

Wind-borne insects mediate directional pollen transfer between desert fig trees 160 kilometers apart

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The question of how far pollen can move between plants has implications for topics as diverse as habitat fragmentation, conservation management, and the containment of genetically modified crops. The monoecious African fig tree *Ficus sycomorus* L. relies on the small, short-lived, night-flying, host-specific fig wasp *Ceratosolen arabicus* Mayr for pollination. We used microsatellite markers to characterize a geographically isolated riparian population of *F. sycomorus* growing along the Ugab River in the Namib Desert, Namibia, together with paternity analysis of seedlings from known mothers, to map pollen movement within this population. In this way we tracked insect movements between individually recognizable trees by means of their pollen cargo and documented the movement of *C. arabicus* between known trees separated by more than 160 km, with a mean distance for confirmed successful pollination events of 88.6 km. The predominant observed movement of pollinators was in a westerly direction, toward the sea, reflecting seasonal nighttime wind direction and the wind-borne dispersal of fig wasps. Our results suggest the existence of an extensive panmictic population of trees that are well suited to overcome the effects of geographical isolation.

Ficus sycomorus | fig wasp | gene flow | spatial structure | pollen dispersal

Gene flow is central in shaping population demography and determining the spatial and temporal scale at which populations respond to selection. The mode of dispersal is an important factor, determining where species persist and how they extend their ranges (1). For most plant species, this is a passive process. However, many plant species depend on insects to transport their pollen, and so the behavior of their particular pollinators determines how far pollen is transported. Pollen flow is commonly skewed toward short distances of just a few meters, reflecting insect behavior and the spacing of the plants (2, 3), but some obligate out-breeding plant species form widely scattered populations, with individuals growing far apart. Long-distance dispersal can be mediated by wind, water, or animal vectors and is normally associated with specific but rare events (4) rather than through frequent long-distance movements. Pollen dispersal distances for some species have been found to range from hundreds of meters (5–11) to kilometers (12, 13). Long-distance pollen flow between fig trees (*Ficus* spp., Moraceae) is particularly well documented (14, 15). Each of the 800 or so species of fig tree is pollinated exclusively by one or a few host-specific fig wasps (Agaonidae) (16). Fig wasps are small, delicate, weak-flying, and short-lived insects (17) that use the wind to carry them between fig trees in open woodland (18) or in rainforests, where many species are transported by the fast-flowing air found above the general canopy (19, 20). Fig trees and their wasps are also effective colonizers of islands, and low levels of genetic differentiation at microsatellite loci between mainland and island populations suggest gene flow can occur over distances of tens of kilometers (15, 21).

Nason et al. (14) were the first to use molecular approaches to detect long-distance dispersal of fig wasps. Their findings, obtained by means of parental reconstruction of single foundress progeny arrays, revealed that incoming pollen at focal trees must have come from many different individuals. Given the low densities of each species, this could only be possible if the wasps were routinely covering distances of more than 10 km. Our article extends the work of Nason et al. by determining the distances traveled by fig wasps between known individual trees. Their study took place in a Central American rainforest, where the tracking of pollen flow between individuals across many square kilometers of tropical forest would present a formidable logistical challenge. We therefore sought an alternative habitat, where all of the trees over a large area could be sampled more easily.

The African fig tree *Ficus sycomorus* L. is widespread throughout Southern Africa, but it is restricted to riverine habitats in the more arid parts of its range. It is a large, monoecious tree that produces crops ranging from a few tens to many thousands of figs that are pollinated by the nocturnal fig wasp *Ceratosolen arabicus* Mayr. Flowering in *F. sycomorus* is largely asynchronous between trees, and synchronous within trees, forcing the fig wasps to fly between trees to find a receptive host. As adult fig wasps survive 48 h or less, this must occur quickly. On entering a fig, the wasp's wings become detached, preventing subsequent dispersal to other trees.

Our study site followed the course of the Ugab River and its White Lady tributary in Namibia (Fig. 1 *A* and *B*), which contains a linearly-distributed and geographically isolated population of *F. sycomorus*. The river flows for ≈ 450 km, crossing the gravel plains of the North Namib Desert and ending at the Skeleton Coast. A rainfall gradient from west to east results in a gradual transformation from extreme desert by the coast to dry savannah habitat inland. With the help of volunteer assistants and guides, we walked three times along the last 253 km of the lower Ugab River, mapping all of the *F. sycomorus* individuals that we found, starting from the river mouth (21°07.5' S, 13°38.7' E) and ending 240-km inland (20°30.5' S, 15°16.0' E). The surveys were carried out during the winter, when prevailing nighttime winds are in an easterly direction (22). Because *F. sycomorus* is restricted to a narrow band on the banks and river

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Table 1. Pollination distances

| Mother tree ID | Nearest neighbor (m) | Number of offspring genotyped | Number assigned paternity | Number of fathers contributing | Minimum observed distance (km) | Maximum observed distance (km) | Mean distance (km) | Standard deviation |
|----------------|----------------------|-------------------------------|---------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------|--------------------|
| 1 | 81,600 | 37 | 19 | 12 | 85.3 | 164.7 | 140.1 | 6.6 |
| 3 | 2,000 | 13 | 5 | 4 | 24.2 | 80.8 | 43.7 | 18.6 |
| 4 | 1,000 | 5 | 2 | 2 | 24.6 | 78.5 | 51.6 | 19.0 |
| 6 | 1,000 | 3 | 2 | 2 | 71.4 | 75.7 | 73.6 | 1.5 |
| 8 | 2,300 | 2 | 1 | 1 | 51.0 | 51.0 | 51.0 | 0.0 |
| 13 | 1 | 1 | 0 | — | — | — | — | — |
| 17 | 1 | 5 | 4 | 4 | 14.2 | 71.5 | 38.3 | 2.7 |
| 28 | 3 | 11 | 2 | 2 | 20.6 | 57.4 | 39.0 | 13.0 |
| 40 | 5 | 2 | 1 | 1 | 27.9 | 27.9 | 27.9 | 0.0 |
| 41 | 5 | 11 | 4 | 3 | 22.3 | 29.1 | 25.9 | 0.1 |

Data are presented by maternal trees, numbered from the coast moving inland, with nearest-neighbor distances for comparison.

the optimal partition was always one when a maximum of between one and five populations was considered. Analysis using Structure v2.2 (29) also suggested a single population.

Paternity analysis of progeny collected over the two seasons of field work was then used to define the patterns of gene flow within this spatially delimited population. We used a categorical paternity analysis method, CERVUS v3 (30), to assign paternity to seedlings grown from the seed collected from individual maternal trees. Paternity analysis of all 90 genotyped seedlings from known mothers resolved the pollen-parent for 40 (Table S4). No occurrence of self-fertilization was detected. All assignments were at high logarithm of odds (LOD) scores (range 3.49–8.14; critical LOD score 3.37) with no mismatches. Five of the offspring left unassigned had multiple matching-paternal genotypes, the remaining 45 had no paternal match for trees in the sample. A range of analysis parameters in CERVUS (see *Methods*) yielded the same outcome. We estimated the number of different pollen donors contributing to offspring for which we could not assign a father by reconstruction of paternal genotypes from the unassigned progeny arrays, and counted the number of different alleles at each locus to reveal the minimum number of donors. Based on locus *MFC2*, which had 21 alleles in the adult sampled population, at least 10 unidentified fathers were needed to account for the Tree-1 offspring and 14 for the whole set of offspring. We also estimated the number of unsampled fathers using locus *Fsyc 6*, which has 10 alleles in the adult sampled population, and this suggested at least four different unsampled fathers for Tree-1 offspring and six for all of the unassigned offspring. Given that only two mature trees within the focal population were not genotyped, the cases of unassigned paternity must reflect the contribution of pollen from trees located outside the study area.

The closest distance between assigned parents was 14.2 km; the furthest pair was separated by 164.7 km (see Table 1) and the mean distance for all confirmed assignments was 88.6 km (SD 54.9, median 79.6 km). These long-distance pollen movements are on an unprecedented scale. The most extreme pollen-movement distances observed came from analysis of seedlings from Tree-1 (Fig. 1C and see Table 1). Thirty-seven genotyped seedlings came from this tree and we assigned paternity to 19 of them. Distances between Tree-1 and the assigned fathers ranged from 85.3 km to 164.7 km (mean 140.1 km, SD 26.2, median 150.8) (see Table 1). A consequence of the geographical location of this tree was that all potential fathers were located toward the east of our study area. Other trees in the population have neighbors both to their east and west, but paternity analysis of progeny from these trees revealed that all but one of the male parents (see Fig. 1D–F for examples) were located to the east of the maternal trees, resulting in predominantly unidirectional

gene flow; nearest neighbors were rarely the fathers of the seedlings.

Fifty-three seedlings were obtained from fruit collected from trees upstream of Tree-1 in the second survey year; of these, 21 were assigned paternity. The distances between mothers and assigned fathers for these seedlings (see Table 1) ranged from 14.2 to 80.8 km, with a mean distance of 42.0 km (SD 22.0, median 29.1 km). The standard deviation of parent-offspring distances σ , a key parameter which, together with effective population density, determines the expected scale of genetic structure in a continuous population, was 97.6 km for all assignments across the two seasons and 12.7 km for the second season alone.

To counter possible effects of false-paternity exclusion because of null alleles in locus *Fsyc13*, we performed the paternity analysis including only five loci. In this way, we were able to resolve the pollen parent of 29 seedlings out of 90, with predominantly unidirectional pollen flow and mean dispersal of 84.3 km including Tree-1 or 43.4 km excluding Tree-1. We also used a simple exclusion approach to estimate the number of offspring for which all putative fathers could be excluded by heterozygous loci alone, therefore avoiding the potential influence of null alleles at any locus. Using this model-free approach, we calculated that there are 22 offspring for which all sampled adult trees can be excluded as fathers.

There was a striking pattern of declining mean pollination distances between trees toward the east of the survey area (see Table 1), reflecting the higher tree densities further inland and the overwhelmingly wind-borne westerly movement of pollinators. Our data may underestimate mean pollination distances between the more inland trees because unidentified distant pollen donors from outside the study area may also be contributing to paternity. We therefore estimated the underlying distribution of these movements using a maximum-likelihood method (31) that allows for a proportion of paternity from outside the study area and compares a variety of forms of the dispersal distribution. However, no model of finite dispersal provided a better fit than the null-model of panmixia, either with or without the outlying Tree-1 included in the dataset. We simulated the expected distribution of paternities under panmixia for each dataset, where we defined the mothers to match the observed data and allowed pollen movement up and down the river with no limit on distance (Fig. 2). The observed distributions of pollination distances are consistent with these expectations because the mean simulated displacement of fathers toward the east of the relevant mothers was 26.6 km, with an observed mean distance of 35.7 km. Moreover, the data obtained in 2001 reveal that 95% of the identified fathers were east of the mothers, a value that was never exceeded in 1,000

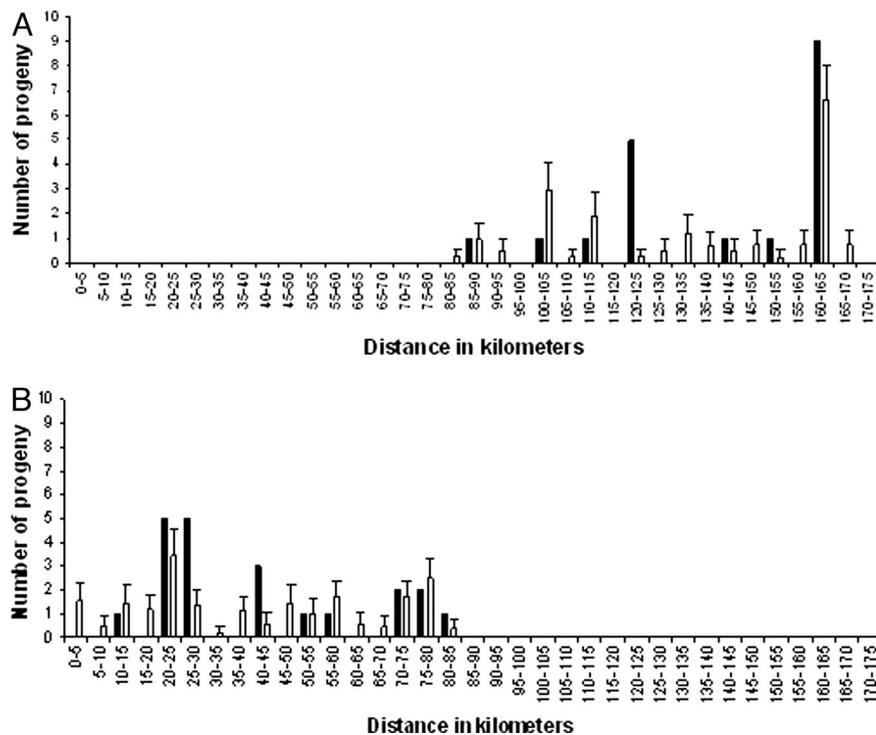


Fig. 2. Distribution of paternity assignments. The observed numbers of progeny assigned (solid bars) and simulated numbers of assignments (\pm SD) (open bars) under the assumption of panmixia, with mothers and numbers of offspring defined by the observed data and fathers chosen at random from sampled trees up- or down-stream of the mother, with no limitation on distance, in 5-km distance increments for (A) year 2000 data (maternal Tree-1 only) and (B) year 2001 data.

simulations, indicating that the null hypothesis of nondirectional pollination can be rejected with $P < 0.001$.

Discussion

Our data demonstrate the movement of individual insects over unprecedented distances and define pollination events between known parent plants over distances >160 km, with a mean dispersal distance of 88.6 km. Within-crop synchrony and between-tree asynchrony in *F. sycomorus*, as is typical of many fig trees, ensured cross-pollination and meant that pollination events were often not between nearest neighbors. We chose this population because of its apparent isolation, but the diversity of genotypes among the seedlings with unassigned paternity cannot be accounted for by the two inaccessible trees within our study. This conclusion suggests that there must be a source of pollen from additional trees located outside our study area, which is also implied by the estimate of unsampled fathers in the paternal reconstruction. It is therefore likely that pollination events of at least the distance to the nearest neighboring water courses of 50 km, but possibly even greater than the observed distance of 164 km, were occurring.

The positive F_{IS} values in the adult population suggest some inbreeding. Although we see no evidence for selfing or biparental inbreeding in our study of contemporary gene flow, it may be that the sampled adult population is a remnant from one of much greater density, where related individuals had greater opportunity to interbreed. Higher densities facilitate the possibility of near-neighbor matings because of the close proximity of trees at the correct stage of the fig reproductive cycle. There is anecdotal evidence for a recent decline in the abundance of fig trees in the study area. Alternatively, selfing may be more common in the summer when fig crops are larger and less synchronized than in the winter, when our sample was collected. Easterly winds in the summer may also reduce pollen flow from inland populations.

Pollination over such long distances has resulted in a single unstructured population that extends for at least 250 km along the Ugab and perhaps much further. *F. sycomorus* shares a monoecious breeding system with the strangler figs studied by Nason et al. (14, 32). Fig wasps associated with monoecious fig species are thought to transport pollen over greater distances than those associated with dioecious fig trees, which are often smaller in size, producing small, asynchronous fig crops (33, 34). Pollen dispersal ranges of 1 to 2 km have been inferred for the dioecious *F. pumila* in China (35), but genetic studies of two pollinators of dioecious fig trees from Indonesia nonetheless found low levels of population subdivision between mainland and island populations over 40 km apart (15), suggesting that the nature of the breeding system is a weak predictor of gene-flow distances in fig trees.

How does *C. arabicus* manage to pollinate *F. sycomorus* trees over 100 km apart? The slow flight speed of fig wasps (36) precludes active, directed flight in the time available during a 48-h adult lifespan. Wind-borne dispersal is therefore required, which offers no control of their necessarily downwind direction of movement. Once fig wasps are close to a tree that is releasing species-specific volatile attractants, they can then seek sheltered areas with low wind speeds that permit directed flight (18, 36). The varying flight heights of fig wasps (34) show that this mode of insect dispersal is not truly passive (37). Predominantly easterly winds occur at night over the central Namib from May to October (22). The winds average ≈ 10 km per hour, but can reach 60 km per hour, making possible dispersal events of more than 100 km within a single night. The observed east-to-west direction of pollination flow reflects these wind conditions. Intriguingly, at other times of the year the predominant nighttime winds in the area are westerly. This meteorological phenomenon leads us to predict a seasonal reversal in the direction of pollen flow between the trees.

Along with other tropical trees, many fig tree populations are being rapidly fragmented (38), but their pollination system and wind-mediated dispersal capabilities (14) may provide them with exceptional resistance to habitat loss, population fragmentation, and large-scale perturbations (14, 39). The recent return of elephants to the Ugab, after being absent for many years, has resulted in the deaths of numerous *F. sycomorus*, resulting in the increasing isolation of the remaining trees, exacerbated by low recruitment. Although the dispersal ability of *C. arabicus* means that isolated down-river individuals will still be pollinated, the likelihood of pollinators successfully finding a fig must scale with down-wind distances between trees, and their population dynamics may limit long-term persistence in the Ugab and other increasingly fragmented landscapes.

Routine dispersal on the scale seen here has implications for population differentiation, local adaptation, and speciation among small insects in general (17). How typical is the dispersal ability of fig wasps? Any slow-flying insects that fly up into an air column will be carried downwind for as long as they remain airborne, but such behavior is not accidental and among rain forest parasitoids (Chalcidoidea), there are clear differences in the preferred flight heights of different families (19), implying differences in behavior that will affect their capacity to be carried by the wind. "Slatkin's paradox" describes the contrast between direct and indirect measures of gene flow seen in many small insects (6, 40). For example, the predominantly flightless planthopper *Tumidagena minuta* shows levels of population differentiation that are comparable with mobile insects, even though most individuals are incapable of flight and so dispersal distances are typically very low (6, 41). The paradox may be resolved if differentiation is low because of recent range expansions or if levels of dispersal are often seriously underestimated. Our extraordinarily high estimates of fig wasp movement, based on genes in the pollen they carry, could not have been achieved by methods such as mark-recapture. This suggests that a resolution of the paradox lies in underestimation of the dispersal abilities of small insects. If this is correct, the lack of genetic differentiation in other insects implies that fig wasp dispersal may not be exceptional.

Methods

Sampling. *F. sycomorus* growing in the study area were numbered and global positioning system coordinates recorded. Young, fresh leaf tissue (5 g) was collected from each tree, the midrib was removed with scissors, and then placed in plastic vials containing 30-ml saturated NaCl-CTAB. Fruit was collected from each tree where possible. Ripe fruits were pressed flat, air-dried in the sun, and placed in paper bags containing silica gel. Seeds were subsequently germinated in a heated glasshouse at 22 °C, 65 to 80% relative humidity. After 3 weeks, seedlings were transferred to pots on capillary

matting containing a 2:1:1 mix of compost (John Innes No. 3), perlite, and grit and maintained at 20 °C.

DNA Extraction and PCR Analysis. DNA extraction and PCR analysis were performed as described in ref. 23.

Microsatellite Markers. Microsatellite genotyping was performed using six loci, five of which were developed for this species (23) and one for a congener (24). Scoring of the genotype data were performed without knowing which offspring belonged to which mother to avoid type-1 errors and controls were included in all runs to allow repetition and ensure reproducibility and reliability of results. For summary statistics relating to these loci see the *SI Text* and *Table S5*.

Population Differentiation and Structure. The data were tested for null alleles and other problems with scoring using Microchecker (42). Estimations of *F* statistics and departures from Hardy-Weinberg Equilibrium were implemented in Genepop v3.4 (43). Population structure was investigated using the programs BAPs 4.3 (28) and Structure v2.2 (29). In Structure, data were analyzed with no population priors under the "no admixture model" and repeated for the "admixture model."

Rousset's genetic distance measure (*a*), which is analogous to the $F_{ST}/(1-F_{ST})$ ratio but using pairs of individuals rather than populations, was calculated in the software SPAGeDi v1.2 (27), such that the slope of the regression with distance provides an estimate of gene-dispersal distances.

Paternity Analysis. Paternity analysis was performed using the program CERVUS v3 (30). We repeated simulations in CERVUS five times and determined the mean critical LOD score. Paternity analysis of the 90 genotyped seedlings with known mothers resolved the pollen-parent for 40 at the 80% confidence level estimated in CERVUS, 5 of which were at the higher confidence level of 95%. For detailed description of the analysis parameters applied see the *SI Text*.

Simulations. We simulated the expected distribution of paternities under panmixia by drawing the observed number of mothers at random from the total set of 79 sampled trees and assigning paternities at random (excluding the possibility of self-fertilization) to give the observed numbers of offspring per mother; the analysis was repeated 1,000 times. We also simulated pollination distances (1,000 replicates) by restricting mothers to those observed and, to test the null hypothesis of nondirectional pollen flow, selected fathers from either east or west of the mother with unlimited distance (see Fig. 2).

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