SEASONAL DEVELOPMENT OF THE INCUBATION PATCH IN THE STARLING

By James A. Lloyd

In many birds, prior to egg laying and incubation, feathers of parts of the ventral surface fall out and the skin becomes highly vascularized and edematous. While the ventral surface is in this condition, it is called an incubation patch. The incubation patch in passerine birds extends from the anterior edge of the breast to the cloaca and laterally to the median edge of the ventral feather tracts. During development of the incubation patch, there is an increase in size and number of capillaries and smaller blood vessels supplying the ventral abdominal region. The increased vascularity gives the skin a deep reddish color. As vascularity increases, the tissue becomes more edematous and there is loss of feather papillae, dermal muscle bundles, and subdermal fat. The edema seen in the fully developed incubation patch is characterized by swelling and disorganization of collagen fiber arrangement, increase in leucocytes, and the presence of large spaces between the tissues. The edema of the fully developed incubation patch can be seen grossly, the skin appearing transparent and highly folded as well as denuded of feathers. The cells of the stratum germinativum of the epidermis multiply rapidly, become larger, and have rounder nuclei than usual. A typical histologic section taken from a nonbreeding bird can be seen in figure 1*a* while figures 1*c* and 1*d* depict sections of fully developed incubation patches. Further descriptions of the incubation patch may be found in Faber, 1826; Lange, 1928; Freund, 1926; Koutnik, 1927; and Bailey, 1952.

The present study comprises part of an investigation designed to determine the effects of environmental stimuli on incubation patch development and presents details of the seasonal development of the incubation patch in the European Starling (*Sturnus vulgaris*). The study was supported in part by Public Health Fellowship MF-10, 043-cl. It is part of a dissertation submitted to the school of Hygiene and Public Health of the Johns Hopkins University in partial fulfillment of the requirements for the degree of Doctor of Science.

METHODS

The procedures used include observations of nesting birds, collection of birds in the field, autopsies with preparation of tissues for histological examination and techniques designed to quantify development of the incubation patch.

Behavioral observations were made on two woodlots. Wooden nest boxes 15 × 6 × 5 inches were marked with identifying numbers and nailed to trees in the area. Birds gained access to a box through a hole three inches in diameter near the top. Examination of the boxes was made possible by means of a hinged roof. Detailed observations were made between dawn and 10:00 p.m. Eastern Standard Time from February through June for three years. Birds were collected throughout the breeding season and samples of skin were taken during each phase of the breeding cycle.

Two hundred and fifty birds, with known behavioral histories, were shot or taken from nest boxes at night. Age and sex were determined by techniques described earlier (Davis, 1960). At autopsy the ventral abdominal surface was examined for loss of feathers, existing feathers were plucked out, and the skin was peeled off with a razor blade and stapled to a piece of paper toweling and placed in 10 per cent formalin for 24 hours.

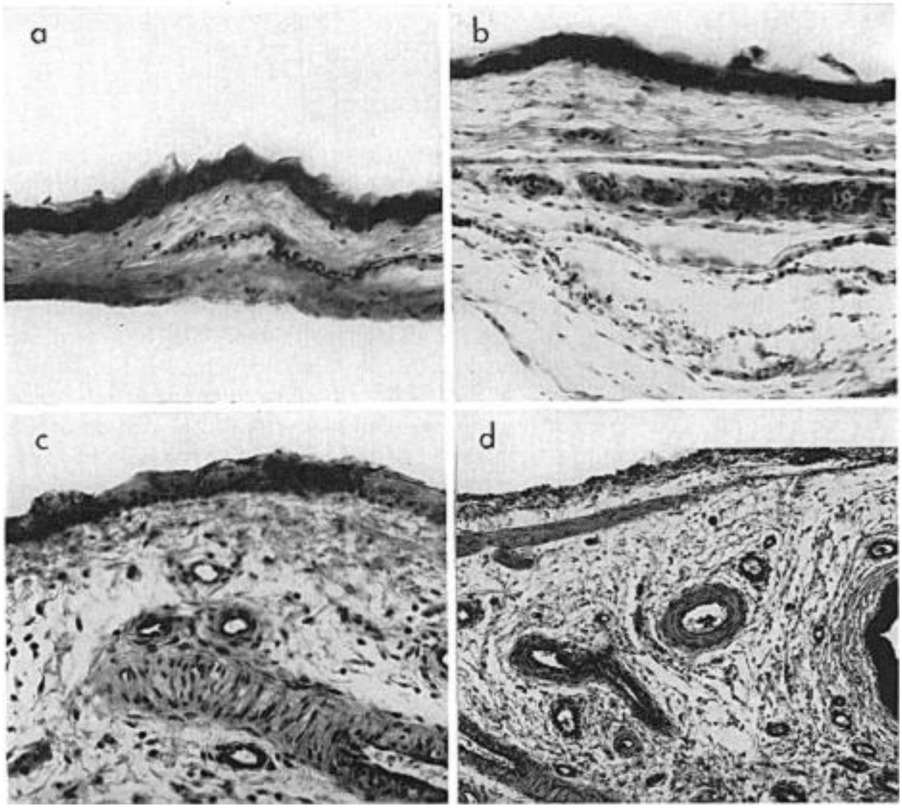

Fig. 1. Sections of skin of brood patch representing different stages of edema; *a*, stage 0, ×300; *b*, 3, ×300; *c*, 5, ×300; *d*, 5, ×125.

A piece of tissue 4 mm.^{2} was removed from the central portion of each fixed skin sample, embedded in paraffin, sectioned at 8 micra and stained with Harris' haematoxylin and eosin dyes. Degree of incubation patch development was determined by measurements of vascularity, edema, and epithelial thickening.

Measurements of vascularity were made on serial sections of the skin samples and involved counting the blood vessels present in each section, totaling the number of sections examined, and calculating a mean number of blood vessels for each section of the tissue. Nine pieces of skin were taken at different locations from each of five males and five females. Examination by analyses of variance of data obtained from these samples indicated that there was not a position effect with respect to vascularity. In order to determine the precision of the counts 50 samples were recounted some time after original counts were made. The original and second counts were compared and a calculated standard deviation of precision was found to be 1.49. Results of these tests are summarized in table 1.

An arbitrary numerical scale was devised to describe the amount of edema present. In this scale 0 indicated no edema (fig. 1*a*). In tissues graded 3, collagen fiber disorganization was evident, spaces were present between the fibers and there was evidence of leucocyte proliferation (fig. 1*b*). Edema of the fully developed

TABLE 1

SUMMARY OF ANALYSIS OF VARIANCE OF DATA OBTAINED FROM COUNTS OF BLOOD VESSELS MADE ON SAMPLES TAKEN AT VARIOUS POSITIONS ON THE VENTRAL ABDOMINAL SURFACE

Source of variation	Degrees of freedom	Mean square	
Between birds	4	930.8	
Between positions	8	4.15	> .05
Observational	32	4.03	
Standard deviation of precision calculated on recounts		1.49	

incubation patch was graded 5 (figs. 1*c*, 1*d*). Regradings of edema were made on sections used for recounts of blood vessels and the second edema gradings corresponded closely with the originals.

The number of cell layers present in the epithelial layer was recorded as a measure of the thickness of the epithelium. In skin of the nonbreeding bird this layer is one cell thick, whereas in the fully developed incubation patch it may contain three or four layers (figs. 1*a*, 1*c*, 1*d*).

In comparing groups of birds a mean value for edema, a mean number of cell layers in the epithelium, and a mean of the average number of blood vessels per section of tissue were calculated for each group. These measures were used as indices to designate the degree of development of the incubation patch and were associated with data gathered at autopsy and from behavioral observations.

RESULTS

Breeding biology of the Starling.—In late February, activity increases in nesting areas as males defend nest sites with loud piercing "scream-like" vocalizations, vigorous wing flapping, chases and mid-aerial fights. Several weeks later courtship of females begins with females investigating nest sites and both sexes engaging in aerial chases, characterized by the close pursuit of one bird by another while making "chattering-like" vocalizations. Nest building is done chiefly by the female, but there may be some participation by the male. Copulation may occur at any time of the year, but it is seen most frequently immediately before egg laying.

Egg laying occurs shortly after mid-April with the laying of one egg each day until the clutch is completed. Incubation begins with the laying of the last egg and is generally done by females, although males may sit on eggs for brief periods while females are absent from the nests. Females also do most of the feeding of the young, but males also participate to some extent.

Young leave the nest about the twenty-first day and wander about on the ground for several weeks, often accompanied by the adult female. Later, young and adults form into large flocks which move into communal roosts. About a month to a month and a half after the first nesting cycle has begun a second one involving fewer birds is started. More detailed descriptions of the seasonal behavior of the Starling may be found in Freitag (1939) and Kessel (1957).

Seasonal development of the incubation patch.—Incubation patches developed in both sexes but the degree of development in the male was never as great as in the female. In mid-March an appreciable increase in numbers, but not in size, of blood vessels occurred in the ventral abdominal surface of females (table 2). No other changes were evident in the skin of the ventral abdominal surface until a day or two

TABLE 2
LEVELS OF INCUBATION PATCH DEVELOPMENT IN BREEDING STARLINGS TAKEN FROM THE FIELD

Date	Females					Males				
	N	DF (per cent)	E	ED	V ± SE	N	DF (per cent)	E	ED	V ± SE
March										
1– 7	11	0	1.0	0	6.3 ± 0.96	9	0	1.0	0	5.5 ± 0.83
8–14	12	0	1.0	0	7.0 ± 0.52	7	0	1.0	0	4.3 ± 0.85
15–21	15	0	1.0	0	17.5 ± 0.92	8	0	1.0	0	7.0 ± 0.84
22–28	15	0	1.0	0	16.2 ± 1.0	9	0	1.0	0	6.0 ± 0.63
29–Apr. 4	10	0	1.0	0	18.5 ± 0.93	10	0	1.0	0	9.0 ± 1.1
April										
5–11	8	0	1.5	0	15.4 ± 1.1	5	0	1.0	0	7.5 ± 0.95
12–18	10	75	3.0	3	16.5 ± 1.6	7	50	2.0	1	10.1 ± 1.4
19–25	20	100	4.0	5	29.1 ± 3.1	10	100	3.0	3	15.5 ± 1.5
26–May 2	10	100	4.0	4	17.7 ± 1.2	8	100	3.0	2	12.2 ± 1.1
May										
3– 9	8	100	4.0	2	12.8 ± 1.9	7	100	2.0	1	7.9 ± 0.95
10–16	10	100	3.0	2	14.5 ± 1.0	5	100	2.0	1	6.5 ± 1.5
17–23	6	100	2.0	2	12.1 ± 1.6	5	100	2.0	1	7.8 ± 1.3
24–31	5	100	2.0	2	8.2 ± 1.2	5	100	1.0	1	6.3 ± 1.1
June										
2– 8	10	100	1.0	1	7.3 ± 0.51	5	100	1.0	1	4.4 ± 1.4

Legend: DF = per cent of sample without feathers on ventral abdominal surface; E = mean number of cell layers in the epithelium; ED = mean edema value; V ± SE = mean number ± STD error of blood vessels per examined section.

before egg laying when the feathers fell out of this area, the tissue became edematous, the epithelium thickened, and the numbers and size of the blood vessels increased (table 2; figs. 1*c*, 1*d*). The incubation patches attained maximal development in the first week of incubation and subsequently there was a gradual disappearance of edema and vascularization coinciding with growth of the young (fig. 2). When young were fledged, the skin of the parents' ventral abdominal surfaces was dry and scaly and remained so until the fall molt when regrowth of feathers occurred in the region. The patch redeveloped if birds became involved in the second nesting cycle.

DISCUSSION

The seasonal development of the incubation patch in the Starling differs somewhat from that of species studied by Bailey (1952). Observing the White-crowned Sparrow (*Zonotrichia leucophrys*), Bailey noted that loss of feathers from the ventral apterium occurred about a day or two before egg laying and that feather loss was followed by an increased vascularity of the region. In the Starling, the sequence of events is somewhat different in that increases in vascularity of the skin of the ventral abdominal surface were observed in females about one month prior to egg laying. At egg-laying time, loss of feathers occurred and there was also a second increase in vascularity which was much greater than the increase observed earlier. Hinde (1962) in studies of the domesticated canary observed that feather loss was closely related to nest building rather than egg laying and that vascularization increased while defeathering was in progress. Selander and Kuich (1963) observed in icterids that increased dermal thickness and vascularity occurred before egg laying, concomitant with feather loss, and that greatest increases in vascularity occurred during egg laying. Dermal thickness in icterids was well developed at the time of egg laying and

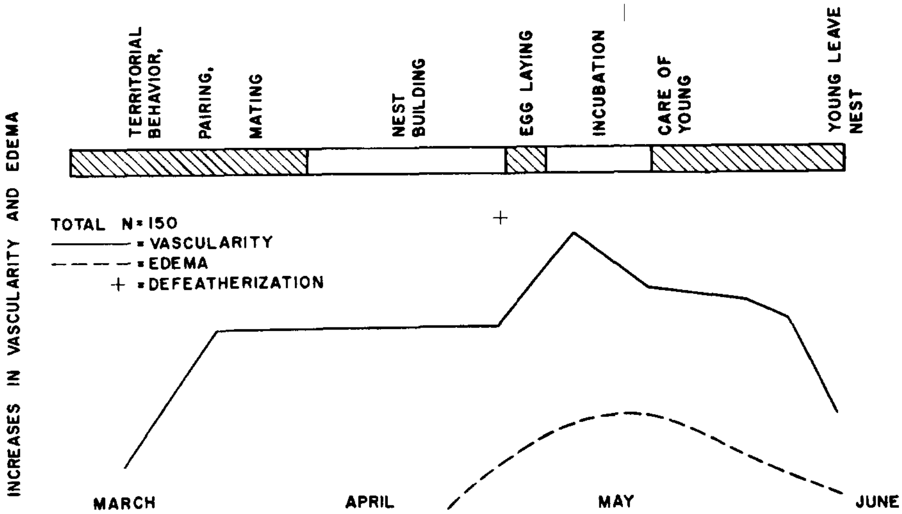

Fig. 2. Relationship of incubation patch development to behavior of the Starling.

increased during incubation. These results parallel closely the observations made on the Starling in the present study.

It is possible that environmental stimuli may affect physiological processes involved in the development of the incubation patch. Seasonal light changes are known to affect gonadal physiology of birds (see Burger, 1947). Effects of photic stimuli on avian gonadal physiology are also known to be enhanced by the presence of birds of the opposite sex and by presence of nest boxes and nesting materials (Burger, 1953). Exposing Starlings to fifteen hours of light a day during winter months resulted in increased vascularity of the ventral apterium. This response was enhanced by the presence of nesting materials (Lloyd, 1964).

The presence of an incubation patch in nesting male Starlings and its absence in unmated males suggests that involvement in certain behavioral patterns may have some influence on the development of the male incubation patch, while other evidence suggests a similar influence in the female. Bailey (1952) did not observe incubation patches in males of species in which males do not incubate. Hinde (1962) observed that vascularization prior to egg laying and edema occurred only in birds which subsequently incubated eggs. Attempts to induce incubation patches in parasitic cowbirds were not successful (Selander and Kuich, 1963). Female Starlings taken after egg laying had occurred always had fully developed incubation patches, but in male Starlings incubation patches were confined to nesting birds. In caged Starlings, females developed incubation patches at the same time as egg laying occurred in the natural population, whereas none of the caged males showed any sign of patch development (Lloyd, 1965). In two instances in the present study copulating pairs were shot and both the male and female were obtained. In both cases, the females had fully developed incubation patches, but the males showed no patch development. Both pairs were taken after egg laying had occurred in the population. These results suggest that in both sexes of the Starling involvement in nesting may have some effect on the activation of physiological processes leading to the development of the incubation patch but that such involvement is more essential for patch development in the male.

SUMMARY

As part of a study of the effects of environmental stimuli on physiological processes associated with incubation patch development, the seasonal development of the incubation patch in the Starling (*Sturnus vulgaris*) was investigated and was related to behavior associated with the breeding cycle.

Increases in vascularity of skin taken from the ventral abdominal surface of females were seen about one month before egg laying began. Marked increases in vascularity, development of edema, epithelial metaplasia and loss of feathers occurred a few days before egg laying, reached peak development about midway through incubation, and progressively declined after eggs hatched. In the male, development of the incubation patch followed a course similar to that of the female but in lesser degree. During the reproductive period, females taken from the study area always had an incubation patch, but incubation patches in males were seen only in nesting birds.

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School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland, April 7, 1964. (Present address: *Research Laboratories, Division of Endocrinology and Reproduction, Albert Einstein Medical Center, Philadelphia, Pennsylvania.)*