Pollination Biology and the Impact of Floral Display, Pollen Donors, and Distyly on Seed Production in *Arcytophyllum lavarum* (Rubiaceae)

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Abstract: In animal-pollinated plants, two factors affecting pollen flow and seed production are changes in floral display and the availability of compatible mates. Changes in floral display may affect the number of pollinator visits and 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 display, availability of neighboring compatible mates, and floral morph on seed production in the distylous herb *Arcytophyllum lavarum*. We found that floral display 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 and the thrum morph produced more seeds. Hand pollination experiments suggest that differences in seed production between morphs are the result of pollen limitation. Future research will elucidate if the higher seed production in thrum flowers is 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 display, Páramo, pollen donors.

Introduction

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 and Baltus, 1987; Klinkhamer and Dejong, 1990; Menges,

1991; Aizen and Feinsinger, 1994; Aizen, 1997). The effect of floral display on pollen flow and female reproductive success varies among pollination systems (Brunet and 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 and Baltus, 1987; Menges, 1991; Aizen and 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 with an intermediate number of open flowers, when pollen arrival per flower is maximized (Geber, 1985; Sih and Baltus, 1987; Andersson, 1988; Schmid-Hempel and Speiser, 1988; Menges,1991; Aizen and 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 and 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 and Barrett, 1992; Lloyd and 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 and 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 and Barrett, 1987; Husband and 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).
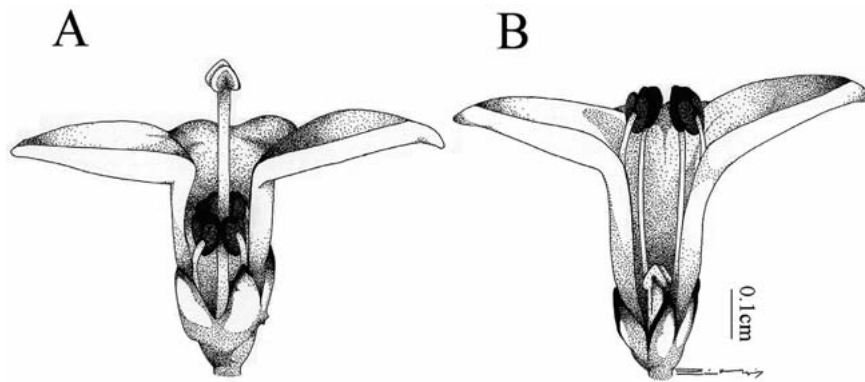
Fig. 1 Longitudinal section of floral morphs in *Arcytophyllum lavarum*. (**A**) Pin, (**B**) thrum. (Illustrations by Erin K. Kuprewicz.)

Arcytophyllum lavarum (Rubiaceae) is a perennial herb endemic to the Central American Páramo, distributed from the north of Panama to Costa Rica (Mena, 1990; Luteyn, 1999). This plant is distylous, therefore two floral morphs are present, long- and short-styled flowers, hereafter named pin and thrum floral morphs (Fig. **1**). *Arcytophyllum lavarum* grows in patches scattered above rock outcrops. Patches are composed of entangled ramets from one or more individuals. This makes it difficult to identify individual plants within each patch. However, patches are discrete units with roots and ramets not interconnected.

Inflorescences are terminal and reach a maximum height of 40 cm from the ground. Pin, thrum, or both floral morphs could compose the flowers offered to pollinators within each patch. We refer to patches composed of both pin and thrum flowers as mixed patches. Although the flowers displayed within patches may belong to more than one individual, pollinator attraction and seed production will be affected by the number of flowers within each patch in a similar fashion to that 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 and the availability of neighbouring genetically compatible mates (represented as the relative abundance of floral morphs within a patch) may affect seed production in pin and thrum morphs of a distylous plant.

To understand some aspects of the pollination biology of *A. lavarum,* as well as how floral display, the availability of pollen donors and distyly affect seed production, the objectives of this research are: (1) to 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 display 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 (9°33′N, 83°45′W). 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 described as a grass páramo (Weber, 1959; Luteyn, 1999). 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 to 0.01 mm.

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 undehisced 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 × 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.,
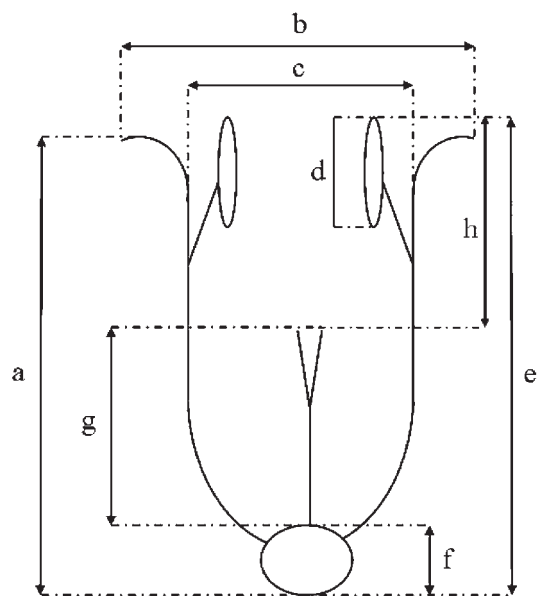
Fig. 2 Floral measurements performed in pin and thrum flowers of *A. lavarum*. a, corolla length; b, corolla diameter; c, corolla tube diameter; d, anther length; e, anther height; f, ovary length; g, style length; h, stigma-anther separation.

1994) (Min – Max number of pollen grains measured per flower = 114 – 444, $N_{pin\,grains}$ = 3840, $N_{thrum\,grains}$ = 4548). We tested for differences in each morphological attribute between floral morphs 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 ($N_{pin\,flowers}$ = 64, $N_{thrum\,flowers}$ = 87). Nectar was collected 24 h after anthesis from previously bagged flowers, using 1 µl micropipettes. 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 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 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 × 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 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 neighbour for each patch. We contrasted these results against the null hypothesis of equal probability of neighbourhood with any patch type using a contingency table.

We explored if there were differences in the size of floral display among patch types as well as in their distance to the nearest legitimate pollen donor. Differences among patch types were determined 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 with a chi-square test.

Effect of floral display on seed production

To explore the effect of floral display on the seed production in *A. lavarum,* we recorded the number of flowers for each patch within the 100 × 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 dot 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 for this test 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 de la muerte 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 between patches).

Nevertheless, the use of this plot is an accurate estimate of seed production at a population level because it included at least ⅓ of the total number of patches present at the study site.

The main issue in our approach is that, inside the plot, seed production in a given patch can be affected by the presence of neighbouring 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 using 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 using 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 display and the corrected mean number of seeds produced per flower. To determine if seed production is maximized at an intermediate floral display, 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 using 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 100×150 m plot we recorded the number of flowers aborted and the mean seed production per flower ($N_{\text{pin patches}} = 69$, $N_{\text{thrum patches}} = 47$, $N_{\text{mixed patches}} = 16$). 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 percentage of flowers 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 numbers 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 an 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 (Figs. **3 a, b**). Stigma-anther separation divides floral morphs into two discrete groups (Figs. **2 h, 3 c**). 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**).

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 the two
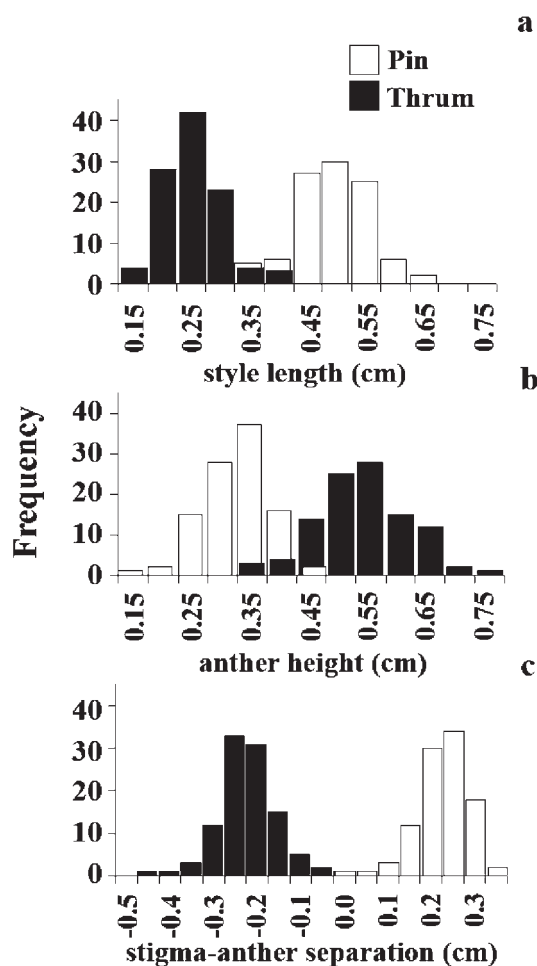
Fig. 3 Frequency distribution of (**a**) style length, (**b**) anther height, and (**c**) stigma-anther separation in pin and thrum morphs from a population of *A. lavarum*.

floral morphs (Table **1**). We were not able to test differences in nectar concentration due to the low volumes of nectar produced per flower.

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 colour changes from pale green to red. Fruits develop completely in *ca.* 1.5 – 2.0 months, then the capsules open and wind-dispersed seeds are shed. Fruits may produce 1 – 13 seeds (Mean ± SD = 4.3 ± 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**).

There was no difference between floral morphs in the number of seeds produced by hand-pollinated flowers (Mean ± SD_{pin} = 4.5 ± 3.8, Mean ± SD_{thrum} = 2.9 ± 2.9, t = 1.3, df = 33, p = 0.2, $N_{pin 1}$ = 22, $N_{thrum 5}$ = 13). Breeding system experiences 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 pre-dispersal seed predators. The behaviour 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 behaviour suggests syrphids as the most likely pollinators of *A. lavarum*.

Patch abundance, spatial distribution, and morph ratio

Pin patches were the most abundant, followed by thrum and mixed patches (χ^2 = 33.13, df = 2, N_{pin} = 70, N_{thrum} = 47, N_{mixed} = 16, respectively, p < 0.001). However, the floral identity of the

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

Floral attribute	Pin		Thrum		t	p
	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]	5 390.5	1 140.7	3 856	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)[e]	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 flowers, df = 78; [c] 15 pin and 15 thrum flowers, df = 28; [d] 18 pin and 20 thrum flowers, all flowers were collected in different patches; [e] 64 pin and 87 thrum flowers from 10 pin and 12 thrum patches.

Table 2 Results from self- and cross-artificial pollinations in *Arcyto-phyllum lavarum* (*N* = no. of flowers, number of patches = 11)

Cross	*N*	Percentage of flowers that set fruit
Pin × pin	17	0
Pin × thrum	24	75
Pin × self-pollination	22	0
Thrum × pin	17	70
Thrum × thrum	14	0
Thrum × self-pollination	16	0

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 ± 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, $N = 133$, $p = 0.327$, $N_{pin} = 70$, Mean ± SD = 19.8 ± 22.5; $N_{thrum} = 47$, Mean ± SD = 19.0 ± 43.3; $N_{mixed} = 16$, Mean ± 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, $N_{pin\ total} = 1676$, $N_{thrum\ total} = 1117$, $p < 0.001$).

Spatial autocorrelation of seed production

Seed production among pin patches was similar for distances closer than 5 m. However, seed production in pin patches was opposite for distances between 25–30 m (Min–Max nearest neighbour = 0.12–12.65 m, Fig. **4**). Seed production in thrum patches was correlated for 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 neighbour = 0.67–20.62 m, Fig. **4**). Pin, thrum and mixed patches had similar seed production when closer than 12 m (Fig. **4**). All the patches displayed a negative autocorrelation at 30–36 m (Min–Max nearest neighbour = 0.12–17.54, Fig. **4**). These results were included in the regression and spatial ANOVA analyses to correct for spatial autocorrelation.

Effect of floral display and morph ratio on seed production

For pin patches, we did not find any relationship between floral display 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**).
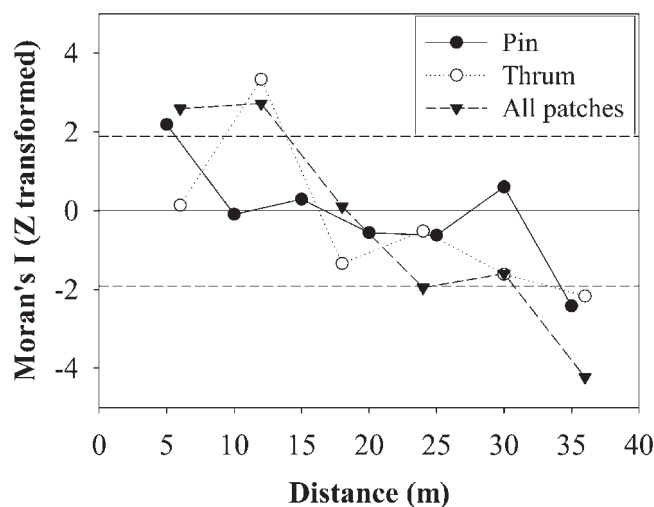
Fig. 4 Changes in the standardized Moran's I statistic for seed production in pin, thrum, and all patches (mixed patches included) at different distances. Dashed lines indicate ± 95 % confidence interval. Dots outside of the confidence intervals represent the distances at which a significant spatial autocorrelation is present ($p < 0.05$).

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

Independent variable	Regression model	*F*	df	r²	*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

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

Independent variable	Seed production	*F*	df	r²	*p*
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, $N_{pin} = 69$, $N_{thrum} = 47$, $N_{mixed} = 16$, $p = 0.005$, Fig. **5a**). Pin patches also produced a lower number of seeds per flower than thrum and mixed patches
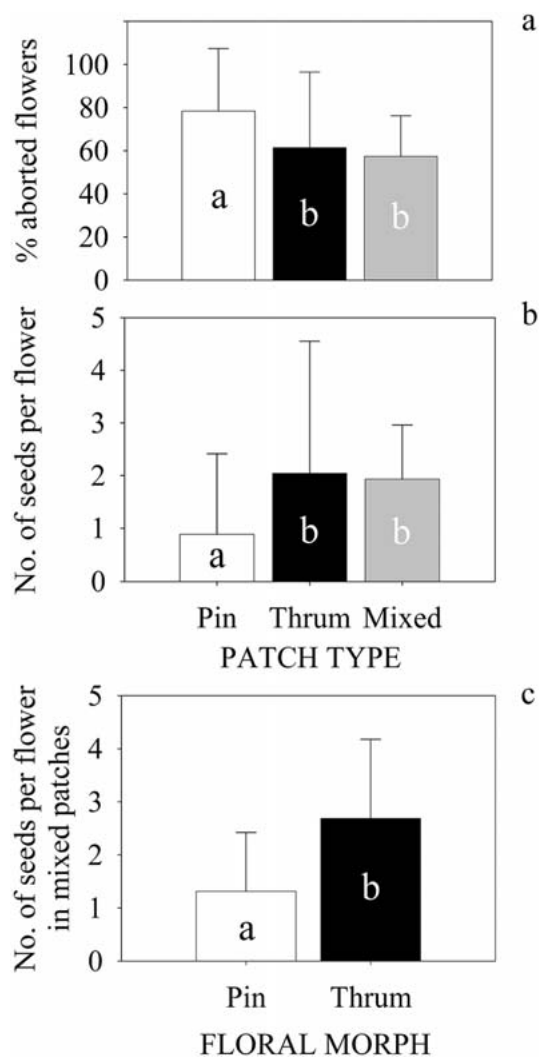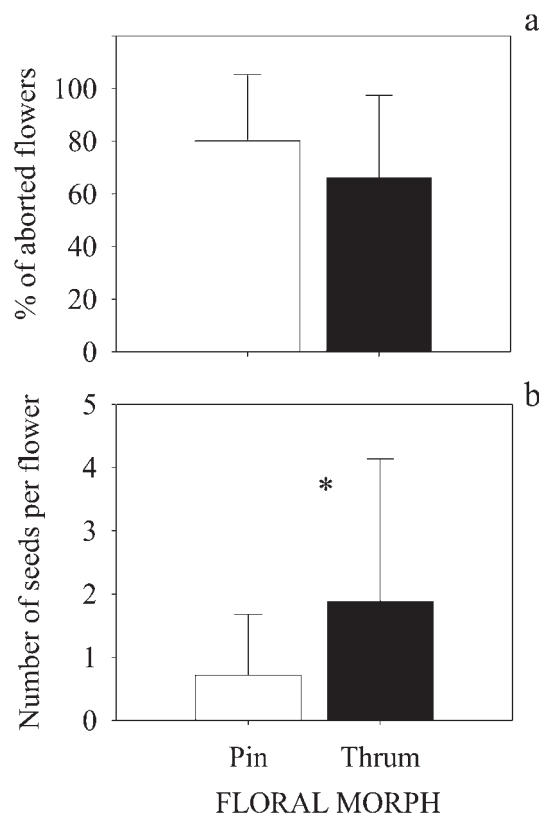
Fig. 5 (**a**) Flower abortion and (**b**) mean number of seed produced per flower in pin, thrum, and mixed patches. (**c**) Female reproductive success for pin and thrum flowers within mixed patches of *A. lavarum* (Mean, SD). Letters group similar categories ($p < 0.05$).

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

(Spatially Adjusted ANOVA: $F = 5.8$, df = 2, $N_{pin} = 69$, $N_{thrum} = 47$, $p < 0.003$, Fig. **5 b**). Within mixed patches, pin flowers also displayed lower seed production ($t = -3.61$, df = 15, $p = 0.003$, Fig. **5 c**). For pairs of pin and thrum patches with equivalent numbers of flowers, the proportion of flower abortion was equivalent between morphs ($t = 1.76$, df = 19, $p = 0.09$, Fig. **6 a**). However, seed production was also higher in the thrum morph ($t = -2.15$, df = 19, $p = 0.04$, Fig. **6 b**).

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 and Koptur, 1993). Most frequently, pistil and anther heights display bimodal distributions, as occurs in *Arcytophyllum lavarum*.

In *A. lavarum* flowers, pin pollen grains are smaller and more abundant than thrum pollen grains, a pattern found in other distylous species (Wyatt and Hellwig, 1979; Ornduff, 1980; Pailler and Thompson, 1997; Ree, 1997; Naiki and Nagamasu, 2003). Pin flowers of *A. lavarum* 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.

Arcytophyllum lavarum displays a self- and morph-incompatible system similar to other heterostylous species (Ree, 1997; De Castro and De Oliveira, 2002; Ornelas et al., 2004 a). In some other heterostylous species, however, the degree of morph compatibility is variable among floral morphs (Dulberger, 1970; Opler, 1975; Ornduff, 1976; Barrett and 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 display did not have an effect on seed production in pin, thrum, and mixed patches of *A. lavarum*. This suggests that the pollen flow may not be sensitive to floral display.

However, as discussed below, 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 and 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 and Bosque, 2003). However, in some species, similar to *A. lavarum,* the pin morph may display higher efficiency as pollen donor (Stone, 1995; Lau and Bosque, 2003; Ornelas et al., 2004a), and the thrum morph may play a major role in seed production (Dommee 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 the higher abortion rate in the pin morph and the higher female reproductive success in the thrum morph of *A. lavarum* are 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. lavarum* is asymmetric, and pollinators are more efficient at delivering pollen from the pin to the thrum morph. Asymmetry in pollen flow has been reported in other distylous species (Ree, 1997; Pailler and Thompson, 1997; Ornelas et al., 2004a; Massinga et al., 2005; Cawoy et al., 2006). In the Rubiaceae *Psychotria suerrensis* (Rubiaceae) pin flowers are more efficient as pollen donors, and thrum flowers are more efficient as pollen receivers (Stone, 1995). Pollen flow is also asymmetric in *Palicourea fendleri* (Rubiaceae), where pollen flow is higher from pin to thrum flowers and thrum flowers are more efficient at delivering pollen to flowers from the same morph (Lau and Bosque, 2003).

Our results suggest that floral display does not have an effect on the seed production of *A. lavarum.* The two floral morphs, however, play 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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