Ultraviolet Reflection of a Male Butterfly: Interference Color Caused by Thin-Layer Elaboration of Wing Scales

Abstract. *Males of the butterfly* Eurema lisa, *like many other members of the family Pieridae, reflect ultraviolet light. The color is structural rather than pigmentary, and originates from optical interference in a microscopic lamellar system associated with ridges on the outer scales of the wing. The dimensions and angular orientation of the lamellar system conform to predictions based on physical measurement of the spectral characteristics, including "color shifts" with varying angles of incidence, of the reflected ultraviolet light. The female lacks such scales and is consequently nonreflectant. The ultraviolet dimorphism supposedly serves as the basis for sexual recognition in courtship.*

Butterflies are the most showy and conspicuous of insects. Although their colors are for the most part pigmentary, some hues, such as the blues and violets, achieve their greatest brilliance by structural means. The familiar "metallic" iridescence of tropical *Morpho* butterflies is an interference color, attributable to thin-layer elaboration of the wing scales (*1*, *2*). Recent studies of the family Pieridae have shown that some species exhibit an iridescent brilliance comparable to that of *Morpho*, but in a region of the spectrum, the near ultraviolet, that is invisible to man and visible to the insects alone (*3*, *4*). Only the males are reflectant, and the dimorphism supposedly provides the basis for sexual recognition in courtship. We here report on the physical characteristics and structural basis of the ultraviolet reflection of *Eurema lisa*, a common pierid from the United States.

To man, the sexes of *Eurema* appear closely similar (Fig. 1A). Both have yellow, black-fringed upper sides and yellowish-green, mottled undersides. The ultraviolet reflection of the male, visible by ultraviolet photography (Fig. 1B) or video-viewing (*4*), is restricted to the yellow portion of the upper surface of the wings. As in many other male pierids, the reflection is directional (*3*, *4*). A pinned male, viewed from above, and illuminated obliquely from one side with an ultraviolet source (300 to 400 nm), "lights up" from the wings of the opposite side only (Fig. 1B). This is suggestive of an interference color, caused by a thin-layer or layering system bearing opposing slants on the wings of the two sides. A coarse estimate of the angle of slant was obtained by viewing males, illuminated vertically from above, with an ultraviolet-detecting television camera (*4*) pointed at different angles and directions to the wing surface. Maximally intense reflection from one side of the butterfly was obtained when the camera was aimed from the opposite side, perpendicularly to the body axis, at about 50 degrees from the wing surface. It follows, by geometrical inference (*5*), that the layering system should be inclined at about 20 degrees to the plane of the wing, with the slope toward the wing base. High-magnification ultraviolet photography (*6*) showed the reflection to be restricted to the outer of the two

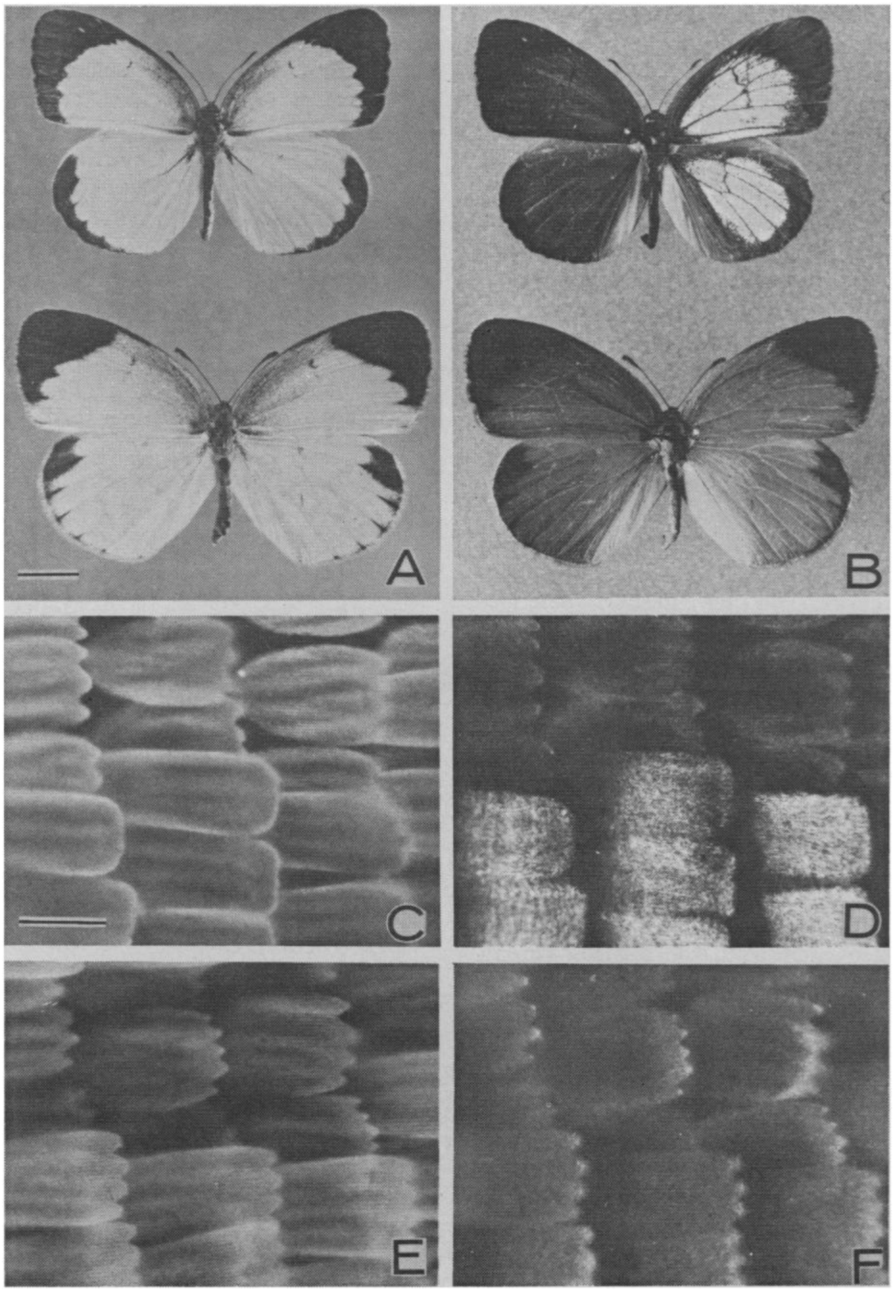

Fig. 1. (A) *Eurema lisa*, photographed in visible light (male, above). (B) Same, photographed in ultraviolet light, obliquely incident from the left (only the right side of the male "lights up"). (C and D) Scales of the upper surface of the right forewing of the male, photographed in visible (C) and ultraviolet (D) light. Only the outer scales (removed in the top region of the pictures) reflect ultraviolet light (note the striate pattern). (E and F) Comparable to the two preceding, but of a female; neither the outer nor the inner scales reflect ultraviolet light. [Reference bars: (A and B) 5 mm; (C to F) 50 μm]

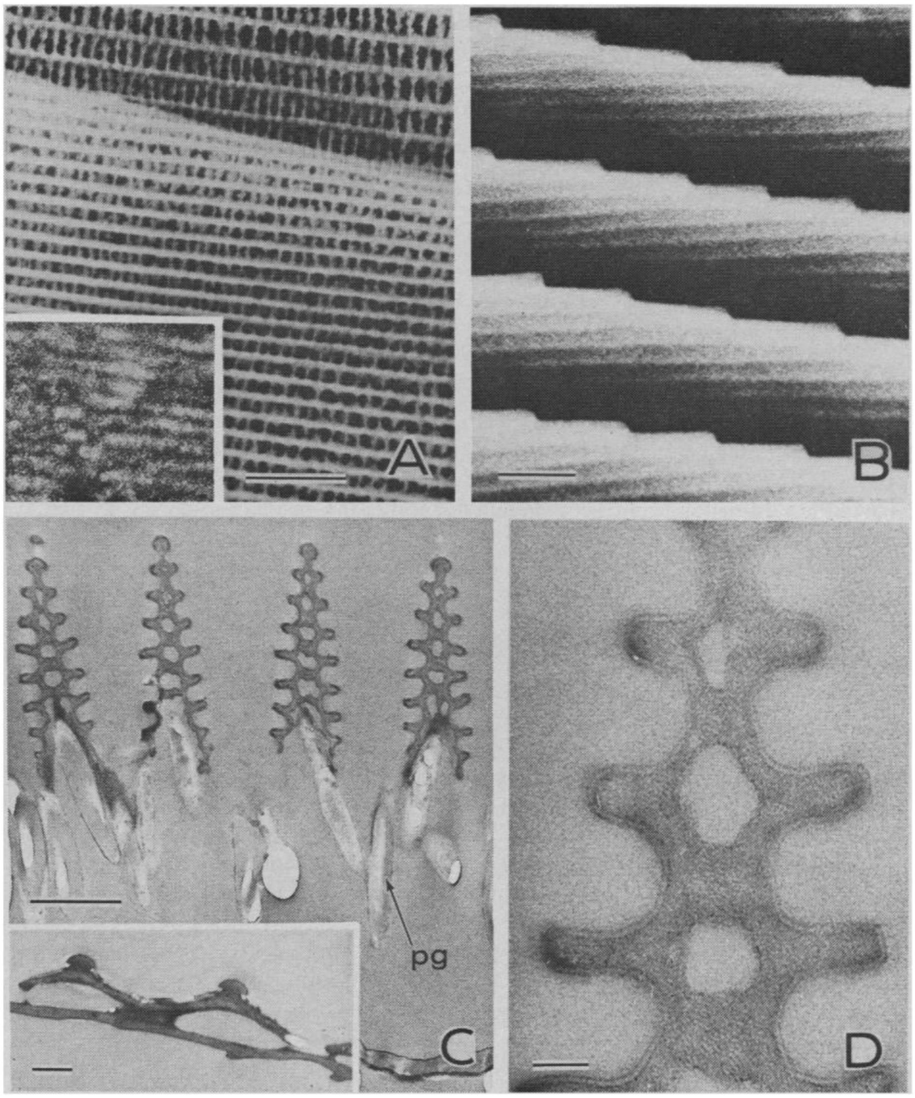

Fig. 2. (A) Scanning electron micrograph of portions of the two types of scale of a male *Eurema*. In the outer, ultraviolet-reflecting scale (bottom), the ridges are more closely spaced than in the inner scale. A highly magnified portion of an ultraviolet photograph of a reflecting scale (inset) shows that the bands of reflection match the ridges of the scale. (B) Scanning electron micrograph of the ridges of an ultraviolet-reflecting scale (viewed obliquely from above), showing the slope and stacking of the lamellae (the base of the scale, and body of the insect, is to the left). (C) Electron micrograph of a transverse section of a reflectant scale, showing the lamellar structure of the ridges. The oval bodies (*pg*) in the matrix beneath the ridges are pigment granules supposedly housing the yellow pigment of the scales. On a nonreflectant inner scale (inset), the ridges are low and lack lamellar flanges. (D) Portion of the ridge of a reflectant scale. [Reference bars: (A) 5 μm; (B and C) 0.5 μm; (D) 0.05 μm]

decks of scales of the wing (Fig. 1, C to F). Since the scales themselves are inserted on the wing with an incline of 10 ± 2 degrees (*7*), the layering system should be built into the scales at an angle of about 10 degrees relative to their plane. Moreover, judging from the parallel banding apparent in the ultraviolet reflection of the individual scales (Fig. 1D), the layering system should be laid out discontinuously in a striate pattern.

Scanning electron microscopy of the upper surface of ultraviolet-reflecting scales showed the presence of parallel ridges (Fig. 2A), seemingly lamellate in substructure (Fig. 2B), oriented lengthwise on the scale with a spacing comparable to that of the bands of reflection in the ultraviolet photographs (Fig. 2A, inset). Electron micrographs of cross sections of the ridges showed each to consist of an A-frame core, with lamellae projecting outward as flanges (Fig. 2, C and D). Viewed longitudinally, the lamellae are slanted toward the base of the scale (Fig. 2B), causing them to be arranged, relative to one another on each side of the core, like partially overlapping shingles on a roof. The stacking is such that a thickness of seven to eight lamellae is consistently maintained along the length of the ridge. The angle of slant of the lamellae relative to the plane of the scale, determined from the electron micrographs, is about 10 ± 4 degrees, in accord with the predicted inclination of the postulated layering system. A reconstructed view of a portion of a scale is shown in Fig. 3. Scales that do not reflect ultraviolet, such as those of the female, and the

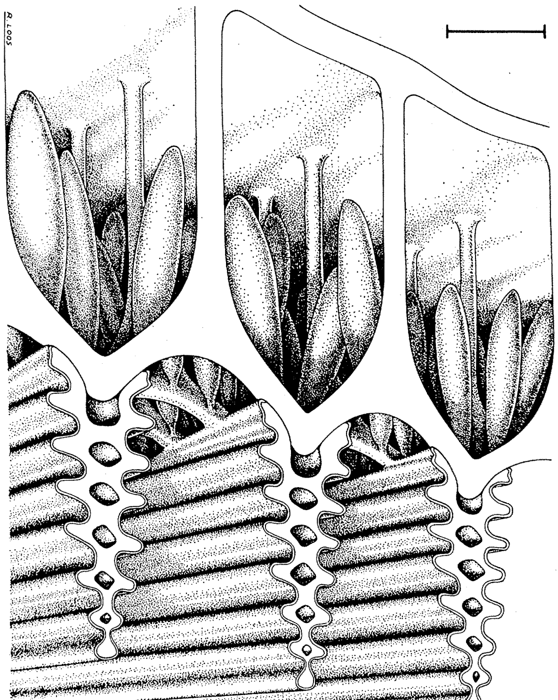

Fig. 3. Reconstructed view of a portion of an ultraviolet-reflecting scale of the male of *Eurema lisa*. The laminate ridges projecting upward from the scale constitute the interference "mirror" responsible for the ultraviolet reflection. The ellipsoid structures that project downward into the air space within the main body of the scale are typical pigment granules, responsible for the visible yellow color of the scale. [Reference bar: 0.5 μm]

inner scales of the male, also have ridges (Fig. 2A, upper part of picture), but these are low and lack lamellae (Fig. 2C, inset).

Optical inferences can be drawn from the morphology of the ultraviolet-reflecting scale. Given the measured average thickness of the lamellae (550 ± 35 Å) and of the air spaces between them (826 ± 42 Å), plus the known refractive index of air (1.0) and the inferred approximate refractive index (*8*) of lamellar cuticle (1.60), it follows that the lamellae and the intervening lamellar air spaces have similar optical thicknesses (lamellae: 880 ± 56 Å; air space: 826 ± 42 Å). Considering the inevitable distortion and consequent error in measurement from the electron micrographs, the disparity in the two optical thicknesses may be considered nil, and a common optical thickness of 858 ± 51 Å for the two types of layer may be assumed. If so, the ridge structure, with its stacked layering of alternating cuticle and air, would function in the manner of a quarter-wavelength interference reflection filter (*9*), which—given the optical thickness common to the layers—should reflect light, perpendicularly incident on the layers, with a constructive interference maximum at 343 ± 20 nm. This was confirmed by reflection spectroscopy. Midportions of forewings of three males, inserted into the spectrophotometer (*10*) with a tilt of 20 degrees, so that the incident light might be expected to impinge near normalcy on the lamellae (*11*), showed ultraviolet reflection with a maximum at 348 ± 2 nm.

It was to be predicted, from the lamellate nature of the interference system, that the spectral characteristics of the ultraviolet reflection should vary—that is, should show "color shifts"—with changes in the angle of viewing (*1, 12*). This was verified empirically, by measuring the spectral reflection of a piece of forewing inserted into the spectrophotometer at different angles of tilt (*11*). As anticipated, wavelengths of maximal constructive interference are longest with light incident on the wing at angles approximating the estimated perpendicular to the lamellae (wing tilt of 20 degrees), and maxima at shorter wavelengths prevail at angles of incidence deviating from either side of this approximate normal (Fig. 4). The computed curve (*13*) that best fits these data (Fig. 4) corresponds to a lamellar slant relative to the wing plane of 18.1 degrees and a single layer with a lamellar optical thickness of 867 Å. The fit of the data indicates a thickness of 535 ± 9 Å and a refractive index of 1.62 ± 0.02 for the cuticular component of the system. This is clearly in line with the morphological measurements and estimates, and leaves no doubt that the lamellate ridges are the source of the ultraviolet interference color of *Eurema*.

The evolutionary convergence implicit in the comparable structural achievements of *Eurema* and *Morpho* (*1, 2*), two butterflies which, despite disparate phyletic standing, have acquired similar lamellar systems for production of interference color, should come as no surprise. The arthropod integument is a plastic and responsive organ, as readily "compliant" to similar selective pressures as it is to differing ones. What is surprising is that in the family Pieridae itself, ultraviolet coloration is produced by two different means. Only some species, including among others the familiar *Colias eurytheme*, have directional ultraviolet iridescence (*3, 4*), presumably caused, as in *Eurema*, by lamellate scales (*14*). In other species, notably some common forms of *Pieris*, there is also an ultraviolet sexual dimorphism that provides the basis for recognition in courtship (*15*), but the difference is achieved by pigmentary (pterins) rather than structural means (*16*). For what reasons, communicative or other, these relatively closely related species should have adopted a duality of solution to a seemingly similar problem, remains obscure. As regards *Eurema lisa* itself, virtually nothing is known about its courtship, and only conjectural propositions could be advanced on how, precisely, the ultraviolet iridescence of the male exerts its signal function during the wing beat. It does seem certain that *Eurema* can see the signal. Other pierids that have been studied are visually attuned to near-ultraviolet light (*16, 17*).

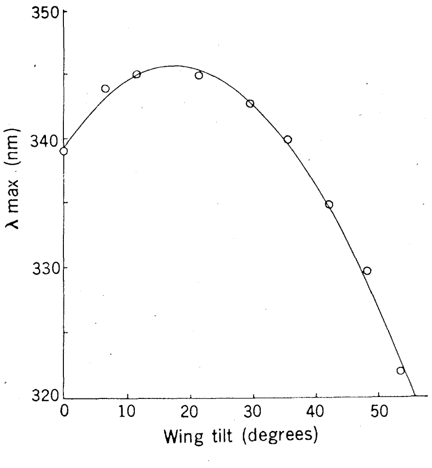

Fig. 4. Wavelength of maximal constructive interference (λ_{max}) plotted as a function of wing tilt. All data points are measurements from a single male forewing. The curve is computed (*13*), and corresponds to a lamellar interference system having an optical thickness of 867 Å (cuticular component with refractive index of 1.62 ± 0.02 and thickness of 535 ± 9 Å) and a lamellar tilt relative to the wing surface of 18.1 degrees.

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5. At maximum intensity, it is assumed that the source and detector are at equal angles from the normal to the reflecting plane(s). Therefore, the bisector of the total angle between the two is the normal to the reflecting plane(s).
6. Photographs were taken with a 40× ultraviolet transmitting mirror lens (Zeiss Jena) and an ultraviolet filter (Zeiss U-5) on Kodak ultraviolet film (type 103-0).
7. Scale insertion angles were measured with a microscope, on thin strips of wing, viewed on edge.
8. Refractive indexes (visible light) of arthropod cuticle range from 1.5 to 1.6 [A. G. Richards, *The Integument of Arthropods* (Univ. of Minnesota Press, St. Paul, 1951)]. For ultraviolet light, refractive indexes are higher, and for predictive purposes a refractive index of about 1.6 for the *Eurema* scale may be assumed.
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10. Beckman dual beam prism spectrophotometer with an integrating sphere reflectance accessory.
11. The axis of tilt was an anteroposterior line across the wing piece, transecting the scales perpendicularly to the ridges. The axis was oriented normally to the incident light, and the rotation involved tilting the wing piece downward from the horizontal (tilt = 0 degrees), as in the downstroke of flight.
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13. In theory, a linear function prevails between the square of the sine of the incident angle and the square of the wavelength of maximum

reflection. Had we had a precise measure of the lamellar angle relative to the wing surface, the incident angle could have been calculated with accuracy by subtraction from the wing angle, and a straight line fit to points based on the data. Calculation of the refractive index and lamellar thickness would have followed (*12*). Instead, we first computed linear functions (sine squared versus wavelength squared) based on assumed lamellar angles varying from 10 to 30 degrees at 0.1-degree increments, and selected the line of best fit, which corresponded to a lamellar slant of 18.1 degrees. The index of refraction and lamellar thickness were inferred from this line (*12*), and provided the basis for construction of the curve in Fig. 4.
14. The ultraviolet-reflecting scales of male *Colias eurytheme* have an ultrastructure comparable to those of *Eurema* (H. Ghiradella, unpublished).
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Moving Visual Scenes Influence the Apparent Direction of Gravity

Abstract. *When an observer views a wide-angled display rotating around his line of sight, he both feels his body tilted and sees a vertical straight edge tilted opposite to the moving stimulus. Displacement of the perceived vertical increases with stimulus speed to reach a maximum (averaging 15 degrees) at 30 degrees per second.*

The observer's visible surround influences his orientation and localization in space. Most investigators of these phenomena have been concerned with the effects of tilted scenes on the apparent vertical. They have interpreted their findings as the outcome of a conflict between spatial coordinates given on one hand by the dominant orientation of visible contours and, on the other, by the direction of gravity (*1*).

We now report that a visual surround rotating around the observer's line of sight induces tilts of the apparent upright ranging up to 40°. The moving visual displays entailed no cues to visual orientation that could conflict with those of gravity. Consequently, the motion as such caused the tilt. Shortly after initiation of motion, the observer both felt his body rolled laterally and saw a stationary edge turned in the direction opposite to the rotation of the display. Accordingly, the effect of the moving surround was equivalent to displacing the direction of gravity in the direction of rotation. It can be accounted for by assuming that neurally encoded signals of visual motion modulate signals from gravireceptors at some level of the nervous system, an interpretation consistent with neurophysiological results (*2*).

In order to measure the effects of seen motion on visual and postural orientation, we designed two experiments in which our subjects were required to readjust continuously the position of either a visual test edge or the orientation of their own body to the apparent vertical. The corrections made to compensate for perceived tilt were continuously recorded.

In the first experiment, the subject monocularly viewed a display restricting his field of view to 130° of visual angle. At its center, a target disk, subtending 32° of visual angle, carried a central fixation point and a straight edge initially set vertical. The disk was mounted on a shaft whose rotation was controlled by the observer. A potentiometer attached to the shaft allowed continuous recording of the observer's settings. Just behind the target disk, mounted on a concentric shaft driven by a variable speed motor (2° to 130° $\sec^{-1}$), was a much larger disk subtending 130° of visual angle. This disk formed a visible ring around the target and was covered with the random pattern of spots that precludes edge orientation cues.

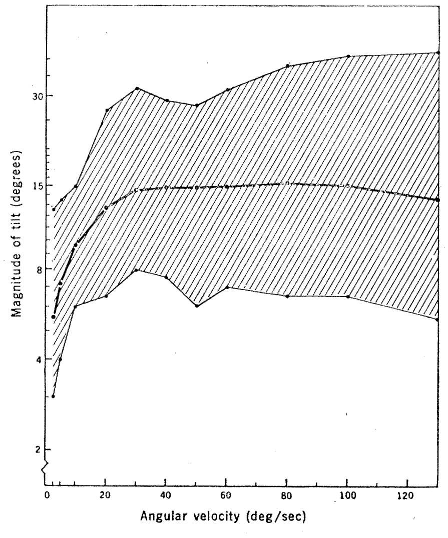

Fig. 1. Magnitude of apparent tilt in relation to stimulus velocity; geometric means on solid line. The shaded region represents the range of single values of the seven subjects. Data for individuals are highly replicable but inter-individual variations are large.

After initiating rotation of the large disk, observers experienced a rapidly increasing tilt of the target edge in the direction opposite to the rotation of the field. The tilt reached its steady state within an average of 18 seconds. When observers were asked to set the target edge to the apparent vertical, the amount of tilt increased with the angular velocity of the ring up to 30° $\sec^{-1}$ and remained approximately constant up to the maximum speed tested of 130° $\sec^{-1}$ (Fig. 1). These settings were obtained in sequence by increasing the angular velocity of the ring every 30 seconds. Among seven subjects the tilt averaged 15° and in one observer, exceeded 40°.

The magnitude of these effects militates against the notion that small torsional eye movements can account for the effects (*3*). In other experiments, tilt increased with stimulus area and stimulation of the more peripheral parts of the retina exerted a disproportionately strong influence on the apparent tilt of the target (*4*). This result ties in with experiments with stimuli that rotate around the vertical axis of an observer; in this case, stimulation of the periphery also dominates the induction of apparent motion of the body (*5*).

To demonstrate corresponding effects on postural orientation and, therefore, to examine the possibility that the tilt effect might not be an exclusively visual phenomenon, we designed a second experiment. Subjects were seated in a moving-base airplane trainer (Link GAT-1). Using a control stick, they were able to adjust the position of the trainer to subjective upright. Continuous repositioning was required to compensate for a lateral tilt disturbance (sum of 16 input frequencies from 0.05 to 0.3 hz) applied to the drive of the trainer. The resulting small but continuous and unpredictable lateral tilts (roll) of the trainer were introduced to force the observer to reindicate repeatedly his postural vertical and to minimize sequential effects. During the first (control) phase, the subject, fix-