173. Bronstein, J L., Gouyon, P.-H., Gliddon, C., Kjellberg, F. and Michaloud, G, *Ecology*, 1990, **71**, 2145–2156.
174. Bronstein, J. L. and Patel, A., *Am J. Bot*, 1992, **79**, 41–48.
175. Milton, K., *J. Ecol*, 1991, **79**, 1–26
176. Milton, K, Windsor, D M., Morrison, D. W and Estribi, M A., *Ecology*, 1982, **63**, 752–762.
177. Anstett, M -C. and Michaloud, G., in preparation.
178. Janzen, D H., *Annu. Rev. Ecol. Syst.*, 1979, **10**, 13–51.
179. Baijnath, H and Ramcharun, S, *Bothalia*, 1983, **14**, 883–888.
180. Bronstein, J. L. and Patel, A., *Am. Midl. Nat.*, 1992, **128**, 397–403.
181. Khadari, B. *et al.*, in preparation.
182. Wilson, E. O., *The Diversity of Life*, W W. Norton & Co., New York, 1992, pp. 424.
183. Gadgil, M. and Vartak, V D., *J. Bombay Nat. Hist. Soc.*, 1975, **72**, 314–320.
184. Patel, A., in preparation.
185. Corlett, R. T., *Biotropica*, 1987, **19**, 122–124.
186 Bawa, K. S., *Annu. Rev. Ecol Syst.*, 1980, **11**, 15–39.
187. Corlett, R. T., *Malay Nature J.*, 1993 (in press).
188. Galil, J. and Eisikowitch, D., *New Phytol.*, 1974, **73**, 515–528.
189. Kjellberg, F., Abdellouahad, T. and Hossaert-McKey, M., in preparation.
190. Verkerke, W., *Experientia*, 1989, **45**, 612–622.
191. Galil, J. and Meiri, L., *New Phytol.*, 1981, **88**, 83–87.
192. Herre, E. A., *Science*, 1985, **228**, 896–898.
193. Kjellberg, F. and Abdellouahad, T., in preparation.
194. Galil, J. and Eisikowitch, D, *Tijd. Entomol.*, 1969, **112**, 1–18
195. Kjellberg, F. and Anstett, M -C., pers. commun.

ACKNOWLEDGEMENTS. This paper was made possible by an NSF Doctoral Dissertation Fellowship (BSR 9112319) to D. M. and A. P. and WWF fellowship No. 6854 to A. P.; by NSF grant No. INT 9218330 to D. M. and Judith Bronstein; and by a CNRS grant No. 93N92/0062 Co-operation CNRS/NSF to M. H.-M. and Finn Kjellberg. Travel to the Symposium on Pollination in the Tropics was made possible by a grant from the University of Miami Biology Department. Many of the ideas in section 'Biogeography of figs and sycophylous insects' of this paper were developed by J.-Y. Rasplus (INRA, Versailles) in a research proposal submitted to the Canopy Raft project of Fondation Canopée. We are very grateful to him for allowing us to benefit from his command of the literature on sycophilous insects, and to detail his as yet unpublished ideas. We thank the Karnataka State Forest Department and its officials who provided research permits to A. P., and to Neelamma Chittiappa and family for their tremendous hospitality. Thanks to M.-C. Anstett, A. Chenuil, R Gunther, and F. Kjellberg for comments on the manuscript. We are grateful to M -C. Anstett, B. Khadari, A. Ware and S. Compton for permission to cite unpublished manuscripts. This paper is Contribution No. 404 from the Program in Tropical Biology, Ecology, and Behavior of the Department of Biology, University of Miami.

Coevolution of plants and animals: Pollination of flowers by primates in Madagascar

W. John Kress

Department of Botany, NHB-166, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA

Observations on the floral biology of the traveller's tree (*Ravenala madagascariensis*) and the ecology of the ruffed lemur (*Varecia variegata*), both endemic to the island of Madagascar, suggest a closely coevolved plant–pollinator relationship. The plant has many specializations for flower visitation by large non-flying animals and the lemurs appear to be dependent on nectar as a food source during specific times of the year. The basal phylogenetic position of *Ravenala* in the family Strelitziaceae, which diversified in the late Cretaceous and early Tertiary, is consistent with the hypothesis that pollination by lemurs is an archaic system in the family whereas bird- and bat-pollination systems are derived in the more advanced genera. These results provide supporting data for earlier hypotheses on the evolution of non-flying mammals as ancient pollinators of flowering plants.

THE origin and explosive diversification of flowering plants during the Cretaceous is often attributed to the simultaneous radiation of insects, which served as critical pollen-dispersal agents[1]. Vertebrates are thought to have played a negligible role in the early evolution of angiosperms because the main groups, which are important pollinators of extant plant taxa (e.g. birds and bats), originated no earlier than the Eocene[2,3]. However, non-flying mammals, such as marsupials and primates which are known nectar feeders, appeared as early as the middle Cretaceous and Paleocene[3–5]. Sussman and Raven[4] have suggested that these non-flying mammals could have been significant pollinators since the uppermost Cretaceous, but were outcompeted in the Tertiary by nectar-feeding birds and bats. Any coevolved relationships between flowering plant species and non-flying mammal pollinators that persist at the present would appear to be '…"living fossils", which have a great deal to tell us about the evolution of both the mammals, including some of our own antecedents, and of the flowering plants'[4].

Many non-flying mammals, such as rodents[5], genets[6], tree shrews[7], procyonids[8], marsupials[5], primates[8–13], and even giraffes[14] feed on nectar or flowers. Among the primates, tamarins (Callitrichidae[8]), monkeys (Cebidae[8-10]), bush babies (Lorisidae[9]), and lemurs (Cheirogaleidae and Lemuridae[4,11-13,15-18]) have been suggested as possible pollinators of angiosperms. However, most reports of flower-visiting by non-flying mammals have shown that the animals destroyed the flowers during feeding and only a very few[5,13] have provided sufficient evidence of actual effective pollination. The investigations reported here provide new data which show (i) that lemurs play a significant role in the pollination of at least one plant species in Madagascar, and (ii) that this association is most likely an archaic, rather than a recently derived coevolutionary system.

Plant morphology and ecology

Investigations were carried out along the northeastern coast of Madagascar in primary wet forest on the island of Nosy Mangabe (15° 30′ S, 49° 46′ E) in the Bay of Antongil. During the rainy season in February 1992 observations were made on the floral biology and flower visitors to *Ravenala madagascariensis* Sonn. (Strelitziaceae: Zingiberales: Monocotyledonae) at Nosy Mangabe where *Ravenala* is a relatively common forest species often found in light gaps. *Ravenala*, called the traveller's tree, is a monotypic genus common at elevations below 1000 m in regions of Madagascar with significant rainfall[19] (Figure 1). It occurs in both primary rain forest and open secondary growth, the former being its probable natural habitat before the influence of man allowed it to invade large tracts of disturbed forest. The flowers of *Ravenala* are borne in large, bracteate inflorescences (to 85 cm in height) which are situated below the crown of banana-like leaves in the axils of the leaf bases. At anthesis the large flowers (Figure 2) are filled with copious ($x = 8.6 \pm 2.7$ ml; $n = 14$), concentrated ($x = 11.8\% \pm 1.2$; $n = 13$), sucrose-dominant ($s/g+f = 1.50 \pm 0.213$; $n = 14$) nectar, which is continuously secreted by each flower for about 24 hours. A typical inflorescence of *Ravenala* will produce an average of 5.2 open flowers per day for five weeks which is equivalent to providing 21.7 kcal of energy per day for a total of 759 kcal over the life of the inflorescence.

Animal behaviour

Observations in 1992 on Nosy Mangabe concentrated on a hillside population of 44 individual plants of *Ravenala* that had a total of 71 inflorescences; four inflorescences on three plants were more critically observed for pollinator activity over six days (59 hours total). Three species of lemur were seen visiting the flowers. *Eulemur fulvus albifrons* (Lemuridae: Primates), the white-fronted lemur, and *Cheirogaleus major* (Cheirogaleidae: Primates), the greater dwarf lemur, were only occasional visitors during the day and night, respectively. Black and white ruffed lemurs, *Varecia variegata variegata* (Lemuridae: Primates), were the primary and dominant animals observed to repeatedly visit the flowers between dawn and sunset (Figures 3,4). During the 40 hours of diurnal observation, individuals of *Varecia* made 57 visits to the four inflorescences under watch (an average of 1.33 visits/h) for a total of 333 mins. A individual lemur can spend up to seven minutes on an inflorescence depending on the number of open flowers. The animals, which clearly carried pollen on their fur, frequently moved between flowers in the same inflorescences, between inflorescences on the same plant and between plants. We saw no other vertebrates or invertebrates frequently visit the flowers of *Ravenala* on Nosy Mangabe. Both pteropodid bats (Pteropodidae) and sunbirds (Nectariniidae) were observed or have been reported from the area[20,21]; however, only the latter occasionally visit these flowers.

Between July 1987 and January 1989 an independent 11-month field investigation was conducted at the same locality on Nosy Mangabe and focused specifically on the social organization and ecology of the black and white ruffed lemur, *Varecia variegata variegata*[22]. This study included 1560 hours of observations of 26 individual animals and showed that the diet of the ruffed lemur consisted mainly of fruit and nectar supplemented by leaves and other items from at least 67 plant species. Nectar was the second most important food item in the diet and on the average accounted for about 22% of feeding time. Two plant species provided the main supply of nectar: *Ravenala madagascariensis* and *Labramia costata* (Sapotaceae). When the latter was in flower, *Varecia* spent more time (74% of feeding records) taking nectar from these flowers than feeding on the fruits or leaves of any other plant species. Complete data on the dietary importance of *Ravenala* nectar for *Varecia* were not available because the total flowering season of this plant was not monitored. However, the results of this study in Madagascar strongly support the 1992 observations that the ruffed lemur is a common visitor to *Ravenala* and other plants which provide nectar for food.

Evidence for pollination

Supporting evidence to prove that an animal is actually pollinating a plant, i.e. transporting viable pollen between flowers that leads to fertilization and seed production, is usually indirect. In the case of *Ravenala*, the ruffed lemurs: (i) consistently and almost exclusively visited the flowers; (ii) visibly carried pollen on their fur between flowers of the same plant as well as between flowers of

Figures 1–4. **1,** Overall aspect of *Ravenala madagascariensis* bordering primary forest west of Irondro, Madagascar (21°23′19″S, 47°56′53″E; voucher: *Kress et al., 92–3177*). The national tree Madagascar, 'ravinala' (Malagasy common name literally meaning 'leaf of the forest') is a tall, single-stemmed or suckering tree with a palm-like trunk that holds the large banana-like leaves high in the forest canopy to heights of 30 m. Note distichous leaf arrangement and position of inflorescences below leaf crown in axils of leaf bases. **2,** Part of inflorescence of *Ravenala* with bract cut away to show large open flower at anthesis. The inflorescence includes from five to 15 strong, congested, protective bracts which enclose 10 to 16 flowers each. A flower (ca. 19 cm in length) consists of an inferior ovary, perianth tube, three yellowish white sepals fused at their base to the perianth tube, three white petals of which two are fused along their margins, six pollen-bearing stamens that are enclosed by the two fused petals, and a stiff, rod-like style terminated by a broad ovoid stigma. The anthers are held under tension inside the perianth which remains closed at anthesis and must be forcibly opened by the animal visitors. Anthers are dehisced at the time of flower opening. Flowers are open both during the day and night but begin to oxidize and turn brown 24–36 hours after anthesis. **3,** A black and white ruffed lemur, *Varecia variegata variegata*, arriving at inflorescence of *Ravenala* in primary forest at Nosy Mangabe. Ruffed lemurs are three to four kg strepsirhines found only in the eastern rain forests of Madagascar. Foraging ruffed lemurs approached the inflorescences of *Ravenala* from the canopy of neighbouring trees. They quickly found unopened or previously opened flowers that were producing nectar. **4,** *Varecia* taking nectar from *Ravenala* with snout thrust inside of open flower. To open a newly emerged flower, the lemur grasped the unopened perianth with its teeth and roughly pulled it from the protective inflorescence bract, but did not break it off. This action sprang the perianth open and released the anthers held under tension which then brushed pollen onto the muzzle and head of the animal. While holding onto other bracts of the inflorescence with its hindfeet, the lemur pulled the lateral sepals apart with one or both forefeet to allow access to the nectar chamber. The snout was then thrust into the center of the flower and nectar was extracted with the tongue. The lemur contacted both the stamens and stigma while feeding.

conspecific plants; (iii) did not destroy the flowers while obtaining the nectar; and (iv) appeared to be highly dependent on nectar as a food source during specific times of the year. Furthermore the plants themselves possess many obvious specializations for visitation by large non-flying animals, such as: (i) inflorescences placed below the crown of the plant making them easily accessible to arboreal animals; (ii) large flowers enclosed in tough, protective bracts that require manual manipulation by a strong pollinator to be opened; (iii) stiff, rod-like styles that withstand the rough handling of the visitors; and (iv) copious, sucrose-dominant nectar that provides an energy-rich, renewable reward for a sizable animal. I believe that these results provide sufficient evidence that this plant species endemic to Madagascar has coevolved with an endemic group of non-flying mammals, the lemurs, as its primary pollinator.

An archaic coevolutionary system?

The proposal that pollination by non-flying mammals is an archaic system which has only persisted in areas of the world where more recently evolved, volant floral visitors are less prominent[4] has as yet not been substantiated. However, the investigations of *Ravenala* directly address this question. *Ravenala* is one of three genera in the bird-of-paradise family, the Strelitziaceae[23]. The other two genera are *Strelitzia*, endemic to southern Africa, and *Phenakospermum*, found throughout tropical South America east of the Andes. Recent investigations of the phylogenetic relationships of the three members of the Strelitziaceae based on gene sequence data[24] present strong evidence that *Ravenala* is the most basal lineage in the family (Figure 5). Although no fossils of the Strelitziaceae have been reported, taxa representing some of the more advanced (e.g. Zingiberaceae) as well as the more primitive (e.g. Musaceae) families of the order Zingiberales are known from the late Cretaceous and early Tertiary[23,25]. It is therefore likely that the Strelitziaceae, a primitive family closely related to the Musaceae, had also originated during the Cretaceous.

Studies on the reproductive biology of the other two genera of the Strelitziaceae have shown that *Strelitzia* is pollinated by sunbirds[26-28] and *Phenakospermum* by phyllostomid bats[29] (Figure 5). These data on pollinators of the three extant genera coupled with the hypothesis on their phylogenetic relationships indicate that the most basal and ancient lineage, represented today by *Ravenala*, has reproductive features suitable for pollination by non-flying mammals, in this case lemurs. Although evidence is meagre, the primate lineage containing lemurs most likely diversified from East African relatives in the early to mid-Tertiary[30,31], which is roughly equivalent to the estimate of the origin of the Strelitziaceae. It is therefore suggested that some ancestor of the lemurs or other early mammal lineage present in Africa during the late Cretaceous or early Tertiary may have been the visitors to flowers of primitive members of this plant family.

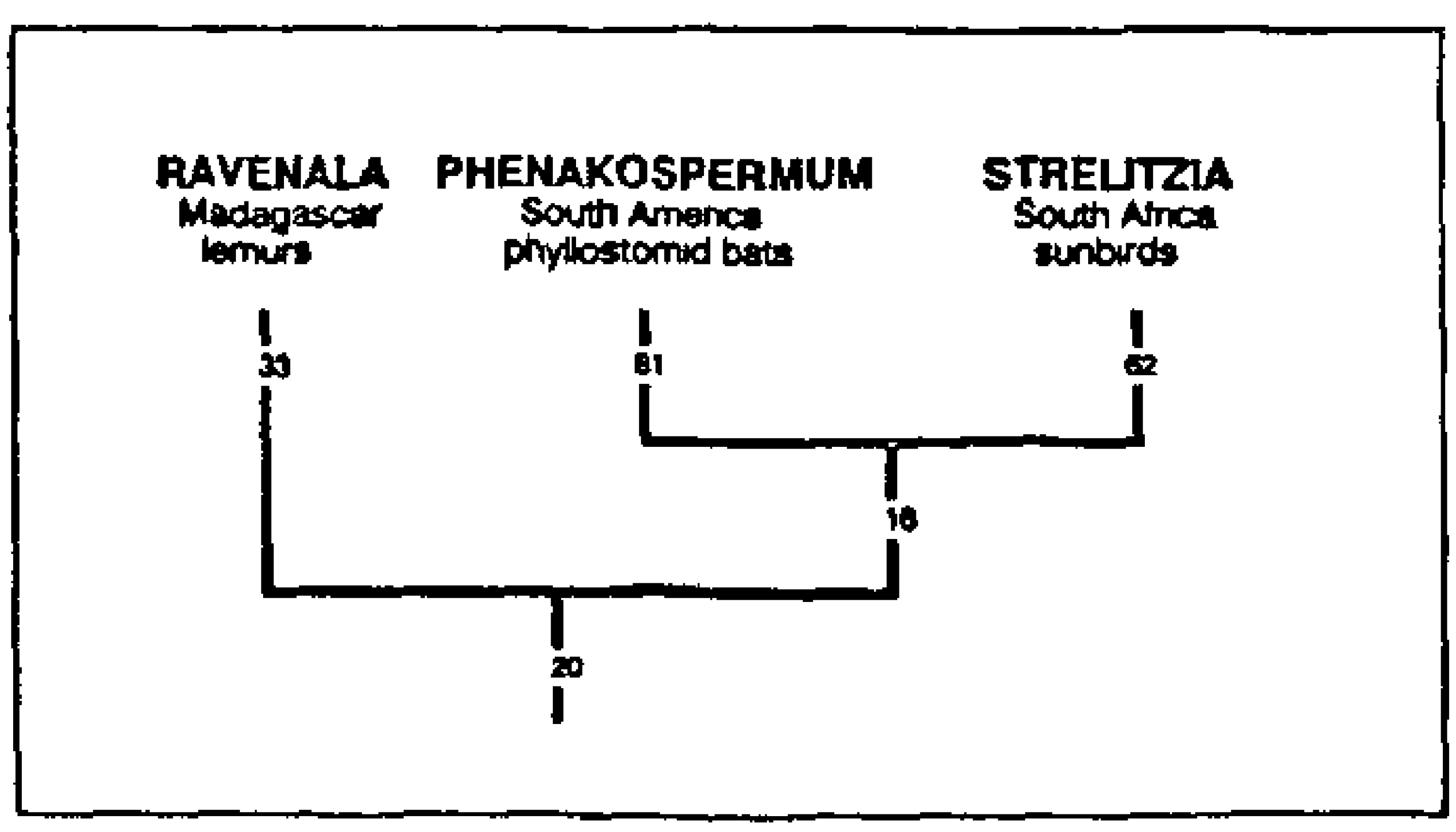

Figure 5. Cladogram representing the phylogenetic relationships of the three genera of the Strelitziaceae. Tree derived from maximum parsimony analysis of sequence data of the chloroplast *rbcL* gene[24]. Numbers along branches indicate nucleotide base pair apomorphies that support each clade. Geographic distributions and primary pollinators for each genus are given under the generic names.

Conclusions

The results of these investigations of *Ravenala* and the Strelitziaceae are consistent with the hypothesis that pollination by non-flying mammals is archaic[4], at least in this family of flowering plants. In the Strelitziaceae, pollination by non-flying mammals, now represented in Madagascar by lemur pollination of *Ravenala*, appears to have persisted in an isolated geographic region that remains depauperate in significant flying vertebrate floral visitors. The two more advanced lineages of the family radiated into areas where birds (i.e. *Strelitzia* in Africa) and bats (i.e. *Phenakospermum* in South America) coevolved with the plants as pollinators.

1. Crepet, W. L. and Friis, E. M., in *The Origins of Angiosperms and Their Biological Consequences* (eds. Friis, E. M., Chaloner, W. G. and Crane, P. R.), Cambridge Univ. Press, Cambridge, 1987, pp. 181–201.
2. Baker, H. G. and Hurd, Jr., P. H., *Annu. Rev. Entomol.*, 1968, **13**, 385–414.
3. Novak, R. M., *Walker's Mammals of the World*, 5th edn., Johns Hopkins Univ. Press, Baltimore, 1991.
4. Sussman, R. W. and Raven, P. H., *Science*, 1978, **200**, 731–736.
5. Rourke, J and Wiens, D., *Ann. Missouri Bot. Gard.*, 1977, **64**, 1–17.
6. Lack, A., *E. Afr. Wildl. J.*, 1976, **15**, 233–234.
7. Nur, N., *Ann. Bot.*, 1976, **40**, 167–177.
8. Janson, C. H., Terborgh, J. and Emmons, L. H., *Biotropica*, 1981, **14** (Suppl.), 1–6.
9. Coe, M. J. and Isaac, F. M., *E. Afr. Wildl. J.*, 1965, **3**, 123–124.
10. Garber, P. A., *Biotropica*, 1980, **20**, 100–106.
11. Tattersall, I. and Sussman, R. W., *Anthropolog. Pap. Am. Mus. Nat. Hist.*, 1975, **52**, 193–216.
12. Hladik, C. M., Charles-Dominique, P. and Petter, J. J., in *Nocturnal Malagasy Prosimians* (eds. Charles-Dominique, P., *et al*), Academic Press, New York, 1980, pp. 41–73.
13. Overdorff, D. J., *Am. J. Primatol.*, 1992, **28**, 191–203.
14. du Toit, J. T., *Afr. J. Ecol.*, 1990, **28**, 63–68.
15. Hladik, C. M. and Charles-Dominique, P., in *Prosimian Biology* (eds. Doyle, G. A., Martin, R. D. and Walker, A. C.), Duckworth, London, 1974, pp. 23–37.
16. Petter, J. J., in *Recent Advances in Primatology* (eds. Chivers, D. J. and Herbert, J.), Academic Press, New York, 1978, vol. 1, pp. 211–223.
17. Hladik, C. M., in *The Study of Prosimian Behavior* (eds. Doyle, G. A. and Martin, R. D.), Academic Press, London, 1979, pp. 307–358.
18. Petter, J. J., Schilling, A. and Pariente, G., *Terre Vie*, 1971, **25**, 287–327.
19. Perrier de la Bathie, H., in *Flore de Madagascar et des Comores* (ed. Humbert), Imprimerie Officielle, Tananarive, 1946, 46e famille, pp. 4–6.
20. Langrand, O., *Guide to the Birds of Madagascar*, Yale Univ. Press, New Haven, 1990.

21. Jenkins, M. D. (ed.), *Madagascar, An Environmental Profile*, IUCN, Gland, Switzerland and Cambridge, UK, 1987.
22. Morland, H. S., Ph.D. thesis, Yale University, 1991.
23. Kress, W. J., *Ann. Missouri Bot. Gard.*, 1990, 77, 698–721.
24. Smith, J. F., Kress, W. J. and Zimmer, E. A., *Ann. Missouri Bot. Gard.*, 1993 (in press).
25. Manchester, S. R. and Kress, W. J., *Am. J. Bot.*, 1993 (in press).
26. Scott-Elliot, G. F., *Ann. Bot.*, 1890, 4, 259–263.
27. Darwin, C., *The Effects of Cross and Self-Fertilization in the Vegetable Kingdom*, D. Appleton & Co., New York, 1895, pp. 371.
28. Frost, S. K. and Frost, P. G., *Oecologia (Berl.)*, 1981, 49, 379–384.
29. Kress, W. J. and Stone, D. E., *Biotropica*, 1993 (in press).
30. Szalay, F. S. and Delson, E., *Evolutionary History of the Primates*, Academic Press, New York, 1979.
31. Savage D. E. and Russell, D. E., *Mammalian Paleofaunas of the World*, Addison-Wesley Publ. Co, Reading, Mass., 1983.

ACKNOWLEDGEMENTS. This invited paper was extracted from a more extensive article that has been submitted to the *American Journal of Botany*. I thank George E. Schatz, Michel Andrianifahanana, and Sally Love for critical help in the field in Madagascar. Hilary Simons Morland generously provided unpublished data from her doctoral dissertation. This work was in part supported by grants from the Research Opportunity Funds and Scholarly Studies Program of the Smithsonian Institution.

Factors affecting pollinator visitation rates: A biogeographic comparison

Richard B. Primack and David W. Inouye*

Biology Department, Boston University, 5 Cummington Street, Boston, MA 02215, USA
* Department of Zoology, University of Maryland, College Park, MD 20742, USA

Careful observations of flower visitors can provide estimates of pollinator visitation rates. Biological communities in varying biogeographic regions were compared using the same techniques. Bees were the predominant pollinators at low elevation sites, whereas flies were the most important pollinators at high elevations. Flower visitation rates varied by a factor of 10 across biological communities, suggesting that pollination activity may be limiting seed production in some places. Visitation rates were highly variable within communities, with temperature, wind speed, and time of the season explaining some of the variation. A picture emerges of relatively unspecialized relationships among the plants and animals involved in pollination, influenced by a variety of environmental and ecological factors.

CAREFUL observations of flowers and their visitors can yield information on the number of visits a flower receives in a certain time period, the *pollinator visitation rate*. Analysis of pollinator visitation rates can give valuable insight into the interaction between plants and their pollinators for those plants that require the services of pollinators to produce seeds. If visitation rates to such species are consistently low, the plants may have low seed production or even be threatened with extinction. Similarly, cultivated plants that need pollinators to produce a seed and fruit crop may have a low crop yield if pollinator visitation to the flowers is low. In the face of such strong selective pressure, flowering plants have evolved a variety of traits that help to attract pollinators, such as nectar production and abundant pollen, or, in some cases, mechanisms for self-pollination and setting seeds in the absence of pollinators. Because most plant species do not grow in isolation, but in a community of potential competitors for the services of pollinators, visitation rates may serve as an indicator of their success in competing with other species for pollinators. Thus, comparisons of visitation rates among sites may shed light on differences in selective pressures among populations or species[1]. Such comparisons can also highlight factors that limit plant reproduction in particular environments.

Another reason for measuring visitation rates is that they can provide insight about temporal variation in availability of pollinator species, either within or among seasons. Data on visitation rates can help in testing hypotheses such as, for instance, that visitation rates to flowers in the early spring are lower than visitation rates later in the growing season (e.g. ref. 2). Measurements of seed set may also provide similar information, but would not give any details about either the species of pollinators or mechanisms involved. Measurements of visitation rates can profitably identify the linkages among pollinators, plants, and subsequent seed set.

One potential pitfall in measurements of visitation rates is the fact that flower visitation may not necessarily translate into transfer of pollen onto the stigma of a flower. Careful observation or even microscopic examination may be required to confirm that a flower visitor is in fact a pollinator, although it is usually fairly obvious when species are foraging as to