

# Asymmetry in pollen flow promotes gender specialization in morphs of the distylous neotropical herb *Arcytophyllum lavarum* (Rubiaceae)

CARLOS GARCÍA-ROBLEDO<sup>1</sup>

<sup>1</sup>*Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, Florida 33124-0421, carlos@bio.miami.edu.*

## Summary

Distyly is a floral polymorphism, characterized by a reciprocal positioning between stigmas and anthers in different flowers, where two floral morphs, long-styled (pin) and short-styled (thrum) occur within the population. Distyly is suggested as one of the routes leading to the evolution of separate sexes in plants. In this evolutionary pathway, pollinators may disrupt the complementarity of pollen transfer between morphs. Consequently, the floral morphs gradually specialize as either male or female. A key process required for gender specialization in distylous plants is a deviation of the realized functional gender (*i.e.* the proportion of genes transmitted to the next generation via pollen donation and seed production) from the potential functional gender (*i.e.* the expected contribution of male and female function to reproductive success from the number of ovules or pollen grains produced by each morph). I selected the distylous herb *Arcytophyllum lavarum* (Rubiaceae) to determine if asymmetry in pollen flow promotes differences in seed production, pollen donation and a discrepancy between the potential and the realized functional genders in pin and thrum floral morphs. Pollen flow in *A. lavarum* is asymmetric and the pin morph is more efficient at performing cross pollination than the thrum morph. Conversely, the thrum morph produced two times more seeds than the pin morph. Male and female contributions to the potential functional gender were equivalent in both morphs. However, the pin morph transmitted more genes through pollen donation and the thrum morph more through seed production than expected from their potential functional gender. These results support the hypothesis that if pollinators consistently promote asymmetric pollen flow between morphs over generations, it is possible that gender specialization may evolve to the extreme of dioecism from an original distylous condition.

**Keywords:** Distyly, evolution of sex, gender specialization, páramo, pollen flow

## Introduction

Plant sexuality is frequently inferred from the ratio of sexual structures such as anthers and carpels or ovules and pollen grains, which may suggest the contribution of male and female functions to reproductive success (Devlin and Stephenson 1987). This estimate of plant sexuality represents the potential functional gender via pollen donation or ovule fertilization, in the hypothetical scenario of all plants attaining their maximum possible male and female reproductive success (Lloyd 1980).

However, actual plant sexuality can be quite different from that predicted by the potential functional gender. In hermaphroditic plants, pollen flow may differ among individuals, with some plants playing a major role as males through pollen donation or as females through pollen reception (Horowitz 1978; Freeman *et al.* 1997). This divergence between the potential and the realized functional gender may promote gender specialization at an ecological scale, and is a required process for the evolution of separate sexes in flowering plants (Lloyd and Webb 1992).

One factor that may promote a discrepancy between the potential and the realized functional gender via pollen flow in hermaphroditic plants is floral polymorphism *i.e.* the presence of flowers with different shapes among individuals (Pannell and Verdu 2006). Heterostyly is a type of floral polymorphism, characterized by a reciprocal positioning between stigmas and anthers in different flowers, where two (distyly) or three (tristyly) floral morphs may occur within the population (Ganders 1979; Kohn and Barrett 1992a; Lloyd and Webb 1992).

Heterostyly is suggested to promote disassortative pollination, *i.e.* higher pollen flow to opposite floral morphs (Darwin 1896; Charlesworth and Charlesworth 1979;

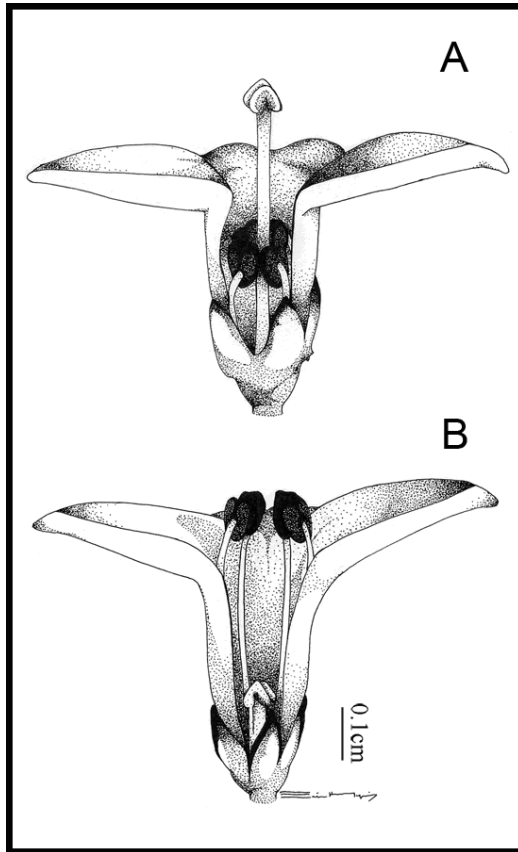
Lloyd and Webb 1992). If pollen flow between floral morphs is symmetric (*i.e.* the probability of arrival of pollen to the opposite morph is equivalent for all floral morphs), and morphs exhibit equivalent potential functional gender, it is expected that the floral morphs within the population will display equivalent male and female reproductive success.

Nevertheless, in some distylous species, one morph may contribute more to the total seed production of the population than the other (Wolfe and Barrett 1987; Husband and Barrett 1992; Ree 1997). Two potential causes for this variation in female reproductive success between morphs are 1) morphological differences such as more ovules or pollen grains present in one of the floral morphs or 2) differences in the realized functional gender between morphs due to a higher efficiency by one of the floral morphs as a pollen donor (Kohn and Barrett 1992a, b; Lloyd and Webb 1992; Oneil 1992; Stone and Thomson 1994).

I selected *Arcytophyllum lavarum* (Rubiaceae), an endemic herb of the Central American paramos ecosystem as a study model. *Arcytophyllum lavarum* is distributed northern Panama to Costa Rica (Luteyn 1999). This plant is distylous, therefore two floral morphs are present, long and short-styled flowers, hereafter referred to as pin and thrum floral morphs respectively (Fig. 1).

To understand how pollen flow affected plant sexuality in the pin and thrum morphs of *A. lavarum*, the objectives of this research were 1) to determine if the floral morphs of *A. lavarum* differed in female reproductive success and 2) to test if the differences in female reproductive success between morphs were a consequence of a) intrinsic morphological characteristics of the floral morphs such as differences in the

number of ovules or pollen grains produced or b) asymmetry in pollen flow between floral morphs.



**Fig. 1.** Cross-section diagram of floral morphs in the distylous Rubiaceae *Arcytophyllum lavarum*. A. long-styled morph (pin) B. Short-styled morph (thrum). Illustration by Erin K. Kuprewicz.

Finally, for each floral morph I examined the degree of divergence between the potential and the realized functional genders. If asymmetric pollen flow is the process producing a discrepancy between the potential and the realized functional genders, this indicates that interactions with pollinators can promote a division of male and female reproductive functions between pin and thrum flowers of *A. lavarum*.

## Materials and methods

### Study site and species of interest

This study was conducted in Costa Rica, Cordillera de Talamanca, Macizo del Cerro de La Muerte, Reserva Natural Tapantí, Cerro Buena Vista (N 9° 33' W 83° 45'). Mean rainfall in the year 2000 was 215 mm, and the average temperature was 14.8 °C (Min – Max = -1.5 – 25.5 °C, F. Valverde unpubl. data). This area is covered with vegetation typical of a grass paramo (Weber 1959). Rock outcrops are abundant, on which the distylous Rubiaceae *Arcytophyllum lavarum* is the predominant plant species (Voucher MO C.Garcia-Robledo 121, determined by C. M. Taylor).

*Arcytophyllum lavarum* K. Schum. ex Standl grows in discrete aggregations scattered on top of the rocks. Ramets within these aggregations may belong to one or more individuals. Hereafter I refer to these aggregations as patches. Pin, thrum, or both floral morphs could comprise the floral display within patches. Pin and thrum patches display equivalent number of flowers. However, patches composed of both floral morphs have larger floral displays than those containing a single morph (García-Robledo and Mora submitted). Patch distribution over the rock outcrops is random, and the probability of having a pin or thrum patch as a neighbor is equivalent for both floral morphs. Inflorescences are terminal and may simultaneously display 1 to 12 flowers that are visited by syrphid flies. Hand-pollination experiments showed that *Arcytophyllum lavarum* is self and morph incompatible and seeds are only produced when flowers are pollinated by the opposite morph (García-Robledo and Mora submitted). When flowers are over-pollinated, pin and thrum flowers produce equivalent number of seeds (García-

Robledo and Mora submitted). This suggests that the difference in seed production between morphs further discussed in this paper is the product of limited pollination.

#### Ovule, pollen and seed production in pin and thrum floral morphs

To determine if pin and thrum morphs differ in their numbers of ovules, I collected 40 flowers of each morph from different patches. I dissected the carpels and recorded the number of ovules per flower. To determine the number of pollen grains produced by pin and thrum flowers, I selected one anther per flower from 15 recently-opened flowers. Each anther was opened on a microscope slide, and all pollen grains were counted. To estimate the total number of pollen grains per flower, the number of grains counted was multiplied by the number of anthers per flower. Differences in ovule or pollen production among morphs were explored using U Mann-Whitney tests. Estimates of ovule and pollen production were included in further analyses of pollen flow between morphs and the potential functional gender of pin and thrum flowers.

To estimate differences in female reproductive success between pin and thrum floral morphs, I measured the mean seed production per flower in 68 pin and 47 thrum patches. In pin and thrum patches with ten or fewer inflorescences, I marked all flowers with a small point of paint on the pedicel. In patches with more than ten inflorescences, I marked all flowers within 10 previously-selected inflorescences (Number of flowers marked: pin = 1004, thrum = 453).

Forty-five days later, I collected the near-ripe fruits, and counted the number of seeds in each fruit. I measured female reproductive success as the mean number of seeds

produced by the previously marked flowers within each patch, assigning a value of zero to aborted flowers. To test for differences in seed production between morphs, I performed a U Mann-Whitney test. Estimates of seed production were included in further analyses of the realized functional gender in pin and thrum flowers.

#### Relative abundances of pin and thrum floral morphs

I determined the relative abundances of pin and thrum morphs by counting all flowers within a 100 X 150 m plot. This is an accurate measure of flower abundances in this population, as this plot included at least 1/3 of all the patches of *A. lamarum* within the study area. Differences in the numbers of pin and thrum flowers were determined by a chi-square test. Estimates of relative abundance were also included in further analyses of pollen flow and the potential and realized and functional genders in pin and thrum flowers.

#### Pollen flow and male reproductive success in pin and thrum morphs

In some distylous species, pollen size differs between pin and thrum morphs. This is a useful characteristic for determining pollen flow between and within floral morphs (Wyatt and Hellwig 1979; Ornduff 1980; Paillet and Thompson 1997; Ree 1997). To determine the degree of pollen size dimorphism between pin and thrum pollen grains of *A. lamarum*, I collected pollen from 18 pin and 20 thrum flowers, each flower came from a different patch ( $N_{\text{pin pollen grains}} = 3840$ ,  $N_{\text{thrum pollen grains}} = 4548$ ), and I obtained the

frequency distribution of pollen size for each floral morph. After determining differences in size and the degree of size overlap between pin and thrum pollen grains, I used pollen size to identify the floral morph of each pollen grain in the following pollen flow experiment:

To test for differences in pollen flow between and within floral morphs, I selected pin and thrum patches with similar floral displays, (i.e. 20 inflorescences,  $N_{\text{pin patches}} = 10$ ,  $N_{\text{thrum patches}} = 10$ , minimum distance among patches = 15 m), and emasculated all flowers within patches. All recently-opened flowers were also emasculated after anthesis (9 to 20 flowers per patch) to exclude from the estimates pollen arriving from the same flower or flowers within the patch (Lloyd and Webb 1992). Pistils of new flowers were collected 24 hours after opening, and fixed in FAA (formalin:acetic acid: 70% ethanol in the proportions 5:5:90 v/v). To record all pollen grains arriving to the pistils, I placed each pistil between two cover slips. I took pictures from both sides of the cover slips with a digital camera attached to a light microscope at 160 X magnification. I recorded in the images the number of pollen grains in each pistil, and the diameter of each pollen grain using the application Sigmascan Image ®.

The diameters of the pollen grains found in the pistils were compared with the estimated frequency distribution of pollen size of each floral morph. Each pollen grain was categorized as pin, thrum or undetermined. Differences in the mean number of pin, thrum or undetermined pollen grains per pistil were tested with a non-parametric two way ANOVA-Scheirer-Ray-Hare test (Sokal and Rohlf 1995).

I also measured the asymmetry in pollen flow within and between morphs by calculating the probability of arrival of one pin or thrum pollen grain to a pin or thrum

pistil (Lloyd and Webb 1992) where  $T_{ij}$  represents the probability of transfer of a single pollen grain of type  $i$  to a stigma  $j$  and  $(av. \text{ Stigmatic load})_{ij}$  is the average number of  $i$  grains on each  $j$  stigma (Equation 1). I used the pollen previously counted in pin and thrum flowers to calculate the mean number of pollen grains per flower in morph  $i$   $(\text{pollen/flower})_i$  (Equation 1). To estimate the relative abundance of each floral morph  $(\text{no. flowers})_i$  and  $(\text{no. flowers})_j$ , I used the flowers recorded within the aforementioned 100 X 150 m plot.

$$T_{ij} = \frac{(\text{av. Stigmatic load})_{ij} \times (\text{no. flowers})_j}{(\text{pollen/flower})_i \times (\text{no. flowers})_i} \quad (1)$$

The levels of disassortative pollination  $E$  (Equation 2), are measured as the deviation of pollen arrival to pistils from that expected with random pollen flow, where  $O$  represents the observed number of pollen grains of a given morph in the pistils collected during the pollen flow experiment.  $R$  represents the expected number of pollen grains per pistil when pollen flow is at random and  $D$  is the expected number of pollen grains in the pistils if pollen flow is totally disassortative (Ganders 1979). Estimates of the probability of pollen arriving to pistils, as well as levels of disassortative pollination between morphs were explored with non-parametric two way ANOVA-Scheirer-Ray-Hare tests (Sokal and Rohlf 1995).

$$E = \frac{O - R}{D - R} \quad (2)$$

Estimates of the asymmetry of pollen flow and disassortative pollination take into account the ratio of pin and thrum flowers in the population as well as the number of pollen grains produced by each floral morph (Ganders 1979; Lloyd and Webb 1992). Therefore, these estimates can determine if differences in pollen arrival between morphs

is a product of the relative abundance of floral morphs or the result of asymmetric pollen flow (Ganders 1979; Lloyd and Webb 1992).

Deviation of the potential from the realized functional gender

Using the estimates of pollen and ovule production (Table 1), I calculated the potential functional gender for pin and thrum morphs of *A. lamarum*. This estimate represents the proportion of genes most likely to be transmitted in each morph through its own ovules ( $G_{pot}$ , Equation 3) or through pollen donation ( $A_{pot}$ , Equation 4), where  $\overline{OV}$  and  $\overline{PO}$  represent the average number of ovules and pollen in morphs  $i$  and  $j$  (modified from Lloyd 1980).

$$G_{pot_i} = \frac{\overline{OV}_i}{\overline{OV}_i + (\overline{PO}_i \times E_{pot_i})} \quad (3) \quad A_{pot_i} = \frac{\overline{PO}_i \times E_{pot_i}}{\overline{OV}_i + (\overline{PO}_i \times E_{pot_i})} \quad (4)$$

*Arcytophyllum lamarum* is self and morph incompatible, so all ovules from morph  $i$  must be pollinated by pollen from the opposite morph  $j$  in order to produce seeds.

Therefore the potential male function is limited by the number of ovules available for pollination in the opposite morph. Assuming that all the ovules of morph  $i$  have equal probability of being pollinated, and all pollen grains of morph  $i$  have the same probability of contributing to pollination, then  $E_{pot_i}$  (Equation 5) is the equivalence factor by which pollen units must be multiplied to adjust  $G_{pot}$  and  $A_{pot}$  to the actual number of ovules available for legitimate pollination  $\overline{OV}_j$  (Equation 5, modified from Lloyd 1980).

Differences in the reproductive potential between morphs were tested by a G test.

$$E_{\text{pot}_i} = \frac{\overline{OV}_j}{\overline{PO}_i} \quad (5)$$

Using the estimates of seed production in pin and thrum patches of *A. lamarum*, as well as the estimates of cross pollination from the pollen flow experiment, I calculated the realized functional genders through seed production of pin and thrum morphs ( $G_{\text{real}}$ , Equation 6) and pollen donation ( $A_{\text{real}}$ , Equation 7). These estimates represent the actual contribution of male and female reproductive functions to the total of genes transmitted to the next generation (modified from Lloyd 1980).  $\text{Seed}_i$  represents the mean number of seeds produced per flower in each patch of morph *i*.  $\overline{PO}_{ij}$  is the average number of pollen grains of morph *i* deposited per patch in stigmas of morph *j* during the pollen flow experiment. (modified from Lloyd 1980).  $\overline{\text{Seed}}_i$  and  $\overline{PO}_{ij}$  are the average seed production and pollen flow estimates per flower for all patches of morph *i*.

$$G_{\text{real}_i} = \frac{\text{Seed}_i}{\text{Seed}_i + (\overline{PO}_{ij} \times E_{\text{real}_i})} \quad (6) \quad A_{\text{real}_i} = \frac{\overline{PO}_{ij} \times E_{\text{real}_i}}{\text{Seed}_i + (\overline{PO}_{ij} \times E_{\text{real}_i})} \quad (7)$$

The equivalence factor by which pollen units must be multiplied to adjust  $G_{\text{real}}$  and  $A_{\text{real}}$  based on the actual seeds sired by the opposite morph *j* is:

$$E_{\text{real}_i} = \frac{\overline{\text{Seed}}_j}{\overline{PO}_{ij}} \quad (8)$$

To quantify the degree at which pollen flow modified plant sexuality in pin and thrum morphs, I calculated the deviation of the realized from the potential functional gender for both female ( $\Delta G$ , Equation 9) and male ( $\Delta A$ , Equation 10) functions. Then,

$$\Delta G_i = G_{\text{real}_i} - G_{\text{pot}_i} \quad (9) \quad \Delta A_i = A_{\text{real}_i} - A_{\text{pot}_i} \quad (10)$$

For each morph, I tested for differences in reproductive success, and the deviation of reproductive success from the reproductive potential using U Mann-Whitney tests.

## Results

### Ovule, pollen and seed production and floral morph abundance

Pin and thrum floral morphs of *A. lamarum* differed in their ovule and pollen grain production. Pin flowers produced on average one more ovule than thrum flowers (Table 1). Pin flowers also produced more pollen grains than thrum flowers (Table 1). The floral morphs also differed in female reproductive success, with thrum flowers producing twice as many seeds as pin flowers (Table 1).

TABLE 1. Differences in gamete and seed production between pin and thrum morphs in *A. lamarum*

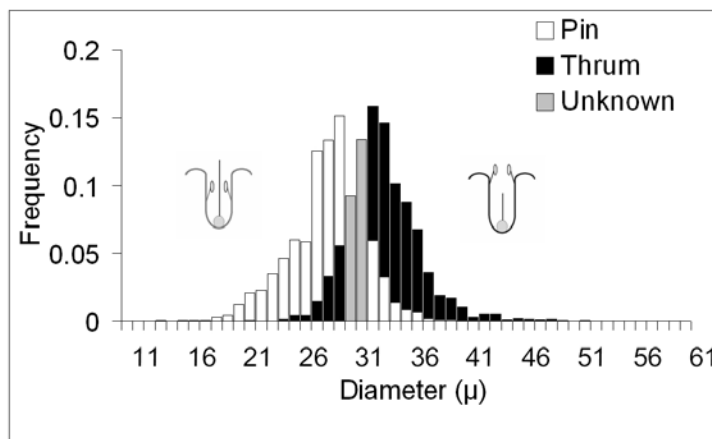
Morph characteristics	Pin			Thrum			U	P
	N	Mean	SD	N	Mean	SD		
No. of ovules per flower	40	11.2	2.3	40	9.7	2.6	495.7	0.003
No. of pollen grains per flower	15	5390.5	1140.7	15	3856	835.3	32	<0.001
Number of seeds per flower	68 <sub>1</sub>	0.89	1.53	47 <sub>1</sub>	2.04	2.51	1093.5	0.003

<sub>1</sub>Number of flowers marked: Pin = 1004, Thrum = 453

Pin flowers were more abundant within the 100 X 150 m plot ( $\chi^2 = 111.8$ ,  $df = 1$ ,  $P = 1676$ , Thrum = 1117,  $P < 0.001$ ). However, as shown by the pollen flow analyses (*see next section*), differences in seed production are the product of asymmetry in pollen flow and not of differences in the abundances of morphs.

## Pollen flow and male reproductive success in pin and thrum morphs

Pin and thrum morphs display pollen grain size dimorphism with some degree of size overlap (Fig. 2). The highest overlap occurred in pollen grains with 31  $\mu$  (89% of overlap) and 32  $\mu$  (81% of overlap). Overlap in other categories was between 0-37% (Fig. 2). In the analyses of pollen flow within and between floral morphs, I assumed that pollen grains with diameters smaller than 31  $\mu$  arrived from pin flowers, and those with diameters larger than 32  $\mu$  arrived from thrum flowers. Pollen grains with sizes ranging from 31 to 32  $\mu$  were recorded as undetermined. Undetermined pollen grains were excluded from the probability and efficiency of disassortative pollination analyses.

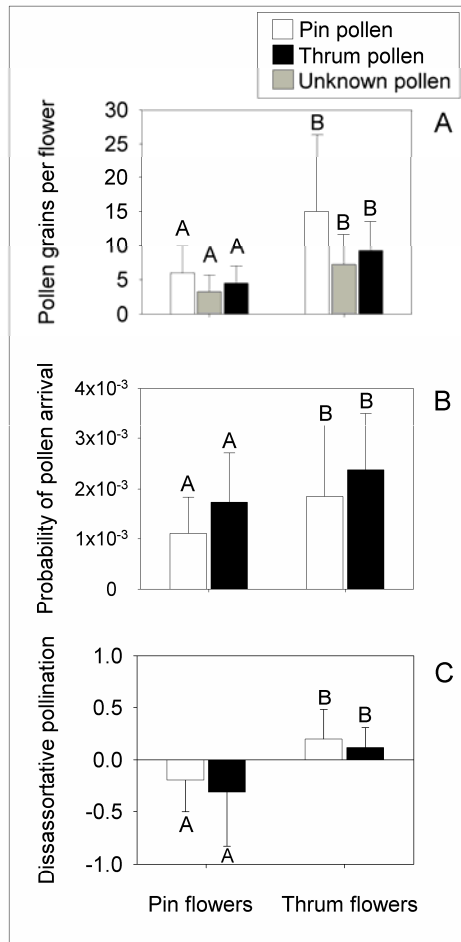


**Fig. 2.** Frequency distribution of pollen size for pin and thrum pollen grains in *A. lamarum*.

The total mean number of pollen grains arriving to pin pistils was lower than the number arriving to thrum pistils ( $H = 13.4$ ,  $df = 1$ ,  $P < 0.001$ , Fig. 3a). However, pistils of each floral morph received an equivalent number of pin, thrum, or undetermined pollen grains ( $H = 4.6$ ,  $df = 2$ ,  $P = 0.09$ , Fig 2a).

The probability of pin and thrum pollen arrival to thrum pistils was higher than the arrival to pin pistils ( $H = 4.0$ ,  $df = 1$ ,  $P < 0.04$ , Fig. 3b). There was no difference in the probability of arrival from different floral morphs within pin and thrum pistils ( $H = 3.1$ ,  $df = 1$ ,  $P = 0.07$ , Fig. 3b).

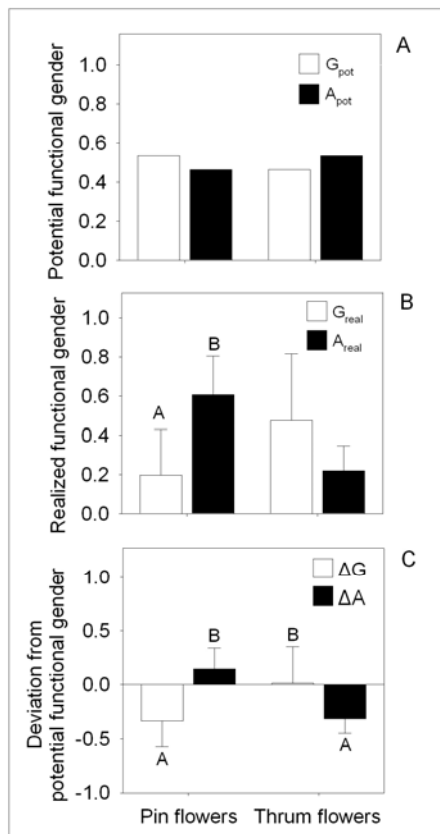
The estimate of disassortative pollination efficiency showed that pin flowers received less pollen, and thrum flowers received more pollen than expected at random ( $H = 12.1$ ,  $df = 1$ ,  $P < 0.001$ , Fig. 3c). Efficiency at receiving pin and thrum pollen was equivalent within both pin and thrum pistils ( $H = 0.32$ ,  $df = 1$ ,  $P = 0.57$ ).



**Fig. 3.** Pollen flow between pin and thrum morphs of *Arcytophyllum lavarum*. A. Number of pin, thrum and undetermined pollen grains in pin and thrum pistils. B. Probability of pin and thrum pollen arrival to pin and thrum pistils and C. disassortative pollination efficiency by pin and thrum pollen grains in pin and thrum flowers of *A. lavarum* (Mean  $\pm$  SD). Letters on the bars group similar categories.

Deviation of the realized from the potential functional gender

Estimates of plant sexuality based on ovule and pollen production showed that ovules and pollen made an equivalent contribution to the potential functional gender in both pin and thrum morphs (Fig. 4a, G test,  $G = 0.64$ ,  $P = 0.42$ ). Estimates of plant sexuality based on seed production and pollen donation suggested that pollen donation contributed to a higher proportion of the realized functional gender of pin flowers than of thrum flowers ( $U = 64$ ,  $N_G = 68$ ,  $N_A = 10$ ,  $P < 0.0001$  Fig. 4b). In the thrum morph, the contributions of pollen donation and seed production to the realized functional gender were equivalent. However, in this morph there was a trend to higher contribution by seeds to the realized functional gender ( $U = 144$ ,  $N_G = 47$ ,  $N_A = 10$ ,  $P = 0.054$  Fig. 4b).



**Fig. 4.** Plant sexuality in pin and thrum morphs of *Arcytophyllum lavarum*. A. Contribution of male and female functions to the potential functional gender. B. Contribution of male and female reproductive functions to the realized functional gender and C. deviation of the realized from the potential functional gender in pin and thrum morphs of *A. lavarum* (Mean  $\pm$  SD). Letters on the bars group similar categories.

The realized functional gender deviated from the potential functional gender in both pin and thrum floral morphs (Fig. 4c). In the pin morph, female contribution was

lower and male contribution was higher than that expected from its potential functional gender ( $U = 46$ ,  $N_G = 68$ ,  $N_A = 10$ ,  $P < 0.0001$ , Fig. 4c). In contrast, for the thrum morph female function contributed more and male function less than expected to its potential functional gender ( $U = 128$ ,  $N_G = 47$ ,  $N_A = 10$ ,  $P = 0.024$ , Fig. 4c).

## Discussion

Similar to other distylous species, the pin morph of *A. lavarum* produced smaller but more abundant pollen grains than the thrum morph (Wyatt and Hellwig 1979; Ornduff 1980; Pailler and Thompson 1997; Ree 1997; Naiki and Nagamasu 2003). Pin flowers of *A. lavarum* also produced on average one more ovule than thrum flowers. However, estimates of the potential functional gender suggested that in both floral morphs, ovules and pollen made equivalent contributions to the total of genes transmitted to the next generation. Equivalence of female reproductive potential between morphs was also supported by hand-pollination experiments. In flowers over-pollinated with pollen from the opposite morph, pin and thrum flowers of *A. lavarum* produced similar numbers of seeds (García-Robledo and Mora Submitted).

Regardless of the equivalent contribution of male and female functions to the potential functional gender in both floral morphs, thrum flowers produced more than twice as many seeds as pin flowers. In other distylous species, similar to *A. lavarum*, the thrum morph may play a major role in seed production (Dommeé *et al.* 1992; Ornelas *et al.* 2004). However, the general finding for most distylous species is that the pin morph may play a major role in seed production (Ree 1997; Lau and Bosque 2003).

Estimates of pollen flow and male reproductive success suggested that thrum flowers were more efficient at receiving both legitimate and illegitimate pollen but were less efficient as legitimate pollen donors. Therefore, pollen flow for thrum flowers was assortative (*i.e.* pollen arrival was more likely to pistils of other flowers of the same morph). Conversely, pin flowers were more efficient as legitimate pollen donors, displaying disassortative pollen flow (*i.e.* pollen arrival was more likely to flowers of the opposite morph). Disassortative pollination by only the pin morph produced asymmetric pollen flow, and a division of reproductive functions between morphs.

Asymmetry in pollen flow between morphs is not a rare phenomenon among distylous species. In emasculated flowers of *Psychotria suerrensis* (Rubiaceae), pin flowers are more efficient as pollen donors, and thrum flowers are more efficient as pollen receivers (Stone 1995). However, differences in pollen flow were not reflected in fruit production which was similar in both floral morphs (Stone 1995). Pollen flow in emasculated flowers of *Palicourea fendleri* is also similar to that reported for *A. lamarum*, *i.e.* a higher legitimate pollen flow from pin to thrum flowers and higher efficiency of the thrum morph as pollen receiver of both pin and thrum pollen (Lau and Bosque 2003).

The main conclusion of this research is that pollen flow promoted a divergence between the potential and the realized functional genders in both morphs of a distylous species. Distyly is suggested as an ancestral state in the evolution of separate sexes in plants, and there is evidence that a shift to dioecy from distyly occurred in several families independently (Ross 1982). In this evolutionary pathway, pollinators may promote an asymmetry in pollen flow that disrupts the complementarity of pollen transfer between the two morphs. As a consequence, the realized functional gender will diverge

from the potential functional gender and the floral morphs will gradually specialize as either male or female (Charlesworth and Charlesworth 1979; Charlesworth 1999).

In *A. lamarum*, asymmetric pollen flow enhanced pollen donation in pin flowers, and therefore seed production in thrum flowers. Pollen flow in *A. lamarum* also promoted a fundamental process required for the evolution of dioecy: a divergence between the potential functional gender and the realized functional gender. These results support the hypothesis that if pollinators consistently promote asymmetric pollen flow between morphs over generations, it is possible that gender specialization may evolve to the extreme of dioecism from an original distylous condition.

**Acknowledgements** I specially thank Federico Valverde, Hilario Abarca and Eliecer Abarca (Estación Biológica Cerro de La Muerte) J. Mora and F. Kepfer for logistic support and F. Mora for assisting during part of the data collection. Image analyses were performed in the Auditory Neurobiology Laboratory, University of Miami and the Entomology Laboratory, University of Costa Rica. S. Tomchik and Dr. Z. (John) Lu provided invaluable advice during image analyses. Charlotte M. Taylor determined the *Arcytophyllum* species. I thank A. C. Villegas, advisor of this project at the Organization for Tropical Studies. Comments by S. Koptur and E. K. Kuprewicz improved this manuscript. Part of the equipment used in this research was provided by IDEA-WILD. This research was supported by the Organization for Tropical Studies – Glaxo fellowship for Latin–American researchers (Fund No.502) to CG. Laboratory and data analyses were supported by the Aldridge Assistantship and the J. McLamore Fellowship, - University of Miami, both to C.G. This project was honored with the Alwyn Gentry Award 2004, Association for Tropical Biology and Conservation.

## References

- Charlesworth D (1999) Theories of the evolution of dioecy. In: Gender and sexual dimorphism in flowering plants (eds) Geber MA, Dawson TE and Delph LF Springer, Berlin, p. 33–60
- Charlesworth D and Charlesworth B (1979) A model for the evolution of distyly. *Am Nat*, 114, 467-498
- Darwin C (1896) The different forms of flowers on plants of the same species. D. Appleton and Company, New York.
- Devlin B and Stephenson AG (1987) Sexual variations among plants of a perfect-flowered species. *Am Nat*, 130, 199-218
- Domme B, Thompson JD and Cristini F (1992) Distyly in *Jasminum fruticans*. An hypothesis for optimal pollination based on variation in intrafloral ecology. *B Soc Bot Fr-Lett*, 139, 223-234
- Freeman DC, Doust L, El-Keblawy A, Miglia KJ and McArthur. ED (1997) Sexual specialization and inbreeding avoidance in the evolution of dioecy. *Bot Rev*, 63, 65–92
- Ganders FR (1979) The biology of heterostyly. *New Zeal J Bot*, 17, 607-635
- García-Robledo C and Mora F (Submitted) Pollination Biology and the impact of floral display, pollen donors and distyly on seed production in *Arcytophyllum lavarum* (Rubiaceae). *Plant Biol*, --, \_\_-\_\_
- Horovitz A (1978) Is the hermaphrodite flowering plant equisexual? *Am J Bot*, 65, 485-486
- Husband BC and Barrett SCH (1992) Pollinator visitation in populations of tristylous *Eichhornia paniculata* in Northeastern Brazil. *Oecologia*, 89, 365-371

- Kohn JR and Barrett SCH (1992a) Experimental studies on the functional significance of heterostyly. *Evolution*, 46, 43-55
- Kohn JR and Barrett SCH (1992b) Floral manipulation reveal the cause of male fitness variation in experimental populations of *Eichornia paniculata* (Pontederiaceae). *Funct Ecol*, 6, 590-595
- Lau P and Bosque C (2003) Pollen flow in the distylous *Palicourea fendleri* (Rubiaceae): an experimental test of the Disassortative Pollen Flow Hypothesis. *Oecologia*, 135, 593-600
- Lloyd DG (1980) Sexual strategies in plants III. A quantitative method for describing the gender of plants. *New Zeal J Bot*, 18, 103-108
- Lloyd DG and Webb CJ (1992) The selection of heterostyly. In: *Monographs in theoretical and applied genetics 15: Evolution and function of heterostyly* (ed Barrett SCH Springer-Verlag, New York, pp. 179-207
- Luteyn JL (1999) *Paramos : a checklist of plant diversity, geographical distribution, and botanical literature*. New York Botanical Garden Press, Bronx, N.Y.
- Naiki A and Nagamasu H (2003) Distyly and pollen dimorphism in *Damnacanthus* (Rubiaceae). *J Plant Res*, 116, 105-113
- Oneil P (1992) Variation in male and female reproductive success among floral morphs in the tristylous plant *Lythrum salicaria* (Lythraceae). *Am J Bot*, 79, 1024-1030
- Ornduff R (1980) Heterostyly, population composition, and pollen flow in *Hedyotis caerulea*. *Am J Bot*, 67, 95-103
- Ornelas JF, Gonzalez C, Jimenez L, Lara C and Martinez AJ (2004) Reproductive ecology of distylous *Palicourea padifolia* (Rubiaceae) in a tropical montane cloud forest. II. Attracting and rewarding mutualistic and antagonistic visitors. *Am J Bot*, 91, 1061-1069

- Pailler T and Thompson JD (1997) Distyly and variation in heteromorphic incompatibility in *Gaertnera vaginata* (Rubiaceae) endemic to La Reunion Island. *Am J Bot*, 84, 315-327
- Pannell JR and Verdu M (2006) The evolution of gender specialisation from dimorphic hermaphroditism: paths from heterodichogamy to gynodioecy and androdioecy. *Evolution*, 60, 660-673
- Ree RH (1997) Pollen flow, fecundity, and the adaptive significance of heterostyly in *Palicourea padifolia* (Rubiaceae). *Biotropica*, 29, 298-308
- Ross MD (1982) Five evolutionary pathways to subdioecy. *Am Nat*, 119, 297-318
- Sokal RR and Rohlf FJ (1995) *Biometry : the principles and practice of statistics in biological research*. 3rd edn. W.H. Freeman, New York.
- Stone JL (1995) Pollen donation patterns in a tropical distylous shrub (*Psychotria suerrensis* Rubiaceae). *Am J Bot*, 82, 1390-1398
- Stone JL and Thomson JD (1994) The evolution of distyly - Pollen transfer in artificial flowers. *Evolution*, 48, 1595-1606
- Weber H (1959) *Los páramos de Costa Rica y su connotación fitogeográfica con los Andes suramericanos*. Instituto Geográfico, San José, Costa Rica.
- Wolfe LM and Barrett SCH (1987) Pollinator foraging behavior and pollen collection on the floral morphs of tristylous *Pontederia cordata* L. *Oecologia*, 74, 347-351
- Wyatt R and Hellwig RL (1979) Factors determining fruit set in heterostylous bluets, *Houstonia caerulea* (Rubiaceae). *Syst Bot*, 4, 103-114