References and Notes

1. J. D. Davis and C. S. Campbell, *J. Comp. Physiol. Psychol.* **83**, 379 (1973).
2. C. L. Hull, J. R. Livingston, R. O. Rouse, A. N. Barker, *ibid.* **44** 236 (1951); D. G. Mook, *ibid.* **56**, 645 (1963); H. D. Hanowitz and M. I. Grossman. *Am. J. Physiol.* **159**, 143 (1949); I. Share, E. Martyniuk, M. I. Grossman, *ibid.* **169**, 229 (1952).
3. C. T. Snowdon, *J. Comp. Physiol. Psychol.* **71**, 68 (1970).
4. R. C. Young, J. Gibbs, J. Antin, J. Holt, G. P. Smith, *ibid.* **87**, 795 (1974).
5. J. A. Deutsch and M.-L. Wang, *Science* **195**, 89 (1977).
6. This valve was made of a Plexiglas tube (1/8 inch inside diameter), which was attached to the implanted stomach tube, bent so as to spill over when the fluid in the vertical tube reached a predetermined height. To prevent the possibility of a siphoning effect, a perforation was made at the top of the bend in the pressure tube.
7. During this initial 20-minute period, the rats drank a mean of 25.8 ml [standard deviation (S.D.) = 4.57] of milk while the cuff was inflated. During the initial 20-minute period of experiment 3, they drank a mean of 24.8 ml (S.D. = 6.7) with cuff uninflated.

8. This work was supported by Academic Senate Grant R-B10 to J.A.D. We thank M. Hayhoe and N. Y. Walton for help with the statistical treatment of the data and Dr. N. M. White for helpful comment.

12 December 1977

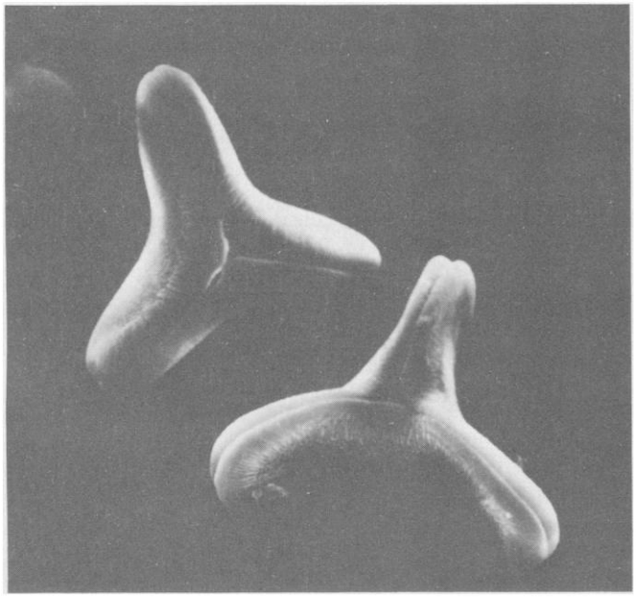
Avian Pollination Studies: A Simple Scanning Electron Microscopic Technique

Abstract. *Identifying ornithophilous plant species utilized by several different flower-visiting birds is simplified by the scanning electron microscope. The technique involves comparing pollen samples taken from the birds' head feathers with pollen reference standards collected in the same area, which simplifies analysis of pollination patterns in a complex community.*

Pollination patterns of avian flower visitors, especially in tropical montane forests, are difficult to study and quantitate by simple observation. We attempted to solve this problem by collecting the pollen carried by hummingbirds and a flower-piercer and identifying the samples with the aid of the scanning electron microscope (SEM). Pollination patterns may be inferred from such data.

Insect (*1*) and bat (*2, 3*) pollination have been studied by analyzing the pollen actually carried by the organisms, but optical microscopy was used in those studies. Our own preliminary attempts to identify pollen with light microscopy proved tedious, and the large quantities of pollen required precluded studying the pollen actually carried by a pollinating bird. However, the small amount of pollen remaining on a bird collected by shooting or mist-netting can easily be retrieved on a small (12 mm^2) piece of double-stick tape. This can be stored in a dust-proof vial, where it will dry spontaneously within a few days, and later prepared for SEM study by coating it with gold. This tape-collecting method is also desirable because the tape can be quickly monitored in the field with a compound microscope, although proper identification of pollen types is obviously impossible at that stage. Study of these pollen collections by SEM makes it possible in most cases to identify the plant or plants the bird visited by comparing the pollen from the bird with pollen standards collected from ornithophilous plants found in the bird's feeding area.

We tested this technique on five species of avian flower visitors in a second-growth montane forest called Yanasacha, near Cuenca, Ecuador (altitude approximately 3000 m). All of the birds collected were hummingbirds except for one masked flower-piercer, *Diglossa cyanea*, which is primarily a nectar robber and a poor pollinator. The pollen grains collected were easily identifiable in the case of the hummingbirds (Figs. 1 and 2), as if they had been shaken directly from the flower. The grains collected from the *Diglossa*, although identifiable, were coated with nectar, which tended to obscure some surface detail and made identification more difficult. The longer bills of the hummingbirds seemed to keep the pollen more nectar-free. The SEM technique is probably less useful for studies of *Diglossa* and related groups because these nectar robbers usually do not become as well dusted with pollen as the trochilids; in fact, the only pollen found on *D. cyanea* was that of *Palicourea aragmatophylla* K. Schum & Kraus, a member of the Rubiaceae whose androecium has very short filaments and hence anthers proximal to the insertion site of the *Diglossa*'s bill. Generally, ornithophilous plants have long filaments, and thus



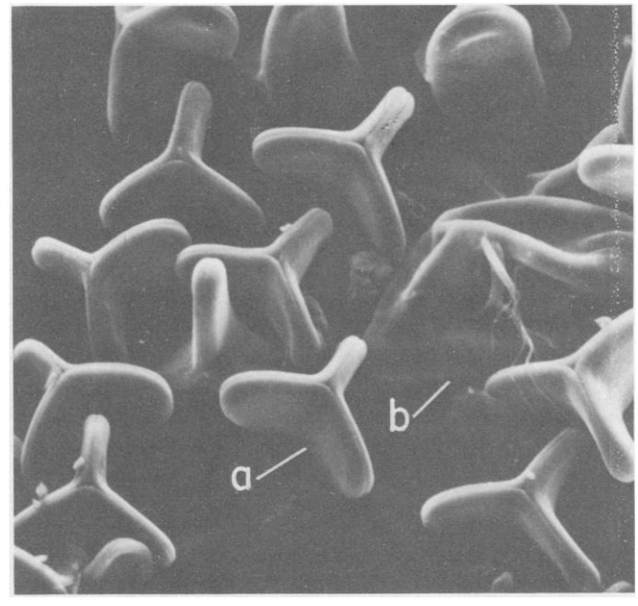


Fig. 1 (left). Pollen standard, *Phrygilanthus longebracteatus* (Desr.) Macbr. (Loranthaceae) (×1000). Fig. 2 (right). Pollen collected from head feathers of *Coeligena iris* (Trochilidae), showing pollen from (a) *Phrygilanthus longebracteatus* and (b) *Fuchsia* sp. (×500).

0036-8075/78/0714-0167$00.50/0

their pollen is not likely to be picked up by flower piercers.

Although our primary purpose was to test SEM analysis for effectiveness, we were able to make some preliminary observations. In all samples one pollen species was in great predominance (90 to 100 percent), possibly reflecting the floral species constancy already known in some hummingbirds' feeding patterns (*4*). Neotropical nectarivorous bats, however, are thought not to display much feeding constancy (*3*).

The advantage of the high resolution of SEM is obvious and was recently shown (*5*) to distinguish easily between the pollen of two species of the genus *Crescentia*, which were difficult to tell apart with a light microscope. Also, the technique previously used to study vector-borne pollen depended on staining the pollen grains. Difficulties in interpretation due to differential uptake of stain by different pollen types was a complicating factor (*1*). Since our SEM technique does not require staining, this variable is eliminated. Finally, the SEM is useful since it is simple to carry out statistical analyses of the bird's feeding behavior and pollination efficiency by scanning the samples even though the bird may carry seemingly invisible amounts of pollen. The double-stick tape loads consistently and completely with pollen, providing an even, rapidly analyzable sample of statistically useful size (1000 to 2000 grains) even when limited quantities of pollen are present. We hope that this technique will be useful in studies of other pollinators, especially those carrying relatively small amounts of pollen.

JEF D. BOEKE
Rockefeller University, New York 10021
FERNANDO I. ORTIZ-CRESPO
Departamento de Biología, Universidad Católica del Ecuador, Quito

References and Notes

1. A. J. Beattie, *Pan Pac. Entomol.* **47**, 82 (1971).
2. E. R. Heithaus, P. A. Opler, T. H. Fleming, *Ecology* **55**, 412 (1974).
3. E. R. Heithaus, T. H. Fleming, P. A. Opler, *ibid.* **56**, 841 (1975).
4. M. Proctor and P. Yeo, *The Pollination of Flowers* (Taplinger, New York, 1972), p. 323; F. Ortiz-Crespo, *Ibis* **116**, 347 (1974).
5. A. Gentry and S. Tomb, in preparation.
6. We thank Drs. J. L. Howland and W. L. Steinhart for use of the SEM. Supported by a Thomas J. Watson Fellowship to J.D.B.

25 January 1978; revised 28 March 1978

Evoked Response Correlate of Symbol and Significate

Abstract. *Changing the source and intensity of the auditory signal to six trained cats responding to meaningful auditory stimuli permits exogenous and endogenous processes in the auditory evoked potential to be separated. For short-latency exogenous processes, latency and amplitude depend on the parameters of the physical stimulus. However, the amplitude and shape of longer-latency endogenous processes are essentially independent of the location and intensity of the signal source and seem to be invariant concomitants of the significance of the signal.*

In a series of papers (*1*), we have presented evidence that the evoked potential (EP) recorded from trained cats is a composite of exogenous processes reflecting afferent sensory input and endogenous processes reflecting central reactions to such input. The results of a wide variety of controls devised to rule out the possibility that the observed endogenous processes were of unspecific origin established that they (i) were not a function of type or level of motivation; (ii) were observed in a variety of different instrumental tasks; (iii) were elicited by visual, auditory, or direct electrical stimulation of brain structures; (iv) appeared in the absence of any visible changes in head or body position or orientation to the stimulus revealed by cinegraphic analyses; (v) were not a reflection of changes in receptor sensitivity or stimulus intensity; (vi) and were not related to response bias or the intention to perform a particular set of motor responses.

In view of this evidence that endogenous processes could not plausibly be attributed to unspecific factors, we concluded that they probably reflected the release of neuronal activity representing the activation of specific memories about the meaning of the afferent input. However, reports in the literature indicated that marked changes in EP waveshapes in untrained animals could result from changes in the spatial relationship between stimuli and exteroceptors (*2*). Reservations about our interpretation would not be eliminated merely by our failure to observe changes in stimulus-receptor geometry when changes in EP waveshape occurred, because our control methods for such effects might have been inadequately sensitive. A better answer to such reservations could be obtained by devising an experimental procedure in which changes in the spatial relationship between exteroceptors and conditioned stimuli with specified meaning were deliberately imposed while we sought invariant features of the endogenous processes. We now report the result of such an experiment.

Six cats, each having 34 electrodes permanently implanted into various brain regions, were differentially trained in a series of stages to obtain food in an L-shaped runway (*3*). Initially the animals were trained to make a free operant response, leave the elevated platform (start), descend to the runway, and proceed to the feeder (goal) at the far end of the L, where a morsel of raw meat was delivered from an automatic turntable if the cat stepped up and touched it with its paw. After learning the free operant response (oscillation, back and forth, from start to goal), the animals were given discrimination training in which food at the goal became contingent upon the presence of a positive conditioned stimulus (CS+)—a click train presented at the rate of two clicks per second (2 sec^{-1}), the "go" stimulus. After acquiring the discriminative performance, the animals were given differential training in which a negative conditioned stimulus (CS−), a 5 sec^{-1} click train, required the animal to "stay" on the starting platform. During both discrimination and differentiation, the animals were required to return to the starting platform and sit down before the next trial began.

Daily sessions of 25 to 50 trials of randomly intermixed CS+ and CS− were run on weekdays after 24 hours of food deprivation. Trials were presented at random intervals averaging 2 minutes. Animals were fed freely on Saturday.

Evoked potentials (EP's) were recorded on magnetic tape during each session, and videotape records were made of each trial. Cross-trial running average EP's were obtained by averaging the first through third, second through fourth, third through fifth (and so forth) responses on all CS+ trials resulting in correct performance.

During training, all stimuli (*4*) were delivered from loudspeaker 1, mounted behind the cat above the starting platform. The secondary component structure of the EP became dramatically altered as the CS+ acquired significance during three stages of training that preceded differential training (Fig. 1). All data are from the medial geniculate bodies because much of our previous data collected in response to visual rather than auditory stimuli showed that both exogenous and endogenous representational pro-

0036-8075/78/0714-0168$00.50/0