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POLLEN DONATION PATTERNS IN A TROPICAL DISTYLOUS SHRUB (*PSYCHOTRIA SUERRENSIS*; RUBIACEAE)[1]

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Selection on male function has been invoked to explain various floral features, including number of flowers, flower size, and flower color. Here I describe two experiments designed to examine the efficiency of distyly in promoting male floral function, as measured by successful pollen dispersal to stigmas. In both experiments, I performed emasculations to control the type of pollen locally available in a natural population of *Psychotria suerrensis,* a tropical shrub. In the "natural-recipients" experiment, I allowed each floral morph to donate pollen on alternate days to emasculated flowers of each morph. In the "paired-recipients" experiment, I attached paired cuttings of each morph to individual donor plants. The results of both methods were consistent. Pollen borne on low anthers (from pin plants) was transferred most efficiently to low stigmas (on thrum plants). Pollen borne on high anthers (from thrum plants) was dispersed in equal amounts to flowers of both morphs. The results suggest that distyly is only partially effective in achieving efficient pollen donation. Male function of pins is enhanced by the polymorphism, but male function of thrums is not. A supplemental pollination experiment illustrates that seed set in this species is predominantly pollen limited, reducing the importance of male function, in comparison with species where seed set is primarily resource limited.

Hermaphroditic flowers fulfill both male and female function. Female function is relatively easy to monitor, and has been the focus of most measures of reproductive success. However, male function of flowers is undoubtedly important. A number of workers have suggested that the low fruit/flower ratio common in flowering plants is due to production of excess flowers to enhance male function (Willson and Rathcke, 1974; Willson, 1979; Bawa and Webb, 1984; Sutherland and Delph, 1984; Sutherland, 1986). Male function has also been thought to shape morphology of individual flowers. In species where seed set is limited by resources and not by pollen, selection for paternal success could lead to morphological changes that are neutral with respect to seed set (Charnov, 1979; Willson, 1979; Queller, 1983; Bell, 1985; but see Wilson et al., 1994). Various floral features have been shown to affect primarily male function, as measured by pollen removal, pollen donation, or donation of fluorescent dyes used as pollen analogs. Among these are corolla color (Schoen and Clegg, 1985; Stanton, Snow, and Handel, 1986), corolla size (Bell, 1985; Young and Stanton, 1990; Campbell et al., 1991), nectar production rates (Mitchell, 1993), and features reducing interference between anthers and stigma (Lloyd and Yates, 1982).

A striking morphological feature that also may affect male function is the balanced polymorphism known as distyly. In distylous species, about half of the plants (pins) have long styles and short stamens, while the remainder (thrums) have short styles and long stamens. Characteristically, the two morphological groups also form mating groups, with each morph able to set seed only when pollinated by the other morph. Distyly has arisen independently at least 23 times in unrelated taxa (Lloyd and Webb, 1992a); therefore the adaptive forces favoring it are presumably important across a spectrum of ecological conditions. Tristyly, a balanced polymorphism with three stylar length morphs, is less common, but appears also to have several independent origins, since it is found in both monocots and dicots (Ganders, 1979). The term "heterostyly" encompasses both conditions.

The adaptiveness of distyly has been investigated almost exclusively in terms of its female function, specifically pollen receipt: do flowers receive more pollen from plants of the other morph than from plants of their own morph? The numerous attempts to address this question have failed to yield a clear result, partly because most did not distinguish between self and outcross pollen loads (reviewed by Ganders, 1979; Barrett, 1990). Intrafloral pollination is not affected by the existence of the polymorphism, but elevates the amount of same-morph pollen found on the stigma. Therefore, when workers neglect to emasculate recipient flowers, they are likely to conclude that the polymorphism is not efficient in promoting pollen transfer, since so much intramorph pollen is found on stigmas. The few studies that have included emasculation of the recipient morph have found intermorph pollen loads in excess of random expectation (Ganders, 1979; Barrett and Glover, 1985; Piper and Charlesworth, 1986).

Distyly could, in fact, be neutral in terms of female function, but be advantageous for male function. That is, intermorph pollen donation could surpass intramorph donation, even if pollen receipt revealed no disassortative pollination. A recent model of the evolution of distyly

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(Lloyd and Webb, 1992b; reviewed by Barrett, 1990) demonstrates that variation in pollen donation could be essential in the origination and maintenance of this floral polymorphism. Earlier models had suggested that the genetic mating groups of distyly were likely to evolve before the morphological features (Charlesworth and Charlesworth, 1979). In contrast, Lloyd and Webb (following Darwin, 1877) suggested the reverse. Their model assumes an "approach-herkogamous" ancestral state, that is, one in which the stigma is exserted beyond the anthers (Webb and Lloyd, 1986). A stylar length polymorphism could then occur if a mutant with a short style were to become established. Conditions for a balanced polymorphism are based on pollen transfer probabilities. Their model shows that if seed set is resource limited, it is sufficient for each morph to be more successful at donating pollen to the opposite morph than to flowers of its own morph. If seed set is pollen limited, the sum of intermorph donation and receipt must be greater than twice the intramorph transfer. In either case, pollen donation is important.

Although the male reproductive success of single individuals is notoriously difficult to measure (reviewed by Snow and Lewis, 1993), assessing the overall success of entire classes of individuals can be less challenging. In heterostylous species, pollen size polymorphisms often allow a direct assessment of the relative success of different morphs in donating pollen to stigmas (Darwin, 1877; Ganders, 1979; Barrett and Glover, 1985). Granted the assumption that pollen donation is an important component of male fitness, the patterns of pollen transfer between flowers of the same and different morphs can be used to assess the efficiency of heterostyly in terms of male function (Kohn and Barrett, 1992b; Lloyd and Webb, 1992b).

The efficiency of pollen transfer in distyly, relative to pollen transfer in a monomorphic population, has been measured in several ways (see Discussion). To consider efficiency from the perspective of male function, one must compare the probability of a pollen grain produced by one morph to arrive at a legitimate (intermorph) vs. illegitimate (intramorph) stigma. If the probability of a pollen grain landing on an intermorph stigma exceeds its probability of landing on an intramorph stigma, then the male function of that morph is enhanced by distyly, since more pollen is donated than would be in a monomorphic population. A finding that distylous populations have more efficient pollen donation than (hypothetical) monomorphic ones would lend support to the Lloyd–Webb model, which suggests that distyly could be favored even in the absence of diallelic mating groups.

The objective of this study was to assess the role of distyly in promoting pollen donation between flowers of a tropical distylous shrub, *Psychotria suerrensis.* This species has a very slight pollen dimorphism (Stone, 1994), making it difficult to determine the morph of origin of pollen grains on stigmas. I therefore restricted the availability of pollen donation type in the population to one morph or the other on alternating days. The stigma counts from emasculated flowers of both morphs allowed me to compare rates of intramorph and intermorph pollen transfer. A second objective was to determine whether seed set was limited by pollen availability or by resources, in view of this issue's importance in the Lloyd–Webb model. If resources limit seed set, then pollen donation is of utmost importance in the model, and pollen receipt is omitted from the critical inequalities. In contrast, if seed set is limited by pollen receipt, then the model considers transfer probabilities for both donation and receipt.

MATERIALS AND METHODS

Study site and species—The research was done in primary lowland rain forest at La Selva Biological Station, in Heredia Province, Costa Rica. La Selva is located at the transition between the central volcanic mountain chain and the Atlantic coastal plain. Its climate falls into the tropical wet forest life zone of the Holdridge classification, receiving ≈4 m of rain a year, with a dry season during February and March (McDade et al., 1994). *Psychotria suerrensis* (Rubiaceae) is a nonclonal understory shrub. It occurs in low elevation wet forests from Nicaragua to Panama (Taylor, 1991). At La Selva, it can be found in relatively isolated populations of 50–100 individuals on level residual soils on ridgetops, separated from other populations by low-lying swamps. The population used for this study is isolated from other populations by a distance of at least 50 m on all sides. Its location is tied into La Selva's GIS data base, and the locations of plants within the population are given in Stone (1994).

Individuals of *P. suerrensis* normally bear 3–15 inflorescences, each opening 0–4 flowers per day during the blooming season, which extends from mid-February to mid-March. Flowers are ≈1 cm long with narrow-tubular corollas and symmetrical placement of floral organs between morphs (Stone, 1994). Pin corolla tubes are slightly, but significantly, longer than thrums (Stone, 1994). The flowers last a single day, opening just before dawn, and wilting at dusk. Anthers dehiscence depends on relative humidity. On dry days, anthers dehisce soon after the flower opens. Anthers on bagged inflorescences do not normally dehisce for several hours after dawn. The flowers secrete small amounts of nectar and are visited primarily by small bees, butterflies, and hummingbirds. Euglossine bees appear to be the most effective pollinators (Stone, 1994). *Psychotria suerrensis* sets seed only when pollinated by the opposite morph (Bawa and Beach, 1983). It has two ovules per flower, and either one or two seeds per fruit.

Pollen counts/anther were made to allow comparisons of stigmatic pollen loads with other published studies. In 1993, I collected anthers from a single flower of 12 plants of each morph. After the anthers dehisced, I added alcohol to the tubes. I counted the number of pollen grains per flower using an Elzone particle counter at SUNY Stony Brook.

Pollen movement to natural recipients: 1991 experiment—In this experiment, I compared pollen donation capabilities of the two morphs by limiting local pollen availability to one morph at a time. Stigmatic pollen loads on emasculated recipients of both morphs allow a comparison of pollen transfer efficiency between each combination of morphs. In *P. suerrensis,* as in some other

Rubiaceous species, pollen dimorphism is slight (Bir Bahadur, 1968; Feinsinger and Busby, 1987; Stone, 1994). I emasculated all individuals of one or the other morph on alternating days to restrict the source of pollen on a given day to the morph with intact flowers. Plants with more than three inflorescences were used both as donors and as focal recipients. Fourteen thrum plants and 11 pin plants in this population had displays of four or more inflorescences. Late in the afternoon preceding treatment, I bagged all inflorescences on these 25 plants to prevent pollinator access. On plants with three or fewer inflorescences, I removed all large flower buds daily; therefore these individuals neither contributed nor received pollen.

Pins and thrums served as donors on alternating days. On days when thrums were donors, I unbagged inflorescences in the early morning and emasculated flowers of all pin plants and flowers on half of the inflorescences of each thrum plant. Pollinators therefore moved thrum pollen to stigmas of emasculated flowers of both morphs. To emasculate pin flowers, I pulled undehisced anthers up through the corolla tube, without slitting the tube. Emasculations were normally completed by 7:00 a.m. On rare occasions where anthers were noted to be already dehisced, I removed all of the open flowers on that inflorescence. In the late afternoon, the stigma from each flower was collected in a separate microcentrifuge tube and subsequently mounted in fuchsin jelly. Stigmas were assigned consecutive numbers, and stigmatic pollen loads were counted in a random order at 100×. On alternate days, I emasculated half of the pins and all of the thrums, to give the donation rates from pin pollen to both morphs. Each treatment was performed for 2 d.

I emasculated half of the flowers on each plant of the donor morph rather than emasculating all of the flowers on half of the plants so that I would not need to make any assumptions about the spatial arrangement of donors and recipients. Note that this is a very conservative test. If intermorph pollen transfer exceeds intramorph, the pattern must be quite strong, since intramorph transfer includes transfer within a single plant. On the other hand, if no difference is found between intra- and intermorph transfer, it is possible that intermorph transfer is more efficient than intramorph, but that this difference is obscured by within-plant movement. To examine the potential effects of geitonogamy on pollen flow patterns, I calculated correlation coefficients between the number of donor flowers open on an individual on a given day and the number of pollen grains received by emasculated flowers on that individual. A high correlation would indicate that within-plant pollen flow substantially increases intramorph transfer estimates.

Plants' floral displays ranged from one to 30 flowers blooming on a given day. Therefore sample sizes would be vastly unequal if stigmatic pollen loads were used as the unit of replication. In addition, stigmatic pollen loads within a plant are not independent. Therefore, I used individual plants as the sampling unit, excluding individuals with fewer than four flowers per treatment. I calculated the mean stigmatic pollen load for each plant by donor type. These means were then used in a Mann-Whitney *U*-test to compare pollen transfer for each combination of donor and recipient morph.

A control treatment consisted of emasculating all flowers in the population in the morning and collecting their stigmas for pollen counts in the afternoon. This control gives an estimate of the sum of two sources of contamination: self-pollination resulting from ineffectual emasculation, and pollen flow from outside of the population. These two sources of contamination would each affect interpretation of the results differently. Pollen flow from outside the population would be likely to accrue to both morphs about equally. Its most likely result, if undetected, would be failure to perceive an effect of the treatments. A Wilcoxon signed-ranks test for paired observations was used to compare plants' mean stigmatic pollen loads on control days, when all flowers were emasculated, to loads on experimental days, when some local flowers served as pollen donors. Contamination by self pollen is most important if it causes systematically inflated estimates of pollen delivery to one morph or the other. A Mann-Whitney *U*-test was used to compare differences between stigmatic pollen loads of the two morphs for the control and experimental treatments.

Pollen movement to paired recipients: 1992 experiment—In 1992, I used another design to reduce variability in deposition due to the spatial arrangement of the two morphs. I attached a pair of test tubes to the stem of five large plants of each morph, which were to serve as the primary donors. On days when thrums were to be donors, I emasculated all pin plants in the population and placed paired emasculated cuttings, one pin and one thrum, in the tubes of the designated donor thrum plants. On alternating days, thrum plants were emasculated and paired cuttings were placed in tubes on the pin donors. This ensured that the two types of recipients were equidistant from plants of the donor morph. Within a pair, cuttings were approximately the same size and had the same number of open flowers, ranging from one to five. Most inflorescences had either one or two open flowers. Casual observation indicated that pollinators visited the inflorescences in the tubes as often as they visited other inflorescences on the plant. Thrums served as donors for 6 d, and pins served as donors for 4 d. I collected stigmas from recipient flowers in the late afternoon and processed them as before. I used a Wilcoxon signed-ranks test for paired observations to compare mean pollen loads per stigma of the two recipient morphs.

As a control, instead of emasculating all flowers in the population, as I had done in 1991, I used a maximum likelihood approach to compare the size-class distributions of the putative donors with that of pollen grains received on stigmas. For the reference distribution, I measured ten pollen grains from each of 16 self-pollinated flowers of each morph. I divided the size-class distribution into seven classes and calculated the proportion of pollen produced by each morph that fell into each size class. On 20 randomly chosen stigmas for each of the four intended transfer types, I measured all pollen grains at 400×. I used the loss function in SYSTAT's nonlinear estimation procedure (Wilkinson et al., 1992) to estimate a contamination parameter that maximized the probability of generating the size-class distribution of pollen found on stigmas for each of the four donor-recipient combinations. The model used was:

$$\text{obs} = \text{total}\cdot[(1 - \text{rho})\cdot\text{ft} + \text{rho}\cdot\text{fp}],$$

where obs = the observed number of grains in a size class on a stigma, total = the total number of grains counted on that stigma, ft = the fraction of thrum grains in that size class, fp = the fraction of pin grains in that size class, and rho = the proportion of nonsource pollen (in this case, for thrums as donors). For days when the intended transfers were intramorph, contamination estimates indicate levels of interpopulation pollen flow. In contrast, for days when the intended transfers were between morphs, contamination estimates include both self-pollination and interpopulation pollen flow, since both the nondonor and recipient morphs come from the same pollen size distribution.

Pollen limitation of fruit set—Preliminary observations indicated that fruit set among individuals was highly variable. Fruit production varies depending on plant size, light environment, and previous fruiting history (Stone, unpublished data). Rather than trying to match experimental and control plants for all of these factors, I used experimental and control inflorescences within plants. To address the possibility that supplementally pollinated flowers set seed at the expense of control flowers within a plant (see Zimmerman and Pyke, 1988), I compared fruit set in control inflorescences with data on fruit set in unmanipulated plants. The data on unmanipulated plants were gathered in two similarly sized populations at La Selva during 1991–1993 by D. Graham as part of a long-term study on the dispersal and regeneration of *P. suerrensis* and other understory shrubs.

The flowers of *P. suerrensis* are sessile on terminal inflorescences. It is very difficult to follow the fate of individual flowers to the fruiting stage; therefore the inflorescence is the only feasible unit of replication for supplemental pollination studies. Three pairs of inflorescences were chosen on six individuals of each morph. Each morning for 15 d at the peak of the flowering season, I hand-pollinated flowers on one inflorescence of each pair and noted the number of flowers on control inflorescences. Inflorescences on three large shrubs of each morph were bagged to provide anthers for hand-pollinations. Within compatible type, no attempt was made to keep track of donor identity. Pollen was applied by sweeping a single anther from the compatible morph across the stigmatic surface. Hand-pollinations were conducted between 6:30 and 8:00 a.m. Unripe fruits were collected in June. Flowers blooming before or after the 15-d period were not counted, so fruit set is recorded as number of fruits per observed flower. Inflorescences with fewer than two flowers blooming during the observation period were omitted from analysis. Fruit set between naturally pollinated and supplementally pollinated inflorescences was compared using a Wilcoxon signed-ranks test. Mean number of seeds per fruit was also compared using a Wilcoxon signed-ranks test.

RESULTS

Emasculation reduced stigmatic pollen loads for both pin and thrum flowers (Fig. 1). Across all treatments excluding the control treatment, emasculated flowers received an average of 24.9 pollen grains ($N = 469$, SD = 39.69), while intact flowers received 44.0 grains ($N = 316$, SD = 66.47). This difference is statistically significant, based on a Wilcoxon signed-ranks test comparing means for intact and emasculated flowers of individual plants ($P < 0.01$, $T_s = 25.5$, $N = 19$). Based on differences between stigmatic pollen loads of emasculated and intact flowers, pin flowers appear to deposit more self-pollen than do thrum flowers (Fig. 1). A Mann-Whitney U-test comparing the magnitude of individual plant's mean differences between emasculated and intact flowers for the two morphs reveals no significance ($P > 0.05$, $U_s = 59$, $N_1 = 11$, $N_2 = 8$); however, the sample size for this comparison is quite small. Geitonogamy did not appear to inflate intramorph transfer levels. The number of donor flowers open on an individual on a given day ranged from zero to 13, with a median value of three. The number of donor flowers on a plant did not affect the number of pollen grains received by emasculated recipient flowers on that plant (Spearmank rank correlation, $r = 0.28$, $P > 0.05$).

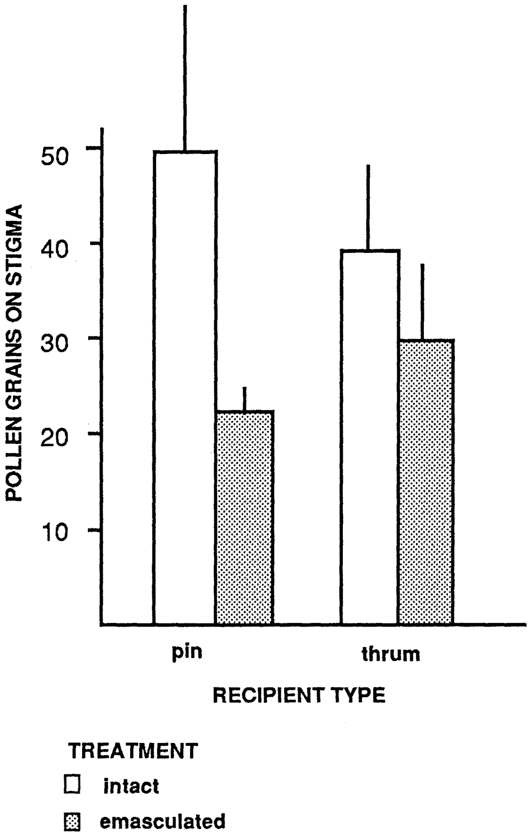

Fig. 1. Mean stigmatic pollen loads of intact and emasculated flowers of individual plants. Intact pollen counts include intrafloral and interfloral transfers from within the same plant and other plants of the same morph. Emasculated counts include interfloral transfer from the same plant and from other plants of both morphs. Values are means from all flowers counted for the 11 thrums and eight pins for which paired comparisons were possible. Vertical lines indicate standard errors.

Pollen import from other populations was substantial. For the control treatment, when all flowers in the local population were emasculated, stigmas received an average of 32.5 pollen grains ($N = 181$, SD = 36.41). During

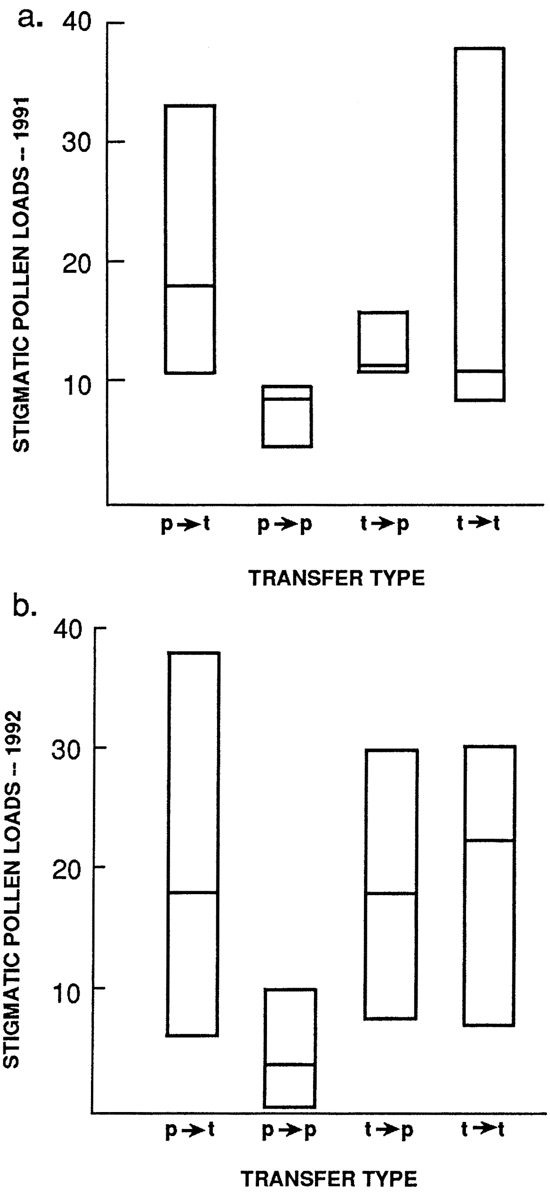

Fig. 2. Stigmatic pollen loads showing all combinations of inter- and intramorph pollen transfer efficiency. All recipient flowers were emasculated. Bars span the distance between the upper and lower quartile, with the median included in the bar. (a) Natural-recipients experiment. Values are medians and quartiles from all flowers counted for the ten thrums and seven pins that had more than four flowers in each treatment. (b) Paired-recipients experiment. Values are medians and quartiles from all flowers counted for each treatment. For pins as donors, N = 20. For thrums as donors, N = 30.

TABLE 1. Mann-Whitney U-test statistics based on mean per-plant stigmatic pollen loads for the natural-recipients experiment. On a given day, local pollen was only from pin or from thrum plants, designated as "donors." All recipient flowers were emasculated. Comparison of the top two rows reveals the effectiveness of distyly for male function; they indicate the extent of disassortative pollen donation. The bottom two rows allow a comparison of female function; they indicate the extent of disassortative receipt.

Donors	Recipients	N_1, N_2	U_s	P
Pins	Pins, thrums	7, 10	59	0.019
Thrums	Pins, thrums	7, 7	25	0.949
Pins, thrums	Thrums	7, 10	30	0.626
Pins, thrums	Pins	7, 7	7	0.025

experimental treatments, when some flowers in the local population remained intact, emasculated flowers received 24.9 pollen grains (N = 469, SD = 39.69). This difference is not statistically significant (T_s = 33, N = 16, two-tailed $P > 0.05$). Population-level emasculation treatments did affect the type of locally distributed pollen. When all flowers were emasculated, mean stigmatic pollen loads did not differ between pins and thrums. Pins received an average of 33.2 grains (N = 72, SD = 43.7), and thrums received an average of 32.1 grains (N = 109, SD = 30.8; U_s = 30.8; N_1 = 7, N_2 = 9, $P > 0.05$). However, when all local pollen of one morph was removed, pollen receipt patterns differed between pins and thrums, as explained below.

Pollen donation ability differed among morph combinations for the natural-recipient experiment (Fig. 2a). Pollen from pins was deposited significantly more on thrum stigmas than on pin stigmas (Table 1). In contrast, pollen from thrums was deposited equally on stigmas of both morphs (Table 1). In terms of pollen receipt, more intermorph pollen tended to be deposited than intramorph pollen; however due to large variances this difference was only significant for pins as recipients (Table 1). Pins received significantly more pollen from thrums than from pins.

Pollen donation ability in the paired-recipients experiment confirms the 1991 natural-recipient results (Fig. 2b). Pollen borne on low anthers was deposited preferentially on low stigmas (T_s = 29.5, N = 20, $P < 0.001$), whereas pollen from high anthers moved equally to both morphs (T_s = 192.5, N = 30, $P > 0.05$). Mean stigmatic pollen loads were not significantly different between years (t-test, $P > 0.05$), and pollen transfer patterns were similar (Fig. 2). The only difference in relative magnitude of pollen transfer was from thrum donors to thrum recipients. In the 1991 natural-recipients experiment, thrums appeared to receive more pollen from pins than from other thrums, although as mentioned above, this difference was not significant. In the 1992 paired-recipients experiment, it appears that thrums received more pollen from other thrums than they did from pins (Fig. 2b), however a Mann-Whitney U-test indicates that this difference was also insignificant. Final interpretation of results from the 1992 experiment depends on pollen size distributions found on stigmas, as explained below.

Pin flowers contained 1,987.4 pollen grains (N = 12, SD = 399.97) and thrum flowers contained 1,870.8 grains (N = 11, SD = 365.15). There was no difference in pollen production between the two morphs (t-test, $P > 0.05$). Thrum pollen was, on average, larger than pin pollen (47.4 μm vs. 39.6 μm; t_s = 16.9; df = 63; $P < 0.001$), but had a larger coefficient of variation (0.104 vs. 0.093; s_v = 0.009; N = 60, $P < 0.001$). Figure 3 shows the size-class distribution of pollen produced by each morph, along with the size-class distribution of grains found on stigmas for each morph as the putative donor. For some transfers, the size-class distribution of pollen measured on stigmas appears the same as the distribution

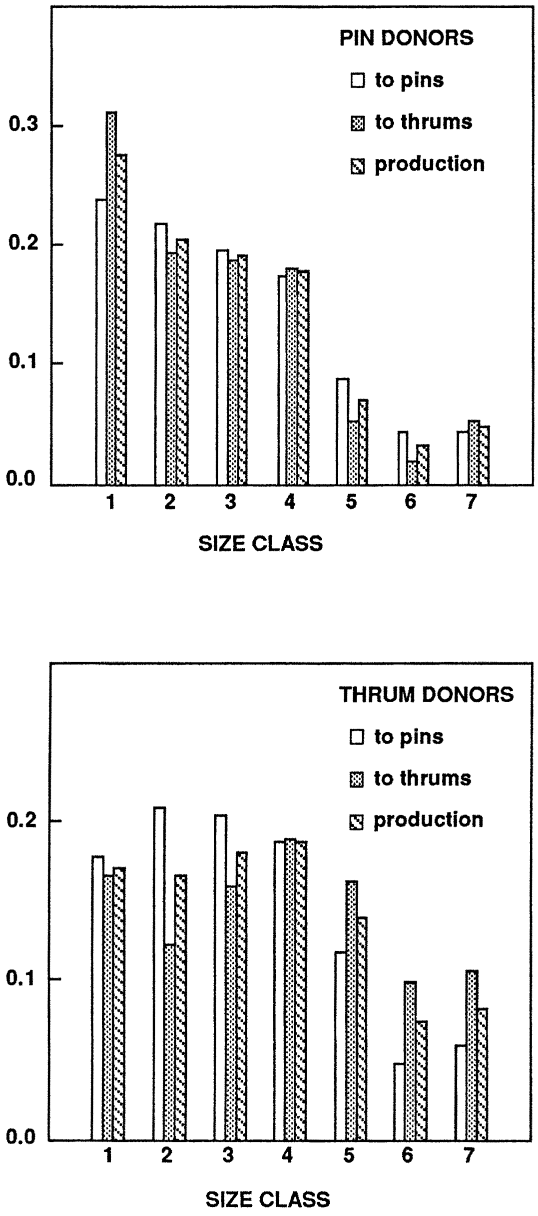

Fig. 3. Pollen size-class distributions from the 1992 experiment. Size-class distributions for pollen found on stigmas on days when pins (top) or thrums (bottom) were designated donors. White bars represent grains found on pin stigmas, gray bars represent grains found on thrum stigmas, and hatched bars represent the size-class distribution of pollen produced by each morph.

produced by the donating morph (e.g., pin to thrum). For other transfers, especially thrum to pin, the fit between the size-class distributions of pollen received on stigmas and pollen production is not good. These qualitative observations are supported by the maximum-likelihood estimates.

The maximum-likelihood estimation of contamination from the 1992 experiment ranged from 15 to 54%. Twenty-four percent of grains in thrum-to-thrum transfers and 29% of grains in pin-to-pin transfers were unlikely to come from either the recipient or the putative donor, and can be attributed to intermorph pollen flow from outside of the population. For pin-to-thrum transfers, 15% of the pollen counted on stigmas was most likely to have come from thrums, either self or outside of the population. For thrum-to-pin transfers, 54% of the pollen counted on stigmas was estimated to have come from pins, either self or outside of the population. Therefore, the rate of thrum-to-pin transfer was substantially overestimated by counts of stigmatic pollen loads. This reinforces the conclusion that pollen is transferred more efficiently from pins than from thrums.

Although both experiments show that pollen transfer from pins is more efficient than transfer from thrums, this does not necessarily translate into a higher level of compatible receipt by thrums than by pins, because more thrum pollen may be dispersed overall. In 1991, it appears that pin-to-thrum transfer may have exceeded thrum-to-pin transfer, but this difference was not significant (Mann-Whitney $U = 347$; $N_1 = 20$; $N_2 = 31$; $P > 0.05$). In 1992, the two rates look equal, but the size-class distributions of pollen grains on stigmas indicate that pin-to-thrum transfer may well have exceeded thrum-to-pin transfer. However, if legitimate transfer to thrums exceeds legitimate transfer to pins, it is apparently not translated into differential fruit production. Unmanipulated individuals of the two morphs produced equal numbers of fruits per inflorescence (Table 2; *t*-test for differences between means, $t = 0.8$, df = 174, $P > 0.05$).

Inflorescences with hand-pollinated flowers set more fruit than inflorescences without supplemental pollination (Table 2; Wilcoxon rank sum $T_s = 59$, $N = 28$, $P < 0.001$). They also had more seeds per fruit (Table 2; Wilcoxon rank sum $T_s = 92$, $N = 30$, $P < 0.01$). The control inflorescences on the plants given supplemental pollination had the same number of fruits per inflorescence as did unmanipulated plants (Table 2; *t*-test for differences between means, $t = 1.5$, df = 186, $P > 0.05$).

TABLE 2. Results of supplemental pollination for inflorescences of *Psychotria suerrensis.* Fruit set is reported per observed flower because flowers at the tails of the flowering season were not counted. Values are means ± standard errors. Sample sizes for the manipulated plants are 30 pairs of control inflorescences and supplementally-pollinated inflorescences across 11 plants. Sample sizes for the unmanipulated plants are 87 pins and 90 thrums, over two populations in 3 yr (D. Graham, unpublished data).

	Fruits/observed flower	Seeds/fruit	Fruits/inflorescence
Pollen added	1.5 ± 0.21	1.6 ± 0.05	11.1 ± 1.20
Control inflorescences	0.8 ± 0.15	1.4 ± 0.05	6.5 ± 0.75
Unmanipulated plants (pins)	—	—	5.2 ± 0.49
Unmanipulated plants (thrums)	—	—	4.7 ± 0.49

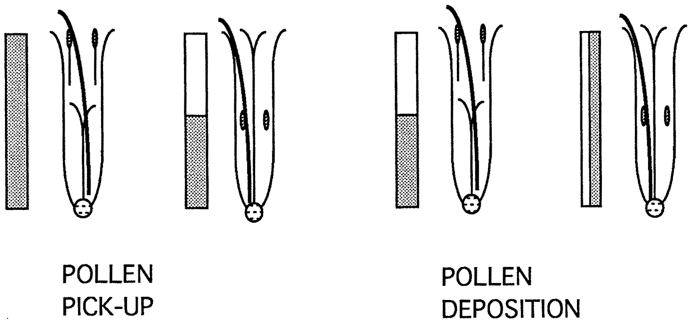

Fig. 4. Possible mechanism for superior pollen transfer probabilities between lower level organs. The curved line in each flower represents a proboscis and the stippled bar to the left of each flower illustrates the portion of the proboscis that is likely to contact the anthers (left panel) or stigma (right panel). The proboscis is likely to contact high-level anthers over the entire portion that enters the corolla. In contrast, only the lower portion of the proboscis is likely to contact low-level anthers. Only the lower portion of the proboscis will contact a low-level stigma. The proboscis may contact a high-level stigma over any part of its length that enters the flower, but, depending on the orientation of the bifid stigmatic lobes, it may also fail to contact the stigma. The net result of these contact probabilities would be accurate pollen transfer between low organs and relatively imprecise transfer between upper-level organs. Diagram adapted from Lloyd and Webb, 1992b.

DISCUSSION

Pollen transfer patterns in *P. suerrensis* were consistent over two flowering seasons and two different experimental techniques. In both cases, distyly served male function with only partial efficiency. Pollen borne deep within the floral tube was efficiently transferred to low stigmas, but pollen borne towards the mouth of the corolla was redistributed equally on both floral morphs.

What causes this pattern of pollen deposition? It seems that the tips of pollinators' mouthparts are placed fairly precisely in the bottom of the narrow floral tube, causing disassortative pollination from low anthers to low stigmas in much the way envisioned by Darwin (1877) for distylous plants in general. Why is pollen produced on high anthers deposited equally on both low and high stigmas? A possible explanation is that the proboscis contacts pin anthers only near the tip, but contacts thrum anthers across the entire portion that is inserted into the flower (Fig. 4, adapted from Lloyd and Webb, 1992b). Either pin or thrum stigmas are then equally likely to contact portions of the proboscis that bear thrum pollen. A similar idea was presented by Ganders (1974), who postulated that greater efficiency in the pollination of thrums than pins is caused by constraints on pollinator orientation imposed by the corolla tube.

Pollen flow from outside of the population was substantial. During the 1991 control treatment, when all flowers in the population were emasculated, stigmas received as much pollen as they did during experimental treatments, when some flowers within the population served as donors. However, patterns of pollen deposition across morph types show that the experimental treatments did influence the local pollen flow patterns. For the control treatment, pins and thrums received equal quantities of pollen. During experimental treatments, in contrast, pins and thrums received different amounts of pollen, depending on which morph in the local population was donating pollen. Thus, although emasculation did not affect the total amount of pollen received, it did influence the type of pollen deposited on stigmas.

Results from the 1992 control treatment support the impression that there were high levels of pollen flow from outside of the population. Estimated interpopulational movement ranged from 24 to 29% of pollen grains received, but this includes only intermorph transfer, so is presumably an underestimate. Contamination due to selfing did differ between morphs. An estimated 54% of grains measured in thrum-to-pin transfers came not from thrums, but from pins. This value includes both self-pollination and pin pollen from outside the population. In contrast, only 15% of pollen measured in intermorph transfers to thrums came from thrums. This value includes both selfing and pollen flow from other populations. Since interpopulational flow was relatively high, we can conclude that contamination of thrums due to unsuccessful emasculations was low. How do the contamination estimates affect interpretation of the results? Contamination from outside the population affected both morphs about equally, and therefore should not have much effect on the relative transfer rates measured for the four combinations of donor and recipient. Contamination by incomplete emasculations does change relative transfer levels, however. Selfing by pins resulted in an overestimate of transfers to pin flowers, implying that thrum-to-pin transfers were significantly lower than the measured values. The high degree of self-contamination by pins does not, however, alter the conclusion that thrum-to-pin transfer exceeds pin-to-pin transfer, since the rate of self-contamination is constant for this comparison.

Comparisons with other species—Asymmetrical pollen donation efficiency between morphs is considered to be common among heterostylous plants (reviewed by Ganders, 1979; Barrett and Glover, 1985). Several authors have concluded that pin plants typically receive disproportionately more illegitimate pollen than thrum plants (Ganders, 1979; Dulberger, 1992). These estimates include self-pollen in most cases, so it is impossible to determine whether the differing proportions of legitimate pollination are simply due to different self-pollination rates. As Ganders (1974, 1979) emphasized, interfloral pollen transfer rates are critical in evaluating the efficiency of the polymorphism.

Comparing pollen transfer efficiency across species is complicated by the various ways that efficiency has been measured. In the simplest type of measurement, pollen grains of each type are counted on stigmas from emasculated flowers of each morph. The polymorphism is considered to serve female function efficiently if legitimate (same-level) pollen loads exceed illegitimate loads. A refinement of this technique takes into account the frequencies of the morphs and their differences in pollen production, so that stigmatic pollen loads are compared with those expected under random transfer (e.g., Ganders, 1974). A third method uses pollen production levels and morph frequencies to calculate the probability of an individual pollen grain of one morph landing on the stigma of any other morph (Lloyd and Webb, 1992b). These pol-

TABLE 3. Three different approaches to measuring efficiency of heterostyly. Data from emasculated recipients of *Jepsonia heterandra* (Ganders, 1974). p to t indicates pin grains found on thrum stigmas, t to p indicates thrum grains on pin stigmas, etc. Method 1 simply reports the mean number of grains found for each transfer type. For method 2, values are the observed number of grains divided by random expectation based on morph frequencies in the population and pollen production rates. Method 3 gives probabilities that individual pollen grains reach a stigma of the specified type (from Lloyd and Webb, 1992b).

Transfer type	Method 1		Method 2		Method 3	
	Value	Rank	Value	Rank	Value	Rank
p to t	572	2	1.49	1	2.71	3
t to p	406	3	1.30	2	4.46	1
p to p	628	1	0.87	3	2.98	2
t to t	117	4	0.56	4	1.29	4

TABLE 4. Conditions for invasion of a monomorphic population by a style-length mutant (from Lloyd and Webb, 1992b). Mean pollen transfer from short-styled to long-styled morph is represented by q_{tp}, rom long-styled to long-styled by q_{pp}, etc. Under particular conditions of limitation of seed set, both inequalities must be met for a balanced polymorphism to become established. The last column indicates whether or not present-day pollen transfer patterns would meet conditions for invasion.

Invading morph	Limitation of seed set	Necessary inequality	Upheld by experiments?
Short-styled	resources	$q_{tp} > q_{pp}$	yes
Long-styled	resources	$q_{pt} > q_{tt}$	1991 yes, 1992 no
Short-styled	pollen	$q_{tp} + q_{pt} > 2q_{pp}$	yes
Long-styled	pollen	$q_{tp} + q_{pt} > 2q_{tt}$	1991 yes, 1992 no

len transfer probabilities can be used to interpret both pollen donation and receipt.

Data from emasculated flowers of *Jepsonia heterandra* (Ganders, 1974) illustrate that the three methods yield mutually inconsistent interpretations (Table 3). The unadjusted stigmatic pollen counts indicate that distyly functions to deliver more legitimate than illegitimate pollen to thrum stigmas, but not to pins. When stigmatic pollen loads are adjusted for pollen production and morph frequency, it appears that both morphs experience disassortative pollination, but that the degree of disassortative pollination is higher for thrums than for pins. Finally, the probabilities for individual pollen grains landing on compatible stigmas indicate that the highest transfer efficiency is from thrum to pin. While the second two methods agree that disassortative pollination occurs, they differ as to the direction of the asymmetry.

The data presented in this experiment are most analogous to the third method. Rather than attempting to correct for differences in morph frequency or pollen production, I controlled pollen source for any given day. Therefore, a comparison of each morph's ability to donate to both types of flowers can be made directly from comparisons of stigmatic pollen loads. Donation efficiency is dramatically higher for thrums than for pins. Based on the pollen probability method, the direction of asymmetry for this species is anomalous. A reanalysis of published stigmatic pollen loads indicates that for nearly all distylous species, thrum pollen has a greater probability of landing on a compatible stigma than does pin pollen (Stone and Thomson, 1995).

A number of studies with tristylous taxa also support the idea that pollen flow between high-level organs usually exceeds transfer between low-level organs. In *Pontederia cordata,* pollen produced by long anthers has the highest probability of encountering a compatible stigma (Barrett and Glover, 1985; Wolfe and Barrett, 1989; Lloyd and Webb, 1992b). In experimental populations of self-compatible *Eichhornia paniculata,* the short-styled morph had the highest male fitness and the long-styled morph was relatively female (Kohn and Barrett, 1992a). The difference in functional gender was directly related to differences in pollen transfer efficiency (Kohn and Barrett, 1992b). In *Lythrum salicaria,* dye transfer among morphs was asymmetric, with more than random expectation of transfer from short-styled morphs to mid- and long-styled morphs (O'Neil, 1992). O'Neil (1992) also showed differences in seed set and morph-specific pollen limitation. In *Psychotria suerrensis,* although pin pollen was transferred more efficiently than thrum pollen, overall rates of disassortative pollination to the two morphs did not differ (because more thrum pollen is captured by stigmas), and fruit production by the two morphs was equal.

Besides the direction of asymmetry in compatible pollen transfer, another apparently anomalous feature of *P. suerrensis* is the equal pin : thrum pollen production ratio. In most taxa surveyed, pin flowers produce significantly more pollen than thrum flowers (Ganders, 1979; exceptions listed by Dulberger, 1992). When pollen transfer efficiencies are calculated on a per-pollen grain basis, the apparent low efficiency of pins as males in many species may just be due to their higher pollen production levels, and consequent lower per-grain redeposition rates. Alternatively, the higher production of pin than thrum pollen in most species may be a result of selection due to lower per-grain redeposition rates of pollen from low anthers.

Implications for evolution of distyly—A recent model of the evolution of distyly (Lloyd and Webb, 1992b; reviewed by Barrett, 1990) suggests that male function may be important in helping a short-styled morph invade an ancestral population of long-styled flowers. Sets of inequalities concerning pollen transfer probabilities define whether or not a balanced polymorphism could subsequently become established after such an invasion (Table 4). The sets of inequalities differ depending on whether seed set is limited by resources or by pollen. Male function is critical in situations where seed set is resource limited. Where seed set is pollen limited, both male and female function are important for a balanced polymorphism to become established.

Presuming that the self-compatible ancestral form had similar morphology and pollen transfer patterns, these results support the plausibility of a short-styled mutant being able to invade a long-styled population due to differential success in pollen transfer, whether seed set were pollen limited or resource limited. However, it is not clear whether conditions for a balanced polymorphism are fulfilled. Results from the 1991 population-level experiment suggest that a pin morph would be favored in a population dominated by thrum plants, but results from the 1992 paired-recipients experiment do not. If conditions prevalent during 1991 prevailed, a thrum-type mutant would spread gradually throughout the population. Fluctuating selection between years could have assisted the establishment of the original polymorphism.

What does the fact that fruit set is pollen limited say about gender-specific selection pressures on *Psychotria suerrensis*? As mentioned above, if fruit set were resource limited, the argument could be made that selection on floral morphology, including the success of an invading mutant, would operate primarily through male function. Since fruit set is pollen limited, it is reasonable to conclude that the function of the polymorphism in pollen receipt is important. The fact that seed set, as well as fruit set, increased with supplemental pollination indicates that per-visit transfer of pollen is important. Nevertheless, the dramatic increase in fruit set resulting from hand-pollination suggests that there were numerous flowers that were never visited. It would be interesting to know whether fruit set would be affected by supplemental pollinations applied only to flowers also visited by pollinators. Efficiency of pollen capture by individual flowers may be less important to female function in this species than overall pollinator attraction.

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