reproduction in *S. grandiflora* males. In contrast, females that were prevented from reproducing were able to re-allocate resources to growth, and produced more stem length on average than males. This re-allocation response may have evolved to reduce delayed costs of reproduction in females over time frames longer than that considered in the present study.

Keywords: demographic costs of reproduction, direct costs of reproduction, fruit production, sexual dimorphism

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Introduction

Females of dioecious plant species generally have greater reproductive allocation than males, and this difference is likely to have significant ecological and evolutionary implications (Darwin 1877; Putwain & Harper 1972; Lloyd & Webb 1977; Wallace & Rundel 1979; Gross & Soulé 1981; Meagher & Antonovics 1982b; Lovett Doust *et al.* 1987; Ågren 1988b; Allen & Antos 1988; Popp & Reinartz 1988; Armstrong & Irvine 1989; Cipollini & Stiles 1991; Korpelainen 1992; Gehring & Linhart, 1993; Cipollini & Whigham

1994; Garcia & Antor 1995). When considering the costs of reproduction, actual allocation of carbon and nutrients at the time of reproduction (often called the direct cost of reproduction) must be distinguished from the long-term effects of reproduction. These long-term costs, variously called indirect, delayed or demographic, are expressed as a loss of growth or subsequent reproduction, or increased chance of mortality, which is incurred due to reproduction. If there is no effect of reproductive allocation on subsequent growth, reproduction or mortality, then reproduction can be looked at as being effectively free, on both ecological and evolutionary scales.

In dioecious species, the delayed costs of female function can be readily separated from those of male function. The evolution of dioecy may be aided by evolution towards different optimal reproductive

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strategies for the sexes (Bateman 1948). This prediction arises specifically because female function is expected to be more costly than male, on both a gamete and episodic basis. Yet, over evolutionary time, selection should reduce delayed costs of reproduction. Therefore, if in dioecious species females have greater delayed costs of reproduction than males, they may face greater selective pressure towards reducing these costs. Several studies have demonstrated equal or greater vegetative growth increments in females, despite greater reproductive allocation (Putwain & Harper 1972; Grant & Mitton 1979; Onyekwelu & Harper 1979; Gross & Soulé 1981; Sakai & Burris 1985; Lovett Doust *et al.* 1987; Lovett Doust & Lovett Doust 1988; Gehring & Linhart 1993; Ramadan *et al.* 1994; Antos & Allen 1999), suggesting that there are differences in assimilation and allocation pattern between sexes that allow females to compensate for greater reproductive allocation.

Delayed costs of reproduction in dioecious species are often inferred from the overall lower frequency of reproduction in females (Bullock & Bawa 1981; Bullock 1982; Meagher & Antonovics 1982a; Bullock *et al.* 1983; Ågren 1988a; Oyama 1990; Cipollini & Stiles 1991; Ataroff & Schwarzkopf 1992; Ibarra-Manríquez & Oyama 1992; Thomas & LaFrankie 1993; Garcia & Antor 1995), from higher mortality rates of females (Meagher & Antonovics 1982b; Ågren 1988a; Bierzychudek & Eckhart 1988; Lovett Doust & Lovett Doust 1988) or from lower growth rates in females (Popp & Reinartz 1988 and references therein; Garcia & Antor 1995). It is not until recently that studies have directly assessed the delayed costs of reproduction in plants of any breeding system (Willson 1986; Reekie & Bazzaz 1987a; Ågren 1988b; Horvitz & Schemske 1988; Snow & Whigham 1989; Fox & Stevens 1991; Newell 1991; Cipollini & Whigham 1994; Delph & Meagher 1995; Antos & Allen 1999). Only two studies report the results of direct manipulations to test for delayed costs in dioecious woody species. In both *Lindera benzoin* and *Salix alaxensis*, field manipulations revealed a trade-off between current reproduction and subsequent reproduction, but not subsequent growth (Fox & Stevens 1991; Cipollini & Whigham 1994). Although the costs of male function are readily separated and assessed in dioecious species, neither study looked for delayed effects in males.

In the present study, the allocation of resources to reproduction, as well as the subsequent long-term costs associated with this allocation for both male and female function in *Siparuna grandiflora* (Siparunaceae), a tropical dioecious shrub, was examined. Biomass and nitrogen allocation were considered, as both of these resources have been shown to be particularly useful in assessing reproductive costs and sometimes differ in the patterns they reveal (Reekie & Bazzaz 1987a; Antos & Allen 1990; Ashman 1994; Cipollini & Whigham 1994; Witkowski & Lamont

1996). Delayed costs were examined by suppressing reproduction in otherwise reproductively active individuals and measuring the effect on subsequent growth and reproduction of both sexes. The study was conducted on naturally occurring plants that had grown and matured in the rain forest. It therefore incorporated the effects of microsite variation, herbivory and competition, and thus can demonstrate not only whether or not there are differences in reproductive allocation and cost, but whether the differences are ecologically significant under field conditions at natural levels of fruit production.

The objectives of the study were to determine whether: (i) females allocate more biomass or nitrogen per reproductive episode than males; (ii) allometric relationships for reproductive allocation differ between the sexes; (iii) there is a long-term cost of reproduction for either sex and, if so, (iv) there is any evidence of evolved mechanisms in either sex to reduce the long-term costs of reproduction.

Methods

STUDY SITE AND SPECIES DESCRIPTION

The study was conducted at the Organization for Tropical Studies, La Selva Biological Station in Costa Rican lowland wet tropical forest (for a detailed site description see McDade *et al.* 1994). *Siparuna grandiflora* (Kunth in Humb. & Bonpl., A. DC., syn *S. tonduziana*, Siparunaceae) is a common understorey shrub in secondary forests of the Sarapiquí region of Costa Rica. The shrub flowers at just over 1 m in stem length, and can reach over 5 m in height. At La Selva, flowering occurs primarily between January and May. Flowers are borne cauliflorously, are 2–4 mm in diameter, and have a reddish colour. Staminate flowers (male) do not bear vestigial pistillate structures, nor do pistillate flowers (female) bear any male structures. Inflorescences are cymose. When mature flowers on either sex dehisce they leave a clear scar, although female flowers may develop into fruits. Immature flowers can be counted as buds. Other *Siparuna* species are reported to be pollinated by Cecidomyid midges (Feil 1992); pollination in *S. grandiflora* has not been observed. The aggregate fruits are 10–25 mm in diameter, mature between June and December, and are dehiscent along irregular lines (Antonio 1983). The pseudo-arilate seeds are bird dispersed (B. Loiselle, personal communication). 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). As both flowers and fruits are red with a white interior, it seems unlikely that they are capable of photosynthesis.

Field methods

Twenty reproductively active individuals of each sex were harvested during the peak flowering season (March–May) in 1994, from secondary forest at La Selva Biological Station for determination of reproductive allocation. Plants were harvested from a single population, and were selected as they were encountered, up to a total of 20 per sex. The total number of harvested plants and the location of the harvest was constrained by the zoning rules of the 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 approximately 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 accurately in the field, five of the harvested plants proved to be 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 (see the Results). Even with a stem length of 20 m, the plants were never more than 6 m tall; rather, they had multiple stems and branches arising from the same main stem. For each harvested plant, stems were sawed off at ground level and total stem length of all branches was summed. The total number of leaves was counted and area determined using a Li-Cor leaf area meter (Li-Cor, Lincoln, NB). Stems and leaves were then dried to constant mass at 70 °C, and weighed to the nearest milligram.

Inflorescences were removed in the field at the time of harvest. Flowers were counted under a dissecting microscope, and total flower number was defined as the sum of immature flower buds, mature flowers and flower scars. Five mature flowers from each individual were collected, dried and weighed to the nearest 0.001 mg to estimate individual flower biomass. To approximate a full season's allocation to flowers, total flower biomass was calculated as the product of total flower number and average biomass per mature flower. Approximately 10 mg of dried flower tissue was collected from each plant for determination of nitrogen and carbon allocation to flowers.

Fruit production by females was estimated by monitoring 103 reproductively active females during the 1995 reproductive season. To estimate total plant size, total stem length was measured non-destructively for each female in June 1995. Total number and diameter of fruits was measured in July and October 1995. Using relationships between size and biomass or nitrogen (described below), estimates of total biomass

and nitrogen allocation to fruit in July and October were calculated. The greater of these two estimates was defined as the minimum biomass or nitrogen allocation to fruit. This is a conservative estimate, as fruits may have matured and dehisced, or been lost for other reasons, between censuses.

The relationship between fruit diameter and biomass or nitrogen content was determined destructively using fruit from female plants not being monitored for fruit production. A total of 93 samples, spanning a size range from 2 to 21 mm diameter, was taken from 22 fruiting females. Each sample was either a single fruit or, for smaller diameters, included several fruit from the same female so that biomass per sample was relatively constant. The diameter of each fresh fruit was measured, fruits were then dried to constant mass, and weighed to the nearest milligram. The diameter to nitrogen relationship was determined from a subset of the fruits used in the biomass relationship (a total of 33 samples from 17 plants). Samples were taken from seven size classes chosen to span the natural size range of the fruits. Five samples were taken from each of six of the size classes (up to 3, 6, 9, 12, 15 and 18 mm diameter), and three samples were taken from the largest size class, 19–21 mm. Each sample for nitrogen analysis consisted of approximately 10 mg of fruit from a single plant.

Analysis

For the harvested plants, allocation to flowering was assessed on both whole plant and per unit size bases using ANOVA. Initial stem length and total above-ground biomass were compared for the sexes using one-way ANOVAs to ensure that the sample was not biased with regard to plant size from the outset. Biomass values were log transformed so that they met the assumptions of normality. Allometric relationships between plant size and flower production were assessed for both sexes using linear regression.

Because flower production could only be determined by removing all inflorescences and counting individual flowers under a dissecting microscope, it was not possible to assess fruit and flower production on the same females. Comparisons between the sexes of resource allocation to reproduction were therefore made based upon fruit production in the 103 surveyed females, and flower production in the 20 harvested males. Although this approach discounts allocation to female flowers that do not develop into fruits, it is the best available comparison of reproductive allocation for the sexes, and is highly conservative. Because of the large difference in sample size, and because the distribution of reproductive allocation differed for the sexes, parametric statistics were deemed inappropriate. Differences in reproductive allocation as a function of total plant size (stem length) between the sexes were tested by boot-

strapping sample means (a total of 1500 bootstrap samples) and comparing accelerated bias-corrected 95% confidence intervals (Dixon 1993; Manly 1997).

DELAYED COST OF REPRODUCTION

Field methods

In January 1995, 72 naturally occurring reproductively active plants of each sex were located to assess the delayed costs of reproduction. As soon as a plant could be sexed (i.e. it had at least one fully mature flower), it was assigned to one of three treatments ($n = 24$ plants per sex per treatment). The treatments were: (i) control, (ii) no-reproduction and (iii) flowers only. Plants were assigned to treatments in the order they were found in the field; thus, males 1, 4 and 7, etc., were controls. Control individuals were not manipulated. For no-reproduction plants, all flowers and buds were removed as soon as sex had been determined. Thus, these plants neither flowered nor set fruit. In some males, a small number of new flower buds was initiated following flower removal and these were also removed. For the flowers-only treatment, flowering was allowed to occur but remaining flowers were removed from males when they had ceased to produce new flowers, and flowers and immature fruits were removed from females as they began to initiate fruits (16 and 17 May). The flowers-only treatment therefore allows the cost of flowering in females to be separated from the cost of fruiting in females. The flowers-only treatment was also applied to males to achieve a fully factorial model, but flowers-only and control treatments were predicted to be effectively equivalent.

For each plant in the study, stem length and leaf number were measured non-destructively in January and June 1995, and again in January 1996. Amount of new stem growth and new leaf production was determined at each census. All plants in the study were censused during the 1996 flowering season to determine whether they were reproductively active in the season following the manipulation. In June 1995, a hemispheric canopy photograph was taken above each plant as a measure of light availability. Photographs were digitally analysed using the Solarcalc package (Chazdon & Field 1987).

Analysis

Data were log transformed so that they met the assumptions of ANOVA. To ensure that the treatments and sexes did not differ in size before the manipulation, preliminary ANOVAs were conducted on initial number of leaves and initial stem length. Likewise, a preliminary ANOVA demonstrated that the sexes and treatments did not differ in light availability (data not shown). ANCOVA was used for analysis of growth in the year following manipulation, because stem and leaf production are expected to be dependent upon initial size. The importance of light availability as a covariate was also explored in a preliminary analysis, but it was not significant and was excluded from subsequent analyses. Stem and leaf production were divided into two 6-month periods by the census method and growth was therefore analysed separately for the two periods. In the ANCOVA model, either new stem or new leaf production was used as the dependent variable, while sex, treatment and initial size (stem length or initial leaf number, respectively) were independent variables. LSD post-hoc tests were used to test for differences among means. All ANOVA and ANCOVAs were done using Datadesk statistical software (Velleman 1996). The effect of the manipulation on reproductive activity in the subsequent (1996) flowering season was analysed using a logistic regression model that tested the effects of sex and treatment on flowering response (PROC CATMOD; SAS Institute Inc. 1985).

Results

REPRODUCTIVE ALLOCATION

Five of the 40 plants harvested for assessment of reproductive allocation were found to be larger than the 2000 cm upper limit set for stem length. Of these, one male (2405 cm) and one female (3500 cm) were extreme outliers in terms of allometric relationships among stem, leaf and flower biomass, because they had disproportionately more stem. The data could not be transformed to normality with these plants included, and as these two plants were larger than 99% of naturally occurring plants, they were excluded from all subsequent analyses. The other three plants ranged from 2092 to 2302 cm stem length, but were not outliers otherwise. There was no difference between the sexes in terms of average stem length or above-ground biomass of plants harvested for this experiment (Table 1). This result was not changed by inclusion or exclusion of the two outlier plants.

Individual flower weight was significantly greater for females than males. Likewise, female flowers contained slightly, but significantly, more carbon than males. The sexes did not differ in floral nitrogen content. Because males had approximately six times as many flowers as females, males allocated significantly more biomass, nitrogen and carbon to flowers than females, when total flower biomass was considered (Table 1). Since flower production is likely to be affected by architectural parameters, flower number per unit stem length and flower biomass per unit above-ground biomass for the sexes were compared. As with total allocation, males had more flowers per centimetre of stem and more floral biomass per milligram vegetative biomass than females (Table 1). When the allometric relationships

Table 1 Means and standard errors for initial size and flower production data. *P*-values are from one-way ANOVA with the main effect of sex. Significance levels were corrected according to sequential Bonferroni test for multiple comparisons. All variables were log transformed for ANOVA. All tests were done following exclusion of two outliers, see the text

Variable	Male		Female		
	Mean	($\pm$ SE)	Mean	($\pm$ SE)	*P*†
Stem length (cm)	679.8	132.1	768.4	119.7	0.418 NS
Above-ground biomass (mg)	302.1	89.6	359.8	65.2	0.142 NS
Individual flower weight (mg)	1.095	0.042	2.418	0.175	<0.001 *
Mg nitrogen per mg flower	0.026	0.001	0.025	0.001	0.110 NS
Mg carbon per mg flower	0.493	0.004	0.514	0.005	0.001 *
Total flower number	1466.8	265.7	243.9	56.2	<0.001 *
Total flower biomass (mg)	1509.6	249.0	614.9	131.6	0.003 *
Total flower nitrogen (mg)	42.0	7.0	17.6	3.8	0.003 *
Total flower carbon (mg)	808.1	124.1	362.6	76.7	0.003 *
Mg flowers per mg above-ground biomass	6.8	0.7	2.00	0.3	<0.001 *
Number flowers per cm stem	2.2	0.2	0.4	0.1	<0.001 *

† *P*-values with asterisks are significant after sequential Bonferroni correction for multiple comparisons.

between plant size and measures of reproductive allocation were considered, flower number, flower biomass and total flower nitrogen were tightly and significantly correlated with stem length for males but not females (Fig. 1).

The regressions to determine the relationship between fruit diameter and either biomass or nitrogen content were highly significant (adjusted $r^2 = 0.949$, $P < 0.0001$ and $r^2 = 0.702$, $P < 0.0001$, respectively; data not shown) and were used to estimate total biomass and nitrogen investment in fruit for females. Carbon content per milligram of fruit did not vary with fruit size, and was not considered in further calculations of fruit production. Fruit production was highly variable, with 34 females producing no fruit (Fig. 2). As in the case of flower production, the correlation between plant size and fruit production in females was weak and not significant (data not shown).

Minimum reproductive biomass allocation for the 103 females surveyed was greater than reproductive allocation for the 20 harvested males (Fig. 3a). The accelerated bias-corrected bootstrap 95% confidence intervals for females did not overlap those of males, and means were greater in females, whether all females or only those that set fruit were considered. Mean minimum reproductive nitrogen allocation in females was also higher than that of males, although the confidence intervals for females slightly overlapped those of males when all females were considered. If the females that set no fruit were excluded, the 95% confidence intervals did not overlap, indicating significantly higher reproductive nitrogen allocation in females (Fig. 3b). Comparison of reproductive biomass and nitrogen allocation yielded the same pattern of results when parametric *t*-tests,

standard bootstrap 95% confidence intervals or simple bias-corrected bootstrap 95% confidence intervals were used.

DELAYED COST

Initially, there were no size differences between the sexes or treatments (control, flowers-only, no-reproduction) in the delayed cost experiment, whether leaf number or initial stem length was used as dependent variable (Table 2). Likewise, growth in the first 6 months following the manipulation did not differ between sexes or treatments (Table 2 and Fig. 4a,b). However, 12 months after the manipulation, leaf and stem production showed a marked impact of both sex and treatment (Table 2 and Fig. 4c,d). Female control plants produced fewer leaves than any of the male treatments, and fewer leaves than female no-reproduction or flowers-only plants, although the difference from the latter was only marginally significant ($P = 0.06$, LSD post-hoc test). There were no significant differences in leaf production among treatments for males, therefore the sex by treatment interaction was significant. Stem production followed a similar pattern to leaf production, although treatment effects were more pronounced. Twelve months after manipulation, female control plants had produced significantly less stem than any of the other treatments. No-reproduction females produced significantly more stem than flowers-only females. Stem production in males was not significantly affected by treatment. The no-reproduction and flowers-only females did not differ significantly from the males, although the difference between no-reproduction males and females was marginally significant (LSD post-hoc test, $P = 0.09$). As was the case with leaf
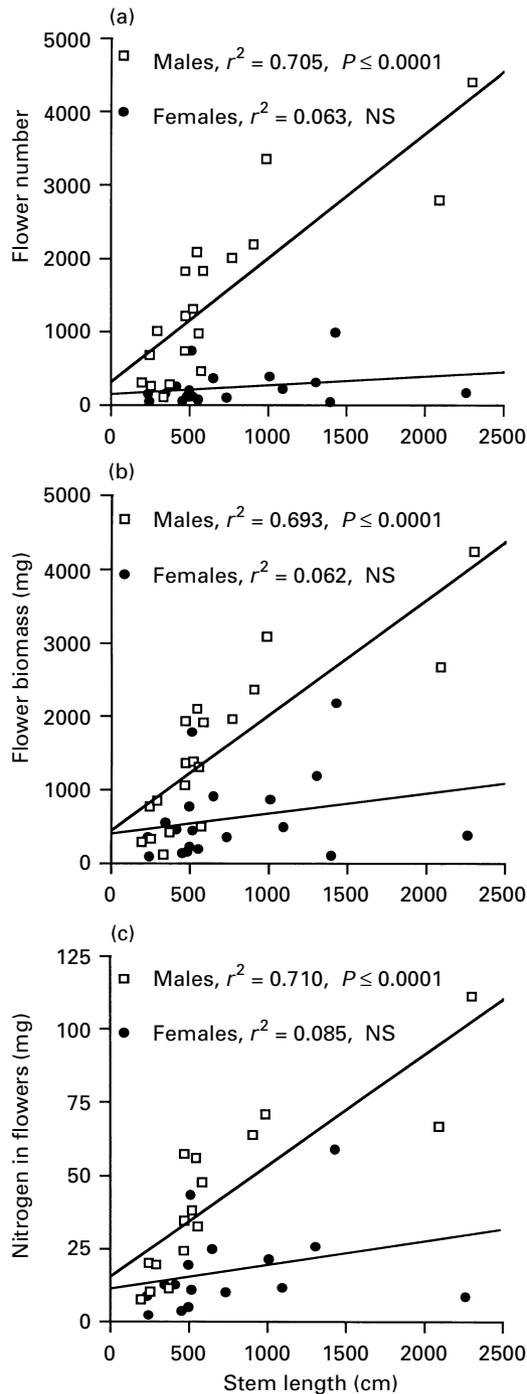
Fig. 1 Correlations between stem length and (a) total number of flowers produced by a plant, (b) total biomass allocated per flower, and (c) total nitrogen allocation to flowers for harvested *Siparuna grandiflora* ($n = 19$ per sex). Equations and statistics are for simple linear regression of the dependent variables (y axis) on stem length.

production, the sex by treatment interaction was significant.

Manipulation of flower and fruit production had pronounced effects on reproductive activity in the subsequent flowering season. Logistic regression analysis revealed both a sex effect on reproductive activity in 1996, and a sex by treatment interaction

(Table 3). These effects are due both to a marked difference between control males and females, as well as to a difference in the way the sexes responded to the manipulation. Nearly 75% of the female controls did not flower in 1996, whereas 95% of the male controls flowered in both years (Fig. 5). Females in either the flowers-only or no-reproduction treatments were much more likely to flower in 1996 than control females were — about 75% flowered in each treatment. Males showed a less marked effect of treatment, although male no-reproduction plants were less likely to flower in 1996 than either controls or flowers-only plants.

Discussion

Because resources allocated to reproduction are not available for allocation to growth or storage, and because a longer term trade-off between allocation to reproduction and allocation to growth is generally assumed, reproductive allocation is often termed a 'cost'. However, even species with substantial allocation to reproduction do not necessarily exhibit delayed costs of reproduction. Populations of *S. grandiflora* exhibit a male-biased sex ratio among reproductive individuals, and males flower more frequently than females (Nicotra 1998). Both the sex ratio bias and the difference in frequency of reproduction suggest that reproductive allocation results in delayed reproductive costs in females (Nicotra 1998). The results of the present study support this hypothesis.

The pattern of reproductive allocation in *S. grandiflora* reflects that generally found in dioecious species: males produce smaller more abundant flowers, and therefore allocate more biomass to flowering, but when fruit production is taken into account females have a higher overall allocation of biomass to reproduction (Lloyd & Webb 1977; Bullock 1984; Armstrong & Irvine 1989; Korpelainen 1992; Meagher 1992; Wheelwright & Bruneau 1992). While there also appears to be a parallel trend towards greater nitrogen allocation by females, the difference was only significant when those females that failed to set fruit were excluded. The difference in nitrogen allocation would have been greater if it was possible to include nitrogen allocated to unsuccessful flowers in females in the estimate of reproductive allocation. The relatively small effect may also indicate that carbon-based resources are more limiting than nitrogen for these plants. It is generally accepted that the 'best' currency to use when assessing reproductive allocation is that which is limiting in the habitat (Reekie & Bazzaz 1987a; Antos & Allen 1990; Ashman 1994). Relatively greater carbon limitation would not be surprising in this system, as the Sarapiquí region has rich volcanic soils, and light is therefore generally more limiting than soil nutrients in the understorey (Denslow *et al.* 1987). Thus, while both nitrogen and biomass are effective currencies for assessing differences in repro-
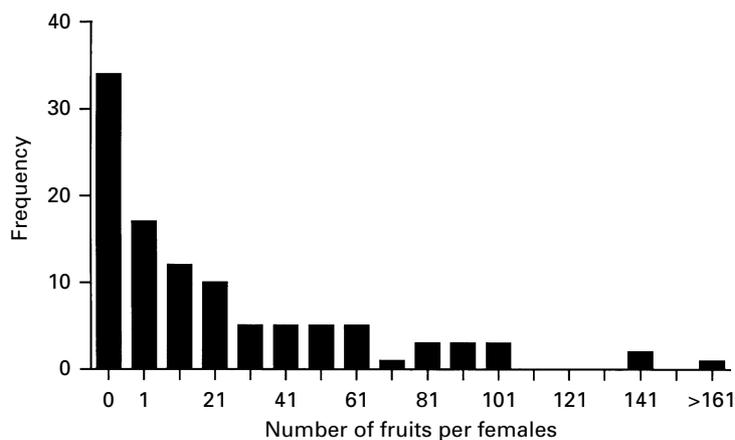
Fig. 2 Frequency distributions for number of fruits produced by naturally occurring females ($n = 103$).
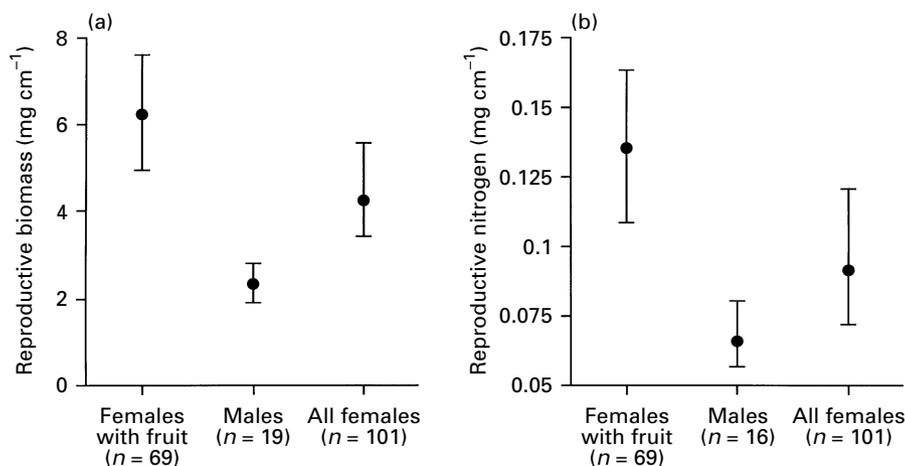

Fig. 3 Comparisons of (a) biomass and (b) nitrogen allocation to reproduction as a function of plant size for female and male *Siparuna grandiflora*. Bootstrap means and 95% accelerated bias-corrected confidence intervals are presented for females that set at least one fruit, for males, and for all females regardless of fruit set. For females, these means represent estimates of biomass or nitrogen in fruit, not including allocation to flowers. Lower sample sizes for nitrogen data reflect missing values due to lost samples.

Table 2 Results of ANOVA on initial size of plants in a delayed cost experiment, and ANCOVAs for effects of manipulation on growth. Growth is divided into 6-month increments of stem and leaf production. Log (initial leaf number) and log (initial stem length) were used as covariates for leaf and stem production, respectively

	Log (initial leaf number) Log (initial stem length)		Sex		Treatment		Sex × treatment		Error	
	MS	*P*	MS	*P*	MS	*P*	MS	*P*	d.f.	MS
	(d.f. = 1)		(d.f. = 1)		(d.f. = 2)		(d.f. = 2)			
Initial size										
Log (leaf number)	–	–	0.042	0.379	0.062	0.317	0.080	0.226	134	0.054
Log (stem length)	–	–	0.000	0.936	0.041	0.353	0.060	0.214	134	0.039
6 months post-manipulation										
Log (leaf production)	5.657	<0.001	0.156	0.120	0.045	0.497	0.016	0.781	120	0.064
Log (stem production)	3.303	<0.001	0.071	0.449	0.104	0.431	0.026	0.811	121	0.123
12 months post-manipulation										
Log (leaf production)	4.750	<0.001	0.317	0.0310†	0.098	0.238	0.392	0.004	117	0.067
Log (stem production)	2.825	<0.001	0.210	0.173	0.504	0.013	0.636	0.004	117	0.112

† Following Bonferroni correction for multiple comparisons, $n = 2$ tests, this effect is only marginally significant. All other effects significant at $P < 0.05$ are also significant following Bonferroni correction.
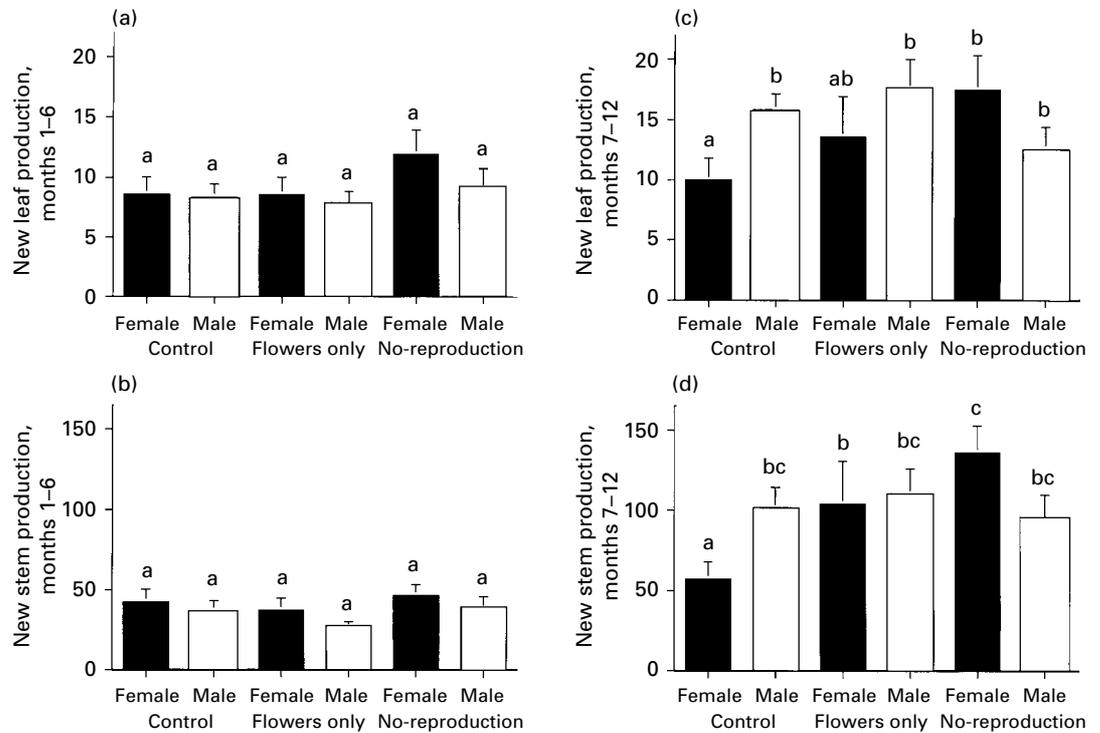
Fig. 4 New stem and leaf production (mean ± SE) following flower/fruit removal experiment. See text for description of treatments. (a) New leaf production and (b) new stem production are for the first 6 months following manipulation. None of these represent statistically significant differences. (c) New leaf production and (d) new stem production are for months 7–12. Different letters above bars indicate significant differences by LSD post-hoc tests.

Table 3 Maximum likelihood analysis of variance table for the effects of sex and treatment on reproductive activity in 1996

Source	d.f.	Chi-square	P
Intercept	1	23.02	<0.001
Sex	1	7.4	0.007
Treatment	2	0.8	0.670
Sex × treatment	2	9.28	0.010
Likelihood	0	–	–

ductive allocation in *S. grandiflora*, the magnitude of the difference in biomass allocation may be more representative of the cost to the plant in terms of limiting resources.

Not only did the sexes differ in absolute reproductive allocation, they also differed in allometric relationships of allocation. Male flower production was consistent on a per unit stem or per unit plant biomass basis, whereas neither female flower nor fruit production was well correlated with measures of plant size. Other studies of reproductive allocation have also shown tighter correlations of size with male reproductive allocation than with female reproductive allocation (Bullock & Bawa 1981; Korpelainen 1992; Gehring & Linhart 1993). The correlation between allocation to reproduction and size may indicate that reproduction in males is supported primarily by cur-

rent photosynthesis. If so, reproductive allocation in males would be directly related to size and photosynthetic potential: two characters that are also likely to be correlated with one another, given that leaf area and stem length are correlated (Nicotra 1998). Thus, in males, allocation to reproduction may be a fixed and dedicated proportion of the photosynthate pool, which cannot be used to enable growth and so does not compromise growth or elicit a measurable delayed cost (Antos & Allen 1999). For females, in contrast, the lack of a strong relationship between size and reproductive allocation, and the negative impact of reproduction on growth, suggest that growth and reproduction are dependent, at least in part, upon the same pool of reserves. This resource pool may draw on stored reserves in addition to newly fixed photosynthate, so that reproductive allocation is likely to be better correlated with levels of stored reserves than availability of photosynthate. Levels of stored reserves will be influenced by history of reproduction and resource availability in addition to current inputs and current size.

Experimental manipulation of flower and fruit production in *S. grandiflora* demonstrated clear delayed costs of reproduction in females, but no such costs for males. In general, other studies have revealed less consistent patterns of delayed costs of female function than shown here for *S. grandiflora*. Among hermaphrodites, results have been mixed (Reekie & Bazzaz 1987b; Horvitz & Schemske 1988; Snow &
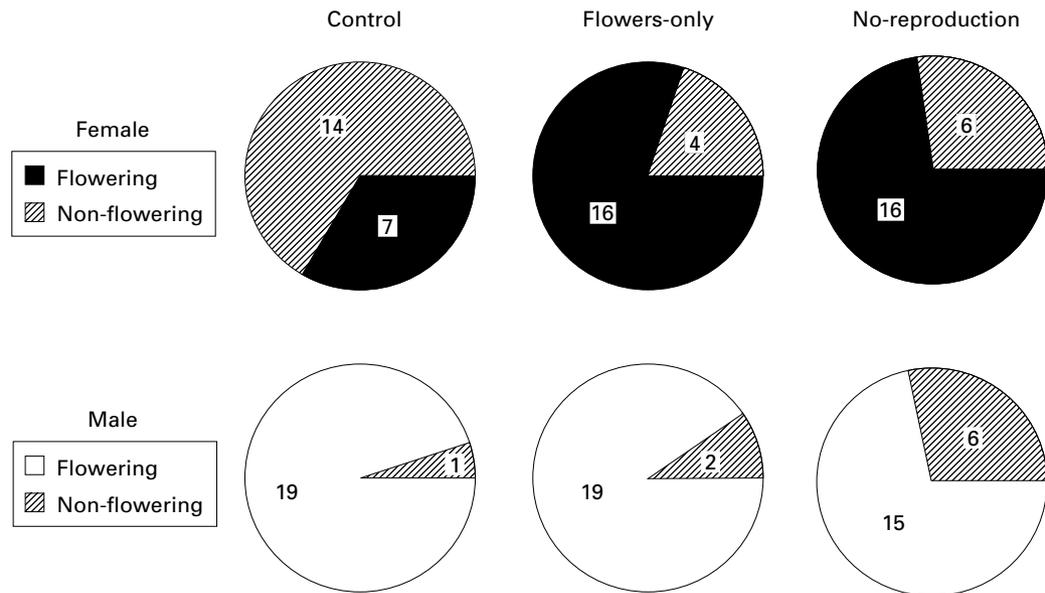
Control   Flowers-only   No-reproduction

Fig. 5 Effect of flower/fruit removal in 1995 on reproductive activity in 1996. Reproductively active females are represented by black, males by white, and plants that did not flower in 1996 by stripes. Numbers in pie diagrams indicate sample sizes. For treatment definitions see the Methods.

Whigham 1989; Primack & Hall 1990; Newell 1991). In dioecious and gynodioecious species, effects on subsequent reproduction (Fox & Stevens 1991; Ashman 1992), subsequent growth (Ågren 1988b) and both reproduction and growth (Delph & Meagher 1995) have been shown. No other studies have directly tested for delayed costs of reproduction in males of dioecious species. Among monoecious and gynodioecious species, delayed costs of male function have been demonstrated in some species but not others (Silvertown 1987; Kohn 1989; Delesalle & Mooreside 1995).

Some of the variability in results of prior studies of delayed costs of reproduction may be due to experimental designs that do not succeed in mimicking biologically realistic conditions. For example, studies that test for delayed costs by manipulating photoperiod or applying hormones (Reekie & Bazzaz 1987b; Saulnier & Reekie 1995) are likely to be less realistic than studies that use manipulations analogous to herbivory or florivory (the present study; Fox & Stevens 1991). Likewise, studies that artificially increase fruit set using hand pollination may illustrate mechanisms of delayed costs by creating extreme cases of resource-demand, but may not be relevant to questions about the evolution of life-history traits or breeding systems (Primack & Hall 1990; Delph & Meagher 1995)

In *S. grandiflora*, the effect of reproduction on growth was not apparent immediately, but developed 7–12 months following the manipulation, when fruits were maturing. The different types of manipulation produced a graduated effect on stem production in females, demonstrating delayed costs of both flower and fruit production. Similarly, the effect on leaf pro-

duction was graduated, although the differences between control and flowers-only plants were less pronounced and not significant. This suggests a different time-scale of response, or perhaps different source–sink relations between reproductive structures, leaves, stems and stored reserves: new leaf production may be less affected by reproduction because leaves are the favoured sink for newly fixed carbohydrates, relative to stems. If reproduction in females occurs only when stored reserves reach some threshold, and reproduction is also subsidized by current photosynthate, it is not surprising that growth increases in females when reproduction is suppressed, and that (in the case of stems at least), this increase was intermediate in the flowers-only plants. Furthermore, the difference in growth between control and no-reproduction plants may result because the negative impact of reproduction on growth is compounded by the opportunity cost of allocation to reproduction: allocation to leaves is an investment in further carbon gain.

If selection to reduce delayed costs has led to sexual differentiation in the way the sexes manage reproductive allocation, and if this pressure is higher on females, because they have greater delayed costs, one might expect the decrease in growth of female controls relative to males to be less than that which would be predicted based upon the greater allocation of biomass to reproduction by females than males. Unfortunately, it is not possible to compare these effects directly using the data in this study. However, the ability of females to re-allocate resources to growth when reproduction was suppressed may be indicative of an evolved difference between the sexes. Such a response may maximize carbon gain in females when reproduction fails due to pollinator limitation

or florivory. If there are differences between the sexes that enable them to reduce delayed costs relative to investment, they are likely to lie in biomass allocation patterns and photosynthetic assimilation patterns. Such differences in assimilation patterns have been implicated in other species in which females achieve equivalent or greater rates of growth despite greater reproductive allocation (see references in the Introduction); however, relationships between growth and photosynthetic assimilation rates have been measured in few dioecious species (Laporte & Delph 1996).

Delayed costs of reproduction were also apparent as decreased subsequent reproduction for females, but not for males. There was no difference between flowers-only and no-reproduction females, suggesting that while flowering alone exacted a cost on stem and (to a lesser degree) leaf production, it did not affect subsequent reproductive attempts. The lack of an effect of flowering on subsequent reproduction in males is not surprising, given the tight relationship between size and flower production discussed before. Were there clear delayed costs of flowering in males, we would expect more variability in the relationship between size and flower production in males. The slight reduction in flowering activity in 1996 in the flowers-only males is contrary to expectations, and may reflect a greater propensity to continue producing flowers following initial flower removal, and therefore a greater effective investment in flowering, although this is speculative.

The level of reproductive failure in the reproductively active females surveyed for fruit production raises a question regarding whether we expect female reproductive allocation and cost to exceed that of males on an episodic or lifetime basis. Lifetime reproductive allocation may be similar for the sexes, since selection to maximize fitness should lead to maximal possible allocation to reproduction in both sexes. Thus, at the population level investment in female function may not exceed male allocation on an episodic basis, whereas among reproductively active individuals it does. This is likely to be the case in *S. grandiflora*, and in other species in which females flower less frequently than males. Other studies of dioecious perennials have demonstrated that there is a threshold of fruit set above which female allocation exceeds male, and that this threshold is generally reached in natural populations (Wallace & Rundel 1979; Gross & Soulé 1981; Ågren 1988b). However, these authors note that the potential for fruit set failure and for less frequent flowering in females could lead to interyear variation in the proportion of females exceeding male reproductive allocation (Wallace & Rundel 1979; Ågren 1988b). Differences in reproductive allocation may be significant to the evolution of sexual differentiation in dioecious species even if female reproductive allocation only exceeds that of males on an individual episodic basis. Such episodes, if they result in significant delayed costs, may be sufficient to set up a selective environment favouring the evolution of sexual differentiation.

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