A STUDY OF VARIATION IN FEATHER PIGMENTS OF THE WRENTIT

By DARL E. BOWERS

Much has been written about the structure and pigmentation of the feathers of birds; see, for example, papers of Strong (1902), Lloyd-Jones (1915), Frank (1939), Lillie and Wang (1941), Watterson (1942), and Auber (1955, 1957). The more recent of Auber's two papers summarizes information on structural colors and unusual pigmentation in the entire class Aves. However, little is found in the literature concerning variation in feather pigmentation of closely related birds at the microscopic level.

The Wrentit (*Chamaea fasciata*), restricted to the west coast of North America, has been subdivided into six races, largely on the basis of color. The birds of northern California and especially those just north of the San Francisco Bay area, form a series of three races with the darkest birds, *C. f. rufula,* along the coast, and the lightest birds, *C. f. henshawi,* interiorward, on the west slope of the Sacramento Valley. A race of intermediate coloration, *C. f. intermedia,* occurs in Sonoma, Napa, and Lake counties between the two extremes. An analysis of the macroscopic color gradients found here have been reported elsewhere (Bowers, 1956, and in press). The colors of the aggregate breast feathers of these birds show a considerable color gamut running from vinaceous-buff to army brown in the terms of Ridgway (1912), or in more precise colorimetric specifications of the Munsell color system (Munsell, 1954), from 10.0 YR 6.3/3.0 to 6.7 YR 4.2/3.5 (pale brown to moderate brown). The color gradation between these extremes runs generally westward from light to dark.

If sample breast feathers from birds of the extreme regions of this color gradient are mounted on microscope slides with piccolyte and cover slips, one notices differences in the degree of pigmentation present in the barbules. These barbules lack barbicels of any kind. Because of this lack, the feathers are extremely soft and fluffy and are called semi-plumes. This makes it relatively easy to observe and measure individual barbules and to make drawings of the location of the pigment granules. Color designations when one is using a microscope (approx. 440×) are difficult to make since there is much color aberration and refraction around edges that confuse the observer's color sense. Pigment granules, taken individually, do appear similar in the different feathers studied. But the distribution of pigment, assumed to be chemically equivalent in the various feathers examined since these birds are all of the same species, shows an interesting correlation with the macroscopic color gradation of the breast feathers viewed en masse. These feathers, the barbules of which contain pigment clumps corresponding to the cells in the cell-chains of which they are composed, are largely transparent horny material. The cortices of barbs and barbules contain the pigment but the medullae of the barbs appear refractory and dark by transmitted light and without color. Pigment in the barbs appears evenly distributed where it can be seen and is not easily measured.

In the darker birds, the brownish pigment clumps are larger and more densely packed than in the lighter birds, with less of the transparent parts of the barbules showing. Figures 1 and 2, drawn with the aid of a camera lucida, show the differences to be seen in these feathers.

In order to obtain a more objective statement of these differences in pigment distribution, series of measurements were made of the pigment masses as they appear under the microscope at an enlargement of approximately 440 diameters (4mm. dry objective and 10x ocular). As one looks at the barbules in this way, one sees the pigment as a dark body within the relatively transparent horny material of the feather. By using an ocular micrometer, one can measure the various dimensions of the color mass and the

barbule itself. One can arrive at a figure that represents the portion of the feather structure filled with pigment as seen in this one view. These data, expressed in percentages, can be compared with data taken from other feathers to bring out differences that exist. Figure 3 shows diagrammatically the measuring scheme. The length "b" is an arbi-

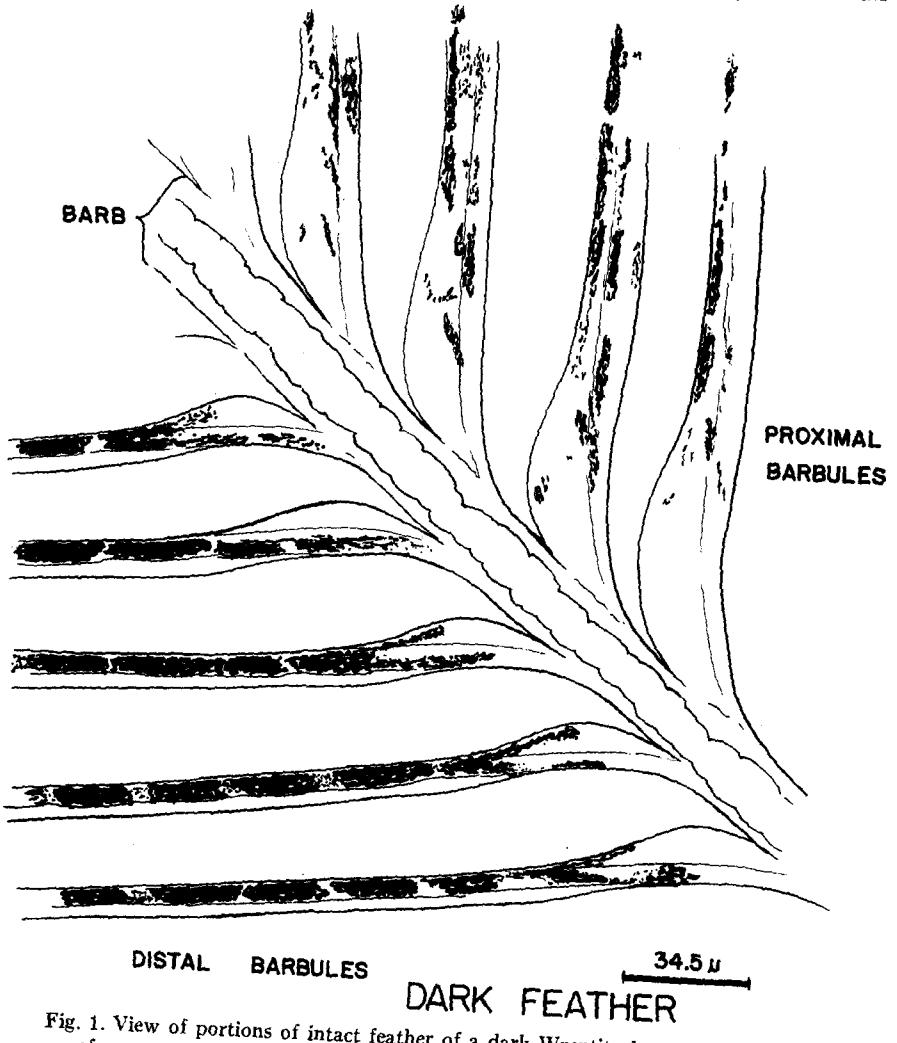

Fig. 1. View of portions of intact feather of a dark Wrentit, drawn with the aid of a camera lucida.

trary unit used for the sake of convenience. From this, xy/ab equals the proportion of the barbule segment pigmented as seen in this two-dimensional projection by transmitted light. There is no need to bring into the measurements the third dimension, since in comparing like measurements in similar sized feathers, such a figure would simply cancel out.

In practice, it became evident that short cuts could be made to facilitate the measuring. The ocular micrometer used is divided into large units appearing 1/30th mm. apart at the magnification used, and each of these is subdivided into ten small units. Barbules

averaged quite consistently two of these small units in width. Since this width (a) is fairly constant in the portions of the barbules measured, the widths of the pigment masses (x) were estimated visually as a proportion of the barbule width and recorded as the nearest tenth of that barbule width. Also, since it was easier to keep one's place in the microscope field by noting the numbered large divisions of the ocular micrometer rather than numbering the individual masses of pigment, the lengths of the masses (y)

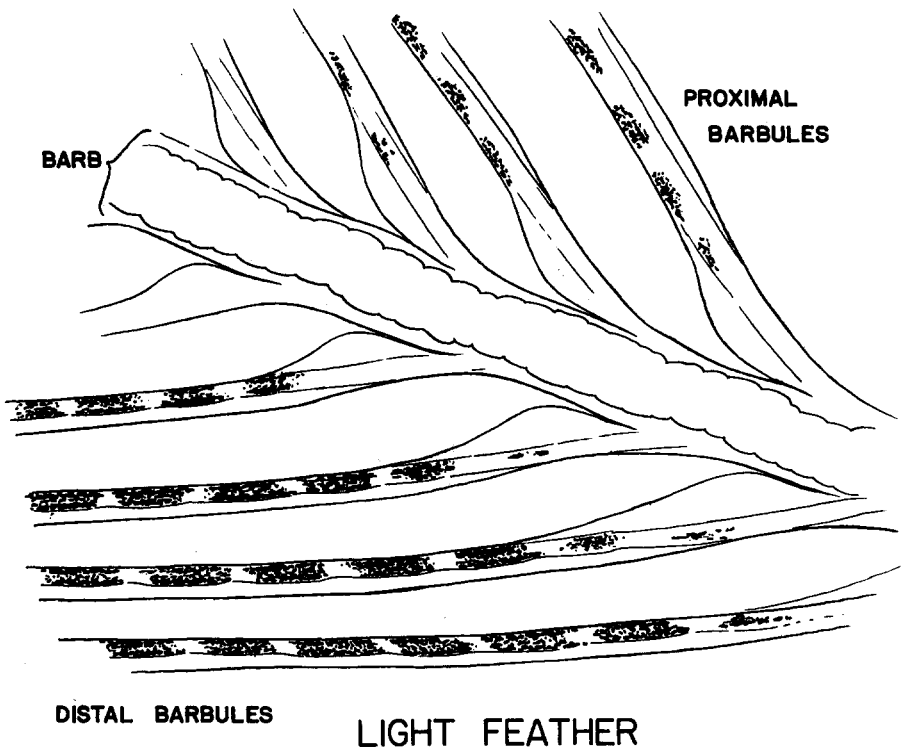

Fig. 2. View of portions of intact feather of a light Wrentit, drawn with the aid of a camera lucida.

were then measured as the number of small units per large unit (b) that were filled with pigment regardless of the number of masses falling within the measuring unit and recorded as the nearest tenth of the large division. The average percentage of each barbule area ($ab = 2 \times 10 = 20$ square units) filled with pigment is given by: xy/abn where n equals the number of "b" lengths measured, or:

$$P = (xa \cdot yb + x'a \cdot y'b + x''a \cdot y''b + \ldots\ldots\ldots\ldots x^n a \cdot y^n b)/abn$$
$$= ab(xy + x'y' + x''y'' + \ldots\ldots\ldots\ldots x^n y^n)/abn$$
$$= (xy + x'y' + x''y'' + \ldots\ldots\ldots\ldots x^n y^n)/n$$

One assumption necessary for this procedure was that the barbules be roughly cylindrical in cross section so that they present a relatively constant diameter to the viewer regardless of their position. As can be seen in the composite drawing, figure 4, this is not true. The barbules are flattened cylinders with a flange protruding along the barbule on the side of the feather away from the bird's body. In viewing intact feathers, the barbules are seen nearly on edge, as shown in figure 4, so that most of the time the

flange did not contribute to the width of the barbules measured. Since most of the barbules measured 6.7 micra (2 units on the micrometer, dimension "a") as seen in the intact feather, it is considered that reasonable consistency was followed in the measurements. Moreover, the same techniques were used in measuring both light and dark feathers, with the same kinds of errors present in both sets of data, and since our purpose here is one of comparison, such errors as there are tend to cancel out in the end.

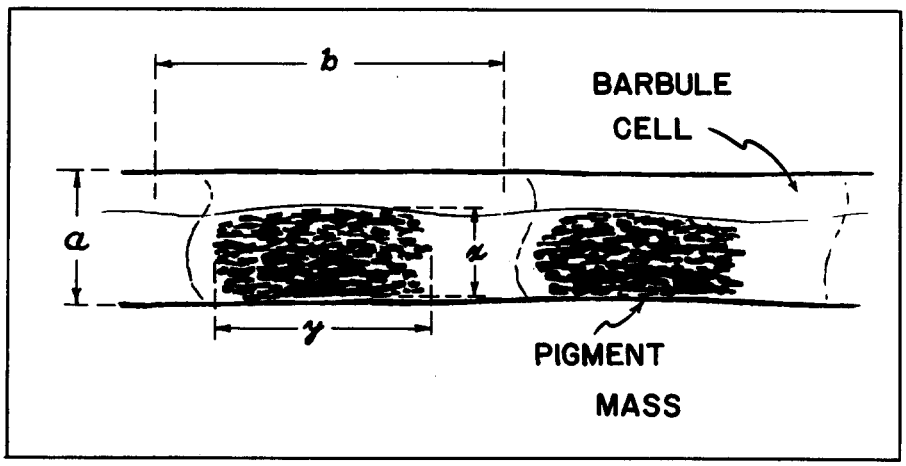

Fig. 3. Diagram of a barbule of the Wrentit showing the pigment measuring scheme.

Another complication is the fact that the pigment masses themselves are not cylindrical but are concave toward the center of the barbules, as can be seen in figure 4 showing cross sections. The view of these clumps in the intact feather was thus not always the same from barbule to barbule. In most cases, the pigment clump was seen next to the side of the barbule as it would be when viewing the barbule from its edge, but varying amounts of overlap of the two extreme edges of the clump may have obscured the true extent of the pigmentation in terms of its dimension "x." Here again, the consistency in measurement technique with the different feathers is called on to offset this possible source of error.

Further difficulties in the method revolved around the fact that the feathers to be measured were not sectioned, but were quite thick in terms of the depth of field available at the magnification used. There was refraction at the boundaries of the feather substance that occasionally confused the observer. The various focal planes possible in such a thick object gave different impressions from level to level. Measurements represent, in some cases, compromises in the appearances of the edge positions.

Pigment clumps are not uniform masses of material, but are composites of pigment granules, oftentimes not closely packed, but with spaces between. Wherever these spaces were obvious, the pigmentation was visually compacted so as to make it more of a "clump" of "uniform" consistency.

There are probably more elegant ways of getting at the amount of pigmentation in these feathers. One that comes to mind is the extraction of pigment from a given feather volume and analyzing the resultant extract with some sort of colorimeter. This method, too, would have its drawbacks and the end results probably would be no more significant than those presented here.

There are some details of the pigmentation pattern that are of interest. The distal barbules, which lie on the side of the barbs closest to the main shaft of the feather, are

more heavily pigmented in all feathers examined than the proximal barbules that lie on the side of the barbs away from the main shaft. This pattern is apparently symmetrically arranged with respect to the main shaft so that each side of the vane is comparable to its opposite.

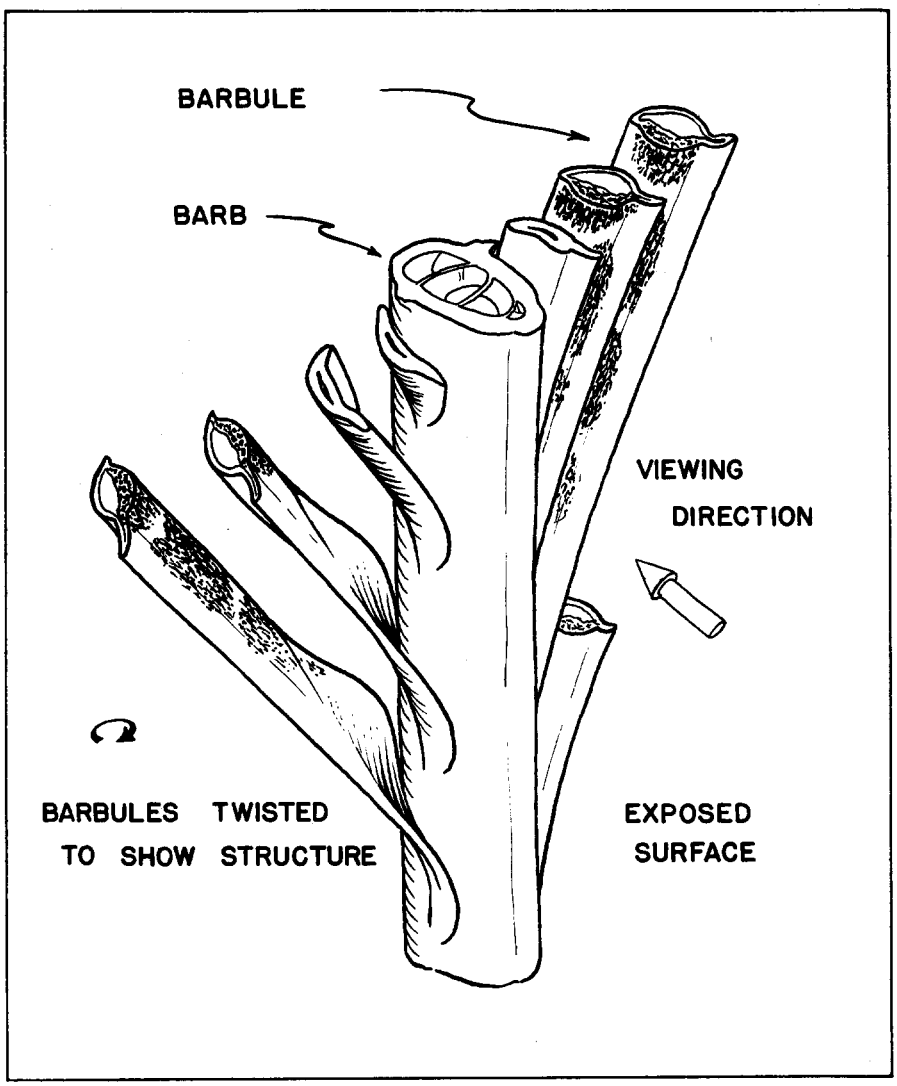

Fig. 4. Composite drawing of a portion of a breast feather of the Wrentit showing cross sections of barb and barbules.

Barbules near the base of the feathers are completely different from those toward the tip. These basal regions appear gray in the intact feather and are usually hidden by overlapping feathers as they lie in place on the breast of the bird. These basal barbules are filled with tear-drop shaped masses of a dark gray or black pigment with their pointed ends toward the base of the structure in which they exist. These barbules are cylindrical in cross section, and the pigment is likewise centrally located and cylindrical in cross

section. There is no apparent difference between the feathers of light and dark birds in this pigmentation (see fig. 5).

A feather from the central breast region of a Wrentit collected in Marin County, December 20, 1951, was used to represent the darkest birds of the color gradient. Another feather from the same breast region of a bird collected in Solano County, Decem-

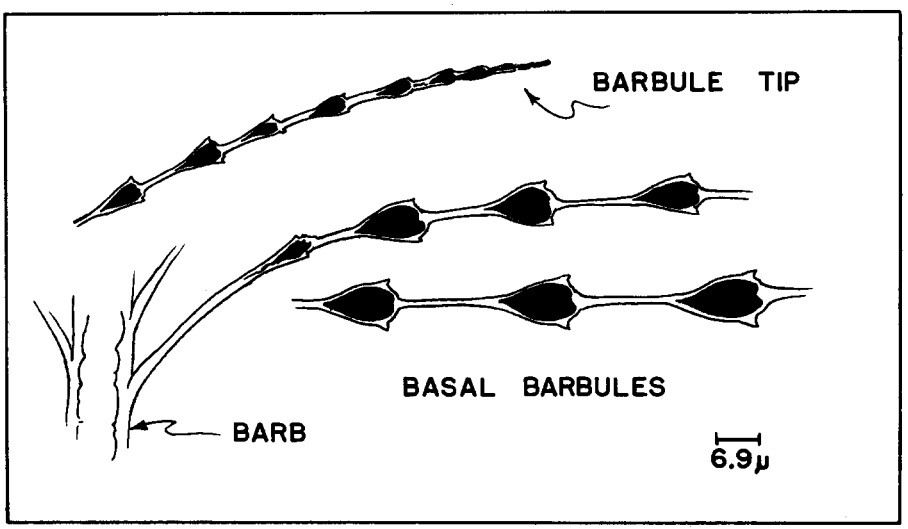

Fig. 5. Drawing of barbules of the Wrentit from the basal part of a breast feather.

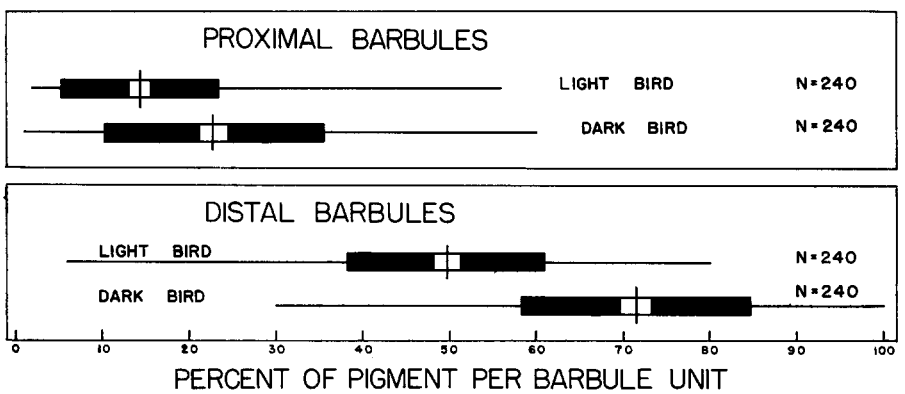

Fig. 6. Dice graphs showing percent of pigment per barbule unit in the Wrentit. Means are represented by vertical lines, standard deviations by solid bars, twice the standard error of the means by the open bars, and the range by the horizontal lines.

ber 11, 1951, was used as a sample of the lightest birds of the color gradient. Both were adult males in fresh plumage having just recently gone through the postnuptial molt. On each of these feathers, the barbules of the fifth and seventh barbs, counting back from the tip of the feather, were measured as a sample of the portion of the feather

showing when in position on the bird. A linear distance of 8000μ (8 mm.) was measured on proximal barbules and an equal length was measured on distal barbules. Approximately half of each of these was from one side of the main vane and half from the other

Table 1

Percentage of Pigment per Barbule Unit

	N units	Range	Mean	Standard deviation
Proximal barbules				
Light bird	240	2–56	14.4±0.6	9.0
Dark bird	240	1–60	22.9±0.8	12.6
Distal barbules				
Light bird	240	6–80	49.7±0.7	11.4
Dark bird	240	30–100	71.6±0.8	13.1

side. The results of these measurements are shown in figure 6 expressed as percentages of barbule unit areas that appeared filled with pigment granules. Table 1 gives the data from which these graphs were drawn.

By inspection of these data, it is evident that there is considerable difference between comparable parts of the two representative feathers. In both proximal and distal barbules, the differences between the two feathers have a P value less than 0.0001. At the microscopic level, pigmentation patterns match those shown in gross aspect of the breast plumage of these birds.

The controlling influences in the birds, very likely of an hereditary nature, can be envisioned as working at the cellular level in the production of more pigment material in those birds living in the more humid and darker environments found along the coast, and conversely in the production of less pigment in those birds living in more arid and lighter environments found inland. It may be postulated that only a few hereditary factors are necessary to bring about these differences which appear to be largely quantitative rather than qualitative. There is little, if any, evidence to substantiate this position for the races of birds, but for other vertebrates, mammals especially, there have been studies to indicate that pelage coloration is under the control of hereditary factors, producing under the agency of natural selection coloration that may be of adaptive advantage in the variously colored backgrounds against which animals are viewed by predators.

SUMMARY

A study has been made of the feather pigment distribution at the microscopic level in two races of the Wrentit (*Chamaea fasciata*). These races represent extremes in coloration from darker birds along the humid coast of northern California to lighter birds interiorward in more arid situations. A simple statistical analysis has been carried out by measuring clumps of pigment in breast feathers and comparing these dimensions with those of the barbules in which the pigment is contained, thus arriving at a figure representing the percent of pigment per unit of barbule length. It has been shown that there is considerable difference in the amount of pigment that can be measured in this way between birds at the extremes of the color gradient. Since this difference appears to be quantitative rather than qualitative, it has been postulated that such variations could be controlled by just a few hereditary factors and that thus the considerable color differences that are observed in the breast plumage of these birds may be produced by a relatively simple genotypic variation.

LITERATURE CITED

Auber, L.
1955. Cortex and medulla of bird-feathers. Nature, 176:1218–1219.
1957. The distribution of structural colours and unusual pigments in the class Aves. Ibis, 99:463–476.
Bowers, D. E.
1956. A study of methods of color determination. Syst. Zool., 5:147–160, 182.
Frank, F.
1939. Die Färbung der Vogelfeder durch Pigment und Struktur. Jour. für Ornith., 87:426–523.
Lillie, F. R., and Wang, H.
1941. Physiology of development of the feather. Physiol. Zool., 14:103–133.
Lloyd-Jones, O.
1915. Studies on inheritance in pigeons. II. A microscopical and chemical study of the feather pigments. Jour. Exp. Zool., 18:453–509.
Munsell, A. H.
1954. (1905). A color notation, ed. 10 (Munsell Color Co., Inc., Baltimore, Md.).
Ridgway, R.
1912. Color standards and color nomenclature (Publ. by author, Washington, D.C.).
Strong, R. M.
1902. The development of color in the definitive feather. Bull. Mus. Comp. Zool., 40:147–184.
Watterson, R. L.
1942. The morphogenesis of down feathers with special reference to the developmental history of melanophores. Physiol. Zool., 15:234–259.

Museum of Vertebrate Zoology, University of California, Berkeley, California, *August 20, 1958.*