Table 1. Frequency of color forms (percent) in a fossil population of *Limicolaria martensiana*, and a comparison with living populations. Streaked and pallids 1 to 3 have been figured and described (*2*). Broken-streaked resembles streaked, but throughout the length of the shell there is a pallid band breaking up the streaks. The ground color of the shells of living snails varies from pale yellow to deep orange and the streaks (where present) are dark brown. The ground color of fossil snails is white and the dark brown markings are faded to pale brown. Kayanja is 11 km from Kabazimu Island, Ishasha Road 16 km, and Rwenshama 30 km. *N*, number.

Color form	Fossil	Living		
	Kabazimu Island (N= 1277)	Kayanja (N= 2840)	Ishasha Road (N= 882)	Rwenshama (N= 841)
Streaked	61.0	54.6	40.6	33.7
Broken-streaked	5.2	8.9	4.2	9.8
Pallid 1	3.9	24.0	7.0	1.9
Pallid 2	28.3	11.9	43.2	37.3
Pallid 3	1.6	0.6	5.0	17.3

from the Katwe explosions. The shells are therefore of the same age as those described from Kichwamba and Equator Road (*3*). They are in large concentrations in the paleosol, and a sample of 1277 was collected and classified into color forms (Table 1).

All the major color forms known from *L. martensiana* occur in the Kabazimu Island fossils and, as in most living populations, the streaked form is the most frequent. Twenty-four living populations of *L. martensiana* were sampled from the same general area of the Western Rift (there are no living snails on Kabazimu Island itself), but only three of these populations resemble the fossil one in the presence of all major color forms, and, in particular, in the presence of broken-streaked, which is an exceedingly local form throughout the range of *L. martensiana* in Uganda. As shown in Table 1, there are differences in the frequencies of the forms between those of Kabazimu Island and the three living populations, and also among the three living populations themselves. Such differences are a feature of polymorphism in *L. martensiana*, and because of them it is not possible to demonstrate evolutionary changes on the basis of past and present frequencies. On the other hand the Kabazimu Island fossils show that five forms of *L. martensiana* are at least 8,000 to 10,000 years old; the fossils do not contain forms that no longer exist. Evolutionary trends in the past 8,000 to 10,000 years have undoubtedly been in repeated adjustment of the frequencies of the forms to local conditions with occasional extermination or spread of a form into a new population and with periods of relative stability.

D. F. Owen
Department of Zoology, Makerere University College, Kampala, Uganda

References and Notes

1. The taxonomy of the tropical African *Limicolaria* is in need of revision, and the name *martensiana* must be regarded as tentative.
2. D. F. Owen, *Proc. Zool. Soc. London* **144**, 361 (1965).
3. ———, *Nature* **199**, 713 (1963).
4. W. W. Bishop, in *Proceedings of the Fourth Pan African Congress on Prehistory*, Leopoldville, 1959 (published 1962), p. 245.

17 January 1966

Analysis of Complex Vascular Systems in Plants: Optical Shuttle Method

Abstract. *The "optical shuttle" is an improved method of photographing with a motion-picture camera, one by one, sequential sections mounted on separate slides. The optical output from two microscopes is combined so that the images can be focused on a single film plane. Slides are photographed alternately through the microscopes. Simultaneous viewing of two successive sections in the microscopes enables initial precise alignment of images. Complex anatomical structures can thus be analyzed with relative ease.*

Recently we have shown that, by photographing with a motion-picture camera, frame by frame, subsequently cut surfaces or serial microtome sections of palm stems, it is possible to analyze a vascular system of far greater complexity than is possible with serial sections alone or with serial photographic records. The method has been used to analyze in quantitative detail the course of vascular bundles in the stem of a small palm, *Rhapis excelsa* (Thunb.) Henry (*1*).

The original apparatus (Fig. 1) incorporates a drawing tube (camera lucida) into the microscope-camera system (*2*). An outline sketch of the section, on a fixed piece of paper, serves to orient in turn a number of succeeding sections in the sequential series so that a fixed point successively occupies the same position in each frame of the film. New drawings have to be made at intervals as alignment gradually deteriorates. Drawings, which include coordinates of photographed positions at regular intervals, are filed as useful references, but essentially all information is "stored" in the film and "retrieved" later by projecting the film in a photo-data analyzer. Films run at 16 to 24 frames per second and can be used to demonstrate essential features to large audiences (*3*); their application in teaching is obvious.

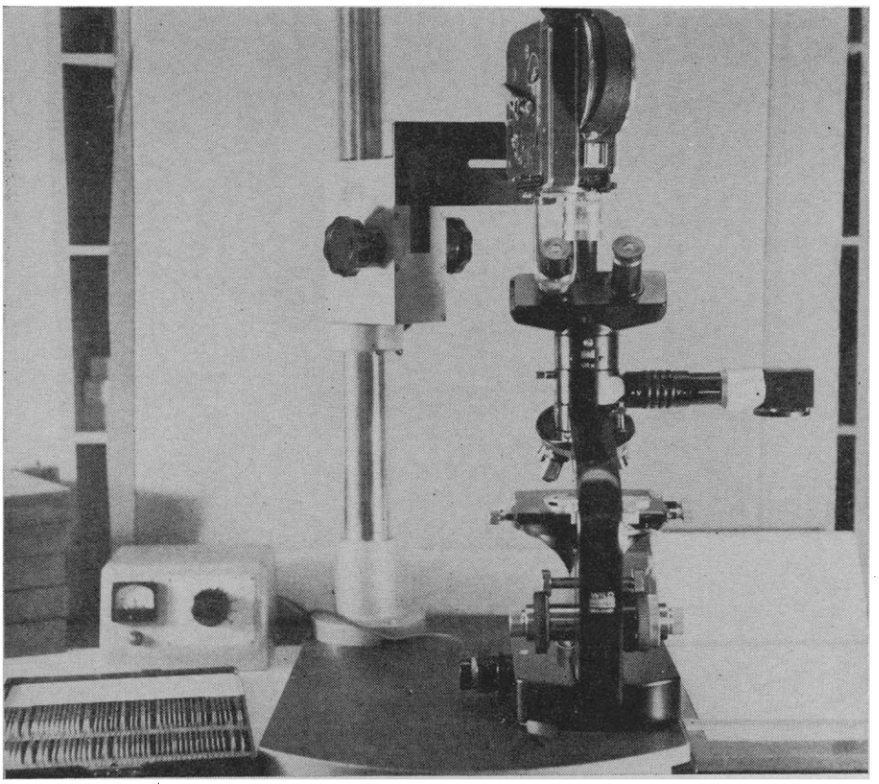
The most critical operation is precise alignment of each succeeding section in the microscope. If each section is mounted on a separate microslide, as is easy with freehand sections of large woody objects cut on a sliding microtome, alignment at the film plane can be accomplished with an "optical shuttle" system. Light output of the two microscopes used is combined by means of a discussion tube in an inverted position (Fig. 2). In its intended use the discussion tube is mounted above a single microscope and divides the optical output so that two observers can simultaneously view and discuss a microscopic preparation. In the inverted position special adapters are necessary so that the male-male and female-female fittings can be joined (*4*).

Successive sections are photographed alternately through each microscope. After selection and photography of one slide by way of one microscope (the other has its light source cut off simply by interposition of a piece of card below the condenser), the next section on the stage of the second microscope is aligned by superimposing its image over that of the first, both images being viewed through the binocular eyepiece. This section in turn, after being photographed, is used to align the third slide in the series, and so on.

Alignment of images may appear to be difficult during the first trials, but with some practice one learns to work quickly. Relative light intensities in the two microscopes can be varied with one of the condenser diaphragms as an aid to the alignment. Photography

itself is done with the condenser diaphragm fully open.

The optical shuttle is faster and simpler than the drawing technique; alignment can be made more precisely, especially at low magnification. With the wider field of view so made available, a larger area of the microscopic section can be analyzed with a single film. The method has already been valuable to us and may well be useful in other fields.



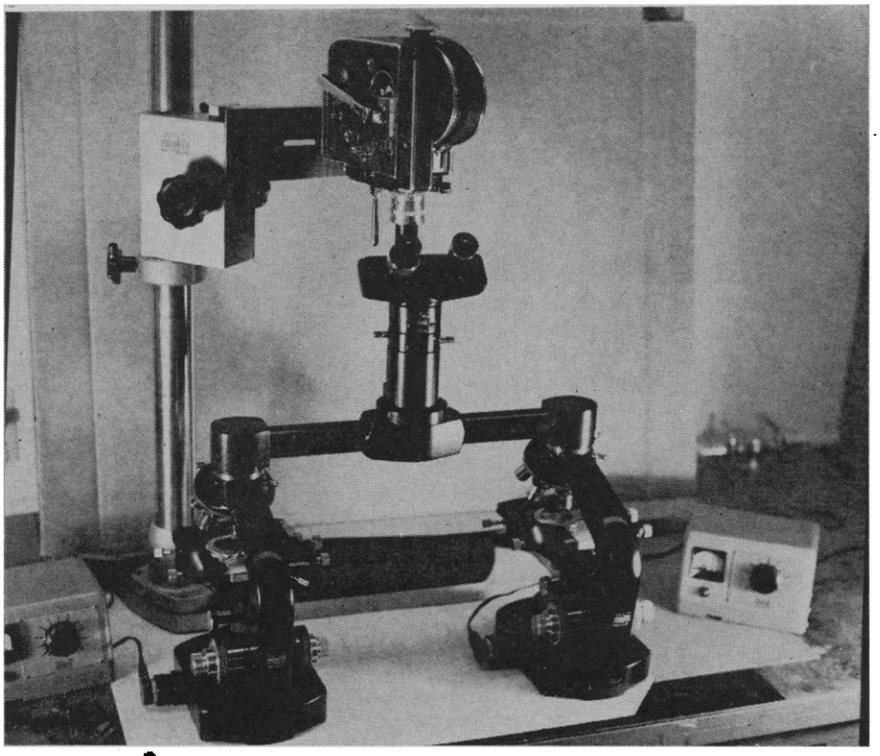


Fig. 1 (top). Frame-by-frame photography of serial sections. Alignment of successive sections is accomplished with the aid of the camera lucida. Fig. 2 (bottom). The optical shuttle assembly.

M. H. ZIMMERMANN
Cabot Foundation, Harvard University, Harvard Forest,
Petersham, Massachusetts 01366
P. B. TOMLINSON
Fairchild Tropical Garden,
Miami, Florida 33156

References and Notes

1. M. H. Zimmerman and P. B. Tomlinson, *J. Arnold Arbor.* **46**, 160 (1965).
2. The method works properly only if comfortable viewing, drawing, and photographing devices can be used simultaneously—only with equipment made by Wild (Heerbrugg, Switzerland) as far as we know, because the camera lucida can be incorporated in the light path. The assembly in Fig. 1 consists of a Wild M20 microscope base with rotating mechanical stage (rather important for ease of alignment of each section), drawing tube, camera tube H, and binocular. Viewing and focusing are done with the binocular; the camera is a Bolex H16REX. These are all standard components.
3. A 12-minute motion picture, representing early investigation of the course of vascular bundles in the stem of the palm *Rhapis*, was presented in the symposium "Long distance transport phenomena," 10th Intern. Botanical Congress, Edinburgh, Scotland, August 1964.
4. The assembly in Fig. 2 consists of two Wild M20 microscope bases with rotating mechanical stages, an inverted discussion tube, camera tube H, and binocular. Separate transformers enable the operator to equalize the light output of the two microscopes. The mounting of the discussion tube in an inverted position is accomplished by two double-male joints and one double-female. We thank the Wild Company which manufactured these pieces especially for us and supplied them free of charge. The special mounting of the discussion tube lengthens the light path above the objectives, and as a result the objectives must be lowered somewhat beyond their normal position. This necessitates use of extension rings between the nose pieces and the 3 × Plane Fluotars. No problem arises, however, with the other low-powered objectives.
5. Part of this work supported by NSF grant GB 2991 to P.B.T.

24 January 1966

Thyrocalcitonin: Cytological Localization by Immunofluorescence

Abstract. *Thyrocalcitonin was detected in the cytoplasm of all epithelial cells of the thyroid gland of the pig, by means of antibody fluorescence. It was present in those cells which normally elaborate thyroglobulin but was not present in the follicular colloid.*

In 1961 Copp *et al.* (*1*) presented indirect evidence that a hypocalcemic principle played a role in calcium homeostasis in the dog. The results of their experiments favored the parathyroid gland as the source of this material, and it was given the name