

**a**, *Rossella*, from a drawing by Schultze (ref. 10). **b**, Magnification of the cross-shaped apex of a *Rossella* spicule. Bar, 1 mm. **c**, A spicule bent to a 90° angle conducts red light from the source to an exposure meter Profisix (Gossen) modified to achieve light-spot measurement. Bar, 2 cm. **d**, Transmission of red light (635 nm) along spicules at different angles of incidence of the laser beam. Measurements were taken, at 30 mm from the apex, from spicules both with (upper plot) and without (lower plot) their cross-shaped apex. For each spicule, the transmission of light is expressed as a percentage of the value measured on the intact spicule aligned with the laser beam. Data represent the means of 10 measurements.

spicules. In the genus *Rossella*, in which there are species 1 m high (part **a** in the figure), these spicules protrude outwards from the sponge surface with a cross-shaped apex (**b** in the figure); the spicules are composed of a very compact opaline inner core and of a multi-stratified outer layer, rich in organic components<sup>2</sup>.

We used pentactinal spicules from *Rossella racovitzae* Topsent 1901 (Porifera: Hexactinellida), sampled at 120 m depth in Terra Nova Bay (Ross Sea, Antarctica) during the PNRA XI Italian Antarctic Expedition (1994–95), to test for optical-fibre properties. We used a Sanyo compact laser beam unit, emitting red light (635 nm) (**c** in the figure) and carried out quantitative measurements on spicules technically bent to a 90° angle, with their cross-shaped apex aligned with the laser beam. The intensity of the conveyed light immediately after the bend, expressed as intensity measured at 25 mm from the cross-shaped apex, reached 65% after 10 mm (35 mm from the apex), decreasing to about 10% after 40 mm (65 mm from the apex).

To verify the role of the cross-shaped apex in conveying the light, we measured the values of light transmission at different angles of incidence to the laser beam

both on intact and on apex-deprived spicules (**d** in the figure). In both kinds of spicules, we detected light transmission up to a 40° angle of incidence to the laser beam, even though spicules deprived of

their cross-shaped apex transmit 70% less light than when the spicule is aligned with the beam. This result suggests that a larger light-capturing surface, as with the cross-shaped apex, enhances light transmission.

Scanning electron microscopy observations on *Rossella racovitzae* show that numerous intact diatoms adhere to its spicules, thereby suggesting the development of shade-adapted diatoms inside this deep-living sponge, as observed in Antarctic demosponges<sup>3</sup>. We believe that diatom survival in these peculiar environmental conditions is helped by this light transfer by siliceous spicules.

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## Distant gene flow in tropical trees

**SIR** — Canopy trees, the main structural elements of tropical rain forests, typically occur at the density of one adult or less per hectare<sup>1–3</sup>. Because these trees are strongly outcrossed<sup>4–6</sup>, their populations are expected to cover large areas of hundreds of square kilometres. These large populations are now being fragmented by deforestation and habitat alteration, but the genetic consequences of fragmentation remain largely unexplored because of the lack of information about effective population sizes and gene flow in canopy trees<sup>7,8</sup>. We have recently succeeded in isolating a relatively new class of genetic markers, simple sequence repeats (SSRs), in tropical trees and have demonstrated their use in addressing central issues in conservation biology<sup>9</sup>. Here, we demonstrate the use of these markers in estimating gene flow and mating parameters in *Pithecellobium elegans*, a large canopy tree from central American rain forests. Our measurement of the distance of pollen flow is among the largest, and exclusion probabilities for assigning paternity the highest reported for any natural population of plant species. Furthermore, our methods allow us to assign paternity to almost any seed collected from the population.

We studied gene flow and mating patterns among 28 adult trees in a forest 'fragment' 7 km west of La Selva Biological Field Station in the Atlantic lowlands of Costa Rica. The 'fragment' (Chilamate) was converted to pasture approximately

15 years ago, but the original trees of *P. elegans* were retained. One edge of the forest 'fragment' (Fig. 1) is separated by less than 30 m from natural, but degraded, forest.

We characterized nine SSR loci in *P. elegans*. Allelic diversity in this population ranges from 5 to 14 alleles per locus for the 28 trees in this site. For this study we used the following five loci: *Pel2* (5 alleles); *Pel3* (6 alleles); *Pel5* (5 alleles); *Pel6* (12 alleles); and *Pel7* (14 alleles). The high allelic diversity and the relatively large number of loci allowed us to identify unambiguously the pollen parents of individual seeds. All adults were uniquely genotyped from DNA isolated from leaves. To identify actual pollen parents and to estimate pollen flow, we analysed 167 seeds from progeny arrays of six trees. Each seed does not represent an independent mating event, because our analysis of paternity revealed that seeds within a pod generally have a single pollen parent (see below). Out of 167 seeds, 110 seeds were from 15 separate pods and the remainder represent seeds from 57 independent mating events.

Two critical attributes of this study allowed for an extremely precise description of the gene flow patterns in *P. elegans*. First, all trees were identified, mapped and genotyped. Second, and more important, the SSR loci provide the highest exclusion probabilities of any set of markers used in any study of natural plant

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populations. The 42 alleles used here give better than a 99% exclusion probability. Thus, we are not restricted to the use of rare alleles and selected individuals, but can examine random pollination events at any location in the population to examine the pollen flow dynamics and population structure. These data are used to characterize mating events at the level of the fruit, the individual tree and the population.

In many outcrossing species there is a substantial degree of pollen competition, and progeny arrays from single fruits are generally half-sibs. Several species in the Leguminosae are known to shed pollen as polyads consisting of up to 32 pollen grains (16 in *P. elegans*) derived from a single meiotic event. A single deposition by a pollinator can possibly ensure full seed production. Of 24 pods examined (15 from Chilamate and 9 others from La Selva), only two showed multiple paternity (two different fathers). Of these two, one had 18 seeds, thus requiring at least two polyads. There was no significant segregation distortion for either the male or female parent of these mating events.

We identified a paternal genotype or haplotype for all 72 mating events. The identification of a single father or the exclusion of all fathers was possible for 70 (97.2%) mating events. Of these 70, 41 (58.6%) of the pollen donors were from the genotyped population and 20 (28.6%) were clearly not from this marked population. Another 9 of the 70 mating events

(12.8%) were either the result of selfing or from a genetically identical pollen donor outside the marked population. These 9 events are progeny from a single individual. Finally, 2 of the 72 matings (2.8%) remain unresolved because the paternal haplotypes could have been derived from two different trees in the mapped population.

These mating events showed that gene flow from known fathers averaged 142 m in our study site (Fig. 2a). This large mean distance of pollen transfer contrasts starkly with the average distance of the nearest neighbours in the population (27 m). A typical leptokurtic distribution of pollination events was not evident in this population. Although one might expect matings to be among neighbouring individuals, pollen contributors to most of the seeds analysed were greater than 10 trees away (Fig. 2b). An obvious explanation for this result is variation in phenology. Flowering is episodic; although some individuals may dominate the pollen pool with massive flower production in some years, they may be minimal contributors to the pool in other years. Thus, the effective population may be considerably smaller than the number of individuals in the site but may cover large physical distances.

The 142 m average gene-flow distance is probably an underestimate of the true gene flow in the population, as our data indicate that many of the mating events are the result of gene flow from outside the site. The longest gene-flow distance covered is approximately 350 m, which may be the longest pollen-flow event precisely characterized in any species.

This species of tree is pollinated by hawkmoths, known to forage over long distances (W. A. Haber, personal communication), as is the case with other common pollinators of tropical trees such as bees<sup>10,11</sup> and bats<sup>12</sup>. Not surprisingly, at least 29% of the matings are from pollen sources outside the marked population.

Mating patterns in plants follow a hierarchical organization. We have shown for the first time how the allelic diversity at SSR loci can be used to characterize mating events at the level of fruit, individual and subpopulation. The most surprising result is that, in contrast to other species<sup>13-15</sup>, most mating events in this species are not with the closest neighbours. Thus forest fragments, as well as isolated trees in pastures, may be stepping stones for gene flow among patches

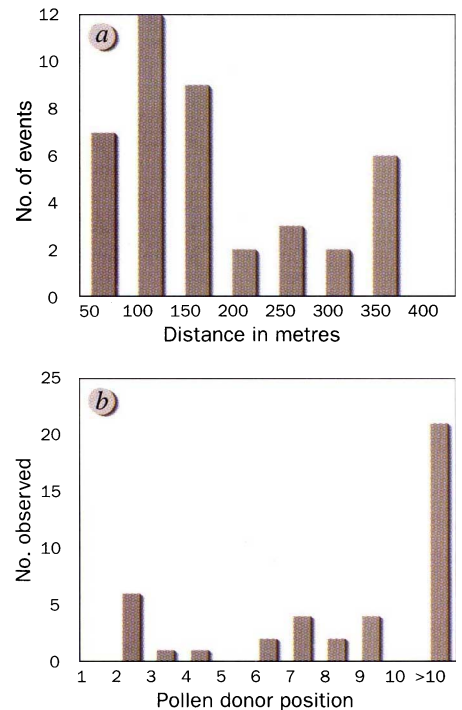


FIG. 2 Relative pollen flow in the Chilamate population of *P. elegans*. The nine probable selfing events are not included. a, Physical distances of pollen sources from the seed parent; b, relative neighbourhood position of the pollen parent. For all defined mating events, the relative position of the pollen donor to the seed plant was determined. The position could range from 1, indicating self-fertilization, to greater than 10, indicating that at least 10 other adults were closer to the seed plant than the pollen donor.

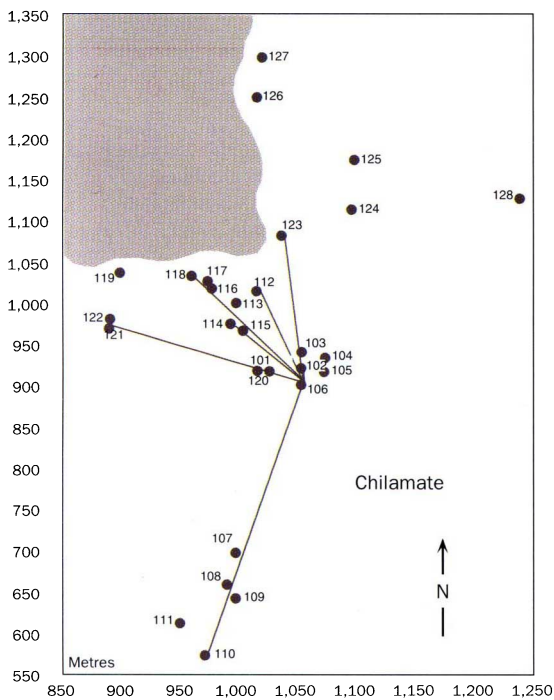


FIG. 1 Physical map of the population of adult *P. elegans* trees and pollen flow events detected to tree 106. Distances estimated with the aid of a rangefinder. Unknown reproductive individuals may exist in partially degraded natural forest (shaded area).

which contribute to the genetic diversity of contiguous undisturbed forests.

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