EXPERIMENTS ON PHOTOPERIODIC REGULATION OF THE TESTIS CYCLE IN TWO SPECIES OF THE THRUSH GENUS *HYLOCICHLA*

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Species of the North Temperate Zone have been used as subjects in most laboratory studies of the timing of migratory and reproductive cycles in wild birds. Rowan (1925, 1929) investigated the effect of artificially lengthened days on the Slate-colored Junco (*Junco hyemalis*), and since his work was reported various species of *Junco* and *Zonotrichia* have been studied extensively (see reviews by Farner, 1959; Wolfson, 1959; Wolfson and Winchester, 1960).

Such birds winter in the North Temperate region, where day length (effective photoperiod) decreases gradually until 21 December, and then increases until 21 June. The changes in physiological condition that occur shortly before the vernal migratory activity commences have been related to the lengthened photoperiods that the birds experience, since these physiological changes have been induced in the laboratory, out of season, by means of treatment with increased daily duration of light.

Rowan's studies led to the hypothesis that increasing day length is the environmental factor that releases migratory behavior in birds. A weakness in this hypothesis has been well stated by Wolfson (1959), who pointed out that birds that winter in the tropics experience very slight alterations in day length, and that birds which winter in the Southern Hemisphere arrive on the wintering grounds at a time when day length is increasing, and depart when it is decreasing. The latter group consequently commences northward migration under day length conditions opposite to those experienced by birds that winter in North Temperate regions. Few studies have been made on such transequatorial migrants. Engels (1959, 1962) has reported results obtained from work on a limited number of Bobolinks (*Dolichonyx oryzivorus*). As the criterion for determining response to experimental day lengths, he used bill color. This turns from dull yellow to black as the male changes from the sexually inactive to the sexually active condition. Engels concluded that in this species post-breeding photorefractoriness exists, which is terminated by short days (10- to 12-hour photoperiods), and that recovery occurs on long days (14-hour photoperiods).

The experiments reported here were designed to determine whether a preparatory phase (photorefractory phase) exists, and if it does to learn something of its nature and regulation, in the thrush genus *Hylocichla*. This genus was selected because it includes both North Temperate (*H. guttata*) and transequatorial (*H. ustulata, H. minima*) migrants. Thus, in a single genus available for study, there are species which have evolved

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similar migratory habits, yet which seemingly are regulated by opposite environmental conditions.

The original plan of the study was to investigate the northward-wintering Hermit Thrush (*Hylocichla guttata*), on the one hand, and the tropical- and transequatorial-migrating Swainson's Thrush (*H. ustulata*) and Gray-cheeked Thrush (*H. minima*), on the other. This plan had to be modified, due to the difficulty of maintaining the latter two species in captivity. Probably significant results were obtained for the Hermit Thrush, but those for the Swainson's Thrush were insufficient to be more than suggestive, and results were entirely lacking for the Gray-cheeked Thrush.

MATERIALS AND METHODS

Hermit Thrushes and Swainson's Thrushes were trapped during late September and early October. They were caught in a drip trap about one kilometer west of Lake Michigan, in a wooded section of Winnetka, Illinois. Birds were removed from the trap within two hours of capture.

After capture the birds were identified, banded, and weighed. Weights were taken on a balance sensitive to 0.1 gm. The birds were kept in Hendryx double breeding cages 50 cm long (this being the dimension of the "front" of the cage) × 27.5 cm wide × 25 cm high, which could be joined at the ends. They were housed in a basement room with artificial light of natural duration until the beginning of the experiment.

Food and water were always available. The basic food was Gaines homogenized dog meal (General Foods Corporation), with enough water added to form a very soupy mixture. It was essential that the food be kept fluid, so that the birds would not damage their bills while prying at a crusty layer. Supplementary food consisted of wild berries in season, sliced green grapes, sliced bananas, *Tenebrio* larvae twice a week, and a commercial preparation for thrushes and Robins, Geisler's Mynah Bird Food (Geisler's Bird Foods, Omaha, Nebraska). The latter was sprinkled daily on top of the dog food. In addition, grit was available from the floor of the cage. The thrushes drank freely from cups that hung on the cage walls. Whenever water was available in larger dishes on the floor, the birds bathed.

From the time of capture, in September or early October, until the beginning of the experimental lighting, the thrushes were kept under the day length they would have received during their normal fall migration, or approximately 13 hours of light. Natural day length was computed using civil twilight, since I have found that thrushes in nature have an equivalent, but not coincident, period of activity. Studies made in northern Lower Michigan show that nesting Veeries become active in the morning about 35 minutes after commencement of civil twilight and settle in for the night about 32 minutes after termination of civil twilight (Annan, 1961). Computations of day length and civil twilight were based on the United States Naval Observatory's "Tables of Sunrise, Sunset, and Twilight" (Eckert and Clemence, 1945).

TABLE 1

COMPOSITION AND TREATMENT OF EXPERIMENTAL GROUPS*
1956–58

Group	Compo-sition	Initial Condition		Alteration		Terminal date
		Date	*Schedule*	*Date*	*Schedule*	
1.	Hg-6/4 Hu-4/0	4 Nov. '56	15L-9D		No change	20 Feb. '57
2.	Hg-6/5 Hu-4/0	4 Nov. '56	9L-15D	5 Jan. '57	15L-9D	20 Feb. '57
3.	Hg-5/2 Hu-7/0	26 Oct. '57	15L-9D		No change	1 Mar. '58
4.	Hg-10/8 Hu-8/1	26 Oct. '57	9L-15D	2 Jan. '58	15L-9D	1 Mar. '58
5.	Hu-6/2	20 Sep.– 1 Oct. '57	13L-11D		No change	1 Mar. '58

* L = light; D = dark; Hg = Hermit Thrush; Hu = Swainson's Thrush. In the second column, the number of birds of each species that started and that completed the experiment is shown. In the fourth and sixth columns, the number of hours of light and darkness in each 24-hour period is given.

The birds were housed in a classroom building on the Evanston campus of Northwestern University. The 9-hour room (lighted from 0700 to 1600 hours) had artificial illumination only. A 7.5-watt bulb remained lit for 10 minutes after the main lights were out and allowed the birds to settle for the night. The main illumination in all rooms was from 40-watt white fluorescent bulbs, with additional exposure in the 15-hour room to natural daylight. Temperature and humidity were not controlled. Temperature fluctuated between 13°C (56°F) and 28°C (about 83°F), remaining near 20°C (about 70°F) most of the time. The birds were disturbed only during cage care or the weekly weighing periods.

At the end of October, 1956, they were divided into two groups. One group was kept on long photoperiods (15 hours of light per day) until the end of February, to test for the existence of a photorefractory period requiring long nights for its termination. Such a photorefractory period would not end under exposure to 15-hour days, and so the birds in this group would not be expected to show any gonadal recrudescence, even though experiencing long days.

The other group was placed on a long night schedule (15 hours of darkness per day), until the first week of January, and then given a 15-hour photoperiod until the end of February. Should a photorefractory period of the sort postulated exist in these birds, the period of long nights would allow it to terminate (as suggested by the work of Wolfson, 1952b). The sub-

TABLE 2

GONADAL RESPONSE, 1956–57*

Bird	*Experimental treatment*	*Autopsy date*	*Gonadal Measurements*			*Stage*
			Testes in mm	*Weight in mg*	*Volume in mm*	
Hg-2	LD	16 Jan. (74)	1.8 × 1.0 1.8 × 0.9	1.2	1.70	-
Hg-18	LD	20 Feb. (109)	1.5 × 1.0 1.3 × 0.8	1.1	1.22	1
Hg-8	LD	20 Feb. (109)	1.9 × 1.0 1.6 × 0.9	1.2	1.68	1
Hg-3	LD	20 Feb. (109)	1.5 × 0.9 1.4 × 0.9	1.2	1.23	1
Hu-9	LD	18 Jan. (76)	1.7 × 1.4 1.7 × 1.1	—	2.82	-
Hg-15	SD-LD	19 Feb. (62 + 46)	10.4 × 5.6 9.2 × 5.7	264.2	327.2	5
Hg-10	SD-LD	20 Feb. (62 + 47)	6.8 × 4.2 7.4 × 4.2	97.6	131.1	5
Hg-16	SD-LD	20 Feb. (62 + 47)	8.8 × 5.0 8.3 × 5.2	177.6	232.7	5
Hu-7	SD-LD	7 Nov. (3)	1.9 × 1.1 1.9 × 1.0	1.8	2.20	1
Hu-14	SD-LD	24 Nov. (20)	2.2 × 1.1 2.0 × 1.2	—	2.90	1

* Species abbreviations are those of Table 1. All birds but Hu-9 were immature in the fall. Two experimental treatments are indicated: LD for birds which were retained on long days (15L-9D) throughout, and SD-LD for birds which were treated with short days (9L-15D) for two months before being put on long days. Under each autopsy date is given the number of days of experimental treatment the bird received. If two treatments were employed, the duration of each is indicated. In testis measurements, the left testis is described first. Weights in mg and volumes in cubic mm are given for the combined testes. Reproductive condition of the testes is given following the criteria of Wolfson (1942).

sequent period of long days would be expected to bring the male birds into a state of active spermatogenesis.

The procedure was repeated in 1957. In addition, a control experiment was run to test responses of birds kept on an "equatorial" day length (13L–11D) throughout the winter. Numbers of birds used and the composition of each group are given in Table 1.

In addition to regular determination of body weight, the amount of fat visible in the region of the furculum, the lower back, and the abdominal region was estimated as *none*, *light*, *medium*, or *heavy*, following the criteria of Wolfson (1942), except that the most conspicuous indication of the *heavy* condition was an overflow of fat from the interclavicular fossa.

TABLE 3

GONADAL RESPONSE, 1957–58*

Bird	*Experi-mental treat-ment*	*Autopsy date*	*Gonadal Measurements*			*Stage*
			Testes in mm	*Weight in mg*	*Volume in mm*	
Hg-44	LD	29 Nov. (35)	1.7 × 1.1 1.7 × 1.0	1.6	1.97	1
Hg-67	LD	12 Feb. (110)	1.8 × 0.9 2.0 × 0.8	1.5	1.43	1
Hg-57	LD	1 Mar. (126)	1.5 × 1.0 1.4 × 0.9	1.6	1.37	1
Hg-58	LD	1 Mar. (126)	1.6 × 1.0 1.2 × 0.8	1.6	1.24	1
Hu-47	LD	29 Nov. (35)	1.9 × 1.2 1.7 × 1.4	1.8	3.17	1
Hu-40	LD	16 Dec. (52)	1.4 × 0.8 1.4 × 0.8	0.8	0.94	1
Hg-47	SD-LD	1 Mar. (68 + 58)	8.6 × 4.6 7.7 × 5.2	161.0	204.3	5
Hg-62	SD-LD	1 Mar. (68 + 58)	8.4 × 5.0 —	143.1	191.0	5
Hg-60	SD-LD	1 Mar. (68 + 58)	5.8 × 3.6 5.0 × 3.7	63.8	75.2	4
Hg-55	SD-LD	1 Mar. (68 + 58)	2.4 × 1.6 2.9 × 1.7	7.8	7.61	regressing
Hg-54	SD-LD	1 Mar. (68 + 58)	10.1 × 5.4 —	256.9	300.4	5
Hu-32	SD-LD	29 Nov. (35)	2.0 × 1.2 1.7 × 1.2	2.0	2.79	1
Hu-25	SD-LD	29 Nov. (35)	2.3 × 1.1 2.2 × 1.2	2.0	3.12	1
Hu-38	SD-LD	21 Feb. (68 + 51)	1.8 × 0.7 1.7 × 0.9	1.1	1.18	1

* Notes as in Table 2.

Measurements were made of the length and width of the testes, using an ocular micrometer. Testes were weighed on torsion balances with sensitivities of 0.2 and 0.02 mg. Volumes of testes were obtained from a table of volumes of ellipsoids. One testis from each bird was prepared for histological study, and the other retained intact. Sections were made at 10 microns, and stained with haematoxylin and eosin. The stage of spermatogenesis of the testis was judged according to the criteria of Wolfson (1942), in which Stage 1 represents the minimum testis, and Stage 5 the testis in breeding condition.

Results

The results are given in Table 2 for the 1956–57 experiments, and in Table 3 for those of 1957–58. At the conclusion of the experimental periods, the Hermit Thrushes that had been kept on long days for 14 weeks had testes in the inactive (Stage 1) condition, and of minimum size. In many birds the testes were smaller than those obtained from immature specimens taken in fall migration.

Hermit Thrushes which had been kept on a schedule of short days for two months, followed by six to eight weeks of 15 hours light per day, had large testes, in Stage 5. One Hermit Thrush (Hg-55) had regressed testes.

Body weight remained uniform from the date of capture until the change was made from short days to long days, and then tended to drop, and to remain low. In spite of this weight loss, which is attributed to conditions of captivity, all of the birds but one retained some fat. The exception was Hg-55, the thrush with regressed testes. Increase in weight, resulting from fat deposition, is considered to be a necessary preparation for migration (see Farner, 1959, for review). The difficulties experienced in keeping thrushes in captivity make weight data of questionable value in this study.

Discussion

The experiments indicate that the Hermit Thrush has a post-breeding period of photorefractoriness, similar to that demonstrated (Wolfson, 1952b) in certain fringillids with which it shares its wintering range. This refractoriness may be terminated by six weeks of long nights, after which the birds will respond by gonadal recrudescence to lengthened photoperiods. The Hermit Thrushes that were kept on long days from October to late February showed no gonadal development, whereas the birds that received six weeks of long nights, followed by long days, reached an advanced stage of gonadal growth, even to the production of motile spermatozoa. These results are similar to those obtained in Slate-colored Juncos and White-throated Sparrows (*Zonotrichia albicollis*) subjected to the same variations of photoperiod. This might be expected, in view of the geographical distribution of the Hermit Thrush in winter.

The Swainson's Thrushes showed a refractory period in the fall, similar to that found in the Hermit Thrushes. Treatment with long days from capture until late February did not induce any gonadal growth in the one bird so treated that survived. On the other hand, the single male Swainson's Thrush that experienced photoperiods which proved stimulatory for Hermit Thrushes did not respond. The sample is obviously too small to be highly significant, and work on Swainson's Thrushes is continuing.

Since the Hermit Thrush has a photorefractory period, and since there is some indication of one in the Swainson's Thrush, it seems probable that the Gray-cheeked Thrush and the Veery have similar periods. Regulation in these species might be expected to differ from that in the Hermit Thrush, since they winter near the equator, where daily dark periods do not exceed 11¼ hours. Even during the fall migration the nights experienced by these birds are shorter than 11¼ hours.

Since in nature the three species of *Hylocichla* that migrate to the tropics winter under relatively short nights, it is likely that 11¼ hours of darkness daily over a long enough time would suffice to dissipate the refractory period. Despite the fact that day length remains constant, the 12¾ hours of light received daily should be effective in bringing the birds into condition for migration. Day length of 12 hours and 25 minutes has been shown to be sufficient for spermatogenesis to resume in the tropical Red-billed Dioch, *Quelea quelea* (Disney *et al.*, 1961; Lofts, 1962).

The majority of the birds of the three species of *Hylocichla* migrating to the tropics winter within the same latitudes. That these birds move north at the same time may indicate that their migratory impulse is triggered by the same physiological and environmental factors. This would be in accord with Wolfson's summation hypothesis (1952a, 1959), which relates the duration of the effective daily photoperiod to the rate of response that occurs in the bird, and consequently to the number of days required to bring the bird into physiological migratory condition. A longer time might be required for the birds wintering in the tropics to complete the preparatory phase of the gonadal cycle, since they experience shorter nights than do birds wintering in the North Temperate Zone. Following this, the progressive phase of the cycle would require a shorter time, since once preparation was complete the tropical-wintering birds would experience longer day lengths than their more northerly-wintering relatives. Such a longer refractory period in transequatorial migrants has been suggested by Farner (1954), and is supported by Engels' (1959) study of captive Bobolinks. Recent work on Bobolinks from Wolfson's laboratory (Wolfson and Westerhoff, 1960) indicates that this refractory period is photoperiodically controlled.

Hermit Thrushes move north through the United States about three weeks before the "tropical" migrant thrushes of the genus *Hylocichla*. Most Hermit Thrushes leave Louisiana by 1 April, while the other species do not reach that state in any numbers until 20 April (Lowery, 1955). At this time the latter have already been on the move for about three weeks. Thus it appears that all four species initiate their northward movement on about the same date. Such coincidence of timing of migration may be explained by a balance that may exist between preparatory and progressive phases of the gonadal cycle. The tropical-wintering thrushes, which receive shorter nights than the Hermit Thrush, would require a longer period of preparation. On the other hand, the Hermit Thrush, prepared for response earlier by long nights, does not experience an equivalent total of light until later in the spring. This balancing of the two variables—an initial requirement for long nights, followed by a requirement for long days—may explain the initiation of migratory behavior in both Temperate Zone and transequatorial groups on about the same date, without the postulation of differing internal mechanisms.

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Summary

Hermit Thrushes showed a refractory period in the fall, during which long days did not result in gonadal response. They responded to various photoperiods in the same ways as White-throated Sparrows and other migratory birds that winter in the southern United States. Male Hermit Thrushes captured in October in the Chicago area were brought into full (gonadal) breeding condition two months ahead of the natural time for free birds, when they were first treated for two months with 15-hour nights and then put on 15-hour days. When no preparatory period of long nights was experienced, Hermit Thrushes kept on 15-hour days did not respond.

Swainson's Thrushes showed a refractory period in the fall similar to that found in the Hermit Thrush, but the sample size was inadequate. Further work on Swainson's Thrush is being done.

An attempt is made to explain the similarity of timing of migration in birds that winter under different photoperiods. It is suggested that the shorter the daily dark period experienced by the birds, the greater the number of days needed for this preparation might be. It is further suggested that the long nights experienced by such birds as the Hermit Thrush bring about readiness to respond to long days several weeks before these long days actually occur. By these means, a single mechanism of photoperiodic regulation may be sufficient to account for the northward spring migration of birds that winter in the Southern Hemisphere, as well as of birds that winter in the southern United States.

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