

**Pollination Biology and the Impact of Floral Offer, Pollen Donor Availability and
Floral Morph on Seed Production in the Distylous Herb *Arcytophyllum lavarum*
(Rubiaceae)**

Carlos García–Robledo^{1,3} and Floria Mora²

¹Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, Florida 33124–0421

²Escuela de Biología, Universidad de Costa Rica, San Pedro Montes de Oca, Costa Rica.

³ Corresponding author; e-mail: carlos@bio.miami.edu

ABSTRACT

In animal-pollinated plants, two factors affecting pollen flow and seed production are changes in the number of flowers available for pollinators and the availability of compatible mates. Changes in floral offer may affect the number of pollinator visits. The availability of compatible mates will affect the probability of legitimate pollination and seed production. Distyly is a floral polymorphism where long-styled (pin) and short-styled (thrum) floral morphs occur among different individuals. Distylous plants frequently exhibit self and intra-morph incompatibility. Therefore changes in morph abundance directly affect the arrival of compatible pollen to the stigmas. Floral morph by itself may also affect female reproductive success because floral morphs may display differences in seed production. We explored the effects of floral offer, availability of neighboring compatible mates and floral morph on seed production in the distylous herb *Arcytophyllum lavarum*. We found that floral offer does not affect the mean number of seeds produced per flower. There is also no effect of the proportion of neighboring legitimate pollen donors on seed production in pin or thrum flowers. However, floral morphs differed in their female reproductive success; the thrum morph produced more seeds. Hand pollination experiments suggest that differences in seed production between morphs are the result of pollen limitation. Higher seed production in thrum flowers can be a consequence of higher availability of pollen donors in the population, or higher efficiency of the pin morph as pollen donor.

Key words: *Arcytophyllum lavarum*; *Costa Rica*; *distyly*; *floral offer*; *Páramo*; *pollen donors*

IN ANIMAL POLLINATED PLANTS, GENE FLOW THROUGH POLLEN DONATION (hereafter pollen flow) is a process that strongly affects plant female reproductive success. A major factor affecting pollen flow is floral display, *i.e.* the number of flowers simultaneously displayed to pollinators by a plant (Schmitt 1983, Sih & Baltus 1987, Klinkhamer & Dejong 1990, Menges 1991, Aizen & Feinsinger 1994, Aizen 1997). The effect of floral display on pollen flow and female reproductive success varies among pollination systems (Brunet & Sweet 2006, Ghazoul 2006). Some studies have found that small floral displays are less attractive to pollinators, resulting in reduced pollinator visits, pollen flow and fruit production (Sih & Baltus 1987, Menges 1991, Aizen & Feinsinger 1994). In other pollination systems pollinator visits increase as floral display size decreases, because the pollinators may aggregate in the few available flowers (García-Robledo *et al.* 2004;2005). In some plant species floral display does not affect pollinator attraction nor plant female reproductive success (Aizen 1997, Kunin 1997, Albert *et al.* 2001).

An alternative effect of floral display on pollen flow and female reproductive success is that fruit or seed set may be high at an intermediate number of open flowers, when pollen arrival per flower is maximized (Geber 1985, Sih & Baltus 1987, Andersson 1988, Schmid-Hempel & Speiser 1988, Menges 1991, Aizen & Feinsinger 1994, Aizen 1997).

In addition to floral display, another factor affecting female reproductive success in plants is the availability of genetically compatible mates within the population (Wyatt & Hellwig 1979, Ishihama *et al.* 2006). One type of system in which compatible mates can be easily monitored is heterostyly, a floral polymorphism characterized by the reciprocal positioning between stigmas and anthers in different flowers. Heterostylous

species present two (distyly) or three (tristyly) floral morphs (Ganders 1979, Kohn & Barrett 1992, Lloyd & Webb 1992). Heterostylous plants frequently exhibit self and intra-morph incompatibility, therefore changes in morph relative abundance will be reflected in the probability of pollen arrival to the stigmas of genetically compatible mates (Charlesworth & Charlesworth 1979, Ganders 1979, Ree 1997).

Seed production in heterostylous plants may vary among floral morphs, one of the morphs contributing more to the total seed production of the population (Wolfe & Barrett 1987, Husband & Barrett 1992, Ree 1997). Differences in seed production between morphs may be a consequence of morphological differences in ovule or pollen production (Stone 1995;1996), intrinsic genetic factors such as partial male sterility in one of the floral morphs (Dominguez *et al.* 1997, Del-Carlo and Buzato 2006), or extrinsic factors such as higher availability of genetically compatible mates or asymmetry in pollen flow between morphs (Stone 1995;1996).

Arcytophyllum lavarum (Rubiaceae) is an endemic plant of the Central American paramos, distributed from the north of Panama to Costa Rica (Mena 1990). This plant is distylous, therefore two floral morphs are present, long and short-styled flowers, here after named as pin and thrum floral morphs (Fig. 1). *Arcytophyllum lavarum* grows in patches scattered above rock outcrops. *Arcytophyllum lavarum* patches may be composed by one or more individuals. Inflorescences are terminal, and reach a maximum 40 cm of height. Pin, thrum, or both floral morphs could makeup the flowers offered to pollinators within each patch. We refer to patches composed of both pin and thrum flowers as mixed patches.

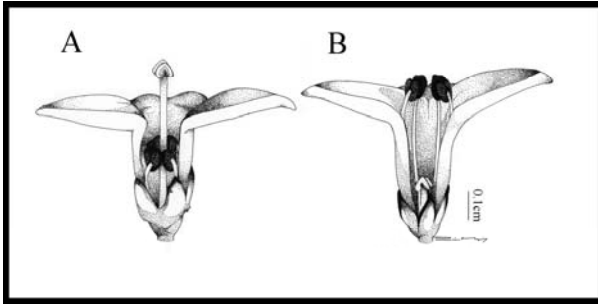


FIGURE 1. Longitudinal section of floral morphs in *Arcytophyllum lavarum*. a) pin, b) thrum. (Illustrations by Erin Kuprewicz).

Although the flowers offered within patches may belong to more than one individual, in this paper we assume that pollinator visits and seed production may be affected by the number of flowers within each patch in a similar fashion as suggested for floral displays in individual plants.

The variation in the number of flowers among patches, as well as variation in the proportion of floral morphs within mixed patches, makes *A. lavarum* a good system to understand how changes in the number of flowers available for pollinators within each patch (hereafter floral offer) and the availability of neighboring genetically compatible mates (represented as the relative abundance of floral morphs within a patch) may affect seed production in pin and thrum morphs of distylous plant. To understand how these factors affect seed production in *A. lavarum*, the objectives of this research are: (1) Report information fundamental to understanding this pollination system such as (a) differences in floral morphology (b) identity of flower visitors (c) levels of self-compatibility and (d) relative spatial distribution and abundance of pin, thrum and mixed patches. (2) To explore the potential effect of floral offer on *A. lavarum* seed production in pin and thrum morphs. (3) To determine in mixed patches how changes in the proportions of floral morphs affect seed production in pin and thrum flowers and (4) to determine whether pin and thrum flowers differ in seed production.

METHODS

STUDY SITE.—This study was conducted between January and August, 2003 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), as reported by the Cerro de La Muerte meteorological station (F. Valverde unpubl. data). This area is covered with the typical vegetation of a grass paramo (see detailed description by Weber 1959). Abundant rock outcrops are present, on which patches of the distylous Rubiaceae *Arcytophyllum lavarum* are very frequent.

FLORAL MORPHOLOGY.—We tested for differences in the level of reciprocal herkogamy and the number of ovules and pollen grains between floral morphs. We collected 101 pin and 104 thrum flowers, each one from a different patch, and measured the following floral attributes: corolla length, corolla diameter, corolla tube diameter, anther length, anther height, ovary length, style length, and stigma–anther separation (Fig. 2). These floral characteristics were measured with calipers, precise near to 0.01 mm.

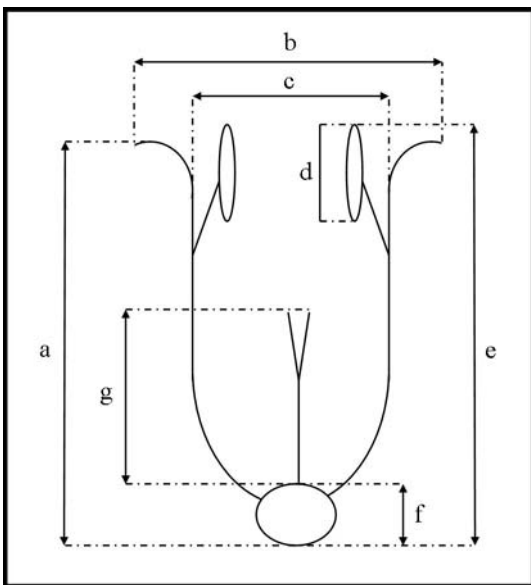


FIGURE 2 *Floral measurements performed in pin and thrum flowers of A. lavarum. a) corolla length b) corolla diameter c) corolla tube diameter d) anther length e) anther height f) ovary length g) style length.*

To determine if there is a difference in the numbers of ovules produced by pin and thrum flowers, we collected from different patches another 40 flowers of each floral morph. Carpels were dissected, recording the number of ovules per flower. Similarly, to determine differences in the number of pollen grains produced by pin and thrum flowers we additionally collected 15 recently-opened flowers of each floral morph. For each flower, one of the four anthers was selected, 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.

To examine differences in pollen size between floral morphs, we dissected indehiscent anthers from 18 pin and 20 thrum flowers collected from different patches. Pictures of the pollen grains were obtained using a digital camera attached to a light microscope at 160 X magnification. Using the digital images, we measured the diameters of at least 114 pollen grains per flower with the application Sigmascan Image® (Link *et al.* 1994) (Min – Max. number of pollen grains measured per flower = 114 – 444, $N_{\text{pin grains}} = 3840$, $N_{\text{thrum grains}} = 4548$). We tested for differences in each morphological attribute between floral morphs by using *t* tests..

FLORAL BIOLOGY AND BREEDING SYSTEM.—To test for differences in nectar production among morphs, we measured the volume of nectar produced per flower in 5 to 11 flowers per patch in 10 pin and 12 thrum patches ($N_{\text{pin flowers}} = 64$, $N_{\text{thrum flowers}} = 87$). Nectar was collected 24 h. after anthesis from previously bagged flowers, using 1 μl micro-pipettes. Volume was calculated using calipers precise to the nearest 0.01 mm. Differences

between the mean volume of nectar produced by each morph was determined by using a t test.

To determine whether or not *Arcytophyllum lavarum* is self and intramorph-incompatible we performed hand pollination experiments. To exclude flower visitors, we covered closed pin and thrum flowers with organdy bags. When the flowers opened, we performed the following crosses: (1) self-pollination, (2) cross-pollination with a flower from the opposite morph, and (3) pollination with pollen from a different patch of the same morph. Organdy bags were removed when the corollas and pistils wilted. Flowers were over-pollinated, covering the entire stigma with all the pollen produced by one anther. The pollen donors used for cross-pollination with the opposite morph and pollination with pollen from the same morph, were located at least 100 m away from the hand-pollinated flowers, in order to reduce the possibility of using pollen from the same genet.

FLOWER VISITORS.— During five consecutive days, from 8:00 to 13:00 h. we performed observations *ad libitum* of all *A. lavarum* flower visitors, monitoring several patches and collecting at least one individual per species for further identification. Some of the flower visitors are difficult to observe due to their small size (*i.e.* length between 1–5 mm). To record small insect visitors, we collected in 10 pin and 10 thrum patches all insects within the corolla tubes (Vouchers: Floria Mora–Kepfer s.n 2003, Entomological collections: UCR and INBIO).

SPATIAL DISTRIBUTION AND PATCH ABUNDANCE.—To determine the abundance and spatial distribution of pin, thrum and mixed patches of *A. lavarum* in Cerro de La Muerte, we recorded the position, floral morph composition, and the number of flowers for each

patch within a rectangular plot of 100 X 150 m. Position of each patch was expressed in Cartesian coordinates. This spatial position was included in further statistical analyses when required to correct for potential spatial-autocorrelation.

Differences in the number of patch types (*i.e.* pin, thrum, or mixed) were determined by using a chi-square test. To determine if similar patch types (*i.e.* pin, thrum and mixed) are aggregated or randomly distributed, we recorded the patch type of the nearest neighbor for each patch. We contrasted these results against the null hypothesis of equal probability of neighborhood with any patch type by using a contingency table.

We explore for differences in the size of floral offer among patch types as well as in their distance to the nearest legitimate pollen donor. Differences among patch types were determined by using one-way ANOVA tests.

To determine if the pin:thrum flower ratio within mixed patches was skewed to one of the floral morphs, we performed a matched-pairs t-test. Differences in the total number of pin and thrum flowers inside the plot were tested by a chi-square test.

EFFECT OF FLORAL OFFER ON SEED PRODUCTION.— To explore the effect of floral offer on the seed production in *A. lamarum*, we recorded the number of flowers for each patch within the 100 X 150 m plot. In pin and thrum patches with less than 10 inflorescences, and for all mixed patches, we marked all flowers with a small point of paint on the pedicel. In larger pin and thrum patches, we marked all flowers within 10 previously-selected inflorescences. Forty-five days later, we collected near-ripe fruits, and counted the number of seeds. We measured for each patch the mean number of seeds produced by the previously marked flowers, assigning a value of zero to aborted flowers.

An alternative design would be to select patches separated by a minimum distance at which it can be assumed independence among patches. However, this approach was not feasible in the population of *A. lavarum* at Cerro Buenavista because (1) the number of patches scattered over the rock outcrops is small and (2) the distance among patches is small (*see* results for information on distances among patches). Nevertheless, the use of this plot is an accurate estimate of seed production because it included at least 1/3 of the total of patches present in this population of *A. lavarum*.

The main issue of our approach is that inside the plot seed production in a given patch can be affected by the presence of neighboring patches. This violation to the assumption of independence must be corrected in the statistical analyses by including the effects of spatial autocorrelation among patches (Legendre 1993).

To determine the distance at which adjacent patches displayed similar seed production, we performed spatial autocorrelations used the Cartesian coordinates and the estimate of seed production for each patch. This is an estimate of the distance required for independent sampling (Cliff and Ord 1981). For these analyses, we grouped the patches selecting 5 m and 6 m lag distances for pin and thrum patches respectively. These lag distances maximized the number of patches per category, as required for the spatial autocorrelation analyses (Cliff and Ord 1981). The semivariance lag for pin and thrum patches were 117.2 and 138.7 m respectively. We used the correlation coefficient Moran's I to quantify the degree of spatial-autocorrelation between patches at each distance category (Cliff and Ord 1981).

The small sample size of mixed patches did not allow us to test for spatial-autocorrelation. Therefore in the following analyses we assumed that the mixed patches

are spatially-autocorrelated at the maximum distance calculated for pin and thrum patches.

We corrected the effect of spatial-autocorrelation on seed production among patches by the Spatial Variate Differencing method (Cliff and Ord 1981). This method uses the spatial position of each patch and the connectivity distance estimated in the spatial-autocorrelations to filter out the spatial structure present in the data (Cliff and Ord 1981). The corrected data were used in the following parametric analyses.

To examine whether a linear relationship exists between patch size and seed production, we performed a linear regression for pin, thrum, and mixed patches between the floral offer and the corrected mean number of seeds produced per flower. To determine if seed production is maximized at an intermediate floral offer, we performed quadratic regressions.

AVAILABILITY OF COMPATIBLE MATES AND SEED PRODUCTION.—To explore an effect of changes in the availability of legitimate mates on the mean number of seeds produced by pin and thrum flowers, we selected all flowers within 16 mixed patches. In each patch, we recorded the number of flowers that set fruit, and the number of seeds produced per flower. Spatial autocorrelation was corrected by the method of Spatial Variate Differencing (Cliff and Ord 1981). The relationship between pin/thrum morph relative abundance within patches, and the mean seed production in each floral morph were tested with lineal regressions.

EFFECT OF FLORAL MORPH ON SEED PRODUCTION.— To determine if flowers within pin, thrum or mixed patches display differences in their seed production, for each patch within

the 100 X 150 m plot we recorded the number of flowers aborted and the mean seed production per flower (N pin patches =69, N thrum patches= 47, N mixed patches = 16).

Differences in the percentage of flowers aborted, and the mean number of seeds produced per flower among patch types were tested with a Spatially Adjusted ANOVA model (Griffith 1978). The percent of flower aborted were arcsin transformed to fulfill the assumption of normality. The maximum autocorrelation distance included in this analysis was selected from a spatial autocorrelation among all the patches within the plot (Lag Distance = 6, Semivariance lag = 138.7 m).

To test for differences in seed production between morphs in mixed patches, we recorded the number of seeds produced by pin and thrum flowers. We tested for differences in seed production between morphs in mixed patches using a matched-pairs t-test.

We additionally tested for differences in seed production between morphs, by selecting pairs of pin and thrum patches with equivalent number of flowers. The objective of selecting patches with equivalent size was to exclude *a priori* unknown effects of patch size on seed production. We selected 20 pairs of pin and thrum patches, each pair with equivalent number of flowers. The distance between selected patches was between 15 - 25 m. The number of flowers within patch pairs ranged from 4 to 47 flowers. We collected the near mature fruits before capsules dehisced, and counted the number of seeds produced. Seed production is not spatially autocorrelated at the selected distance among patches (*see* results) therefore we compared the proportion of aborted flowers within patch pairs, and the mean number of seeds produced per patch using Matched-Pairs t-tests.

RESULTS

FLORAL MORPHOLOGY.—In addition to the obvious differences in pistil and anther lengths, pin and thrum flowers of *A. lavarum* differ in other morphological characteristics. The corolla length and the corolla tube diameter are larger in thrum flowers (Fig. 1, Table 1). In this population, the lengths of some pistils and anthers overlap within and between floral morphs (Fig. 3a and b). Stigma–anther separation divides floral morphs into two discrete groups (Fig. 3c). Pin flowers produce an average of 1.5 more ovules than thrum flowers (Table 1). Pin flowers produce more pollen grains, but the pollen size is smaller in pin than in thrum flowers (Table 1). There is no difference between morphs in corolla diameter, anther length and ovary length (Table 1).

TABLE 1. Differences of floral attributes between pin and thrum flowers in *A. lavarum*.

Floral attribute	Pin		Thrum		<i>t</i>	<i>P</i>
	Mean	SD	Mean	SD		
Corolla length (cm) ^a	0.42	0.07	0.45	0.06	-3.712	< 0.001
Corolla diameter (cm) ^a	0.55	0.07	0.56	0.09	-0.666	0.51
Corolla tube diameter (cm) ^a	0.11	0.03	0.15	0.03	-7.552	< 0.001
Anther length (cm) ^a	0.06	0.02	0.06	0.02	0.641	0.52
Anther height (cm) ^a	0.35	0.07	0.56	0.08	-20.055	< 0.001
Ovary length (cm) ^a	0.08	0.05	0.09	0.04	-0.831	0.40
Style length (cm) ^a	0.50	0.08	0.27	0.05	24.776	< 0.001
No. of ovules per flower ^b	11.2	2.3	9.7	2.6	2.724	0.008
No. of pollen grains per flower ^c	5390.5	1140.7	3856	835.3	4.203	< 0.001
Pollen size (μm) ^d	28.6	1.9	33.3	1.6	-8.101	< 0.001
Nectar production (μl)	0.04	0.05	0.03	0.02	0.523	0.607

Sample size: ^a 104 pin and 100 thrum flowers, *df* = 202, ^b 40 pin and 40 thrum ^c, *df* = 78, 15 pin and 15 thrum flowers ^d, *df* = 28. 18 pin and 20 thrum flowers. All flowers were collected in different patches.

FLORAL BIOLOGY AND BREEDING SYSTEM.— Inflorescences of *A. lavarum* may offer 1–12 flowers simultaneously to pollinators (Mean ± SD = 2.7 ± 1.7, *N* = 1023 inflorescences). Flowers begin to open in the morning and anthers can shed pollen

immediately after the flower opens, or during the next day. Nectar production was equivalent between both floral morphs (Table 1). We were not able to test differences in nectar concentration due to the low volumes of nectar produced per flower.

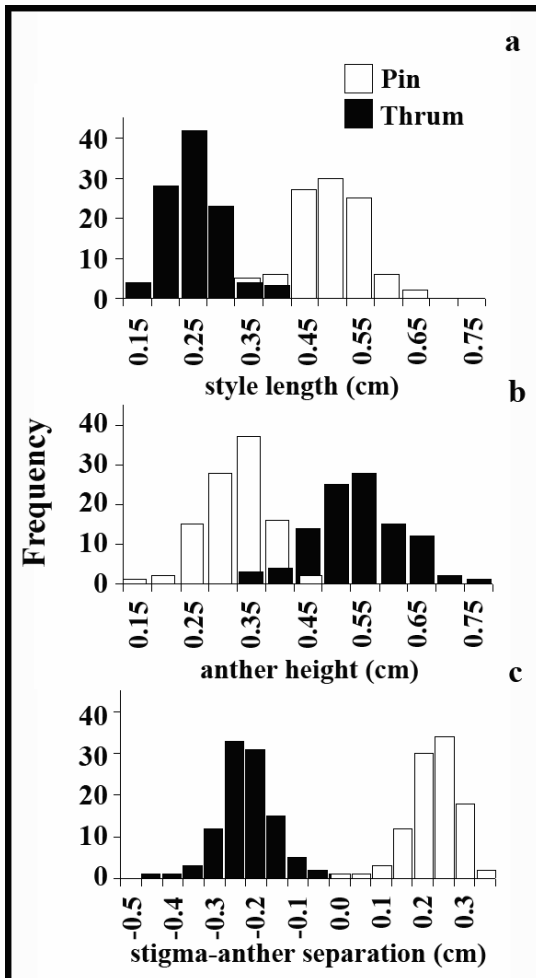


FIGURE 3. Frequency distribution of a) style length b) anther height and c) stigma–anther separation in pin and thrum morphs from a population of *A. lavarum*.

After the second day of flower opening, the pistils begin to turn dark brown, and the petals start to fade. If pollination was successful, the ovary starts to grow and its color changes from pale green to red. Fruits develop completely in ca. 1.5 – 2 mo, then the capsules open and wind–dispersed seeds are shed. Fruits may produce 1 – 13 seeds (Mean \pm SD = 4.3 \pm 1.8, N = 462 fruits).

In the experiments of self, cross and intra-morph hand pollination, only inter-morph crosses set fruit (Table 2). For these inter-morph crosses, 25 percent of pin flowers and 30 percent of thrum flowers aborted (Table 2).

TABLE 2. Results from self and cross artificial pollinations in *Arcytophyllum lavarum* (n = no. of flowers, Number of patches = 11)

Cross	n	Percent of flowers that set fruit
Pin X Pin	17	0
Pin X Thrum	24	75
Pin X Self pollination	22	0
Thrum X Pin	17	70
Thrum X Thrum	14	0
Thrum X Self pollination	16	0

There was no difference between floral morphs in the number of seeds produced by hand-pollinated flowers (Mean \pm SD $_{pin}$ = 4.5 \pm 3.8, Mean \pm SD $_{thrum}$ = 2.9 \pm 2.9, t = 1.3, df = 33, P = 0.2, $N_{pin\ s}$ = 22, $N_{thrum\ s}$ = 13). Breeding system results show that *A. lavarum* displays a self and morph-incompatible breeding system.

FLOWER VISITORS.— Flowers of *A. lavarum* were visited by small Diptera (1-5 mm) from the families Chironomidae, Scatopsidae and Sciaridae. Other small flower visitors include chrysomelid and curculionid beetles, aphids and thrips. These flower visitors stay inside the corolla feeding on pollen or nectar, walking or flying to nearby flowers.

chrysomelid beetles were observed feeding on pollen from anthers. Larvae of Geometridae feed on pollen and are also are pre-dispersal seed predators. The behavior of these flower visitors suggests that they might not play a major role in the pollen

transfer in *A. lavarum*. Syrphidae flies are also flower visitors of *A. lavarum*. Syrphids may fly frequently among patches visiting multiple flowers both within and among patches. This behavior suggests syrphids as the most likely pollinators of *A. lavarum*.

SPATIAL DISTRIBUTION OF PATCHES AND MORPH RATIO.— Pin patches were the most abundant, followed by thrum and mixed patches respectively ($\chi^2 = 33.13$, $df = 2$, $N_{\text{pin}} = 70$, $N_{\text{thrum}} = 47$, $N_{\text{mixed}} = 16$, $P < 0.001$). However, the floral identity of the nearest patch (*i.e.* pin, thrum or mixed) does not deviate from that expected at random ($\chi^2 = 4.1$, $df = 4$, $N = 133$, $P = 0.38$). Therefore, in spite of differences in patch abundance among patch types, floral morphs are not spatially aggregated.

Mean distance to the nearest potential legitimate pollen donor was equivalent for pin, thrum and mixed patches ($F = 1.5$, $df = 2$, $N = 133$, $P = 0.21$, Mean \pm SD: pin = 2.0 ± 1.4 m, thrum = 2.6 ± 3.0 m, mixed = 1.6 ± 1.5 m). The mean number of flowers per patch was also similar among patch types ($F = 1.12$, $df = 2$, $N = 133$, $P = 0.327$, $N_{\text{pin}} = 70$, Mean \pm SD = 19.8 ± 22.5 ; $N_{\text{thrum}} = 47$ Mean \pm SD = 19.0 ± 43.3 ; $N_{\text{mixed}} = 16$ Mean \pm SD = 32.0 ± 24.6).

The pin:thrum flower ratio in mixed patches is not skewed to any floral morph ($t = 0.602$, $df = 15$, $N = 16$, $P = 0.55$) and ranges from 1:23 to 13:1. When comparing the total number of flowers from each morph, pin flowers were more abundant ($\chi^2 = 111.8$, $df = 1$, $N_{\text{pin total}} = 1676$, $N_{\text{thrum total}} = 1117$, $P < 0.001$).

SPATIAL AUTOCORRELATION OF SEED PRODUCTION. — Seed production among pin patches was similar at distances closer than 5 m. However, seed production in pin patches was opposite at distances between 25-30 m (Min - Max Nearest Neighbor = 0.12 – 12.65 m, Fig. 4). Seed production in thrum patches was correlated at a longer distance than pin

patches (6-12 m. Fig. 4). Thrum patches displayed opposite patterns of seed production at 30 – 36 m, as suggested by a significant negative autocorrelation (Min - Max Nearest Neighbor = 0.67 – 20.62 m, Fig. 4).

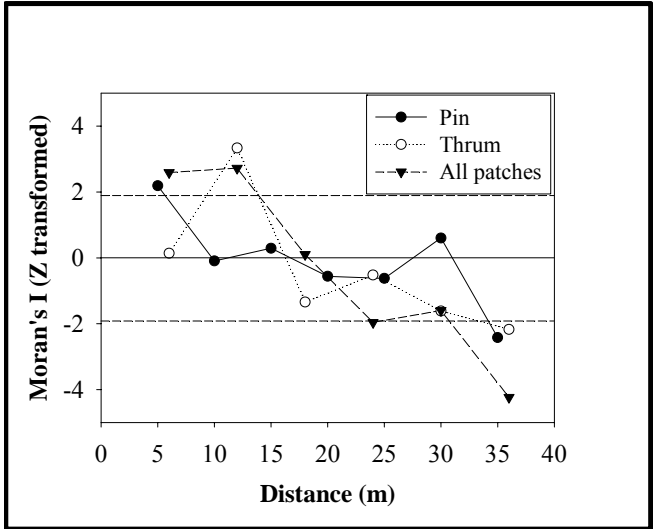


FIGURE 4. Changes of the standardized Moran's I statistic for seed production in pin, thrum and all patches (mixed patches included) at different distances. Dashed lines indicate $\pm 95\%$ Confidence Interval. Dots outside of the confidence intervals represent the distances at which is present a significant spatial autocorrelation ($P < 0.05$).

Pin, thrum and mixed patches had a similar seed production when closer than 12 m (Fig. 4). All the patches displayed a negative autocorrelation at 30 – 36 m (Min - Max Nearest Neighbor = .0.12 – 17.54, Fig. 4). These results were included in the regression and Spatial ANOVA analyses to correct for spatial autocorrelation

TABLE 3. Regression analyses for the effect of the number of flowers per patch on seed production. Data was corrected for spatial autocorrelation using the Spatial Variate Differencing method (Cliff and Ord 1981).

Independent variable	Regression model	F	df	r ²	P
Pin patches	Linear	1.99	1, 67	0.014	0.16
	Quadratic	1.08	2, 66	0.032	0.34
Thrum patches	Linear	0.05	1, 45	0.01	0.51
	Quadratic	0.54	2, 44	0.54	0.59
Mixed patches	Linear	1.51	1, 14	0.1	0.24
	Quadratic	1.4	2, 13	0.179	0.27

EFFECT OF FLORAL OFFER AND MORPH RATIO ON SEED PRODUCTION.— For pin patches, we did not find any relationship between floral offer and the mean number of seeds produced per flower (Table 3). Similar results were obtained for thrum and mixed patches (Table 3). In mixed patches, we did not find any relationship between the pin/thrum morph ratio and the seed production for pin flowers (Table 4). Seed production in thrum flowers was also independent of the pin/thrum morph ratio within mixed patches (Table 4).

TABLE 4. Regression analyses for the effect of floral morph ratio in mixed patches on seed production. Data was corrected for spatial autocorrelation using the Spatial Variate Differencing method (Cliff and Ord 1981).

Independent variable	Seed production	<i>F</i>	df	r ²	<i>P</i>
Pin/Thrum morph ratio	From pin morph	0.437	1, 24	0.03	0.52
	From thrum morph	0.862	1, 14	0.058	0.37

EFFECT OF FLORAL MORPH ON SEED PRODUCTION.— Flower abortion and seed production differed among pin, thrum, and mixed patches. The proportion of aborted flowers in pin patches was higher than in thrum and mixed patches (Spatially Adjusted ANOVA: $F = 5.4$, $df = 2$, $N_{pin} = 69$, $N_{thrum} = 47$, $N_{mixed} = 16$, $P = 0.005$, Connectivity = 36 m, $\rho = -0.99$, Fig. 5a). Pin patches also produced a lower number of seeds per flower than thrum and mixed patches (Spatially Adjusted ANOVA: $F = 5.8$, $df = 2$, $N_{pin} = 69$, $N_{thrum} = 47$, $P < 0.003$, Connectivity = 36 m, $\rho = -0.99$, Figure 5b). Within mixed patches, pin flowers also displayed a lower seed production ($t = -3.61$, $df = 15$, $P = 0.003$, Fig. 4c). For pairs of pin and thrum patches with equivalent number of flowers, the proportion of flower abortion is equivalent between morphs ($t = 1.76$, $df = 19$, $P = 0.09$, Fig. 6a). However

seed production was also higher in the thrum morph ($t = -2.15$, $df = 19$, $P = 0.04$, Fig. 6b).

DISCUSSION

In *Arcytophyllum lavarum*, both pistil and anther heights overlap within floral morphs. The degree of overlap of pistils and anthers within floral morphs varies among distylous species, ranging from species in which no overlap is present, to species in which a reciprocal pistil:anther height occurs (Richards & Koptur 1993). Most frequently, pistil and anther heights display bimodal distributions, as occurs in *Arcytophyllum lavarum*.

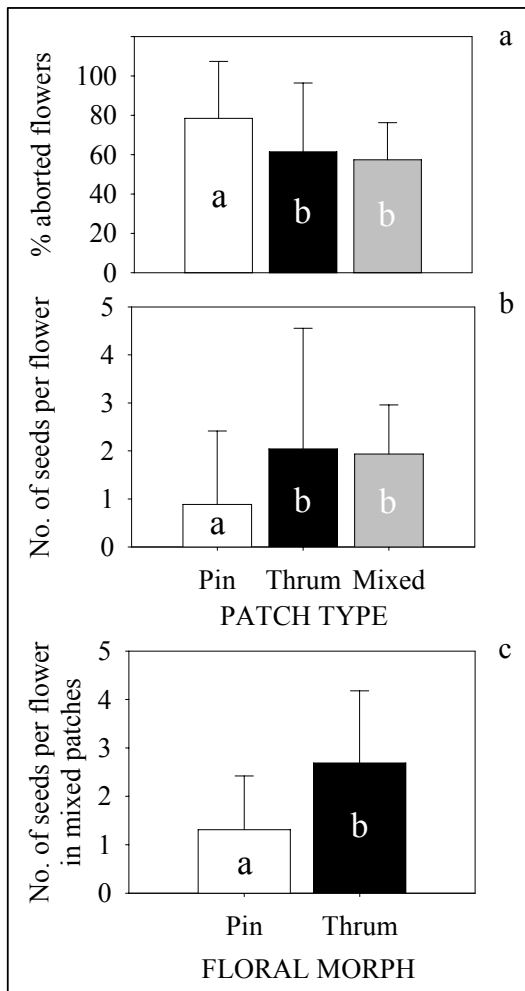


FIGURE 5. a) Flower abortion and b) mean number of seed produced per flower in pin, thrum and mixed patches. c) Female reproductive success for pin and thrum flowers within mixed patches of *A. lavarum* (Mean, SD). Letters group similar categories ($P < 0.05$).

In *A. lamarum* flowers, pin pollen grains are smaller and more abundant than thrum pollen grains, a pattern found in other distylous species (Wyatt & Hellwig 1979, Ornduff 1980, Pailler & Thompson 1997, Ree 1997, Naiki & Nagamasu 2003). Pin flowers of *A. lamarum* also produce one more ovule on average than thrum flowers. However, when over-pollinated in the hand-pollination experiments, pin and thrum flowers produced equivalent numbers of seeds. This suggests that although the pin morph is able to potentially produce one more seed than thrum flowers, pin and thrum morphs display equivalent female reproductive success when not limited by pollen arrival.

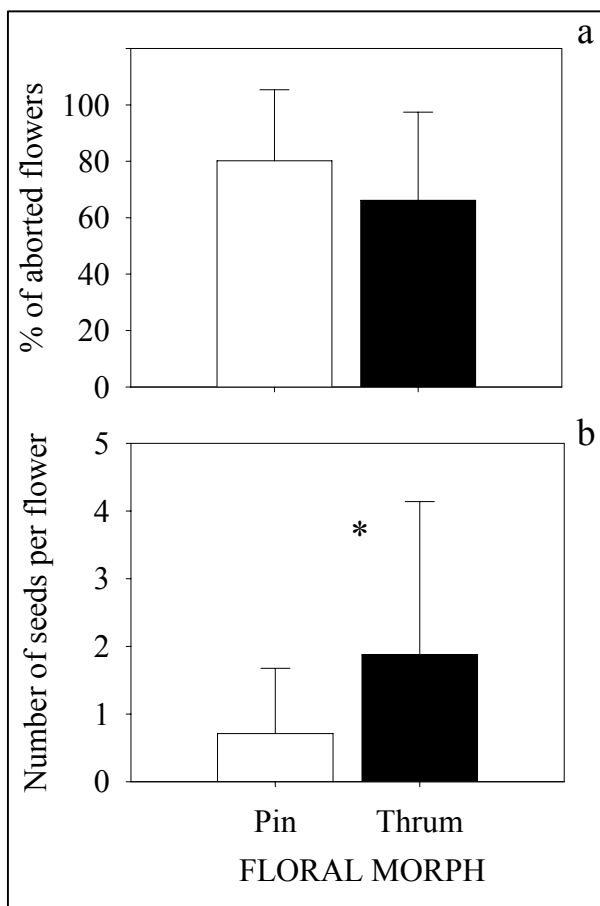


FIGURE 6. a) Flower abortion and b) mean number of seed produced per flower in pairs of pin and thrum patches of *A. lamarum* displaying equivalent number of flowers (Mean, SD) * $P < 0.05$.

Arcytophyllum lavarum displays a self and morph incompatible system similar to other heterostylous species (Ree 1997, De Castro & De Oliveira 2002, Ornelas *et al.* 2004a). In some other heterostylous species, however, the degree of morph compatibility is variable among floral morphs (Dulberger 1970, Opler 1975, Ornduff 1976, Barrett & Glover 1985, Dommee *et al.* 1992). The degree of morph compatibility may differ not only at the species level, but also among populations. For example, the distylous Rubiaceae *Palicourea petiolaris* has both morph compatible and incompatible populations along its geographical range (Sobrevila *et al.* 1983).

Changes in floral offer did not have an effect on seed production in pin, thrum and mixed patches of *A. lavarum*. This suggests that the pollen flow and arrival to pistils may not be sensitive to floral offer. However, as discussed next, pollen arrival is sensitive to floral morph identity. We found that within mixed patches of *A. lavarum*, a higher proportion of pollen donors did not produce an increase in seed production per flower in the opposite floral morph. In other pollination systems such as the distylous Rubiaceae *Houstonia caerulea* and the style-length dimorphic *Narcissus assoanus* (Amaryllidaceae), deviations from the 1:1 proportion between morphs may produce an increase in the female reproductive success of the less relatively abundant morph (Wyatt & Hellwig 1979, Thompson *et al.* 2003). In *A. lavarum*, the mean distance between mixed patches to potential donors is only 1.6 m. thus, even when the number of legitimate pollen donors within a patch is small, other potential donors are available at a very short distance.

In this population of *A. lavarum*, thrum flowers produced more seeds than pin flowers. Floral morphs in other distylous species may also display differences in seed

production. The general finding is that the pin morph may play a major role in seed production (Lloyd and Webb 1992, Ree 1997, Lau & Bosque 2003). However, in some species the pin morph may display higher efficiency as pollen donor (Stone 1995, Lau & Bosque 2003, Ornelas *et al.* 2004a), and the thrum morph may play a major role in seed production (Domme *et al.* 1992, Ornelas *et al.* 2004b).

In hand-pollination experiments, pin and thrum flowers produced equivalent numbers of seeds when not pollen limited. This suggests that higher female reproductive success in the thrum morph of *A. lamarum* is a consequence of pollen limitation. In this population, pin flowers are more abundant. Therefore, higher seed production in thrum flowers may be the result of more pin pollen available for legitimate pollination. It is also possible that pollen flow between morphs in *A. lamarum* is asymmetric, and pin flowers are more efficient delivering pollen to the thrum morph. Asymmetry in pollen flow has been reported in other distylous species such as the Rubiaceae *Psychotria suerrensii* (Rubiaceae), where pin flowers are more efficient pollen donors, and thrum flowers are more efficient as pollen receivers (Stone 1995). Pollen flow is also asymmetric in *Palicourea fendleri* (Rubiaceae), where flow is higher from pin to thrum flowers and thrum flowers are more efficient delivering pollen to flowers from the same morph (Lau & Bosque 2003).

Our results suggest that floral offer does not have an effect on the seed production of *A. lamarum*. The two floral morphs, however are playing different roles in seed production. Our current research is focusing on understanding the mechanisms that increase female reproductive success in the thrum morph. Future experiments on the pollen flow between floral morphs will elucidate if the higher female reproductive

success in thrum flowers is a product of a high number of genetically compatible pollen donors at the population level, or the result of higher efficiency of the pin morph as a pollen donor.

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