ANNUAL TESTICULAR CYCLE AND BILL COLOR CHANGE IN THE EASTERN AMERICAN GOLDFINCH

PAUL C. MUNDINGER

THE avian testicular cycle of seasonally-breeding birds consists of three phases: a regeneration phase when the tissues of the regressed gonad are reorganized, an acceleration phase characterized by development and growth of the testis, and a culmination phase in which the birds are responsive to certain environmental end stimuli that result in their nest building and gamete deposition (Marshall, 1961). Among wild populations of north-temperate passerines such a cycle has been demonstrated by histological examination of the testis in only a few early nesting species (e.g. Blanchard, 1941; Bullough, 1942; Blanchard and Erickson, 1949; Threadgold, 1956a, 1956b; Marshall and Coombs, 1957). Comparative study of the cycles of more species, particularly late nesters, is desirable. An analysis of seasonal changes in external characters that reflect seasonal changes in the gonad may provide an indirect method of determining the testis cycle in other species quickly. Bill color and postnuptial molt are well-suited for such an analysis as, in certain species, they are readily measurable from museum skins. Both characters have been correlated with changes in the titer of one or more of the gonadal hormones (Witschi and Fugo, 1940; Kobayashi, 1954a, 1954b, 1954c, 1958; Witschi, 1961; Engels, 1962), and Marshall (1961) associates the onset of the postnuptial molt with early regeneration phase and the termination of postnuptial molt with the onset of acceleration phase.

The Eastern American Goldfinch, *Spinus t. tristis,* exhibits both a seasonal change in bill color and a late breeding season, and is principally single-brooded. Throughout the northern part of its range this goldfinch generally does not nest until July and later (Walkinshaw, 1938; Stokes, 1950; Nickell, 1951; Berger, 1968), and breeding behavior occurs seasonally later in this species than in other passerines nesting in northeastern North America. Field experiments conducted in Ithaca, New York revealed that not until late June or early July do female goldfinches begin gathering cotton artificially supplied them in their nesting habitat since early June, and they do not begin collecting thistle papus, a major natural nesting material, until July or, more rarely, in the last week of June (Mundinger, 1968). This correlates temporally with the onset of nest construction. In a study field in Ithaca only about 6 percent (2 of 35 nests) of all nests were initiated as early as the period mid-June to 1 July in 1963-65. Likewise the males do not engage in the defense of a geographically fixed area with flight song display and other territorial behaviors until nest construction begins or, in some cases, only a few days

to a week earlier (Drum, 1939; Mundinger, 1968). The American Goldfinch was therefore selected as the subject of a study with three separate but related purposes: to explore the hormonal control of the seasonal bill color change; to determine, from testis histology, the annual testicular cycle of this late nesting passerine; and to demonstrate that the external characters, bill color and molt, can be used to infer an annual testicular cycle.

BILL COLOR

Both sexes of the American Goldfinch exhibit an annual bill color change. In the spring the dark winter bill color of juveniles and adults gradually changes to a yellow-orange nuptial coloration. This change begins at the base of the bill and progresses distally. After the dark-billed young fledge in late summer or early fall, the bills of the adults again became darkened by melanin deposition. My purpose was to investigate experimentally the possible hormonal control of this bill color change.

MATERIALS AND METHODS

Wild caught American Goldfinches of both sexes were used. The males in Experiments I and II were identified as postjuveniles (i.e. in their first winter); the ages of the females, and the males in Experiment III, are not known. The birds were housed in either 30 × 30 × 12-inch (Experiment I), 42 × 21 × 42-inch (Experiment II), or 9 × 10 × 15-inch (Experiment III) cages, and were given food (French's Parakeet mix; French's Conditioning Food, lettuce) and water ad libitum. For Experiments I and II the birds were assigned at random to treatment and cage, and each cage had every treatment and each sex represented. Three experiments were run, each requiring different methods.

Experiment I. Intact birds subcultaneously injected with sex and gonadotropic hormones.—Twenty-four birds, housed in four cages, were grouped, three males and three females per group. Included in each group were all treatments: 0.1 cc sesame oil/day; 2 μg testosterone proprionate (Schering Oreton) in 0.1 cc sesame oil/day; 0.5 μg estradiol benzoate (Schering Progynon B) in 0.1 cc oil/day; 10 μg LH (Armor lot T 10708) in 0.1 cc water/day. The birds were injected subcultaneously on the inner aspect of the thighs.

The birds were captured in late February and early March 1964, and most birds evidenced early stages of melanin loss at the base of their bills. On the assumption that this was ultimately due to the increasing natural photoperiods (which reached 11.2 hours daylight on 1 March), the birds were maintained on short days, 8 hours light–16 hours dark, for a week before and throughout the experiment. The experiment ran 20 days, 16 March–10 April 1964. At the beginning and end of the experiment closeup color photographs of the head and bill of each bird were taken with a Nikon F, Auto Micro Nikkor 55mm/f 3.5 lens, Kodachrome II type A film and photoflood lamps. Weekly measurements of melanin loss along the culmen (to nearest 0.1 mm) were made with dividers and millimeter rule; weekly comparisons of bill color to Ridgway's (1912) color standards were also made.

Experiment II. Intact birds given intramuscular injections.—Bills of the newly captured birds used in Experiment I evidenced some natural melanin loss as early

as late February. This, together with some mortality (possibly caused by subcutaneous accumulation of oil) during the experiment prompted this second experiment.

Five males and five females were captured 14–25 October 1966 in Oxford, Ohio. The birds were kept in two cages, at room temperature, and were maintained on a natural, late fall, photoperiod regime. The experiment was carried out in Oxford during the period 3 November–12 December 1966.

From 3–22 November the birds were given 0.02 cc oil injected into the pectoralis every 4th day. Treatments and dosages were: testosterone proprionate (Towne, Poulson, Co.) 500 μg in 0.02 cc sesame oil (5 birds); control, 0.02 cc sesame oil (5 birds). Bill color change was determined by measuring the amount of melanin loss along the culmen, using a divider and a millimeter rule. Measurements were taken 3 November (base line), 20 November, and 12 Decembr.

Experiment III. Bilateral castration.—Seven recently captured males, in breeding plumage and with bright yellow-orange bills, were bilaterally castrated 7–10 July 1965. The color of their bills was noted on 7 July (base line), 30 July, and 12 August.

Results and Discussion

The results are summarized in Tables 1–3. As analyzed by the median test (Siegel, 1956: 111), the Experiment I scores in Table 1 gave a statistically significant difference ($P = 0.01$) between testosterone treatment

TABLE 1

Bill Color Ranking Following Hormone Treatment[1]

Treatment	Bird	Scores evaluating response		
		Obs. 1	Obs. 2	Mean
Testosterone	RW	3	3	3
Testosterone	BP	3	3	3
Testosterone	GP	3	3	3
Testosterone	O	3	3	3
Testosterone	BY	3	2.5[2]	2.75
Testosterone	Bb		Died before experiment ended	
Estradiol	W	3	2.5[2]	2.75
Estradiol	BB	2	2	2
Estradiol	Gb	2	1	1.5
Estradiol	Y	1	2	1.5
Estradiol	R		Died before experiment ended	
Estradiol	WW		Died before experiment ended	
LH	P	3	3	3
LH	PP	3	2.5[2]	2.75
LH	YG	2	1.5[2]	1.75
LH	YB	2	1.5[2]	1.75
LH	G	1	2	1.5
LH	GB	1	2	1.5
Control	BG	2	2	2
Control	YP	1	1.5[2]	1.25
Control	PG	1	1	1
Control	b	1	1	1
Control	GG		Died before experiment ended	
Control	B		Died before experiment ended	

[1] Results of Experiment I. Scoring: 1 = very little, or no bill color change; 2 = a detectable but moderate change; 3 = an obvious bill color change.

[2] When the observer could not decide between two categories a midvalue was taken.

TABLE 2
MELANIN LOSS ALONG CULMEN[1]

Treatment	Bird		Extent of melanin loss in mm		
			3 Nov.	20 Nov.	20 Dec.
Testosterone	b	♀	0.0	1.5	3.5
Testosterone	G	♀	0.0	1.1	3.0
Testosterone	GW	♀	0.0	1.5	3.9
Testosterone	RW	♂	0.0	2.7	4.0
Testosterone	P	♂	0.0	2.1	3.8
Control	O	♀	0.0	0.0	0.0
Control	B	♀	0.0	0.0	0.0
Control	YR	♀	0.0	2.0	3.0[2]
Control	g	♂	0.0	0.0	0.0
Control	Y	♂	0.0	0.0	0.0

[1] Results of Experiment II. Extent of melanin loss was measured distally from culmen base.
[2] There is 0.5 chance that the only control bird to show a response (YR) was accidentally injected with testosterone on the first day of injections.

and control. At the 0.05 significance level estradiol and LH were not statistically different from the control. The results of Experiment II support this. As analyzed by the Fisher exact probability test (Siegel, 1956: 96) the difference in the extent of melanin loss along the culmen between the testosterone and the control group is significant ($P = 0.025$). Thus testosterone proprionate significantly reduces melanin in the rhamphotheca. This demelanization is rapid, visible in approximately 1 week (Figure 1), and sensitive to low levels (2 μg/day) of hormone.

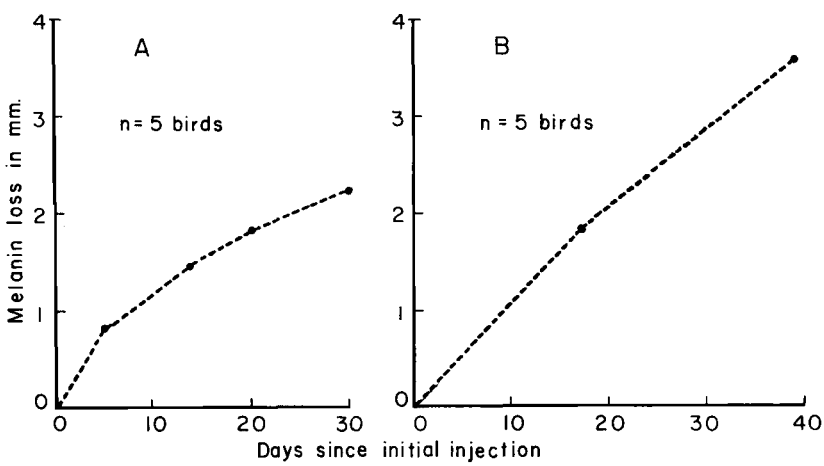

Figure 1. Average rate of melanin loss along culmen base in response to exogenous testosterone. A, Experiment I results (dose: 2 μg/day subcutaneously). B, Experiment II results (dose: 500 μg IM over days 1–20).

TABLE 3
BILL COLOR CHANGE IN BILATERALLY CASTRATED *S. T. TRISTIS*[1]

Bird	Bill color		
	7–10 July	30 July	12 August
	(Castrated)	(~ 3 weeks post-op)	(4–5 weeks post-op)
g	Orange	Dark for 3 mm	Entirely dark
YW	Orange	Dark for 2–3 mm	Entirely dark
b	Orange	Dark for 2–3 mm	Entirely dark
O/p	Orange	Dark for 2 mm	Entirely dark
G	Orange	Dark for 2 mm	Entirely dark
O	Orange	Dark at base (difficult to measure)	Died
p[2]	Orange	Orange	Orange

[1] Results of Experiment III.
[2] Incomplete castrate.

The corollary, that melanin deposition in the bill is due to testosterone withdrawal, is supported by the results of Experiment III. Six of the seven castrated males evidenced darkening of their bills within 3 weeks after castration (Table 3). The seventh male retained the orange bill color indicative of the breeding condition and on autopsy proved to be incompletely castrated. Additional support is provided by the five birds that were injected with testosterone proprionate in Experiment II. These birds received no exogenous testosterone subsequent to 22 November 1966. By 26 January 1967, more than 2 months after the final testosterone injection, all five of these birds evidenced renewed melanin deposition. I interpret this redeposition of melanin as a consequence of the withdrawal of the exogenous testerostone and of the lack of endogenous testosterone production under the short winter photoperiods the birds experienced.

The springtime bill color change in the American Goldfinch consists of both a melanin loss and a deposition of carotenes. The melanin loss is caused by testosterone proprionate, but I could not establish a comparable correlation between hormone treatment and carotene deposition. Difficulty in measuring objectively the degree of carotene accumulation may be responsible for this, and the question of the control of carotenes remains unanswered. In the succeeding pages, a change in bill color will refer only to change in melanin distribution.

TESTICULAR CYCLE

The next step was to determine the naturally occuring testis cycle and to see if bill color changes and the testis cycle are correlated under natural conditions.

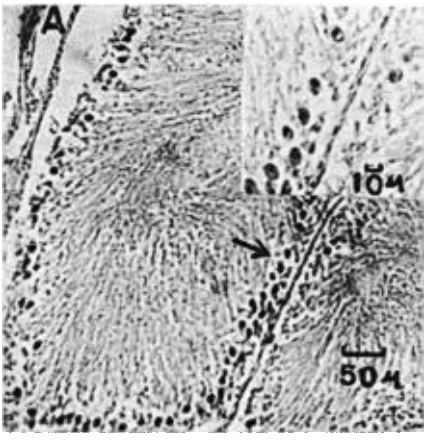

Figure 2. Testis in stage 0 illustrating the distinctive ring of large cells at the periphery of the seminiferous tubule (100×). Inset, enlargement (200×) of the cells in the region indicated by the arrow.

MATERIALS AND METHODS

Histological analysis.—The gonads of wild male goldfinches, juveniles and adults, were used. The testes of 56 birds, collected in the field from 1961-65 were usually fixed within 1 hour after death or surgical removal. Four birds, two road kills and two originally collected for other purposes, were stored at freezing temperatures for several weeks prior to removal and fixing of their testes. All specimens were obtained within a 20-mile radius of Ithaca, New York.

The histological treatment of the testes approximated Marshall's technique (1952). To detect the lipid content of the Leydig cells or lipid content of seminiferous tubules, one testis (per bird) was fixed either in formal-calcium, neutral formalin, or, in rare instances, in Bouin's solution; embedded in 20 percent gelatin; freeze sectioned at 10–15μ; stained with the lipophyllic stain oil red 0 and counterstained with 1 percent hematoxylin. For examinations of cellular detail the contralateral testis was fixed in Bouin's solution, paraffin embedded, sectioned at 4–8μ, and stained with Harris' hematoxylin and eosin. For each individual male two well-stained, midline sections (one stained with oil red 0, and the other with hematoxylin and eosin) were selected for measurement of the following parameters (for parameters 1–4 individual average values were calculated): 1) seminiferous tubule lipid content—the lipid content of five tubules, randomly selected from a single section, was measured with an ocular micrometer at 430×. 2) Leydig cell lipid content—the lipid content of five interstitial regions was measured with an ocular micrometer at 430×. For a given individual the interstitial regions measured were chosen at random with the exception that they were approximately equal in surface area when measured with a grid micrometer. 3) Seminiferous tubule diameter—five tubules (circular in cross-section) were measured at 430× with an ocular micrometer. 4) Composition of interstitium—the relative percentage of total interstitial area covered by each of the three predominant interstitial cell types (fibroblasts, mature Leydig cells, and juvenile cells that are undifferentiated precursors of fibroblast and Leydig cells) was estimated. For each male, five randomly chosen interstitial areas, approximately

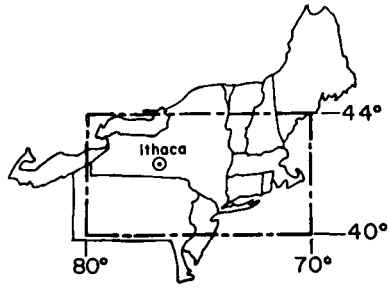

Figure 3. Latitudinal and longitudinal boundaries of collection area for museum skins.

equal in area, were measured at 430× and 970× with a grid micrometer. 5) Spermatogenic development—measured with an 8 stage index. Stages 1–7 correspond to the spermatogenic index described by Blanchard (1941). I added an eighth stage (stage 0) describing a regressing testis. A testis in stage 0 (found in but one male) has enlarged seminiferous tubules, degenerating sperm, and a ring of large cells just inside the tubule periphery (Figure 2). This obvious ring of cells may be equivalent to the unique epithelial cells that appear in the regressing Rook, *Corvus frugilegus,* testis (Marshall and Coombs, 1957). Stage 1 represents the small, fully regressed testis, stages 2–6 represent progressive stages in development, and stage 7 represents the mature, breeding testis.

Analysis of external characters.—Bill color and molt were measured from study skins of the U. S. National Museum, the Cornell University collection, and a collection made personally for this study. The specimens spanned the years 1844–1967. Only males collected in an area of northeastern United States surrounding Ithaca were used (Figure 3), because the annual testis cycle inferred from these data was to be compared to a testicular cycle derived from the histological analyses of the testes of Ithaca birds. Bill color was measured as the extent of melanin absent from the culmen, measured to the nearest 0.1 mm with dial calipers. One caliper jaw was placed on the culmen base and the other was extended out to the point on the culmen where melanin pigments first became visually obvious (Figure 4). For late summer and fall specimens showing redeposition of melanin, a written description of the pattern of depostion supplemented similar culmen measurements. In addition to bill color, the body plumage of each specimen was assigned to one of the following categories: 1) winter plumage, 2) onset of prenuptial molt, 3) heavy prenuptial

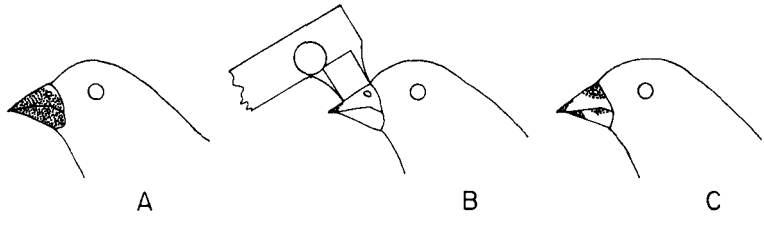

Figure 4. Seasonal changes in bill color. A, early spring specimen. B, early summer specimen (the method of caliper measurement is illustrated). C, fall specimen, illustrating several loci of melanin deposition.

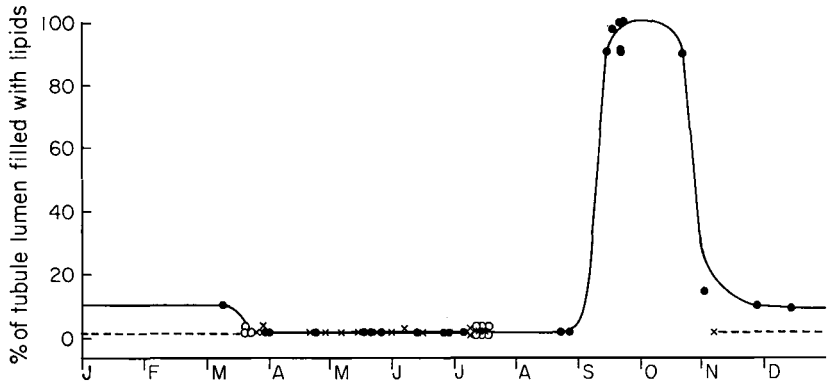

Figure 5. Seasonal changes in tubule lipids. •, adult male; ×, juvenile or first-year breeding male; O, age unknown.

molt, 4) terminal prenuptial molt, 5) breeding plumage, 6) onset of postnuptial molt, 7) heavy postnuptial molt, and 8) terminal postnuptial molt. Age classes of males in winter plumage were identified by the color of wrist feathers, which are olive on birds of the year and yellow on birds that have experienced at least one postnuptial molt.

RESULTS

Figures 5–9 present the results of the histological analyses; seasonal changes of external characters are presented in Figure 10. On the basis of these data and of supporting field observations the following phases and their durations were inferred.

Culmination phase, July and August.—The culmination phase of the testis cycle is the period of nest building, insemination, and ovulation; a period when the birds are sensitive to environmental end stimuli that permit the initiation and completion of the nesting cycle (Marshall, 1955, 1961). This phase is primarily determined by behavioral parameters, although the testes must have reached a breeding condition capable of sperm production.

Mature sperm and maximal testis size are attained at the very end of June and early July (Figures 6 and 9). This date corresponds to the initial appearance in the breeding population of three behaviors characteristic of the onset of the culmination phase—the males' territorial behavior, the females' responsiveness to and subsequent collection of nesting material, and nest construction by the females. As mentioned earlier these behaviors first began to appear irregularly in Ithaca populations in late June and are seen with increasing frequency throughout July. Thus both behavioral and gonadal data support the conclusion that the culmination phase of the great majority of the breeding birds does not

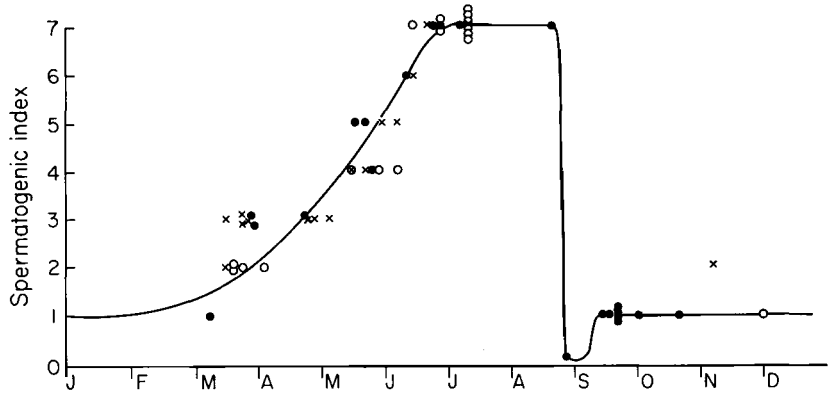

Figure 6. Seasonal changes in spermatogenic development. See Figure 5 for legend.

begin until after 1 July. Subsequently behavior characteristic of the culmination phase is observed in the population well into late August or even early September. This is due to several factors: renesting following nest desertion or destruction; a small percentage of the population beginning second broods (Mousley, 1932; Stokes, 1950; Mundinger, 1968); and possibly a few physiologically delayed birds (Stokes, 1950). In Ithaca fledging in the primarily single-brooded American Goldfinch also occurs in the latter half of August and in early September. This and the attendant cessation of active breeding behavior marks the end of its culmination phase.

Regeneration phase, late August/early September to late October/early November; late August/early September to March.—The onset of the regeneration phase is clear. At the end of August and first part of September the testes exhibit many major changes: degeneration and loss of mature sperm (stages 0 and 1, Figure 6); massive steatogenesis of the seminiferous tubules (Figure 5); a new generation of undifferentiated interstitial cells (Figure 7); and, externally, a pronounced darkening of the bill and the onset of the postnuptial molt (Figure 10).

The termination of the regeneration phase and the simultaneous onset of the acceleration phase are not so easily determined. In late October and early November the termination of the postnuptial molt and a small melanin loss from the base of the bills of some males (Figure 10) and a precipitous reduction in tubule lipids (Figure 5) suggest that the testes of at least some birds are rehabilitated and have begun to recrudesce. Other histological parameters do not show comparable changes until March when all parameters, except seminiferous tubule diameters, show changes indicative of testes in the acceleration phase. These March

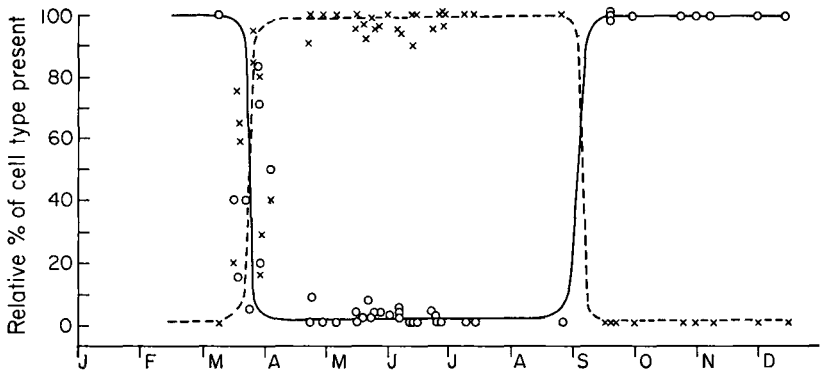

Figure 7. Seasonal changes in the differentiation of the interstitium. O, juvenile cell contribution to the interstitium; ×, fibroblast and mature Leydig cell contribution to the interstitium.

changes include the loss of residual tubule lipids (Figure 5), onset of spermatogenesis (Figure 6), maturation of the interstitium (Figure 7), and the presence of lipids in Leydig cells followed by a gradual depletion of the lipids (Figure 8). Externally there is a gradual and continuous melanin loss from the bills of the entire male population (Figure 10). The bill color and testicular changes noted in late October or early November and then again not until March suggest that the onset of the progressive testicular development of the acceleration phase is variable, occurring primarily in October/November and in March. During the intervening winter months there seems to be a hiatus in gonad develop-

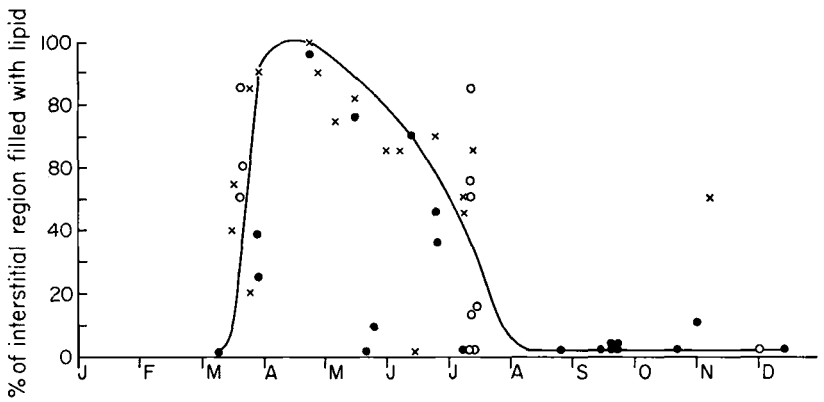

Figure 8. Seasonal changes in interstitial lipid content. The curve represents a general trend only for variation from May through July is great (e.g. see sample collected 10 July). See Figure 5 for legend.

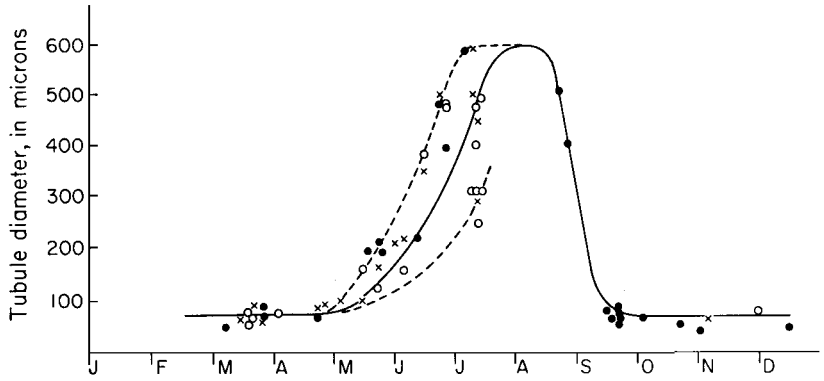

Figure 9. Seasonal changes of seminiferous tubule diameter. See **Figure 5 for** legend.

ment that halts further testicular recrudescence for some males and delays the onset of recrudescence for other males. This apparent winter hiatus in gonad development may be due to environmental factors such as short photoperiods, or could be an artifact of the small winter sample sizes of this study.

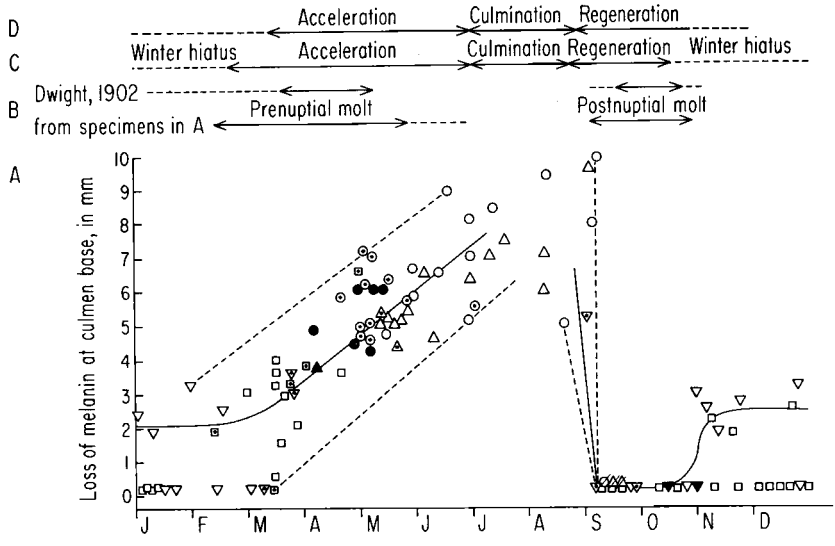

Figure 10. A, Seasonal changes in extent of culmen demelanization, and condition of plumage. Symbols: ▽ adult, □juvenile-winter plumage; △adult, ○ first-year male-breeding plumage; ▽ ⊡ beginning molt; ▲ ▼ ● heavy molt; ◬ ⊙ molt ending; / indicates melanization of bill. B, Calendar of molts in *S. t. tristis*. C, Annual testis cycle derived from seasonal changes in bill color and molt. D, Annual testis cycle derived from histological changes of the testes (from Figures 5–9).

Acceleration phase, late October/November through June; or March through June.—The two different dates derived for the onset of this phase correspond to the dates inferred for the termination of the regeneration phase. Then from March through June there is a continuous testicular development (Figures 6–9). By late June or early July the enlarged testes and the production of mature sperm indicate attainment of full breeding potential. Throughout spring the change in bill color is also continuous and by June the bill has lost almost all of its dark coloration (Figure 10). Both males and females typically retain a bit of melanin (about 1 ± 1 mm) at the bill tip. The extent and intensity of this residual dark tip is variable, thus bill color cannot be used to indicate precisely the end of the acceleration phase for a given bird. Behavioral data are necessary to infer the beginning of the next culmination phase.

DISCUSSION

It is now possible to compare the testis cycle of the extremely late nesting and single-brooded *S. t. tristis* to the cycles of a few earlier nesting passerines. A population of the Rook, which breeds in March, was studied with methods similar to my own (Marshall and Coombs, 1957). Threadgold (1956a, 1956b) analyzed annual changes in testis histology in the Jackdaw, *Corvus monedulus*. Gonad and changes in bill color in the April- and May-nesting British Starling, *Sturnus vulgaris*, were studied by Bullough (1942); and several workers have studied the May- and June-nesting Gambel's Sparrow, *Zonotrichia leucophrys gambelii* (Blanchard and Erickson, 1949; King et al., 1966; DeWolfe, 1967; Morton et al., 1969). Other studies were excluded because either the study was done in captivity or the populations were multibrooded (e.g. Blanchard, 1941; Selander and Hauser, 1965).

The testis cycles of these single-brooded species are presented in Figure 11. It is clear that the onsets of all of the phases of the American Goldfinch cycle occur later in the year than in the cycles of earlier nesting species. In other respects the testis cycle of the American Goldfinch appears to be similar to most of the other species. For example, if the duration of the constituent phases for breeding populations are compared, then all five species have a regeneration phase of about 7- to 10-weeks' duration. Gambel's Sparrow has the briefest regeneration phase, which may be due to sampling error as no gonads were collected in September, or it may indicate a greater degree of synchronization in the breeding population of this high latitude migrant. The culmination phases of the American Goldfinch and three of the other four species is also on the order of magnitude of 2 months, with the culmination phase of the

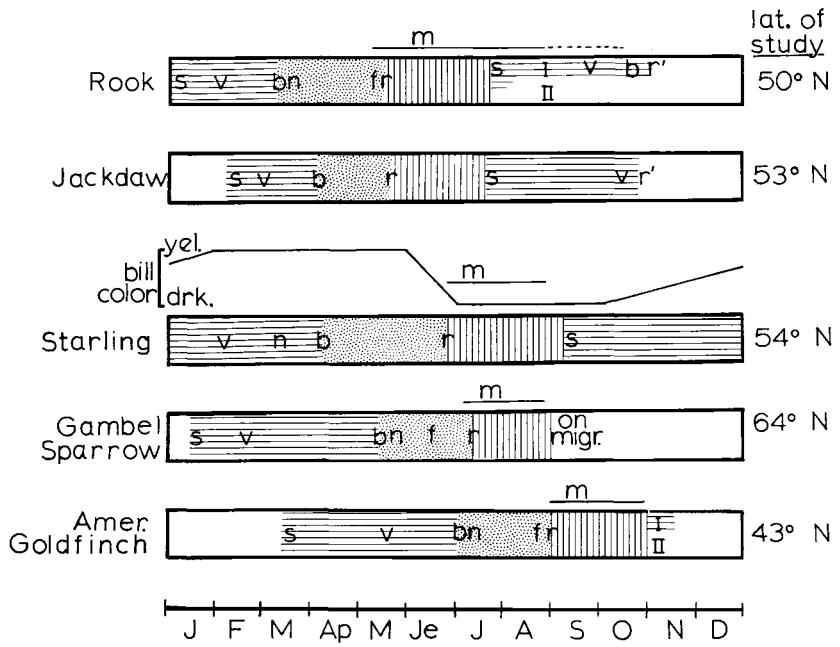

Figure 11. Annual testis cycles of five single-brooded passerines. Horizontal lines represent acceleration phases; small dots represent culmination phases; and vertical lines represent regeneration phases. Cycles are my interpretations of original studies (references to original studies in text). Latitudes of the original stuly areas are indicated. Symbols: s = spermatogenic onset (change from stage 1 to stage 2 of index), v = onset of logarithmic growth of testis volume, b = breeding testis (mature sperm and maximum volume attained), n = onset of nest building, f = fledging, r = testis regression (decreased volume and/or steatogenesis), m = post-nuptial molt, migr = migration, I and II represent two categories of gonadal re-crudescence (see text).

British Starling, which is occasionally double-brooded, being a few weeks longer.[1]

If both culmination and regeneration phases of single-brooded species are each about 2 months long, this leaves an acceleration phase of 8 months. There is some evidence that testis development in the Starling is gradual and continuous throughout this period (Bullough, 1942). However this may not hold for other species. Figure 11 shows that following

[1] Multi-brooded populations would have longer culmination phases. Within one species, *Zonotrichia leucophrys,* the culmination phase of a single-brooded population (*Z. l. gambelii*) is about 2 months, in a double-brooded population (*Z. l. pugetensis*) it is about 3 to 3½ months, and in a triple-brooded population (*Z. l. nuttalli*) it is 4 to 4½ months long (Blanchard, 1941; Blanchard and Erickson, 1949).

the regeneration phase, male Rooks and American Goldfinches fall into two groups. The testes of group I males recrudesce in the fall, but this is then followed by a hiatus or a secondary testicular regression during the winter. Following the regeneration phase males in Group II evidence a hiatus in gonad development for several months. Their gonad recrudescence first becomes evident in January (Rook) or March (American Goldfinch). If this interpretation is correct the period of progressive and continuous gonadal development can be substantially less than 8 months. Further study is needed to determine the extent and degree of any interspecific differences in the duration of the acceleration phase.

In sum the comparison of the five single-brooded, north-temperate passerines points up the basic similarities in their testis cycles. The distinguishing feature in the cycle of the late nesting American Goldfinch is the peculiarly late seasonal timing of all its constituent phases.

A broader interspecific comparison of testis cycles was hindered by insufficient comparative data. But the demonstration that the testis cycle can be determined relatively quickly by examination of seasonal changes in external characters may alleviate this problem. In *Spinus t. tristis* the testis cycle inferred from the seasonal changes in molt and bill color is essentially identical to one based on testis histology (compare Figure 10C to Figure 10D). Also the seasonal change in the Starling's bill color, which is controlled by testosterone, is rather well-synchronized with the phases of its cycle (Figure 11), even though Bullough's (1942) temporal measurements of change in bill color were neither precise nor quantified. Presumably cycles for other avian species can be inferred in a similar manner although certain requirements must be met. These would include the availability of a large and seasonally representative museum skin collection, external characters that adequately reflect change in the physiology of the gonad-pituitary axis, and external characters that are temporally stable. The change in bill color of *S. t. tristis* involves both melanin and carotene pigments, and it is indeed fortunate that the stable melanin pigments were demonstrably under the control of testosterone since carotenes fade from the bills of museum specimens.

Irrespective of the ultimate value of external characters as measures of gonad activity, histological examination of testes remains essential. A cycle inferred from changes in external characters should at least be checked against a small sampling of testes collected at critical points in the inferred cycle. And for those species that lack external characters suitable for analysis, the determination of a cycle is dependent upon a large sampling of testes. Some comments on histological parameters are therefore appropriate.

Five parameters were used in this study. Three of these, tubule lipids (Figure 5), spermatogenic development (Figure 6), and differentiation of the interstitium (Figure 7) measure one or more of the phases, and taken together they establish the calendar of the entire annual testis cycle. The other two parameters, Leydig cell lipid content and tubule diameter, appear to be less useful due to the variation evident (Figures 8 and 9). Testis size, which can be measured as tubule diameter, testis diameter, or testis volume, deserves special comment as it is a parameter often used as an index of reproductive condition.

Size alone can be misleading. For example, enlarged testes do not necessarily indicate that the individual bird was in breeding condition. Male goldfinches either in late acceleration phase or entering the regeneration phase (i.e. stage 0) have testes comparable in size to testes collected at the height of the breeding season. And at the other extreme small testes are not necessarily regressed or quiescent, for many progressive changes occur in the testis months before the gonad enlarges grossly. This points up another feature of testis size—that the onset of the logarithmic growth phase of the testis is usually not correlated with the onset of the acceleration phase. In *S. t. tristis* the logarithmic growth phase occurs about 2 months after the beginning of the acceleration phase. Similar results have been reported in the domestic chicken (Kumaran and Turner, 1949) and White-crowned Sparrow, *Zonotrichia leucophrys,* (Blanchard, 1941), and a similar lag is also evident for all the species in Figure 11. However, in certain instances a minor increase in size, which occurs well before the onset of the logarithmic increase in size, may be temporally correlated with the onset of the acceleration phase. Such minor growth is only detectable when precise and quantitative techniques are employed (e.g. Bullough, 1942). In sum it appears that unless sufficient care is taken, dependence on the parameter testis size may lead to significant error in estimating a gonad cycle.

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SUMMARY

The annual testis cycle of the Eastern American Goldfinch, *Spinus t. tristis,* was determined by histological examination of testes of wild birds. Three parameters, tubule lipids, spermatogenic development, and differentiation of the interstitium, adequately describe the cycle. Two other parameters, lipid content of the Leydig cells and testis size, were less reliable.

Seasonal changes of the external characters, bill color and molt, correlate well with the annual testes cycle. Experiments demonstrate that the bill color response is sensitive to low levels of testosterone and a measurable effect is visible in about a week. Thus bill color is an effective bioassay of testicular activity. The annual testis cycle of the American Goldfinch can be inferred from the calendar of its natural bill color change and molt. Annual gonad cycles should be inferrable from changes in external characters in other species too.

The testis cycle of *S. t. tristis* is compared to the cycle of four other passerines. In the unusually late nesting Eastern American Goldfinch all of the constituent phases of the cycle are later, as compared with those of the four earlier nesting species.

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Section of Neurobiology and Behavior, Division of Biological Sciences, Cornell University, Ithaca, New York 14850. Present address: Rockefeller University and New York Zoological Society, New York, New York 10021. Accepted 15 April 1971.