

POLLEN DISPERSAL IN LOW-DENSITY POPULATIONS OF THREE  
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**Abstract.**—Studies of mating patterns of tropical trees, typically involving common species, have revealed that most species are outcrossed and that, in some cases, a significant fraction of outcross pollen moves long distances. We evaluated mating systems and effective pollen dispersal for three hermaphroditic, insect-pollinated Neotropical tree species, *Calophyllum longifolium*, *Spondias mombin*, and *Turpinia occidentalis*, all of which occurred at low adult densities at the study site. Mating patterns were estimated for each maternal tree within 84-ha populations of *C. longifolium* and *S. mombin* in 1992 and 1993 and within a 50-ha population of *T. occidentalis* in 1993. Each population was 100% outcrossed. Multilocus paternity exclusion analyses indicated that in *C. longifolium*, a minimum of 62% of effective pollen moved at least 210 m. For *S. mombin*, estimates of apparent pollen flow greater than 300 m were 5.2% and 2.5% in 1992 and 1993, respectively. For all species, pollen dispersal was strongly affected by the spatial distribution of reproductive trees. Where flowering adults were evenly spaced, a large fraction of effective pollen moved at least a few hundred meters and well beyond the nearest reproductive neighbors. Conversely, where flowering trees were clumped, the majority of matings were among near neighbors. The minimum area required to encompass a natural breeding unit was estimated for each population.

The large intertree distances characteristic of many tropical tree populations have long fascinated evolutionary biologists. Early workers, perplexed by the broad spacing often observed among conspecifics, predicted that such trees should be predominantly self-fertilizing or inbred (e.g., Corner 1954; Baker 1959; Fedorov 1966). This expectation was based on the following generalizations. First, unlike the primarily wind-pollinated temperate flora, tropical woody species are almost exclusively animal pollinated (Frankie 1975; Janzen 1975; Opler et al. 1980). In view of the high tree species diversity and structural complexity of the rain forest, it was assumed that these pollinators would be unlikely to move reliably among widely spaced conspecifics (Corner 1954; Baker 1959; Fedorov 1966). It was also observed (though in unnatural settings) that tropical trees flower

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asynchronously within populations (Corner 1954; Fedorov 1966). Thus, opportunities for outcrossing among tropical trees, particularly over long distances, were expected to be limited.

Based on his studies of dipterocarp forests in Southeast Asia, Ashton (1969) advanced the contrasting view that, although selfing may be common, ample opportunity exists for outcrossing in tropical trees. The generalist nature of many tropical pollinators, as well as the highly synchronized flowering characteristic of dipterocarp forests, were cited among the reasons for this expectation (Ashton 1969). Indeed, more recent observations of the reproductive phenology of tropical trees have revealed that flowering within natural populations is largely synchronized and governed by seasonal processes (e.g., Frankie et al. 1974). Further support for the view that widely spaced tropical trees are outcrossed came from Janzen's (1971) observation that female Euglossine bees, important pollinators in the Neotropics, regularly "trapline" over tens of kilometers in their daily foraging for nectar and pollen. Janzen (1971) cited additional reports that other Neotropical pollinators, including hummingbirds, sphyngid moths, and other large bees, regularly move long distances among conspecific plants, shifting to new host species as they become available.

Despite the challenge presented by these opposing views, relatively few studies have focused on mating patterns of tropical trees. Direct investigations of tropical tree breeding systems, involving surveys of floral morphology and/or hand pollination experiments, revealed that obligate outcrossing is the chief mode of reproduction in these species (Ashton 1969; Bawa 1974; Kalin Arroyo 1976; Opler and Bawa 1978; Chan 1981; Koptur 1984; Lack and Kevan 1984; Bawa et al. 1985*b*; Kevan and Lack 1985; Dayanandan et al. 1990). High outcrossing rates in tropical trees are achieved largely through a combination of widespread self-incompatibility in hermaphroditic species and a high frequency of dioecy (22% in a Costa Rican dry forest [Bawa 1974], 23% in a Costa Rican moist forest [Bawa et al. 1985*b*], and 26% in a mixed dipterocarp forest in Sarawak [Lewis 1942]).

In the first analysis of the mating system of a tropical tree species using genetic markers, O'Malley and Bawa (1987) estimated a population outcrossing rate of 95% for *Pithecellobium pedicellare* in a Costa Rican rain forest. Subsequent analyses have revealed high rates of outcrossing in many other hermaphroditic tropical trees (O'Malley et al. 1988; Murawski and Hamrick 1991; Murawski et al. 1994). Though mixed mating systems, in which a significant proportion of progeny arise through selfing, have also been found (e.g., *Cavanillesia platanifolia* [Murawski et al. 1990; Murawski and Hamrick 1992*a*], *Ceiba pentandra* [Murawski and Hamrick 1992*b*], and *Shorea trapezifolia* [Murawski et al. 1994]), such species appear to be relatively uncommon. For two species of Bombacaceae with mixed mating systems (*C. platanifolia* and *C. pentandra*), outcrossing rates of individual trees were positively correlated with the number of flowering conspecific neighbors (Murawski et al. 1990; Murawski and Hamrick 1991, 1992*b*). Furthermore, for these and six other tropical species, deviation from random mating was greater in more spatially isolated trees, which presumably received pollen from fewer donors (Murawski and Hamrick 1991).

Studies of patterns of pollen movement in tropical woody species are few, but

in some cases they have revealed that a significant fraction of outcross pollen moves over long distances. For two butterfly-pollinated species, the herb *Cnidocolus urens* and the vine *Psiguria warscewiczii*, work with fluorescent dye particles indicated pollen movement over distances of 31 m and up to 600 m, respectively (Webb and Bawa 1983; Murawski 1987). Analogous work with the hummingbird-pollinated shrub *Malvaviscus arboreus* indicated pollen flow over 225 m between flowering plants (Webb and Bawa 1983). More recently, genetic markers and paternity exclusion techniques have been used to infer directly patterns of effective pollen movement in populations of tropical trees. Using this approach, other researchers (Hamrick and Murawski 1990) found that, in populations of the hermaphroditic canopy trees, *Platypodium elegans* and *Tachigali versicolor*, 20% of successful pollen moved at least 750 m, and 25% moved at least 500 m, respectively. Both of these species (Leguminosae: Papilionoideae) are pollinated by bees that presumably forage over extensive areas (Janzen 1971). Similar work with a third Neotropical tree species, *Cordia alliodora*, which is likely pollinated by long-tongued bees and/or butterflies (Croat 1978), showed that, although the majority of effective pollen movement was restricted to about 75 m, a small but significant proportion moved up to 280 m (Boshier et al. 1995).

Most studies of mating patterns of tropical trees have focused on conspicuous species. Much less is known about tropical tree species that normally occur at very low densities (e.g., one adult per 10 ha), for which reproduction is presumably more challenging. For low-density populations, a key question is whether cross-pollination is regularly accomplished or whether selfing is more common as a result of the limited availability of outcross pollen.

For instance, in an indirect study of mating systems of lowland rain forest trees in Costa Rica, Bawa et al. (1985b) found an unusually high frequency of self-compatibility among low-density hermaphroditic species. It was suggested (Murawski and Hamrick 1991) that the underlying incompatibility mechanisms and constraints on selfing normally found in tropical trees may be relaxed in species that typically occur at extremely low densities. On the other hand, if reproduction in low-density populations is primarily by outcrossing, as it is for the more common tree species, then how far does pollen move among flowering adults? More specifically, are maternal trees mating primarily with nearest neighbors, or are they receiving the bulk of their pollen from donors located farther away? If the dispersal of outcross pollen is highly localized (i.e., restricted to nearest neighbors), then the potential for inbreeding in these populations should be considerable (Fedorov 1966; Bawa et al. 1985b), especially if seed dispersal is also limited.

In this study, we examine the mating system and patterns of effective pollen movement of three hermaphroditic tree species that occur at low adult densities in the tropical moist forest of Barro Colorado Island (BCI), Republic of Panama. In contrast to tropical tree species that have been analyzed previously, these three species are pollinated by a variety of small insects, including bees, beetles, flies, wasps, moths, and butterflies (Bawa et al. 1985a). Although the behavior of these insects as pollinators is largely unknown, their potential for long-distance pollen movement is presumably less than that for the larger tropical bee species

that have been studied more intensively (Frankie et al. 1976; Bawa 1977; Bawa et al. 1985a).

For each study species, the following questions were asked: Are reproductive individuals primarily selfed or outcrossed? If they are outcrossed, what is the pattern of effective pollen movement among individuals within the population? Specifically, what proportion of pollen movement is restricted to nearest neighbors versus that over longer distances? What is the effect of the spatial distribution of reproductive adults on the mating system and patterns of effective pollen movement? How do the mating system and the breeding structure of the population vary between reproductive events? Finally, what is the minimum area required to encompass a natural breeding unit?

#### STUDY SPECIES

*Calophyllum longifolium* Willd. (Clusiaceae) is a large, polygamous canopy tree known from moist forests of Panama, Colombia, Surinam, Brazil, and Peru (Croat 1978) (here, *polygamous* refers to individuals possessing both unisexual and bisexual flowers). Its flowers, produced in vast numbers, are small, greenish-white, fragrant, and characterized as primitive and open (Croat 1978). In Panama, flowering occurs principally during the rainy season (October–November), with fruits maturing throughout the late dry and early rainy seasons (March–July; Croat 1978). The green, single-seeded fruits are dispersed by mammals (primarily bats; Yazquez-Yanes et al. 1975) but may also be moved by water (Ridley 1930). At the study site, seedlings and saplings of *C. longifolium* are common, but adults occur at a density of about one tree per 3.5 ha.

*Spondias mombin* L. (Anacardiaceae), also a canopy tree, occurs naturally throughout tropical America and as an introduced species in tropical Africa and the East Indies (Croat 1978). In Panama, the small, white, actinomorphic flowers of *S. mombin* are typically bisexual and occasionally pistillate (Croat 1978). Flowering occurs primarily from March to June, with fruits maturing from July to October (Croat 1978). The fleshy, tasty mesocarp of the multiseeded fruit is an important food source for a wide range of frugivores, some of which may serve as effective seed dispersers (Smythe 1970; Heithaus et al. 1975; Yazquez-Yanes et al. 1975). On BCI, *S. mombin* is frequent in secondary forest and along lake edges, but it is restricted within the old forest to an adult density of approximately one tree per 6 ha.

*Turpinia occidentalis* G. Don subsp. *breviflora* Croat (Staphyleaceae) is a smaller tree found occasionally in subcanopies of moist forests of southern Mexico through Colombia and the West Indies (Croat 1978). The flowers of *T. occidentalis* are tiny, fragrant, white, and bisexual (Croat 1978). In Panama, flowers are present mainly from April to June, with fruits maturing from July through September (Croat 1978). Fruits are yellow at maturity and contain several small orange-brown seeds that are dispersed by arboreal frugivores and secondarily by terrestrial mammals (Croat 1978). Although juveniles at the study site are common (about one individual with >1-cm diameter at breast height [dbh] per 0.6 ha), adults occur at a much lower density (one tree per 2.2 ha).

## MATERIAL AND METHODS

*Study Site and Field Methods*

We sampled populations of *Calophyllum longifolium*, *Spondias mombin*, and *Turpina occidentalis* on BCI, the largest island (16 km<sup>2</sup>) in Lake Gatun. Lake Gatun was formed after the damming of the Chagres River during the construction of the Panama Canal from 1907 to 1914. The vegetation on BCI is semievergreen, moist tropical forest, including both primary and secondary stands (Knight 1975; Croat 1978). Our study site was the 50-ha Forest Dynamics Project (FDP) plot, managed by the Smithsonian Tropical Research Institute (fig. 1) (Hubbell and Foster 1983). The Smithsonian Tropical Research Institute maintains an extensive database on the demography and sizes of all woody species occurring on the FDP plot, updating the information every 5 yr through recensusing. Using this database and published records of fruiting phenology (e.g., Croat 1978; Foster 1992), we selected three species that occur at low adult densities on the FDP plot and that produce accessible seeds. For the two larger species, *C. longifolium* and *S. mombin*, we surveyed a 100-m-wide border around the plot perimeter (previously established by J. L. Hamrick and D. A. Murawski). This approach increased the total study area to 84 ha for those two species. All adults found within this zone were tagged, measured for diameter at breast height, scored as with or without fruit, and mapped relative to the 50-ha FDP plot.

*Calophyllum longifolium* and *S. mombin* were sampled in both 1992 and 1993, whereas sampling of *T. occidentalis* was restricted to 1993. All potentially reproductive individuals within the study area were scored for the percentage of their crown that contained fruit. During the longer 1993 field season, the total reproductive output of individual trees (from 0% to 100% crown) was determined by repeatedly observing the canopy and the ground surrounding each tree throughout the period of fruit maturation.

Dispersion analysis for reproductive trees was performed for each species according to the nearest-neighbor method of Clark and Evans (1954). The index of dispersion ( $R$ ) was calculated as the ratio of observed to expected mean distances between nearest reproductive neighbors, the latter calculated on the assumption that trees are distributed randomly. Values of  $R$  less than 1.0 indicate clumping, whereas those greater than 1.0 indicate a more even distribution.

Using a wrist slingshot, we sampled leaf material from each adult and subadult within the study area for each species (i.e., either 84 ha or 50 ha). Subadults were included in the sampling, as estimates of minimum size (dbh; FDP data) at sexual maturity are not always accurate. Thus, all individuals of *C. longifolium*  $\geq 18$ -cm dbh according to the 1990 plot census were sampled. Similarly, we sampled all individuals of *S. mombin* and *T. occidentalis* recorded in 1990 as  $\geq 20$ -cm and  $\geq 15$ -cm dbh, respectively. Sampled tissue was freeze-dried on BCI for 48 h following an earlier method (Hamrick and Loveless 1986) and shipped in airtight plastic bags to the University of Georgia within 2 wk of sampling. At the lab, all freeze-dried material was held at  $-70^{\circ}\text{C}$  until enzyme extraction was performed. Fruit or seed was collected from the ground beneath each reproductive adult and either freeze-dried on BCI (for *C. longifolium*, about one-third embryo) or

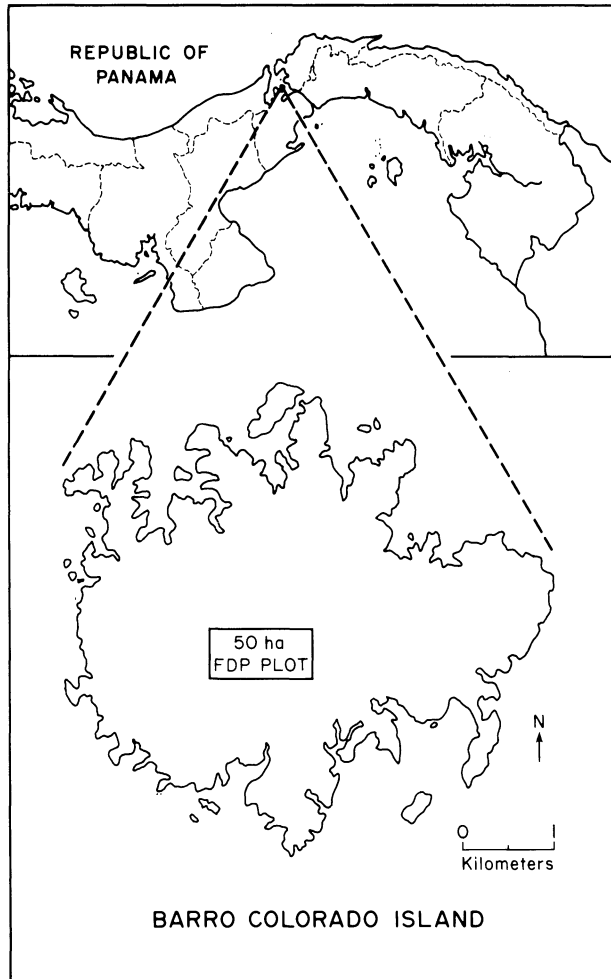


FIG. 1.—Republic of Panama with Barro Colorado Island, located approximately in the center of the Panama Canal. The location of the 50-ha Forest Dynamics Project (FDP) plot on Barro Colorado Island is also shown.

shipped directly to the University of Georgia for germination under standard greenhouse conditions (single seeds of *T. occidentalis* and cleaned, air-dried endocarps of *S. mombin*).

Enzyme extraction followed the procedure of Mitton et al. (1979). All material was crushed in liquid nitrogen with a pinch of clean sea sand. An extraction buffer was added, and the resulting solution was absorbed onto filter-paper wicks and stored at  $-70^{\circ}\text{C}$  before electrophoresis.

#### *Electrophoresis and Statistical Analysis*

For all adults and progeny sampled, we employed starch-gel electrophoresis to determine genotypes at all polymorphic loci showing high resolution. Five gel/

TABLE 1

POLYMORPHIC LOCI RESOLVED FOR THREE SPECIES OF TROPICAL TREES  
AND THE GEL/ELECTRODE BUFFER SYSTEMS USED FOR EACH

Buffer System	<i>Calophyllum longifolium</i>	<i>Spondias mombin</i>	<i>Turpinia occidentalis</i>
7	...	...	AAT-1(2)
	...	...	AAT-2(2)
	...	...	MNR-1(2)
8	LAP-1(3)	DIA-1(2)	DIA-1(2)
	PGI-2(2)	FE-1(2)	PER-1(2)
	...	LAP-1(2)	PGI-2(2)
	...	PGI-2(4)	TPI-1(2)
10	6PGD-1(3)	...	...
11	MDH-1(2)	PGM-1(2)	MDH-1(2)
	...	...	IDH-1(2)
6	CE-2(2)	...	...
	FE-2(2)	...	...
	PER-1(2)	...	...

NOTE.—Descriptions and recipes of buffer systems can be found in Soltis et al. (1983). The number of alleles at each locus is shown in parentheses. Abbreviations: AAT, Aspartate aminotransferase; CE, colorimetric esterase; DIA, diaphorase; FE, fluorescent esterase; IDH, isocitrate dehydrogenase; LAP, leucine aminopeptidase; MDH, malate dehydrogenase; MNR, menadione reductase; PER, peroxidase; 6PGD, 6-phosphogluconate dehydrogenase; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; and TPI, triosephosphate isomerase.

electrode buffer systems were used to resolve five to nine polymorphic allozyme loci, depending on the species (table 1). Based on observed levels of genetic diversity at each locus, multilocus exclusion probabilities were calculated for each population (Brown 1989). An exclusion probability indicates the resolving power of paternity data and is defined as the mean probability of excluding a randomly selected male as the father of a given offspring from a randomly chosen maternal individual (Chakraborty et al. 1988).

The mating system, or proportion of selfing versus outcrossing, was estimated for each species using the multilocus mixed-mating model of Ritland and Jain (1981; Ritland 1990). This maximum-likelihood method provides single- and multilocus estimates of outcrossing rate, as well as estimates of pollen allele frequencies at both the population and individual levels. Standard errors for each value were estimated based on the construction of 100 bootstrap data sets, following the methods of Ritland and Jain (1981). Model assumptions are discussed by Ritland and Jain (1981).

The distribution of pollen movement to each outcrossed adult was evaluated using fractional paternity exclusion analysis (Devlin et al. 1988). For each maternal tree, the straight-line distance to the nearest edge of the study area was determined and treated as the radius of a hypothetical circle encompassing the "known neighborhood" for that tree (fig. 2). The known neighborhood was there-

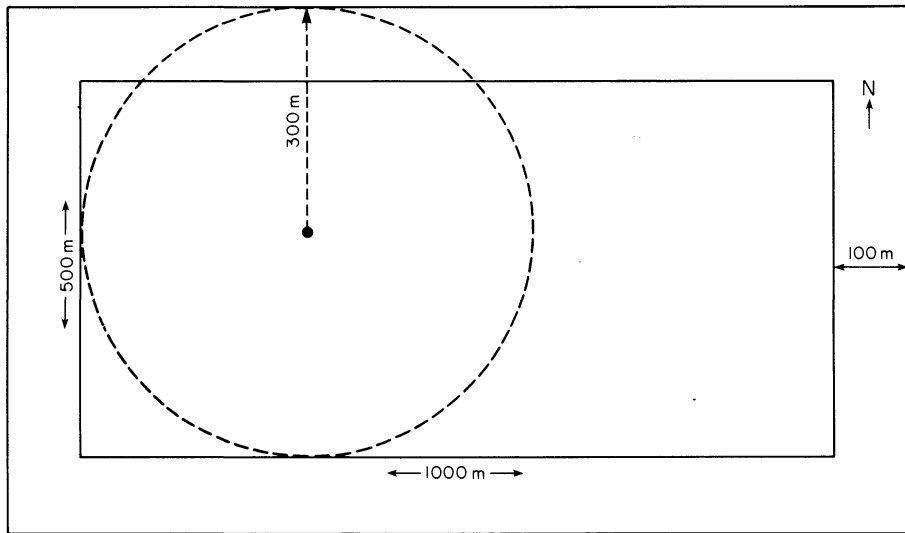


FIG. 2.—Schematic of an example of a “known neighborhood” for the single-tree paternity exclusion analyses conducted on the 50-ha FDP plot and surrounding 100-m-wide border zone.

fore the maximum circular area surrounding each maternal tree within which all potential pollen donors had been located and genotyped. More centrally located maternal trees had larger known neighborhoods, the largest possible neighborhood being 700 m in diameter (38.5 ha in area) for trees located near the center of the east-west midline of the FDP plot. A series of paternity exclusion analyses was performed for all progeny sampled from each maternal tree. In the first analysis, only the maternal tree was included in the pool of potential paternal parents. Subsequent analyses included additional trees in the paternal pool, one at a time, from the nearest neighbor outward until the edge of the known neighborhood was reached. After each analysis, the proportion of progeny whose genotype could not be assigned to any tree in the paternal pool was recorded. Apparent pollen flow was estimated as the proportion of total pollen flow for which no donor within the paternal pool could be identified given the available genetic data (Devlin and Ellstrand 1990). Since this approach evaluates levels of apparent pollen movement only, it provides conservative, minimum estimates of pollen dispersal distances to each reproductive adult over the spatial scale of its known neighborhood. In addition to the above analyses, further analyses were conducted in which all known potential fathers occurring within a zone (“extended neighborhood”) of 100–150 m beyond the known neighborhood were included. Estimates of incoming apparent pollen flow from beyond the extended neighborhood may be inflated, if unidentified reproductive adults occurred in unsurveyed areas.

A second means of examining pollen flow was used for *S. mombin*. Three reproductive adults were each heterozygous for a rare marker allele. These individuals were treated as pollen donors, and the distribution of their rare alleles



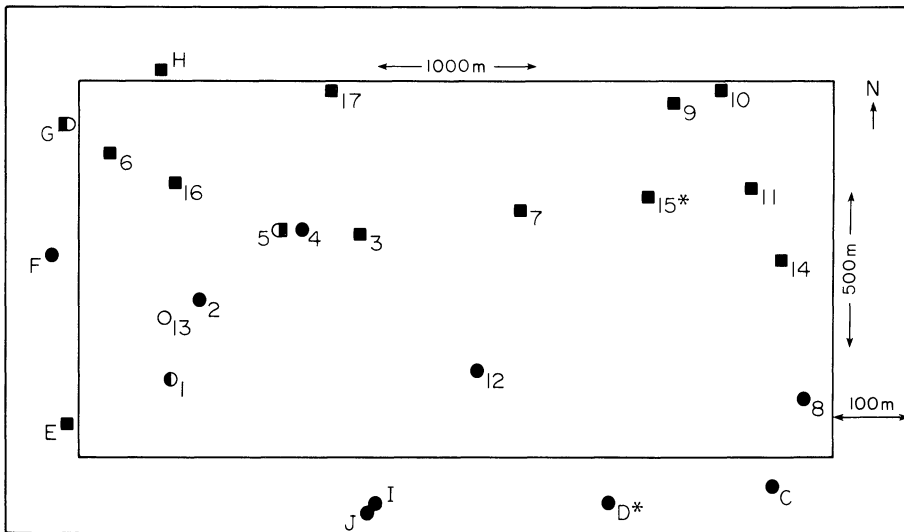


FIG. 3.—The 84-ha plot indicating the locations and identifications of all 25 adults of *Calophyllum longifolium* (i.e., all individuals with  $\geq 30$ -cm dbh). Symbols are in two halves, the left and right halves depicting reproductive activity in 1992 and 1993, respectively. Circles denote reproductive individuals (*solid circles* for moderately to highly reproductive trees; *open circles* for minimally reproductive trees). Squares denote nonreproductive adults. Asterisks indicate adults alive in 1992 that died before the 1993 fruiting season.

was examined in progeny sampled from surrounding adults. Also, at each of three loci, an allele was found in the sampled progeny that was absent among reproductive adults within the study area. Variation in the frequency and distribution of each immigrant allele among sampled families was examined for insights into patterns of long-distance gene movement in *S. mombin*.

Based on the observed distributions of effective pollen movement, we estimated the minimum area occupied by a natural breeding unit of each species. This was defined as the area within which, on average, 95% of the pollen received by a centrally located adult originates.

## RESULTS

### *Spatial and Reproductive Patterns*

*Calophyllum longifolium*.—The minimum size required for reproduction in *Calophyllum longifolium* is about 30-cm dbh (FDP data). Fruit production by adults less than 49-cm dbh, however, was limited or absent in both years. A total of 25 adult (fig. 3) and six subadult trees were sampled from the 84-ha study area. Of the 25 adults present in August 1992, 23 survived to the following fruiting season. In 1992 and 1993, respectively, about half (14 and 12) of these adults did not reproduce, and a few (one and four) were minimally reproductive (i.e., with 10% of their canopy containing fruit), which left only 8–10 highly reproductive trees

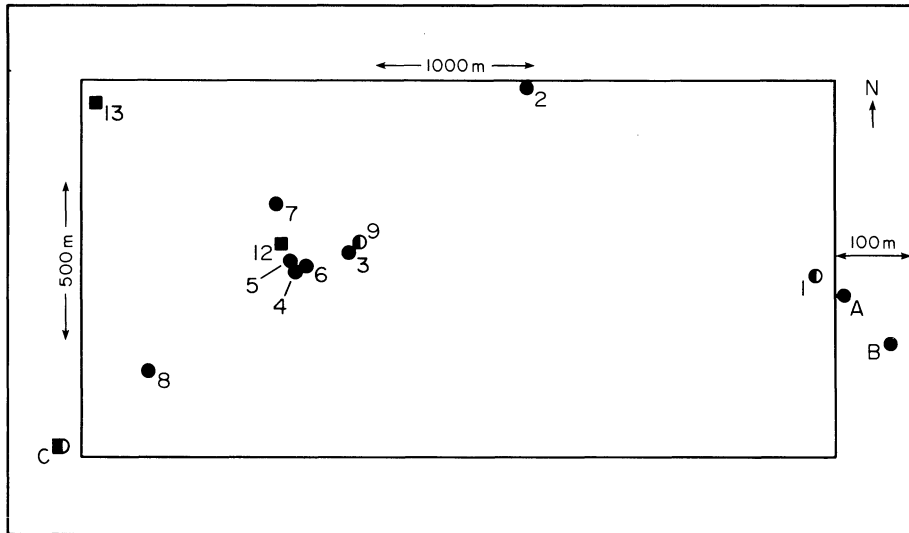


FIG. 4.—The 84-ha plot indicating the locations and identifications of all 14 adults of *Spondias mombin* (i.e., all individuals with  $\geq 30$ -cm dbh). Symbols are in two halves, the left and right halves depicting reproductive activity in 1992 and 1993, respectively. Circles denote reproductive individuals (solid circles for moderately to highly reproductive trees; open circles for minimally reproductive trees). Squares denote nonreproductive adults.

(50%–100% of the crown with fruit). Total fruit production in these trees was consistent across the two study seasons. In sum, the density of *C. longifolium* adults with substantial reproduction was only about one tree per 10 ha. The spatial distribution of reproductive trees was slightly clumped (index of dispersion,  $R = 0.89$ ), with a mean nearest-neighbor distance between reproductive trees of 99.8 m. Two adults, I and J, were tightly clustered (fig. 3). Removing these two trees from the dispersion analysis resulted in a slightly hyperdispersed distribution ( $R = 1.06$ ) with a mean reproductive nearest-neighbor distance of 108.4 m.

Up to 60 seeds were collected from each reproductive tree per season. Excluding minimally reproductive trees, means of 32 and 56 mature single-seeded fruits per tree were collected in 1992 and 1993. The larger sample sizes in 1993 were due to a longer field season in that year, which permitted greater opportunity to collect from trees with slightly different phenologies.

*Spondias mombin*.—As for *Calophyllum longifolium*, the minimum size of sexually mature adults of *Spondias mombin* is approximately 30-cm dbh (FDP data). A total of 14 trees of adult size occurred within the 84-ha site, 12 of which reproduced during the study period (fig. 4). Eleven adults produced fruit during both reproductive seasons, whereas the twelfth, a small adult, fruited possibly for the first time in 1993. In sum, the observed density of reproductive *S. mombin* in the study area was approximately one tree per 7 ha. The distribution of repro-

ductive *S. mombin* adults was clumped (fig. 4;  $R = 0.65$ ), with a mean nearest-neighbor distance for reproductive adults of 86.5 m. The two adults that failed to reproduce in either year had heavy vine loads, which could have restricted flowering. In addition to the 14 adult trees, five subadults between 20- and 29.9-cm dbh (1990 census) were sampled for electrophoretic analysis.

Fruiting of reproductive trees involved nearly 100% of the crown with few exceptions. In 1992 and 1993, about 150 mature fruits were collected from the ground beneath each reproductive adult. Ultimately, 576 seedlings from 10 families and 430 seedlings from 11 families were analyzed from the 1992 and 1993 fruit crops, respectively. The additional family sampled in 1993 was permitted by the longer field season that year, which overlapped with fruit drop of all adults.

*Turpinia occidentalis*.—We observed and sampled *Turpinia occidentalis* in 1993 only. Although the minimum size of sexually mature trees was previously reported as 30-cm dbh (FDP data), we found evidence of low fruit production by individuals as small as 17-cm dbh, which were consequently classified as adults. A total of 30 trees  $\geq 15$ -cm dbh (1990 census) were sampled from the 50-ha study area, including 23 adults (fig. 6, below). Dispersion analysis of all potentially reproductive trees (i.e.,  $\geq 17$ -cm dbh) revealed a slightly clumped spatial distribution ( $R = 0.95$ ), with a mean reproductive nearest-neighbor distance of 67.1 m. Fruit production varied considerably among individuals, with seven failing to fruit altogether. Of the 16 remaining trees, eight showed minimal fruit yield ( $< 10\%$  of canopy), with most of the fruit crop restricted to the largest trees. Five of the six largest adults had crowns 50%–100% full of fruit, whereas reproduction by the sixth tree was apparently restricted by vines. The five highly reproductive trees occurred in two clusters (i.e., no individual was spatially isolated; fig. 6, below).

We collected from 34 to approximately 800 seeds from the seed shadows of each reproductive tree. Germination rates were fairly low across all families, in large part because of infestation of some seeds. Ultimately, 172 progeny from six families were electrophoresed (6–43 progeny each; mean = 28).

#### *Genetic Variation and Exclusion Probabilities*

The quality of allozyme expression varied among species and tissue types. The number of resolvable loci was greatest in freeze-dried embryos of *Calophyllum longifolium* and fresh and freeze-dried leaves of *Turpinia occidentalis*, whereas it was somewhat reduced in all other lyophilized material and fresh *Spondias mombin*. For all three species, particularly *C. longifolium* and *S. mombin*, accurate scoring of some loci was restricted to seed/seedling material, because of stronger expression in these tissues. Maternal genotypes at these loci were, therefore, inferred based on genotypic ratios in progeny arrays (Brown and Allard 1970). In all, seven polymorphic loci (with two or three alleles each) were scored in *C. longifolium*, five (with two to four alleles each) in *S. mombin*, and nine (each with two alleles) in *T. occidentalis* (table 1). Based on observed levels of allozyme variation, exclusion probabilities calculated for each population and season were as follows: *C. longifolium*, 0.67 and 0.74 in 1992 and 1993, respectively; 0.53 for *S. mombin* in both years; and 0.78 for *T. occidentalis* in 1993.

### *Mating System*

All three populations were essentially 100% outcrossed. No notable variation in mating system was found within individuals between reproductive seasons or among fruiting adults within populations. Therefore, there seems to be little effect of year or local density of reproductive adults (i.e., number of near neighbors) on individual outcrossing rates. Here we report multilocus and mean single-locus estimates of population outcrossing rates, along with standard errors for each based on 100 bootstraps.

*Calophyllum longifolium*.—Estimates of the mating system were based on a set of eight reproductive adults in each year. Multilocus estimates of outcrossing rate ( $t_m$ ) were very similar over the 2 yr at  $1.030 \pm 0.085$  (mean  $\pm$  SE) and  $1.031 \pm 0.035$  in 1992 and 1993, respectively. Mean single-locus estimates ( $t_s$ ) for the two seasons were lower,  $0.974 \pm 0.062$  and  $0.948 \pm 0.042$ , which possibly indicates biparental inbreeding (Brown 1989).

*Spondias mombin*.—Multilocus estimates of population outcrossing rate were  $t_m = 0.989 \pm 0.163$  and  $t_m = 1.304 \pm 0.108$  in 1992 and 1993, respectively, based on 10 and 11 families sampled in the 2 yr. Mean single-locus estimates were  $t_s = 0.996 \pm 0.166$  in 1992 and  $t_s = 1.280 \pm 0.124$  in 1993. The high variances associated with these estimates may be due to skewed allele frequencies at three of the five loci used.

Additional evidence that adults of *Spondias mombin* were completely outcrossed was observed in the progeny genotype data of the three reproductive adults heterozygous for a rare allele. No individuals homozygous for the rare allele were found among the progeny sampled in either year (156, 129, and 64 progeny each, pooled across years).

*Turpinia occidentalis*.—Population-level estimates of outcrossing rate based on all six families with appreciable progeny arrays were  $t_m = 1.006 \pm 0.090$  and  $t_s = 1.071 \pm 0.109$ .

As unequal family sizes can negatively bias multilocus estimates of the outcrossing rate (Shaw et al. 1981), mating system analyses were repeated for each species minus the one or two smallest families in each set. The resulting estimates of population outcrossing rate were elevated slightly.

### *Patterns of Effective Pollen Movement*

*Calophyllum longifolium*.—In each reproductive season, a substantial proportion of pollen moved over distances of at least a few hundred meters and well beyond the nearest reproductive neighbors. Averaging the results of the paternity exclusion analyses over both years for the five most centrally located maternal trees (414 total progeny), we found that 62% of all successful pollen moved at least 210 m (table 2), which is more than twice the mean distance between nearest reproductive neighbors. For all but two maternal trees (trees I and J) for which the nearest reproductive neighbor occurred within the known neighborhood, from 40% to 91% of all pollen received came from trees located beyond the nearest neighbor. Trees I and J occurred just 2 m apart at breast height (fig. 3). In sharp contrast to the high levels of moderate-distance pollen movement observed

TABLE 2

MINIMUM ESTIMATES OF APPARENT INCOMING POLLEN FLOW (%APF) FROM BEYOND THE KNOWN AND EXTENDED NEIGHBORHOODS FOR FIVE CENTRALLY LOCATED MATERNAL ADULTS OF *CALOPHYLLUM LONGIFOLIUM*

Material ID	Number of Progeny	Radius of Known Neighborhood (m)	%APF	Radius of Extended Neighborhood (m)	%APF
1	41	200	48.8	316	9.8*
2	90	260	38.9	382	6.7
4	43	300	48.8*	375	9.3*
8	121	140	73.4	290	46.4*
12	119	212	77.3	230	23.7
Means	82.8	209.5†	62.1†	...	...
	73.3‡	237.9‡	57.3‡	...	...

NOTE.—The extended neighborhood for each maternal tree includes the known neighborhood plus an additional area up to 150 m wide that includes the next nearest known pollen donor(s). Values are pooled over two reproductive seasons except where noted.

\* Estimate for one reproductive season only.

† Means are weighted by family size.

‡ Mean values if tree 8 (with smallest known neighborhood) is excluded.

among the more widely distributed trees, paternity exclusion results showed that each of these highly reproductive adults could have sired 73% (I) and 72% (J) of the progeny of its nearest neighbor. Thus, where reproductive trees occurred randomly, pollen flow ranged over at least a few hundred meters and well beyond the nearest reproductive neighbors. Conversely, in the single instance where adults were clustered, a very large fraction of matings were between nearest neighbors. The observed distribution of effective pollen flow was highly consistent over both years of the study.

To evaluate total apparent pollen flow on a broader scale, we performed exclusion analyses that treated the 50- and 84-ha populations as pollen sinks. Analyses involving all maternal trees on the 50-ha FDP plot and the same adults in the pool of potential fathers revealed 24.1% and 35.2% apparent pollen flow coming from beyond the 50-ha border in 1992 and 1993, respectively. When the paternal pool was expanded to include all reproductive adults in the 84-ha area, the estimated level of gene flow from beyond the study plot was 5.8% and 6.2% in these years. Consequently, in 1992 and 1993, approximately 18.3% and 29.1% of the progeny from maternal trees within the 50-ha plot may have been sired by adult trees within the 100-m zone surrounding the FDP plot. Finally, when all families within the 84-ha area were analyzed and all reproductive trees within the same area were included in the paternal pool, total apparent pollen flow from beyond the 84-ha plot was 5.0% and 7.7% in 1992 and 1993, respectively. Averaged over the 2 yr, maternal trees located within the 50-ha FDP plot received about 6% of their total incoming pollen from outside the 84-ha study area, whereas mothers located within the 100-m border received approximately 8% (9.3% in 1993 with increased sampling).

*Spondias mombin*.—The 84-ha *Spondias mombin* population was character-

TABLE 3

DISTRIBUTION AND FREQUENCY OF LONG-DISTANCE POLLINATION EVENTS (IMMIGRATION) WITHIN AND AMONG SIX ADULTS OF *SPONDIAS MOMBIN* CLUSTERED NEAR THE CENTER OF THE FDP PLOT

MATERNAL ID	NUMBER OF PROGENY SAMPLED		NUMBER WITHOUT FATHERS		PERCENTAGE IMMIGRATION	
	1992	1993	1992	1993	1992	1993
4	96	60	1	1	1.04	1.67
5	...	74	...	3	...	4.05
6	69	60	11	0	15.94	0
7	48	35	1	3	2.08	8.57
3	62	37	4	1	6.45	2.70
9	73	60	1	0	1.37	0
Means	70	54	3.6	2.3	5.17	2.45

NOTE.—Results shown are of paternity exclusion analyses for 2 yr including all reproductive trees within the 84-ha plot in the pool of potential fathers. Ellipses indicate no sample.

ized by a low but substantial level of apparent pollen flow over distances of at least 300–350 m. A cluster of six reproductive trees located in a seasonal swamp along the east-west midline of the study plot (fig. 4) permitted quantification of apparent pollen flow over this range of distances. Treating the swamp cluster as a pollen sink, paternity exclusion analyses revealed that a minimum of 5.2% (of 348 pooled 1992 progeny) and 2.5% (of 326 pooled 1993 progeny) of the apparent pollen flow originated from outside the 84-ha plot. Within this group, the proportion of progeny per maternal tree sired by unknown donors ranged from 0% to roughly 16% (of 69 progeny) in 1992 and 0% to almost 9% (of 74 progeny) in 1993 (table 3). The seemingly haphazard distribution of apparent pollen flow events among mothers within this relatively small area, as well as within mothers between years, suggests that long-distance pollen movement in this population is highly idiosyncratic.

Paternity exclusion analyses were also performed for the entire population of maternal trees within the 84-ha plot, including all known potential fathers in the paternal pool. Viewing the study population as a whole, a minimum of 4.2% (24/576 pooled 1992 progeny) and 2.3% (10/430 pooled 1993 progeny) of all matings resulted from immigrant pollen from outside the 84-ha area. Unlike *Calophyllum longifolium*, adults of *S. mombin* at the edge of the study area received no more detectable immigrant pollen than did more centrally located trees. This observation, however, may be an artifact of the lower exclusion probability for *S. mombin*.

At three of five polymorphic loci, we found an allele in the progeny that did not occur in the adults within the 84-ha plot. Each progeny carrying a foreign allele therefore represented a pollen flow event from a paternal tree located outside the 84-ha area. Even though all alleles at polymorphic loci were included in the multilocus analysis, examination of the on-plot distribution and frequency of each foreign allele in sampled families allowed additional insights into patterns of long-distance pollen flow. At PGI-2 in 1992, the foreign allele (PGI-2c) was

present in very low frequencies in families sampled throughout the 84-ha plot (one to three progeny from each of five families). In 1993, the same allele was sampled in the progeny of only two maternal trees (two progeny of tree 7 and three progeny of tree B). Ironically, these two maternal trees were located 849 m apart (fig. 4). Observation of a second immigrant allele (FE-1b) was restricted to 1992 and in one family only (in seven of 69 progeny), which was located in the cluster of trees in the swamp. A third foreign allele, LAP-1b, appeared in both years and was restricted to progeny from the swamp cluster. The LAP-1b allele was observed in 1992 in each of the four swamp cluster families sampled (nine progeny; from one to five per family), and it was found in 1993 in three families in that group (five progeny; one, one, and three per family). Based on the observed frequencies of LAP-1b (the most common immigrant allele), estimated rates of apparent gene flow from beyond the 84-ha border to the swamp cluster (>314-m distance) were 2.6% and 1.5% in 1992 and 1993, respectively.

Based on the pooled results of the single-tree paternity exclusion analyses, a high proportion of successful pollen moved short distances between mates, usually among members of a cluster. Pooling data across all but the two most isolated maternal trees, we found that  $90\% \pm 3.6\%$  of the progeny of a given mother were sired by the nearest neighbor (or two nearest neighbors in the tight cluster of adults including trees 4, 5, and 6; fig. 4). The observed pattern of effective pollen movement was consistent over both years of the study.

Analysis of the distribution of rare marker alleles about paternal parents indicates that pollen dispersal among adults of *S. mombin* was typically restricted to near neighbors. Because each donor was heterozygous for a rare allele, the total proportion of progeny sired by a donor on a particular maternal tree was determined by multiplying by two the proportion of offspring carrying that allele. This method should provide unbiased estimates of pollen dispersal among members of a cluster, though not necessarily over longer distances (i.e., the probability of sampling a rare allele decreases with increasing distance from the source). Each of the three pollen donors included in this analysis (trees 1, 4, and 6) occurred in an area of clumped reproductive adults (two groups of three and six trees each; fig. 4). The rare marker alleles were detected in progeny of adults within a radius of 75, 87, and 138 m from the three donor trees (fig. 5a–c). The only exception to this pattern was the observation in 1993 of one progeny sampled from tree 2 (60 total progeny sampled) with a rare allele for which the two only known sources occurred at distances of 392 and 465 m (fig. 5b, c). As tree 2 occurred 103 m from the nearest edge of the study area, it is possible that the donor of the rare allele was an off-plot adult located closer than either of the two known donors. However, the complete absence of the rare allele in all but one of 110 progeny sampled from tree 2 over the 2 yr makes this latter scenario unlikely.

The distribution of the three rare marker alleles among sampled families was consistent over the 2-yr period. In all cases, the rare allele was present in each family within the cluster that included the donor tree. The sole exception to this pattern occurred in 1993, when we failed to find DIA-1b from tree 1 in any of 19 progeny sampled from tree B located in the same cluster (fig. 4). Dispersal of rare alleles decreased as a function of distance between parents within a cluster

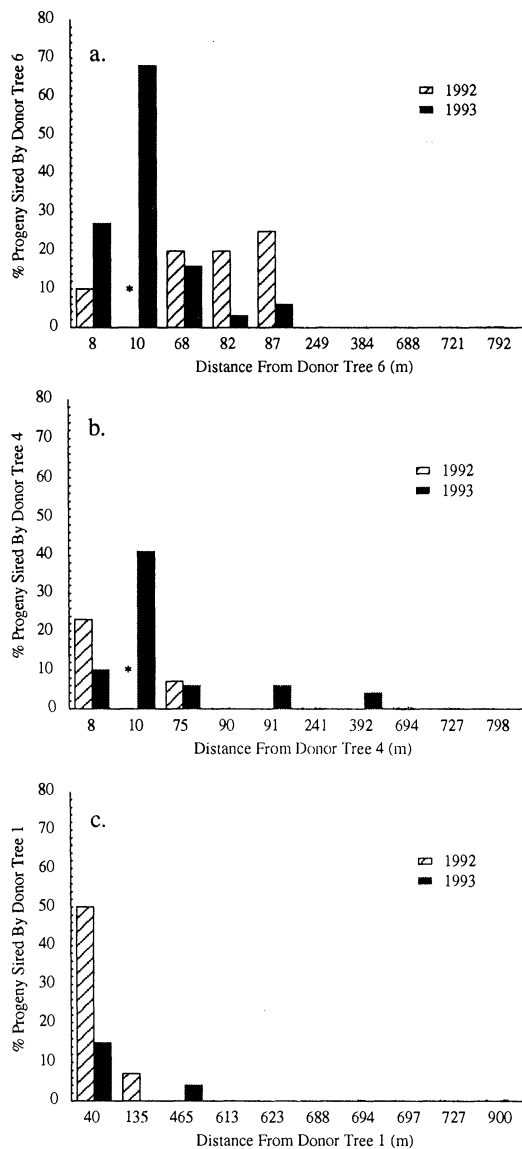


FIG. 5.—Effective pollen dispersal from three individual donor trees of *Spondias mombin* as indicated by rare marker alleles over two reproductive seasons. *a*, Tree 6 using PGI-2e; *b*, tree 4 using DIA-1b; *c*, tree 1 using DIA-1b). Zero values are indicated by the absence of a vertical bar, while missing data are indicated by an asterisk.



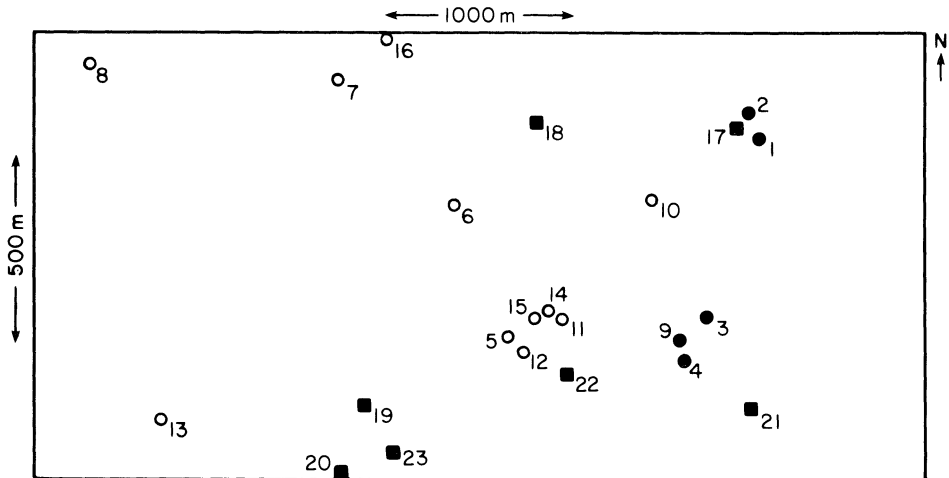


FIG. 6.—The 50-ha FDP plot indicating the locations, identifications, and fruiting activity in 1993 of all 23 adults of *Turpinia occidentalis* (i.e., all individuals with  $\geq 17$ -cm dbh). Circles denote reproductive individuals (solid circles for moderately to highly reproductive trees; open circles for minimally reproductive trees). Squares denote nonreproductive adults.

( $r^2 = 0.25$ , slope =  $-0.061$ ,  $F = 9.88$ ,  $df = 1$ ,  $P = .004$ , pooled across pollen donors and years; fig. 5a–c). However, the frequencies of rare alleles sampled within families varied between years (fig. 5a–c).

*Turpinia occidentalis*.—The five highly reproductive adults of *Turpinia occidentalis* occurred in two clusters, 35 and 57 m in diameter, located approximately 235 m apart (fig. 6). Paternity exclusion analyses indicated that the majority of successful pollen moved among individuals within clusters but that a fraction of the matings occurred over longer distances. Mating between the two adults of the more northerly cluster (trees 1 and 2), located 35 m apart, was such that each could have sired up to 62.5% (20/32) and 89.5% (17/19) of the progeny of its nearest neighbor. Pooling progeny over both individuals, 27.5% (14/51) resulted from apparent pollen flow from beyond these two trees. In the second cluster, composed of three adults, even higher proportions of progeny resulted from near-neighbor matings. For two of these maternal trees, 100% of the progeny sampled (72 total) could have been sired by one or two of the other adults, which were located within 42 m of the maternal tree. For the third adult (tree 4), however, pollen flow over longer distances was detected. Paternity analyses indicated that the nearest neighbor (tree 9, 21 m away) could have sired up to 83.7% (36/43) of progeny sampled from tree 4. Including the second-nearest neighbor (tree 3, 57 m away) in the analysis accounted for 93% of the progeny sampled. Adding the third-nearest neighbor (tree 11, 145 m away), which was well outside the cluster and slightly outside the known neighborhood for tree 4, accounted for all but one, or 97.7%, of the sampled progeny. Thus, pooling progeny over all three maternal trees, we found that less than 1% (1/115) resulted from apparent pollen flow from beyond the cluster of nearest neighbors and over a minimum distance of 130 m.

## DISCUSSION

Although patterns of pollen movement varied among these three Neotropical tree species, they also had many features in common. First, these species, like the majority of tropical trees examined to date, are insect pollinated and predominantly outcrossed (e.g., O'Malley and Bawa 1987; O'Malley et al. 1988; Murawski and Hamrick 1991, 1992; Murawski et al. 1994). These trees probably experience high rates of intracrown pollination. The high observed rates of outcrossing, however, do not necessarily indicate self-incompatibility. *Spondias mombin*, the only study species examined previously for its breeding system, is reportedly self-incompatible in Costa Rica (Bawa 1974; Bawa and Opler 1975). If the other two species are self-compatible, their high outcrossing rates might be due to preferential abortion of selfed seeds (Dayanandan et al. 1990; Ramirez 1993). For instance, adults of *Calophyllum longifolium* experienced heavy fruit abortion throughout the fruit maturation period (E. Stacy, personal observation). Although we sampled and electrophoresed embryos from aborted fruits of *C. longifolium*, analysis of the mating system was impossible because of insufficient enzyme activity.

Another characteristic of the breeding system common to the three study species is that a substantial proportion of pollen moved at least a few hundred meters, and possibly well beyond that range, in each population. Actual pollen flow is expected to be substantially greater than the levels of apparent pollen movement detected. For the three species, the probability of exclusion ranged from 0.53 to 0.78. As a result of these modest values, the single-tree paternity exclusion analyses were unable to exclude some of the potential but not actual fathers for a portion of the progeny analyzed. As more potential fathers were included in the paternal pool in each analysis, starting with the nearest neighbor and proceeding outward, our inability to exclude nonfathers resulted in the underestimation of the distances between members of a mating pair. Moreover, our inability to exclude a higher proportion of pollen donors within the study plot decreased the number of detectable pollen flow events from off-plot fathers. The ratio of total to apparent gene flow for populations with higher exclusion probabilities ( $>0.80$ ) is 2–3:1 (Devlin and Ellstrand 1990). In contrast, with a lower exclusion probability (e.g., *S. mombin*), estimated total gene flow would be greater, possibly three to four times the apparent rate. As a result, the paternity exclusion analysis estimates of pollen dispersal distances reported here should be conservative underestimates of their actual values.

Estimates of gene movement into the 84-ha *S. mombin* population based on rare marker alleles are also conservative. First, reported rates of immigrant pollen flow over 300 m (2.6% in 1992; 1.5% in 1993) are underestimates that can be translated to total gene flow by dividing by the marker allele frequencies in the donor population. For example, if the global frequency of a marker allele is 0.20, estimated total gene flow in 1992 would be 13% (2.6%/0.20). Second, our analyses of pollen movement within the 84-ha study plot based on rare marker alleles in individual donors revealed only one case of pollen flow over a few hundred meters (392 or 465 m; fig. 5*b*, *c*). Certainly, many more cross-pollinations occurred over

this range of distances. However, as the frequency of a rare allele in the pollen pool is initially low and decreases with increasing distance from the donor, the likelihood of detecting long-distance pollen movement by this method is low given the progeny sample sizes obtained.

To our knowledge, no studies exist on the pollination behavior of the diverse array of small insects that occur in Neotropical rain forests. The capacity for long-distance pollen movement by such species has been suggested to be limited, however, because of their restricted foraging ranges (Frankie et al. 1976; Bawa 1977). In this study, we identified substantial levels of pollen movement over 300 m for two species pollinated by diverse small insects. Because of the conservative nature of our estimates and because the size and shape of the study plot limited detection of pollen flow to less than 350 m, actual pollen dispersal over distances greater than 350 m undoubtedly occurred but could not be quantified.

Previous work on the mating patterns of tropical trees has also revealed substantial levels of pollen flow over relatively long distances (e.g., Hamrick and Loveless 1989; Hamrick and Murawski 1990). These studies, however, focus on species primarily pollinated by bees (small to large). In one (Hamrick and Loveless 1989), it is estimated that 25% of progeny arising within an isolated cluster of five adults of *Tachigali versicolor* resulted from pollen flow from at least 500 m outside the group. In a second study (Hamrick and Murawski 1990), patterns of pollen movement were assessed over a 3-yr period for *Platypodium elegans* occurring in low reproductive adult density (8–12 individuals) on the 50-ha FDP plot. Using fractional paternity methods, at least 20% of the pollen movement within this population is estimated to have occurred over distances of 750 m. As both pollinator type and experimental design differ among our study and these investigations, it is difficult to assess the effect of pollinator type on patterns of pollen movement directly. However, because the *P. elegans* study was also conducted on the 50-ha FDP plot and 100-m border zone, a comparison can be made of the level of apparent pollen flow received by maternal trees located within the 50-ha plot from trees located outside the 84-ha area. For *P. elegans*, minimum estimates of long-distance gene flow via pollen into the 50-ha plot are 17.5%, 36.1%, and 40.1% for three consecutive years (Hamrick and Murawski 1990). The same estimates for *C. longifolium* are 5.8% and 6.2% for two seasons; for *S. mombin* (with a lower exclusion probability), 4.3% and 2.0% for the same 2 yr. The greater estimates of moderate- to long-distance gene flow in the bee-pollinated *P. elegans* are consistent with the view that tropical bees are capable of moving pollen over greater distances than are smaller insects.

Overall patterns of pollen flow were strongly affected by the spatial distribution of reproductive trees. Where flowering adults occurred in clusters, a large fraction of matings were among nearest neighbors (although the proportion of near-neighbor matings is overestimated by our methods, as explained earlier). Conversely, where reproductive trees were spaced more evenly, the majority of pollen dispersal occurred over larger areas, often bypassing the nearest reproductive trees. For each species, we assumed that flowering activity was restricted to those individuals producing fruit, however minimal. Because of the clumped distributions of *S. mombin* and *T. occidentalis*, violation of this assumption would

not affect the observed patterns of pollen movement for either population. If, however, substantial flowering occurred in adults of *C. longifolium* classified as nonreproductive, the estimated distances of pollen movement would be upwardly biased. Flowering of *C. longifolium* adults classified as nonreproductive was likely nominal or absent. Reproduction was assessed even for trees with minimal fruiting activity. In 1993, all adult trees were monitored for evidence of reproduction beginning early in the period of fruit maturation, and flowering was confined to those adults classified as reproductive.

Whether the strong association observed between the spatial distribution of flowering trees and the degree of near-neighbor mating can be generalized to tropical trees pollinated by other kinds of vectors remains to be seen, as we are not aware of similar studies for comparison. Our results, however, may be consistent with a previous observation (Murawski and Hamrick 1991) that trees occurring at low density appear to receive pollen from relatively few donors (i.e., one or a few near neighbors). In a related study based on pollinator flight patterns and movement of marker dyes, Linhart (1973) concludes that pollen flow in two hummingbird-pollinated species of *Heliconia* was strongly influenced by the spatial dispersion of flowering individuals. Where flowering shrubs were clumped (*H. latispatha*), the majority of pollen movement was within the cluster, whereas where shrubs were scattered (*H. acuminata*), a linear relationship between distance and pollen flow was observed (Linhart 1973). The similarity between the observed patterns of hummingbird- and small-insect-mediated pollen movement suggests that, in general, foraging behavior of tropical pollinators (and hence pollen dispersal patterns) is strongly affected by the spatial distribution of flowering plants.

Examination of mating patterns on a finer scale revealed some inconsistencies in pollen flow. For example, apparent pollen dispersal from individual donors of *S. mombin*, as indicated by rare marker alleles, was typically restricted to members of a cluster. The distribution of rare alleles was, however, erratic across years over any given distance, as well as across families within a year. Such variation in mating patterns within clusters of flowering trees could be explained by lowered fitness of offspring resulting from biparental inbreeding (assuming near neighbors are related), asynchronous flowering phenologies of neighboring trees, or inconsistent pollinator behavior. Although genetic relatedness of neighboring adults cannot be ruled out, spatial autocorrelation studies of other tropical species have shown that genetic relatedness of adult-sized neighboring trees is extremely low (Hamrick et al. 1992). Slight differences among the flowering periods of near neighbors is a more likely explanation, because initial fruit drop was not completely simultaneous among adults at the study site (E. Stacy, personal observation). It seems, therefore, that slightly asynchronous flowering phenologies and variable pollinator behavior are the most likely causes of the inconsistencies observed in pollen movement among neighboring trees.

The appearance of immigrant marker alleles from donors outside the 84-ha plot also was idiosyncratic, in terms of both frequency and distribution among families of *S. mombin*. Immigrant alleles were detected no more frequently in progeny sampled near the edge of the plot than in families sampled from the plot center.

In fact, the majority (76%) of all detected foreign alleles occurred in families in the swamp cluster (roughly 350 m from the nearest plot border), although at variable frequencies among families. As the probability of sampling rare alleles decreases with distance from the source (here, a minimum of 300 or 350 m), variation in frequency of immigrant alleles in sampled families could be explained, in part, by sampling error. It seems equally likely, however, that pollen movement by small insects between widely spaced trees, or clusters of trees, is highly erratic. The only consistent pattern with respect to immigrant alleles was observed for LAP-1b, the most common immigrant allele. The LAP-1b allele was found in 1992 in all families sampled within the swamp cluster, and it was observed in 1993 in three of the five families sampled there. This finding may indicate that once a cluster of flowering trees is discovered by foraging pollinators, most or all individuals within the group are visited.

From our analyses, we suggest the following generalizations for low-density tropical trees pollinated by diverse small insects. In populations characterized by a high degree of clumping (e.g., *S. mombin* and *T. occidentalis*), a large proportion (up to 90%) of the matings are among nearest neighbors, with a smaller but substantial fraction occurring over distances of a few to possibly several hundred meters. Conversely, where the spatial distribution of trees is more regular (e.g., *C. longifolium*), a large proportion of outcrossed pollen moves well beyond the closest reproductive neighbors. This general pattern may be explained by the foraging behavior of small insects. If, unlike many of the larger tropical pollinators, small insects do not trapline regularly among widely dispersed conspecific plants, movements by these insects between trees might be more haphazard. Where flowering adults occur in clusters, neighboring conspecifics would be readily discovered and visited. Where flowering trees are more widely dispersed, however, locating conspecific individuals could be more difficult. As a result, these pollinators may inadvertently bypass nearest neighbors in their search for pollen and/or nectar.

Based on the mating patterns observed for each species, we estimate the smallest area required for a natural breeding unit, defined as the minimum area within which 95% of the pollen received by a centrally located adult originates. Using *C. longifolium* as a model of an evenly dispersed population with a low density of reproductive adults, we suggest that a natural breeding unit would extend a minimum of 60 ha. For populations characterized by clumping of reproductive trees (e.g., *S. mombin* and *T. occidentalis*), a natural breeding unit would occupy at least 40 ha. These conservative estimates should be useful in the development of management strategies for tropical forests wherever the preservation of normal population dynamics and mating patterns in uncommon tree species is desirable (e.g., in the design of forest reserves). On a dour note, however, these values suggest that forest fragmentation, which is widespread throughout tropical regions, likely results in significantly reduced breeding neighborhoods for many trees when isolated fragments are on the order of 60 ha or smaller.

These results should apply to a broad range of Neotropical forest species. Because the general patterns of gene movement were consistent across species and years, mating patterns observed in this study should be representative for

other low-density tropical trees pollinated by an array of small insects. Our finding of appreciable levels of moderate-distance pollen movement in all three populations indicates that this class of pollinators is effective in transferring pollen among widely dispersed tropical trees. It has been estimated that approximately 30% of the tree species in a Costa Rican rain forest are pollinated by diverse small insects, including small bees (Bawa et al. 1985a). Although such figures for other tropical forests are unavailable, this group of insects is likely to be involved in the pollination of a significant fraction of woody species occurring throughout the Neotropics.

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