SEED MASS AND SEEDLING PERFORMANCE WITHIN EIGHT SPECIES OF *PSYCHOTRIA* (RUBIACEAE)

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Abstract. Large seeds have been hypothesized to confer survival advantages in the shaded forest, and small seeds to confer growth advantages in open habitats. We explored these hypotheses using experimental studies in both field and controlled conditions. Our experiments examined intraspecific effects of seed mass on seedling demography in eight sympatric woody species of *Psychotria*. We compared the effects of seed mass on seedling size (biomass), maximum relative growth rate (RGR), the proportion of emerged seedlings that survived until one year of age, and the proportion of sown seeds reaching the stage of one-year-old seedlings in gaps compared with shaded rain forest habitats, as well as in contrasting greenhouse light conditions. For each species, seeds were classified into four seed mass categories and introduced to three pairs of shaded forest gap sites. In the greenhouse, emerged seedlings from different seed mass categories were grown in contrasting light environments. A positive effect of seed mass on seedling survival was observed in six of seven species in the shaded forest but only in two species in gaps. Final seedling biomass was positively related to seed mass in five species in the shaded forest, but only in two species in gaps. In some species, RGR decreased with seed mass. Consequently, the biomass achieved by small-seeded seedlings with relatively fast growth rates was similar to that of seedlings derived from large seeds. The probability of recruitment increased with seed mass in four of seven species in the shaded forest, but only in two species in gaps. Negative effects of seed mass on recruitment occurred only in one species, and only in gaps. The positive effects of seed mass on seedling biomass and survival observed under shaded greenhouse conditions were weaker than those observed in the shaded forest. Overall, our results indicate that: (1) seed mass influences recruitment in natural forest habitats, (2) larger seeds confer higher probability of recruitment in the shaded forest by producing seedlings with larger size and higher probability of survival, and (3) in natural habitats ecological factors in addition to light (e.g., seed predators) operated selectively on seedlings emerging from seeds differing in mass.

Key words: life histories; Los Tuxtlas, Mexico; Psychotria; Rubiaceae; seedling demography; seed mass; seed size variation; shade tolerance; tropical rain forest.

INTRODUCTION

Seed mass may influence Darwinian fitness of plants. Seed mass may affect the probability of dispersal, as well as survival and growth during early life cycle stages (Foster 1986, Wheelwright 1988, Westoby et al. 1992). Several studies have shown that low resource availability (e.g., Salisbury 1974, Augspurger 1984*a,* Leishman and Westoby 1994*a, b*), natural enemies (Augspurger 1984*b,* Armstrong and Westoby 1993, Dalling and Harms 1999), physical damage (Clark and Clark 1991), and leaf litter (Vázquez-Yanes et al. 1990) operate differentially on seedlings that emerge from seeds of different mass.

Large seeds have been proposed to enhance the survival of seedlings in light-limited environments (Foster 1986). Maternal resources can be transformed into larg-

er seedlings or can be used to maintain a positive carbon balance. In contrast to survival, RGR frequently is negatively correlated with seed mass (e.g., Shipley and Peters 1990, Marañón and Grubb 1993, Kitajima 1994, 1996), suggesting the existence of an intrinsic relation between RGR and seed mass. One prediction emerging from this relation is that in the shade, both large- and small-seeded seedlings do increase little in biomass, but in open habitats, large-seeded seedlings can be overgrown by small-seeded seedlings. Experimental studies have examined effects of seed mass on survival and RGR under controlled conditions (e.g., Gross 1984, Saverimuttu and Westoby 1996, Walters and Reich 2000). However, the ecological consequences of variation in seed mass for seedling performance have seldom been explored in natural settings, where a diverse suite of environmental conditions affects seeds and seedlings.

Seed mass may represent an adaptation to shaded environments. This hypothesis has been claimed on the

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basis of correlative analysis between the mean seed mass of species and the environment where seedlings successfully become established (Foster and Janson 1985, Hammond and Brown 1995, Metcalf and Grubb 1995). Comparative studies that have incorporated phylogenetic affinities of species offer contradictory evidence, however. Kelly (1995) and Grubb and Metcalfe (1996) suggested that seed size/habitat correlations found among tropical trees do not reflect recent adaptive processes but result from inheritance of seed mass from ancestral to recent lineages (for example, from families to genera). In contrast, Hewitt (1998) reported evidence that within genera of temperate trees large seeds can be an adaptation to shaded forest habitats.

We explore the ecological effects of seed mass on seedling recruitment using intraspecific variation in seed mass found within congeneric species. Our approach was taken to control for other attributes varying among species and to assess if existent variation in seed mass determines differential recruitment (early survival and growth) of seedlings in contrasting forest habitats (gaps and shaded closed canopied sites). We studied eight woody sympatric species of *Psychotria* in the tropical forest at Los Tuxtlas, Mexico. To isolate the effects of light environment on seed/seedling performance, we performed greenhouse experiments. Finally, using survival, growth, and recruitment probability (the transition probability from sown seed to 1-yr-old seedling) as measures of offspring fitness, we explore the variation in selective regimes between habitats. We address the following questions that aim to detect general functions attributable to seed mass, as well as to describe changes in selective regimes operating on seed mass variation: (1) Does variation in seed mass affect survival, growth, and recruitment of seedlings during the first year under natural conditions? (2) Do larger seeds offer greater advantages in survival, but not in growth under low light conditions? (3) Under high light conditions, do smaller seeds offer greater advantages in growth but not in survival, as predicted by a presumed intrinsic negative relationship between RGR and seed mass? (4) Are habitat-specific selective regimes, acting on traits related to seed mass, similar across species?

Methods

Study site and species

The study was conducted at the Los Tuxtlas Tropical Biological Field station in southeast Mexico, from December 1992 to October 1994. Eight woody species of *Psychotria* (Rubiaceae) were selected: *P. limonensis* K. Krause, *P. graciliflora* Benth, *P. chagrensis* Standley, *P. papantlensis* (Oersted) Hemsley, *P. flava* Oersted former Standley, *P. faxlucens* Lorence and Dwyer, *P. simiarum* Standley, and *P. veracruzensis* Lorence and Dwyer. These species coexist in the forest understory and some of them clearly use habitats differing in light

availability (Paz et al. 1999). Among species, mean mature seed mass ranges from 0.007 g (*P. limonensis*) to 0.38 g (*P. faxlucens*), and within-species seed mass varies by a factor of two to four. Birds disperse the fleshy drupes from November to January.

Field experiment

Experimental plots.—Three 1-yr-old treefall gaps with projected open canopy area (sensu Brokaw 1982) of ~290–320 m² were selected. Close to each gap (<80 m away), we selected a shaded forest plot of 320 m² where typical dominant species of mature forest were present. Thus, the experiment included three pairs of gap-shade forest plots (hereafter called sites). All plots were located on the same hillside, with a common orientation and with a terrain slope of <10° (more details in Paz et al. 1999). The field experiment included seven species (the previously mentioned group, except *P. veracruzensis*).

Experimental seeds and design.—The natural variability in seed mass present within each species was evaluated using a minimum sample size of 600 seeds. Paz et al. (1999) provide details of methods used to obtain and process the experimental seeds. Seed mass variation was then evenly partitioned into four quartile-based seed mass categories. Twenty-eight treatments, formed by combinations of seven species and four seed mass categories, were applied to each experimental plot (gap, shaded forest). To diminish effects of microenvironmental heterogeneity inside each plot, all treatments were applied to 35 quadrats (rectangles of 1.8 × 1.0 m), spatially arranged in a grid. Within each quadrat, two seeds representing each treatment were placed on the soil surface in random positions generated by a grid of 20 × 20 cm, resulting in a total of 70 seeds per plot for each treatment and 56 seeds per quadrat. To reduce the effects of soil heterogeneity and to avoid contamination with nonexperimental *Psychotria* seeds, the original soil column (5 × 5 × 5 cm) where each experimental seed was laid was replaced with soil collected from a single forest site distant from any mature *Psychotria* adult. There was a total of 1680 seeds per species, resulting in 11 760 seeds sown in the entire experiment. To estimate the probability of survival from emerged seedling to one year of age, we used all the seedlings of a given species that emerged during the entire experiment. To estimate the probability of transition from sown seed to 1-yr-old seedling, we used only half of the experimental seeds. The other half of the experimental seeds were protected from animals until they emerged as seedlings, as a procedure to address questions treated in another study (see Paz et al. 1999).

Monitoring program for seedlings.—We followed the fate of each emerged seedling for a one-year period, beginning when the epicotyl was visible above ground. There were between 72 and 167 emerged seedlings per species, seed mass category, and habitat type, account-

ing for a total of 7357 experimental seedlings. For each species, seedlings that emerged from the different seed mass categories were considered as cohorts. After one year, each of the surviving seedlings was harvested entire and the total individual dry mass was recorded after seedling material was dried for 72 h in a 70°C oven.

Greenhouse experiments

Experiment 1.—This experiment included five species, *P. limonensis*, *P. papantlensis*, *P. flava*, *P. faxlucens* (all used in the field experiment), and the small-seeded *P. veracruzensis*. Seeds from the smallest and the largest seed mass categories used in the field experiment were germinated in shade (similar to the closed forest), using sterilized and water-saturated forest soil. Individual seedlings 5–7 d old, with expanded cotyledons, were transplanted to plastic black pots each containing 3.5 L of sterile soil collected from a single forest site. After one week, these seedlings were placed in one of three light environments: "high," "intermediate," and "low." The high and intermediate light environments were established in a greenhouse outside the forest, by covering eight 1-m^3 metallic frames with green nylon mesh (9-mm^2 apertures). For the high light environment we covered four frames with a single mesh layer, and for the intermediate light environment we covered four frames with three mesh layers. Four pairs of high and intermediate light frames were established as blocks along the axes of main light variation in the greenhouse. Due to initial logistic problems establishing the low light environment in the same greenhouse, we built another greenhouse inside the forest by covering a 3-m^3 wood frame with a layer of green nylon mesh (9-mm^2 apertures). Inside the frame, the understory vegetation was removed and experimental pots were arranged in four randomized blocks.

To characterize the environment of the greenhouse treatments, we obtained light and temperature measurements. At the start of the experiment, photosynthetic photon flux density [PPFD], red to red far light ratio (R/RF), and minimum and maximum temperatures (T_{min}, T_{max}) were measured during three cloudless days (details of the protocol are given in Paz et al. 1999). The mean (± 1 SE) daily values per light environment were: (1) low light, PPFD = 4.1 ± 0.6 mol·m^{-2}·d^{-1}, $T_{max} = 26.1 \pm 0.4$°C, R/RF = 0.36 ± 0.04, (2) intermediate light, PPFD = 41.5 ± 7.5 mol·m^{-2}·d^{-1}, $T_{max} = 30.6 \pm 1.4$°C, R/RF = 1.10 ± 0.17, and (3) high light, PPFD = 117.3 ± 7.5 mol·m^{-2}·d^{-1}, $T_{max} = 33.4 \pm 1.0$°C, R/RF = 1.20 ± 0.10.

Five to ten seedlings of each species and seed mass category were randomly assigned to each frame within blocks. This yielded a total of 20–40 seedlings per species, seed mass category, and light environment. Seedlings that died during the first week were replaced. Within each frame, pots were rotated continuously and watered to soil saturation every three days. After one year, surviving seedlings were harvested entirely, dried in an oven for 72 h at 70°C, and weighed with a precision of 0.0001 g.

Experiment 2.—The physical separation of the low light environment from the others in the greenhouse could introduce confounding factors into the experiment. To explore this possibility, one year later we carried out a second experiment establishing light environments in the same greenhouse outside of the forest. In this experiment, three species (*P. limonensis*, *P. papantlensis*, and *P. flava*), the same seed mass categories used in experiment 1, and two light environments were employed. Light environments were established again by covering frames with shade mesh, and seedlings and physical variables were obtained following the same procedures used in experiment 1. The low light environment was characterized by PPFD (mean ± 1 SE) = 13.1 ± 2.33 mol·m^{-2}·d^{-1}, $T_{max} = 28.1 \pm 0.5$°C, and R/RF = 0.6 ± 0.05, and the high light environment by PPFD = 105.7 ± 9.33 mol·m^{-2}·d^{-1}, $T_{max} = 32.7 \pm 1.2$°C, and R/RF = 1.23 ± 0.2. Three pairs of low and high light frames were spatially arranged as random blocks. In this experiment, 10 seedlings per species, seed mass category, and light environment were monitored and survivors were harvested entirely one year after. To determine the effects of seed mass on the initial seedling biomass, we harvested 12 5-d-old seedlings with fully expanded cotyledons representing each species and each seed mass category. These seedlings were obtained following the procedure described in experiment 1.

Statistical analysis

Survival and recruitment.—For each species, seedling survival per seed mass category per plot (hereafter referred as "survival") was estimated as the proportion of emerged seedlings that survived for one year. Similarly, the probability of recruitment per seed mass category per plot (hereafter referred as "recruitment") was estimated as the proportion of the initial sowed seeds reaching the stage of 1-yr-old seedlings, which is equivalent to the product of the proportion of emerged seedlings (from Paz et al. 1999) times the probability of seedling survival for one year. We used a logistic ANCOVA model to test for effects of seed mass, and effects of interactions between seed mass and habitat, on survival and recruitment of each species in the field. In this model, we assumed binomial errors for survival and recruitment (McCullagh and Nelder 1989). In such models, the mean seed mass per category was used as a continuous variable, habitat was a categorical variable, and sites were blocks. Following Aitkin et al. (1989), the significance of each term was evaluated using the log-likelihood ratio chi-square, calculated as twice the difference in log-likelihood between the full model and the model without the term to be tested. As a measure of the goodness of fit of the models to the data, we used the log-likelihood ratio for

the residual deviance of the full model. A significant χ^2 value suggests a poor fit (Aitkin et al. 1989). For each species, to explore the strength and direction of seed mass effects on survival and recruitment within each habitat, we performed single linear regression analysis for each habitat. Because in some species we knew that predation and litter cover have opposing effects on the survival of seeds of different mass (Paz et al. 1999), in those cases where data suggested a quadratic trend between variables, we also tested a quadratic model.

Paz et al. (1999) found effects of seed mass on the time of emergence in two species, *P. papantlensis* and *P. flava*. We ran ANCOVA and regression analyses using the time of emergence as a covariate for all measurements of seedling performance of these species. We did not find any case where the inclusion of the covariate resulted in a change of the significance level of any term related to seed mass. For this reason, we ruled out the possibility of indirect effects of seed mass due to the time of emergence, and thus reported statistics from models without the covariate. In the greenhouse experiments, survival per seed mass category per light level was estimated as the proportion of final survivors, pooling all seedlings for each treatment. For each species, the effects of seed mass category and light level on seedling survival were tested using a two-factor log-linear model (Crawley 1993). All logistic models (ANCOVAs, regressions) and log-linear analyses were performed using the GLIM 4.0 statistical package (Francis et al. 1993).

Growth.—We used the individual total-final dry biomass (g), and the individual maximum-annual relative growth rate (in grams per gram per year) to quantify seedling growth for each species. Following Hunt (1978) and using a one-year interval, the RGR_{max} was calculated as the logarithm of the ratio (final seedling dry biomass) : (seed dry mass without seed coat). Mean values of dry seed mass (excluding the seed coat) per category were calculated from a random sample of 100 seeds per seed mass class.

We used an ANCOVA model for each species and variable to test the main and interactive effects of seed mass and habitat type on growth in the field. Mean seed mass per category was used as the continuous regressor variable, habitat was a categorical factor, and site was used as a blocking factor. Because seed mass was a fixed factor split inside the experimental units for habitat (gap, shaded forest plots), a split-plot model, with appropriate errors, was used to test each model term. Following Milliken and Johnson (1984), the error for habitat type was the mean squares due to the site × habitat type interaction, and the error for the seed mass and seed mass × habitat was the residual error of the model. To avoid pseudo-replication problems we used the mean values of growth per seed mass category per site as response variables.

We performed single regression analyses for each species in each habitat to explore the strength and direction of seed mass effects on growth variables within each habitat. For three species, *P. chagrensis*, *P. papantlensis*, and *P. simiarum*, variances of biomass and RGR remained heterogeneous between habitats after several variable transformations. In these cases, we did not conduct ANCOVAs, we performed only single linear regressions for each habitat. In none of these cases did we find significant regressions in both habitats; thus, it was not necessary to test for slope differences between habitats.

The effects of seed mass and light environment on growth variables were similar between the two greenhouse experiments (1 and 2). This allowed us to conclude that effects due to location on seedling performance in experiment 1 were less important than light environment effects. In addition, we found that the structure of paired blocks of high-intermediate light environments accounted for <5% of the total variance of any growth variable. Therefore, the main and interaction effects of seed mass and light on seedling growth were explored assuming a full design with light environment applied to blocks randomly distributed in space.

The effects of seed mass and light environment on growth variables were tested using a two-factor split-plot ANOVA for each species. In these models, light environment was the plot factor and seed mass category was a fixed factor split within light treatment. Factors were tested using proper error terms following Milliken and Johnson (1984). To avoid nonindependence between seedlings growing in the same frame, we used the mean value per frame per species per seed mass category as a single data point. All models using normally distributed errors were carried out using the GLM procedure in SAS version 6.12 (1999).

Results

Survival.—Seedling survival in the field was significantly affected by seed mass ($P < 0.05$) in six of the seven species studied, in at least one habitat (Fig. 1; summary of ANCOVAs in Appendix A). In all six cases, survival was positively related to seed mass (Fig. 1). For four species, the effects of seed mass differed between habitats ($P < 0.05$), being positive in the shaded forest (Fig. 1). There were no observed seed mass effects for *P. simiarum* either in gaps or in shaded forest (Fig. 1). Seed mass effects were similar in those cases where these effects occurred in both habitats, as indicated by slopes and R^2 values (Fig. 1).

Light environment in the greenhouse affected seedling survival. In both greenhouse experiments, for all species except *P. flava*, mean survival was significantly lower in the low light than in the intermediate and high light environments ($\chi^2 > 19.7$, $P < 0.0001$, for each species; Fig. 2). In addition, no seed mass effects on survival were observed in intermediate and high light
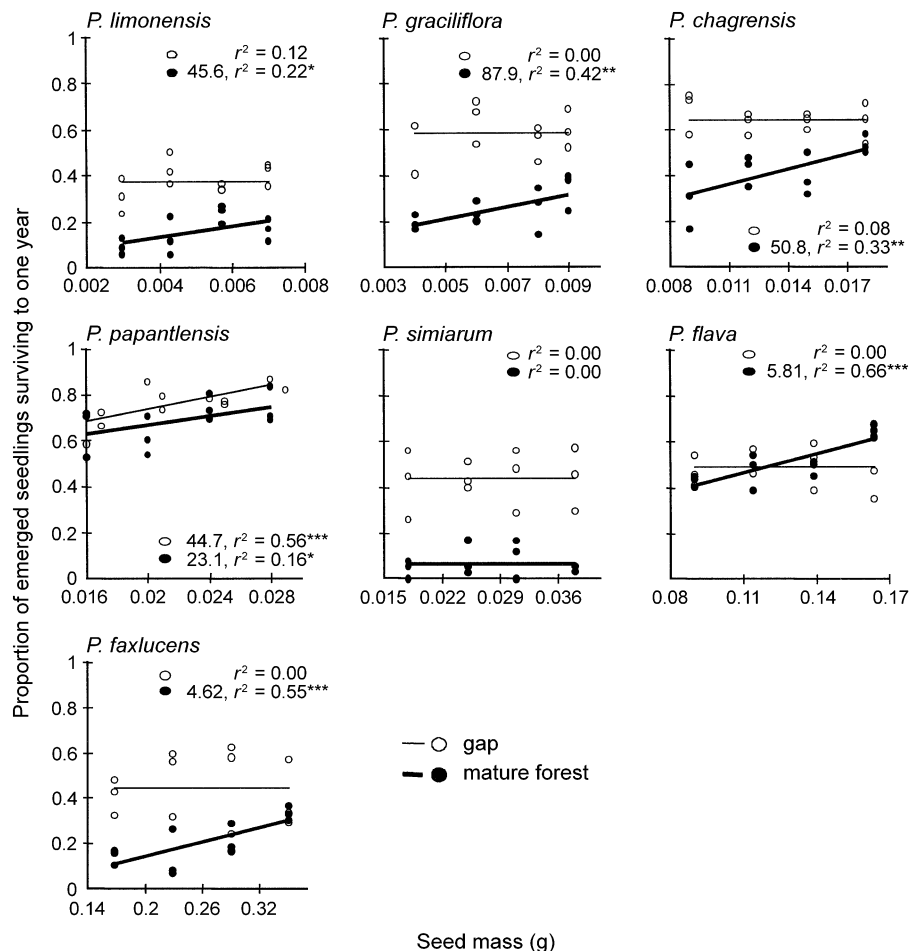
FIG. 1. Seedling survival and seed mass in seven species of *Psychotria* under experimental field conditions at Los Tuxtlas, Mexico. Species appear in order of increasing mean seed mass from top left to bottom right. Each point represents the mean survival probability among all seedlings emerged from a given seed mass class monitored in a particular gap or shaded forest site. The slopes (in logit scale) and r^2 values (deviance accounted by seed mass/deviance accounted by a constant model) of logistic regressions are given for each habitat. Significant regressions are indicated as: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. In nonsignificant regressions, horizontal lines indicate the mean value of seedling survival across the entire seed mass range, and no slope value is reported. In cases where ANCOVA models indicated similar slopes between habitats (see *Methods: Statistical analysis: Survival and recruitment*), both slopes are reported.

environments (Fig. 2). In contrast, in the low light environment of experiment 1, survival was higher in the largest seed mass category for *P. limonensis* and *P. faxlucens* (Fig. 2).

Growth.—Larger seeds produced larger seedlings. In all species, initial seedling biomass was positively related to seed mass. The ratio between the mean dry biomass of seedlings representing category four and category one ranged between 1.3 and 2.0, and differed from a 1.0 ratio at $P < 0.01$ in all species.

In the field, after one year of growth, seedling biomass was still positively related to seed mass in five of seven species in at least one habitat, and was independent of seed mass in two species (Fig. 3). We found significant ($P < 0.05$) seed mass effects in three of four species tested with ANCOVAs, but only in one species, *P. graciliflora*, did such effects differ between

habitats (details in Appendix B). For those species in which we did not run ANCOVA, single regressions indicated positive seed mass effects only in the shaded habitat in *P. chagrensis* and *P. papantlensis*, but no seed mass effects were detected in *P. simiarum* (Fig. 3). Overall, effects of seed mass on seedling biomass were more frequent in the shaded habitat than in gaps, yielding five and two cases, respectively (Fig. 3).

In the field, RGR declined with seed mass in five species in at least one habitat, and was independent of seed mass in two species (Fig. 4). There were significant ($P < 0.05$) seed mass effects on RGR in two of four species tested with ANCOVAs, but only in *P. graciliflora* did such effects differ between habitats (see details in Appendix B). In this species, the effect of seed mass was significant only in gaps (Fig. 4). For those three species in which ANCOVA was not applied,
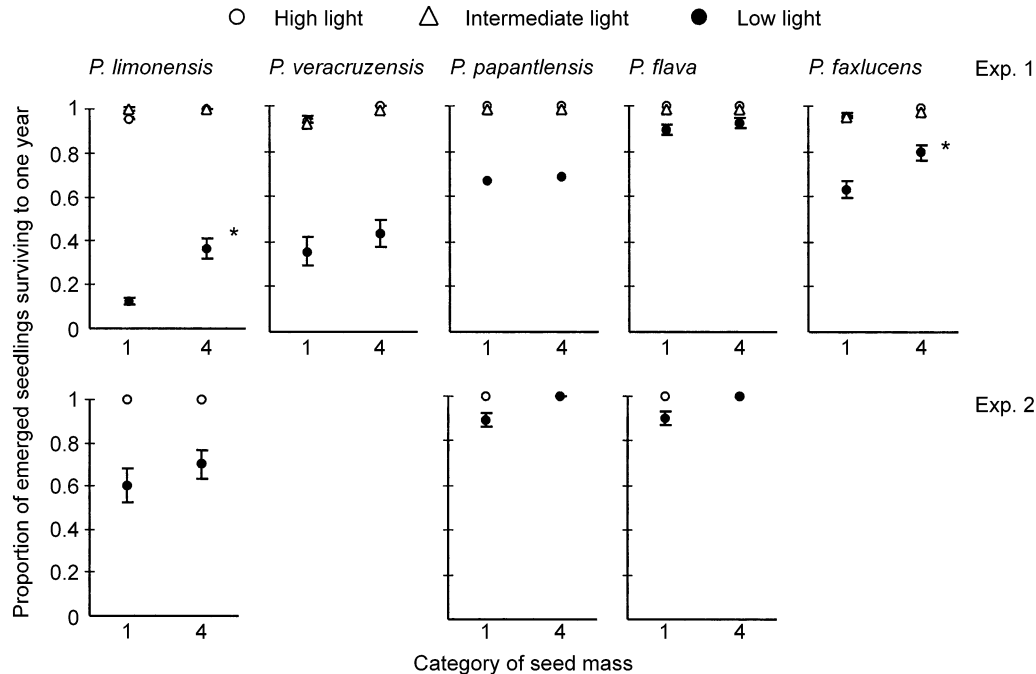
FIG. 2.   Effect of seed mass and light environment on seedling survival after one year, in five *Psychotria* species from two experiments in greenhouse conditions at Los Tuxtlas, Mexico. Means ± 1 SE are shown (vertical bars). Small (indicated as 1) and large (indicated as 4) seed mass classes are, respectively: 0.003 g and 0.007 g for *P. limonensis*, 0.010 g and 0.021 g for *P. veracruzensis*, 0.017 g and 0.029 g for *P. papantlensis*, 0.090 g and 0.164 g for *P. flava*, and 0.168 g and 0.351 g for *P. faxlucens*. An asterisk indicates significant differences between seed mass categories within each light environment ($P < 0.05$, *G* test).

single regressions detected significant negative seed mass effects in *P. chagrensis* and *P. papantlensis*, only in the shaded habitat, and in *P. simiarum* only in gaps (Fig. 4). Overall, in both habitats we found the same frequency of species (three) with significant seed mass effects on RGR. When such effects occurred for the same species in both habitats, RGR decreased with seed mass at similar rates (Fig. 4).

In the greenhouse, final seedling biomass was positively related to, or independent of, seed mass (Fig. 5). The strength of this relationship varied with species and with the light environment. ANOVAs showed significant ($P < 0.02$) seed mass effects in three of five species in experiment 1, and in two of three species in experiment 2 in at least one light environment (Appendix C). For most of these cases, seed mass effects were restricted to the low light environment (see seed mass × light environment interactions in Appendix C, and contrasts in Fig. 5). The only exception was *P. limonensis* in experiment 1, for which ANOVA did not detect a significant interaction between seed mass and light.

In the greenhouse, RGR and seed mass tended to show a negative relationship in all species (Fig. 5). In experiment 1 ANOVAs detected significant ($P < 0.01$) seed mass effects on RGR in all species tested (Appendix C). A significant seed mass × light environment interaction was found only in *P. papantlensis* and *P.*

faxlucens in experiment 1, and *P. papantlensis* and in *P. flava* in experiment 2 (Appendix C). Contrasts within each light condition indicated significant seed mass effects on RGR in eight cases in high and intermediate light levels and in four cases in low light (Fig. 5). Together these results indicate that RGR was more strongly associated with seed mass in high light than in low light conditions.

Recruitment.—Seed mass affected recruitment in six of seven species in at least one habitat (Fig. 6). Seed mass effects varied in direction and strength both among species and habitats. In four species, *P. limonensis*, *P. chagrensis*, *P. papantlensis*, and *P. faxlucens*, recruitment tended to increase with seed mass, principally in the shaded forest (Fig. 6). Among these species, ANCOVAs detected a significant ($P < 0.05$) interaction with habitat in *P. flava* and *P. faxlucens* (Appendix D). In these cases, regressions were significant only in the shaded forest (Fig. 6). In *P. limonensis* and *P. papantlensis*, there was no significant seed mass × habitat interaction (Appendix D). However, the individual regressions showed significant positive seed mass effects in the shaded forest and only marginal positive effects in gaps (Fig. 6). In *P. flava*, the effects of seed mass on recruitment differed ($P < 0.05$) between habitats; recruitment was negatively related with seed mass in gaps but independent of seed mass in the shaded forest (Fig. 6). In *P. simiarum*, we observed a
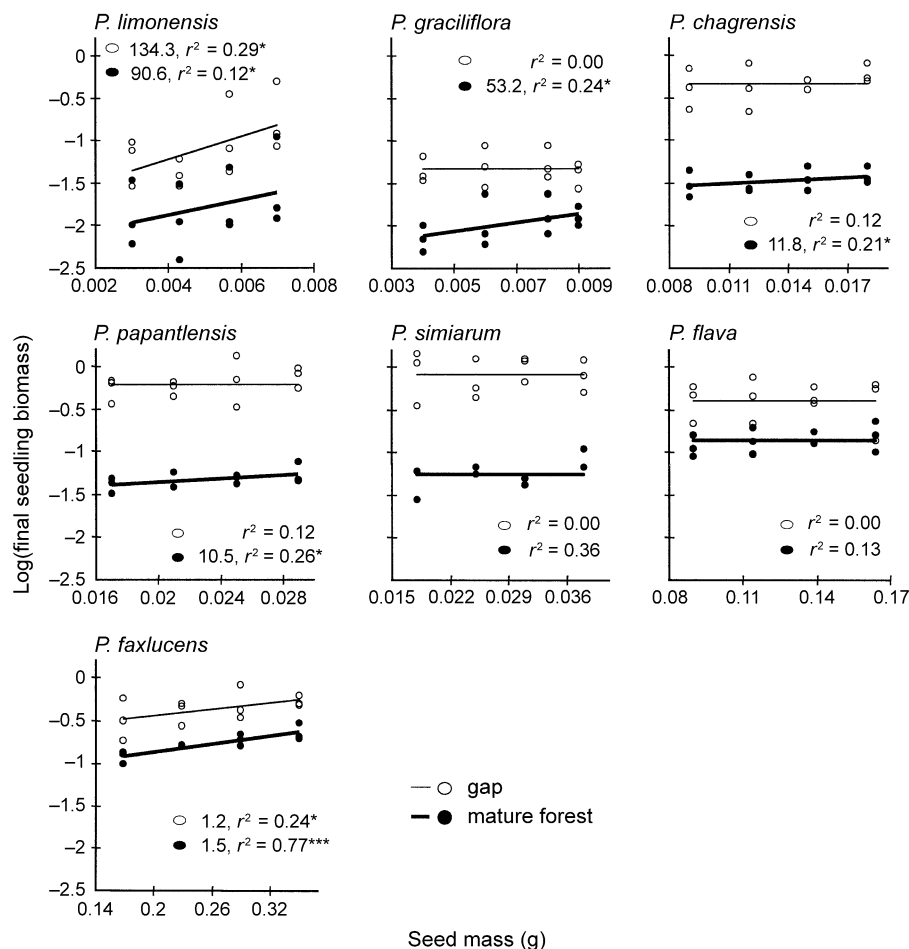
FIG. 3. Effect of seed mass on seedling biomass (measured in grams) after one year of growth for seven species of *Psychotria* under experimental field conditions at Los Tuxtlas, Mexico. Each point represents the mean biomass among all seedlings emerged from a given seed mass class monitored in a particular gap or shaded forest site. The slopes and r^2 values of regressions are given for each habitat; slopes significantly different from zero are indicated as: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Horizontal lines indicate the mean value of the seedling biomass across the entire seed mass range. Other specifications are as indicated in Fig. 1.

quadratic trend, where maximum recruitment occurred for seeds of intermediate masses in both habitats ($P < 0.02$, Fig. 6). Overall, we detected positive significant seed mass effects on recruitment in four species in the shaded forest, but only marginal effects in two species in gaps. Again, when seed mass effects occurred for a species in both habitats, they were expressed with similar intensities, as indicated by regression slope or R^2 values (Fig. 6).

DISCUSSION

Effects of seed mass on seedling performance in light-contrasting habitats

Survival.—Our study supports the hypothesis that within species larger seeds generally confer survival advantages to seedlings in light-limited environments, but less often in habitats that are not light limited. These results are consistent with previous studies that explored the consequences of intraspecific seed mass variation for early seedling survival (Gross 1984, Howe et al. 1985). Seed mass effects on seedling survival in the shade were more prevalent and stronger under natural than under greenhouse environments. These results suggest that other ecological factors, such as physical damage, pathogens, herbivores, and competitors, interact with low light to reduce the survival of seedlings derived from small seeds (see also Foster 1986). The occurrence of positive effects of seed mass in gaps, detected in one species, but not at all in the greenhouse high light environment, could result from a purported progressive reduction in light availability as the gaps close.

Survival of large-seeded seedlings under the shade in the natural habitats may have been influenced by long-term maintenance of initial advantages in seedling size. Large initial seed sizes may provide seedlings
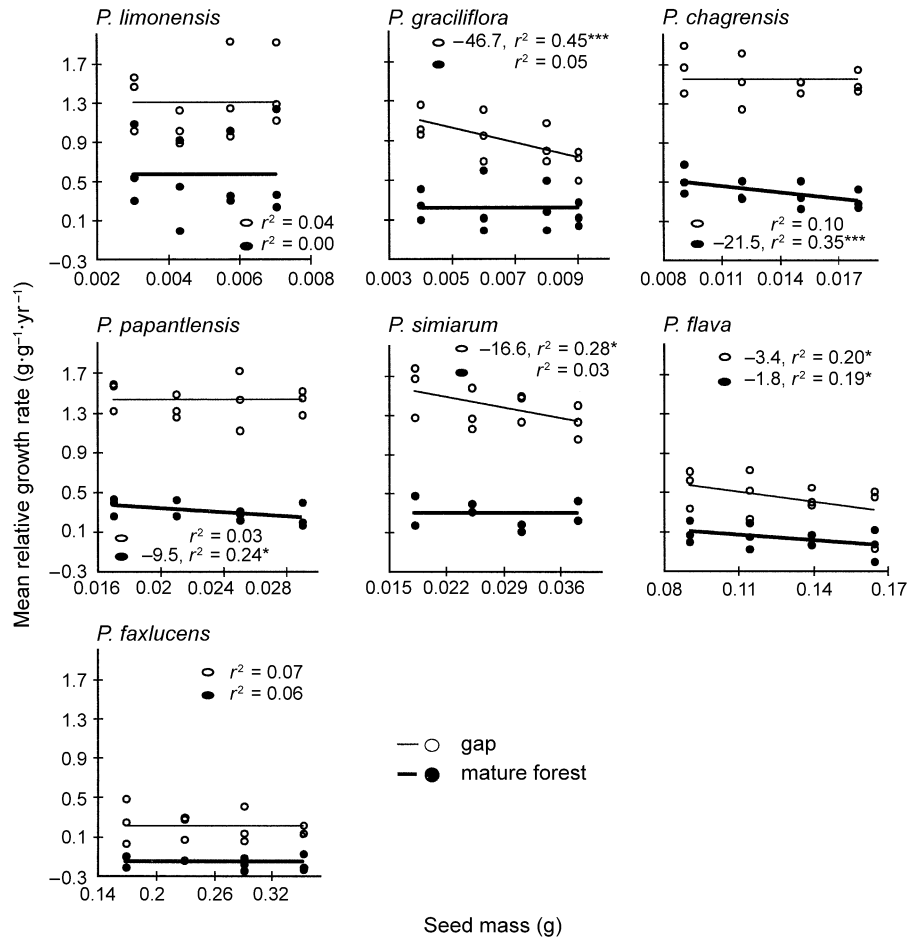
FIG. 4.   Effect of seed mass on seedling RGR, for seven species of *Psychotria* under experimental field conditions at Los Tuxtlas, Mexico. Each point represents the mean RGR among all seedlings derived from a given seed mass class monitored in a particular gap or shaded forest site. The slopes and r^2 values of regressions are given for each habitat; significant values are indicated as: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Horizontal lines indicate the mean relative growth rate across the entire seed mass range. Other specifications are as indicated in Fig. 1.

with a greater vigor and thus tolerance for physical and biotic mortality risks (Sarukhán 1984). In *Psychotria* the biomass of recently emerged seedlings was positively correlated with seed mass, and such ranking persisted after a year of growth in most species in the shaded forest, but not in gaps.

The reserves within *Psychotria* seeds do not necessarily provide direct metabolic support that enhances seedling survivorship in the closed forest throughout their first year. Naturally, a larger amount of seed maternal resources can enhance the ability of seedlings to support metabolic carbon demands in light conditions close to the compensation point (Thompson 1987), or to recover from events of carbon loss, such as damage by herbivores or falling debris (Foster 1986, Harms and Dalling 1997, Dalling and Harms 1999). In *Psychotria*, however, the presence of nonreserve photosynthetic cotyledons, and the rapid development of a pair of true leaves within two months after emergence, suggest a short period of direct metabolic dependency

on maternal reserves (see Kitajima [1996] for discussions on this point).

In addition to maternal reserves, seedling survival in deeply shaded habitats can be enhanced by increased resource allocation to defense, support, and storage functions. Such allocation might occur at the cost of a reduced allocation to potential growth rate (Coley 1988, Chapin et al. 1990, Kitajima 1996, Kobe 1997, Walters and Reich 2000). Large seeds might be associated with a low carbon economy. In most of the species studied, large-seeded seedlings had higher survival than small-seeded seedlings in the shaded forest, but lower potential RGR in high light greenhouse conditions. In addition, the production of new leaves by *Psychotria* seedlings that suffered natural 100% leaf loss occurred frequently among individuals derived from large, but not small seeds (H. Paz, *unpublished data*). In large-seeded seedlings, photosynthates may be disproportionately allocated to storage structures other than cotyledons and leaves.
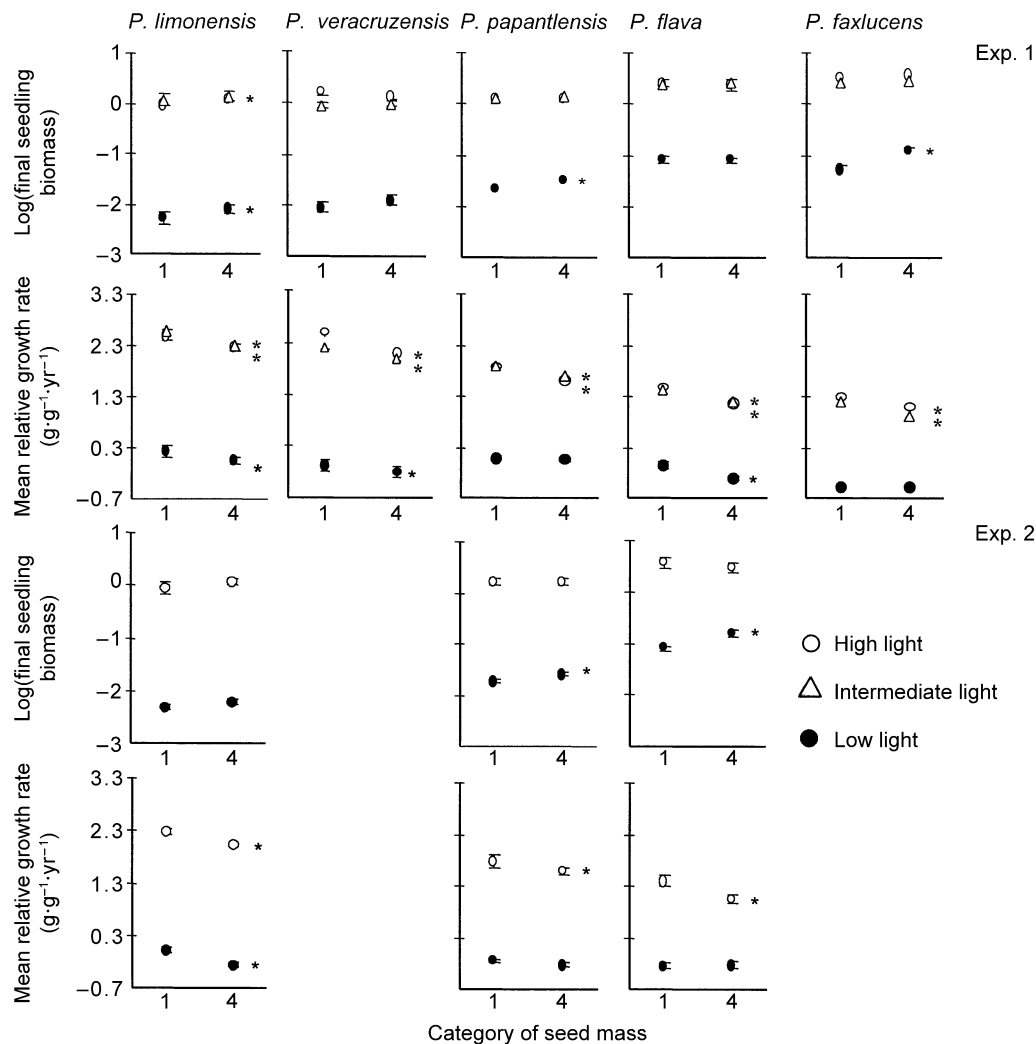
FIG. 5. Effect of seed mass and light on seedling growth variables in five *Psychotria* species from two greenhouse experiments conducted in Los Tuxtlas, Mexico. Means ± 1 SE are shown (vertical bars). Final seedling biomass was measured in grams. Small and large seed mass categories for each species are indicated by 1 and 4, respectively. Values of dry mass per seed category are the same as indicated in Fig. 2. The asterisk indicates significant differences in growth between seed mass categories within each light environment ($P < 0.05$, Tukey's hsd test).

Growth.—Our data support the concept of a negative relationship between seed mass and RGR (e.g., Jurado and Westoby 1992, Marañón and Grubb 1993, Swanborough and Westoby 1996, Reich et al. 1998). Such relationships can be partly explained by seedlings emerging from large seeds having relatively low light capture area per unit mass of photosynthetic tissue (Marañón and Grubb 1993, Kitajima 1992, 1996, Walters and Reich 2000). Thicker cotyledons and leaves have been suggested to enhance shade tolerance by increasing physical defenses against herbivores and by reducing the metabolic costs of maintaining a large leaf area ratio (Kitajima 1996).

The high RGR values of small-seeded seedlings tended to compensate for the initial size advantages conferred by large seed mass. This effect decreased in mag-

nitude from high to low light availability and from controlled to natural conditions. Under greenhouse high light conditions, higher RGR values in small-seeded seedlings fully compensated for the initial seedling size hierarchy in all five species tested (seed mass was correlated with RGR but not with final seedling biomass). In contrast, in the shaded forest we found full compensation only in one species out of seven studied. Slow biomass gain rates operating in the shade may buffer the expression of potential interseedling RGR variation attributable to seed mass differences. Evidence for such a buffering effect has been found previously in temperate herbs (Gross 1984) and trees (Walters and Reich 2000). The lack of compensation for the seedling size hierarchy in the shaded forest could also result from higher mortality rates of intrinsically fast-growing seedlings.
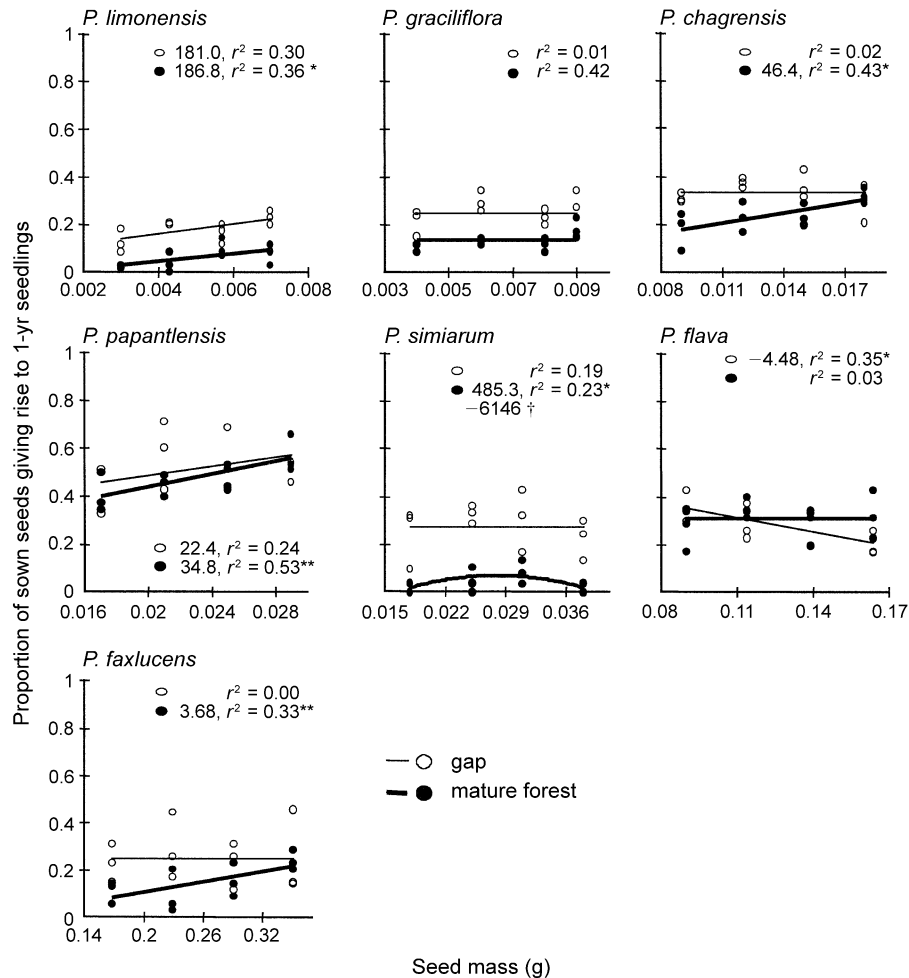
FIG. 6. Effect of seed mass on recruitment probability, for seven species of *Psychotria* under experimental field conditions at Los Tuxtlas, Mexico. Each point represents the mean probability of recruitment among all sown seeds in a given seed mass class, in a particular gap or shaded forest site. The slopes (in logit scale) and r^2 values (deviance accounted by seed mass/deviance accounted by a constant model) are given for each habitat. Significant regressions are indicated as: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Horizontal lines indicate the mean recruitment across the entire seed mass range. For *P. simiarum*, the dagger (†) indicates a quadratic coefficient (see *Methods: Statistical analysis: Survival and recruitment*). Other specifications are as indicated in Fig. 1.

Selection regimes on seed mass between light habitats

Our results support the view that seed mass influences recruitment (our best estimate of offspring fitness) in natural populations. We observed seed mass effects on the probability of recruitment in six of seven species studied. Analysis of the variation of the frequency, direction, and strength of seed mass effects among species and habitats suggest three trends concerning selective regimes on seed mass. First, larger seeds tended to have higher recruitment, although this trend was not universal and was even reversed in two cases. Second, the importance of seed mass as a determinant of recruitment was clearly higher in the light limited habitat, as indicated by a larger number of species with significant seed mass effects. Nonetheless, in

species where seed mass effects occurred in both gap and shade habitats, the intensity of selection was similar in both habitats. Third, negative effects of seed mass on recruitment were restricted to just one species in gaps. This negative effect of seed mass on recruitment resulted not from limits to emergence or survival under conditions of high solar radiation, as discussed by Foster (1986), but from a greater risk of removal of larger seeds in gaps (Paz et al. 1999). Selection favoring larger seeds was more pervasive, and more habitat-specific, during seedling growth/survival than during emergence. These observations suggest that a full understanding of the consequences of seed mass on recruitment needs to take into account all demographic sieves through which a seed passes until it becomes an established seedling.

An analysis of the selection pressures operating on seed mass variation needs to take into account other aspects not treated in the present study. For example, the largest seeds may maximize the probability of recruitment of offspring but not necessarily parental fitness, because investment in a large number of small seeds can result in a larger number of successful offspring (Smith and Fretwell 1974). Also, in *Psychotria*, as in many other rain forest plant species, a higher recruitment probability in gaps than in the shaded forest clearly indicates a high selective premium to reach gaps. Therefore, to estimate the contribution to fitness of offspring dispersed to each habitat type, it is necessary to assess dispersal probabilities as a function of seed mass. If dispersal of seeds is negatively related to seed mass (e.g., Howe 1993) we may expect that selection for dispersion counteracts selection for large seeds during recruitment.

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APPENDIX A

A summary of logistic ANCOVAs evaluating the effects of seed mass and habitat on the probability of seedling survival one year after emergence in seven species of *Psychotria* in field conditions at Los Tuxtlas tropical rain forest, Mexico, is available in ESA's Electronic Data Archive: *Ecological Archives* E084-010-A1.

APPENDIX B

A summary of ANCOVAs evaluating the effects of seed mass and habitat on seedling biomass and relative growth rate one year after emergence in seven species of *Psychotria* in field conditions at Los Tuxtlas tropical rain forest, Mexico, is available in ESA's Electronic Data Archive: *Ecological Archives* E084-010-A2.

APPENDIX C

A summary of ANOVAs evaluating the effects of seed mass and light environment on seedling biomass and relative growth rate one year after emergence in species of *Psychotria* in greenhouse experiments 1 and 2 is available in ESA's Electronic Data Archive: *Ecological Archives* E084-010-A3.

APPENDIX D

A summary of logistic ANCOVAs evaluating the effects of seed mass and habitat on recruitment probability (transition from sown seed to 1-yr-old seedling) in seven species of *Psychotria* under field conditions at Los Tuxtlas tropical rain forest, Mexico, is available in ESA's Electronic Data Archive: *Ecological Archives* E084-010-A1.