Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances

Rémy S. Pasquet*†‡§, Alexis Peltier¶, Matthew B. Hufford‡, Emeline Oudin‖, Jonathan Saulnier‖, Lénaic Paul‖, Jette T. Knudsen**, Hans R. Herren*††§, and Paul Gepts‡

*International Centre of Insect Physiology and Ecology, PO Box 30772, Nairobi, Kenya; †Institut de Recherche pour le Développement, Département Ressources Vivantes, 213 Rue La Fayette, 75480 Paris Cedex 10, France; ‡University of California, Department of Plant Sciences/MS1, Section of Crop and Ecosystem Sciences, 1 Shields Avenue, Davis, CA 95616-8780; ¶Maisha Trust, PO Box 34304 (00100), Nairobi, Kenya; ‖Institut National Agronomique Paris-Grignon, Ecologie des Populations et Communautés, 16 Rue Claude Bernard, 75231 Paris Cedex 05, France; **Lund University, Department of Ecology, Chemical Ecology and Ecotoxicology, Ecology Building, SE-223 62 Lund, Sweden; and ††Millenium Institute, 2200 Wilson Boulevard, Suite 650, Arlington, VA 22201-3357

Contributed by Hans R. Herren, July 10, 2008 (sent for review January 9, 2008)

Foraging range, an important component of bee ecology, is of considerable interest for insect-pollinated plants because it determines the potential for outcrossing among individuals. However, long-distance pollen flow is difficult to assess, especially when the plant also relies on self-pollination. Pollen movement can be estimated indirectly through population genetic data, but complementary data on pollinator flight distances is necessary to validate such estimates. By using radio-tracking of cowpea pollinator return flights, we found that carpenter bees visiting cowpea flowers can forage up to 6 km from their nest. Foraging distances were found to be shorter than the maximum flight range, especially under adverse weather conditions or poor reward levels. From complete flight records in which bees visited wild and domesticated populations, we conclude that bees can mediate gene flow and, in some instances, allow transgene (genetically engineered material) escape over several kilometers. However, most between-flower flights occur within plant patches, while very few occur between plant patches.

cowpea | radio-tracking | *Vigna unguiculata* | *Xylocopa flavorufa*

Both solitary and social bees provision their broods by central-place foraging from their nest. Nesting females return several times to the nest during a given day after foraging bouts. Therefore, the investigation of bee flights is essential to understand their ecology and mobility. Foraging success is determined by habitat size and the amount and variety of forage that a bee utilizes. As the flight range of bees will determine the minimum resource density that can sustain a nest, knowledge of flight range is important for designing strategies for bee conservation when their plant resources are threatened or fragmented (1, 2). Likewise, knowledge of bee flight range is important for bee-pollinated plants, because flight range governs the distance over which pollen can be transported. Additionally, precise measurement of pollinator flight range has recently become imperative because of concern over the spread of engineered genes through pollen-mediated gene flow from genetically modified crops into conventional agriculture and wild relatives (3).

In insect-pollinated plants, pollen movement, rather than movement of seeds, is generally the main component of gene flow (4, 5). When measured by the proportion of progeny that contains an immigrant gene from a given source, gene flow declines rapidly with increasing sink population size and spatial isolation (6). Long-range dispersal events are excluded by methods used in quantitative dispersal studies (7), which means that measuring dispersal from a source almost always truncates the actual dispersal curve. In predominantly autogamous bee-pollinated species, source and sink trials fail to detect gene flow events beyond a few meters (5, 8–9). Paternity testing (10), although more informative, follows the same principles and has the same distance limitations (11). At the landscape level, the difficulty of directly measuring gene flow has led to the common use of indirect measures extrapolated from genetic frequency data according to Wright's island model of population genetic structure (12, 13). These measures are variants of F_{ST}, a standardized measure of the genetic variance among populations, and are used to solve for Nm, the number of migrants successfully entering a population per generation. However, the translation of F_{ST} into an accurate estimate of Nm is controversial (11, 14, 15). Therefore, genetic data should be complemented with direct observations and documentation of pollinator-mediated transport (13, 16). Such data allow for interpretation of spatial and temporal heterogeneity in movement patterns across a species' range and measurement of the impact of environmental attributes, such as habitat patchiness and resource quality on movement patterns. More fundamentally, this information allows an interpretation of gene flow in an ecological context (14).

Although genetic results suggest that inter-population pollen dispersal by pollinators is more extensive than previously believed, little is known about the phenomenon, especially when compared with intra-population pollinator movements. Flight capabilities remain poorly resolved for most pollinating agents, including bumblebees. Successful pollen transfer between trees separated by a distance ranging from 10 to 84 km have been recorded, but pollinators are known to generally forage at distances well below their maximum flight potential (1, 17). Until now, every technique used to assess bee foraging range has had strong limitations. The waggle dance decoding (18) in honeybees (e.g., 19, 20) is restricted to the few species of the genus *Apis*. Mark-recapture techniques (e.g., 21, 22) cannot be used if nests are not accessible. Identification of sister bees through microsatellite markers (23) does not work with nonsocial species. Harmonic radar (24) has been used to track individual flying honeybees and bumblebees over hundreds of meters. However, bees become undetectable behind obstacles or beyond 700 m. Direct radio-tracking, although a standard technique with mammals and birds, has rarely been used with arthropods and has never been attempted with pollinators (25–30).

Author contributions: R.S.P. designed research; R.S.P., A.P., M.B.H., E.O., J.S., L.P., and J.T.K. performed research; R.S.P. and E.O. analyzed data; R.S.P., M.B.H., J.T.K., H.R.H., and P.G. wrote the paper.

The authors declare no conflict of interest.

§To whom correspondence may be addressed. E-mail: rpasquet@icipe.org or hh@millennium-institute.org.

This article contains supporting information online at www.pnas.org/cgi/content/full/0806040105/DCSupplemental.

Table 1. Summary of the bee flights recorded

		Kaskazi season		Kusi season	
	Total	Many flowers (≈50,000)	Few flowers (≈1,000)	Rainy days	Sunny days
Number of flights	134	28	34	41	31
Flight length, m					
Maximum	6,040	6,040	2,340	1,390	5,000
Median	720	1,495	600	570	660
Average	1,014	2,092	759	585	887
Minimum	50	200	80	50	170
Median/maximum	0.12	0.25	0.26	0.41	0.13

Here we used insect-pollinated cowpea and radio-tracking of pollinators to determine pollinator movements and their implication for long-distance pollen flow. This research was triggered by the imminent release of an insect-resistant, genetically engineered cowpea in Africa (31[‡‡]) where a cowpea crop-weed complex exists (32, 33). If insect-resistance genes would enhance fitness of cowpea and were to escape through pollinator-mediated gene flow, they could make the wild progenitor of cowpea more competitive and increase its weediness. Additionally, introgression of an insect-resistance gene into wild cowpea populations could trigger a selective sweep in the genomic region of the gene, thereby reducing genetic diversity of potential importance for cowpea crop breeding.

The carpenter bee *Xylocopa flavorufa* (DeGeer) is one of the main cowpea pollinators in coastal Kenya, where wild and domesticated cowpea are found. This large, solitary bee has a very fast and powerful flight, which rules out most conventional techniques used to study foraging flights. In addition, its nests are usually burrowed in barely accessible dead branches located high in trees, thereby preventing a mark-recapture study. Furthermore, the hilly and wooded landscape (supporting information (SI) Fig. S1) does not allow for the use of the harmonic radar technique. However, we show here that *X. flavorufa* individuals do fly well while carrying a very small radio-transmitter. Using this method, we assessed both the distance between the bee's nest and one of its foraging locations and how many places the bee visits during a single foraging trip.

Results

A total of 134 *X. flavorufa* return flights were recorded (Table 1). Flight distances ranged from 50 to 6,040 m, with a median of 720 m. Of the flight distances observed, 64% were between 200 and 1,000 m. When comparing homing tests with direct radio-tracking, the homing tests revealed that carpenter bees have a potential flight range of around 10 km, which is well beyond the longest foraging flight recorded in this study (Fig. S2).

During the Kaskazi seasons (December to March, with predominant northeast winds), flights were much longer in February 2004 than in March 2005 and February 2006 (one-way ANOVA, log of distances, df = 1, MS = 1.983, F = 11.97, $P < 0.001$), even though weather conditions were quite similar (see Table 1, Table S1, Fig. 1). In 2004, in addition to natural wild cowpea stands, several trials with wild cowpea, wild-domesticated F_1 hybrids, and progenies were ongoing within the field station, with around 1,000 plants covering 4,000 m^2. In 2005 and 2006, there were only two small plots of wild cowpea, with ten plants each, and up to 20 scattered natural wild plants elsewhere. Considering an approximate number of 10 to 50 flowers per plant, the number of flowers may have been 10,000 to 50,000 in 2004 versus 200 to 2,000 in 2005 and 2006.

During the Kusi seasons (April to November, with predominant southeast winds), flights were longer during sunny days than during rainy or cloudy days (one-way ANOVA, df = 1, MS = 0.463, F = 4.15, $P = 0.045$; see Table 1 and Fig. S3). The direction of flights was independent of the dominant wind direction. In fact, despite differences in the prevailing wind direction during the Kaskazi (predominantly from the northeast) and Kusi (predominantly from the southeast) seasons, the frequency of flights in the eight different 45° sectors around the station was not significantly different (χ^2 test, $P = 0.717$) during these seasons.

In each of the three tracked foraging bouts (from nest exit to nest return), the bee visited two wild cowpea patches and a cowpea field during the same flight. On all three occasions, the bee visited the same domesticated cowpea field but different wild cowpea patches, the distance between wild cowpea patches and the cowpea field being at least 50 m (Fig. 2).

Discussion

This study of bee foraging flights demonstrates that radio-tracking can be used effectively to measure pollinator foraging distances. Smaller transmitters are rapidly being developed: our transmitters were half the weight of those used by Lorch *et al.* in 2000 (25), 0.1 g lighter than those used by Hedin and Ranius in 2002 (28), and very similar to those used by Wikelski *et al.* (29) during their 2005 dragonfly tracking campaign. Smaller and more powerful transmitters will likely be manufactured in the future, allowing the technique to be used with even smaller pollinators. In our case, although individual bees may have been hampered by the weight of the radio-transmitter and the length of the transmitter antenna during the few complete flights recorded, the foraging distances measured using return flights were not biased by the weight of the transmitter because the bees did their outgoing flight from the nest to the flowers without any extra weight.

Nest Locations and Flight Length. This is the first study to determine the origin (nests) of pollinators of a specific plant population. With two exceptions, all of the nests were outside or at the edge of the rain forest, which lines the south fence of the field station (see Fig. S1). No nest was found deep within the rain forest. This is in agreement with results from Anzenberger (34), who observed *X. flavorufa* nests at the fringes of woodlands and in larger tree groups in Tanzania. Only two flights below 100 m were recorded (80 m during a sunny morning and 50 m during a rainy morning), although 20 nests were less than 100 m away from flower patches where other bees were captured. Bees from these closely-located nests were captured in the central part or on the opposite side of the station (see Fig. 1 and Fig. S3). This result is consistent with results obtained with bumblebees (24, 35) and honeybees (36, 37), which indicate that bees usually do not forage very close to their nests (1), even if this should not be true for every bee species (38).

Results from homing tests were similar to results obtained with other pollinators. Honeybee flights are typically less than

[‡‡]Higgins TJ, Popelka C, Ishiyaku M, Pasquet R, Mignouna J, Bokanga M, Huesing J, Murdock L, Biotechnology, breeding and seed systems for African crops, March 26–29, 2007, Maputo, Mozambique, (abstr).

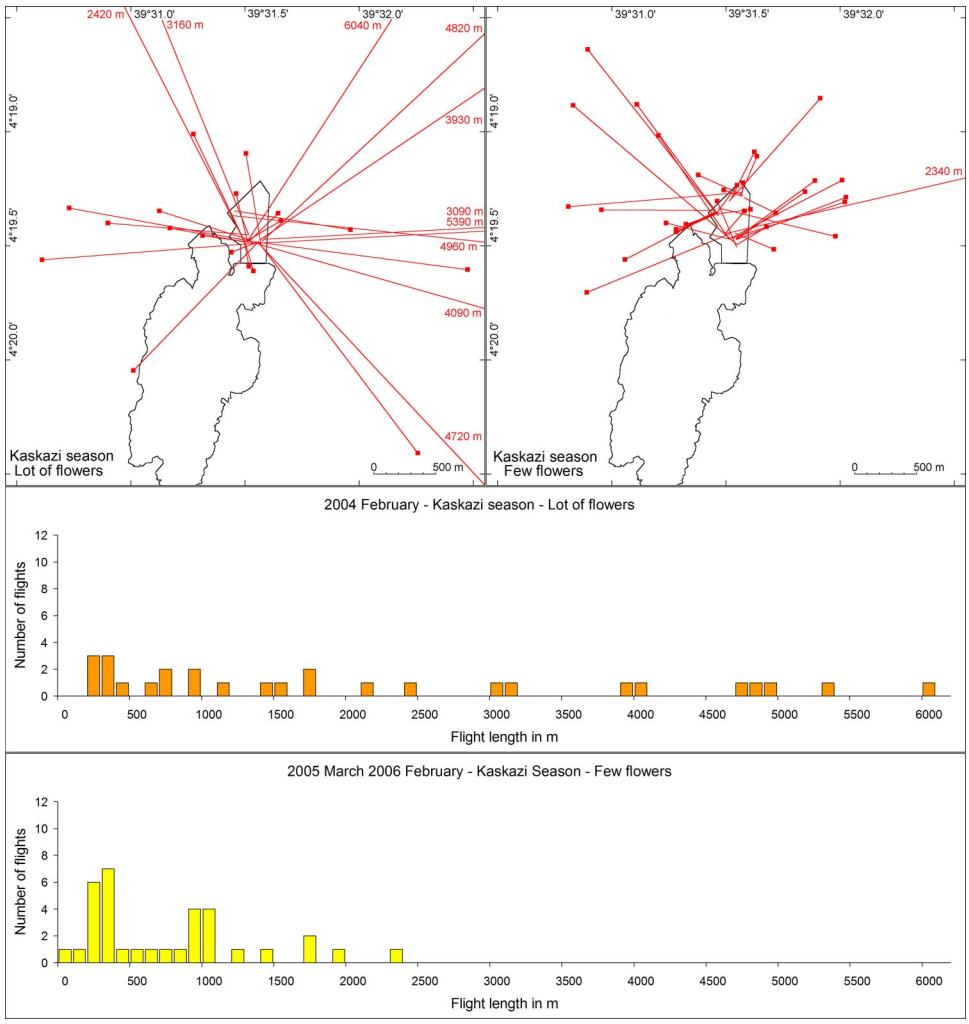

Fig. 1. Bee flights recorded during the Kaskazi season (predominant northeast winds) in 2004 with high density of flowers at the field station (*Left*), and in 2005 and 2006 with low density of flowers at the field station (*Right*). Nests are indicated by red squares. Outlines indicate the field station and the Muhaka rain forest, south of the field station.

2-km long, but have a potential range of 10 km (36, 39). *Bombus terrestris* has a 10-km potential range (40) and its estimated foraging range varies between 0.6 and 2.8 km (41). Our data fit Roubik's model (1), with most foraging taking place between one third and one half of the foraging range. However, while this model fits perfectly during poor conditions (bad weather or low reward levels), in better conditions involving either sunny weather or high reward levels, long flights are more numerous than predicted by this model. Flights were much longer when larger numbers of flowers were available for foraging within the study area, as was the case in February 2004. Several studies have indicated that an increased floral display attracts more pollinators (42–45). However, such studies were never combined with foraging range assessment. Here, we show that in 2004 the higher number of foraging carpenter bees was a result of an increase in visits by bees from remote locations. The higher floral reward (10,000–50,000 available flowers versus 200–2,000) presumably justified visits from longer distances. Therefore, we can expect that the floral display of cowpea fields with dense synchronized flowering (for example, in breeding lines or commercial cultivars) are likely to attract bees from farther away than smaller, less dense patches of wild cowpea. Poor weather conditions are known to affect bee activity (1), which was confirmed in the present study where bees flew significantly shorter distances during poor weather conditions, even with roughly similar flower densities (see Table 1). The shortest foraging distances (less than 100 m) were recorded during such poor weather conditions.

The longest flights (11 flights longer than 2.5 km) apparently took place when bees were pushed by the wind, which means that the bee, loaded with pollen or nectar, had to fly against the wind on its way back to the nest. This seems to contradict Roubik's (1) assessment that a symmetrical distribution of foragers around the home base is to be expected in a relatively flat area, while bees in steep terrain, to avoid difficult returns while loaded, are expected to fly longer distances uphill than downhill. No correlation was found between direction of bee flight and wind, probably reflecting that wind is usually very calm around 6 AM (see Table S1).

Flight Length and Pollen Flow. The three complete foraging bouts recorded lasted between 60 and 92 min. This is somewhat long, but still within the range of flight durations without a transmitter (at the same time of the day): 13 to 188 min with a median of 30 min for 15 *X. flavorufa* foraging bouts (E.O., personal observation). From personal observation (R.S.P.), we can roughly estimate that *X. flavorufa* visits one flower every 10 s within a flower patch (visit duration = 7.1 ± 0.3 s, n = 121; within patch flight duration = 3.1 ± 1.6 s, n = 92) and that the duration of

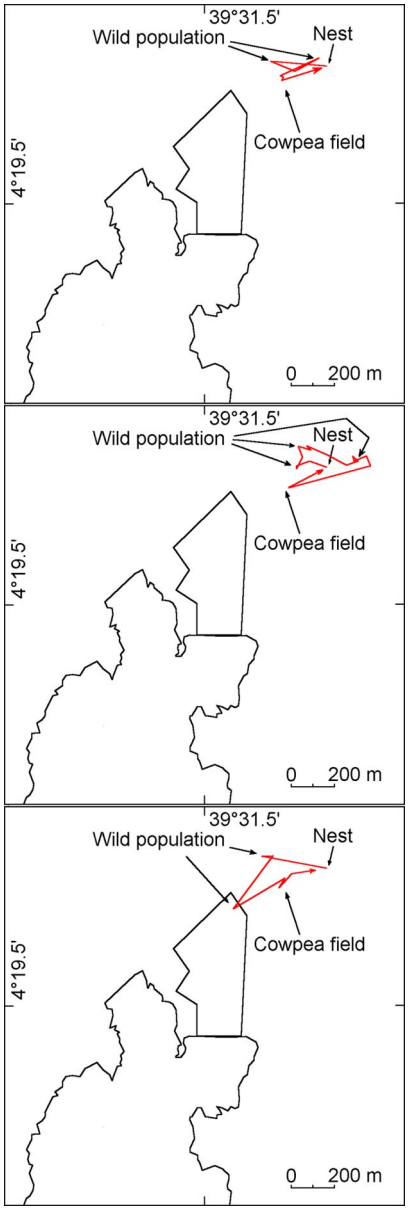

Fig. 2. Flights recorded from nest to nest. From top to bottom: August 29, 2006, 92 min; August 30, 2006, 85 min; October 23, 2006, 60 min.

the trips between flower patches are not longer than a few minutes, considering the speed of these bees. Therefore, if we consider our recorded foraging bout, the *X. flavorufa* bee potentially visited around 350 (third 1-h foraging bout) or 500 (first and second 1.5-h foraging bout) flowers distributed within two wild flower patches and a domesticated field during a trip. In such foraging bouts, the large majority of flights between flowers take place within plant patches, with only two flights taking place between patches. These results are consistent with the classical observation that most bee flights occur within flower patches (e.g., 16).

If we consider the potential domesticated to wild cowpea gene flow, only one flight was between wild and domesticated cowpea (in the three recorded bouts, the field was the last place visited before returning to the nest). This ratio will increase slightly when wild plants are adjacent to domesticated cowpea fields, which is often the case in coastal Kenya. It should also increase much more in situations where wild cowpea plants are weeds

Fig. 3. *Xylocopa flavorufa* taking off (*Upper*) and returning to its nest (*Lower*).

within domesticated fields, as in West Africa (46). However, if the closest wild plant patch is 50 m from the domesticated field, as in the present foraging bout tracked, few wild-to-domesticated or domesticated-to-wild flights versus numerous wild-to-wild and domesticated-to-domesticated flights make the probability of getting wild-domesticated progenies very low.

This particular foraging behavior with many within-patch flights versus very few between-patch flights fits results obtained from isozyme studies of the three wild cowpea populations that occur in the 1.5-km radius around the bee nests used for the study of complete foraging bouts. In this study, 10 isozyme loci were used and $N_m = (1-F_{ST})/4F_{ST}$ values ranged between 1.5 and 2.5 (I. Y. Rabbi and R.S.P., unpublished data), while t_S values observed in a larger wild cowpea population a few kilometers away indicated outcrossing rates higher than 30% during the most favourable months (E. Kouam and R.S.P., unpublished data). Therefore, we observed a fairly high level of gene flow within wild populations, while F_{ST} indices suggested poor gene exchange between populations.

The combined results of the longest nest-flower distances and the three complete flights tracked (over shorter distances) suggest that *X. flavorufa* has the potential to move pollen over several kilometers and between wild and domesticated populations of cowpea. However, if wild and domesticated plant patches are at least 50 m away, as in the area where we performed the tracking of three complete flights, the very few flights between-patches makes the probability of pollen movement between domesticated and wild plants low.

This is especially important, considering that pollinator flights mediate pollen dispersal and enable gene escape from domesticated plants. With regard to genetically engineered cowpea in Africa, these results indicate that although pollen movement beyond a few hundred meters has a low probability, strict isolation by distance may not be feasible, as large areas cannot be screened and guaranteed to be free of wild or weedy cowpea plants. Confined field trials should be conducted outside Africa if adventitious gene escape is to be strictly avoided, as wild cowpea is widely distributed over the African continent south of the Sahara (32). If biological containment is not implemented, deploying a genetically engineered cowpea in Africa may mean that transgene escape to wild cowpea populations is inevitable.

Materials and Methods

Study Species and Site. Cowpea, *Vigna unguiculata* (L.) Walp. is the main native legume crop of Africa. *Vigna unguiculata* var. *spontanea* (Schweinf.) Pasquet is the wild relative and the progenitor of the domesticated cowpea var. *unguiculata*. Although the crop is predominantly inbred, both wild and domesticated cowpea are sexually compatible and do exchange genes (32, 33).

In coastal Kenya, wild cowpea populations are mostly encountered on sandy soils from several geological formations, such as Mazeras sandstone and Mariakani sandstones from the Permo-Triasic, Magarini sands from the Pleistocene, and Kilindini sands from the Pliocene. The Muhaka International Centre of Insect Physiology and Ecology field station (32 km south-southwest of Mombasa, Kenya, 4°19.5′ S 39°31.5′ E) is situated on the Magarini sands geological stratum. Wild cowpea populations are located north and east of the field station, within a distance of a few hundred meters.

Tracking Technique. Individuals of *X. flavorufa* were able to carry a 0.35-g LB-2N radio-transmitter, including a 14-cm antenna (four times the length of the bee) (Holohil System), which is equivalent to a third of their average weight (1.01 ± 0.15 g, $n = 21$). We captured and recorded the GPS position of bees foraging on cowpea flowers at sunrise within the field station. A radio-transmitter was affixed with a sticky paste (Plastofix from Plasto) on the dorsal part of the thorax, after which each bee was released and allowed to return to its nest (Fig. 3). Because the size of the nest entrance is about the size of the bee's body, the transmitter is dislodged and falls to the ground when the bee enters its nest (see Movie S1). Because the transmitter was affixed only after the bee arrived at its foraging site, the extra weight did not influence our foraging range results. However, the extra weight may have reduced the amount of forage that the bee carried back to its nest and the duration of the foraging bout. The range of the transmitters (around 200 m at soil level) and the large potential target area (around 300 km^2) necessitated an aircraft for locating transmitters. We used a Piper Colt PA22 or a Cessna 206 and a hand-held TRX-3S receiver (Wildlife Materials International). We flew along a spiral path starting from the field station (foraging point) and extending up to 10 km from the field station to recover the signals from each transmitter. From aircraft GPS records, ground teams recovered the transmitters, checked for the presence of nests (in some instances the bees lost their transmitters while in flight), and recorded the exact GPS position of the transmitter and the corresponding nest. Three tracking efforts were carried out during the Kaskazi season, with predominant northeast winds (December to March): February 3–14, 2004 (7 transmitters, 2 detectors), March 10–13, 2005, and February 21–25, 2006 (15 transmitters, 4 detectors). Three tracking efforts were also carried out during the 2004 Kusi season, with predominant southeast winds (April to November): June 26 to July 2, October 11–15, and November 11–17 (17 transmitters, 4 detectors).

Homing Tests. Most nests were inaccessible, but from five nest groups located close to the field station we were able to perform 22 homing tests. Bees leaving their nests were captured and marked with colored tags, then released at varying distances from their nests and checked upon return to their nests. Ten individual bees were used and some completed up to four consecutive homing tests.

Complete Foraging Bout Tracking. From a group of nests located in one small tree, we were able to track three complete foraging flights. A transmitter was affixed to the bee when it left its nest and its whole foraging bout was followed. Although very informative, the number of such flights was limited because very few low-positioned nests were available, and bees quickly learned how to dispose of their transmitter either during their flight soon after departure or by returning immediately to the nest. In some instances, bees flew too far, too fast, or to inaccessible areas (six incomplete flights recorded). In one instance the track was complete but the bee did not visit a single cowpea patch. The area surveyed during the tracking of these foraging bouts included several wild cowpea patches and a 5,000-m^2 field with a mix of cowpea and watermelon. In this area, no wild cowpea plants were growing less than 50 m from the cowpea field.

ACKNOWLEDGMENTS. We thank our Piper Colt pilot Allan Hurt from Kijipwa Aviation Limited and all of the people who searched for transmitters: Athumani Mohamed Gunia, Omari Juma Mwamguta, George Okoth Nyabach, Mohamed Masudi Koja, Loya Suleiman Mohamed, Hadi Masudi Mwachidzayo, Daniel Ogesi Manoah, and the late Sapaya Welemba. We thank David W. Roubik for his comments on the manuscript and suggestions. This study was funded by United States Agency for International Development Grant 551-0138-01 (via the International Institute of Tropical Agriculture) and the Rockefeller Foundation Grant 2000 GI 087. M.B.H. was partially supported by a Jastro-Shields award of the International Agricultural Development graduate group at University of California Davis.

1. Roubik DW (1989) *Ecology and Natural History of Tropical Bees* (Cambridge Univ Press, Cambridge).
2. Cresswell JE, Osborne JL, Goulson D (2000) An economic model of the limits to foraging range in central place foragers with numerical solutions for bumblebees. *Ecol Entomol* 25:249–255.
3. Ellstrand NC (2003) Current knowledge of gene flow in plants: Implications for transgene flow. *Philos Trans R Soc B* 358:1163–1170.
4. Ennos RA (1994) Estimating the relative rates of pollen and seed migration among plant populations. *Heredity* 72:250–259.
5. Fenster CB (1991) Gene flow in *Chamaecrista fasciculata* (Leguminosae). I. Gene dispersal. *Evolution* 45:398–409.
6. Handel SN (1983) Pollination ecology, plant population structure, and gene flow. In *Pollination Biology*, ed Real L (Academic, Orlando), pp 163–211.
7. Grant V (1985) The problem of gene flow on a geographical scale. *Zh Obschei Bio* 46:20–31.
8. Ferreira JL, *et al.* (2007) Gene flow in common bean (*Phaseolus vulgaris* L.). *Euphytica* 153:165–170.
9. Schuster I, *et al.* (2007) Soybean gene flow in the Western Region of Paraná (Translated from Portugese). *Pesqui Agropecu Bras* 42:515–520.
10. Adams WT, Griffin AR, Moran GF (1992) Using paternity analysis to measure effective pollen dispersal in plant populations. *Am Nat* 140:762–780.
11. Sork VL, Nason J, Campbell DR, Fernandez JF (1999) Landscape approaches to historical and contemporary gene flow in plants. *Trends Ecol Evol* 14:219–224.
12. Weir BS, Hill WG (2002) Estimating F-statistics. *Annu Rev Genet* 36:721–750.
13. Slatkin M (1985) Gene flow in natural populations. *Annu Rev Ecol Syst* 16:393–430.
14. Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: Limitations, lessons and new directions. *Trends Ecol Evol* 13:202–206.
15. Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: F-ST not equal 1/(4Nm+1). *Heredity* 82:117–125.
16. Levin DA, Kerster HW (1974) Gene flow in seed plants. In *Evolutionary Biology* vol. 7, eds Dobzhansky T, Hecht MK, Steere WC (Plenum Press, New York), pp 139–220.
17. Ghazoul J (2005) Pollen and seed dispersal among dispersed plants. *Biol Rev* 80:413–443.
18. von Frisch K (1967) *The Dance Language and Orientation of Bees.*(Harvard Univ Press, Cambridge, MA).
19. Beekman M, Ratnieks FLW (2000) Long-range foraging by the honey-bee, Apis mellifera L. *Funct Ecol* 14:490–496.
20. Steffan-Dewenter I, Kuhn A (2003) Honeybee foraging in differentially structured landscapes. *Proc R Soc Lond Ser B* 270:569–575.
21. Gary NE (1971) Magnetic retrieval of ferrous labels in a capture-recapture system for honey bees and other insects. *J Econ Entomol* 64:961–965.
22. Kreyer D, Oed A, Walther-Hellwig K, Frankl R (2004) Are forests potential landscape barriers for foraging bumblebees? Landscape scale experiments with Bombus terrestris agg and Bombus pascuorum (Hymenoptera, Apidae). *Biol Conserv* 116:111–118.
23. Knight TM, *et al.* (2005) An interspecific comparison of foraging range and nest density of four bumblebee (Bombus) species. *Mol Ecol* 14:1811–1820.
24. Osborne JL, *et al.* (1999) A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. *J Appl Ecol* 36:519–533.
25. Lorch PD, Gwynne DT (2000) Radio-telemetric evidence of migration in the gregarious but not the solitary morph of the Mormon cricket (*Anabrus simplex*: Orthoptera: Tettigoniidae). *Naturwissenschaften* 87:370–372.
26. Janowski-Bell ME, Horner NV (1999) Movement of the male brown tarantula, *Aphonopelma hentzi* (Aranae, Theraphosidae), using radio telemetry. *J Arachnol* 27:503–512.
27. Riecken U, Raths U (1996) Use of radio telemetry for studying dispersal and habitat use of *Carabus coriaceus* L. *Ann Zool Fennici* 33:109–116.
28. Hedin J, Ranius T (2002) Using radio telemetry to study dispersal of the beetle. *Osmoderma eremita*, an inhabitant of tree hollows. *Comp Electron Agric* 35:171–180.
29. Wikelski M, *et al.* (2006) Simple rules guide dragonfly migration. *Biol Lett* 2:325–329.
30. Rink M, Sinsch U (2007) Radio-telemetric monitoring of dispersing stag beetles: implications for conservation. *J Zool* 272:235–243.
31. Popelka JC, Gollasch S, Moore A, Molvig L, Higgins TJV (2006) Genetic transformation of cowpea (*Vigna unguiculata* L.) and stable transmission of the transgenes to progeny. *Plant Cell Rep* 25:304–312.
32. Pasquet RS, Baudoin JP (2001) Cowpea. *Tropical Plant Breeding*, eds Charrier A, Jacquot M, Hamon S, Nicolas D (Science Publishers, Enfield), pp 177–198.

33. Coulibaly S, Pasquet RS, Papa R, Gepts P (2002) AFLP analysis of the phenetic organization and genetic diversity of. *Vigna unguiculata* L. Walp. reveals extensive gene flow between wild and domesticated types. *Theor Appl Genet* 104:358–366.
34. Anzenberger G (1977) Ethological study of African carpenter bees of the genus *Xylocopa* (Hymenoptera, Anthophoridae). *Z Tierpsychol* 44:337–374.
35. Dramstad WE, Fry GLA, Schaffer MJ (2003) Bumblebee foraging: Is closer really better? *Agric Ecosyst Environ* 95:349–357.
36. Visscher PK, Seeley TD (1982) Foraging strategy of honey bee colonies in a temperate deciduous forest. *Ecology* 63:1790–1801.
37. Meade DE (1991) Effective foraging ranges of feral colonies. *Am Bee J* 131:778.
38. Darvill B, Knight ME, Goulson D (2004) Use of genetic markers to quantify bumblebee foraging range and nest density. *Oikos* 107:471–478.
39. Breed MD, McGlynn TP, Sanctuary MD, Stocker EM, Cruz R (1999) Distribution and abundance of colonies of selected meliponine species in a Costa Rican tropical wet forest. *J Trop Ecol* 15:765–777.
40. Goulson D, Stout JC (2001) Homing ability of the bumblebee Bombus terrestris. *Apidologie* 32:105–112.
41. Chapman RE, Wang J, Bourke AFG (2003) Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators. *Mol Ecol* 12:2801–2808.
42. Batra SWT (1993) Opportunistic bumble bees congregate to feed at rare, distant alpine honeydew bonanzas. *J Kans Entomol Soc* 66:125–127.
43. Ohashi K, Yahara T (1998) Effects of variation in flower number on pollinator visits in Cirsium purpuratum (Asteraceae). *Am J Bot* 85:219–224.
44. Goulson D, Stout JC, Hawson SA, Allen JA (1998) Floral display size in comfrey, Symphytum officinale L. (Boraginaceae): Relationships with visitation by three bumblebee species and subsequent seed set. *Oecologia* 113:502–508.
45. Cresswell JE, Osborne JL (2004) The effect of patch size and separation on bumblebee foraging in oilseed rape: Implications for gene flow. *J Appl Ecol* 41:539–546.
46. Rawal KM (1975) Natural hybridization among wild, weedy and cultivated Vigna unguiculata (L.) Walp. *Euphytica* 24:699–707.