olism for temperatures beyond this range. I assume that variations in perching metabolism due to song, preening, and other activities are negligible, at least relative to the overall metabolic differences between perching and flight.

10. The value I used is intermediate between the values for hovering flight metabolism obtained by Lasiewski (*9*) and Pearson (*1*). See also D. G. Raveling and E. A. LeFebvre, *Bird-Banding* **38**, 97 (1967); M. Berger, J. S. Hart, O. Z. Roy, *Z. Vergl. Physiol.* **66**, 201 (1970); F. R. Hainsworth and L. L. Wolf, *Science* **168**, 368 (1970). On the basis of studies of the flight metabolism of the budgerigar by V. A. Tucker [*J. Exp. Biol.* **48**, 67 (1968)], I doubt that metabolic differences between hovering and forward flight will qualitatively affect the conclusions drawn here.
11. G. H. Orians, *Ecol. Monogr.* **31**, 285 (1961).
12. J. A. Verner, *Condor* **67**, 125 (1965).
13. Once located, flowers can be utilized repeatedly with no energy expended in searching; each individual insect requires search and pursuit.
14. D. L. Martinsen, *Ecology* **50**, 505 (1969).
15. Work supported by an NSF predoctoral fellowship. I thank O. P. Pearson, F. A. Pitelka, L. L. Wolf, and R. C. Lasiewski for helpful advice and criticism.

8 February 1971; revised 28 May 1971

Identification of Each Human Chromosome with a Modified Giemsa Stain

Abstract. *Differential staining of human chromosomes can be obtained when the pH of Giemsa stain is changed to 9.0 from the usual 6.8. Such staining permits identification of all homolog pairs and distinct regions within chromosome arms. In most instances, the pattern is quite similar to that obtained with quinacrine mustard fluorescence staining. Certain regions, such as the paracentric constrictions in chromosomes A1 and C9, and the distal end of the long arm of the Y chromosome stain differently with the Giemsa 9 technique. The technique is considerably simpler than the quinacrine mustard fluorescence technique and identification of homologs is also easier than in cells stained by the latter.*

The characterization of human chromosomes has been greatly improved by two recently described techniques. The quinacrine mustard (QM) fluorescence technique described by Caspersson *et al.* (*1*) permits the identification of autosomal homologs and the sex chromosomes. The second technique, differential staining of heterochromatic areas after treatment with NaOH, ribonuclease, and standard saline citrate, permits the identification of regions with repetitious DNA as well as the recognition of certain homologs (*2*). We now report a simple modification of the Giemsa stain which produces a banding pattern very similar to that observed with QM fluorescence staining; it also stains certain heterochromatic areas differentially.

A banding pattern similar to that seen with QM fluorescence staining or differential staining of the centromere regions of certain human chromosomes was occasionally observed in our laboratory with routine orcein or Giemsa staining. A systematic study of certain of the variables involved in the Giemsa staining procedure was therefore undertaken in order to develop a more regularly informative staining technique. Cells were cultured for 68 to 70 hours in a complete McCoy's 5A medium with 15 percent fetal calf serum, harvested by treatment with hypotonic KCL (0.075 mole/liter), fixed in a mixture of methanol and acetic acid (3 : 1), spread, and air-dried by blowing. Several aspects of the Giemsa staining procedure were studied in detail. The *p*H of the Na_2HPO_4 buffer and stain was varied from *p*H 5.0 to 12.0 by the addition of citric acid or sodium hydroxide to the buffer. Two milliliters of stain and 2 ml of buffer solution were then added to 96 ml of water. The *p*H was determined with a Beckman Zeromatic *p*H meter. No consistent banding pattern was noted below *p*H 9.0; apparent chromosomal damage was induced above *p*H 10.0, and less clear and less consistent banding patterns were observed. The duration of staining in Giemsa at *p*H 9.0 also proved to be important. When the slides were stained for 1 to 2 minutes, the predominant staining was in the centromere regions; occasional staining of the secondary constriction regions of A1 and E16 was also observed (*3*). When the slides were stained for 4 to 10 minutes, a reproducible banding pattern similar to that seen with QM

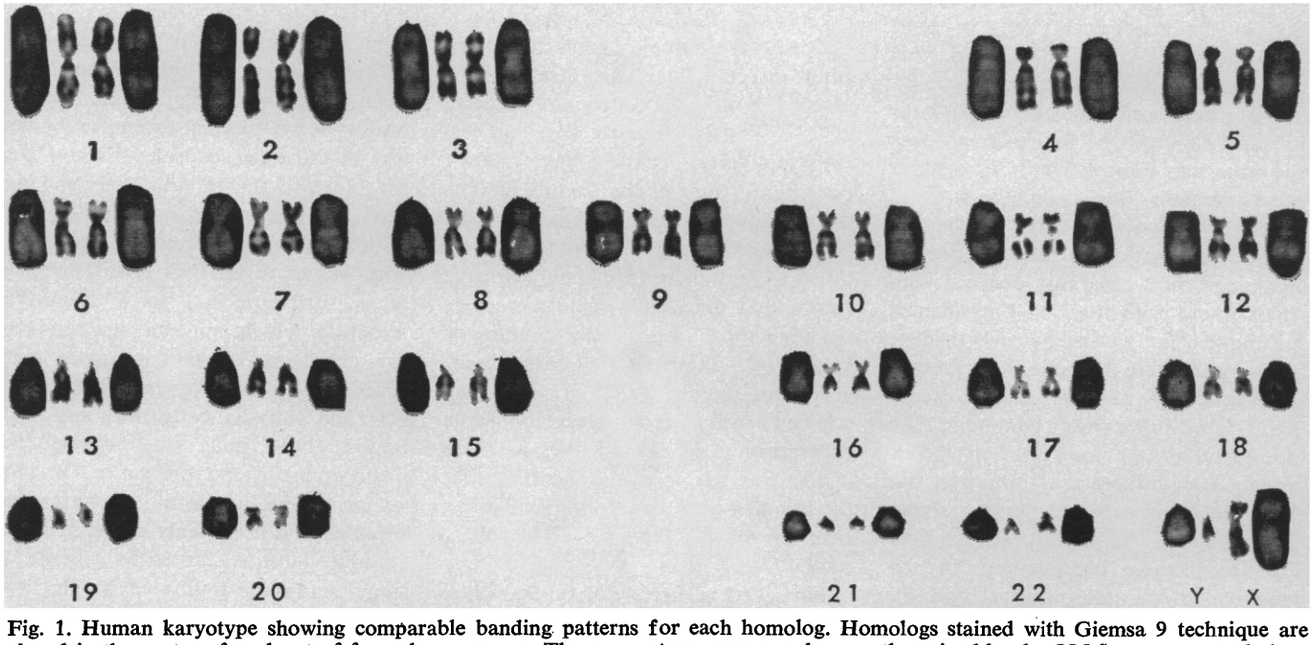

Fig. 1. Human karyotype showing comparable banding patterns for each homolog. Homologs stained with Giemsa 9 technique are placed in the center of each set of four chromosomes. The same chromosomes subsequently stained by the QM fluorescence technique are placed adjacent to these. The comparable banding patterns for homologs and for both techniques are evident.

fluorescence staining was observed in cells that were in mid-metaphase (Fig. 1). With a staining time of 15 to 30 minutes, uniform staining with Giemsa was observed. The molarity was varied from .003 to 0.14 mole/liter by the addition of 2 to 98 ml of 0.14*M* Na_2HPO_4 buffer in a total of 100 ml of staining solution. The *p*H was held constant at 9.0, and the concentration of Giemsa was 2 ml per 100 ml of final solution in these experiments. No qualitative effect of varying the molarity was observed on the staining pattern, but the time of staining necessary to obtain the banding pattern was increased from 5 minutes to 30 minutes. Preincubation of the slide in phosphate buffer at *p*H 9.0 for 2 to 30 minutes before a 5-minute staining period did not alter the banding pattern.

On the basis of these observations we now use the following procedure. Slides are stained for 5 minutes, washed in cold running tap water for 1 minute, and air dried. The stain consists of 2 ml of Harleco Giemsa Blood Stain Azure A stock solution, 2 ml of 0.14*M* Na_2HPO_4 (5 g of $Na_2HPO_4 \cdot 12\ H_2O$ per 100 ml of H_2O) and 96 ml of H_2O. This solution is adjusted to *p*H 9.0, if necessary. The phase of contraction of the metaphase cell is particularly important. Cells which are in very early metaphase show a discrete chromomere pattern; identification of homologs, however, is difficult in these cells. Cells which are in very late metaphase are also difficult to use for homolog identification. In mid-metaphase cells, homologous pairs can be identified in the majority of cells in good preparations. We have termed this technique Giemsa 9 staining.

Fifty cells were studied in this fashion and also by QM fluorescence after the stain was removed with 70 percent ethyl alcohol; dual karyotypes were prepared as shown in Fig. 1. The important landmarks for identification of homologs were generally identical with those found with the QM fluorescence technique. That was particularly evident in group C where the same areas showed an absence of staining. A few exceptions, however, warrant comment. The distal end of the long arm of the Y chromosome fluoresces brightly, but with the Giemsa 9 technique it shows a decrease in the density of staining. The paracentric constrictions in the long arm of chromosomes A1 and C9 show only minimum QM fluorescence; Giemsa 9 produces dark staining near the centromere of both chromosomes, and only the more distal parts of the constriction regions stain lightly. Other less pronounced differences will be reported after comparison of specific regions in a larger number of cells (*3*).

The striking similarities in banding patterns obtained by Giemsa 9 and the QM fluorescence techniques suggest that both stains may be acting in a similar fashion, although the mechanism of action of neither stain is clearly understood. Since there is less staining in secondary constriction regions with both techniques, it is possible that the intensity of staining may be due in part to variations in DNA content or to the availability of the DNA. The regions where particularly bright fluorescence is often obtained, such as the centromere of A3, the satellite regions in groups D and G, and the distal end of the long arm of the Y chromosome, may be exceptions and the brightest fluorescence may result from interaction with guanine-rich areas as suggested by Caspersson *et al.* (*1*).

It is likely that this technique, which is simpler than the QM fluorescence method, will prove extremely useful in routine human cytogenetics for homolog identification, for the recognition of regions of exchange in translocations, and for the more precise characterization of certain variations in arm length. The combination of the two techniques will probably provide more information than either technique alone.

SHIVANAND R. PATIL
SUZANNE MERRICK
HERBERT A. LUBS

Department of Pediatrics, University of Colorado Medical Center, Denver, 80220

References and Notes

1. T. Casperson, L. Zech, C. Johansson, *Exp. Cell Res.* **62**, 490 (1970); ———, E. J. Modest, *Chromosoma* **30**, 215 (1970).
2. M. L. Pardue and J. G. Gall, *Science* **168**, 1356 (1970); K. W. Jones, *Nature*, **225**, 912 (1970); F. E. Arrighi and T. C. Hsu, *Cytogenetics*, in press; F. H. Ruddle and T. R. Chen, in *Perspectives in Cytogenetics*, S. W. Wright and B. Crandall, Eds. (Thomas, Springfield, Ill., in press.
3. H. A. Lubs, unpublished data.
4. Research supported by PHS grant HD05079-01 and contract NIH-71-2096-0172.

1 June 1971; revised 18 July 1971

Insect Photoreceptor: An Internal Ocellus Is Present in Sphinx Moths

Abstract. *Adult sphinx moths lack external ocelli. In* Manduca sexta *and other anocellate moths structures homologous to ocelli have been observed. Histological examination of such a structure in* Manduca sexta *has shown structural similarities to ocelli of other insects. Electrophysiological studies revealed a response to light stimuli similar to the electroretinogram of external ocelli. The evidence strongly suggests that the structures are internal ocelli.*

Ocelli are simple eyes found in adult insects. They are incapable of perceiving form but they do perceive changes in light intensity (*1, 2*). Electrophysiological studies have shown that illumination of the ocellus inhibits the continuous discharge which occurs in the ocellar nerve during darkness (*3*). Further, behaviorial studies have shown that the ocelli are important in the regulation of the daily rhythm of insects (*4*). External ocelli are absent in sphinx moths (*1, 3, 5*). This fact coupled with the presence of large superposition compound eyes in these insects has led to the suggestion that the function of the ocelli has been taken over by the compound eyes in sphinx moths (*1, 5*).

However, in a study of the morphology of the nervous system in the adult tobacco hornworm (*Manduca sexta* Johanssen) a pair of internal ocelli have now been observed. They are located in the same anatomical position as the ocelli of other insects, except that the ocellar nerve does not extend to the vertex of the head capsule (Figs. 1 and 2A). Instead the retinula cells and the ocellar nerve are enclosed in and supported by a membranous tube filled with hemolymph (Fig. 2B). This tube is in turn attached to a transverse structure which appears analogous to the transverse muscle associated with antennal circulation in cockroaches (*1*). The retinula cells thus are positioned about half way between the protocerebrum and the vertex. Examination of the cuticle of the vertex has revealed that it is evenly pigmented and without lenslike structures. Observations made on moths taken in light traps have revealed the presence of similar structures in ten different species of sphinx moths, one saturnid,