

## Temperature Modulation of Photoperiodically Induced LH Secretion and Its Termination in Japanese Quail (*Coturnix coturnix japonica*)

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Long days induced LH release in photosensitive Japanese quail and ambient temperature did not affect this process. Temperature also did not affect the levels of circulating LH concentrations at the steady state of LH release on long days. On the other hand, low ambient temperature was required to reduce circulating LH to a nonbreeding level together with the change of the photoperiod from long to short days; the changes of the photoperiod without low ambient temperature induced a decrease of circulating LH only to a certain level (1-2 ng/ml) which could maintain reproductive activity. The results also indicated that there were three levels of circulating LH in quail which were tentatively designated as a nonbreeding level (<0.5 ng/ml), a basal breeding level (1-2 ng/ml), and a steady breeding level (ca. 4 ng/ml). By photostimulation, levels of circulating LH increased to the steady breeding level directly and gradually, or leveled off after overshooting it. Photoperiodic changes from long to short days under moderate temperature resulted in a decrease in circulating LH to the basal breeding level which, however, could keep the gonad and the accessory sex organs active. Photoperiodic changes under cold ambient temperature reduce circulating LH to the nonbreeding level at which the gonad and the accessory sex organs regressed completely. These results suggest that ambient temperature is involved in the mechanism controlling an annual reproductive cycle, especially at the termination of the reproductive activity, in Japanese quail. © 1990 Academic Press, Inc.

It is well established that day length is the major environmental variable in control of LH secretion in many avian species of mid and high latitudes (Farner and Gwinner, 1980). Information of the day length is transmitted through the hypothalamic neurosecretory LHRH system (e.g., Hattori *et al.*, 1986); thus long days initiate LH release in sexually immature and in sexually quiescent birds (Robinson and Follett, 1982; Follett, 1981; Follett and Nicholls, 1984; Wada, 1979, 1981; Hatanaka and Wada, 1988). However, stimuli that initiate a decrease of circulating LH seem to be different in different species. Some species show "absolute photorefractoriness" to long days during which levels of circulating LH start to decline at a time when the day length is still long enough, and others show

"relative photorefractoriness" in which a decrease in circulating LH is initiated by a decrease to a relatively shorter day length (for review, see Nicholls *et al.*, 1988).

It is said that the Japanese quail is a relative photorefractory species (Robinson and Follett, 1982; Follett and Nicholls, 1984), and that a decrease in day length suppresses LH release and terminates the reproductive activity. However, experimental results always annoy us that short days do not always suppress LH release enough to reduce cloacal activity in the Japanese quail in our laboratory. We obtain quail from a commercial source and do not make any selection against photoperiodic response before experiments. Recently, however, we confirmed that circulating LH and the size of the cloacal protrusion de-

crease to reproductively quiescent levels in our Japanese quail in September under natural environments (in preparation).

It is thus natural to consider that factors other than the photoperiod are involved in suppression or termination of LH secretion together with short days in this species. Studies of the effect of other environmental cues on avian gonadal activity are rather sparse in the literature. Of the possible environmental cues controlling gonadal activity, ambient temperature has been studied in several avian species: starlings (Burger, 1948), sparrows (Kendeigh, 1941), white-crowned sparrows (Farner and Mewaldt, 1952; Lewis and Farner, 1973), and Japanese quail (Oishi and Konishi, 1978). In these experiments, however, reproductive activity is estimated by testicular weight or the size of the cloacal protrusion, and no detailed study concerning the effect of temperature on gonadotropin secretion is present.

Thus the purpose of the present experiment is to make clear the effect of ambient temperature on LH secretion (1) at the phase of photoperiodic induction, (2) at the phase in which circulating LH is maintained, and (3) at the phase in which the photoperiod is shortened.

## MATERIALS AND METHODS

### Animals

Male Japanese quail (*Coturnix coturnix japonica*), 3 weeks old, were purchased from a commercial source. Throughout the experimental periods described below, except for two groups in Experiment 1, the birds were kept in environmental chambers with constant ambient temperature. They were raised under a short day of 8L:16D (lights on at 0800 hr) until 5–6 weeks of age, when they showed full somatic growth but were still sexually immature. Ambient temperature was basically  $19 \pm 1^\circ$  except for the  $25^\circ$  group in Experiment 1. Food and water were constantly available.

They were then transferred to each experimental condition. No selection on the basis of responsiveness of gonadal activity to alternating long and short photoperiods was made beforehand.

Blood samples of 0.2–0.3 ml were collected at 1000

hr basically every day at the beginning and every other day thereafter. The samples were taken by venipuncture at the wing vein with heparinized capillary tubes. The plasma was separated and stored at  $-20^\circ$  until assay.

Cloacal protrusion areas were measured with a ruler to the nearest millimeter at the time of blood collection.

### Experimental Schedules

*Experiment 1. Effects of combinations of day length and seminatural ambient temperature on LH secretion.* Three groups of birds were used in this experiment. The first group ( $n = 7$ ) was transferred to long days of 16L:8D (lights on at 0800 hr) at  $25^\circ$  and stayed there for 40 days. The second ( $n = 7$ ) and third ( $n = 6$ ) groups experienced 16L:8D or 8L:16D with  $25^\circ$  (in the environmental chamber) or low ambient temperatures (see below), successively, as shown in Fig. 1.

To expose the birds in the second and third groups to lower temperatures, individual cages were transferred into a light-tight box which was ventilated with a motor-driven fan placed in the corner of a building, not air conditioned, from January 12 to April 2, 1986. Temperature in the box during the experimental period was monitored with a maximum–minimum thermometer and recorded at blood collection (temperature range from 4 to  $16^\circ$  during the experimental period). Illumination of the inside was regulated with a timer.

*Experiment 2. Effects of various fixed ambient temperatures on LH secretion under long days and short days.* Three groups of male quail were used for this experiment. The first group ( $n = 20$ ) was kept under 8L:16D at  $25 \pm 1^\circ$  and the second ( $n = 10$ ) and third groups ( $n = 7$ ) were kept under 8L:16D at  $19 \pm 1^\circ$  before photostimulation. In the third group, the ambient temperature was reduced to  $8 \pm 1^\circ$  a week before photostimulation.

The photoperiod was then changed to long days of 16L:8D under the previous ambient temperature for 30 days. After 30 days of photostimulation, the birds returned to 8L:16D under the same temperature conditions.

*Experiment 3. Effect of short days and low temperature on LH secretion.* To change both day length and temperature, two groups of mature birds (3 months old) which had been kept under 16L:8D at  $19^\circ$  were used. Group 1 ( $n = 7$ ) was transferred to 8L:16D at  $9 \pm 1^\circ$  and Group 2 ( $n = 6$ ) was transferred to the short days at  $19^\circ$ .

### Radioimmunoassay

Plasma concentrations of LH were determined in 50- $\mu$ l sample volumes, in duplicate, using the radioim-

monoassay method described by Hattori and Wakabayashi (1979). Chicken LH (fraction IRC-2, Gunma) was used for reference preparations and a preparation of chicken LH (fraction AGCHDS112312A) was used for iodination. The antiserum (AH-MH No. 1) was raised against chicken LH (fraction IRC-2, Gunma). Results are expressed in terms of nanograms per milliliter of a chicken LH fraction IRC-2.

Intraassay and interassay coefficients of variation were 6.43 and 8.30%, respectively.

### Statistics

Single comparisons of means were made using Student's *t* tests, paired where appropriate. For multiple comparisons, an analysis of variance (ANOVA) was first performed, and this revealed significant variation ( $P < 0.05$ ); pairwise comparisons of means were made using Student's *t* test. Differences were considered significant when  $P < 0.05$ . Throughout the text, means are given  $\pm 1$  SEM.

## RESULTS

### *Experiment 1. Effects of Combinations of Day Length and Seminatural Ambient Temperature on LH Secretion*

In birds transferred to 16L:8D at 25° levels of circulating LH increased rapidly to  $8.64 \pm 1.69$  ng/ml after 10 days of photostimulation. Then the levels of LH decreased to  $3.24 \pm 0.81$  ng/ml by Day 20 and leveled off (3–4 ng/ml) during the next 20 days (Fig. 1, upper panel).

Circulating LH in birds kept under 8L:16D at 25° increased to  $2.84 \pm 0.76$  ng/ml by Day 4 and the level was maintained by Day 20. Their cloacal protrusions developed to a full mature size by Day 20. However, in the birds kept under 8L:16D at low temperature (4–12°), circulating LH stayed at a low level (less than 0.5 ng/ml) for 20 days (Fig. 1, middle panel, first session) and the cloacal protrusion did not develop.

After 20 days, the photoperiod was changed to 16L:8D. Circulating LH began to increase to  $8.28 \pm 1.73$  ng/ml by Day 28 in the birds at low temperature as was seen in the group of 16L:8D at 25°. In the 25° group, long days also induced an increase of LH, but the increase, although signifi-

cant, was not as drastic as that observed in the birds at 16L:8D, 25° (Fig. 1, middle panel, second session). The cloacal protrusion of the birds in the lower temperature group developed to the mature size by Day 40 and that in the 25° group maintained its full mature size.

Temperature changes from high to low and those from low to high under 16L:8D did not induce any significant change in circulating LH. Levels of LH in both groups were 3–4 ng/ml (Fig. 1, middle panel, third session). The cloacal protrusion of both groups maintained the full mature size.

Short days initiated a gradual decrease of the level of circulating LH in the low temperature group to the immature level ( $0.51 \pm 0.07$  ng/ml by Day 80); the cloacal protrusion of these birds regressed completely. In the 25° group, there was a slower decrease but LH levels stayed around 2 ng/ml ( $2.33 \pm 0.49$  ng/ml by Day 80; Fig. 1, middle panel, fourth session). The cloacal protrusion of these birds was active with secretion.

Actual ambient temperatures in the cage during the experiment are shown in Fig. 1, lower panel. Ambient temperatures were between 4 and 16° during the experimental period.

### *Experiment 2. Effects of Various Fixed Ambient Temperatures on LH Secretion under Long Days and Short Days*

Photostimulation by 16L:8D induced a rapid LH release under any ambient temperature of 25, 19, and 8° (Fig. 2). Profiles of LH increase were not different among the three groups, even though the amplitudes were different; the higher the ambient temperature, the higher the maximum LH concentrations. The cloacal protrusion developed to a matured size in all the groups.

When the photoperiod was changed to 8L:16D after 30 days of photostimulation (arrows in Fig. 2), significant decreases in circulating LH were observed in all the

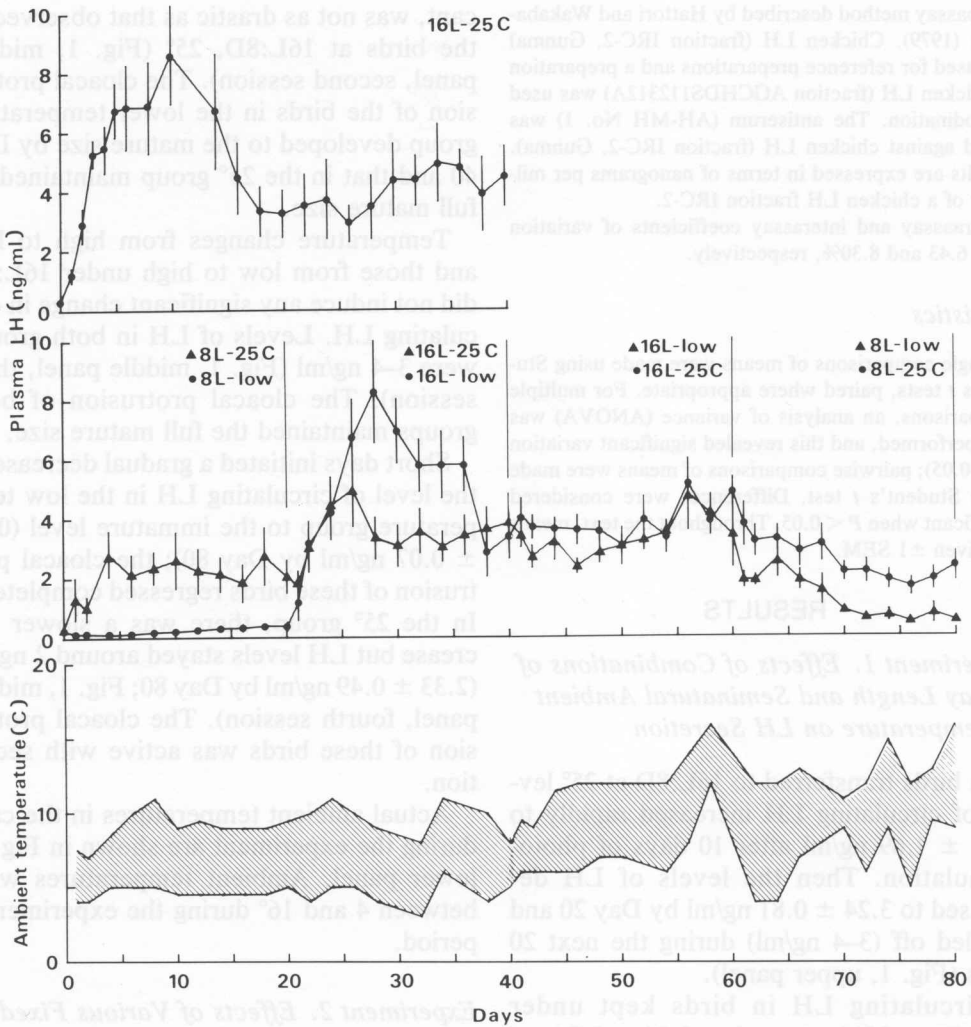


FIG. 1. Changes in circulating LH of male Japanese quail kept on long days of 16L:8D or short days of 8L:16D with different ambient temperature conditions. The upper panel shows changes in circulating LH on 16L:8D at 25°. The middle panel shows changes in circulating LH in two groups of birds: the first group was kept under 8L:16D-25° 16L:8D-25° 16L:8D-low temperature, and 8L:16D-low temperature (solid triangles) and the second group was kept under 8L:16D-low temperature, 16L:8D-low temperature, 16L:8D-25° and 8L:16D-25° (solid circles). The lower panel shows changes in actual ambient temperatures during the experimental period designated as low temperature in the middle panel (the shaded area represents the difference between the highest and lowest temperatures).

groups at the beginning. However, LH concentrations in the 25 and 19° groups did not decrease completely to the basal level but maintained median levels; the cloacal protrusion of the birds in these groups was active. On the other hand, LH concentrations in the 8° group decreased to the basal level

(0.33 ± 0.10 ng/ml) and the cloacal protrusion regressed completely.

Mean concentrations of circulating LH in the 25 and 19° groups by Day 59 and Day 57 were 1.54 ± 0.25 and 1.38 ± 0.28 ng/ml, respectively. Detailed inspection of changes in LH in each bird of the 25 and 19°

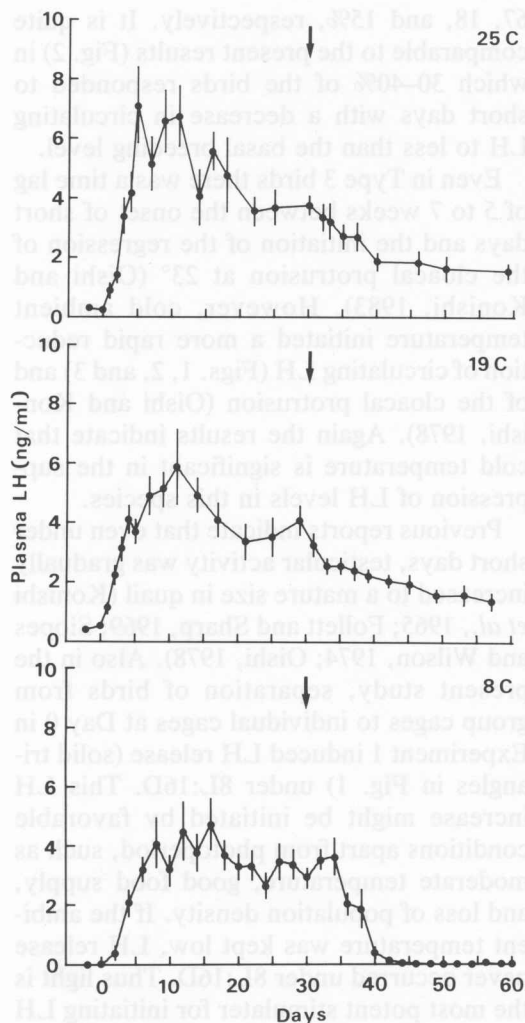


FIG. 2. Changes in circulating LH of Japanese quail kept on long days of 16L:8D and on short days of 8L:16D at different fixed ambient temperatures (25, 19, and 8°). Arrows indicate the day of change from long days to short days.

groups showed that LH decrease to a level less than 1 ng/ml was found in 7 out of 20 and 4 out of 10, respectively. The cloacal protrusion of these birds was regressing or regressed.

### Experiment 3. Effect of Short Days and Low Temperature on LH Secretion

In this experiment, both the ambient tem-

perature and the photoperiod were reduced at the same time in Group 1 (from 16L:8D, 19° to 8L:16D, 9°). LH concentrations decreased steadily to the basal level ( $0.33 \pm 0.06$  ng/ml). However, circulating LH in Group 2 (control) in which only the photoperiod was shortened (from 16L:8D, 19° to 8L:16D, 19°) decreased only to  $1.84 \pm 0.25$  ng/ml, even though the decrease was significant (Fig. 3).

The cloacal protrusion of the birds in Group 1 regressed completely to the non-breeding state, but that in Group 2 remained active.

### DISCUSSION

The present experiments show that, in Japanese quail, changes of a photoperiod from long days to short days are not always enough to suppress LH secretion to a reproductively quiescent level and that low ambient temperature, together with the change of the photoperiod, is required for full suppression of LH secretion. However, low ambient temperature does not affect photoperiodically induced LH release, although it may modulate the amplitude of the LH levels. The results also indicate that there seem to be three distinct levels in circulating LH, namely a nonbreeding level (less than 0.5 ng/ml), a basal breeding level

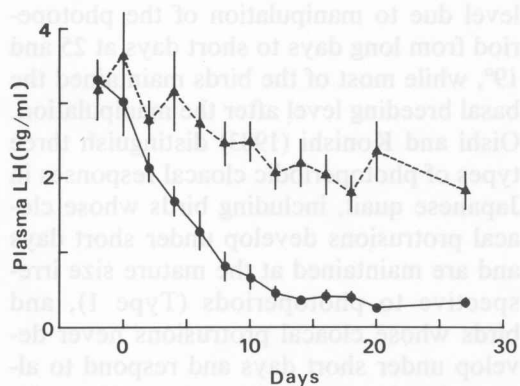


FIG. 3. Changes in circulating LH after transfer from 16L:8D at 19° to 8L:16D at 9° (solid line) or to 8L:16D at 19° (broken line).



(1–2 ng/ml), and a steady breeding level (about 4 ng/ml).

The present results support the conclusion of Oishi and Konishi (1978) and extend it in terms of circulating LH. They measured the size of the cloacal protrusion in Japanese quail under various combinations of day length and temperature; when the temperature was lowered from 23 to 10° on 8L:16D, the cloacal protrusion reduced significantly. This involuted protrusion attained mature size after elevation of the temperature to 23°. The cloacal size did not decrease in birds on 16L:8D when the ambient temperature was lowered. They concluded that ambient temperature and the length of photoperiod affect the testicular activity and that the effect of the length of the photoperiod is predominant. The present results indicate that the day length is certainly predominant when the LH release is induced; as is shown in Figs. 1 and 2, any ambient temperature tested did not affect the LH secretion. The changes of photoperiods from 16L:8D to 8L:16D also induce a significant decrease in circulating LH to the basal breeding level which, however, could maintain the cloacal protrusion at the mature size. To reduce circulating LH to the nonbreeding level, cold ambient temperature was necessary.

Some birds in Experiment 2 showed a decrease of circulating LH to the nonbreeding level due to manipulation of the photoperiod from long days to short days at 25 and 19°, while most of the birds maintained the basal breeding level after the manipulation. Oishi and Konishi (1983) distinguish three types of photoperiodic cloacal responses in Japanese quail, including birds whose cloacal protrusions develop under short days and are maintained at the mature size irrespective to photoperiods (Type 1), and birds whose cloacal protrusions never develop under short days and respond to alternating long and short days (Type 3). Type 2 is intermediate between Types 1 and 3. The frequencies of Types 1, 2, and 3 are

67, 18, and 15%, respectively. It is quite comparable to the present results (Fig. 2) in which 30–40% of the birds responded to short days with a decrease in circulating LH to less than the basal breeding level.

Even in Type 3 birds there was a time lag of 5 to 7 weeks between the onset of short days and the initiation of the regression of the cloacal protrusion at 23° (Oishi and Konishi, 1983). However, cold ambient temperature initiated a more rapid reduction of circulating LH (Figs. 1, 2, and 3) and of the cloacal protrusion (Oishi and Konishi, 1978). Again the results indicate that cold temperature is significant in the suppression of LH levels in this species.

Previous reports indicate that even under short days, testicular activity was gradually increased to a mature size in quail (Konishi *et al.*, 1965; Follett and Sharp, 1969; Siopes and Wilson, 1974; Oishi, 1978). Also in the present study, separation of birds from group cages to individual cages at Day 0 in Experiment 1 induced LH release (solid triangles in Fig. 1) under 8L:16D. This LH increase might be initiated by favorable conditions apart from photoperiod, such as moderate temperature, good food supply, and loss of population density. If the ambient temperature was kept low, LH release never occurred under 8L:16D. Thus light is the most potent stimulator for initiating LH release under any condition, but other environmental factors act permissively in combination.

Some argue that this gonadal growth under short days is due to domestication, because the Pullman strain at Washington State University did not show any growth of the cloacal protrusion as long as they were kept under short days (Follett and Farner, 1966). However, Kawahara (1973, 1976) reported that wild quail showed wide variation in the number of days required for sexual maturation suggesting the variation in photogonadal response in this species. This variation may be intrinsic which is a reflection of their habitat and is actually

due to being heterologous in sensitivity to cold temperature. Quail are short-distant migrants but could easily shift to being residents if the environmental conditions were satisfactory.

In a series of experiments, Follett and co-workers demonstrated that short days were enough to terminate LH secretion in Japanese quail (for example, Urbanski and Follett, 1982a, b), which is different from the present results. They used castrated birds and their argument was based on the observation of the decrease of the levels of circulating LH. As was shown in this experiment, circulating LH actually decreased to a certain level by a change of day length from long to short days, but the level is still high enough to maintain the reproductive activity. Castration makes it difficult to distinguish between the changes from the basal breeding level to the non-breeding level and the changes in circulating LH within breeding levels. An important point we would like to know is the actual mechanism for decreasing circulating LH which relates to the termination of reproductive activity but not to the relative decreases in circulating LH.

Follett and Nicholls (1984, 1985) demonstrated that long-term thyroxine injections to thyroidectomized quail before the change of photoperiod induced gonadal regression (estimated by the size of the cloacal protrusion). They suggest that a functional thyroid gland is required to be relative refractory in quail so that only a small decrease in photoperiod is necessary to switch off the reproductive system. It has been demonstrated that ambient temperatures affect thyroid function (for review, see Assenmacher, 1973) and cold temperature induced thyroxine or triiodothyronine secretion in birds (Bobek *et al.*, 1980; Kühn and Nouwen, 1978; Oishi and Konishi, 1978). Suppression of LH secretion by cold temperature in the present experiment might be due to activation of the thyroid gland. However, thyroxine treatment had

only a transient effect on the suppression of circulating LH, even though it caused molting (Wada, unpublished observation). This problem remains to be solved with more precise experiments.

No detailed experiment has been done on the effect of diel variation of temperatures rather than constant temperature. Such experiments will provide clues to understanding the mechanism of termination of a breeding season.

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