

《Research Note》

Different Photoperiodic Responses in Four Lines of Japanese Quail

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Organisms measure day length to better adapt to seasonal changes in the environment; this phenomenon is called photoperiodism. The Japanese quail has a highly sophisticated photoperiodic mechanism and is an excellent model for the study of photoperiodism. Various lines of quail have been established during the domestication process. In the present study, we examined the effect of long day (LD) followed by short day (SD) on testicular weight in four lines of quail (L, AMRP, NIES-Br, and WE). When the quail were raised under SD conditions, testicular development was suppressed in all examined lines. The speed of the LD-induced testicular development of NIES-Br line was faster than that of AMRP line, while the speed of the SD-induced testicular regression of L line was significantly faster than that of WE line. These quail lines provide excellent model to uncover the underlying mechanism of seasonal testicular regression.

Key words: photoperiodism, seasonal reproduction, testis

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Introduction

Organisms measure changes in day length (photoperiod) to adapt to seasonal changes in the environment. This mechanism enables the animals to ensure their offspring are born when the climate is moderate and food is abundant. This adaptation to day length is called photoperiodism. Among various vertebrate species, the Japanese quail (*Coturnix japonica*) has been proved to be an excellent model animal for studying photoperiodism because of its rapid and dramatic response to photoperiodic changes (Follett and Sharp, 1969; Nicholls *et al.*, 1983; Follett *et al.*, 1998). For example, testicular mass in Japanese quail is maintained at its minimum size when the quail are raised under short-day (SD) conditions (i.e., non-breeding conditions). However, once they are transferred to long-day (LD) conditions, their testes grow rapidly, typically increasing by more than a hundred-fold within 4 weeks. This dynamic change is in marked contrast with that of mammals (i.e., several-fold) (Dawson *et al.*, 2001). Hence, birds are often

used as experimental animals in the photoperiodism research. In previous studies, we have uncovered the signal transduction pathway regulating seasonal testicular development and regression from photoreceptor to neuroendocrine output (Yoshimura *et al.*, 2003; Nakao *et al.*, 2008; Nakane *et al.*, 2010; 2014; Ikegami *et al.*, 2015).

Recently, we investigated the mechanism of seasonal testicular regression using commercial quail (Ikegami *et al.*, 2015). We noticed that the response of quail to SD conditions varied among quail obtained from different commercial dealers. The genetic background of quail obtained from commercial dealers is not controlled. In the present study, therefore, we evaluated the effect of LD followed by SD conditions in four lines of quail (L, AMRP, NIES-Br, and WE) to demonstrate the line differences in seasonal responses. L line is selected for its low antibody response against inactivated Newcastle disease virus (Koyama *et al.*, 2005; Takahashi *et al.*, 1984), whereas AMRP has a panda plumage (wild type with white spots), which is associated with the endothelin receptor B2 (*EDNRB2*) (Miwa *et al.*, 2007). NIES-Br is a Brazilian line selected for meat production and large body weight, and WE is a standard line used to evaluate endocrine disruptors (Shibuya *et al.*, 2005; Yamashita *et al.*, 2011). All of these lines have been maintained in closed colonies for long time.

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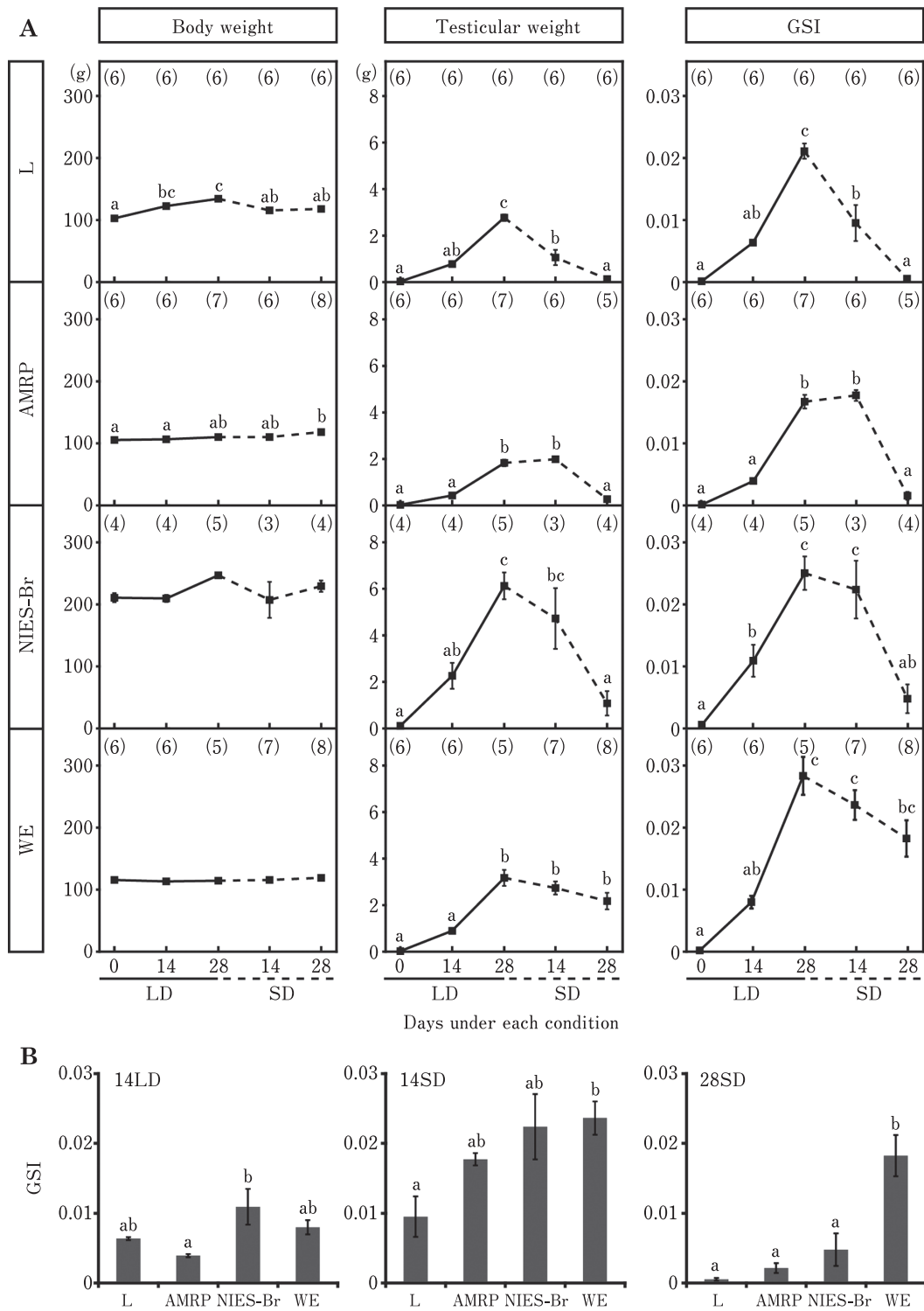


Fig. 1. The effect of changing day length on body weight, testicular weight, and gonadal-somatic index (GSI) in four quail lines (L, AMRP, NIES-Br, and WE). (A) Changes in body weight (left), testicular weight (middle) and GSI (right) in quail transferred from SD to LD (solid line) and then to SD (dashed line). (B) Statistical analysis among the four lines of quail at each condition. All values are reported as the mean \pm s.e.m. Lowercase letters (a, b, c) indicate significant differences based on one-way ANOVA Scheffe's post hoc test ($P < 0.05$). $n=3-8$. Numbers in parentheses indicate the number of animals used.

Materials and Methods

Animals and Sampling Procedures

Four-week-old quail from the L, AMRP, NIES-Br, and WE lines were used to investigate the effect of SD and LD on testicular mass. The 4 week-old quail were maintained under SD conditions (6 h light 18 h dark; 6L18D) for 4 weeks in light-tight boxes in a room at a temperature of $23 \pm 1^\circ\text{C}$. At 8 weeks of age, the quail were transferred to LD conditions (20L4D) for 4 weeks, and then again back to SD conditions. Birds were sacrificed with isoflurane and testes were collected from 3–8 males 3 h after dawn. Because the body weight in NIES-Br differed from that of the other three lines, gonadal-somatic index (GSI) was used as an indicator of the effect of photoperiod on testes. GSI was determined as the ratio of a paired testicular mass to whole body weight (Tilton *et al.*, 2003). This study was approved by the Animal Experiment Committee of Nagoya University.

Data Analysis

Data were analyzed with one-way ANOVA and Scheffé's post hoc test to determine the significance level. All values are reported as the mean \pm s.e.m. Speed of testicular development and regression was evaluated by comparing GSI at 14 days after transferred into LD conditions, and 14 days and 28 days after transferred into SD conditions, respectively.

Results and Discussion

In the present study, we evaluated the effect of LD followed by SD conditions on testicular weight in four lines of quail to demonstrate the line differences. Because the body weight was markedly different among the lines, we also measured the body weight and calculated the GSI. Body weight in the NIES-Br line was approximately 1.8-fold greater than in the other lines (Fig. 1A). In general, quail are known to enter puberty by 8 weeks of age when they are raised under LD condition. Furthermore, four lines of quail used in the present study are confirmed to start egg laying from 6 to 7 weeks of age (Dr. Mikiharu Nakano, Avian Bioscience Research Center, Nagoya University, personal communication)(Shoda, 2006). In the present study, however, quail were raised under SD condition to suppress gonadal development. As a result, suppression of testicular development was observed in all examined lines (Fig. 1A). Once the quail had been transferred to LD conditions, testes developed into fully mature size within 4 weeks in all lines (Fig. 1A) (Follett and Maung, 1978; Ikegami *et al.*, 2015). The speed of testicular development of NIES-Br was faster than that of the AMRP line (Fig. 1B). The quail were then exposed to SD conditions, which induced testicular regression. We found differences in the SD induction of testicular regression among the four lines (Fig. 1A, B). Testicular regression of L line was much faster than that of the WE line, whereas AMRP and NIES-Br lines showed intermediate response (Fig. 1A, B). It is of note that marked testicular regression was not observed in the WE line. These results clearly demonstrate differences in the SD induction of testicular regression among the lines.

Recently, we have reported the effect of SD and low temperature on testicular regression (Ikegami *et al.*, 2015). Low temperature stimulus accelerated SD-induced testicular regression by inducing meiotic arrest and germ cell apoptosis, and shutting down the hypothalamus-pituitary-gonadal (HPG) axis. However, we have also reported the involvement of other organs such as thyroid gland, liver and muscles in this process. The line differences in the testicular regression may originate from all of these tissues and thus their underlying mechanisms remain unknown. Recently, a draft sequence of the quail genome was published, and microsatellite analysis revealed differences in the genetic variability and diversity of quail lines (Kawahara-Miki *et al.*, 2013). Genetic analysis of the four lines of quail augmented by the results from the present study will contribute toward understanding the underlying mechanisms of seasonal testicular regression in future studies.

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