THE NATURAL TERMINATION OF THE REFRACTORY PERIOD IN THE WHITE-CROWNED SPARROW

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In a number of the avian species in which testicular development can be evoked by increased daily photoperiods it has been found that such development cannot be sustained indefinitely and that there is a period following each cycle of development during which increased daily photoperiods are ineffective. This period is commonly designated as the refractory period. Much of the available information concerning this phenomenon has been reviewed by Burger (1949) and Miller (1954). In the course of experiments in our laboratories on the photoperiodic control of gonadal and migratory cycles in the Gambel race of the White-crowned Sparrow, *Zonotrichia leucophrys gambelii,* it has been necessary to ascertain quite precisely the time of natural termination of the refractory period. The results of our studies of this phenomenon now prove to be supplemental to those obtained by Miller (1954) on this subspecies in his investigation of the refractory period in three subspecies of *Z. leucophrys* and in the Golden-crowned Sparrow, *Z. coronata*. However, since our experiments involved numbers of *Z. l. gambelii* considerably greater than were available to him, it would appear useful to publish some of our data and observations. The investigations reported here were supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

THE EXPERIMENTS

The experimental and control birds were captured with Japanese mist nets in the vicinity of Pullman, Washington, during September and in the Snake River Canyon about 15 miles from Pullman in October. The birds were held in large outdoor aviary cages until the experimental treatments were begun. The environmental temperatures to which these were subjected were similar to those of the wild controls but usually were 3°C. lower. The six experimental groups were placed in experimental cages in a well ventilated indoor aviary according to the schedule and in the numbers indicated in table 1. Each cage received 40 to 70 foot candles of continuous light from incandescent lamps for 15 hours per day. The temperature of the aviary could not be controlled. Consequently the temperature to which the later groups were subjected during the 5-week period of treatment were lower than those to which the earlier groups were subjected (table 1). However, our earlier studies (Farner and Mewaldt, 1952) indicate that whereas these lower temperatures might result in a slightly lower rate of photoperiodic response, the time at which the response begins is not altered. Water, grit, and nutritionally adequate food were provided *ad libitum*. Weights were obtained and recorded weekly; weekly inspections of the plumage were made and the observations recorded. After 34 days the birds of each group were killed and autopsied; the gonads were placed in alcohol-formaldehyde-acetic acid fixing mixture. After thorough impregnation with the fixing mixture, the testes were weighed rapidly on a Roller-Smith precision balance to the nearest fifth of a milligram. For histologic examination one testis from each male was imbedded in paraffin and sectioned at 8 micra; the sections were stained with acid hematoxylin and yellowish eosin.

RESULTS AND DISCUSSION

The responses, as indicated by changes in testicular weights and spermatogenic activity, are summarized in table 2. The first-year and adult birds are not treated separately since we found no differences in the time of termination of the refractory period.

In this respect our observations are in accord with those of Miller (1948, 1954) on *Z. coronata* and *Z. leucophrys,* as well as with those of Wolfson (1952) on the Slate-colored Junco (*Junco hyemalis*), and, apparently also, with those of Schildmacher (1938) on the European Redstart (*Phoenicurus phoenicurus phoenicurus*). In this respect it is interesting to note that Riley (1936) found young male English Sparrows (*Passer domesticus*) to be non-refractory in fall, an observation consistent with that of Davis (1953) who has reported cases of gonadal development among first-year English Sparrows in late spring. However, Vaugien (1952) has found young English Sparrows

Table 1

Experimental Groups

Group	Period of treatment	Total number	Adult males	1st-year males	Mean temperature in °C
A	Sept. 17–Oct. 22	9	1	2	25
B	Sept. 27–Oct. 31	12	2	4	22
C	Oct. 11–Nov. 14	12	1	4	18
D	Oct. 25–Nov. 28	12	3	6	15
E	Nov. 8–Dec. 12	12	2	6	15
F	Jan. 1–Feb. 4	15	6	6	10
Control	....	8	3	5	....

to be refractory in summer. With respect to *Z. l. gambelii,* it still remains to be established whether there is an earlier period in which the young males will respond to increased photoperiod; here, the possibility of a pituitary response without an accompanying gonadal response should be considered. At present it can only be said that the young males in fall show refractoriness at least superficially identical with that of the adult males and that this refractoriness terminates at about the same time.

In the histologic aspects of our investigations we first studied a normal series of testes from wild-taken birds and from birds held in large cages under natural conditions of light and temperature. An ocular micrometer was used to obtain a series of measurements of seminiferous tubule diameters and diameters of nuclei of Leydig cells. These testes were found to conform closely with the description of testicular development in this race published by Blanchard and Erickson (1949). However, we were unable to delineate their stages 1 and 2 which differ only in the presence of Leydig cells in the latter. It is possible that our disagreement is a matter of what should be designated as Leydig cells. In wild specimens taken in fall we found scattered nuclei which, although somewhat smaller in diameter (3.5–4.1 micra compared to 5.5–6.0 micra in late winter and spring), otherwise resemble those of the Leydig cells as described by Blanchard (1941) and Blanchard and Erickson (1949). Certainly this is a matter which requires further investigation; very possibly the technique employed by Marshall (1951) would produce enlightening results. For the purposes of this paper we are retaining the stages of testicular development proposed by Blanchard (1941) except that we combine stages 1 and 2, designating this "combined" stage as 1–2. The histologic data from the experimental groups are summarized in table 2. The differences between the adult and first-year testes, both in the experimental and control groups (except in weight in fall and winter), are slight, as noted also by Blanchard and Erickson (1949) and consequently have not been treated separately.

Our data (table 2) indicate that the time at which refractoriness terminates, allowing for individual variations, comes during a period encompassing the last two weeks of October and the first week of November. This is almost identical with the conclusion reached by Miller (1954:16).

Although our data are only suggestive, it appears possible that the refractory period in *Z. l. gambelii* may not be a single phenomenon. The basis for this suggestion is the apparently greater diameters of the nuclei of the Leydig cells without accompanying tubular responses in groups A, B, and C as well as in some of the individuals in group D compared to those of birds obtained in the wild during the same period (table 2). The mean of nuclear diameters of the Leydig cells from wild birds is significantly smaller

Table 2

Testicular Responses to 15-Hour Daily Photoperiods

Group	Weight response				Mean diameter seminiferous tubules in micra	Mean diameter Leydig cell nuclei in micra	Stage of testicular development[4]
	Positive[1]	Negative	Range; mg.	Mean			
A	0	3	0.8–1.8	1.3	54	4.1	1–2 only
B	0	6	0.8–1.6	1.1	45	4.0	1–2 only
C	0	5	0.6–3.0	1.4	58	4.5	1–2 only
D	3[2]	6[3]	1.2–50	16	62[5], 245[6]	3.9[5], 5.3[6]	6 in 1–2[5]; 2 in 4[6]; 1 in 5[6]
E	8	0	17–140	47	226	5.3	7 in 4; 1 in 6
F	12	0	14–210	104	298	4.6	1 in 3; 3 in 4 5 in 5; 3 in 6
Control	0	8	0.4–3.6	1.7	49	3.8	1–2 only

[1] Combined weights of both testes greater than 10 mg.
[2] 2 first-year, 1 adult. [3] 1 first-year, 5 adult.
[4] According to Blanchard (1941) except that stages 1 and 2 are not distinguished separately (see text).
[5] Negative weight response. [6] Positive weight response.

($P<0.05$) than the mean for those experimental birds in which the seminiferous tubules showed no ($P>0.05$) response. An alternative explanation, which could be studied experimentally, might be that of a slower response of the tubules to gonadotropins. However, since the development of the tubules and the development of the Leydig cells (Nalbandov, Meyer, and McShan, 1951) apparently involves different gonadotropic hormones, the possibility of separate states of refractoriness with respect to the production or release of individual gonadotropic hormones merits consideration.

Our observations of the plumage of the experimental birds show, with considerable individual variation in extent and pattern, a rather consistent molt response to the 15-hour daily photoperiods. Without regard to extent of the molt, and including both males and females, the positive responses were as follows: group A, 9 out of 9; B, 12 out of 12; C, 12 out of 12; D, 11 out of 12; E, 12 out of 12; F, 14 out of 15. We have found the prenuptial molt in wild controls and caged controls with natural daily photoperiods to begin in mid-March. These results are apparently similar to Miller's (1954) observations on a small series of *Z. leucophrys* and his (1948, 1954) extensive series of *Z. coronata*. There is certainly ample reason to believe, as Miller (1954) suggests, that this experimentally induced molt is not associated with the gonadal response. However, these data do suggest the possibility that the pituitary, although refractory with respect to the light-induced release of gonadotropic hormones, may not be refractory with respect to other light-induced functions, assuming, of course, that the pituitary is involved in the mechanism of the molt induced by increased daily photoperiods. Obviously the mechanism of the induction of this molt must be a subject of further research.

Because our experiments were designed primarily to ascertain the time of natural termination of the refractory period as indicated by testicular response, we unfortunately do not have data on fat deposition for direct comparison with the observations of Miller (1954). However, it may be of some interest to comment on trends in the weights of the experimental males. In considering these trends it is necessary to bear in mind that the relationship between changes in body weight and changes in the amount of fat deposited, as indicated by our unpublished data involving quantitative lipid extraction, is not necessarily a simple one. In general it may be said that groups A and B showed no real tendency toward increases in weight. In group C, in which there were no positive testicular responses, several of the birds showed an initial increase in weight of the order of 2 to 3 grams; in most of these there was a subsequent decline, often to a final weight lower than the initial weight, as the molt developed. In this respect it should be noted

Table 3

Body-weight Responses to 15-Hour Daily Photoperiods

Group	Initial weight; mean in grams	Greatest recorded increase in weight; mean in grams	Difference between final weight and initial weight; mean in grams
A	30.0	0	—1.0
B	25.9	—0.4	—1.8
C	28.4	2.0	—0.4
D[1]	29.4	0.7	—0.6
D[2]		1.7	—3.1
E	27.4	2.7	0.5
F	25.5	3.4	2.6

[1] Negative testicular response. [2] Positive testicular response.

(Farner, Mewaldt, and King, 1954) that both caged and wild birds under natural conditions do not show increases in weight in spring until after the conclusion of the prenuptial molt in late March or early April; there is a strong positive correlation between this increase in weight and the quantity of deposited lipids. The changes in weights in group D, for those with positive and negative testicular responses alike, were similar to those of group C. In groups E and F, particularly in the latter, the tendency toward increased weight appeared to be more pronounced and to persist more steadily throughout the 5-week period despite the development of the molt. These observations suggest the possibility that increased daily photoperiod may cause an increase in weight somewhat before the end of the refractory period, as indicated by testicular response, and that the effect of increased photoperiod, with respect to weight, may increase as a funcion of time after the end of the refractory period. They also suggest that molt and weight responses, which are sequential under natural conditions in spring, can develop simultaneously under experimental conditions.

CONCLUSIONS

On the basis of increases in testicular weights and tubular diameters in response to 15-hour daily photoperiods, it may be concluded that the refractory period in male *Zonotrichia leucophrys gambelii* reaches its natural termination, with individual variations, during the last two weeks of October and the first week of November. Apparently there are no essential differences between first-year and adult birds in this respect. A variable molt is usually induced by increased daily photoperiods in both refractory and non-refractory individuals.

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