

Pollen-mediated gene flow and differential male reproductive success in a tropical pioneer tree, *Cecropia obtusifolia* Bertol. (Moraceae): a paternity analysis

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We used a likelihood-based paternity analysis to examine pollen-mediated gene flow within and among populations of *Cecropia obtusifolia*, a dioecious, wind-pollinated, tropical pioneer tree. We used allozyme data collected from leaf samples of adult trees and seeds from known mothers in an 8.64 ha study plot located in pristine forest in the Los Tuxtlas range in southern Mexico. Trees within the study plot have a patchy distribution. Four populations (including the reference population) were in natural forest areas, and these were paired with four ‘acahual’ populations (populations growing on human-disturbed sites). The paired sites were located from 1 to 43 km from the reference population, with a ninth (acahual) population 100 km away. We addressed the following questions: (1) Do males from the reference population contribute differentially to reproduction within the stand?; (2) How do intermate distance and patch affiliation affect pollination within the reference population?; (3) What are the relative male reproductive contributions from within and outside the study population? Male reproductive contributions were significantly uneven within the reference population ($P < 0.001$). We demonstrated modest isolation by distance and an effect of patch affiliation for mating pairs within the reference population ($P \leq 0.001$), independent of the male-specific differences. The addition of the eight outpopulations to the model showed that 37% of the offspring in the reference population were probably fathered by males from the other populations. We observed a strong isolation by distance effect for these populations, but long-distance pollen flow is enough to have a strong homogenizing effect on the regional gene pool. None of the acahual populations appears to have contributed pollen. Paternity analysis yields a more detailed view of the effects of pollen-mediated gene flow than had emerged from previous F_{ST} analyses.

Keywords: *Cecropia obtusifolia*, paternity analysis, pollen-mediated gene flow.

Introduction

Research on population genetic structure can help to elucidate the evolutionary dynamics of a species that exhibits different dispersal patterns in natural and disturbed environments. In plants, pollen and seed dispersal may vary among populations, depending on the availability of dispersal agents and the sizes and spatial distributions of individuals within the population. Species may show fine-scale genetic structure if only local individuals sire seeds and if

seed dispersal is restricted, but long-distance pollination and/or seed dispersal can result in genetic homogenization over large areas (Wright, 1946).

Many studies of gene flow in plants have shown that pollen flow is the major contributor to gene flow (e.g. Schaal, 1980; Loveless & Hamrick, 1984; Fenster, 1991; Nason & Hamrick, 1997). Pollen flow has been measured by examining pollinator movements, but actual gene flow has often been underestimated (Handel, 1982; Fenster, 1991). Recent studies with genetic markers demonstrate that gene flow is much more extensive than previously thought (Hamrick & Murawski, 1990; Eguiarte *et al.*, 1992;

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Burczyk *et al.*, 1996; Stacy *et al.*, 1996; Nason & Hamrick, 1997). For example, in two insect-pollinated tropical canopy trees, pollen flow occurred from as far away as 1 km, although most matings were between nearest neighbours (Hamrick & Murawski, 1990). Most tropical canopy trees are widely spaced, and one would expect long-distance pollen and seed dispersal. In keeping with that expectation, tropical canopy trees often show minimal population genetic structure (Hamrick & Murawski, 1990; Eguiarte *et al.*, 1992).

A similar pattern is expected for pioneer tree species colonizing tree-fall gaps and artificially disturbed sites. Pioneer trees often grow close together, but the gaps themselves are spatially dispersed. Wright (1940) expected colonizing species to show population genetic structure; new populations are started by few individuals and are relatively isolated from founding populations. More recent theory shows that a regular extinction/recolonization dynamic, with some migration, can augment gene flow, thus reducing divergence among such populations. The balance between gene flow/extinction/recolonization determines the ultimate population structure (Slatkin, 1987; Wade & McCauley, 1988; Gaggioti, 1996).

Alvarez-Buylla & Garay (1994) showed that *Cecropia obtusifolia* Bertol., a dioecious wind-pollinated tropical pioneer tree, had little population genetic structure over distances from 1 to 107 km, as measured by F_{ST} , and they postulated widespread gene flow. Fine-scale genetic analyses, however, have shown significant fine-scale structure at the intrapopulation level for seedlings, juveniles and adults (Alvarez-Buylla *et al.*, 1996; Epperson & Alvarez-Buylla, 1997). This structure has been interpreted as resulting from the clustering of genetically correlated progeny of individual mother trees, because of limited animal-based seed dispersal within single populations. However, previous studies of *C. obtusifolia* had shown that although the great majority of seeds fall within 30 m of the source, they can be found as far as 100 m from the mother (Alvarez-Buylla & Martínez-Ramos, 1990). Another study found seeds of *C. obtusifolia* up to 500 m from the nearest seed source (J. Laborde, pers. com.), and as bats are one of the primary dispersers, seeds may occasionally move considerably further. Seed dispersal could have a significant impact on gene flow, but because most seeds are dispersed close to the parent plant, populations are expected to show some fine-scale genetic structure. The degree to which such genetic correlation of single-tree progeny

might also be caused by localized pollination is unknown.

In contrast to the pattern predicted by seed dispersal, pollen flow could be substantial in *C. obtusifolia*, and males might contribute to a regional pollen cloud that could homogenize the species over large regions. We might expect little genetic structure and certainly no evidence of isolation by distance. Such a pattern would be similar to that described for some conifers (Friedman & Adams, 1985). In white spruce (*Picea glauca* (Moench) Voss.), for example, male reproductive success was influenced more by the amount of pollen that the male contributed to the pollen cloud, rather than by the male's proximity to potential mates (Schoen & Stewart, 1986).

Pollen flow in *C. obtusifolia*, both within and among populations, can be studied directly with parentage analysis, using polymorphic genetic markers. Most studies do not employ enough polymorphic markers to assign each offspring categorically to a single male (e.g. Chakraborty *et al.*, 1988), but, even in the absence of categorical assignment, the genetic likelihood of any particular male being the father of an offspring can be evaluated (Meagher & Thompson, 1987; Devlin *et al.*, 1988; Roeder *et al.*, 1989; Adams *et al.*, 1992; Milligan & McMurry, 1993; Smouse & Meagher, 1994). Roeder *et al.* (1989) and Smouse & Meagher (1994) have developed likelihood-based approaches to estimate male reproductive contributions, based on fractional allocation of paternity. We extend the model to the case of multiple populations, in an attempt to answer the following questions about pollen-mediated gene flow in *C. obtusifolia*: (1) Do males from the reference population contribute differentially to reproduction within the stand?; (2) How do intermate distance and patch affiliation (measures of mate separation) affect pollination?; (3) What are the relative male reproductive contributions from both within and outside the study population?

Methods

Species and study site

This data set is from previous genetic and demographic studies on *C. obtusifolia* (Alvarez-Buylla & Martínez-Ramos, 1992; Alvarez-Buylla & Garay, 1994). *Cecropia obtusifolia* colonizes tree-fall gaps and abandoned pasture lands in south-eastern Mexico. We sampled from a total of nine discrete populations, eight in the Los Tuxtlas Range (all of the populations in the range), and one from outside

the Los Tuxtlas Range (Fig. 1). The reference population (the F1 population) is an 8.64 ha study plot of pristine forest at the 'Los Tuxtlas' Field Station, in and around which *C. obtusifolia* trees established within natural tree-fall gaps of different ages before the population was sampled. This reference population includes the permanent 5 ha study plot used in previous demographic studies (Alvarez-Buylla & Martínez-Ramos, 1992). In the reference population, all trees were assigned (x,y) map coordinates. Each tree could also be assigned to one of 12 patches, representing identifiable tree-fall canopy gaps (see figure in Alvarez-Buylla *et al.*, 1996). This reference population was used to determine whether males had differential reproductive success, and if so, whether their success was related to distance from females or patch affiliation with females.

In addition to the reference population, eight other populations were surveyed to determine whether there was any evidence for 'isolation by distance' in pollen flow among populations. Three of the other eight populations (F2–F4) occur in natural tree-fall gaps and four (A1–A4) occur in nearby 'acahuales', dense stands of *C. obtusifolia* on artificially perturbed sites. These sites were paired (A1 with F1, A2 with F2, A3 with F3, A4 with F4) at different distances, from 1 to 43 km, from the reference population (Fig. 1). We also sampled one

acahual site (A5) at 100 km, outside the Los Tuxtlas Range, where no pristine forest remains. We sought evidence of differential pollen contributions by natural and acahual populations. Detailed descriptions of the reference population, species, and study plot can be found in Alvarez-Buylla & Martínez-Ramos (1992) and Alvarez-Buylla & Garay (1994).

Sampling and electrophoretic procedures

Leaf samples were collected from all adults in the reference population, 47 males and 41 reproductive females, found growing under natural conditions in and around the permanent study plot. From each adult female in the reference population, we collected infructescences, and randomly selected and germinated seeds from each infructescence. We randomly selected 30 seedlings from each mother, and used a few leaves from each for enzyme extractions and electrophoretic assays. We also determined the genotypes of 60 random adult trees (30 female and 30 male trees) from each of the other eight populations. See Alvarez-Buylla & Garay (1994) for detailed collection, processing and storage procedures.

Horizontal starch gel electrophoresis was used in all cases. We assayed all samples for eight polymorphic loci: *AcPH* (one locus), *FE* (two loci), *GOT*

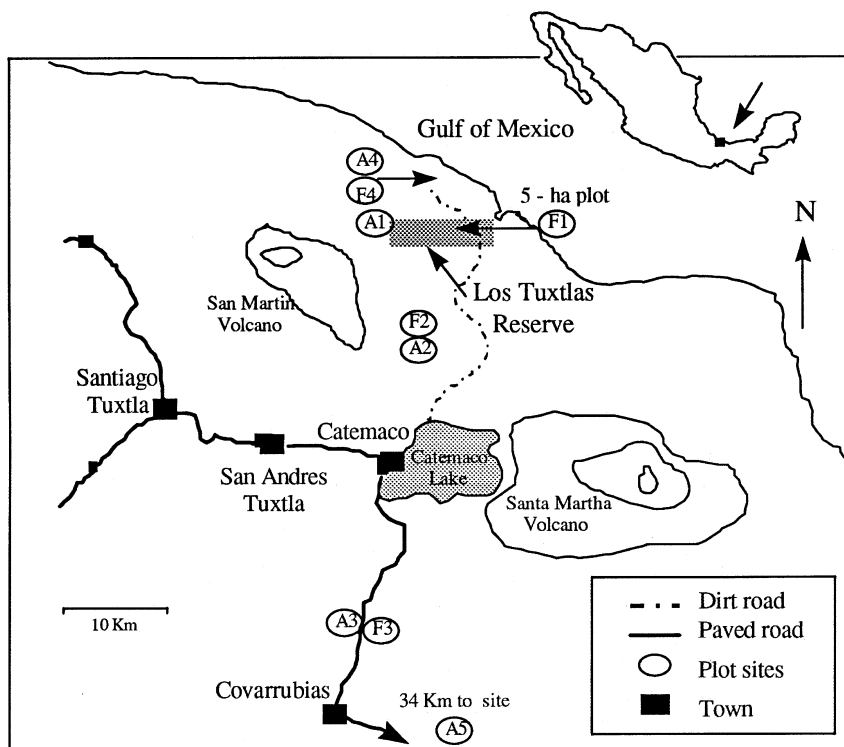


Fig. 1 Map of the Los Tuxtlas range in the State of Veracruz (México). Collection sites shown by ellipses: A (1–5), 'acahuales', human-disturbed sites; and F (1–4), pristine rain forest sites. F1 corresponds to the 8.64 ha reference plot.

(two loci), *LAP* (one locus) and *PGM* (two loci). Buffer systems and staining protocols are given in Alvarez-Buylla & Garay (1994).

Data analysis

Exclusion analysis For analyses within the reference population, we generated a matrix of the Mendelian probabilities for the genotype of each offspring, given the genotype of its (known) mother and the genotype of each of the potential male parents, using a program written by T. Meagher (Rutgers University). For the among-population analyses, where the interest was in the male reproductive contributions of whole populations, each population's male gamete pool was treated as a single entry for the matrix of Mendelian probabilities (see Chakraborty *et al.*, 1988 for a description of exclusion probability matrices). For the reference population, all 47 males have been genotyped and enumerated; for the other eight populations, the 30 males represent a sample of the gene pool. Building this matrix of Mendelian probabilities assumes that inheritance is regular and Mendelian, that there is no selection during the gametic phase, and that males have equal probabilities of mating with individual females. This last assumption is relaxed later, for example, by accounting for distance between particular males and females.

Reproductive heterogeneity We used the matrix of Mendelian probabilities to estimate the relative reproductive success of males, using the likelihood-based approach described by Smouse & Meagher (1994) and embedded in the program CHAMLAMB (available from P.E.S.). This model used fractional assignment of paternity when there was more than one possible father. The model for relative reproductive success of the k th male, irrespective of female, was

$$\ln \lambda_k = \alpha_k \quad \text{for } k = 1, \dots, 47. \quad (1)$$

In this model, α_k is a constant for the k th male, and the λ s (relative male reproductive contributions) must sum to unity. If the reproductive contributions of all 47 males were the same, then all of the λ s would be identical, and the variance in the α s (or λ s) would be zero; otherwise, the variance will be positive. The program starts by assuming the null hypothesis, that each male has equal reproductive success, and then iterates until a maximum likelihood solution is achieved (Smouse & Meagher, 1994).

We tested the departure of the estimated α values from constancy by comparing the variance of the actual results to a distribution of variances of the null hypothesis α values, calculated by randomly assigning offspring to fathers. This is similar to the approach of Smouse & Meagher (1994). The whole experiment was run 1000 times to generate a random distribution of variances for the α values. The actual result was compared with this null distribution to determine the strength of the evidence for male reproductive heterogeneity.

Isolation by distance and patch We adapted the likelihood analysis to test whether intermate distance had any predictable effect on male reproductive success. This analysis is embedded in a program called BETADIST (available from S.R.K.), which calculates Euclidean map distances between individual males and females, and uses those distances to gauge the effect of intermate distance on relative male reproductive success for a particular female, using the model

$$\ln \lambda_{jk} = \gamma \times DIST_{jk} \\ \text{for } j = 1, \dots, 41 \text{ and } k = 1, \dots, 47, \quad (2)$$

where λ_{jk} is the relative reproductive contribution of the k th male to the progeny of the j th female, considered to be a predictable function of the physical separation between them. The γ -coefficient, expected to be negative from 'isolation by distance' theory, measures the strength of that isolation. The analysis begins with the null hypothesis value of $\gamma = 0$, and then iterates γ until the likelihood is maximized.

A similar model was used to test the effect of patch affiliation, whether trees had grown up in the same tree-fall gaps or not,

$$\ln \lambda_{jk} = \delta \times PATCH_{jk} \\ \text{for } j = 1, \dots, 41 \text{ and } k = 1, \dots, 47, \quad (3)$$

where δ measures the strength of the association of patch affiliation for mating pairs. We generated a matrix of ones and zeros; zero if a male and female occurred in the same patch, one if they occurred in different patches. The isolation-by-distance and patch models both measure mate separation, but the former is a smooth function of distance, whereas the latter uses a binary decision variable (ones and zeros). We also tested the model with distance and patch together (eqn 4, below), and we compared the likelihood with those derived from the distance

model (eqn 2) and the patch model (eqn 3) to separate the component for ‘distance effects’ from that for ‘patch effects’.

$$\ln \lambda_{jk} = (\delta \times PATCH_{jk}) + (\gamma \times DIST_{jk})$$

for $j = 1, \dots, 41$ and $k = 1, \dots, 47$. (4)

The distance and patch models have unimodal likelihood distributions, so a single optimum is expected from the analysis. To test the significance of the isolation-by-distance results, we shuffled the map positions of males, and then recalculated the γ value, on the null premise that if distance between mates has no effect, then the map positions of the males are irrelevant, and we should have an average γ -estimate of zero. We did this 1000 times, generating a null distribution of γ values to compare with the result from the data. We tested the isolation-by-distance model within the reference population, as well as for the whole set of populations. The Euclidean map distances were calculated between individual females and gene pools (pooled male genotypes from each population). The significance of the patch affiliation results was determined by scrambling patch affiliations of males and recalculating the δ value. The δ value should be zero if patch affiliation does not affect male reproductive success.

Combination models We next combined the effects of distance (or patch) and male-specific reproductive effects into a joint model, specifying

$$\ln \lambda_{jk} = \alpha_k + (\gamma \times DIST_{jk})$$

$j = 1, \dots, 41$ and $k = 1, \dots, 47$. (5)

Once again, the relative reproductive contribution of a given male depends on the female, through the distance function, but here we allow for male-specific effects (the α_k) that do not depend on inter-mate distances or on the female in question. The strategy is to optimize the likelihood simultaneously for the α s and γ . We have developed a program called FULLBETA (available from S.R.K.) that begins with the null hypothesis values for both α s and γ , and iterates to a maximum likelihood solution. A comparison of likelihoods derived from eqn (5) with those from eqns (1) and (2) permits decomposition of the ‘differential reproductive contribution’ signal into separate components for ‘male effects’ and ‘distance (or patch) effects’, respectively.

To test for the significance of the separate male effects, after accounting for the distance (or patch)

effect (and hence the relative spatial positions of the males), we assigned spatially averaged λ values to different males and proceeded as with the other resampling problems. We carried out 100 random assignments of offspring to fathers to generate a null distribution of variances against which to compare with the data. To test the significance of the distance (or patch) effects, we held male effects constant, scrambled the spatial positions of the males, and then recalculated the γ value. This is analogous to using Type III sums of squares. We repeated this 100 times to obtain a null distribution of gamma values to compare with the result from the data.

Results

Within-population analyses

Genetic exclusion This study first addresses the factors that potentially affect male reproductive success in *C. obtusifolia* and then determines the pattern of pollination within and among populations. The exclusion probabilities for each locus were $LAP \approx 0.114$, $GOT1 \approx 0.178$, $GOT2 \approx 0.527$, $FE1 \approx 0.038$, $FE2 \approx 0.167$, $PGM1 \approx 0.275$, $PGM2 \approx 0.532$, $ACPH \approx 0.181$ (Jamieson, 1994). The multilocus exclusion probability was 0.923 (Chakraborty *et al.*, 1988). An examination of genetic exclusions shows that most pollination (1100/1210 seeds) could have occurred within the reference population, but at least 9% of the seeds (110/1210) must have been fathered by males from outside populations; there were simply no males in the reference population that could have fathered them. Many of the remaining 1100 individuals could also have been sired by males from outside the reference population, of course, so it follows that effective pollen immigration is *at least* 9%. Two seeds had rare genotypes that could not have been fathered by any of the males in the study, but 1208/1210 could be accounted for by at least one male in the study. We shall return to the 1208 seeds later, when we discuss long-distance pollen flow.

The within-population models were examined for the 1100 offspring that could have been fathered by the 47 males in the reference population. Male reproductive success within the reference population was very uneven, and male-specific effects were large (Fig. 2). Four of the 47 males apparently contributed no paternity. The ratio of largest λ_k to smallest contributors, among the 43 remaining males, was $\approx 60:1$. The variance of α_k was 46, as compared to the average random variance of 15.

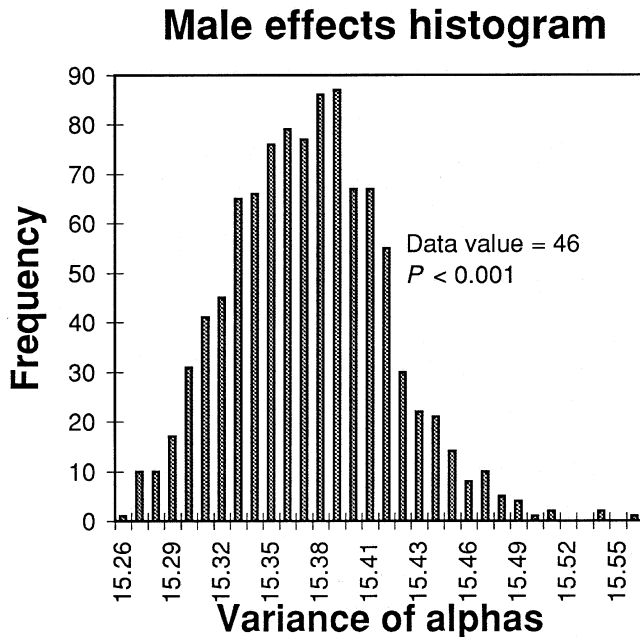


Fig. 2 Male-specific effects for the 47 males of *Cecropia obtusifolia* within the reference population. The histogram represents the null distribution of variances of alphas from each of 1000 random sampling runs. The data value is several standard deviations out in the tail.

Male reproductive contributions thus appear to be very uneven.

Intermate distance and patch Both the isolation-by-distance model and the patch affiliation model are measuring mate separation. There was a modest but significant isolation-by-distance effect within the reference population ($\gamma = -2.97$; $P \leq 0.001$); as the distance from a particular female increased, male reproductive success decreased (Fig. 3). Averaged over females, however, the ratio of largest to smallest male contribution (λ) was only about 2:1. This is a smaller ratio than seen from the male-specific effects model, but there is still compelling evidence for isolation by distance.

The patch effect was much stronger than the distance effect within the reference population ($\delta = -1.66$; $P \leq 0.001$). Males in the same patch as a particular female had higher reproductive success with those females than did males from other patches. This patch effect is partially confounded with the distance effect, because males and females in the same patch are likely to be closer together than males and females in different patches. Testing the two effects together, however, showed that both patch and distance have effects on male reproductive

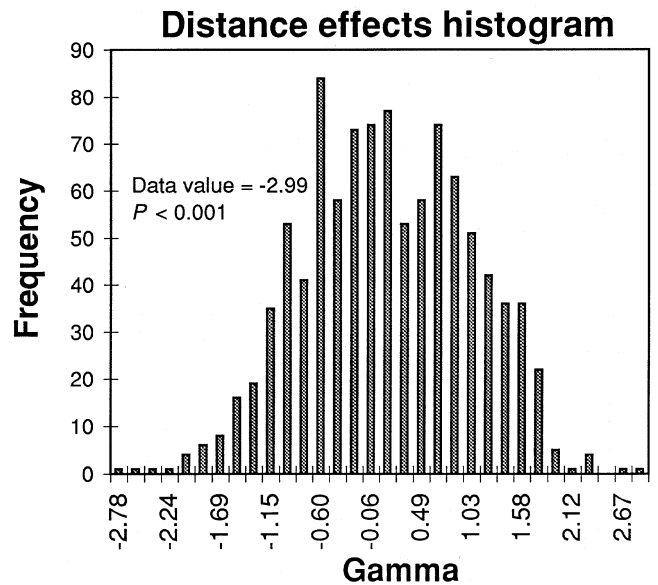


Fig. 3 The effects of intermate distance on male reproductive success in *Cecropia obtusifolia*. The histogram represents the null distribution of gammas after random shuffling of the spatial positions of the males 1000 times.

success within the reference population ($\delta = -1.35$; $\lambda = -1.89$; $P \leq 0.001$).

Combination models Analysis of the distance plus male-effects model, described in eqn (5), demonstrates that distance does not have a significant effect on reproductive success, after accounting for specific male effects (Table 1). The patch plus male-effects model, on the other hand, shows that both patch and male effects are affecting reproductive success. The specific male effects represent the larger component of the variation, and they are *not* an artifact of males' patch locations within the stand. The precise distance from male to female is not as important as whether males and females are in the same patch.

Among-population analyses

Pollen flow Average male gene pool frequencies were used for analyses of pollen flow among populations, and all 1208 offspring were included. The addition of the eight 'outpopulations' to the male-specific effects model yielded some interesting inferences. We know from the within-population analysis that at least 9% of the offspring in the reference population *must* have been fathered by males from other populations, but even with only the 1100 offspring that could have been fathered by the males

Table 1 Results of paternity analysis for the 47 males within the F1 population of *Cecropia obtusifolia*. Variances of male-specific α values, intermate distance effects (γ), and patch affiliation effects (δ) are presented, along with the log-likelihood of each model and the probability of these log-likelihoods being different from their null hypothesis values

Source of variation	Degrees of freedom	Coefficient estimates	Log-likelihood	P value
Null model	47	$\sigma_{\alpha}^2 = 0.0, \hat{\gamma} = 0.0, \hat{\delta} = 0.0$	-5943.78	
Male effects	46	$\sigma_{\alpha}^2 = 46.64$	-5789.81	<0.001
Distance	1	$\hat{\gamma} = -2.97$	-5914.96	<0.001
Patch	1	$\hat{\delta} = -1.66$	-5905.16	<0.001
Distance + Patch	2	$\hat{\gamma} = -1.89, \hat{\delta} = -1.35$	-5879.57	<0.001
Male effects + Distance	47	$\sigma_{\alpha}^2 = 60.58, \hat{\gamma} = -1.74$	-5786.49	<0.001
Male effects + Patch	47	$\sigma_{\alpha}^2 = 27.89, \hat{\delta} = -2.43$	-5704.36	<0.001

in the reference population, inclusion of the other gene pools indicates that an additional 9.6% of the offspring were probably fathered by males from elsewhere, raising the total to 18.6%. With the additional 108 excluded offspring added to the among-population analysis, the evidence tips strongly in favour of heavy pollen flow from outside the reference population (37%), in keeping with *a priori* expectations and the results of previous population structure analyses of these same populations (Alvarez-Buylla & Garay, 1994). The allowance for gene flow from other populations did not change the relative reproductive contributions of males within F1; it merely reduced their collective contribution.

Isolation by distance F4 (at 6 km) accounted for 27.1% of the offspring, followed by F2 (at 14 km), which accounted for 9.6%, but F3 (at 43 km) contributed very little pollen, if any (Fig. 4). Remarkably, none of the acahual populations, at any distance, appears to have contributed pollen to the F1 population, so we used only the natural populations for the distance model. Pollen contribution declines with distance from the reference population (Fig. 4). It is worth noting here that the distances involved are three orders of magnitude larger than for the within-population analyses, and the distance effect is consequently larger. The tail-probabilities for the cross-population analysis are less compelling. For the 'isolation by distance' test, there are only four populations and only $4! = 24$ permutations of their distance order from F1, but the result is the most extreme of those possible from random permutation, implying a *P* value of $1/24$ (0.04). The juxtaposition of an almost perfect 'isolation by distance' model on the natural populations and an apparent failure of the acahuals to make any contribution, represents a strong demonstration that the regional pollen cloud

is far from homogeneous. Pollen-mediated gene flow is heavy, but it is a function of geographical distance. Natural populations appear to contribute, whereas acahuals seemingly do not.

Discussion

The present study concentrated on patterns of pollen-mediated gene flow, by using paternity analysis on seed drawn from known mothers. Males within the reference population varied greatly in their relative reproductive successes, and patch affiliation affected pollination within the reference population. Spatial effects have also been found within populations of *Raphanus sativus* L. (Ellstrand & Marshall, 1985), *Cordia alliodora* (Ruíz and Pavón) Oken (Boshier *et al.*, 1995), *Spondias mombin* L.

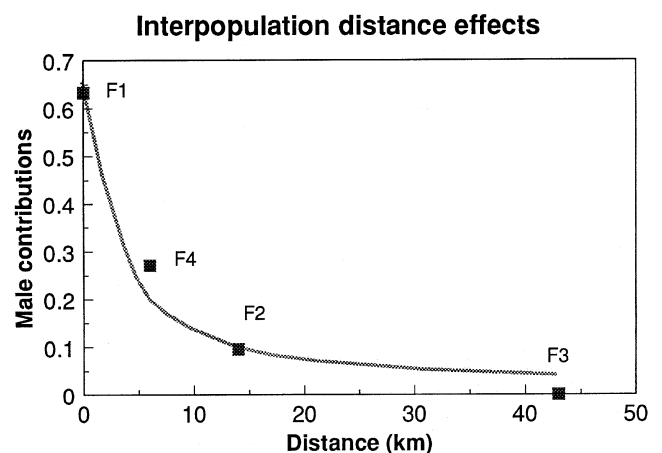


Fig. 4 The effect of distance on pollen contributions from multiple natural populations of *Cecropia obtusifolia*. The curve represents the predicted contributions from these populations, based on an isolation-by-distance analysis, and the points are the directly estimated contributions.

(Stacy *et al.*, 1996) and *Chamaelirium luteum* L. (Meagher, 1986). The impact of patch affiliation in this species reinforces the importance of the history of gap formation within a population as a later determinant of fine-scale genetic structure.

F -statistics analysis (Alvarez-Buylla *et al.*, 1996) showed that *Cecropia obtusifolia* populations exhibit significant fine-scale genetic structuring at the intra-population level, particularly for seedlings ($F_{ST} = 0.063$). That structure almost surely represents the impact of clumped seed dispersal, but it probably also includes an element of isolation by distance in pollen dispersal as well. The genetic clumping evident in seedlings is not evident among mature trees ($F_{ST} = 0.002$) (Alvarez-Buylla *et al.*, 1996). That same demographic pattern is even more evident with spatial autocorrelation analyses (Epperson & Alvarez-Buylla, 1997). Clearly, local patch structure among adults will lead to a pollination pattern that contributes to microspatial genetic structure among the resulting offspring. As the stand matures, it suffers progressive mortality, and there will be less genetic structure among surviving saplings, still less among young adults, and almost none among mature adults. Thus, the age-specific 'structure-pattern' is consistent with the demography of the species.

Although there was a strong pattern of isolation by distance among populations of *C. obtusifolia*, pollen was still received from more than 10 km away. Consequently, even relatively isolated populations should receive pollen from widely separated populations. The fact that 37% of the offspring in the reference population were probably fathered from other populations means that pollen flow should have a profoundly homogenizing influence on the regional gene pool (Wright, 1946). This result fits with the observation that F_{ST} values were small, $0.014 < F_{ST} \leq 0.060$ (Alvarez-Buylla & Garay, 1994). A similar pattern has been found in wind-pollinated loblolly pine (*Pinus taeda* L.) seed orchards (Friedman & Adams, 1985). Nearest neighbours made the greatest male reproductive contributions, but the background cloud of pollen from surrounding populations accounted for up to 36% of the offspring. Distance effects were not tested for the pollen cloud in that study, but here we were able to do so, discovering that the 'pollen cloud' is not homogeneous. It exhibited isolation by distance for natural populations and (apparently) did not involve the acahuals at all.

The fact that the acahuals contributed nothing to paternity within the reference population is a striking result. Female trees in acahuals do produce

seeds, indicating successful pollination. We have evidence that pollination is needed for seed-set, and have not detected any apomixis. One possibility is that the phenology of trees in acahuals and primary forest gaps is different, but we have observed trees with mature male and female flowers in both acahual and primary forest populations at the same time. This matter clearly deserves further attention.

The most likely explanation is that the physical structure of the populations themselves influences pollen flow patterns. Tree density in acahual populations can be 80 times greater than that in the natural tree-fall gaps (Alvarez-Buylla & Garay, 1994), and mature trees in such secondary sites are notably shorter than those growing in natural tree-fall gaps. Turbulent air flow within dense populations might be different from that in the more open natural stands of the primary forest, and it is possible that the pollen is simply 'bottled up' in the acahual stands.

If there were greater barriers to pollen flow in or out of acahuals, we would expect more evidence for population structure among them than among primary forest patches. Alvarez-Buylla & Garay (1994), examining both pollen- and seed-mediated gene flow, have estimated that F_{ST} among pairs of sites within the Los Tuxtlas range is equal to 0.0099, and they found no evidence for isolation by distance. On the other hand, F_{ST} values were highest when comparing the most isolated site (A5) with any of the others (mean $F_{ST} = 0.0142$), and the largest F_{ST} values (mean acahual = 0.0168 vs. mean forest = 0.0148) were found when acahuals were compared among themselves, suggesting that they are genetically more isolated (F_{ST} values were back-calculated from table 5 in Alvarez-Buylla & Garay, 1994).

We would also expect, if gene flow into acahuals were restricted, that there would be higher inbreeding coefficients within acahual stands than within primary patches. Averaging fixation indices from Alvarez-Buylla & Garay (1994), we find that mean F values are very similar ($F = 0.071 \pm 0.112$ and $F = 0.073 \pm 0.096$, for acahuals and primary patches, respectively), so the evidence is inconclusive. Another possibility is that acahuals do not produce as much pollen as do natural populations. We do not presently have the data on pollen production or movement into or out of acahuals that would be needed to test this hypothesis.

There are several possible reasons for the differences in inference obtainable from the paternity analysis and the population structure analysis. One possibility is that because our paternity analysis

examines only pollen-mediated gene flow, extensive gene flow via seeds might obscure the pattern observable with population structure analysis. Perhaps the animal vectors that disperse the seeds of *C. obtusifolia* have foraging behaviours that do not follow an isolation-by-distance pattern (see Alvarez-Buylla *et al.*, 1996 and citations therein). That seems highly unlikely, however, because the dispersers would all seem to have maximum foraging distances.

The other possibility is that paternity analysis is more sensitive to patterns of gene flow. *F*-statistics have two limitations: (i) they rely on the overall pattern of variation for a single generation — the analysis measures only the long-term genetic structure ‘consequences’ of gene flow, because the analysis assumes a global drift/migration equilibrium for populations; (ii) standard analyses are averaged over loci, taking no account of the cross-population disequilibria that inevitably accompany population structure.

Parentage analysis, although requiring a great deal more work, has two advantages: (i) it uses information on two generations, linked through the mothers — the effects of pollen flow are measured directly and the analysis makes no assumptions about population equilibria; (ii) the analysis uses multiple-locus gametic data on all entries, and is exquisitely sensitive to the cross-population disequilibria that accompany population structure. Small differences in allele frequencies become magnified when they are translated into gametic frequencies. With parentage analysis, we need more information, but we can use it to advantage.

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References

- ADAMS, W. T., GRIFFIN, A. R. AND MORAN, G. F. 1992. Using paternity analysis to measure effective pollen dispersal in plant populations. *Am. Nat.*, **140**, 762–780.
- ALVAREZ-BUYLLA, E. R. AND GARAY, A. A. 1994. Population genetic structure of *Cecropia obtusifolia*, a tropical pioneer tree species. *Evolution*, **48**, 437–453.
- ALVAREZ-BUYLLA, E. R. AND MARTÍNEZ-RAMOS, M. 1990. Seed bank vs. seed rain in the regeneration of a tropical pioneer tree. *Oecologia*, **84**, 314–325.
- ALVAREZ-BUYLLA, E. R. AND MARTÍNEZ-RAMOS, M. 1992. Demography and allometry of *Cecropia obtusifolia*, a neotropical pioneer tree — an evaluation of the climax–pioneer paradigm for tropical rain forests. *J. Ecol.*, **80**, 275–290.
- ALVAREZ-BUYLLA, E. R., CHAOS, A. C., PIÑERO, D. AND GARAY, A. A. 1996. Demographic genetics of a pioneer tropical tree species: patch dynamics, seed dispersal, and seed banks. *Evolution*, **50**, 1155–1166.
- BOSHIER, D. H., CHASE, M. R. AND BAWA, K. S. 1995. Population genetics of *Cordia alliodora* (Boraginaceae), a neotropical tree. 3. Gene flow, neighborhood, and population substructure. *Am. J. Bot.*, **82**, 484–490.
- BURCZYK, J., ADAMS, W. T. AND SHIMIZU, J. Y. 1996. Mating patterns and pollen dispersal in a natural knobcone pine (*Pinus attenuata* Lemmon.) stand. *Heredity*, **77**, 251–260.
- CHAKRABORTY, P., MEAGHER, T. R. AND SMOUSE, P. E. 1988. Parentage analysis with genetic markers in natural populations. 1. Paternity exclusion and expected proportions of offspring with unambiguous paternity. *Genetics*, **118**, 527–536.
- DEVLIN, B., ROEDER, K. AND ELLSTRAND, N. C. 1988. Fractional paternity assignment: theoretical development and comparison to other methods. *Theor. Appl. Genet.*, **76**, 369–380.
- EGUIARTE, L. E., PÉREZ-NASSER, N. AND PIÑERO, D. 1992. Genetic structure, outcrossing rate and heterosis in *Astrocaryum mexicanum* (tropical palm): implications for evolution and conservation. *Heredity*, **69**, 217–228.
- ELLSTRAND, N. C. AND MARSHALL, D. L. 1985. Interpopulation gene flow in wild radish, *Raphanus sativus*. *Am. Nat.*, **126**, 606–616.
- EPPERSON, B. K. AND ALVAREZ-BUYLLA, E. R. 1997. Limited seed dispersal and genetic structure in life stages of *Cecropia obtusifolia*. *Evolution*, **51**, 275–282.
- FENSTER, C. B. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae). I. Gene dispersal. *Evolution*, **45**, 398–409.
- FRIEDMAN, S. T. AND ADAMS, W. T. 1985. Estimation of gene flow into two seed orchards of loblolly pine (*Pinus taeda* L.). *Theor. Appl. Genet.*, **69**, 609–615.
- GAGGIOTI, O. E. 1996. Population genetic models of source–sink metapopulations. *Theor. Pop. Biol.*, **50**, 178–208.
- HAMRICK, J. L. AND MURAWSKI, D. A. 1990. The breeding structure of tropical tree populations. *Pl. Sp. Biol.*, **5**, 157–165.

- HANDEL, S. N. 1982. Dynamics of gene flow in an experimental population of *Cucumis melo* (Cucurbitaceae). *Am. J. Bot.*, **69**, 1538–1546.
- JAMIESON, A. 1994. The effectiveness of using co-dominant polymorphic allelic series for (1) checking pedigrees and (2) distinguishing full sib pair members. *Anim. Genet.*, **25**, 37–44.
- LOVELESS, M. D. AND HAMRICK, J. L. 1984. Ecological determinants of genetic structure in plant populations. *Ann. Rev. Ecol. Syst.*, **15**, 65–95.
- MEAGHER, T. R. 1986. Analysis of paternity within a natural population of *Chamaelirium luteum*. I. Identification of most-likely parents. *Am. Nat.*, **128**, 199–215.
- MEAGHER, T. R. AND THOMPSON, E. A. 1987. Analysis of parentage for naturally established seedlings of *Chamaelirium luteum*. *Ecology*, **68**, 803–812.
- MILLIGAN, B. G. AND McMURRY, C. K. 1993. Maximum likelihood analysis of male fertility using dominant and codominant genetic markers. *Mol. Ecol.*, **2**, 257–283.
- NASON, J. D. AND HAMRICK, J. L. 1997. Reproductive and genetic consequences of forest fragmentation: two case studies of Neotropical canopy trees. *J. Hered.*, **88**, 264–276.
- ROEDER, K., DEVLIN, B. AND LINDSAY, B. G. 1989. Applications of maximum likelihood methods to population genetic data for the estimation of individual fertilities. *Biometrics*, **45**, 363–379.
- SCHAAL, B. A. 1980. Measurement of gene flow in *Lupinus texensis*. *Nature*, **284**, 450–451.
- SCHOEN, D. J. AND STEWART, S. C. 1986. Variation in male reproductive investment and male reproductive success in white spruce. *Evolution*, **40**, 1109–1120.
- SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. *Science*, **236**, 787–792.
- SMOUSE, P. E. AND MEAGHER, T. R. 1994. Genetic analysis of male reproductive contributions in *Chamaelirium luteum* (L.) Gray (Liliaceae). *Genetics*, **136**, 313–322.
- STACY, E. A., HAMRICK, J. L., NASON, J. D., HUBBELL, S. P., FOSTER, R. B. AND CONDIT, R. 1996. Pollen dispersal in low-density populations of three Neotropical tree species. *Am. Nat.*, **148**, 275–298.
- WADE, M. J. AND McCAULEY, D. E. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.
- WRIGHT, S. 1940. Breeding structure of populations in relation to speciation. *Am. Nat.*, **74**, 232–248.
- WRIGHT, S. 1946. Isolation by distance under diverse systems of mating. *Genetics*, **31**, 39–59.