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Research Article

Sex-specific flowering patterns and demography of the understorey rain forest tree *Iryanthera hostmannii* (Myristicaceae)

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Abstract

Sexual dimorphism in dioecious plants is a common phenomenon that has received widespread attention, yet the implications for reproductive function and fitness remain poorly understood. Using data from a long-term study of a population of 839 dioecious *Iryanthera hostmannii* (Myristicaceae 'nutmeg') trees in a large permanent plot in a lowland tropical rain forest, we examined the effects of greater investment in reproduction by females compared to males for various aspects of life history. Although male trees often produced more inflorescences than females, total dry mass of flowers was roughly equal in two out of three years for both sexes, implying that any investment differential lies in fruit production. There was no difference in the 12-year relative growth rate of males and females, suggesting that females can compensate somehow for their greater reproductive investment, although there were weak suggestions that mortality might have been greater in females. Male flowers opened slightly earlier in the day than female flowers and were short-lived, lasting at most two nights compared to up to four nights in females. Understanding the interacting effects of resource availability (studied here) and pollen movement (currently unknown in *Iryanthera*) on reproduction is essential in terms of life history theory. Knowledge of reproductive biology is key in considering the ecology and conservation of tropical forest communities.

Key-words: Amazonian Ecuador, dioecy, nutmeg, phenology, pollination biology, reproductive ecology, Yasuní National Park.

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Introduction

In a finite world, organisms must allocate acquired resources in a way that maximizes fitness. Resources allocated to growth, survival or reproduction often cannot be reassigned to another function nor serve a dual function. Thus, in plants, trade-offs often exist, such as between allocating to height growth, permitting increased carbon gain, or to seed production, ensuring that reproductive fitness is not zero. Because reproduction is the ultimate goal of any individual, the 'cost' of reproduction, usually expressed as a decrease in future growth or survival, has received particular attention from biologists and is central to life history theory.

Studies of life history are often comparative, for example examining differences in resource allocation between annual and perennial plants. Those studies, however, suffer from the fact that such comparisons are between organisms that differ greatly in life history strategy. To better understand how the cost of reproduction affects life history strategy and in particular how reproduction is influenced by the resources allocated to it, we would ideally compare individuals that allocate much to reproduction with those that allocate little, within a single population of related individuals. Dioecious species, those which separate male and female function between different individuals, offer just such a natural experiment. Species with this breeding system usually exhibit sex-related differences in reproductive investment, with females generally allocating more to any one reproductive event than males [1, 2, 3]. Because sex is separated on different plants, male and female functions are independent of each other, and populations of dioecious plants therefore provide an excellent opportunity to examine the influence of reproductive costs on life history strategy.

Many of the observed differences in life history strategy between males and females are caused by this difference in investment in reproduction and have been known for a long time [1, 2]. For example, males often flower more frequently than females [4, 5], flower earlier (both in terms of phenology and size or age) [6, 7, 8, 9], and have faster growth and lower mortality [7, 10]. These strategies in turn usually give rise to male-biased sex ratios [11]. Further, sexual dimorphism in vegetative organs has been observed [2, 12, 13], and each sex may occupy different 'sub-niches' within the same environment, usually with females occurring more frequently in high- resource patches [14, 15]. However, these patterns are not observed consistently among all dioecious species, and results from such studies are often inconclusive regarding the influence of resource allocation on reproduction [16]. This confusion may in part be because the trade-off between reproduction and other resource sinks can vary markedly from year to year because of fluctuating resource availability over time [17, 18, 11, 19], and investment in reproduction may vary across temporal, spatial and morphological scales. Long-term studies are necessary to understand these effects. Furthermore, many studies are on short-lived herbaceous plants, and data are lacking for many tropical tree species, even though dioecy is more common in tropical systems than in temperate ones [20].

Dioecious species not only provide a natural experiment to examine how resource allocation patterns differ between the sexes, but also allow us to examine how selection acts differently on males and females to maximise their reproductive fitness [10, 21]. These selection pressures, in turn, may influence allocation patterns. For example, competition between males will favour males that flower at as small a size as possible, because this will likely increase their number of offspring compared to males that delay flowering. Equally, males that start to open their flowers just before the bulk of female flowers open will be selected for because the pollen from these males would likely reach females before pollen from late-opening males. Most previous work on dimorphism in flowering phenology has focused on differences at the whole population level, i.e. do males flower earlier in the season than females? Little work has been carried out on daily phenological patterns at the individual or flower level [22].

In a long-term study of reproduction, growth and demography of the Myristicaceae, a dioecious tropical tree family, Queenborough et al. (2007) [19] documented a large differential in reproductive investment between the sexes in *Iryanthera hostmannii*. The dry mass of each female fruit was several orders of magnitude greater

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than a single male flower (1600mg versus 0.48mg). At the tree level this difference was less, but females still invested an order of magnitude more dry mass in reproduction than males (mean total fruit mass per female tree was 91g, whereas mean total flower mass per male tree was 1.4g). This lower cost of reproduction in males resulted in higher flowering frequency of males leading to male biased flowering sex ratios [19]. However, the growth rate analyses presented were limited to only six years of data, and too few individuals died to attempt any analysis of mortality. Since then [19], six more years of demography data have become available. Re-analysing these expanded data will allow us to better address the question of whether higher allocation to reproduction by females is expressed in the long-term as lower growth rates and greater mortality of females.

Furthermore, Queenborough et al. (2007) [19] did not document female investment in flowers and thus did not present a complete picture of the costs of reproduction in this species. Questions remain over any difference in male and female investment in flowers and whether there are differences in flower or inflorescence structure. Given that male reproductive investment is solely in pollen-bearing flowers, we would expect males to invest more in flowering than females because females also have to produce fruit. Further, male and female trees may differ in their flowering phenology, for example in how many flowers reach anthesis/receptivity per night, what time of night they do so, and how long they remain available.

Queenborough et al. (2007) [19] also examined the spatial distribution of male and female trees to look for evidence of spatial segregation - do males and females occupy different 'micro-habitats' in the environment? Where this has been documented in other species, females tend to occur in high-resource patches and males in low-resource patches [23]. In tropical rain forest, such gradients of resource availability are rare and no evidence of this phenomenon has been found. Here we extend Queenborough et al.'s (2007) analyses to include the distribution of non- reproductive trees in order to determine whether trees become reproductive only in high-resource patches.

In this study, therefore, we first examine flower and inflorescence structure of males and females, the spatial patterns of males, females and non-reproductives, and growth and mortality over twelve years in a common western Amazonian understorey dioecious tree species. We quantify differences in allocation to reproduction between the sexes in relation to strategies to maximize reproductive output and survival. Second, we test for differences between males and females in daily patterns of flower development. Specifically, we addressed the following hypotheses: (i) females invest fewer resources in flowering than males, however, (ii) total female investment in reproduction is greater than total male investment, (iii) female and non-reproductive trees have overlapping spatial distributions, (iv) growth rates are higher in males, (v) mortality rates are higher in females, (vi) sex ratios are male-biased, and (vii) male flowers open earlier and close earlier than female flowers.

Methods

Study Site

Yasuní National Park and Biosphere Reserve and the adjacent Huaorani Ethnic Reserve form a 1.6 million ha protected area in Amazonian Ecuador (Fig. 1). While human influences are currently sparse but increasing, most of the park is undisturbed wilderness of lowland tropical rain forest [24]. Rainfall and temperature are aseasonal, with a mean annual rainfall of 2,800 mm and mean monthly temperature of 25–27ºC. A 25-ha permanent forest dynamics plot (FDP) is located inside the park (0º 41' S, 76º 24' W, 216-248 m a.s.l. [24]). We use data from the first and third censuses (1994-1997 and 2006-2007).

Study Species

Iryanthera hostmannii (Benth.) Warb. is a small understorey tree in the family Myristicaceae (nutmeg). It is one of 15 species of Myristicaceae that co-occur on the Yasuní FDP [19] and is the 29th most abundant tree species on the plot [24]. *Iryanthera hostmannii* is dioecious [19]. The fruits are woody capsules, containing a

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single arillate seed, dispersed by guans (*Penelope jacquacu* and *Pipile pipile*) and motmots (*Baryphthengus martii*) [25, 26]. Flowers are small and yellow-green in colour. They are borne on ramiflorous paniculate inflorescences emerging from leaf axils. Several flowers develop from nodes along the inflorescence. The male perianth is 3-lobed with the filaments fused into a column with three anthers. Female flowers are slightly larger and more fleshy with a single ellipsoid ovary. Pollinators of paleotropical Myristicaceae are usually small nocturnal insects such as beetles that are deceived into pollinating females, although nothing is currently known about the pollinators of *Iryanthera* [27].

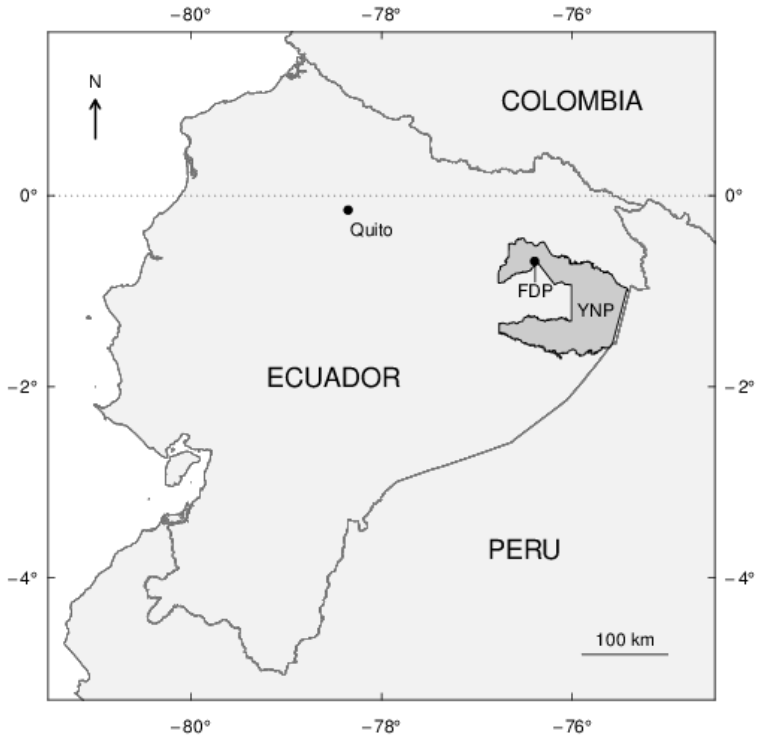


Fig. 1. Location of study site, Yasuní National Park (YNP), and the Yasuní forest dynamics plot (FDP), in Ecuador.

Data Collection

To quantify investment in flowers and determine the sex of individual trees, we counted the number of inflorescences on all 839 individuals of *Iryanthera hostmannii* within the Yasuní FDP in July-August of 2002, 2003 and 2004. For every tree we examined at least one inflorescence every year and sexed the tree from flowers. Status (alive/dead) and diameter at breast height (1.3m; DBH) of each tree were taken from the first and third FDP census. Annualized growth rates over 12 years (1997-2009) were calculated as: DBH1 – DBH0 / time1 – time0.

To monitor the phenology of individual flowers, in August 2003 we selected two large male and two large female trees that lay within or close to the FDP, each with many inflorescences. Previous studies have documented that Myristicaceae flowers open in the late afternoon and early evening [28]. We therefore undertook a preliminary census of *I. hostmannii*. On 13 and 14 August 2003, we monitored inflorescences every two hours from 06:00 to 22:00, and confirmed that flowers opened in the late afternoon. Therefore, we focussed attention on this period for the next 10 nights. From 16:00 hours we censused inflorescences every hour until no more flowers opened. We then recensused all flowers between 06:00 and 08:00 hours the following day to check for flowers that opened during the remainder of the night. We censused 13-25 inflorescences per tree and at every census we recorded the state (closed, open, wilting or dead) of each bud and flower (Table 1, Fig. 2).

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In August 2003 we also collected inflorescences from three female and four male trees from outside the FDP. We collected two or three inflorescences per tree and a total of 85 female flowers and 404 male flowers. We removed the flowers from the inflorescences and oven-dried them. The total dry mass of flowers from each individual was calculated, because flowers were too small to weigh individually. We calculated mean flower mass from these group means. The number of flowers and nodes per inflorescence were calculated from the flower phenology data above.

Table 1. Flowering of two *Iryanthera hostmannii* male and female individuals over several nights. For each night of observations, we show the number of buds and the number and proportion of buds that opened.

	Night	# Infl.	# Buds	# Open	% Open		# Infl.	# Buds	# Open	% Open
Male 1						Female 1				
	1	14	458	47	10.3		25	142	22	15.5
	2	14	420	42	10		25	121	36	29.8
	3	14	378	33	8.7		25	95	14	14.7
	4	14	248	49	19.8		25	88	12	13.6
	5	14	202	46	22.8		25	82	3	8.5
	6	14	128	28	21.9		25	73	5	4.1
	7	14					25	77		6.5
Male 2						Female 2				
	1	13	245	32	13.1		16	49	4	8.2
	2	13	223	32	14.3		16	47	12	25.5
	3	13	200	30	15		16	43	11	25.6
	4						16	23	4	17.4
	5						16	19	5	26.3
	6						16	16	2	12.5

Data analysis

We compared reproductive investment between male and female trees in a variety of ways. We tested for greater mass of individual female flowers using a t-test. We tested for a greater number of flowers per inflorescence node in males using a generalised linear model (GLM) with Poisson errors. We tested for a greater number of nodes per inflorescence in males using a GLM with quasipoisson errors because of overdispersion in the data. Significance of each GLM was assessed using a Chi-squared likelihood ratio (LR) test to compare models with and without sex. We tested for a greater natural logarithm number of open flowers per inflorescence in males and a greater number of open flowers per night in males using ANOVA, and compared models with and without sex using an F-test.

We tested for earlier flower opening times in males using ANOVA adapted to circular data [29]. Significance was determined with a likelihood ratio test [30]. According to the 24-hr clock, 00:01 is at the opposite end of the day from 23:59. In reality, however, there is no natural start or end point to the time of day and thus quantities that vary on a diurnal cycle should be analysed as circular variables, where the time difference between the 23rd and first hour is recognized to be two hours, not 22 [31]. Thus, the timing of daily peaks in flower opening was quantified as mean angles (0–360º, roughly equivalent to hour of the day) and the

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dispersion of flowering (i.e. the length of the flowering period) was quantified as circular standard deviations. These two metrics can be displayed on circular histograms as a 'mean vector' with a specified length and direction, where vector length is an inverse function of the standard deviation [29].

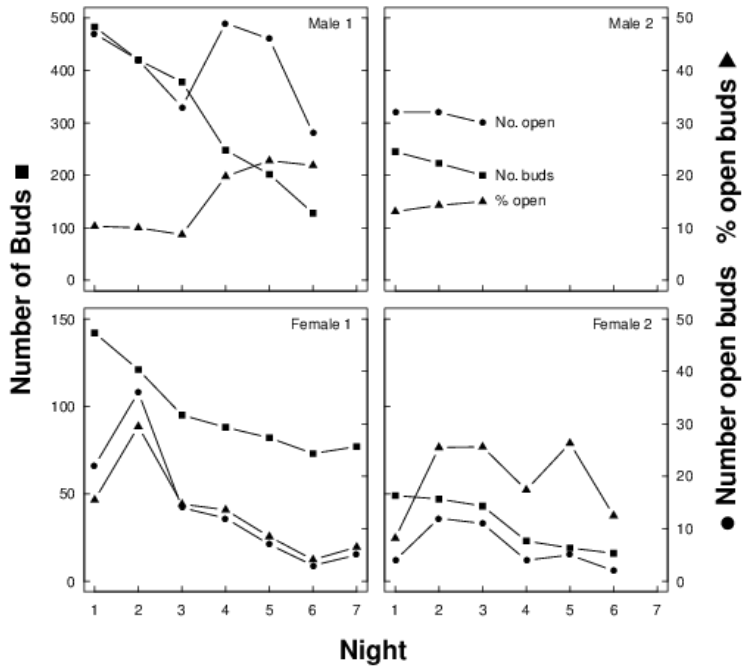


Fig. 2. Phenology of flower observations for two male (top) and two female (bottom) *Iryanthera hostmannii* trees (See Table 1).

We tested for a greater number and mass of inflorescences in males using a GLM with Poisson error structure. We tested for higher growth rates in males over 12 yrs, also using a linear model, and higher survival of males over 12 years using logistic regression. We used a non-linear Poisson error structure where appropriate because of right-skewed count data (e.g. number of flower per node, number of inflorescences), with a large number of low values, or logistic regression for binomial data. The full models for inflorescence number and mass, growth and mortality took the following form: response as a function of sex and DBH, with DBH as a covariate and an interaction with sex. In this way, we tested for a difference between males and females in both the intercept and slope of the response variable as a function of DBH. Significant effects in these models were determined from Chi-squared likelihood ratio tests. The spatial distributions of males, females and non-reproductives were compared using Ripley's K statistic, with 95% confidence envelopes calculated using the 'envelope' function in the R-package Spatstat [32]. Significant differences from a 1:1 sex ratio within DBH classes were tested with a G-test [33]. All analyses were conducted in the base statistical package R 2.10.1 [34] unless stated otherwise.



Fig. 3. *Iryanthera hostmannii* flowering branches. A) Female, B) Male. Scale bar = c.1cm. Credit: Robin Foster, The Field Museum, Chicago, USA.

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Results

Flower and inflorescence structure and investment

Iryanthera hostmanii flower and inflorescence structure were quite different between the sexes (Fig. 3 and 4, Table 2). Female flowers had over twice the mass of male flowers (t-test: df = 2.644, t = 5.75, P = 0.015; Fig. 4A). Female inflorescences also had one more flower per node than male inflorescences (LR χ^2 = 28.3, P < 0.001; Fig. 4B). Conversely, female inflorescences were shorter than male inflorescences and had fewer than half the number of nodes of male inflorescences (LR χ^2 = 65.2, P < 0.001; Fig. 4C). However, mean total dry mass per inflorescence appeared slightly greater in females than males (although we could not statistically test this difference because these data were calculated from single mean values per sex; Fig. 4D).

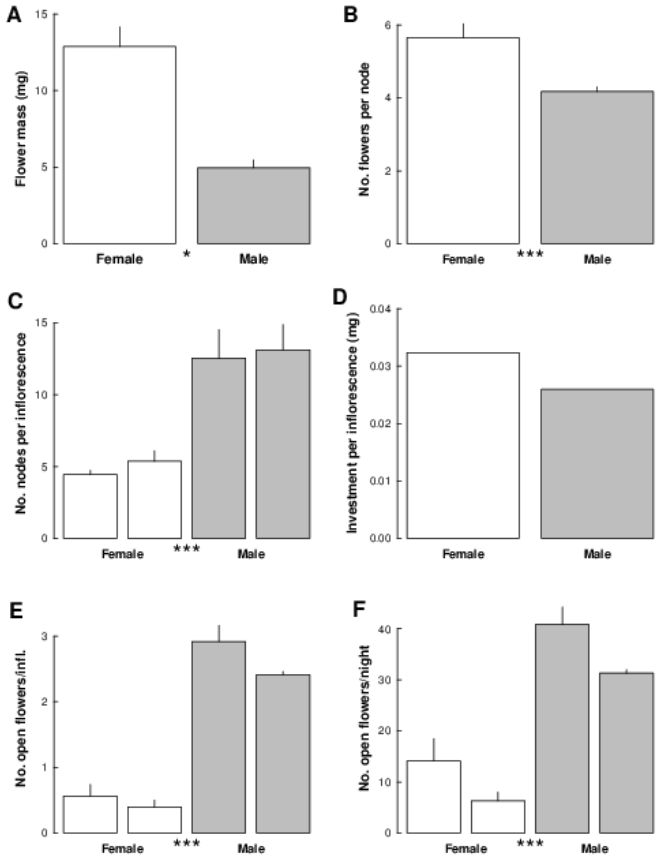


Fig. 4. Differences in flowering patterns between male (filled) and female (open) *Iryanthera hostmannii* trees. A) Mass of flowers, B) Number of flowers per node, C) Number of nodes per inflorescence, D) Number of inflorescences per tree, E) Number of flowers opening per inflorescence, F) Number of flowers open per night. Data: A & B: data from inflorescences collected from three female and four male trees, pooled; C, E & F: data from two female and two male trees monitored for flower phenology, treated separately; D: mean values per sex calculated from A, B & C. Significant differences between the sexes are indicated by asterisks: * P < 0.05, *** P <0.001.

At the tree level, there was a significant relationship between the number of inflorescences and tree size (DBH) in all three years (2002: LR χ^2 = 7241.9, P < 0.001; 2003: LR χ^2 = 8723.7, P < 0.001; 2004: LR χ^2 = 6886.8, P < 0.001; Fig. 5). Furthermore, there was a significant interaction between sex and DBH in this relationship: both the intercept and slope of the number of inflorescences as a function of DBH for males were significantly different from those of females (2002: LR χ^2 = 53.8, P < 0.001; 2003: LR χ^2 = 201.4, P < 0.001; 2004: LR χ^2 = 44.3, P < 0.001). In 2002 and 2003 the intercept of male trees was significantly lower than that of female trees, indicating that males produced fewer inflorescences on average. However, in all three years, male trees had a steeper slope than female trees, indicating that male trees produced more inflorescences than females at larger sizes (Fig. 5A-B). In 2004, male trees had a significantly larger intercept, indicating that they produced more inflorescences on average (Fig. 5C).

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Table 2. Investment in flowers by male and female *Iryanthera hostmannii*. Values: mean ± SE.

	Male	Female	N_{male}	N_{female}
Flowers per node	4.2±0.4	5.7 ±0.1	243	83
Nodes per inflorescence	12.5 ±1.9	4.4 ±0.5	19	17
Flowers per inflorescence	52	25		
Investment per inflorescence (g)	259.4	323.3		

In terms of inflorescence dry mass, again there was a significant relationship between dry mass and DBH in all three years (2002: LR χ^2 = 206.5, P < 0.001; 2003: LR χ^2 = 249.9, P < 0.001; 2004: LR χ^2 = 194.2, P < 0.001; Fig. 5D-F). However, only in 2003 was there a significant interaction between sex and DBH in this relationship (2002: LR χ^2 = 1.6, P = 0.209; 2003: LR χ^2 = 6.06, P = 0.014; 2004: LR χ^2 = 1.3, P = 0.25). In 2003, females had a significantly higher intercept than males, but males had a significantly steeper slope than females, indicating that females invested more dry mass in inflorescences per tree than males at smaller sizes, but this difference disappeared at larger size classes (Fig. 5E).

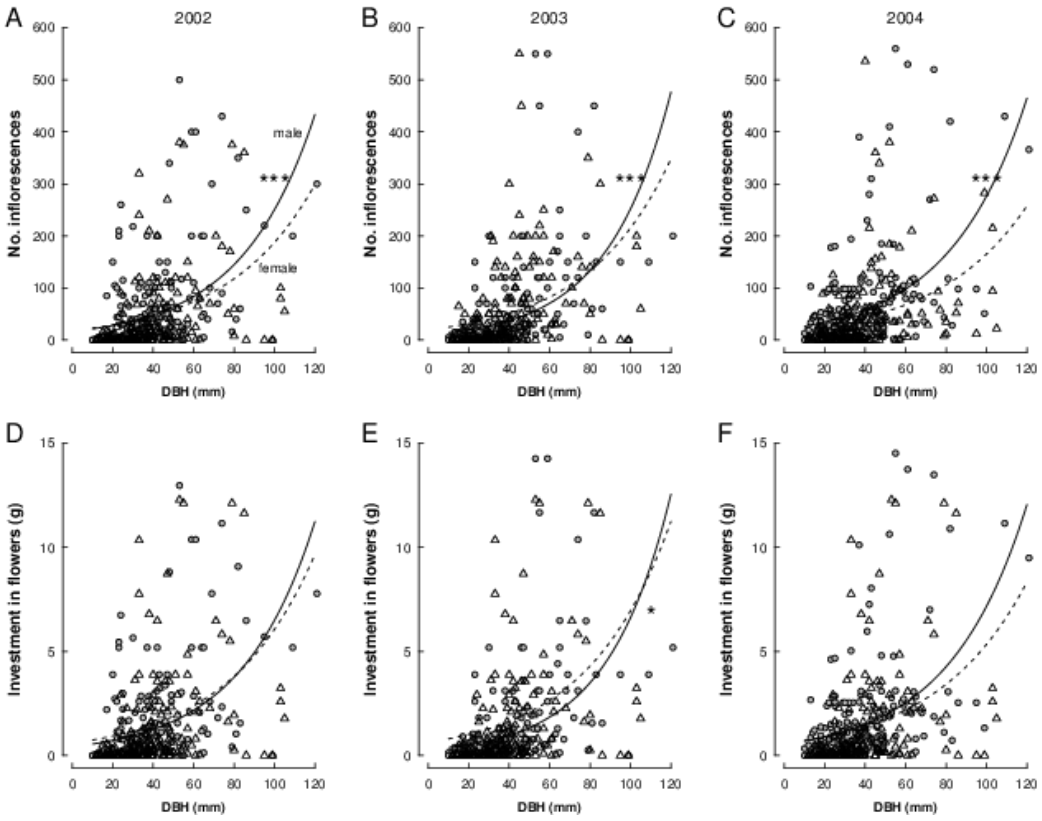


Fig. 5. Differences in inflorescence number and mass between male (filled circles, solid line) and female (open triangles, dashed line) *Iryanthera hostmannii* trees over three flowering seasons. A-C) Number of inflorescence per tree in 2002, 2003 and 2004. D-F) Estimated total mass of inflorescences (g) per tree in 2002, 2003 and 2004. Significant differences between the sexes (ANCOVA with DBH as a covariate) are indicated by asterisks: * P < 0.05, *** P <0.001.

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Demography

Monitoring survival and growth over 12 years, rather than six as in Queenborough et al [19], revealed fewer insights than we had hoped. Only seven trees that were reproductive between 2002 and 2004 had died by 2009. However, six of these were female and only one was male. Although this hints that females may suffer higher mortality than males, the sample size remains too small to give a robust estimate.

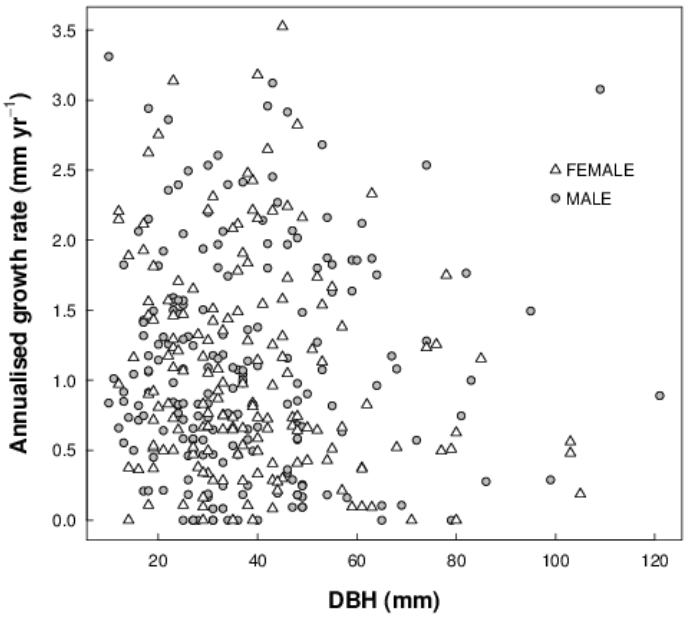


Fig. 6. Annualized growth rates of male (filled circles) and female (open triangles) *Iryanthera hostmannii* trees over 12 years.

In terms of growth, there was no significant relationship between relative growth rate and tree DBH (LR χ^2 = 0.99, P = 0.19), nor was there a significant difference between the sexes (LR χ^2 = 0.22, P = 0.54; Fig. 6). Thus, male and female trees of all sizes had equivalent growth rates over 12 years between the two censuses.

Table 3. Sex ratios of *Iryanthera hostmannii* over three years on the Yasuní FDP.

Year	Nrep	Male proportion	*G*	*P*
2002	295	0.56	4.16	0.04
2003	315	0.54	1.99	0.16
2004	196	0.63	12.9	0
Cumulative	370	0.54	2.44	0.12

Sex ratio

The population of *Iryanthera hostmannii* in the Yasuní FDP that flowered over 2002, 2003 and 2004 contained more male trees (200) than female trees (170), although this cumulative (total) sex ratio was not significantly different from 1:1 (G = 2.43, P = 0.12). However, flowering sex ratios were significantly male-biased in 2002 and 2004 (Table 2), and also within one specific size class. In the 20-30 mm DBH size class, significantly more male trees flowered than female trees in 2002 and 2004 (2002: G = 8.40, P = 0.004; 2004: G = 5.96, P = 0.015; Fig. 7).

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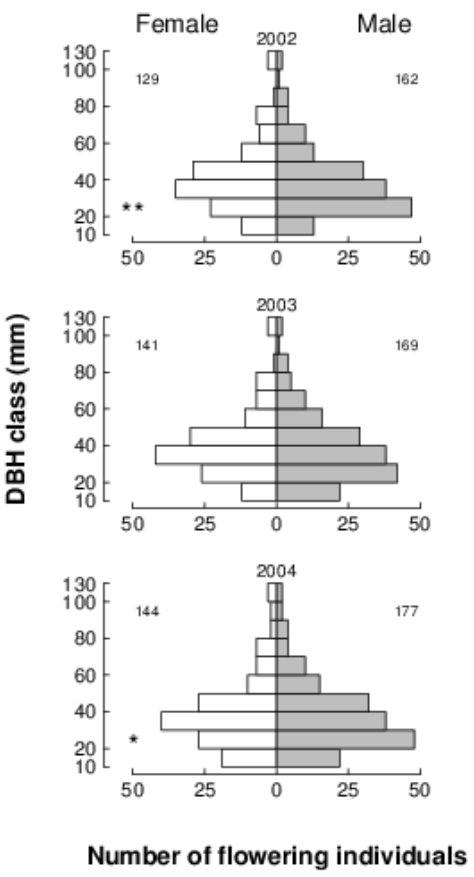


Fig. 7. Sex ratios in increasing size (DBH) classes in *Iryanthera hostmannii* in the Yasuní FDP between 2002 and 2004. Total number of males (filled) and females (open) are indicated on each side. Significant differences from a 1:1 sex ratio within a DBH class are indicated by asterisks: * P < 0.05, ** P <0.01.

Spatial distribution

Male and female *Iryanthera hostmannii* were distributed randomly throughout the FDP, indicated by the fact that the observed values of L(r) did not exceed the confidence envelopes (Fig. 8A,B), and as in [19] there was no significant aggregation or over-dispersion of males relative to females (Fig. 8C). There was also no evidence that trees become reproductive only in certain areas of the plot, as reproductive trees were distributed randomly with respect to non-reproductive trees (Fig. 8D).

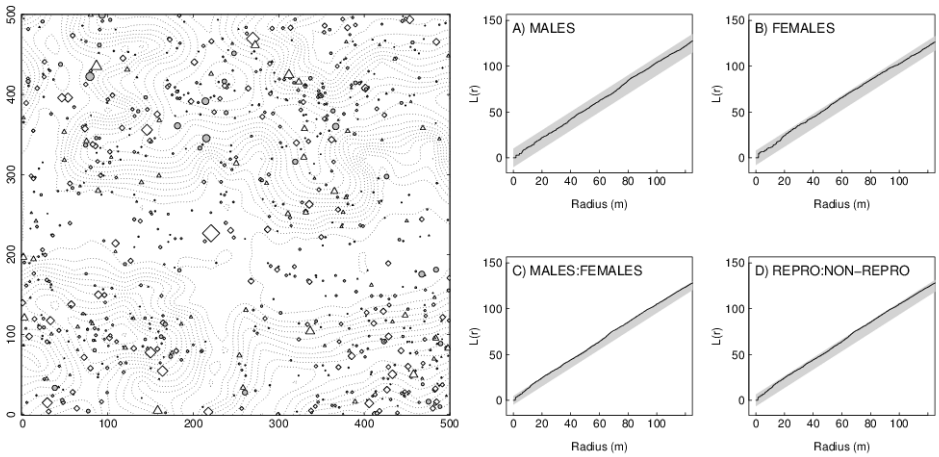


Fig. 8. The spatial pattern of male, female and non-reproductive *Iryanthera hostmannii* trees in the Yasuní FDP. The left panel shows a map of the 25ha plot, with males (filled circles), females (open triangles), and non-reproductives (open diamonds). The size of the symbol is proportional to the DBH of the tree. The right panel (A-D) shows results from point pattern analyses (Ripley's K). 95% confidence intervals are indicated by the shaded bar; if the black line lies out of these it indicates aggregation (above) or over-dispersion (below).

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Flower phenology

Iryanthera flowers did not start to open until after 16.00. Most flowers opened between 17.00 and 20.00, with only a few opening later (Fig. 9). Male flowers opened significantly earlier than female flowers, although there was more variation in peak opening time in males (circular ANOVA with likelihood ratio test: df = 1, $\chi 2 = 6.626$, $P < 0.05$). Furthermore, male flowers were much shorter-lived than female flowers, remaining open less than two days before abscising, although again there was considerable variation among trees (ANOVA, sex: df = 1, SS = 4083, MS = 4083, F = 4.9, P = 0.026; tree: df = 1, SS = 102254, MS = 102254, F = 123.8, P < 0.001; Fig. 10). Female flowers, on the other hand, were open and receptive twice as long before either wilting and abscising or beginning to mature into fruit.

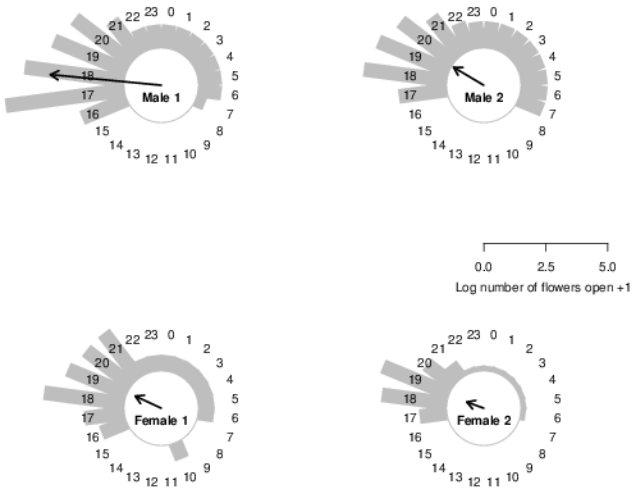


Fig. 9. Flower opening times of two male and two female *Iryanthera hostmannii* trees. Circular bar charts show the number of open flowers per hour (24 hr clock). Arrows show the mean opening time (angle) and standard deviation (length). Note the log scale.

In terms of the number of flowers opening, male trees opened three times more flowers per inflorescence than females (F-test: F = 53.2, P < 0.001), and three times more flowers per inflorescence per night (F = 56.4, P < 0.001, Table 1, Fig. 4E and F).

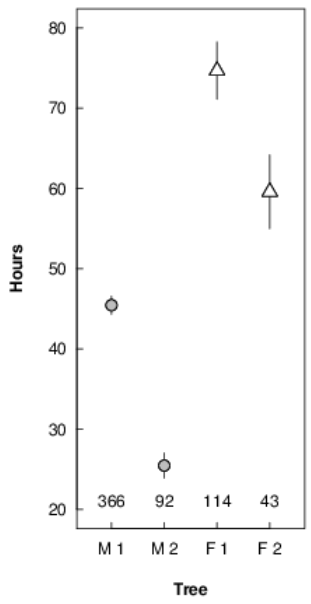


Fig. 10. Mean flower life time for two male (filled circles) and two female (open triangles) *Iryanthera hostmannii* trees in Yasuní. Error bars = SE. Sample sizes (# flowers per tree) indicated at the bottom.

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Discussion

Total reproductive investment is greater in female than in male *Iryanthera hostmannii* because females produce fruit, a single one of which is equivalent to the mass of over 3,000 male flowers. This differential investment was expressed by males as longer inflorescences with more flowers and shorter flower phenology, and potentially greater tree longevity. These differences in turn were associated with male-biased flowering sex ratios in small size classes. This study provides a more complete picture of the effect of this difference in resource allocation to reproduction than previously published [19].

Investment, resource and pollen availability

Previous studies of Myristicaceae have documented significant differences between the sexes in terms of flower size and number [35, 36, 37], such as we found in this study. Males are under selection pressure to effect pollen transfer to females. Competition between males in our case has led to possibly earlier flower opening in the day than females, as well as increased flower number: male inflorescences were longer than female inflorescences and had more flowers, and large males also produced more inflorescences than females. Males that produce more flowers are likely to have greater reproductive fitness than males that produce fewer. However, even small males produced some flowers. Given the short distances that the likely pollinators of this species move [27], as well as the understorey environment in which *Iryanthera hostmannii* exists, proximity to a female may be just as important as flowering intensity to ensure the successful transfer of pollen [38]. However, given the often orders-of-magnitude difference in flower number between males and females observed in multiple-ovaried flowers of species of other families [39, 40, 41], the difference in flower number in *Iryanthera*, with the single-ovaried flowers, was not that great. Females can only produce one offspring per flower, and thus may increase flower number to account for this.

An alternative mechanism to increase pollen availability would be for each male flower to produce more pollen, likely leading to larger male flowers. This would entail a whole suite of problems associated with the deceit pollination that occurs in *Myristica*, and likely *Iryanthera* as well [27]. Selection on reproductive structures has also been posited to explain dimorphism in canopy structure [42] and leaf size [43]. However, because inflorescences in *Iryanthera* are ramiflorous, with multiple inflorescences emerging from a single branch, there is little scope for selection pressure to influence these traits here.

Total investment in flowers, as measured by dry mass, was equivalent in males and females in two out of three years of our study. This parity implies that, in general, the difference in reproductive investment between males and females is predominantly in the fruiting stage (at least in terms of carbon). It is likely that only those females with access to abundant resources, or resources stored from previous years, may proceed to set fruit [44, 45]. Resolution of this question will require long-term studies of resource and pollen availability combined with detailed quantification of growth, mortality and reproduction.

Further investigation will also be required to determine whether the same resources limit reproduction in both sexes. For females of many tropical species, carbon is the most likely [46]. However, in the case of the Myristicaceae, each seed is surrounded by a fatty, nutrient-rich aril, and resources such as nitrogen may also limit female reproduction [47]. Thus, dry mass may not be the best measure for comparing resource allocation between the sexes.

Furthermore, resources will only limit reproduction in females if pollen is not limiting [48]. We have previously found evidence of pollen limitation in this species (Queenborough, *unpublished data*). Determining whether resources or pollen limit any one reproductive event, and thus enabling a robust examination of how resources are allocated, is essential for life history theory.

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Long-term costs of reproduction

Evidence for long-term demographic costs of reproduction in woody plants is equivocal [16, 49], apart from the few species of monocarpic tree [50]. Evidence of negative effects of reproduction on growth has come from tree-ring dendrochronological studies or species that produce one fruit per branch, such as some gymnosperms [51], but by definition these studies only include those individuals that survived a long time. Individuals that suffered extremely negative effects of reproduction on growth (i.e. those that died) are lost. Thus, only prospective studies of individuals of known sex will provide robust estimates of the long-term effects of reproduction on growth and survival. Unfortunately, the currently available data cover too short a period of many species' life cycles, and good estimates of long-term growth and mortality are difficult to obtain [49]. There is good evidence from some species, however, that female physiological processes cannot increase output, and that photosynthesis and growth rates decrease with increased costs of fruit production [10]. The true long-term costs of reproduction in many species remain unknown.

Floral phenology

Most previous work on dimorphism in flowering phenology has focused on differences at the whole plant level, i.e. do males flower earlier in the season than females? Less work has been carried out explicitly on daily patterns of phenology at the flower level [22]. Competition between males would suggest that males should flower earlier in both instances, ensuring that pollen is available and is transferred efficiently from males to females. Theory would also predict that males might flower for longer than females, ensuring that they have the potential to pollinate every last female. Evidence for these predictions is equivocal, however, with some studies finding earlier male flowering [9, 52] and some earlier female flowering [53]. In our study, we found a slightly earlier flower opening time in males, but with much overlap between the sexes.

Conclusions and Implications for Conservation

Inter-sexual variation in plant form and function has been a fertile topic of investigation since Darwin [54]. Dioecious species provide a natural experiment with which to study how high (in females) vs. low (in males) allocation to reproduction affects life history. In this study, we found significant variation in allocation to flowering by males and females among years, although investment in flowering was broadly equivalent among sexes. Thus, higher female allocation to reproduction comes primarily as fruit. Key questions remain about whether resources or pollen limit fruit set, and how variation in resource and pollen availability influence reproduction, growth and survival over long time periods meaningful to the lifetime of a tree .

Our results suggest that large areas of forest need to be conserved to maintain viable populations of trees, as well as to supply adequate food to frugivorous animals, given the variation in individual flowering within this population. Further, we highlight the lack of knowledge of pollinators in the family. Maintaining intact and undisturbed ecosystems is essential if humans are ever to fully understand the complexity of multi-level interactions between and among plants and animals.

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