5 percent because, on acrylamide-gel electrophoresis, the cross-linked material was found not to travel in a 7.5 percent gel, an indication that the molecular weight was 300,000 or greater. Under the same sedimentation conditions α chains isolated by ion exchange and molecular sieve chromatography had a molecular weight 99,500.

Amino acid compositions were determined on an automatic instrument by the single column method (*8*) for the cross-linked component isolated from the carboxymethyl cellulose column and for the original guinea pig skin collagen (Table 2). The cross-linked fraction has a composition most closely resembling the sum of two $\alpha 1$ chains and one $\alpha 2$ chain, $(\alpha 1)_2(\alpha 2)_1$.

The technique of introducing cross-links into collagen dispersions was used effectively by Veis and Drake (*2*) to demonstrate the formation of both inter- and intramolecular cross-links in solutions containing free molecules and aggregates. This procedure, modified to remove aggregates, has been used here to prove the mixed chain composition and predominance of $(\alpha 1)_2(\alpha 2)_1$ distribution within collagen molecules of mammalian skin, proposed by Piez *et al.* (*1*).

Recently, however, Miller and Matukas (*9*) have obtained evidence for the existence of a native collagen molecule in chick xiphoid cartilage, which contains three $\alpha 1$ chains, thus renewing interest in the possible heterogeneity of collagen in terms of chain distribution in individual molecules. The formaldehyde cross-linking method has now been used in our laboratory (*10*) to separate and quantitate the two different collagen species in cartilage. The technique of formaldehyde cross-linking in molecular dispersions of collagen can be used to explore aspects of collagen heterogeneity in different tissues, during embryonic development, and in disease states.

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Distal Lobe of the Pilifer: An Ultrasonic Receptor in Choerocampine Hawkmoths

Abstract. *Evidence is presented for a novel mechanism mediating the auditory sense in the Choerocampinae (Lepidoptera: Sphingidae). Vibrations in the scale-free medial wall of the enlarged labial palp segment are transmitted by contact to the distal lobe of the pilifer, a derivative of the labrum, and are there transduced into afferent impulses in the labral nerve.*

Hawkmoths of the subfamily Choerocampinae react behaviorally to ultrasound. That the enlarged labial palps characteristic of this group are concerned in ultrasonic reception (*1*) was shown by recording second-order spike responses in neural units within the cervical connectives. Lateral deflection of both palps from their resting position, or amputation of their bulbous distal portions, reduces sensitivity 50- to 100-fold, but neither this treatment nor total removal of both palps completely abolishes the acoustic response. A number of experiments exploring the possibility that sound energy throws the labial palps into vibration about their cranial articulations confirmed the amplifying property of the palps but yielded no definite information about the receptor site (*2*). No structures that might serve as the receptor mechanism could be discovered either within the palps or near

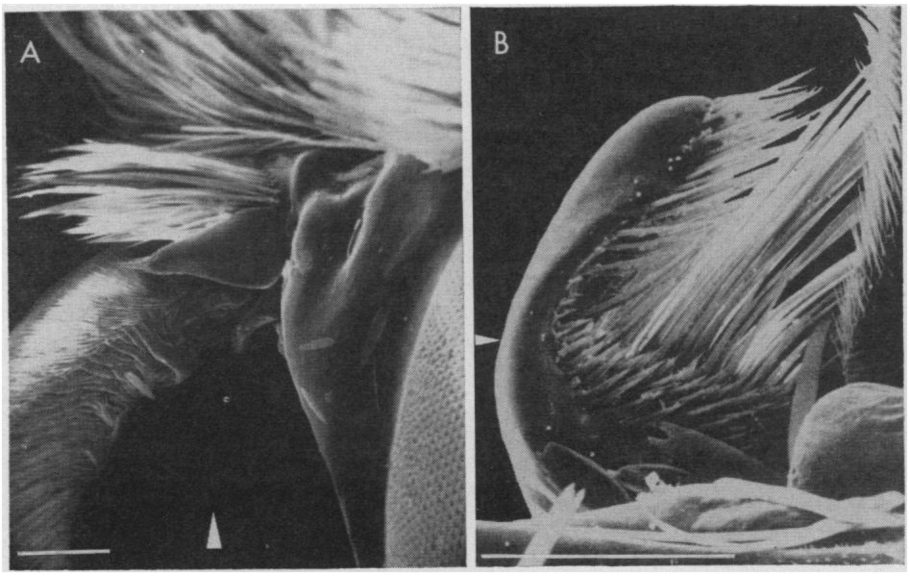

Fig. 1. The left pilifer of *Celerio lineata* exposed by removal of the labial palp (*5*). The arrow in each picture indicates the approximate angle from which the other picture was taken. The scales represent 0.2 mm. (A) Lateral view of anterior region of the head. The proboscis extends downward to the left; a part of the left compound eye is at lower right. Long sensilla chaetica from the basal part of the pilifer are visible near the apex of the distal lobe. (B) Ventral view of the same region. The background has been retouched. Lateral is to the left of the frame; the lower surface of the proboscis is visible at upper right. Several detached scales lie near the base of the distal lobe. When the labial palp is fully adducted some part of its medial surface appears to make contact with the smooth, convex, lateral surface of the distal lobe (at the left in B). The concave medial surface of this lobe bears short sensilla chaetica that do not touch the proboscis base (right). Long sensilla on the basal part of the pilifer (visible also in A) make contact with the proboscis.

their articulations, and no acoustic response could be detected in the palpal nerves.

This led to a search for other structures to which the labial palps might transfer their vibrations. In most lepidoptera the pilifers are small, sclerotized protuberances situated on either side of the base of the proboscis and bearing anteromedially directed bristles which are displaceable by movements of the proboscis. Uniquely in the Choerocampinae (*3*), the distal lobe of the pilifer is thin-walled and somewhat flattened mediolaterally, making it elastically flexible to pressure applied toward the midline. In side view the distal lobe of the pilifer appears bluntly triangular, its lateral, convex surface being completely smooth. Its rounded apex is somewhat skewed or twisted relative to the broadly pyramidal base which is firmly united to the cranial wall slightly ventral to the tentorial pits (Fig. 1A). The concave medial surface of the distal lobe bears setae up to its apex. In the Choerocampinae these setae are much shorter than those on the broad basal portion and are thus incapable of touching the base of the proboscis (Fig. 1B). Histological sections show that the longer basal setae, and possibly some of those of the distal lobe, are typical sensilla chaetica. When the labial palps are in their normal position, the pilifers are entirely concealed. The distal part of each is nested in a triangular, minutely setose depression at the "hilum" of the bean-shaped contour of the medial palpal wall (Fig. 2). This depression is bounded on one side by a narrow ridge along the anterior border of the scale-free area, and either this ridge or some part of the depressed surface of the palp is probably in contact with the convex surface of the distal lobe of the pilifer.

Experiments with *Celerio lineata* (Fab.) and *C. euphorbiae* (Linn.), in which the response in the cervical interneurons is used as a criterion of acoustic sensitivity, suggest that airborne ultrasound of suitable frequency can set up vibrations in the medial wall of the palp which can activate a receptor in the pilifer, generating impulses that are then transmitted via the labral nerve to the tritocerebrum. Evidence for this arrangement is summarized below. Passive abduction of the palp breaks its contact with the pilifer and nullifies the amplifying effect, causing a 50- to 100-fold drop in sensitivity. Ablation of the distal half of the second palpal segment has the same effect. After displacement or removal of the palps, acoustic sensitivity can be almost completely restored by substitution of an artificial membrane such as a small "flag" of thin Mylar film attached to a pin so that its free tip just touches the smooth convex surface of the distal end of the pilifer. The residual acoustic sensitivity remaining after removal of the palps is abolished by destruction of the distal parts of both pilifers. Abrupt mechanical displacements of small amplitude applied directly to the pilifer lobes elicit descending impulses in the second-order units previously activated by acoustic stimulation. Finally in *C. lineata* an electrode in contact with the labral nerve near its root in the tritocerebrum registered synchronous spike trains when the moth was exposed to a sequence of ultrasonic pulses.

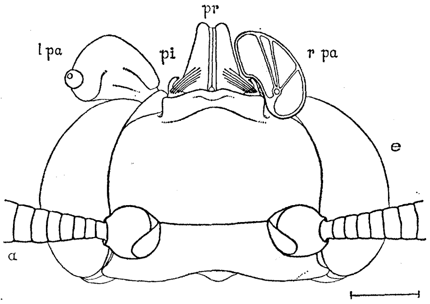

Fig. 2. Dorsal view of the denuded head of *Celerio lineata*. The left labial palp (*l pa*) has been deflected laterally, exposing the distal lobe of the left pilifer (*pi*). The right labial palp (*r pa*) is in its fully adducted position and has been transected obliquely at the level of the distal lobe to show the region of apposition between the distal lobe and the medial wall of the second palpal segment. The extensive air sac enclosed by the thin walls of the palp is traversed by nerves and blood channels sheathed in tracheal epithelium. The compound eyes (*e*), antennae (*a*), and base of the proboscis (*pr*) are shown as points of reference. The scale represents 1 mm.

The mechanical or interface portion (analogous to an eardrum) and the receptor portion of this acoustic system are derived from different appendages belonging to separate regions of the head—the labial and labral segments, respectively. Effective detection of ultrasound depends upon casual external contact between these two portions. This unusual acoustic mechanism presents several interesting possibilities. The ease with which the palp and pilifer can be disengaged and reengaged allows the acoustic function of either part to be measured independently or reversibly modified experimentally while the other part remains entirely intact. The original state can be reestablished time after time, with reproducible results, in the same preparation. The accessibility of the rugged yet highly sensitive pilifer might make it a convenient sensor for testing the performance of thin membranes exposed to ultrasonic frequencies.

The second-order response in the cervical connectives or prothoracic ganglion remains stable for many hours while the system is exposed to 10-msec pulses of 30 khz at two per second. Afference from both right and left pilifers converges upon the interneuron system, making the acoustic sense nondirectional at this level (*2*). At the most favorable frequency (roughly 20 to 60 khz) acoustic sensitivity is comparable to that of the noctuid tympanic organ (*4*). The best frequency is probably determined largely by the characteristics of the palp. Optimum sensitivity in this frequency range suggests that the choerocampine acoustic sense serves to alert these hawkmoths to the presence of nearby echolocating bats or to birds (*2*). It is remarkable that the acoustically modified palp and pilifer have been evolved in only one subfamily (four species tested) of the Sphingidae (*2*), although many members of the other four subfamilies are similar in size, flight pattern, and feeding habits.

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5. The scanning electron micrographs of Fig. 1 were made by J. D. Geller and J. Costa of Photometrics Inc., Lexington, Massachusetts. Supported by an NIH career award and grant SI-00947 to K. D. Roeder. Herndon Agee, U.S. Department of Agriculture, Cotton Insects Laboratory, Florence, South Carolina, supplied some of the moths used in this study.

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