GONADAL RESPONSES OF BROWN-HEADED COWBIRDS TO LONG DAYLENGTH

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Experimental work with several species has shown that the gonads of wild birds develop from sexual inactivity into breeding condition when the birds are kept on increased hours of daily light in the winter months before the natural breeding season. The effect of the long daily photoperiods on the experimental birds is thought to be similar to the gradual gonadal development in wild birds during the slowly increasing daylengths of spring. The birds of temperate regions have probably evolved the photoperiodic response rather than a response to other environmental conditions because of the greater predictive value of daylength in timing the breeding season (Farner, 1959, 1964). Energy is conserved by remaining in a sexually inactive condition until a few weeks before the breeding season. The breeding seasons of the birds are thought to be timed to the occurrence of abundant food for their young, and daylength in early spring provides a reliable source of information about the coming of spring. As the time of nesting of host species is determined by daylength, it is of interest to know whether the development of the gonads in spring in brood parasites is determined to the same degree by daylength. The Brown-headed Cowbird (*Molothrus ater*) was studied to compare the gonadal response of a brood parasite to long daylengths with the photoperiodic responses of nonparasitic species. In addition, the significance of the refractory period as a timing factor in the annual cycle of temperate-zone songbirds was evaluated by a comparison of the breeding seasons and the timing of the refractory period in four species of North American icterids.

METHODS AND MATERIALS

Brown-headed Cowbirds were caught in central California and were held captive in outdoor aviaries in Berkeley, California. All of the birds were identified as members of the small race of cowbirds (*M. a. obscurus*), which is resident in central California throughout the year. First-year males were aged by the presence of retained juvenal coverts under the wing. Unpolished brown rice was fed *ad libitum*, and this food was supplemented with canary seed, rodent meal, and ground dry dog food. Water was available for drinking. After the birds had been held captive on natural daylengths for at least a week, some of them were released into experimental cages and were given 17 hours of light each day (LD 17:7). One of these experimental cages, located in Strawberry Canyon, was 8 by 7 by 7 feet and was illuminated by six incandescent lamps of 75 watts each. The other cage, located at the Animal Behavior Research Station of the University of California, was 8 by 8 by 7 feet and was illuminated by four five-foot fluorescent lamps. Control birds were released into outdoor aviaries on natural daylengths. The birds were laparotomized before and after each experiment, and the left gonads were measured to the nearest 0.1 mm with calipers inserted into the body cavity. Testis volumes were calculated from the expression for an ellipsoid, $V = \frac{4}{3} \pi a b^2$ where a is half the length and b is half the width of the testis. The diameters of the largest ovarian follicles were measured in the females.

RESULTS AND DISCUSSION

Development of the Gonads in Spring

The annual cycle of gonadal activity of wild cowbirds was studied for four years by dissection of birds taken in the field. Several hundred males and females were collected in all months of the year in central California. The annual cycle of the Brown-headed Cowbirds in California will be described in detail elsewhere, but the main features may be summarized here. At the end of winter nearly all birds molt many of the feathers of the head. Both first-year and adult males retain minute testes less than 2.0 mm in length until March, and females show no ovarian development until late March. During the breeding season the testes of most males are larger than 100 mm^3. The laying season extends from the middle of May into late June in the dry inner coast range of California and into July in the irrigated Central Valley. The gonads of the birds regress in July and August, and the postnuptial molt extends from August to October.

Captive cowbirds were kept in Berkeley in 1964 and in 1965 to determine the gonadal cycle in captivity. The birds were kept in large outdoor aviaries and were laparotomized at about monthly intervals. Captive birds have an incomplete prenuptial molt of the head feathers in February and early March. Data on the growth of the testes of the captives in spring are shown in figure 1. In March the testes of four adult males and of four first-year males were all shorter than 2.5 mm and were all smaller than 10 mm^3. By May the left testis of most of the males was nearly 100 mm^3 in volume, and the birds retained enlarged testes into June. Males in the outdoor cages sang from April through June. Of the two males that were kept through August on natural daylength, one (YP) had minute testes 1.7 mm long on 31 August and the other male (YW, an adult) had a yellow, regressing testis 4.0 mm long. Four adult females were caged with the males and were laparotomized at irregular intervals in spring. In winter in 1964 the largest ovarian follicles of the captive females were only 0.6 mm in diameter; on 29 March they ranged from 0.8 to 1.0 mm; and by 29 April they ranged from 0.9 to 1.7 mm. In August the largest ovarian follicles in each bird were 1.0 mm or smaller. The times of gonadal development in early spring and of regression in summer in the captive male and female cowbirds were similar to the times of gonadal development in wild birds. Although the breeding season of the Brown-headed Cowbirds coincides with the breeding seasons of most of its hosts, some species of hosts such as the Song Sparrow (*Melospiza melodia*) nest before the brood parasites are in breeding condition in spring.

Gonadal Response to Long Daylength

The effect of long days on gonadal development before the breeding season was studied by holding cowbirds on long daylengths in the winter. Seven male and five female cowbirds were kept on 17 hours of daily light in Strawberry Canyon from 8 December 1963 to 3 January 1964. Another group of four males and four females was caged on natural daylength in an outdoor aviary during the same period. On 16 December several of the males in the 17-hour group sang. The effect of the long photoperiod on the gonads is recorded in table 1. Mean testis size of the birds on long daylengths increased from 1.14 mm^3 to 12.5 mm^3, while mean testis size of the birds on natural daylength increased only slightly from 1.05 mm^3 to 1.61 mm^3.

The rates of growth of the testes of the cowbirds may be compared with the rates of growth of testes of birds of different body size in an expression of growth

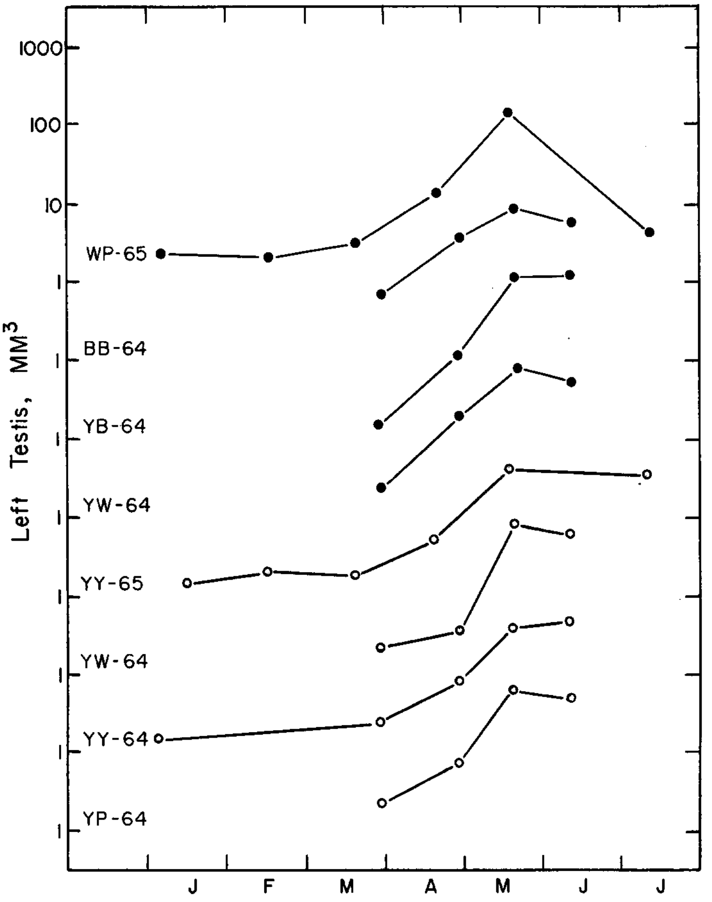

Figure 1. Seasonal variation of testicular volume in captive Brown-headed Cowbirds exposed to natural daylength in spring in Strawberry Canyon, Berkeley, California, displayed in overlapping semilogarithmic plots. Each curve depicts data from a single individual (solid symbols = adults, open symbols = first-year birds) identified by the code at the left.

as a ratio of the size of the testes before and after photostimulation. The expression for a growth-rate constant (k) is $k = \log_{10} (W_t/W_o)\ (1/t)$, where W_o is the initial testis volume or weight and W_t is the weight or volume t days later, and has been applied to several species of birds by Farner (1964). The growth-rate constants k for the testes of the Brown-headed Cowbirds on 17-hour daily photoperiods ranged from 0.033 to 0.068 and averaged 0.045. By interpolation, this value also appears to describe approximately the rate of testicular growth in cowbirds studied by Middleton and Scott (1964), although the photoperiod was shorter (LD 15½:8½) in the latter experiments. In general, the rate constants for cowbirds in my investigations were somewhat lower than those estimated by Farner (1964) for several species of nonparasitic songbirds exposed to 17-hour daily photoperiods. Rigorous comparisons among the sets of data are difficult to make, however, because different methods were used to quantify testicular growth.

TABLE 1

EFFECT OF LONG DAYS ON THE TESTES OF BROWN-HEADED COWBIRDS

Bird	Age	Testis length, mm		Testis volume, mm³		Growth-rate constant, *k*
		Initial[a]	Final[b]	Initial[a]	Final[b]	
Experimental group, LD 17:7						
A	Adult	1.6	3.4	1.21	10.3	0.037
B	Adult	1.8	3.8	1.86	13.5	0.033
C	Adult	1.6	3.5	1.42	13.4	0.049
D	First-year	1.4	3.5	0.60	15.5	0.054
E	First-year	1.4	3.6	1.06	15.9	0.068
F	First-year	1.5	2.7	0.96	6.85	0.033
G	First-year	1.4	3.3	0.89	12.6	0.042
Control group, natural photoperiod (LD 11:13)[c]						
H	Adult	1.5	1.6	0.96	1.42	0.006
I	Adult	1.5	1.8	0.96	1.86	0.010
J	First-year	1.4	1.6	1.06	1.42	0.006
K	First-year	1.6	1.7	1.21	1.75	0.006

[a] 8 December 1963.
[b] 3 January 1964.
[c] Approximate photoperiod, including civil twilights.

The mean growth-rate constant for cowbirds is similar to the mean values found in Tricolored Blackbirds, *Agelaius tricolor* (k = 0.048), and Red-winged Blackbirds, *A. phoeniceus californicus* (k = 0.054), under similar conditions (1965b). The slight differences among the rate constants are not statistically significant. From these data it seems evident that daylength is an important influence on the seasonal development of the testes of cowbirds. This influence does not appear to differ quantitatively in two nonparasitic species of icterids that breed in the same area.

Female cowbirds on 17 hours of light per day developed somewhat enlarged ovaries. On 8 December the largest ovarian follicles in the five experimental females were 0.7, 0.7, 0.6, 0.6, and 0.6 mm in diameter. On 8 January the largest follicles were 1.8, 1.4, 1.6, 1.4, and 1.3 mm in diameter in the same birds. The ovaries of the females on long daylengths were about as large as the ovaries of wild birds taken in April. The largest ovarian follicles in the control birds were 0.7, 0.6, 0.6, and 0.6 mm in diameter on 8 December, and were 1.0, 0.8, 0.7, and 0.7 mm in diameter on 7 January. The development of the ovaries of cowbirds kept in winter on long daylengths to a condition resembling that of wild birds in spring suggests that the long days of spring are responsible for the initial increase in the size of the ovaries of cowbirds at that time. Other species of birds may be affected in a similar way. Farner *et al.* (1966) and King *et al.* (1966) have shown that the initial stages of ovarian growth in the White-crowned Sparrow (*Zonotrichia leucophrys gambelii*) can be induced by long daily photoperiods, but that gonadal maturation, unlike the case in males, does not occur with significant frequency in captive females.

The parasitic Brown-headed Cowbirds are stimulated to undergo gonadal development by some of the same environmental conditions as are their hosts. For example, an important host of cowbirds along the central coast of California is the White-crowned Sparrow, in which testicular growth in spring is under the influence of long daylengths (Miller, 1954; Farner, 1959). As a result of the common photoperiodic response in early spring the cowbirds breed at about the same time as their nesting hosts. However, the photoperiodic response of the cowbirds probably did not evolve as a special adaptation to brood parasitism. The control of sexual cycles by daylength is an adaptation present in most northern temperate-zone songbirds that have been studied in this respect. The cowbirds may have inherited their photoperiodism from their nonparasitic ancestors, or they may have developed the response as a brood parasite independently and under the same selective pressures as in songbirds that raise their own young.

Effect of the Availability of Nesting Hosts

During the breeding season the sexual activity of brood parasites may be determined in part by the availability of suitable host-nests in which to lay eggs. The large variation in clutch size and in the number of eggs laid in a breeding season in a single locality suggests that Brown-headed Cowbirds are indeterminate layers (Payne, 1965a). Since indeterminate layers are birds that vary clutch size in adaptive response to environmental resources, it seems likely that the number of eggs laid by cowbirds is influenced by the availability of host-nests. To test this idea in captive birds, pairs of cowbirds were caged with varying numbers of empty sparrow nests, nests with eggs of other species, nests with both host-eggs and cowbird eggs, and nesting Canaries (*Serinus canarius*). Unfortunately the cowbirds did not lay eggs in captivity, but the following observations may be relevant to the design of future investigations.

One pair of adult Brown-headed Cowbirds was caged alone in an outdoor cage (8 by 7 by 7 feet) from December 1962 to March 1963. A pair of Canaries was released into the cage in March. The Canaries built seven nests and laid eggs in them seven times between March and August, but the cowbirds paid little attention to the nests except for brief inspections by the female. The two pairs were kept together in the same cage through the spring of 1964, and in this year the Canaries built four nests and laid in them. Two other pairs of cowbirds were isolated in large flight cages in January 1964, and a pair of Canaries and also extra nests were kept with each pair of cowbirds from March to August. A fourth pair of cowbirds was kept in a flight cage with empty nests from January through August 1964. The pairs of cowbirds were isolated from each other by opaque partitions between the cages. All male cowbirds sang and were seen bowing to the females in the precopulatory display. No copulations were observed in captive birds. The males in the cages with the Canaries sang and displayed more frequently than did the male with no nesting hosts. Although the Canaries built 16 nests, no cowbird eggs were found in the Canary nests or in the other nests.

The cowbirds were laparotomized on 30 April 1964. The male with no Canaries in the cage had a left testis 3.7 mm long (19.8 mm^3). The males with nesting Canaries had left testes 5.8 mm long (70.0 mm^3), 5.6 mm long (47.0 mm^3), and 6.4 mm long (83.9 mm^3). The female with no Canary had an ovarian follicle 1.4 mm in diameter, while the three females with the Canaries had ovarian follicles of 1.1, 1.1, and 1.6 mm in diameter. By late July none of these cowbirds had laid eggs, and it seemed unlikely that they would lay later in the summer. Laparotomy on 24 July showed a testis 5.2 mm in length in the male with no Canaries, and the males with the nesting Canaries had testes 5.6, 4.8, and 4.6 mm long. The female with no hosts had an ovarian follicle 1.2 mm in diameter, which was no smaller than the ovarian follicles (1.2, 1.2, and 1.0 mm) in the other females. Apparently the captive males were in breeding condition but the females were not.

Refractory Period

At the end of the breeding season the gonads of most species of birds living in temperate regions regress and remain minute and inactive until the next spring. During the first weeks or months of this inactive period, adult birds of most species are unresponsive to long experimental daylengths. The period that follows gonadal regression at the end of the breeding season and in which adult birds are unresponsive to long photoperiods is known as the refractory period. The duration and phasing of the refractory period varies among species and even among some races of the same species (Miller, 1954, 1960; Marshall, 1961). The refractory period is considered to be an adaptation that inhibits sexual activity until the following spring and that thus conserves energy at the other times of the year when an effort at breeding would be unsuccessful.

Onset of the refractory period. Brown-headed Cowbirds that had been held in captivity from two weeks to 10 months on natural daylength were laparotomized on 8 July 1965. Birds with testis sizes ranking first, third, fifth, and so on were then released into the experimental aviary in Strawberry Canyon, where they were exposed to 17 hours of light per day, beginning on 8 July. The males with an even rank were returned to the outdoor aviary. One adult female was kept with each group. The testes of six of the seven males on 17-hour daily photoperiods were initially either in breeding condition or partly regressed and ranged in length from 4.3 to 9.0 mm. The other male in the group (WP) had regressed in captivity from breeding condition in late May (fig. 1) to 2.1 mm on 8 July.

The cowbirds were again examined on 9 August. The lengths of the testes at autopsy are given in table 2. The testes of birds kept on 17-hour daily photoperiods averaged significantly larger than the testes of birds on natural summer daylengths ($P < 0.01$, *t*-test). The birds in the 17-hour group that had enlarged testes in July also had enlarged testes in August, and the testis lengths of these six birds ranged from 4.9 to 7.5 mm. Cowbirds with enlarged testes were all responsive to the experimental long daylengths, as all controls had minute, regressed testes. The other male in the 17-hour group (WP) had a minute left testis, 2.0 mm long. The onset of the refractory period in Brown-headed Cowbirds evidently begins with the complete regression of the testes in summer. While one male had entered the refractory period by the first half of July, most cowbirds probably become refractory in late July or early August, when the testes regress to an inactive condition.

Termination of the refractory period. Cowbirds were examined in late summer and early autumn to determine the time of the year when the refractory period ended. Birds that had been kept on natural daylengths from six weeks to six months were laparotomized on 24 September 1964. Two adult males and three adult females were then switched to 17 hours of daily light at the Animal Behavior Research Station. Four males and five females were kept on natural daylength. The birds were laparotomized again on 21 October. One of the males on long days did not respond to the extra light; the left testis changed but little from an initial 1.8 mm to 1.7 mm in length. This bird was evidently still in its refractory period through October. The left testis of the other adult cowbird increased in length from 1.8 to 5.0 mm. Both birds were in a late stage of molt at the beginning of the experiment, and both had the outer two pairs of primaries incompletely grown. On 21 October the molt had been completed in the unresponsive bird, but the outer primary was still ensheathed in the bird with the large testis. The four males on natural daylength all had testes

TABLE 2

TESTIS REGRESSION IN BROWN-HEADED COWBIRDS IN RELATION TO DAYLENGTH

Photoperiod	n	Testis length, mm[a]		
		Max.	Min.	Mean ± 95% limits
LD 17 : 7[b]	7	7.5	2.0	5.61 ± 1.65
Natural[c]	6	2.2	1.9	2.02 ± 0.105

[a] On 9 August 1965.
[b] 8 July–9 August 1965.
[c] Approximately, including civil twilights, LD 15½ : 8½ initially, 14½ : 9½ finally.

smaller than 1.8 mm long in October. The largest ovarian follicle of one female on long daylengths increased from 0.7 to 1.2 mm, but the other two females developed no ovarian follicles larger than 0.7 mm. The largest ovarian follicles of the females on natural autumnal daylengths (four adults and one first-year bird) ranged from 0.5 to 0.7 mm in diameter. Evidently some individuals of both sexes have passed out of the refractory period and are gonadally responsive to long days by early October.

The four males and three of the females that were held on natural daylength through early autumn were caged on 17 hours of daily light from 21 October to 18 November. The testes of the two adult males increased in length from 1.6 to 3.5 mm and from 1.5 to 3.2 mm, and the testes of the two first-year males increased from 1.5 to 2.2 mm and from 1.4 to 3.7 mm in length. The ovaries of the females increased only slightly, as the original diameters of the largest ovarian follicles were 0.5, 0.6, and 0.6 mm and the diameters in November were 0.7, 0.7, and 0.8 mm. The small response in the females in November suggests that the refractory period may not have been completed by late October in all individuals. The first-year cowbirds had not passed through a postbreeding refractory period, as these young birds had not yet lived through a breeding season. The response of the young birds rather indicates that the photoperiodic response has developed in cowbirds at the age of four or five months.

Marshall (1961) has suggested that the differences among species of birds in the timing of the annual cycles are caused mainly by differences in the annual "internal rhythms," particularly in the most readily measured aspect of such a rhythm, the refractory period. If the breeding seasons of songbirds of the temperate zones are indeed determined directly by the duration and phasing of the refractory period, then one would expect closely related species with different annual cycles to have correspondingly different refractory periods. However, a comparison of the breeding seasons and the timing of refractory periods in the icterids suggests no such correlation. Several species of North American icterids with different annual cycles are now known to terminate the refractory period at the same time of the year. Red-winged Blackbirds (*Agelaius phoeniceus californicus*) are resident throughout the year in central California and breed in the spring, and Tricolored Blackbirds (*A. tricolor*), resident in the same area, breed in spring and often also in autumn. In both species gonadal development and darkening of the bill and mouth lining begins in January. Males of both of these species are unresponsive to long days until October (Payne, 1965b). Brown-headed Cowbirds in the present study retain small gonads and pale bills and mouth linings until March, yet their refractory period terminates at the same time as in *Agelaius*. Finally, the Bobolink (*Dolichonyx oryzivorus*) is a transequatorial migrant that is not known to become sexually active on its wintering grounds during the increasing daylengths of the springtime of the southern hemisphere, but it nevertheless recovers from its refractory period by October or November (Engels, 1962, 1964). Although these four species have different annual cycles, the refractory periods of all of them end at the same time in autumn. The differences in the breeding seasons of these icterids are apparently not caused by differences in the time of recovery from the postbreeding refractory period. An alternative explanation of the different breeding seasons of temperate-zone icterids is that environmental cues in addition to daylength are important as proximate factors in the control of gonadal cycles and breeding seasons, and that the nature of significant environmental cues is related to the ecology of each species (Payne, 1965b).

SUMMARY

Brown-headed Cowbirds (*Molothrus ater obscurus*) were studied in central California. Both wild birds and captives held on natural daylength in spring remain sexually inactive until March. Males held on 17 hours of light per day during winter developed large testes, while controls on natural short days remained sexually inactive. The mean testicular growth rate of cowbirds on 17 hours of light per day for 26 days was not statistically different than the rates in related nonparasitic icterids studied under the same conditions. Female cowbirds on long days developed ovarian follicles as large as 1.8 mm in diameter, and females kept on short days had no follicles larger than 1.0 mm in diameter. The development of the gonads of birds on long daylengths suggests that the long days of spring control the gonadal development of cowbirds in this season.

Cowbirds that were paired in captivity did not breed in the presence of nesting hosts. Female cowbirds in captivity did not develop mature ovaries.

Brown-headed Cowbirds became unresponsive to long daylengths in summer at the time of regression of the gonads. They recover from the refractory period in October. The late time of gonadal development in spring is not associated with a prolonged refractory period, but it is delayed until the completion of the partial prenuptial molt in late winter.

The refractory periods of four species of North American icterids having different breeding seasons are terminated at the same time of year. These data do not support the idea proposed by Marshall (1961) that species differences in breeding seasons are caused primarily by differences in an internal rhythm and its refractory phase.

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