Previous-year reproduction reduces photosynthetic capacity and slows lifetime growth in females of a neotropical tree

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Females of dioecious plant species typically invest more in reproduction than males because they produce seeds, fruits, and associated structures in addition to flowers. If females are unable to compensate by up-regulating rates of photosynthesis or by reproducing less frequently than males, their greater reproductive investment may result in reduced growth or higher mortality. Here we provide evidence of the cost of reproduction in *Ocotea tenera* (Lauraceae), a dioecious neotropical tree common in lower montane forests of Monteverde, Costa Rica. Over periods of 12–21 years, females grew more slowly than males in a natural population and in two experimental plots where we were able to control for genotype, age, habitat, and reproductive history. Simultaneous measurements of 10 matched pairs of sibling trees of the opposite sex but same age demonstrated that the photosynthetic capacities of females were 13% lower than those of males. Among females, photosynthetic capacity was negatively correlated with fruit production during the most recent reproductive season but not with lifetime fruit production. Sexual size dimorphism in adult *O. tenera* trees appears to be a nonadaptive consequence of trading off recent reproduction against maintenance of the photosynthetic apparatus, with long-term negative effects on growth.

Dioecious plants, species in which male and female flowers are borne on separate individuals, offer an opportunity to assess the costs of reproduction by examining how sexual differences in reproductive effort affect physiology, growth, and life history traits (1, 2). Greater allocation of resources toward reproduction by one sex (typically females) may result in sex-specific differences in the production and consumption of fixed carbon at the whole-plant level, with both short-term consequences (e.g., photosynthetic acclimation) and long-term consequences (e.g., fecundity, growth, and survival). In some dioecious species, the costs of reproduction are reflected in the fact that females are less likely to survive in stressful habitats, which results in spatial segregation of the sexes (3, 4). In other species (5, 6), females can co-occur with males but must defray the costs of reproduction by delaying reproductive maturity or by reproducing less frequently than males (7, 8).

Although it is now well established that females of many dioecious plant species invest more carbon, nitrogen, and other resources in reproduction than males (2), few studies have linked physiological performance with morphological or demographic consequences or taken into account the influence of recent reproductive effort, much less lifetime reproductive effort, particularly in long-lived trees (9). Moreover, it has been difficult to generalize about the sex-specific costs of reproduction because of differences in the results of laboratory versus field studies and between studies of herbaceous versus woody plants. For example, females tend to be larger than males in herbaceous plants grown in greenhouse experiments (10). In contrast, in woody plants observed under natural conditions (4, 6, 11), males are more commonly larger than females (7). In field studies, spatial segregation of the sexes can confound interpretations of sex-specific differences in photosynthesis or growth because of differences in habitat quality. In addition, field studies rarely take into account such factors as plant age, genotype, and reproductive history. The question thus remains whether sexual dimorphism in plants reflects an adaptive response to selection for different phenotypic optima for males versus females (e.g., larger size favored in males because of intrasexual competition for access to females) or reflects nonadaptive trade-offs or constraints because of sex-specific differences in the cost of reproduction (9).

Here we provide evidence of the cost of reproduction in *Ocotea tenera*, a dioecious tree in the avocado family (Lauraceae) found in the lower montane moist forests of Monteverde, Costa Rica (12). We monitored growth rates and reproduction of individual trees in a natural population and in three experimental plots over 12- to 21-year periods. Simultaneous measurements of photosynthetic capacity of opposite-sex sibling trees that had been observed from seed germination through sexual maturity allowed us to quantify sex-specific physiological differences while controlling for genotype, age, habitat, and lifetime reproductive history.

Materials and Methods

Monitoring and Analyses of Growth Rates. Observations of growth and reproduction in a natural population of *O. tenera* began in 1981. The sample initially included every reproductively mature *O. tenera* tree found within 10 m of transects in the study site (n = 36 males and 47 females). At the start of the study, trees were ≈10–30 years old (as estimated later based on growth rates of known-age trees in experimental plots). After several years, observations were focused on a subsample of 29 trees (12 males and 17 females, chosen on the basis of their location near existing study sites) in the natural population that were monitored every year until 1992 and sporadically thereafter through 1997. Because water is rarely limiting in the lower montane moist forests and because male and female *O. tenera* co-occur in the same habitats (13), differences between the sexes in water relations were unlikely (4, 12).

Experimental plots were established in 1981 (plots T1 and T2) and 1984 (plot H) with seedlings of known maternity (n = 14 maternal genotypes) planted in a Latin square design beneath scattered canopy trees in small clearings within natural forest. More than 90% of the mortality in the experimental plots occurred within the first or second year after seedlings were planted and was chiefly due to falling trees and branches. The young age at which mortality occurred (3–4 years before reproductive maturity) and its random source suggest that mortality was independent of sex. Nonetheless, the sex ratio at maturity was male-skewed in the experimental plots. Sixty-two of the seedlings survived until 2002 (n = 39 males and 23 females; X^2_1 = 4.21, P = 0.04, χ^2 test). Trunk diameter (measured with calipers at a height of 1 m) and fruit production were measured in February or March every year from 1981 until 2002 (except

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for 1995 and 1998–2001). Trunk diameter was strongly correlated with other measures of plant size (e.g., height, $r^2 = 0.90$; $n = 23$, $P < 0.001$, linear regression). Each year, fruits were counted individually on all trees.

We modeled the relationship between age and diameter in the longitudinal datasets by using linear mixed-effects models (14), with diameter as the dependent variable, tree number as a random effect, and age as a fixed effect.

Collecting Samples and Measuring Photosynthetic Capacities. We measured photosynthesis of freshly collected leaf samples as the rate of oxygen evolution at light and CO_2 saturation, which is a common method of determining photosynthetic capacity (15). Measurements of photosynthetic capacity were performed on leaf samples of 10 sibling pairs of male and female trees matched for age, maternal genotype, and habitat, with three replicates per tree. The pairs represented seven different maternal genotypes. Three haphazardly selected branchlets from different parts of each tree were collected and stored for no more than 70 min in plastic bags containing a moist paper towel. Counting from the tip of the branchlet, we selected the third to fifth fully expanded leaf.

Collections were made in March 2002, ≈6 months after flowering and just as fruits were ripening. Thus, our measurements were made at a time of year when fruits were still carbon sinks and when nutrient allocation to reproduction might have its greatest negative effect on photosynthetic capacity because of nutrient limitation. Leaf discs from sibling pairs were examined in parallel by using identical oxygen electrodes. Replicate measurements from a single tree alternated between electrodes. There was no effect of storage time, leaf number, or electrode on photosynthetic capacity ($P > 0.25$, ANOVA). Although there was a tendency for the leaves of females to be slightly longer and wider than those of males, specific leaf area (m^2/kg of dry weight) was similar (mean leaf length and width, $t_9 = 2.12$, $P = 0.06$, paired t test; mean specific leaf area, $t_9 = 0.79$, $P = 0.45$). Photosynthetic oxygen evolution was measured from leaf discs (2.92 cm^2) with an oxygen electrode (Model LD-2, equipped with a LS-2 light source, Hansatech, King's Lynn, U.K.). Leaf temperature was held constant by circulating gravity-fed 19–20°C water around the electrode chamber. Measurements were performed at saturating light intensity (1,750–1,800 μmol of photons m^{-2}·s^{-1}) and CO_2 concentration once photosynthesis had reached steady state (i.e., when the rate of oxygen evolution achieved linearity). Leaf discs were exposed to a 15-min four-step progressive increase in light intensity before measurements. The rate of respiratory oxygen consumption was determined immediately after determination of the net rate of photosynthesis by darkening the leaf disk. For each tree, we averaged the measurements from the three different leaves to determine photosynthetic capacity. Replicate measurements from the same tree were similar [repeatability (16) of photosynthetic capacity = 0.46]. Finally, we tested for differences between the sexes with paired t tests comparing the photosynthetic capacities of siblings of opposite sex.

Results

Life History Traits of Males and Females. Males and female *O. tenera* trees had similar life history traits. Both sexes reached reproductive maturity at the same age in the experimental plots (mean ± SD = 4.9 ± 1.4 years; $t_{62} = -0.045$, $P = 0.96$, t test). Thereafter, both males and females flowered every year during the rainy season. Mortality rates were low (<0.004 year^{-1}) for trees that reached reproductive maturity, and they did not differ between the sexes. Over the course of the study, only two mature males and three females died in the experimental plots; in the natural population, 5/36 males and 13/47 females died ($P = 0.18$, Fisher's exact test).

Males produced ≈20 times as many flowers as females over the course of the flowering season, and their flowers were ≈30% larger (unpublished data). Nonetheless, investment in the di
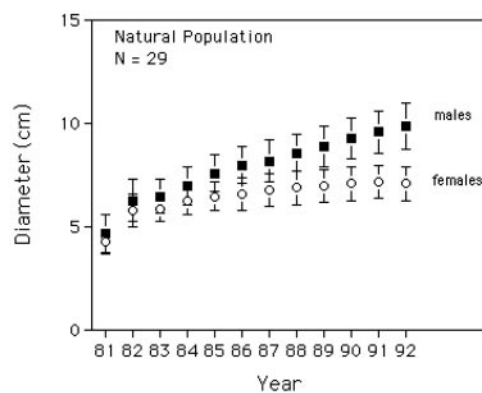

Fig. 1. Trunk diameter of male and female *O. tenera* trees in a natural population. Females (○) grew significantly more slowly than males (■) over a 12-year period. Results are means ± SE.

minutive (3–4 mm) flowers was relatively low for both sexes. Total reproductive costs were greater in females because in addition to flowers they annually produced numerous bulky single-seeded fruits and maintained them for six months from seed set until dispersal. On average, fruits weighed 6.6 g and seeds weighed 3.8 g (17). Annual fruit crops ranged from a few fruits to >1,000 fruits on a single tree. [Some large male trees occasionally produced fruits (18, 19), but never more than one to five, far fewer than similarly sized females.] Fecundity was positively correlated with tree size ($r_{s_{21}} = 0.78$, $P = 0.0004$, Spearman rank correlation). Over the course of 12 years, individual females in the natural population produced as much as 35 kg of fruits (mean ± SD = 6.3 ± 10.5 kg). During the first 18–21 years of their lives, female trees in experimental plots produced an average of 5.1 ± 4.4 kg of fruits.

In addition to carbon costs, fruits and seeds of *O. tenera* are well provisioned with nitrogen, a limiting nutrient in many environments, particularly in the tropics; nitrogen made up 2.3% (dry weight) of the protein-rich exocarp alone (17). Thus, some females invested as much as 2.2 kg of nitrogen in fruit pulp over a 12-year period. Unlike males, females also paid the costs of building and supporting reproductive structures, such as thickened pedicels (each weighing ≈0.16 g of dry weight, 60 times the mass of individual flowers), which protect the fruits and serve to display them to large avian seed dispersers, such as resplendent quetzals (*Pharomachrus mocinno*) and emerald toucanets (*Aulacorhynchus prasinus*) (17).

Growth Rates of Males Versus Females. In the natural population of reproductively mature *O. tenera* trees, including the large initial sample, males were slightly larger than females when our study began in 1981 ($t_{81} = 2.60$, $P = 0.01$). In the subsample of trees measured annually through 1992, the mean size of males was larger than that of females in 1981 but not significantly so ($t_{27} = 0.38$, $P = 0.71$, t test). However, 12 years later, males were 28% larger on average than females (Fig. 1). A significant fixed effect between age and sex in our linear mixed-effects models indicated that males grew more rapidly than females ($t_{348} = 2.88$, $P = 0.004$, natural population).

Males also grew significantly faster than females in the experimental plots over 12- to 21-year periods (Fig. 2; $t_{649} = 3.27$, $P = 0.001$, linear mixed-effects models on combined data). Small but significant size differences between the sexes were evident in one of the three experimental plots (plot H) 1 year before trees were reproductively mature (Fig. 2; $t_{29} = 2.34$, $P = 0.03$, t test). Across all three plots, males averaged 20% larger within a year of reproducing for the first time ($t_{63} = 2.05$, $P = 0.044$). When we analyzed each experimental plot separately, there was a
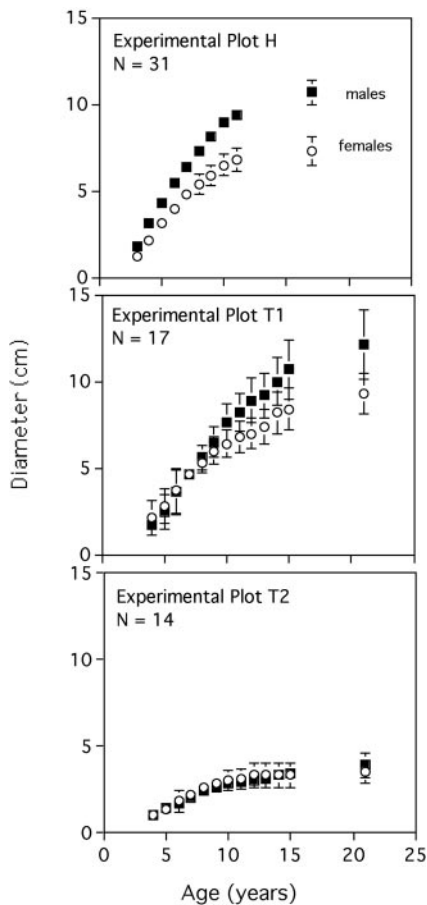
Fig. 2. Trunk diameter of male and female *O. tenera* trees in three experimental plots. Females (○) grew significantly more slowly than males (■) over periods of 18–21 years in the experimental plots overall and in plots H and T1 analyzed separately but not in plot T2, which was deeply shaded. Results are means ± SEM.

significant fixed effect between age and sex in plot H (t_{282} = 3.01, P = 0.003) and a marginally significant effect in plot T1 (t_{210} = 1.82, P = 0.071). By using a repeated-measures ANOVA, we found a significant interaction between age and sex in both plots ($F_{9,261}$ = 5.657, P < 0.0001, plot H; $F_{12,180}$ = 2.146, P = 0.016, plot T1). Seeds that produced males were no different in size than seeds that produced females (t_{23} = 0.30, P = 0.77). Thus, there was no evidence that sex-specific size differences among adult trees were due to maternal effects.

In plot T2, which was deeply shaded and where growth rates were only one-third of those in the other two plots, there was no significant fixed effect between age and sex (t_{166} = 0.31, P = 0.76; $F_{12,144}$ = 0.269, P = 0.99, repeated-measures ANOVA). Reproductive maturity in plot T2 was delayed by 1.8 years, and cumulative fruit production over a 21-year period was less than half that of plot T1 (t_{11} = 2.46, P = 0.03, t test).

Photosynthetic Capacity of Males Versus Females. Studies of long-lived woody species have demonstrated that females often photosynthesize at higher rates than males (4, 6, 7, 11). In such species, females presumably are able to up-regulate photosynthesis to cover the higher carbon costs of fruit production (4, 20). However, when we compared sibling males and females of the same age growing under the same conditions in the field during the end of the fruiting period, we found that females did not up-regulate photosynthetic rates in response to the demands of
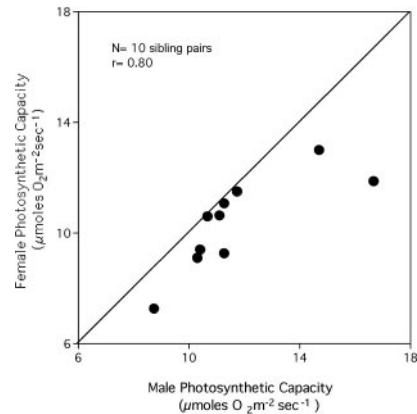

Fig. 3. Photosynthetic capacity (expressed as the rate of oxygen evolution per unit of leaf area) of 10 sibling pairs of *O. tenera* trees. The line indicates equal photosynthetic capacities of males and females. In each pair, males had higher photosynthetic capacity (P < 0.001, binomial test). Photosynthetic capacities of siblings were positively correlated (r = 0.80, linear regression).

reproduction. In fact, females had photosynthetic capacities that averaged 13% lower than their male siblings (females, 10.4 ± 1.7 μmol of O_2 m^{-2}·s^{-1}; males, 11.7 ± 2.3 μmol of O_2 m^{-2}·s^{-1}; t_9 = 2.92, P = 0.017). In each pair, males had higher photosynthetic capacity than their female sibling (P < 0.001, binomial test). Photosynthetic capacities of siblings were positively correlated (r = 0.80, P = 0.004, linear regression; Fig. 3). Similarities in rates of photosynthesis between close relatives suggests the possibility of a heritable component to this physiological trait.

In some plant species, photosynthetic activity of maturing fruits, sepals and other reproductive structures supports a substantial fraction of the overall energy costs of reproduction (21). The exocarp of *O. tenera* fruits that were full-sized but still green had photosynthetic capacities of 4.7 ± 1.7 μmol of O_2 m^{-2}·s^{-1} (n = 4). In mature fruits, which turn black upon ripening, photosynthetic capacities were only half that level. These rates of photosynthesis, which were measured at optimal temperature and under saturating light and CO_2, were <50% of the instantaneous respiration rates for both ripe and unripe fruits.

Photosynthetic Capacities and Previous Reproduction. Photosynthetic capacities of individual female trees were strongly and negatively correlated with the number of fruits produced in the most recent reproductive season (Fig. 4). When we adjusted for the size of trees (with residuals of a regression of tree diameter versus fruit crop size), the correlation was also significant (r_s = −0.80, P = 0.01, Spearman rank correlation). However, neither lifetime fruit production nor lifetime fruit production adjusted for tree size was correlated with photosynthetic capacity (P > 0.67, Spearman rank correlation).

Discussion

The dioecious neotropical tree *O. tenera* has several traits that would indicate especially high costs of reproduction in females (1, 2). Females produce fruits that are much larger than those of typical bird-dispersed woody plants in the temperate zone (22, 23). Producing such bulky fleshy fruits should be particularly costly because of the small size at which females reach reproductive maturity (<2-cm trunk diameter and <2-m height). Fruit pulp and seeds in the Lauraceae also have unusually high concentrations of nitrogen (17). Although the Central American lower montane moist forest soils where *O. tenera* grows are relatively fertile compared with most tropical soils (12), the costs of producing nitrogen-rich fruits in most tropical habitats may still be high compared to the temperate zone.
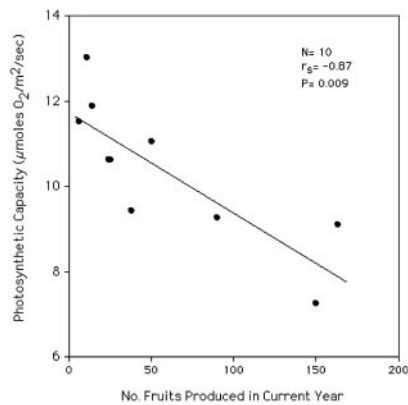
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Fig. 4. Photosynthetic capacity (expressed as the rate of oxygen evolution per unit of leaf area) of individual female *O. tenera* trees as a function of the number of fruits produced in the current year. Females that had recently invested heavily in reproduction had significantly lower photosynthetic capacities (P = 0.009, Spearman rank correlation).

The first question we asked in this study was whether there was any evidence that reproductive costs were reflected in the life history traits of *O. tenera*. Unexpectedly, females and males reached reproductive maturity at the same age. Also, both sexes flowered annually and females set fruit each year. Thus, females did not defray the cost of reproduction by delaying reproductive maturity or by reproducing less frequently than males. Nor did females suffer higher mortality rates as a result of their greater reproductive investments. Despite slower growth rates and smaller sizes in female trees, the species' relative shade tolerance allowed even heavily shaded and stunted trees to persist (see Fig. 2, plot T2). Thus, we found no indication of a demographic cost of reproduction.

Nonetheless, our study revealed three independent types of evidence for other costs of reproduction in *O. tenera*: (*i*) sex-specific differences in long-term growth rates, (*ii*) sex-specific differences in photosynthetic capacity, and (*iii*) negative correlations between photosynthetic capacity and recent reproductive investment. Female trees grew more slowly than males in a natural population monitored over a 12-year period. Females also grew more slowly than males in two experimental plots monitored over 18- to 21-year periods. In a third experimental plot, the mean size of females was smaller than that of males, but differences were not significant because growth rates were so slow and reproductive maturity was so delayed in the heavily shaded site. The most likely proximate causes for slower growth rates in female *O. tenera* are the direct effects of reallocating carbon from growth to reproduction and the indirect effects of lower photosynthetic capacity caused by the investment of nutrients, such as nitrogen, in fruits rather than in the photosynthetic apparatus (2, 7).

Perhaps the strongest evidence for a cost of reproduction comes from a significant negative correlation between photosynthetic capacity and recent reproductive investment. Such a result also suggests that a female's photosynthetic capacity is determined by her ability to mobilize nutrients to maintain the photosynthetic apparatus (24). Interestingly, the effect is apparently relatively short-term because there was no correlation between photosynthetic capacity and lifetime reproductive investment. Presumably, females are gradually capable of replenishing nutrients expended in a given year's fruit production. Our finding that photosynthetic rates of even green (unripe) fruits were less than half of fruit respiration rates demonstrates that photosynthesis by fruits cannot cover construction and overall respiration of the exocarp alone, let alone the entire fruit with its large nonphotosynthetic seed. Thus, photosynthetic activity of reproductive structures in *O. tenera* is insufficient to pay the high costs of reproduction borne by females.

We were able to avoid a number of potentially confounding effects in this study. By comparing plants grown from seeds collected at the same time from known trees, we could control for age and genotype. Moreover, sex-specific differences in growth rates and photosynthetic capacities could not be attributed to habitat differences for two reasons. First, previous studies (13) have shown that there was no spatial segregation of the sexes in the natural population of *O. tenera*. Second, measurements of photosynthesis were conducted on plants grown in a common garden in a natural habitat.

Very few studies of the cost of reproduction in trees have attempted to control for possible maternal effects. For example, males could grow more quickly than females because of greater initial investment in seeds that produced male plants. In the case of *O. tenera*, maternal effects are unlikely because we found no difference in the size of seeds that produced male versus female plants.

Our results suggest that female *O. tenera* trees trade off fruit production against photosynthetic capacity over the short-term, with negative consequences for growth over the long-term. Nitrogen allocated to fruits and seeds is unavailable for investing in Rubisco, the most abundant enzyme in leaves (25), and other photosynthetic proteins (24). The costs of reproduction are exacerbated because females not only have less fixed carbon to allocate to growth and maintenance but also a lowered potential for future photosynthetic carbon gain. Limitations on the availability of critical nutrients combined with the extra expense of producing large nutrient-rich fruits may explain why females were unable to up-regulate photosynthesis, in contrast with several temperate-zone tree species (4, 20). This constraint may be particularly acute in bird-dispersed plants that produce large nutrient-rich fruits and in tropical trees growing on nutrient-poor soils.

Sexual dimorphism in *O. tenera* reflects a constraint rather than an adaptation: Male trees are larger at maturity than females not because of sexual selection for larger size in males but rather because males do not have to invest as much in reproduction as females and consequently have more resources available for photosynthesis and growth. Given that fruit crop size and the number of seeds dispersed are positively correlated with tree size, diminished photosynthetic capacity and long-term growth may reduce lifetime reproductive success in female *O. tenera*.

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