

PHOTOPERIODIC RESPONSIVENESS OF EQUATORIAL AND TEMPERATE-ZONE STONECHATS¹

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Abstract. The gonads of equatorial stonechats in East Africa (African Stonechats, *Saxicola torquata axillaris*) develop in anticipation of the short rainy season, the major reproductive period. After the breeding season, gonads regress and birds undergo a complete molt. This annual cycle of reproduction and molt is based on an endogenous circannual rhythmicity that is highly sensitive to photoperiodic changes. By exposing birds to sinusoidal day-length variations with an amplitude of 7 hr, simulating in shape and amplitude those occurring at 47.5°N, gonadal and molt cycles could be synchronized to periods of either 12 or 6 months. In this respect, equatorial birds behaved essentially like temperate-zone stonechats (*S. t. rubicola*). In both subspecies, photoperiodic synchronization results from seasonal changes in responsiveness to photoperiodic stimuli, reflected in the alternation between a state of photosensitivity and a state of photorefractoriness. When exposed to a 6-month photoperiodic cycle with an amplitude of only 1 hr and 10 min, birds of both subspecies no longer synchronized their rhythms with it. Under these conditions, the two subspecies differed in the way testis size changed after the initial phase of testicular growth: whereas the testes of most of the temperate-zone birds stayed enlarged for 6 to 11 months, those of most equatorial birds started to regress within only 2 to 3 months. This difference probably reflects evolutionary adjustments of the two subspecies to the different photoperiodic conditions prevailing in their respective breeding grounds: the equatorial birds appear to initiate refractoriness under shorter photoperiods than do the temperate-zone stonechats. It is not clear as yet whether the very small photoperiodic changes that occur at the equator are sufficient to synchronize circannual rhythms of birds in this region. Possibly the photoperiodic responsiveness of equatorial stonechats is only the unavoidable consequence of gene flow from adjacent, more northerly or more southerly populations.

Key words: circannual rhythms, photoperiodic responsiveness, refractoriness, *Saxicola torquata*, Stonechats, tropical birds.

INTRODUCTION

Since the classical studies of Rowan (1926), an abundance of data has revealed that the annual cycles of reproduction, molt, migration, and other functions of high-latitude birds are rigidly controlled by seasonal variations in photoperiod. In these birds, photoperiodic changes either act directly as causal stimuli, initiating or terminating certain seasonal states (Farner and Follett 1979), or as zeitgebers, synchronizing an endogenously programmed circannual rhythmicity (Aschoff 1955, Gwinner 1990). The extent to which day-length changes also are involved in the control of annual cycles in the tropics, where 80% of all birds live, is still a matter of debate. The amplitude of the annual photoperiodic cycle (i.e., the difference be-

tween the longest photoperiod in summer and the shortest one in winter) declines as latitude decreases, to become less than a few minutes at the equator. These changes are heavily masked by day-to-day variations in cloud cover, which may lead to considerable changes in effective day-length (Dittami and Gwinner 1985). In view of this situation, many authors have expressed the opinion that photoperiod cannot be an important stimulus controlling reproductive cycles in birds breeding close to the equator (Chapin 1932, Miller 1965, Epple et al. 1972). However, changes in cloud cover may not only amount to "noise" that obscures the small "astronomical" variations in day-length, but they also may, in connection with the waxing and waning of the rainy seasons, cause systematic changes in effective day-length that are possibly larger than the astronomical ones (Dittami and Gwinner 1985). So far, only a few attempts have been made to determine the min-

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imum effective amplitude of a photoperiod cycle (Lofts and Murton 1968, Epple et al. 1972, Gwinner and Dittami 1985). Recently, Hau et al. (1998) have found that Spotted Antbirds (*Hylophylax naevioides naevioides*) in Panama can exhibit gonadal growth in response to an increase of photoperiod as small as 17 min. Mammals, likewise, may be quite sensitive to small photoperiodic changes (Wayne and Rissman 1991, Heidemann and Bronson 1993).

In view of such low photoperiodic discrimination thresholds, it can no longer be assumed that even birds living very close to the equator do not use photoperiodic information for the control of their annual cycles. Indeed, several studies indicate the presence of photoperiodic response mechanisms in a number of tropical bird species (reviewed in Gwinner and Dittami 1985). In one of them, hereafter called the African Stonechat, *Saxicola torquata axillaris*, the timing of postjuvenile molt could be strongly affected by photoperiod: molt started earlier and lasted longer in birds exposed to an equatorial photoperiod than in birds exposed to a (longer) temperate-zone photoperiod. Qualitatively similar responses were observed in individuals of the European subspecies of the Stonechat, *S. t. rubicola*, hereafter called the European Stonechat (Gwinner et al. 1983). A subsequent study (Gwinner and Dittami 1985) suggested that photoperiod also affects the annual cycle of reproductive competence in equatorial stonechats. Breeding is seasonal in these birds (Dittami and Gwinner 1985) and the underlying annual cycle in gonadal size and function is controlled by an endogenous circannual rhythmicity that runs for up to 10 years in a constant equatorial photoperiod (Gwinner and Dittami 1990, Gwinner 1996). Whereas the period of this rhythm usually deviates from 12 months under such constant conditions, it was found to be very close to 12 months in a group of birds that was exposed to a sinusoidal photoperiodic cycle mimicking that at 47.5°N. Again, European Stonechats responded in a similar manner (Gwinner and Dittami 1985). This result strongly suggested that photoperiodic cycles are in principle capable of synchronizing the circannual rhythms of both subspecies. From this, a number of intriguing questions arose, three of which are addressed in the present investigation.

First, we test the hypothesis, derived from

previous preliminary results, that a high-amplitude photoperiodic cycle can act as a zeitgeber of circannual rhythms of equatorial stonechats. For that purpose, annual gonadal and molt cycles were studied in male stonechats kept for up to 27 months in a photoperiodic cycle with an amplitude corresponding to 47.5°N. The period of this cycle was either 12 months or 6 months (Experiment 1 and 2, respectively).

Second, we attempt to characterize the nature of the photoperiodic response system through which synchronization is achieved. In all temperate-zone birds yet investigated, the basis for synchronization is a seasonally changing responsiveness to photoperiodic stimuli. The most conspicuous expression of this is the spontaneous termination of photosensitivity, the state during which long days induce gonadal development or maintain active gonads: the birds become refractory at the end of the breeding season (Nicholls et al. 1988). To determine whether equatorial stonechats also go through such a state of photorefractoriness, males and females were transferred at various stages following gonadal regression to continuous light—an extremely long photoperiod—and their gonadal responses were measured (Experiment 4).

The third and final goal of the present study is to evaluate the possibility that African Stonechats living in their tropical environment use photoperiodic information for synchronizing their circannual rhythms. To test this, male stonechats were exposed to a photoperiodic cycle with a low amplitude characteristic of 10°N, but with a period of only 6 months (Experiment 3).

In all four experiments, the responses of African birds were compared with those of conspecifics from Europe.

METHODS

African Stonechats were collected in April as nestlings near Nakuru, Kenya (0°14'S, 36°0'E), and transported within two weeks to Andechs, Germany. European Stonechats were collected in May, June, and July near Vienna, Austria (47°40'N, 36°30'E) and immediately moved to Andechs. The birds were hand-raised (Gwinner et al. 1987) and subsequently kept in individual cages (54 × 30 × 38 cm) in temperature-controlled chambers at 20 ± 3°C. Unless stated otherwise, light intensity was about 300 lux during

the light cycle and about 0.01 lux during the dark cycle.

At intervals of about 1 week, the birds were checked for the occurrence of molt. The state of body molt (BM) was assessed by noting whether molt had occurred in any one of 19 different feather tracts on the bird's body. The tracts were the same as those described in Berthold et al. (1970) except for the "eye ring" and the "belly," which were neglected in the present study. The onset of body molt was defined as the first day on which molting was detected in more than five feather tracts. Flight-feather molt (FM) was assessed by noting the state of each growing primary and secondary according to the system described by Newton (1966). The onset of flight-feather molt was defined as the mean date between the last check without molt and the first check with molt. The period of the rhythms of body molt (τ_{BM}) and flight-feather molt (τ_{FM}) was calculated as the interval between successive molt onsets.

Every 3 to 8 weeks the birds were laparotomized and testicular width or the diameter of the largest ovarian follicle was determined, usually to the nearest 0.1 mm. Ovarian follicles smaller than 1 mm were estimated to either 0.5 or 0.8 mm. In the birds of experiments 1 to 3, the onset and end of a testicular cycle, determined by interpolation, were defined as the times at which testicular width reached a value of 1.5 mm during testicular growth and regression. The duration of the testicular growth cycle (D_T) is the interval between onset and end of testicular growth. The period of the testicular rhythm (τ_T) is defined as the interval between successive onsets of testicular growth.

EXPERIMENT 1

The eight male African and seven male European Stonechats were kept from an age of about one month under an artificial photoperiod simulating that at 47.5°N. Because Austrian Stonechats are migrants that winter in the Mediterranean area, the photoperiodic simulation was correspondingly adjusted, assuming that the birds reach 45°N on October 4, 42.5°N on October 11, and their 40°N wintering area on October 18. Between October 19 and February 27, birds were exposed to the photoperiodic changes occurring at 40°N. Subsequently, the photoperiodic conditions during their homeward migration were simulated, assuming that the birds reach

42.5°N on February 28, 45°N on March 7, and their 47.5°N breeding grounds on March 14. The simulation was accomplished by re-adjusting the light-on and -off times at weekly intervals. For the simulation of photoperiodic conditions, the time interval between the onset of civil twilight in the morning and the end of civil twilight in the evening was used, assuming that the day-length effective for photoperiodic reactions corresponds closely to this interval. Thus, the longest photoperiod was 17 hr 20 min, the shortest 10 hr 20 min, and the range 7 hr.

The mean hatching date of the African birds was April 24, that of the European birds (which were from second and replacement broods) July 9. To standardize the photoperiodic simulation, we assumed that the European birds had hatched at the same time as the African birds; thus the initial photoperiod was set for the European birds to the photoperiod occurring on April 24. At about this time, the first young hatch in central European populations (Glutz von Blotzheim and Bauer 1988). The experiment lasted 30 months. Around month 28, sample size was reduced to six in the African and to five in the European group. Preliminary results from this experiment have been reported previously (Gwinner and Dittami 1985).

EXPERIMENT 2

The eight male African and eight male European Stonechats in this experiment had initially been kept for at least 16 months, either in a constant equatorial photoperiod (six African birds) or under the natural photoperiodic conditions at 47.5°N (two African birds, eight European birds). The testicular and molt cycles of all birds had been followed by repeated laparotomies and molt checks. By October 29, the start of experiment 2, all birds had regressed gonads and their postnuptial molt was either just completed or in its final stage. The birds were exposed to a photoperiodic cycle corresponding to that of experiment 1, except that its duration was only 6 instead of 12 months. As a consequence, the birds experienced two full photoperiodic cycles within the 13 months of the experiment. As in experiment 1, the longest photoperiod was 17 hr 20 min, the shortest 10 hr 20 min, and the range 7 hr.

EXPERIMENT 3

At the end of experiment 2, when all birds had completed their second testicular cycle and were

in the process of postnuptial molt, eight African and seven European Stonechats were exposed to a photoperiodic cycle mimicking in its general shape and amplitude the cycle occurring at 10°N. However, as in experiment 2, the duration of this cycle was only 6 months. The longest photoperiod was 12 hr 37 min, the shortest 11 hr 27 min, and the range 1 hr 10 min. The experiment lasted 15 to 16 months, except for four African birds which were removed after 11 to 12 months.

EXPERIMENT 4

In temperate-zone passerine birds, refractoriness usually sets in at the end of the breeding season when gonads regress, and extends through postnuptial molt into autumn and early winter (Nicholls et al. 1988). To test whether African and European Stonechats also become refractory, male and female birds were transferred at four specified times after completion of the breeding cycle (see below) to constant bright light (300 lux), an extremely long day, where they were kept for 26 days. Immediately before and at the end of bright light exposure, testicular or follicular size was determined by laparotomy.

Postnuptial molt was previously found to be a reliable marker for the birds' annual cycle (Gwinner 1991). Therefore, birds were transferred individually to constant light according to their molting stage or the time after completion of molt, to form the following groups:

Group 1. Immediately after the shedding of the 3rd primary; i.e., early during molt. Africans: five males, six females.

Group 2. Immediately after the shedding of the 9th primary; i.e., late during molt. Africans: 10 males, 5 females; Europeans: 6 males, 4 females.

Group 3. 30–35 days after completion of molt. Africans: 7 males, 10 females; Europeans: 4 males, 1 female.

Group 4. 60–65 days after completion of molt. Africans: five males, seven females; Europeans: three males, four females.

Birds were fed with our standard diet consisting of 25% commercial insect food (Eckrich), 30% hard-boiled eggs, 15% bread crumbs, 21% curds, 3% beef heart, and 6% ground egg shells, plus about 10 mealworms per day. Once a week, the drinking water was enriched with vitamins.

The data did not significantly deviate from a

normal distribution (Kolmogorov-Smirnov-Test). Therefore, parametric statistics were used.

RESULTS

EXPERIMENT 1

The birds of both subspecies underwent a postjuvenile body molt during the first autumn of their lives (Figs. 1 and 2, upper diagrams; Table 1). Subsequently, they went through two cycles of testicular growth and regression during each of the two successive photoperiodic springs and summers. Each gonadal cycle was followed by a complete postnuptial molt.

Circannual period values (τ_T , τ_{FM} , and τ_{BM}) were determined separately for each individual. τ -values were close to 12 months, indicating that the rhythms were synchronized with the photoperiodic cycle. No differences were found between the two subspecies in the period of the rhythms of testicular size and flight feather molt (τ_T , τ_{FM}). However, the period of the rhythm of body molt (τ_{BM}) was slightly shorter in the African than in the European birds (one-way ANOVA; $F_{1,9} = 14.6$, $P < 0.01$). To test for differences in the duration of the testicular cycles (D_T), a repeated measures MANOVA was used. Whereas no significant differences were found between subspecies, there was a highly significant difference in both subspecies in the duration of the two successive cycles ($P < 0.001$; Table 1).

Although the patterns of the gonadal and molt cycles were similar in the two subspecies, inspection of Figures 1 and 2 suggests that interindividual variability is higher in the African than in the European birds. Indeed, the period of the rhythm of flight feather molt (τ_{FM}) and the durations of both testicular growth cycles (D_T) were significantly more variable in the African than in the European subspecies (τ_{FM} : $F_{1,10} = 36.8$, $P < 0.001$; D_T : $F_{1,12} = 44.9$, $P < 0.001$). One African bird (arrow in Fig. 1) showed a particularly unusual pattern: its testicular growth during the second and third cycle started much earlier than in the other birds, i.e., before the winter solstice, and thus even before the photoperiod had begun to increase. In this bird, gonadal regression at the end of the second gonadal cycle, as well as the subsequent molt, also occurred earlier than in the other birds; i.e., the whole gonadal cycle was advanced relative to the photoperiodic cycle.

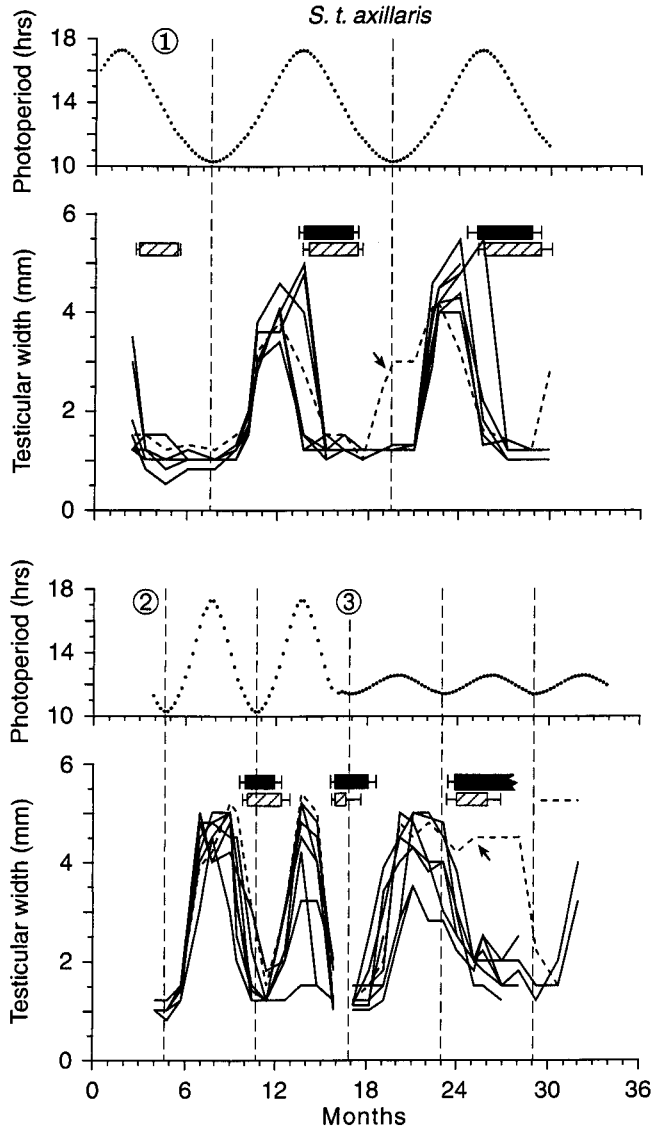


FIGURE 1. Changes in testicular width of individual birds and mean occurrence of molt (with SD; black bars: flight feather molt; hatched bars: body molt) of the African Stonechats in experiments 1, 2, and 3 (numbers encircled), kept under the photoperiodic cycles indicated by the dotted lines. The arrow in the upper diagram points to the testicular growth curve of the individual bird whose gonads developed and regressed earlier than those of the other birds of experiment 1. The horizontal dashed line in the right part of the lower diagram indicates the occurrence of body molt in the bird (arrow) that maintained enlarged testes much longer than its conspecifics in experiment 3. Black bar with fringed end in lower right: no data on molt termination.

EXPERIMENT 2

As in experiment 1, all birds of experiment 2 went through two testicular growth cycles, each of which was followed by a molt of flight and body feathers (Figs. 1 and 2, left part of lower

diagrams; Table 1). As is consistent with the short photoperiodic cycle of 6 months, the intervals between successive onsets of testicular cycles (τ_T) and molts (τ_{FM} , τ_{BM}) also were shortened to about 6 months.

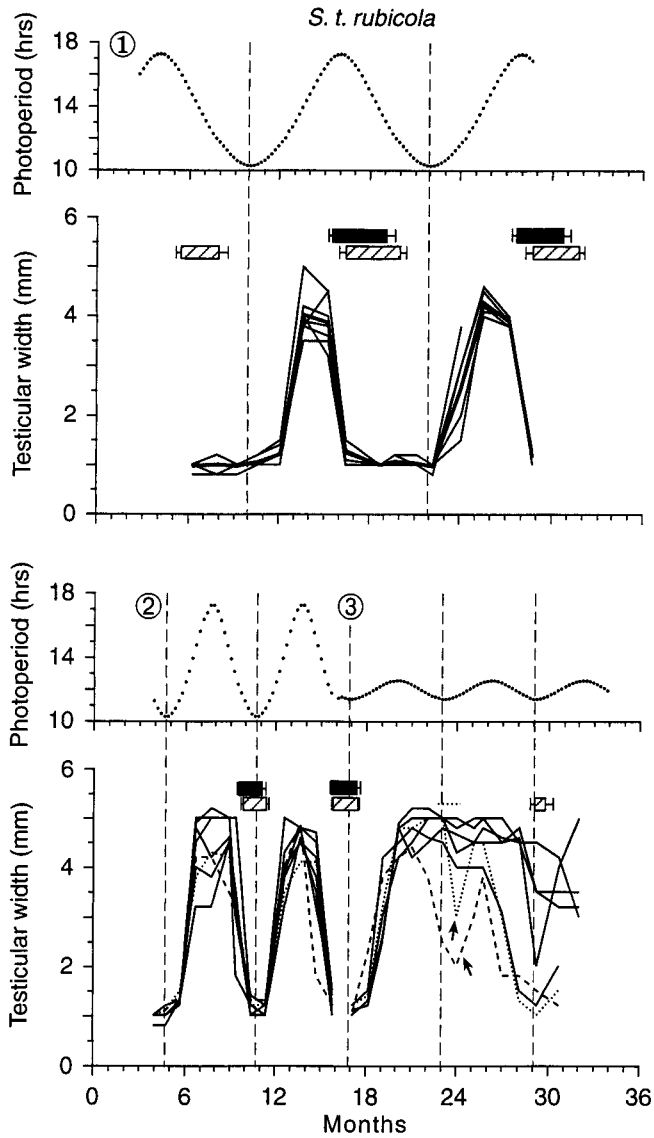


FIGURE 2. Information as in Figure 1, but for the European Stonechats. The dotted horizontal line in the lower diagram indicates the occurrence of body molt in one of two individuals (arrows) whose testes partially regressed during the first photoperiodic trough.

The two subspecies were indistinguishable with regard to the period of their testicular, body molt, and flight feather molt rhythms (τ_T , τ_{BM} , τ_{FM}). In a repeated measures MANOVA, no significant effects between either subspecies or cycles could be detected (Table 1).

As in experiment 1, interindividual variability was greater in the African than in the European subspecies regarding the period of the rhythm of

flight feather molt (τ_{FM}) as well as the duration of each of the two testicular growth cycles (D_T) ($F_{1,12} = 42.0$, $P < 0.001$ for τ_{FM} ; $F_{1,14} = 37.8$, $P < 0.05$ for D_T). Furthermore, the period of the testicular rhythms (τ_T) also was more variable in the African birds than in their European counterparts ($F_{1,14} = 185.3$, $P < 0.001$; Table 1).

Inspection of Figures 1 and 2 suggests that gonadal growth cycles and molt occurred later

TABLE 1. Mean \pm SD period in days (in parentheses: sample sizes) of the rhythm of testicular size (τ_T), body molt (τ_{BM}), and flight-feather molt (τ_{FM}), as well as the duration of testicular cycles (D_T) in the African (*S. t. axillaris*) and European (*S. t. rubicola*) Stonechats of experiments 1 and 2. P_M : level of significance at which means differ from each other; P_V : level of significance at which variances differ from each other.

	<i>S. t. axillaris</i>		<i>S. t. rubicola</i>		Between subspecies		Between testicular cycles	
	P_V	P_M	P_V	P_M	P_V	P_M	P_V	P_M
Experiment 1								
τ_T	333.08 \pm 29.22 (6)		328.29 \pm 17.15 (6)					
τ_{BM}	356.00 \pm 9.45 (6)		375.20 \pm 4.60 (5)			<0.01		
τ_{FM}	349.67 \pm 20.39 (6)		370.60 \pm 3.36 (5)			<0.001		
D_T	135.72 \pm 35.72 (8)	170.43 \pm 45.11 (6)	1st cycle 122.81 \pm 5.33 (7)	2nd cycle 166.31 \pm 16.91 (5)		<0.001		<0.001
Experiment 2								
τ_T	168.67 \pm 23.82 (7)		175.49 \pm 1.75 (8)			<0.001		
τ_{BM}	177.38 \pm 2.97 (8)		180.63 \pm 4.06 (8)					
τ_{FM}	182.00 \pm 10.37 (7)		187.71 \pm 1.60 (7)			<0.001		
D_T	142.92 \pm 10.39 (8)	135.01 \pm 35.70 (7)	1st cycle 135.29 \pm 4.66 (8)	2nd cycle 126.54 \pm 5.81 (8)		<0.05		

relative to the photoperiodic cycles in the birds of experiment 2 as compared to those of experiment 1. Table 2 shows that the differences between the phase of the longest day on one hand and the phases of maximal testicular width and onset of molt on the other hand (the phase-angle difference between the synchronizing and the synchronized rhythms) is significantly smaller under the shorter than under the longer photoperiodic cycle.

EXPERIMENT 3

As in experiments 1 and 2, the testes of the birds in this experiment became enlarged during the first photoperiodic spring and summer (Figs. 1 and 2, right part of lower diagrams). During the following photoperiodic cycle, however, the testicular size of the birds of both subspecies no longer followed the low-amplitude cycle of day-length. In the African group, the testes of all but one bird slowly decreased, reaching low values during the second photoperiodic cycle. While testis size decreased, all birds began to molt. During the third photoperiodic cycle, the testes developed again, suggesting either synchronization with every second photoperiodic cycle or the expression of the endogenous circannual rhythmicity. The gonads of one bird (arrow) stayed enlarged for more than 8 months before they regressed during the second reduction of photoperiod; at that time the bird started to molt, much later than the other birds of this experiment. In the European group, the testes of all birds showed an initial increase. Subsequently, those of four individuals stayed enlarged until the declining phase of the second photoperiodic cycle, when they partially regressed. The testes of one of these birds increased again during the photoperiodic spring of the third cycle. The two other birds (arrows) responded to both the first and the second decline of photoperiod with partial gonadal regression, accompanied in all but one case by a partial body molt.

EXPERIMENT 4

The birds of the two subspecies were indistinguishable in their response to constant bright light (Fig. 3). The size of the testes and follicles stayed close to baseline in groups 1 and 2, which were transferred to bright light during the molting season, but increased significantly in groups 3 and 4, which were transferred to bright light after molting had been completed (two-way AN-

TABLE 2. Phase-angle differences in days (means \pm SD) between the phase of maximal testicular width and the onset of flight feather molt on one hand and the phase of the longest day on the other hand, in African (*S. t. axillaris*) and European (*S. t. rubicola*) Stonechats, as a function of zeitgeber period. Positive values indicate that the respective phases of the testicular or molt rhythms lead the photoperiodic rhythm, negative values that they lag the photoperiodic rhythm. *U*-values and significance levels from a Mann-Whitney *U*-test are given.

	Duration of photoperiodic cycle		<i>U</i>	<i>P</i> <
	12 months	6 months		
<i>S. t. axillaris</i>				
Maximal testicular width	51 \pm 25.8	-1 \pm 7.4	94.0	0.001
Onset flight-feather molt	14 \pm 19.6	-63 \pm 7.4	69.0	0.001
<i>S. t. rubicola</i>				
Maximal testicular width	77 \pm 13.4	2 \pm 13.4	55.0	0.001
Onset flight-feather molt	10 \pm 8.4	-60 \pm 0	55.0	0.001

OVA; males: $F_{2,39} = 52.6$, $P < 0.001$; females: $F_{2,36} = 19.0$, $P < 0.001$; data from groups 1 and 2, as well as 3 and 4, pooled).

DISCUSSION

A major result of these experiments is that the circannual cycles of gonadal size and molt of African Stonechats originating from an equatorial population can be synchronized with large-amplitude sinusoidal photoperiodic cycles just as easily as those of European conspecifics from the temperate zones. As previously

demonstrated, a rhythm in testicular size and molt persisted in most African and many European birds kept under a constant equatorial photoperiod (Gwinner and Dittami 1990). Under such constant conditions, the period of these rhythms usually deviated slightly from 1 year. In contrast, when the African Stonechats of the present study were exposed to a 12-month temperate-zone photoperiodic cycle, the period of the testes and molt rhythms of all birds synchronized to the photoperiodic cycle. Moreover, when the photoperiodic cycle was

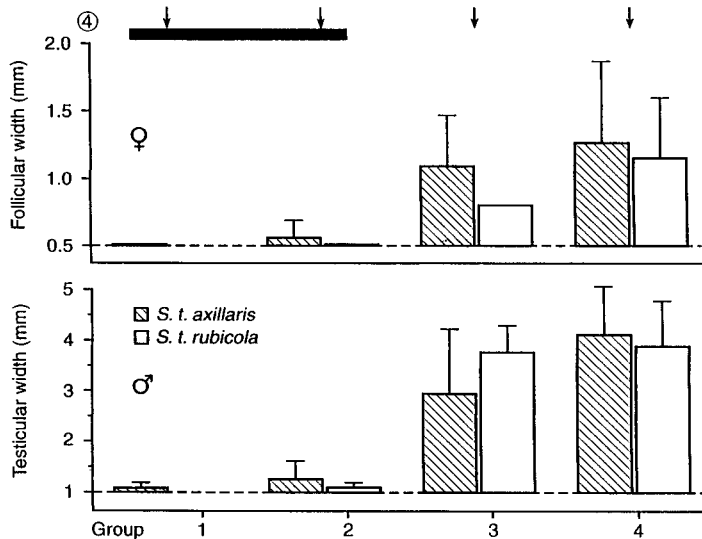


FIGURE 3. Mean testicular and follicular width (with SD) of the four groups of African (hatched bars) and European Stonechats (open bars) in experiment 4. Birds were transferred to constant bright light at four phases of their annual cycle (arrows), defined in relation to their postnuptial molt (black bar); gonad size was determined after 26 days of bright-light exposure. The horizontal dashed lines in both graphs indicate initial mean follicular and testicular width, measured prior to the transfer.

shortened to 6 months, the gonadal and molt rhythms were shortened accordingly. Only minor differences were found between the two subspecies in circannual pattern. Taken together, the results of experiments 1 and 2 strongly suggest that a large-amplitude photoperiodic cycle can be an effective zeitgeber, synchronizing circannual rhythms not only in temperate-zone but also in equatorial stonechats.

The tentative conclusion that photoperiod is a potential circannual zeitgeber for both subspecies is supported by the fact that the phase-relationship between the biological rhythms and the photoperiodic cycle was different under the long and short photoperiodic cycles. The onset of both testicular growth and molt occurred later relative to the photoperiodic cycle if the period of the photoperiodic cycle was 6 months than if it was 12 months (Table 2). This kind of relationship is typical of the performance of self-sustaining oscillators under the influence of a driving oscillator; it has been confirmed in many studies on circannual (Gwinner 1986) and, particularly, circadian rhythms (Aschoff and Pohl 1978).

In temperate-zone birds, synchronization of reproduction and molt rhythms by annual photoperiodic cycles is usually achieved through seasonal changes in responsiveness to photoperiod. A characteristic feature of this mechanism is the seasonal switch from a state of photorefractoriness, during which the hypothalamic-pituitary-gonadal axis cannot be activated by long photoperiods, to a state of photosensitivity during which long days regain their stimulatory action and can induce gonadal recrudescence. The results of experiment 4 indicate that this switch from a state of refractoriness to a state of photosensitivity occurs in both subspecies of stonechats around the time of molt completion.

Although the results discussed so far did not reveal any major differences in photoperiodic responsiveness of the two subspecies, experiment 3 strongly suggests that there may be subspecies differences in the mechanisms that induce the refractory state. In this experiment, following the initial phase of testicular recrudescence, most European birds retained large gonads for an unusually long period of time, i.e., up to 11 months. Following complete or partial gonadal regression, some birds molted their body plumage, about 1 year after the beginning of the experiment. In contrast, with the

exception of one individual, the African Stonechats started to regress their testes within 2 to 3 months after having reached maximal testicular size, and in most birds testes regressed to baseline levels. Subsequently, all these birds started to molt body and flight feathers about 7 months after the beginning of the experiment. The most likely interpretation of this difference is that the critical photoperiod for the induction of photorefractoriness (which in most avian species requires long days; Nicholls et al. 1988) is longer in the European than in the African birds. This is consistent with the longer photoperiod to which European birds are normally exposed in summer, the time at which refractoriness sets in. Conversely, individuals of the tropical subspecies must initiate photorefractoriness under shorter day-lengths than their temperate-zone conspecifics. This implies that there has been selection on the photoperiodic system of equatorial birds, resulting in a shortening of the critical photoperiod required for the induction of refractoriness.

It should be emphasized at this point that the latter interpretation does not necessarily imply that photoperiodic information is used by these birds for the synchronization of their circannual cycles. Instead, particular photoperiodic conditions may be required at certain phases for the circannual rhythmicity to persist. This distinction is consistent with the fact that photoperiod is known to act in two ways on circannual rhythms: as a factor synchronizing circannual rhythms and as a factor permitting their expression (Gwinner 1986, 1996). Evolutionary adjustments of critical photoperiods controlling refractoriness also have been shown in equatorial migrants, e.g., flycatchers (Gwinner 1996).

Another feature in which birds of the two subspecies differed from each other in a conspicuous manner is the greater interindividual variability of the African birds in the periods of circannual rhythms (i.e., those of flight feather molt in experiments 1 and 2, and that of testicular size in experiment 2), as well as in the duration of testicular growth cycles (both experiments). This indicates that the African birds responded in a more variable way to photoperiodic changes than the European birds. In contrast to the situation under changing photoperiodic conditions, the timing of gonadal activity and molt was found to be less variable in African than in European Stonechats when the birds were kept in

a constant equatorial photoperiod (Gwinner 1991). The latter result suggests that the tropical stonechats are more dependent on endogenous circannual factors than their temperate-zone conspecifics, whereas the results of the present study suggest that the temperate-zone stonechats are more responsive to photoperiodic stimuli than the tropical birds.

The finding that equatorial stonechats can respond to a changing photoperiod corroborates previous results indicating photoperiodic effects in African Stonechats and other species of tropical birds. In African Stonechats, the timing and duration of postjuvenile molt are affected by photoperiod in a way similar qualitatively, although not quantitatively, to the effects on the European subspecies (Gwinner et al. 1983). In addition, the tendency to express circannual rhythms of gonadal size and molt under constant conditions depends upon the day-length (Gwinner 1996). Finally, even details of reproductive timing, such as the interval between successive broods, can be modified by photoperiod (König and Gwinner 1995). As in stonechats, gonadal growth or regression, molt, and other seasonal phenomena of many other tropical birds have been experimentally synchronized with photoperiod (see Gwinner and Dittami 1985, Hau et al. 1998).

In regions several degrees off the equator, photoperiod may play a role in timing annual reproductive cycles. Hau et al. (1998) have recently shown that Spotted Antbirds, *Hylophylax naevioides*, in Panama can respond with gonadal growth to a single increase in photoperiod as small as 17 min, suggesting that even birds breeding at a latitude of only about 3° might be able to time their annual cycles by photoperiodic changes. On the other hand, photoperiodic synchronization seems to be quite unlikely in truly equatorial birds like the African Stonechats of the present study. Even under the favorable assumption that such birds use the day-length measured between the onset and end of civil twilight in the morning and evening, respectively, for synchronizing their circannual rhythms, the annual amplitude of the photoperiodic cycle at the equator is less than 4 min. In addition, these small changes are heavily masked by cloud conditions, which may cause differences in effective light intensity from one day to the next, much larger than the maximal theoretical photoperiodic amplitude over the course of a year (Dit-

tami and Gwinner 1985). On the other hand, as mentioned in the Introduction, it seems conceivable that cloud cover causes systematic changes in effective day-length that may be larger than the astronomical ones (Dittami and Gwinner 1985). Unfortunately, data on the seasonal pattern of actual day-length, measured close to the equator, seem to be very scarce.

The birds of experiment 3, which were exposed to a photoperiodic cycle with a low amplitude of only 1 hr 10 min, failed to synchronize their gonadal and molt cycles in a normal 1:1 fashion. It must be emphasized, however, that in this experiment the period of the photoperiodic cycle was 6 months rather than 12 months as in nature. It might therefore have been beyond the range of synchronization, and a period closer to 12 months might have synchronized the rhythms. That even the 6-month photoperiodic cycle was not entirely ineffective is indicated by the fact that although the African birds failed to follow the second photoperiodic cycle with their gonadal rhythms, the testes of the two birds kept long enough under experimental conditions started to grow during the third photoperiodic cycle, concomitantly with the increase of day-length. This suggests 1:2 synchronization to every second photoperiodic cycle, a phenomenon frequently found among circannual and, particularly, circadian systems exposed to weak zeitgebers (Aschoff 1960, Aschoff et al. 1980, Gwinner 1986). Future experiments in which birds are exposed to photoperiodic cycles closer to 12 months (e.g., 10 months) should reveal the minimal photoperiodic amplitude required for synchronizing circannual rhythms in equatorial birds and thus eventually permit an answer to the question of whether photoperiod is a zeitgeber for these birds at the equator.

Although the present results do not rule out the possibility that equatorial stonechats use day-length information to synchronize their circannual rhythm, it seems appropriate to search for possible alternative explanations for their capacity to respond to high-amplitude photoperiodic cycles. In the following, three possibilities will be discussed.

(1). *Coincidental reaction of a control mechanism that normally responds to other stimuli.* Tropical birds may be equipped with a mechanism that normally perceives zeitgeber stimuli other than day-length but happens to be suscep-

tible to photoperiodic changes as well. Circadian mechanisms are known to be involved in the regulation of annual cycles, and it is conceivable that in tropical birds these mechanisms are normally affected by non-photoperiodic stimuli and also are responsive to photoperiod (as they are in temperate-zone species), even though the photoperiodic stimuli are not used by the tropical species in their natural habitat. Factors of this type may include nonphotic stimuli, as well as photic stimuli other than day-length. Among the latter might, for instance, be the light intensity during daytime, which can be expected to change with the alternations between dry and rainy seasons. Indeed, in the European Starling *Sturnus vulgaris* and other species, light intensity and photoperiod seem to be to a certain extent synergistic; that is, a particular photoperiod with a low light intensity is interpreted as being shorter than the same photoperiod with a higher light intensity (Bissonnette 1937, Burger 1949, Bentley et al. 1998). Some recent evidence suggests that circannual rhythms of African Stonechats may in fact become synchronized by seasonal changes in light intensity, even if photoperiod is kept experimentally constant (Gwinner and Scheuerlein 1998).

(2). *A phylogenetic relic.* The second possible explanation for the photoperiodic responsiveness of the African Stonechat is that the population now breeding at the equator originated at higher latitudes, where the amplitude of the photoperiodic cycle is large enough for birds to use it for the timing of their seasonal activities. According to this hypothesis, individuals may at some time have colonized equatorial regions and retained their photoperiodic system as a relic, because there was no selection pressure against it. Inconsistent with this hypothesis is the finding of experiment 3, namely that equatorial stonechats differed from their temperate-zone conspecifics with regard to the duration of the photoperiod inducing gonadal regression. That is, under a photoperiod of about 12 hr most equatorial birds were able to rapidly regress their gonads, whereas most European birds did so only with a very long delay; this suggests an adaptation of the African birds to the local equatorial short-day conditions. Interindividual variability in seasonal timing was larger in the African than in the European birds of experiment 1; this outcome might have resulted from the absence of selec-

tion pressures on timing properties of photoperiodic mechanisms. Also consistent with this interpretation is the fact that African Stonechats almost certainly evolved from taxa living at higher latitudes. Although the breeding range of the Stonechat extends over much of Europe and Asia and covers wide areas in Africa, the species most probably emerged somewhere in the Palearctic region, where most related species occur (Voous 1960, Hall and Moreau 1970, Glutz von Blotzheim and Bauer 1988).

A particularly attractive hypothesis was that our equatorial birds may be descendants of European migratory populations that happened to be unable to leave their former wintering grounds and colonized them permanently as a result (Gwinner 1991). This kind of colonization of former winter quarters by migrants has indeed been demonstrated for other species (Schüz 1971). Long-distance migrating populations of the Asiatic subspecies Siberian Stonechat *S. t. maura* migrate far into Africa and winter as far south as Ethiopia, less than 500 km from the northern edge of the *S. t. axillaris* breeding range. *S. t. maura* also bears some morphological similarities to *axillaris*. However, a molecular genetic study, specifically carried out to determine whether *maura* is more closely related to *axillaris* than is *maura* to *rubicola*, failed to support this hypothesis (Wittmann et al. 1995). On the basis of nucleotide sequences in a 300 bp fragment of the mitochondrial cytochrome-*b* gene, the genetic distance between *axillaris* and *maura* is about the same as that between *axillaris* and *rubicola*. Moreover, the genetic distance is so large (5.1 to 5.7% sequence divergence) that it was suggested that the three subspecies should actually be considered different species (Wittmann et al. 1995).

(3). *The result of gene flow.* The final and perhaps most likely hypothesis, which is consistent with all findings of the present study, states that the strong photoperiodic responsiveness of the equatorial stonechat represents the unavoidable consequence of gene flow from more northern or southern African Stonechat populations into the equatorial population. The subspecies *S. t. axillaris* occurs from 5°N to about 10°S in East Africa (Keith et al. 1992). In particular, it seems possible that the photoperiodic mechanism evolved in populations exposed to day-length changes large enough to be useful for seasonal timing and that it spread from these regions to-

wards the equator as a result of dispersal and interbreeding with lower-latitude populations. In this case, equatorial populations cannot avoid receiving photoperiodic genes and may retain them in their gene pool. Seen from an evolutionary perspective, genes for photoperiodic responsiveness might even be adaptive for birds living at the equator, as their offspring might disperse to latitudes where photoperiodic changes are large enough to be useful for seasonal timing. A critical evaluation of this hypothesis would require information about the rate and distance of dispersal, which is unfortunately not yet available.

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