PIGMENTATION OF EGGS: VARIATION IN THE CLUTCH SEQUENCE

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THE material herein reported upon comprises a collection of some twenty clutches of eggs of the Laughing Gull (*Larus atricilla*) collected in 1952 from the saltings east of Stone Harbor, New Jersey, being the same collection that was analyzed for *shape* in a previous report (Preston and Preston, 1953), and a collection of twenty-two clutches, of three eggs each, of the Common Tern (*Sterna hirundo*), collected in 1953 from the high-level sand-flats south of Stone Harbor, being the same collection analyzed for *shape* in another previous report (Gemperle and Preston, 1954). Some reference is made briefly to a small, and inadequate, collection of eggs of the Least Tern (*Sterna albifrons*) collected in 1953 south of Stone Harbor and north of Anglesea, New Jersey.

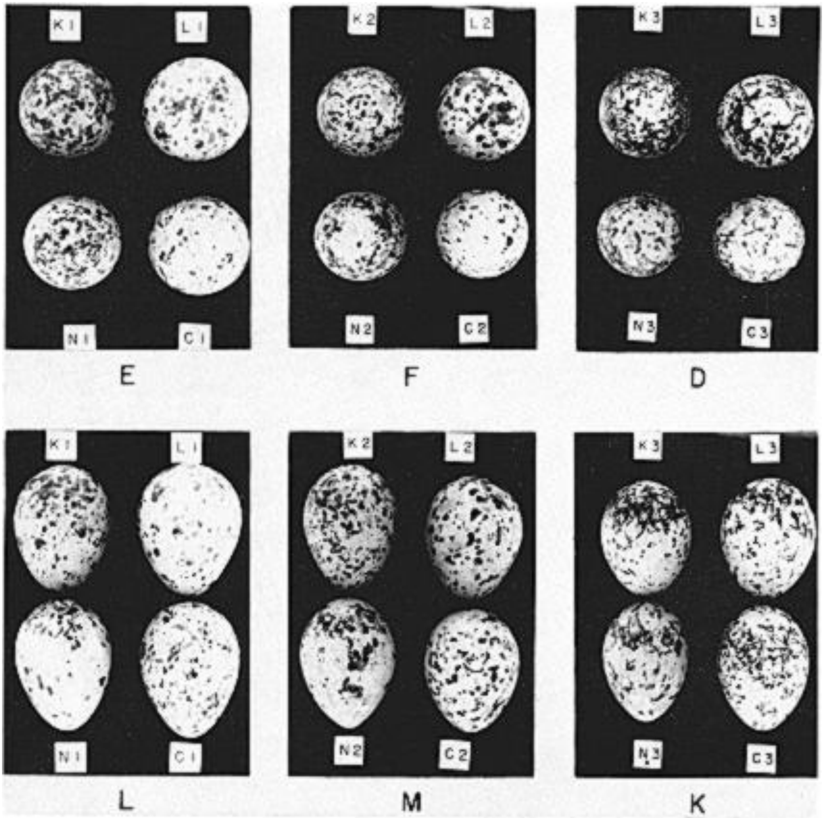
It was observed, as these eggs were collected, that the last egg typically differed from the others not only in shape but also in pigmentation. It seemed advisable to report on this, but pigmentation does not lend itself so readily to exact quantitative description as shape does. Apparatus was developed to try to describe the arrangement of pigment on a quantitative basis, but since the results are capable of fairly satisfactory treatment on the basis of personal judgment, a method we have used with success in other fields, especially when several individuals expressed independent judgments, the present paper reports briefly on that basis. This appears the more desirable, since no apparatus was developed for assessing the *shape* of the individual spots, and though such an apparatus is imaginable, it was not made.

In Plate 2, Figure D shows an axial view of four terminal (third in a clutch of three) eggs of the Laughing Gull, showing how definitely the spots are arranged in a wreath or crown with a bare center. Figure E shows the four "first" eggs of the same clutches, and Figure F shows the four "second" eggs.

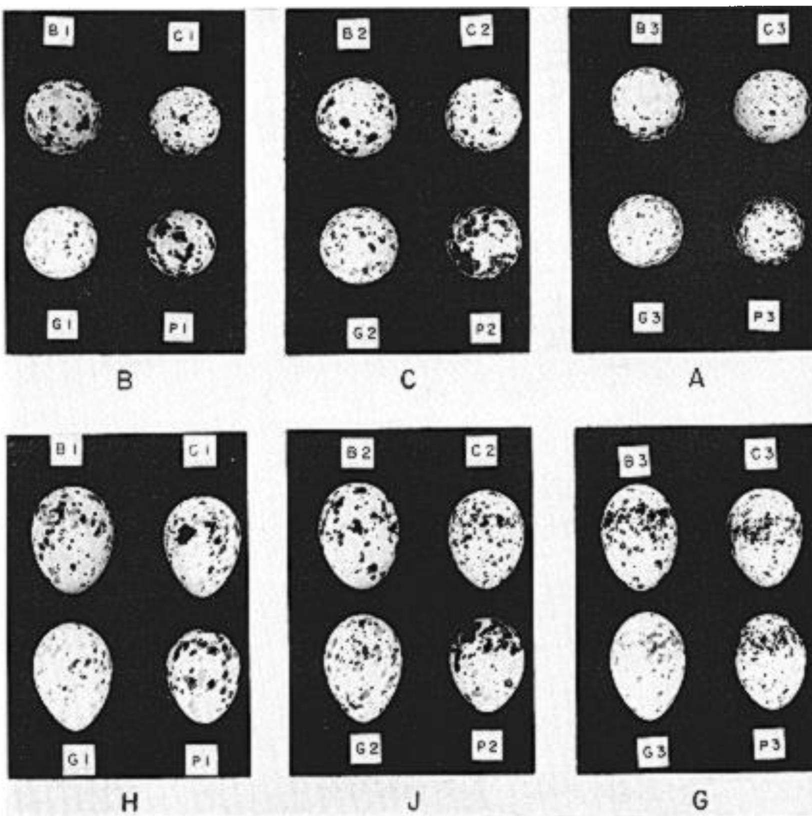
In Plate 3, Figure A shows an axial view of four terminal eggs of the Common Tern. Figure B shows the corresponding "first" eggs, and Figure C the corresponding "second" eggs.

Not all nests show the phenomenon so clearly, but it is frequent enough that it is worth some brief discussion.

In Plate 2, Figure K shows the more conventional aspect (profile view) of the same terminal eggs of the Laughing Gull. Figure L shows



EGGS OF THE LAUGHING GULL. (E) Paraxial and (L) profile views of the first eggs in clutches K, L, N, and C. (F) Paraxial and (M) profile views of the second eggs of the same clutches. (D) Paraxial and (K) profile views of the third (terminal) eggs of the same clutches.



EGGS OF THE COMMON TERN. (B) Paraxial and (H) profile views of the first eggs in clutches B, C, G, and P. (C) Paraxial and (J) profile views of the second eggs of the same clutches. (A) Paraxial and (G) profile views of the third (terminal) eggs of the same clutches.

the corresponding first eggs, and Figure M the corresponding second eggs.

Figures G, H, and J do for the Common Tern what Figures K, L, and M do for the Laughing Gull.

It will be observed, most easily in the conventional views, and more especially in the Laughing Gull eggs, that many of the "spots" are elongated, vermiform markings, and that this is particularly the case with the terminal eggs.

The present paper is concerned with the question whether there is a significant difference between the first, second, and third eggs in respect either of the vermiform nature of the markings or the tendency of the spots to form a definite "wreath." The fact that sometimes the spots are uniformly distributed and sometimes tend to form wreaths has been a matter of occasional comment for generations past. So far as I know, the variation has not previously been ascribed to the position of the egg in the clutch-sequence.

Since the judgments reported below are subjective, it was thought advisable to use as observers persons having no great knowledge of birds' eggs or the results expected, but otherwise of high intelligence and trained scientific minds. It is not to be expected that two or more observers will agree closely with one another, but it *is* to be expected that each observer will be reasonably *self* consistent. If several observers were used, it would be necessary to run an "analysis of variance" on the findings, to discover how much of the variation was due to observers and how much to eggs. This seemed too ambitious a project in the present instance. In the case of the first observer reported below, all the Laughing Gull eggs were presented to her once (some sixty of them) and seventeen of them were presented twice without her knowledge. She was quite consistent on her ratings of these seventeen eggs.

LAUGHING GULL EGGS

(a) How much of the overlay-pigmentation consists of spots and blotches, and how much of "scribble-marks"?

The observer was Mrs. Effie Young, a graduate chemist: the interviewer was Miss Mary Gemperle, also a graduate chemist. The test material in the first test consisted of fifteen clutches of three eggs each, known to be complete clutches, of the Laughing Gull. The eggs were presented one at a time, with no comparison material available, and the order was random. As previously mentioned, seventeen of the eggs were presented twice, with very good consistency. Table 1 summarizes the results.

TABLE 1

LAUGHING GULL EGGS. PROPORTION OF SPOTS AND SCRIBBLES

	Mrs. Young's Estimates (in per cent)		
Position in the Clutch	*"Spots and Blotches"*	*"Scribble Marks"*	*Standard Deviation*
First	84.2	15.8	27.6
Second	67.3	32.7	37.2
Terminal	16.9	83.1	23.3

Note: The Standard Deviations here reported have little real meaning, because the distribution is not Gaussian, and in some cases three-quarters of the total variance is contributed by a couple of non-conforming eggs, so that in the absence of these two eggs, the standard deviation would fall to one-half its value.

The same observer now operated in the same fashion on four clutches of two eggs each, from nests that were left long enough to make sure that no third egg was going to be laid. Table 2 summarizes the results.

TABLE 2

	Observer's Report (in per cent)	
Position in Clutch	*"Blotches"*	*"Scribble Marks"*
First	94.5	5.5
Terminal	63.7	36.3

This group is too small for satisfactory statistical analysis. So far as it goes, it suggests that the terminal egg of a two-egg clutch resembles the second egg of a three-egg clutch rather than the terminal egg of a three-egg clutch. This is rather surprising in view of our (1953) finding that the terminal egg of a two-egg clutch resembles in *shape* the terminal egg of a three-egg clutch, not the second egg thereof.

A second observer, Mr. R. Daum, a college student with no knowledge whatever of previous work on the subject, was tested by myself (FWP) some twelve months after the test of Mrs. Young. The eggs were presented in random order, and the test was repeated using another random order. Frequently the observer repeated his estimate exactly, but differences of five or ten percentage points were common, larger deviations being rare. Table 1A gives the outcome.

The results for the two-egg clutches are given in Table 2A. Again each egg was presented twice. The test was actually made by mixing

TABLE 1A

LAUGHING GULL EGGS. PROPORTION OF EQUIAXIAL SPOTS AND BLOTCHES (IN PER CENT). MR. DAUM'S ESTIMATES ON THREE-EGG CLUTCHES

Position in Clutch	*First Test*	*Second Test*	*Average Result*	*Mrs. Young's Estimate*
First	81.0	85.8	83.4	(84.2)
Second	72.1	77.6	74.8	(67.3)
Third	30.6	31.4	31.0	(16.9)

TABLE 2A

LAUGHING GULL EGGS. MR. DAUM'S ESTIMATES (IN PER CENT)

Position in Clutch	*Proportion of Spots and Blotches Average of Two Tests*	*c.f. Mrs. Young's Estimates*
First	80	94.5
Second	77	63.7

the eggs in with the three-egg clutches, but the results are reported separately.

It is clear that the "personal equation" has come into play in a somewhat consistent fashion: the two observers have interpreted either their instructions, or their observations, somewhat differently. They agree, however, on all essential points, viz., that the third egg differs greatly from the other two in pigmentation: that the second differs a little from the first, in the direction of adumbrating what the third will be like: that in a two-egg clutch (so far as our small sample shows) the second egg tends to resemble the first rather than the second, contrary to its behavior in the matter of *shape*.

(b) Do the spots, blotches, and scribble-marks form a recognizable crown or wreath? How well developed is it?

Table 3 reports Mrs. Young's findings on this point.

TABLE 3

WREATH FORMATION

Fifteen Three-Egg Clutches		*Four Two-Egg Clutches*	
First Egg	Mere trace of a wreath	First Egg	Trace
Second Egg	More definite trace	Second Egg	Trace
Third Egg	Fairly well developed		

Thus in this respect also the second egg of a three-egg clutch is nearer to the first egg than it is to the third egg, a phenomenon that

QUANTITATIVE ESTIMATES OF THE DISTRIBUTION OF PIGMENTATION

The methods so far outlined in this paper represent subjective judgments of unbiased observers. In other fields we have found such techniques often surprisingly consistent and very useful, but most ornithologists and other men of science would prefer quantitative information, and some would prefer instrumental information.

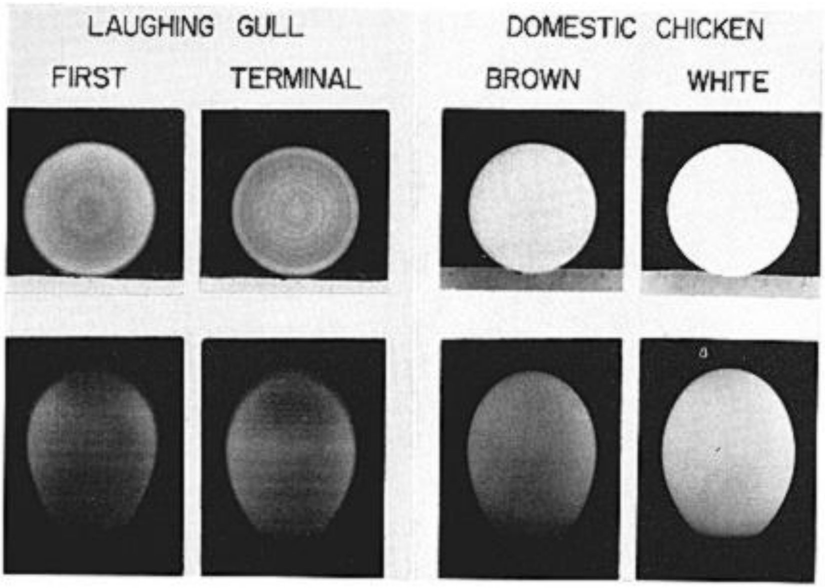
One method by which quantitative results could be obtained is this: we might mount the egg in such a way that it can rotate on its axis, and we might draw real or imaginary pencil lines on it to represent "parallels of latitude." Then we could measure quantitatively how much of a given parallel passes through dark spots and how much is over relatively light background or "ground-color." If this were done for a sufficient number of parallels and for a sufficient number of specimens, we could report a distribution curve of (spot) pigmentation characteristic of the first, second, and third eggs of a sequence.

A modification of this might consist in taking photographs and ruling pencil lines on the photographs.

Such methods are time-consuming but might very likely prove quite accurate. What we actually did was somewhat more ambitious, and somewhat less easily reduced to an absolute measure of pigmentation, but theoretically capable of greater accuracy.

Pigmentation Profiles of Eggs.—These were obtained as follows: four clutches of Laughing Gull eggs that showed well the differences between pigmentation of the first and last eggs, viz., clutches K, L, N, and C, were chosen (these are the same that are illustrated in the "still" pictures), and the first and last eggs of the clutch were used. A special device had been made that permitted us to mount the egg, supported only by a tiny area at the small end, with its axis vertical and big end up. After the egg had been "trued up" so that its own axis coincided closely with the axis of the device, it was rotated at constant speed around this axis by a small gear motor, the egg making about one revolution per second. The egg was illuminated by a somewhat elaborate system of lights, translucent diffusing screens, and matt reflecting screens, so as to destroy all obvious "high lights," and make the egg appear uniformly illuminated all over. This uniformity was sufficient for visual purposes, but, photometrically, truly uniform illumination is perhaps impossible and was not achieved here. The background was black velvet, out of focus.

The camera, the gear motor, the illuminants, the screens and accessories were mounted in a specially-made apparatus, so that the same positions, degree of illumination, distribution of illumination,



PARAXIAL (*top*) AND PROFILE VIEWS OF ROTATING EGGS. Left to right, a composite photograph of the first eggs in clutches K, L, N, and C of the Laughing Gull; a composite photograph of the third (terminal) eggs of the same clutches; and brown and white eggs of the domestic chicken.

and so on could be accurately recovered, in spite of the necessity for taking photographs horizontally and vertically, and in spite of the necessity for changing eggs. Although absolute perfection was not obtained, we believe that the photographs can validly be compared with one another to illustrate the points of the present paper.

The purpose of rotating the egg was to smear out individual spots into a circular ring, as seen from above along the direction of the axis, or into a horizontal band as seen from a horizontal position. When egg K_1 had been photographed, L_1 was substituted and without moving the camera or film, its image was photographed on top of that of K_1. Then N_1 and C_1 followed, so that we had a composite picture of all four eggs, the total exposure being divided equally among them. The final result is an averaging out of the individual spots of all four eggs. One photograph was taken vertically (axially) and consists of a pattern of diffuse circular rings. A second was taken horizontally, and shows the outline of an egg, with a pattern of horizontal bands. See Plate 4.

The same experiment was repeated for the third eggs of the same clutches, viz., K_3, L_3, N_3, and C_3. In addition we obtained negatives of a white chicken egg and a brown chicken egg, of substantially the same size. All told we now had eight negatives.

These were taken to the Physics Department of the University of Pittsburgh, where Dr. T. M. Donahue and Dr. W. M. Benesch ran transmission curves on an automatic Leeds and Northrup microdensitometer. The curves were generally obtained in duplicate, by running the instrument both forward and backward across the film, and the results agreed extremely well, and showed a great deal of detail. This detail is irrelevant to our purposes and appears to represent an ability on the part of the instrument to pick out the traces of many individual spots, in spite of the rotation. It seems also to have detected, in the longitudinal "profile" views, the hole through which the egg was blown, since this is, photometrically, essentially a black spot.

Figure 1 shows the curves obtained from the (negative) photographs of the profiles (longitudinal views) of rotating eggs. A is for a brown egg of a domestic chicken, B is an average, or composite, of four "first" eggs of the Laughing Gull, and C represents the terminal eggs thereof.

Since the detail is somewhat beside the point in the present paper, we give in Figure 2 a smoothed-out version of these curves, emphasizing the major features.

Microdensitometer curves were run also on the paraxial photo-

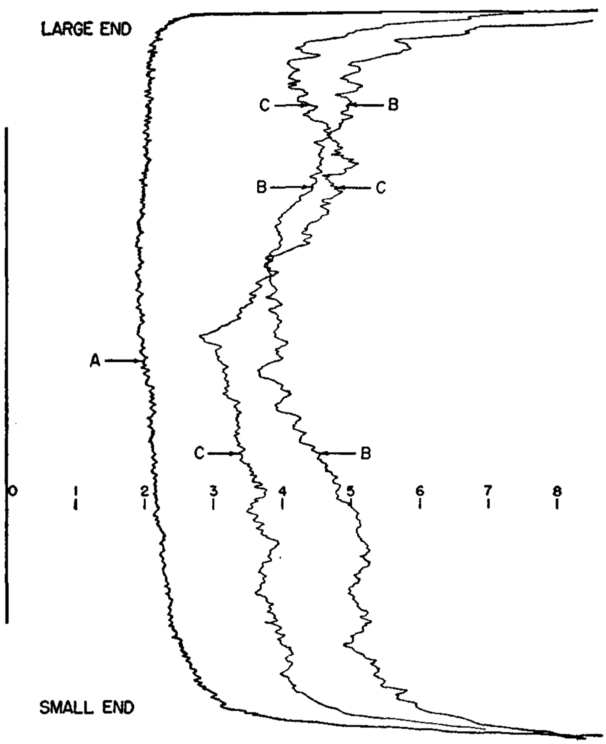


FIGURE 1. Microdensitometer curves from top to bottom of the longitudinal profiles of rotating eggs. A. Brown egg of the domestic chicken. B. Composite of the first eggs of clutches K, L, N, and C of the Laughing Gull. C. Composite of the third (terminal) eggs of the same clutches.

graphs, and some of these are shown in Figure 3, while the smoothed-out versions are given in Figure 4.

The scale of intensity which the instrument actually records is a little complicated. What the microdensitometer actually measures is the amount of *blackening* in the *negative* of the egg. It does this by passing a fine pencil of light through the negative and measuring the fraction of the light that is *transmitted*. Thus if I_0 is the original intensity, i.e., the intensity of light in the complete absence of the photographic film, and if I is the intensity that is transmitted, what we record is the fraction I/I_0. This has the value of zero if the negative is absolutely black and opaque, and the value unity if the negative is absolutely transparent.

For many physical measurements, it is convenient to work with the "extinction coefficient," which we may call "z," and the instrument is calibrated in these terms. The scale at the foot of Figures 2 and 4 is a scale of z. Its relation to the transmission is given by

$$I/I_0 = e^{-z} \qquad \text{or} \qquad \log_e (I/I_0) = -z \tag{1}$$

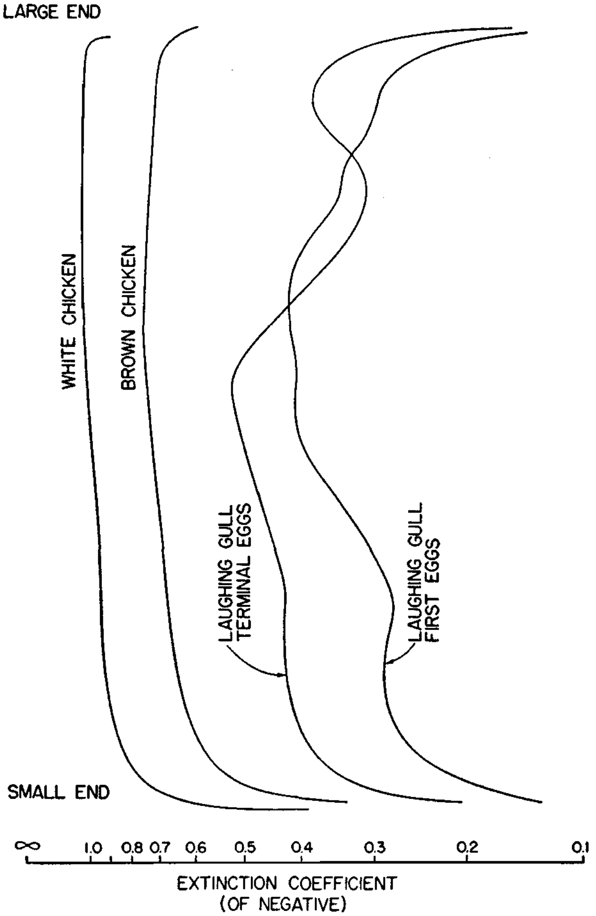


FIGURE 2. The same curves as in Figure 1, but simplified and smoothed out, and the curve for a white egg of a domestic chicken added.

	Extinction Coefficient	*Per Cent of Light Transmitted*
For example, if	$z = \infty$	$I/I_0 =$ 0
	$z = 1.0$	36.8
	$z = 0.8$	44.9
	$z = 0.6$	54.9
	$z = 0.4$	67.0
	$z = 0.2$	81.9
	$z = 0.1$	90.5

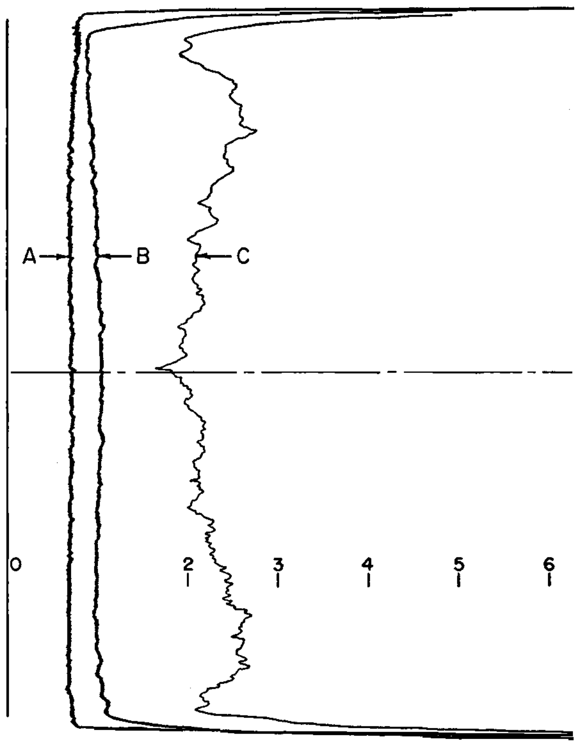


FIGURE 3. Microdensitometer curves from paraxial photographs of the large ends of rotating eggs. A. White egg of the domestic chicken. B. Brown egg of the domestic chicken. C. Composite of the third (terminal) eggs of clutches K, L, N, and C of the Laughing Gull.

The fraction of light transmitted through the *negative* is the fraction that would be blocked by a perfect *positive*, and hence, subject to all the peculiarities of the photographic process, is an indication of the intensity of pigmentation of the eggs. The relationship, as we have said, is not simple, and in particular is not a direct measure of the number or size or concentration of spots; for the spots themselves are not absolutely black, the background is far from being white, and the illumination is not absolutely even, nor identical in the axial and profile views.

The important point is that the various eggs may be compared with one another with some approach to quantitative accuracy. The further the curve moves to the right, the greater the degree of pigmentation of the egg.

Note that at the extreme margins (the margins of the circle in the axial view and the top and bottom in the profile view) there are two further sources of inaccuracy. First, the eggs, though rotating nearly true on their axis, did not rotate absolutely true, and secondly, the eggs are not all of absolutely identical size, so that, where com-

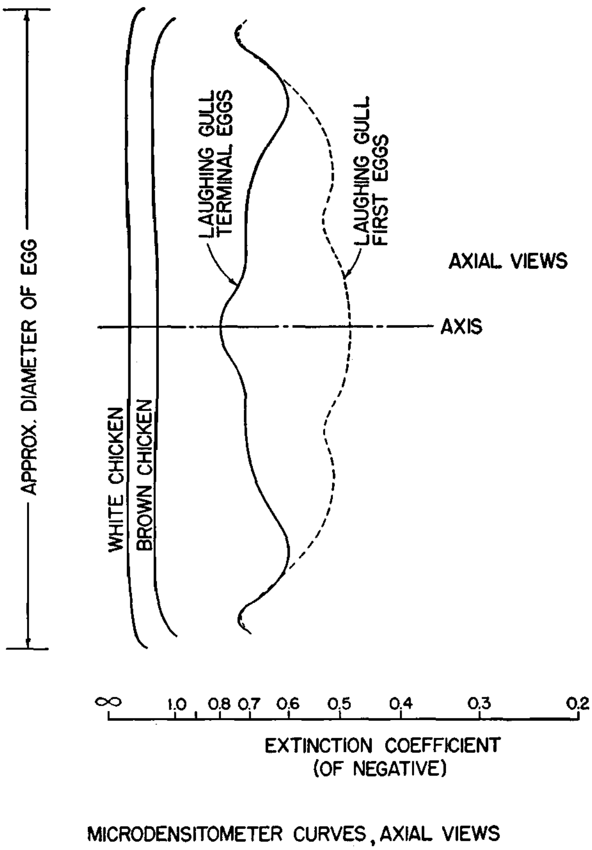


FIGURE 4. The same curves as in Figure 3, but simplified, smoothed out, and corrected for light distribution. A smoothed-out graph for the first eggs of clutches K, L, N, and C of the Laughing Gull is added.

posite or "average" pictures are involved (and this means all rotating gull egg pictures), we were sometimes photographing eggs and sometimes black velvet.

Referring now to Figures 2, 3, and 4, it is clear that while the brown chicken egg is somewhat "darker" than the white egg, the Gull eggs are considerably darker than the chickens'. But what is more interesting is the fact that on the whole the curve for the "last" eggs of the Laughing Gull lies to the left of the curve for the "first" eggs. This means that it is markedly less pigmented, a point that no one had noticed by visual observation. We knew that the spots were arranged differently and that the individual spots were of a different shape, but had not recognized that there was substantially less total pigment.

We may note further that the curves cross, and that there is a zone where the third egg is markedly *more* pigmented than the first. This zone extends (as seen in side view) from about one-eighth to about one-third of the length of the egg, measured from the blunt end. This is the zone of the "wreath" around the large end.

Referring next to Figure 4, the axial view, we see that over the "pole" or center of the blunt end, the third egg is much lighter in pigment than is the first egg. This is due, in part at least, to the egg being "bald on top" and substantially freer of spots in this area than the first egg, but presumably the background may also average a little lighter. A curious thing, if it were valid for a large number of eggs and not merely for four, is that the last egg tends to show a dimple in the extreme center where the first egg shows a definite bulge. This might have a physiological interpretation and not be a mere accident.

Once more referring to Figure 2, it appears possible, after allowing for the slightly less illumination "south of the equator," that there is a trace of a wreath, in both first and terminal eggs, but more definitely in the first, south of the equator as well as north of it.

The samples, of course, are not large enough to warrant any broad conclusions, and the above notes merely indicate that a quantitative tool is available for much more detailed examination of the intensity of pigmentation and its distribution than is possible from visual inspection.

I wish to acknowledge the help of Professor T. M. Donahue and Professor W. M. Benesch, both of the Physics Department, University of Pittsburgh, in running the microdensitometer curves; the help of Mr. T. R. Schuerger of the Preston Laboratories in preparing the negatives from which the curves were made, and the *negatives* of Plates 2 and 3; and my wife's help in the field with the eggs and in the Laboratory typing and experimenting. The *positives* were prepared by Mr. Paul Wolfe, of Butler, in all cases. The tracings from which Figures 1 to 4 were made were prepared by Mr. R. R. Lehnerd, of the Laboratory.

SUMMARY

In our own collections of the eggs of Laughing Gulls and Common Terns, and probably somewhat generally with these species and some others, the last egg of a clutch of three tends to show a very definite "wreath" or "crown" of spots circumscribing the large end

of the egg: that is to say, the spots are arranged in a belt "north of the equator" of the egg, but leaving the "north pole" area, the big end of the egg, essentially free from spots, while the first egg of a clutch tends to have the spots uniformly distributed all over the egg. The second egg tends to be intermediate but more nearly resembles the first than the third. Further, as is particularly evident in the Laughing Gull, the individual spots tend to be equiaxed in the first egg, but elongated into sausage-shaped marks and even "scribbles" in the third egg.

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