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OUTCROSSING AND POLLINATOR LIMITATION OF FRUIT SET: BREEDING SYSTEMS OF NEOTROPICAL *INGA* TREES (FABACEAE: MIMOSOIDEAE)

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Many hermaphroditic flowering plants produce many more flowers than fruit. Low fruit set from a large number of potential fruit may result from a variety of factors. In animal-pollinated species, a large floral display may be advantageous in attracting pollinators (Gentry, 1974; Willson and Rathcke, 1974; Schaffer and Schaffer, 1979; Stephenson, 1979; Augspurger, 1980; Udovic, 1981), especially if the density of plants is low, the duration of bloom of the species is short, and/or there are many other species blooming simultaneously. If pollinators visit only some of the flowers, not all flowers will receive pollen, and fruit set without fertilization will not take place in plants that are non-agamospermous and without automatic selfing; in this way the plants can be pollinator-limited, by virtue of low visitation (Bierzychudek, 1981). Incompatibility systems can cause pollinator-limitation of a more complex nature: the fruit set of a self-incompatible species will be limited by the kinds of visitors, and the kinds of visits (intraplant movements resulting in intrafloral selfing or geitonogamy versus interplant movements resulting in xenogamy) (Arroyo, 1976). Interspecific pollinator movements can result in stigma-clogging, with foreign pollen impairing the adherence and germination of pollen of the correct species (Levin and Anderson, 1970; Wissel, 1977; Waser, 1978). Allelopathic effects of foreign pollen may also depress fecundity of some species (Sukada and Jayachandra, 1980; Thompson et al., 1981). Even if all flowers on a plant are visited, and pollinated with pollen appropriate for fertilization, the plant may be unable to mature every fruit because of resource, spatial, or physical restrictions. Resource limitation may cause abortion of some developing ovules or ovaries (Stephenson, 1981), and can provide a logistical basis for mate choice in plants (Janzen, 1977; Willson, 1979) and sibling competition between developing embryos (Kress, 1981).

Inga is a large genus of neotropical legume trees that have alternate, parapinnately compound leaves (often with foliar nectaries: Léon, 1966; Bentley, 1977; Koptur, 1984) and large floral displays (Croat, 1978; Koptur, 1983). The showy white inflorescences are composed of many flowers that have reduced perianth parts and many white stamens; the stamens are basally connate, forming a tube within which nectar accumulates. The open flowers are visited by a wide array of visitors of which bats, hummingbirds, hawkmoths, butterflies, and settling moths are pollinators (Snow and Snow, 1972; Salas, 1974; Toledo, 1975; Feinsinger, 1976, 1978; Koptur, 1983). *Inga* pollen grains are clustered into globular polyads of 16 to 32 grains; the polyad is dispersed as a unit. Most *Inga* produce few fruit in relation to the number of flowers they bear (Léon, 1966; pers. observ.). The focus of this study is the factors influencing fruit set in some Costa Rican species of *Inga*. The floral biology, flowering and fruiting phenology of these *Inga* species is described by Koptur (1983). The breeding systems of these trees are elucidated here as a basis for understanding patterns of flowering, and

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interpreting pollinator movements in terms of potential fertilization of *Inga* flowers. The opposing evolutionary constraints of massive flowering to attract pollinators and the resulting excessive self-pollination are conflicts common to many tropical trees (Bawa, 1983). In this study I consider the importance of pollination phenomena in limiting fruit set in *Inga* and possibly many other species.

MATERIALS AND METHODS

The study area is in Puntarenas Province, Costa Rica, situated between 1,320 m and 1,600 m elevation, and includes two forest types and the transition zone between. The lower areas consist largely of forest remnants bordering cleared pastures in the communities of Santa Elena and Monteverde. The transition area is included in a large tract of privately owned forest contiguous with the Monteverde Cloud Forest Biological Reserve, a large area of pristine forest, which includes the uppermost locations. The sites have been described in detail (Buskirk and Buskirk, 1976; Feinsinger, 1976; Powell, 1979; Lawton and Dryer, 1980; Hartshorn, 1983).

Seven species of *Inga* were studied: *Inga brenesii* Standl., *I. densiflora* Benth., *I. longispica* Standl., *I. mortoniana* J. Léon, *I. oerstediana* Benth. ex. Seem., *I. punctata* Willd., and *I. quaternata* Poeppig. Voucher specimens are in the herbaria of the University of California, Berkeley (UC), the Missouri Botanical Garden (MO), and the Museo Nacional de Costa Rica (CR). All these species are canopy or subcanopy trees. Trees used in the study were growing at the forest's edge, along trails, or in open pastureland, as the flowers of these individuals were accessible. Flowers were reached by climbing trees, using a ladder, or occasionally from the ground. There are biases that may have arisen by working on semi-isolated trees (altered pollinator activity, resource availability) resulting in false (perhaps) estimates of actual fruit numbers in nature.

To determine pollen/ovule ratios, polyads were mounted in polyvinyl lactophenol cotton blue, and the pollen grains were separated by applying pressure on the cover slip. The number of pollen grains per polyad was counted under a compound microscope. Twenty five–30 polyads from 2–5 individuals per species were counted. Ovules were dissected from ovaries and counted under a dissecting microscope. Twenty five–40 ovaries from 2–5 individuals per species were dissected. The pollen ovule ratio (P/O) for each species was calculated using the formula shown in Table 1.

Activity of visitors was observed at flowers of selected *Inga brenesii* and *I. punctata* individuals for 10 min every 30 min over 8-h periods on several different days. Visitor frequency and the number of flowers visited in each foraging bout were noted where possible, and in some cases it was possible to watch where the visitors went after they left the individual tree under observation.

The amount of pollination in the field was measured by looking for polyads on stigmas of randomly collected flowers (both fresh and recently wilted) from 3–5 individuals of all the species. The flowers were preserved in ETOH or FAA and not examined until later, so that some polyads may have fallen off if not germinated. The measure is therefore a conservative estimate on two counts: fresh flowers may have had additional pollination opportunities prior to wilting, and ungerminated polyads may have been disturbed prior to counting.

Crossing experiments to investigate the breeding systems of six species were performed on inflorescences bagged with Pollen-Tector heavy paper bags (Carpenter Paper Co., Des Moines, IA). Inflorescences were bagged the day before the opening of flowers to be hand-pollinated. Flowers that were open previously were removed at the time of bagging. Approximately equal numbers were used for each treatment on each tree. Flowers were first emasculated, and polyads from either different flowers on the same tree (self-pollination) or from flowers on two or three other trees (cross-pollination) were applied to the receptive stigmas. All flowers pollinated on an inflorescence received the same kind of pollen; only 1–4 flowers were pollinated per inflorescence, as it was found (see below, maximum fruit set capacity) that maximum fruit set did not exceed 4–6 fruit per inflorescence. Pollen was collected from flowers that had opened inside bags; stamens were removed with clean forceps and put in glassine envelopes. Polyads were visible on the hairs of fine paint brushes that were used to place polyads on stigmas. Care was always taken to apply at least three polyads to a stigma. On other bagged inflorescences on the same trees, newly-opened flowers were left untouched to test for autogamy (automatic selfing). An additional group of unbagged inflorescences on the same trees were unobtrusively tagged and left untouched for monitoring fruit set on "open pollinated" flowers, in order to estimate the actual amount of successful fertilization that occurs under natural conditions.

The fertilization effectiveness of pollen from different distances away from stigmatic parent was investigated for *Inga brenesii* and *I. punctata.* Pollen was collected from three sources (Self, Near-Cross, and Far-Cross—2 or 3 individuals at each distance) from flowers newly opened in bagged inflorescences. Pollinations were performed within 3–4 h of collecting the pollen, and all three types of crosses were done more-or-less simultaneously (same tree, same time, same day) so that all conditions were as similar as possible. For "near" cross-pollinations, the pollen source tree was at a distance less than .5 km (.2–.4 km) from the stigmatic parent; for "far" cross-pollinations, the pollen-parent was at a distance more than 1 km away (1–3 km).

The maximum fruit-set capacity of an inflorescence was determined in *Inga brenesii* by cross-pollinating every flower on ten inflorescences as the flowers opened over 3–10 days. Pollen from a distant source was applied (these were all "far" crosses).

Interspecific cross-pollinations were performed between simultaneously blooming *Inga* species: *I. brenesii* × *I. oerstediana*; *I. brenesii* × *I. punctata*; *I. punctata* × *I. densiflora*; *I. brenesii* × *I. mortoniana*. 20–30 crosses were performed on at least two individuals of each species in all cases.

In all experiments, inflorescences were re-bagged for 3–5 days after pollination, until the non-fertilized flowers began falling. Inflorescences with flowers remaining (presumably fertilized) were tagged and labeled, and subsequent fruit development was monitored on a monthly basis. When fruits appeared fully mature, they were removed and opened to see if they had mature seeds; only then was a flower counted as successful in producing a fruit. All seeds germinated after sowing unless they had been damaged in the pod by insect larvae.

In order to examine more closely the nature of the incompatibility system, the styles of *I. brenesii* flowers pollinated according to the preceding regime (40/treatment) were fixed in lactophenol at 2-h intervals from the time of pollination (2 h, 4, 6, 8, 12, and 24 h). These styles were cleared and stained with aniline blue using the method of C. Johnson and D. Mulcahy (adapted from Ramming et al., 1973, Stain Tech. 48:133–134). Pollen tube growth was observed using a fluorescence microscope.

RESULTS

Pollen and Ovule Counts, P/O Ratios

All of the *Inga* species have 8 polyads per anther (therefore, 8 polyads per stamen), as reported by Léon (1966) and Elias (1981). Previously (Léon, 1966) it had been reported that the number of pollen grains per polyad was either 16, 24, or 32, presumably constant within a species. I found considerable variation in this number for *Inga* species with greater than 16 pollen grains per polyad (Table 1—*I. brenesii, I. densiflora, I. oerstediana, I. punctata, I. quaternata*). Variation in the number of pollen grains per

TABLE 1. Pollen ovule ratios. Numbers given are $\bar{x}$ ± standard deviation. Sample size is 25–40 for each count. P/O = pollen ovule ratio; p.g. = pollen grains.

	$\bar{x}$ number stamens/flower	$\bar{x}$ number pollen grains/polyad	$\bar{x}$ number ovules	P/O*	$\frac{\text{\# p.g./polyad}}{\text{\# ovules}}$
I. brenesii	92.07 ± 14.50	33.8 ± 4.48	21.13 ± 2.72	1178.22	1.60
I. densiflora	29.65 ± 7.32	30.5 ± 3.06	17.50 ± 2.09	413.41	1.74
I. longispica	80.29 ± 12.16	16.0 ± .00	12.71 ± 1.50	808.59	1.26
I. mortoniana	50.00 ± 11.83	16.0 ± .00	16.80 ± 1.93	380.95	.95
I. oerstediana	60.02 ± 7.39	31.3 ± 1.92	18.07 ± 2.52	830.38	1.73
I. punctata	37.00 ± 6.90	23.2 ± 1.76	19.40 ± 1.35	353.98	1.20
I. quaternata	35.30 ± 4.99	21.8 ± 4.53	19.80 ± 1.62	310.93	1.10

* Pollen ovule ratio figured by formula $\text{P/O} = \dfrac{\bar{x}\ \text{\# stamens} \times \dfrac{\text{8 polyads}}{\text{stamen}} \times \dfrac{\bar{x}\ \text{\# pollen grains}}{\text{polyad}}}{\bar{x}\ \text{\# ovules}}$.

polyad in *Acacia* species has been described by Leach and Whiffin (1978) and Ali and Quaiser (1980), associated with hybridization. Guinet (1981) also describes asymmetric 28-grain polyads for some *Inga* species.

Mean ovule numbers range from 12.7 in *I. longispica* to 21.1 in *I. brenesii* (Table 1). Pollen/ovule ratios are all within the same order of magnitude, but the ratios of *I. brenesii, I. longispica,* and *I. oerstediana* are roughly twice that of the other species. These three species have the greatest number of stamens as well. The final column in Table 1 shows the mean number of pollen grains per polyad divided by the mean number of ovules for each species. The data in this column address the question: Can one polyad be enough to fertilize all the ovules and form a full-seeded fruit? In all cases (except *I. mortoniana,* which is .95) the ratio is greater than one, indicating that if the pollen grains are all of a compatible type, all the ovules could be fertilized with one polyad.

Pollinator Activity

Inga brenesii has flowers that open continuously throughout the day and night (Koptur, 1983), and both hummingbirds and hawkmoths are abundant and effective pollinators. Hummingbird activity is higher during daylight hours than is hawkmoth activity during evening and night hours (Table 2), but both of these kinds of visitors visit a large number of flowers. The number of visits per flower per day for each visitor type was estimated by multiplying (the mean # visits during the 10 min observation periods) × (6 periods/h) × 12 h of activity per day) × (the mean number of flowers per visit) to obtain the estimated number of flowers visited each day, which was then divided by the number of open flowers counted on the study trees. Hummingbirds provide an estimated 4.1 visits/flr·day, hawkmoths .7, and skippers .1; a total of close to five visits per flower per day. Chances are that very few flowers go unvisited! Feinsinger (1976) reports hummingbird visits to this species average 1.68 visits/flr·day, (from 1.24–5.09).

Inga punctata has most of its flowers opening around dusk, but a small proportion have early morning anthesis (Koptur, 1983). This accounts for the increased importance of hawkmoth pollinators (an estimated 2.02 visits/flr·day) over hummingbirds (.4). Pollinators furnish an estimated total of 2.4 visits/flr·day, ample for most flowers to receive pollen. Feinsinger (1976) reported a figure of .12 visits/flr·day for hummingbirds on *Inga* sp. 2 (later identified as *I. punctata*).

TABLE 2. Visitor activity on two *Inga* species. Numbers given are: $\bar{x}$ ± standard deviation (range). Fifteen observation periods averaged for each visitor type; flower counts averaged for 20 visits/visitor.

Visitor	# visits during 10 min. obs. period	Hours of activity	# flowers per visit	Estimated # flowers visited each day	# flowers on tree	# visits per flower per day
Inga brenesii						
Hummingbirds (diurnal)	4.5 ± 2.6 (1–9)	12	36.1 ± 26.5 (4–91)	11,696	2,860	4.09
Hawkmoths (crepuscular and nocturnal)	2.7 ± 2.3 (0–8)	12	10.2 ± 8.8 (1–32)	1,983	2,860	.69
Skippers (diurnal)	.3 ± .4 (0–2)	12	9.2 ± 6.8 (1–21)	199	2,860	.07
Total for *brenesii* visitors						4.85
Inga punctata						
Hummingbirds (diurnal)	1.2 ± 1.0 (0–4)	12	6.4 ± 6.9 (1–18)	553	1,440	.38
Hawkmoths (crepuscular and nocturnal)	3.7 ± 3.2 (0–10)	12	10.9 ± 5.6 (2–22)	2,904	1,440	2.02
Total for *punctata* visitors						2.40

Polyads on Stigmas

Approximately 66% of the stigmas on *Inga brenesii* had at least one polyad; approximately 49% of *I. punctata* stigmas were pollinated. The other *Inga* species were found to have approximately half of the stigmas with polyads (*I. oerstediana* 66%; *I. longispica* 57%; *I. mortoniana* 48%; *I. quaternata* 46%) except for *I. densiflora,* which had only 28% (Table 3). These are conservative estimates (see Methods), so it is likely that even more flowers than these received polyads.

Breeding Systems

Hand-pollinations were performed on six of the seven *Inga* species, and the results (Table 4) indicate that these species are self-incompatible, using the criteria of Bawa (1974) and Zapata and Arroyo (1978). For *I. quaternata* no fruit was set with either self- or cross-pollination; it is likely that the two individuals used were close relatives, and therefore incompatible (see "Pollen source distance," below). Fruit set on open-pollinated (control) flowers can be compared with that on hand-pollinated flowers (Table 4). These figures provide an estimate of the amount of effective outcrossing that occurs in nature; few flowers that receive pollen set fruit. In the inflorescences of *Inga brenesii* in which every flower was cross pollinated (25–40 flowers), fruit set was never more than six (3–6), from 10–15% within each inflorescence.

Interspecific crosses were all unsuccessful, indicating that the species tested are unlikely to be interfertile. No foreign polyads were seen to germinate on *Inga* stigmas. Pollen grains in all polyads examined were entirely viable (100% staining dark with lactophenol cotton blue). The variable number of pollen grains per polyad in some *Inga* species may be an indication of hybridization, but it seems unlikely in view of these crosses.

Both self and cross *I. brenesii* polyads germinated. In all cases of germination, the pollen tubes penetrated the stigmatic surface and grew into the style. In no preparation, however, did the pollen tubes reach the ovules. Apparently, fertilization occurs after 24 h from the time of pollination. In approximately 50% of the self-pollinations, self pollen tubes grew the length of the style, indicating that the incompatibility system is ga-

TABLE 3. Polyads on stigmas of *Inga* flowers at Monteverde. Flowers were sampled randomly from 3–5 individuals per species, and preserved prior to counting.

Species	# flowers with No polyad	1 polyad	2	3	>3	Total # flowers examined	Proportion with polyads
I. brenesii	50	65	16	12	6	149	.66
I. punctata	83	46	23	6	6	164	.49
I. densiflora	118	24	10	6	5	163	.28
I. longispica	86	80	28	3	3	200	.57
I. mortoniana	65	38	14	4	4	125	.48
I. oerstediana	49	71	14	4	6	144	.66
I. quaternata	29	17	6	1	1	54	.46

metophytic in nature, and that inhibition may occur in the ovary.

Pollen Source Distance

The results of experimental pollinations with pollen from sources at varying distances from the stigmatic parent were examined with contingency table analysis (BMD stat. pack.; Sokal and Rohlf, 1982), to take possible variation between individuals into account. For *Inga brenesii* (Table 5), there is a significant interaction between pollination treatment and fruit set, selfs yielding the lowest, near crosses intermediate, and far crosses the highest fruit set. For each tree taken separately, this difference is upheld (Freeman-Tukey deviates all significant).

Inga punctata shows the same overall result (Table 6); however, only three of four trees show greater fruit set with "far" pollen. The fourth tree set very little fruit at all; this may have resulted from weather conditions unfavorable to fertilization (cool, misty) on the days of pollination.

The same trend was shown in pollinations done on two individuals of *I. densiflora* (sample sizes smaller): cross pollinations between neighboring individuals, .3 km apart, failed to yield fruit; whereas crosses with pollen from a source 1.5 km away yielded 25% fruit.

DISCUSSION

At one time it was assumed that because of the enormous diversity and complexity of tropical wet forests, and the large interindividual distances in many species of tropical forest trees, most species would be self-compatible and inbred (Corner, 1954; Baker, 1959; Federov, 1966). Ashton (1969) was perhaps the first to suggest that pollen and seed dispersal occur over large distances in tropical forests, and that rain forest trees are largely outcrossed. Janzen (1971) demonstrated that pollinating Euglossine bees moved great distances in tropical forests; subsequent studies (e.g., Stiles, 1975; Frankie et al., 1976; Linhart and

TABLE 4. Results of hand pollinations. Self- and cross-pollinated flowers were bagged prior to opening, emasculated, and hand-pollinated. Control flowers were not bagged and not hand-pollinated, but were left open for visitors. Fruit set is # fruit/# flowers.

		Self-pollinated		Cross-pollinated		Control	
	# trees	*N*	Fruit set	*N*	Fruit set	*N*	Fruit set
I. brenesii	11	319	.01	205	.35	960	.03
I. densiflora	2	88	.01	79	.25	505	.05
I. mortoniana	2	33	.03	53	.49	480	.02
I. oerstediana	3	65	.02	67	.12	325	.05
I. punctata	4	130	.02	146	.20	360	.02
I. quaternata	1	50	.00	52	.00	430	.03

TABLE 5. Pollen source distance experiment with *I. brenesii.* Near Cross (Near X) is with pollen from a source at a distance of less than .5 km from the stigmatic parent; Far Cross is from a source greater than 1 km away.

Tree	Cross	Flower fate		Total # flowers	% fruit set	Pearson χ^2	*d.f.*	Probability
		w/fruit	w/o fruit					
1	Self	0	39	39	0			
	Near X	8	42	50	16	21.26	2	.0000
	Far X	11	14	25	44			
	Total	19	95	114	—			
2	Self	0	39	39	0			
	Near X	3	30	33	9	20.49	2	.0000
	Far X	8	12	20	40			
	Total	11	81	92	—			
3	Self	1	33	34	3			
	Near X	0	15	15	0	19.41	2	.0001
	Far X	18	30	48	38			
	Total	19	78	97	—			
4	Self	0	40	40	0			
	Near X	5	44	49	10	9.10	2	.0106
	Far X	5	17	22	23			
	Total	10	101	111	—			
5	Self	1	45	46	2			
	Near X	2	33	35	6	31.37	2	.0000
	Far X	25	35	60	42			
	Total	28	113	141	—			
6	Self	0	30	30	0			
	Near X	3	23	26	11	15.98	2	.0003
	Far X	10	16	26	38			
	Total	13	69	82	—			
7	Self	1	175	176	<1			
	Near X	10	60	70	14	45.09	2	.0000
	Far X	11	23	34	32			
	Total	22	258	280	—			
All trees	Self	3	401	404	<1			
	Near X	31	247	278	11	175.14	2	.0000
	Far X	88	147	235	37			
	Total	122	795	917	—			

Mendenhall, 1977) have revealed that many tropical forest pollinators are capable of moving large distances between individuals of the same species. Investigations of many species of lowland (semi-deciduous) dry forests of Costa Rica (Bawa, 1974), lowland wet forest of Costa Rica (Bawa, 1979), and lowland semi-deciduous forest in Venezuela (Zapata and Arroyo, 1978) have discovered that the majority of hermaphroditic species are self-incompatible. A recent investi-

TABLE 6. Pollen source distance experiment with *I. punctata.*

Tree	Cross	Flower fate		Total	% fruit			
		w/ fruit	w/o frt	# flowers	set	Pearson χ^2	*d.f.*	Probability
1	Self	0	29	29	0			
	Near X	0	16	16	0	16.16	2	.0003
	Far X	10	22	32	31			
	Total	10	67	77	—			
2	Self	1	37	38	3			
	Near X	1	16	17	6	5.76	2	.0561
	Far X	7	31	38	18			
	Total	9	84	93	—			
3	Self	0	29	29	0			
	Near X	1	19	20	5	3.24	2	.1980
	Far X	0	35	35	0	(inflated value due to small expecteds)		NS
	Total	1	83	84	—			
4	Self	2	32	34	6			
	Near X	2	9	11	18	6.71	2	.0348
	Far X	12	29	41	29			
	Total	16	70	86	—			
All trees	Self	3	127	130	2			
	Near X	4	60	64	6	23.95	2	.0000
	Far X	29	117	146	20			
	Total	36	304	340	—			

gation of montane tropical cloud forest in Venezuela (Sobrevila and Arroyo, 1978) found that a lower proportion of hermaphroditic species possess self-incompatibility systems than species of lowland forest, a consequence, perhaps, of weather-influenced unpredictability of pollination pattern.

The *Inga* species studied here in montane cloud forest of Costa Rica are all self-incompatible. Arroyo (1976) suggested that self-incompatibility in massively flowering tropical trees has evolved as a response to excessive geitonogamy (self-pollination effected by pollinators moving between flowers on the same tree). *Inga* fits a typical pattern for a mass-flowering tree, in having a large number of small, generalized flowers that attract many species of pollinators. Although I did not observe the penetration of pollen tubes into ovules in any of my preparations, the suggestion is that the incompatibility reaction takes place in the ovary. Bawa (1979) suggests that such pollen-ovule incompatibility may occur in many tropical trees; Kress (1983) reports a similar situation in *Heliconia* species, tropical herbs.

Self-incompatibility is more widespread in woody than herbaceous legumes (Arroyo, 1981). The tribe Ingeae is a largely woody group, and all species previously tested have proven self-incompatible. Of the three (out of 5) mimosoid tribes in which some experimentation has been done, all contain some self-incompatible species. The phenomenon of few fruit set from many flowers has been noted in other mimosoid legumes besides *Inga*: *Parkia clappertoniana* (Baker and Harris, 1957), *Prosopis*

(Solbrig and Cantino, 1975; Simpson et al., 1977), *Calliandra* (Cruden et al., 1976; Cruden, 1977).

Pollen grains dispersed as a unit (in polyads, or pollinia) provides great efficiency of fertilization if the pollen grains are of a compatible nature—one good polyad can potentially fertilize all the ovules needed to fill a fruit. Cruden (1977) has called this strategy "sweepstakes reproduction." The *Inga* pollen/ovule ratios are low for a typical obligately out-crossing species (Cruden, 1977), but are very similar to those Cruden found for species of *Calliandra,* another mimosoid legume with polyads. *Asclepias* have pollinia, and exhibit low fruit sets in many situations (Willson and Price, 1980). Kress (1981) argues that this strategy reduces intra-ovary competition between sibling seeds as all are sure to be genetically similar. Pollen packaging in polyads assures high seed set if one compatible unit is transferred. These sorts of pollination systems may allow a plant to recognize and choose between mates more readily than in situations where the number of pollen grains reaching a stigma is highly variable. Here the most important factor may be finding maximum compatibility. Clearly, more experimentation needs to be done before these speculations can be tested.

What factors limit fruit set in *Inga*? Lack of pollinator visitation is not the explanation: not only were approximately half of the stigmas of the various species found to have polyads on them, but observations of all visitors to two species gave estimates of visits per flower per day in excess of 1, indicating that most flowers are probably visited. A number of other plants have been shown to have natural pollinations much in excess of fruit set. In milkweeds (requiring only one pollinium for fruit set), only 9.6% of *Asclepias syriaca* flowers set fruit, although 95% of the flowers had received ample pollination (Moore, 1947); *A. solanoana* fruit set was only 3%, although there was an average of 1.73 pollinia per flower (Lynch, 1977). From 73–93% of flowers on individuals of *Calliandra callistemon* were pollinated (as evidenced by presence of polyads on stigmas), but fruit set in this species was only 5.2% (Cruden et al., 1976).

Resource limitation alone does not explain the low levels of fruit set in *Inga.* This factor was controlled by doing different pollinations on the same individual trees; hand cross-pollinations with pollen from far sources were much more successful than "open pollinations" on the same trees, indicating that it was not for lack of energy reserves that fruit were not forming. Although an *Inga* inflorescence may bear up to 40 flowers, the maximum number of fruits matured with multiple optimal hand cross-pollinations was six (more usually, 4 or 5); there may be physical/spatial restrictions on how many large fruit will fit on one peduncle. Resource limitation has been found to be important in *Asclepias* species (Willson and Price, 1980): when fertilizer was added, fruit set increased; with defoliation and shading, fruit set decreased. *Catalpa speciosa* responded to defoliation with lower fruit set due to increased fruit abortion (Stephenson, 1980). Baker (1970) suggested that resource limitation of fruit production is not infrequent among tropical woody plants. There are many examples of resource limitation in cultivated plants (reviewed by Stephenson, 1981) and reports of increasing fruit set with fertilizing at the time of flowering. Overall fruit set of *Inga* would probably not be enhanced by application of soil fertilizers, etc. . . , unless the flowers receive increased numbers of compatible polyads (see below).

Inga are pollinator-limited (sensu Bierzychudek, 1981), as open-pollinated flowers and inflorescences have much lower fruit set than those flowers and inflorescences cross-pollinated by hand. The limitation is not strictly a result of geitonogamy; it is determined also by the distance of the source of cross-pollen from the stigmatic parent. The mechanism of this phenomenon in *Inga* has not yet been elucidated; it may be that flowers outcrossed with remote pollen donors are selectively matured. Selective abortion of fruits with less than average seed numbers has been found in other plants, including the annual legume, *Cassia fasciculata* (Lee and Bazzaz, 1982*b*). Further experimentation on *Inga* is needed before anything definite can be said regarding selective maturation—the strategies may well be different in long-lived perennials and annuals. What can be asserted is: since crosses are more successful when pollen comes from a substantial distance, the most effective pollen transfer results from pollinators that move not only from tree to tree, but to trees some distance away.

Many *Inga* pollinators are known to be strong fliers, and are likely to travel substantial distances in their daily foraging bouts. Hummingbirds have been implicated in traplining by Stiles (1975), and are capable of moving long distances. Frankie et al. (1976) found that territorial behavior by *Centris* bees in crowns of *Andira inermis* cause more inter-tree movement by other bees; territorial and aggressive behavior of hummingbirds on *Inga* may cause others to move greater distances than they might otherwise, curtailing not only geitonogamy by the others, but also cross-pollination of near neighbors. Sphingidae moved *Lindenia rivalis* pollen hundreds of meters (Linhart and Mendenhall, 1977); it is conceivable that pollen may be carried on hawkmoth bodies for days, during which time they could move quite far (W. A. Haber, pers. comm.). Hesperiidae are strong, fast, and direct fliers, and are important in cross-pollination of many plants (Klots, 1976). All of these visitors can serve as effective pollinators, but in many cases they transfer incompatible pollen from near neighbors.

This sort of pollinator limitation (many selfs and near-neighbor pollinations not setting fruit) may be common in tropical trees. Open-pollinated fruit set in all *Inga* species here was less than 5%; Bawa's (1974) survey of many lowland species revealed many legumes, and plants of other families also, to have low open-pollinated fruit set (less than 10%), which could result from a variety of factors. However, some of his cross-pollinations (performed with pollen from several sources, presumably near) yielded low amounts of fruit—e.g., *Enterolobium cyclocarpum* (Mimosoideae) with crosses yielding only 28% fruit; *Caesalpinia eriostachys* 10%; *Lonchocarpus eriocarinalis* 28%; *Hemiangium excelsum* 17%; *Hirtella racemosa* 18%; and *Godmania aesculifolia* 14% (Bawa, 1974). A likely explanation for this phenomenon is that cross-pollinations between genetically similar individuals are less fecund than those between genetically distinct individuals.

Inga population structure is the result of gene dispersal effected not only via pollen movement, but also by seed dispersal. Seed dispersal of *Inga* is performed by mammals (monkeys, squirrels) and perhaps by birds (pers. observ.). Dispersers eat the sweet pulp surrounding the seeds, and drop the seeds (which germinate almost immediately). Animals feeding in the parent tree may drop many seeds directly below, resulting in offspring clustered around the parent tree. Seed dispersal may also occur to a great distance when whole fruits are carried off by dispersers that may range far and wide, but clumps of relatives may be deposited where the frugivore sits and feeds. It is common to see a dense local group of *Inga* seedlings of the same age and size on the forest floor, far from any potential "parent" tree, providing circumstantial evidence that this type of dispersal may be occurring. If this is indeed the case, crosses between adult individuals close together are likely to be crosses between close relatives. This could be why far-crosses are more fruitful than near-crosses in the *Inga* species examined. Price and Waser (1979; Waser and Price, 1983) found the "optimal outcrossing distance" of the herbaceous perennials *Delphinium nelsonii* and *Ipomopsis aggregata* to be between 1 and 100 m, determined by the interaction of restricted pollen and seed dispersal. If there is an optimal distance for outcrossing in *Inga,* it is likely to be greater than 10 m because of the greater distances between individuals in nature, as well as greater distances involved in pollen and some sorts of seed dispersal.

The large floral displays of *Inga* species are successful in attracting many visitors away from other species of flowering plants in the forest, but may be advantageous in other ways as well. Interspecific hand-crosses yielded no fruit, and I saw no germination of foreign polyads on *Inga brenesii* stigmas; however, foreign polyads may interfere with the attachment and germination of polyads of the appropriate species. The same visitor species (from hummingbirds to hawkmoths to skippers) will visit whatever *Inga* species have flowers open at their foraging time, and although a certain amount of separation is afforded simultaneously blooming, sympatric *Inga* species by virtue of differences in floral behavior (flower opening time, anther dehiscence, nectar secretion; Koptur, 1983), some interspecific cross-pollinations are inevitable. Self-polyads and incompatible cross-polyads can also serve to "clog" stigmas (Waser, 1978), and visitors were frequently observed to visit many flowers on the same tree, and/or move to the nearest neighbor tree after leaving the first tree. The many thousands of flowers produced by an *Inga* individual may be needed to provide enough stigmas so that at least a few flowers will be successfully fertilized.

The findings of this study underscore the importance of long-distance pollinators in the reproductive biology of tropical trees. Self-incompatibility is the rule rather than the exception among tropical trees that have been studied; where complex self-incompatibility systems are involved, the movement of pollen between individuals is not always the only requirement for fertilization. Reasoning that inter-individual distances are maximized, and the density of conspecific plants low as the result of selection over evolutionary time by seed predators and other enemies (Janzen, 1970; Connell, 1978), long-distance pollinators are clearly necessary for any fruit to be produced in self-incompatible species. Recent quantitative studies (Hubbell, 1979, 1980; Hubbell and Foster, 1983) indicate that hyperdispersion is not usual, but rather than individuals of most species are clumped. If clumps are comprised of individuals with a higher degree of relatedness than that found between clumps, there should be selection for adaptations in self-incompatible tree species to support the energetic needs of exceptionally long-flying visitors, and to offer these rewards over an extended period of time. The perfection of this strategy is seen in species with very large flowers opening a few at a time over a long period, taking advantage of specialized "trapliners." Phylogenetic constraints may prevent many species from adopting this strategy—an alternative available to many (e.g., the mimosoid legumes like *Inga*) is to produce large numbers of relatively unspecialized flowers that are visited by a variety of pollinators over an extended blooming season. Most of these flowers will be visited, but the vast majority of these will not receive pollen suitable for fertilization. Since most of these plants exist within a clump of close relatives, these costly features may be worthwhile for maintenance of genetic variability in the species.

SUMMARY

Species of *Inga* characteristically have large floral displays, but few of the flowers set fruit. Seven species were studied in lower montane wet forest at Monteverde, Costa Rica (*Inga brenesii, I. densiflora, I. longispica, I. mortoniana, I. oerstediana, I. punctata,* and *I. quaternata*) to elucidate breeding systems and determine what factors limit fruit set.

Observations of visitor activity (hawkmoths, hummingbirds, and skippers) on *Inga brenesii* and *I. punctata* indicated that there was no shortage of pollination.

Examination of stigmas of flowers collected from all species showed that far more flowers had received pollen than normally set fruit. Hand pollination of six spp. revealed them to be self-incompatible. Observations of pollen grain germination and pollen tube growth in *I. brenesii* indicated its incompatibility system is gametophytic. Pollen/ovule ratios were much lower than those usually associated with self-incompatible species, but this may be explained by the greater efficiency of fertilization provided by having pollen in polyads. None of the species that bloom simultaneously were cross-compatible. Intraspecific cross-pollinations in *I. brenesii, I. punctata,* and *I. densiflora* were more successful when the pollen sources were more than 1 km away from the stigmatic parent than when pollen sources were less than .5 km away. Long-distance pollinator movements are therefore likely to be of greatest consequence in fruit setting. The low fruit set of *Inga* is a result of a special case of pollinator limitation: much more geitonogamy and unsuitable xenogamy is effected by pollinators than optimal outcrossing to greater distances. This phenomenon may be widespread among tropical trees; long-distance pollinators may be even more important than formerly thought. The flowering strategy of *Inga* has evolved to attract many pollinators over extended periods of time, while the breeding systems ensure maintenance of genetic variability despite much selfing and crosses between near neighbors.

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