Photoperiod-Induced Changes in Cloacal Gland Physiology and Testes Weight in Male Japanese Quail Selected for Divergent Adrenocortical Responsiveness[1]

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ABSTRACT In quail, activation of the hypothalamic-pituitary-adrenal axis has been linked to depression in the hypothalamic-pituitary-testicular axis, and cloacal gland development and foam production are known to be androgen dependent. Therefore, photoperiod manipulations that induce gonadal growth (long day) and involution (short day) were used to examine changes in cloacal gland physiology in male quail from lines selected for a reduced (low stress, LS) or exaggerated (high stress, HS) plasma corticosterone response to brief restraint. Line differences in cloacal gland area (CAREA) and volume (CVOL), the proportion of individuals that produced cloacal gland foam (PICF), and the intensity of cloacal gland foam production (CFP) were initially assessed in adult males maintained on stimulatory, long photoperiods of 14L:10D. Thereafter, these variables were re-examined weekly in the same males during their subsequent exposure to 3 wk of short days (6L:18D) followed by return to long d for 3 more wk. Line differences in testes weight relative to body weight (RTW) were also measured at the end of the study. CAREA and CVOL were markedly greater in LS than HS males grown under long days. As expected, these indices of cloacal gland size were greatly reduced upon exposure to short days in both lines. However, line differences (LS > HS) in CAREA and CVOL were maintained during the first 2 wk of exposure to short days. Line differences in the depressed cloacal gland size measures were no longer evident by the third week on short days as well as after the first week of relighting wherein there were no further depressions in gland size of either line. However, by the end of 2 wk of rephotostimulation and thereafter, marked line differences (LS > HS) in CAREA and CVOL re-emerged. Photoperiod-induced line effects on CFP generally mimicked those of CAREA and CVOL. The PICF was marginally different ($P < 0.07$) between the lines (LS > HS) only after 1 wk of light reduction. After 3 wk of rephotostimulation, RTW was nearly 25% greater in LS quail. The results suggest that selection for reduced adrenocortical responsiveness in Coturnix has resulted in certain reproductive advantages in middle-aged, adult males maintained on stimulatory photoperiods as well as in such adults subjected to photocastration and subsequent rephotostimulation.

(*Key words*: cloacal gland, Japanese quail, light, stress, testes)

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INTRODUCTION

Japanese quail are extremely photosensitive, and in males the degree of sexual development largely depends on the existing photoperiod as it relates to recent photoperiodic history. For example, increases in cloacal gland size and foam production, as well as testicular volume and weight, are well-documented consequences of long day photostimulation in growing, male Japanese quail (Tanaka et al., 1965; Sachs, 1967; Siopes and Wilson, 1975; Chaturvedi et al., 1992, 1993). In contrast, growth under or transfer of sexually developed birds to short days is associated with regression of cloacal gland and gonadal development and function (Delville et al., 1985; Chaturvedi et al., 1992, 1993). Light shift experiments have also demonstrated that transfer of such sexually regressed males back to long days results in regrowth and restored

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Abbreviation Key: CAREA = cloacal gland area; CFP = cloacal gland foam production; CVOL = cloacal gland volume; G_x = generation; GnRH = gonadotropin-releasing hormone; HPT = hypothalamic-pituitary-testicular axis; FSH = follicle-stimulating hormone; HS = high stress; LH = luteinizing hormone; LS = low stress; PICF = proportion of individuals that produced cloacal gland foam; PTW = combined testes weight; RTW = relative testes weight; TW = testes weight.

function of the testes and cloacal gland (Domjan, 1987; Chaturvedi et al., 1993).

Cloacal gland hypertrophy and foam production are androgen dependent and are highly positively correlated with testes size and sexual activity (Coil and Wetherbee, 1959; McFarland et al., 1968; Sachs, 1969; Siopes and Wilson, 1975; Oishi and Konishi, 1983; Delville et al., 1984; Domjan, 1987). Thus, measurement of cloacal gland size and expression of cloacal gland foam are considered excellent nondestructive indicators of male gonadal development.

In avians, the administration of corticosterone (Deviche et al., 1982; Joseph and Ramachandran, 1993) or adrenocorticotrophin (Edens, 1987) and activation of the hypothalamic-pituitary-adrenal axis by various nonspecific systemic stressors (Deviche, 1983; Edens, 1987) have been associated with depression in the hypothalamic-pituitary-testicular (HPT) axis that, in turn, presents a plausible explanation for the observation of stress-induced inhibition of male reproductive functions. Specifically, Deviche et al. (1982) showed that exogenous corticosterone partially blocked photoinduced development of the cloacal gland. Moreover, in Leghorn chicks, hypercorticalism induced by exogenous corticosterone led to a reduction in testis weight and poor histological organization (Joseph and Ramachandran, 1993). In quail, the administration of adrenocorticotropin and social stress treatment induced decreases in body and relative testes weights (Edens, 1987), and, in general, plasma luteinizing hormone (LH) and testosterone levels are reduced during acute and chronic stress states in avian males (Deviche, 1983).

At a “snapshot” interval of rapid sexual development (42 d of age), we showed that Japanese quail selected for reduced (low stress, LS) rather than exaggerated (high stress, HS) plasma corticosterone response to brief restraint have cloacal glands of larger size, and more LS quail produce a greater amount of cloacal gland foam at this time (Satterlee et al., 2002). In a follow-up study, cloacal gland development and foam production occurred sooner in LS than in HS quail, and line differences (LS > HS) in cloacal gland size and testes weight persisted well into adulthood (Marin and Satterlee, 2004). Thus, it appears that both the onset of puberty and adult sexual development may be advanced in the LS genotype. In addition, we have shown that LS quail adults have a higher copulatory efficiency as estimated by the number of times that copulation sequences are completed once they have been initiated (Marin and Satterlee, 2003).

The present study was conducted to determine whether or not the previously demonstrated adult quail stress line differences in cloacal gland and testes size shown by Marin and Satterlee (2004) persisted during periods of light-induced gonadal involution and subsequent regrowth. Changes in cloacal gland area (CAREA) and volume (CVOL), the proportion of individuals that produced cloacal gland foam (PICF), and the intensity of cloacal gland foam production (CFP) were monitored in fully sexually developed males of the LS and HS lines that had been maintained on long days (14L:10D) followed by brief exposure to short d (6L:18D) and return to long days. Line differences in body weight (BWT), testes weight (TW), and testes weight relative to body weight (RTW) were also measured at the end of the relighting period.

MATERIALS AND METHODS

Genetic Stocks and Husbandry

Male Japanese quail (*Coturnix japonica*) from 2 lines, LS, and HS, for plasma corticosterone response to brief mechanical immobilization (Satterlee and Johnson, 1988) were studied. The more recent genetic history (from generation G_{13} to G_{29}) that verifies maintenance of divergent adrenocortical responsiveness to the genetic selection stressor in these lines is discussed in detail elsewhere (Satterlee et al., 2000, 2002).

The quail studied were taken from a larger population of approximately 600 hatchlings per line of generation G_{29} (n = 96; 48 males per line). Egg incubation, chick brooding, feeding, and lighting procedures were similar to those described elsewhere (Jones and Satterlee, 1996), with the exception that chicks were brooded from d 1 in mixed-sex, mixed-line groups of approximately 100 chicks within each of 12 compartments of a brooder battery[4] modified for quail. During brooding, birds were fed a quail starter ration (28% CP; 2,800 kcal of ME/kg) and water ad libitum. In order to maintain the line identity of each bird, leg bands (placed on chicks at hatching) were replaced with permanent wing bands at 21 d of age.

At 32 d of age, quail were sexed by plumage coloration, and 180 LS and 180 HS birds (12 cages of 15 quail, 10 females + 5 males, per line) were housed within 2 four-tier cage batteries[5] (each battery comprising 12 colony-style laying cages). Individual cage dimensions were 102 × 64 × 27 cm (length × width × height). Occupants of each cage represented a different family within a line. At 32 d, birds were also switched to a breeder ration (21% CP; 2,750 kcal of ME/kg) with feed and water continued ad libitum. The daily photostimulatory cycle was 14L:10D (approximately 280 lx during the lighted portion of the day); lights-on was at 0600 h and lights-off was at 2000 h daily. Daily maintenance and feeding chores were done at the same time each day (0800 h).

At 29 wk of age, 48 males of each selected line, representing 4 breeder males from each of the 12 families within a line, were randomly selected for study. Cloacal gland measurements (see below) were made in each male before housing them in a four-tier pedigree cage[5] unit. The cage unit contained 48 cages, each measuring 51 × 15 × 27 cm (length × width × height). In each cage, along with 2 experimental quail (1 LS + 1 HS), a third, randombred male (hatched and managed under the identical

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[4] Model 25-D, Petersime Incubator Co., Gettysburg, OH.

[5] Alternative Cage Designs, Alternative Design Manufacturing and Supply, Inc., Siloam Springs, AR.

conditions described for the selected line males; see above) was housed.

Upon housing at 29 wk, birds were exposed to daily short day lengths of 6L:18D for 3 wk (or until 32 wk of age). Lights-on remained at 0600 h, and lights-off was reset to 1200 h. The original long day length cycle of 14L:8D was re-established beginning at 32 wk of age and then maintained for an additional 3 wk (or until 35 wk of age).

Traits Measured

From 29 to 35 wk of age, cloacal gland size measurements (length and width, mm) were made weekly in LS and HS males using a digital caliper. This period encompassed the initial measurement (at 29 wk; a time at which the birds had been previously maintained on long day), subsequent measurements (30 to 32 wk of age) during placement on short day, followed by exposure to long day (measurements made during 33 to 35 wk of age). The CAREA and CVOL were calculated from the cloacal gland size measurements according to the formulas proposed by Siopes and Wilson (1975) for CAREA (i.e., gland length × width) and Chaturvedi et al. (1993) for CVOL ($4/3 \times 3.5414 \times a \times b^2$, where $a = 0.5 \times$ long axis, and $b = 0.5 \times$ short axis). In addition, 2 measures of CFP were made weekly from 29 to 35 wk of age in the LS and HS males: PICF and intensity of CFP. The CFP was quantified by subjective scaling of the amount of foam ejected upon manual expression (squeezing) of the foam gland, using a scale of 1 (no foam expressed) to 5 (maximum amount of foam expression) (Satterlee et al., 2002).

At the end of the study (at 35 wk of age and following 3 wk of relighting), birds were weighed (BW) and killed by cervical dislocation, and their testes were removed by blunt dissection for determination of TW. Body weight and TW measurements were made using digital scales that weighed to the nearest 0.01 and 0.001 g, respectively. Each individual's TW and BW were used to calculate RTW. In addition, at 35 wk and within each line, the proportion of individuals having a combined testes weight < 2.4 g (PTW < 2.4) was determined. This milestone was chosen by identification of those birds within the entire population (considering all LS and HS quail) that possessed a reduced TW that qualified them as members of the bottom 15% of the population.

Statistical Analyses

Cloacal gland area, CVOL, and CFP were subjected to repeated measure ANOVA that examined the main effects of line (LS and HS), time of sampling as influenced by photoperiod (29, 30, 31, 32, 33, 34, and 35 wk of age; the repeated measure), and their interaction. In order to better fit the assumptions of the ANOVA, CFP values were transformed to ranks (Shirley, 1987). Where appropriate, LSD tests were used for posthoc comparisons. To evaluate line differences in PICF and PTW < 2.4, the proportion test (Analytical Software[6]) was used to compare the number of birds that were producing foam (successes) and the number of birds with a testes weight < 2.4 g (failures) as a proportion of total sample size. Line differences in BW, TW, and RTW at 35 wk of age were detected using one-way ANOVA considering the main effect of line.

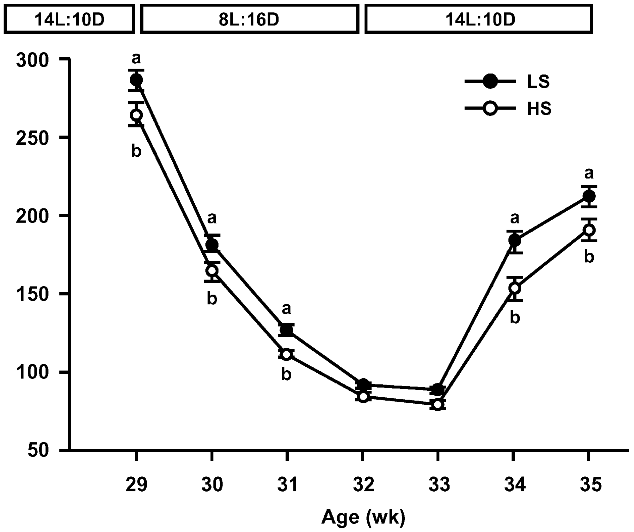

FIGURE 1. Mean (± SE) cloacal gland area (CAREA) in photostimulated low stress (LS) and high stress (HS) Japanese quail subjected to short day lengths followed by long days.

RESULTS

Cloacal gland area was affected by line (LS > HS, $F_{1,84} = 11.9$, $P < 0.001$) and quail age (photoperiod treatment; $F_{6,504} = 547.1$, $P < 0.0000001$) and these treatments showed a marginal interaction ($F_{6,504} = 1.9$, $P = 0.08$) on CAREA. Figure 1 depicts the mean (± SE) CAREA of LS and HS quail held on long day lengths (initial measurement at 29 wk of age), followed by exposure to short days (wk 30 to 32) and then returned to long days (wk 33 to 35). Mean CAREA values were markedly greater ($P < 0.0003$) in LS than HS males grown on long days to 29 wk of age. Cloacal gland area was dramatically decreased by subsequent exposure to short days in both lines. However, the line difference (LS > HS) in CAREA persisted during the first 2 wk of exposure to short days (30 wk, $P < 0.003$ and 31 wk, $P < 0.008$). Line differences in the depressed CAREA measures were no longer evident by the third week (32 wk of age) on short days as well as after the first week of relighting (33 wk), wherein there were no further depressions in CAREA of either line. However, by the end of 2 wk of rephotostimulation (34 wk of age) and thereafter, marked line differences (LS > HS, P range: < 0.0004 to 0.0000001) in CAREA re-emerged.

In support of the CAREA findings, CVOL was similarly affected by line (LS > HS, $F_{1,84} = 11.8$, $P < 0.001$), quail

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[6] Analytical Software **[AUTH QUERY: please provide software version and distributor name for Analystical Software]**.

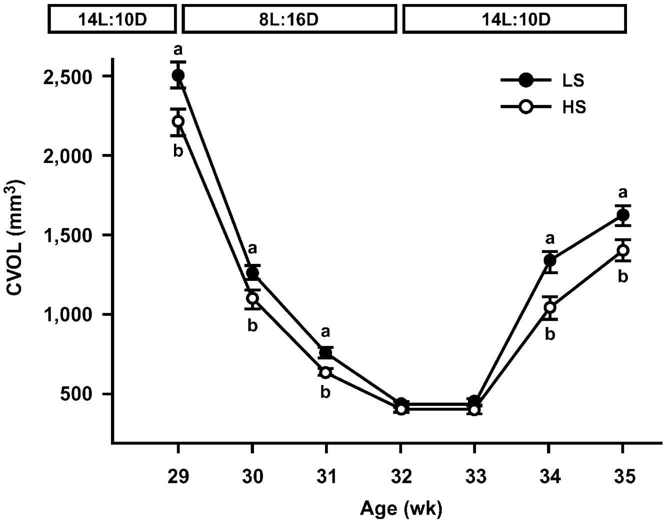

FIGURE 2. Mean (± SE) cloacal gland volume (CVOL) in photostimulated low stress (LS) and high stress (HS) Japanese quail subjected to short day lengths followed by long days.

age (photoperiod treatment; $F_{6,504} = 490.1$, $P < 0.0000001$), and the interaction of these treatments ($F_{6,504} = 2.6$, $P < 0.02$). Mean (± SE) CVOLs for LS and HS quail during the 7 weekly time intervals of measurement are depicted in Figure 2. Cloacal gland volume differences in response to the photoperiod manipulations mimicked those described for CAREA. Specifically, CVOL was markedly greater ($P < 0.000002$) in LS than in HS males grown on long days to 29 wk of age. The line difference (LS > HS) persisted during the first 2 wk of exposure to short days (30 wk, $P < 0.008$ and 31 wk, $P < 0.04$). No line differences in depressed CVOL were detected by the third week on short days (32 wk) and after the first week of relighting (33 wk). However, marked line differences (LS > HS) in CVOL re-emerged by the end of 2 (34 wk, $P < 0.000005$) and 3 (35 wk, $P < 0.0008$) wk of rephotostimulation.

Nearly all of the quail, regardless of line, were in foam production at the 2 times of greatest photostimulation: at the start (29 wk of age) and end (35 wk of age) of the experiment (Figure 3). The PICF decreased dramatically following 1 wk of light reduction (at 30 wk) wherein only a marginal line difference (LS > HS, $P < 0.07$) in foam production was detected. By the end of the second and third weeks of treatment with short days, as well as following the first week of relighting, essentially no LS and HS quail were in foam production. After 2 wk of relighting (at 34 wk), there were approximately 8% more LS than HS quail that had regained the ability to produce foam ($P = 0.20$).

Because almost no quail were found to be in foam production during 31 to 33 wk of age, these times were removed from the repeated measures ANOVA used to detect line and sampling time effects on CFP. The abbreviated analyses showed that, like CAREA and CVOL, CFP was affected by line (LS > HS, $F_{1,84} = 4.6$, $P < 0.03$) and quail age ($F_{4,336} = 156.7$, $P < 0.0000001$). There was no interaction of these 2 treatments. Mean (± SE) CFP for LS and HS quail during the 7 weekly time intervals of measurement are depicted in Figure 4. Line differences (LS > HS) in CFP were only evident at the 2 times of greatest photostimulation: at the start (29 wk of age, $P < 0.07$) and end (35 wk of age, $P < 0.007$) of the experiment.

Mean (± SE) BW at end of the experiment did not differ between LS (139.4 ± 1.5 g) and HS (142.5 ± 1.8 g) quail. However, TW at this time (35 wk of age, following 3 wk of relighting) was greater ($F_{1,85} = 8.1$, $P < 0.005$) in LS than in HS males. Thus, RTW was greater ($F_{1,85} = 14.4$, $P < 0.0003$) in LS quail (0.013 ± 0.001) than in their HS counterparts (0.009 ± 0.001). The proportion of individuals having a combined testes weight < 2.4 g (PTW < 2.4) at 35 wk was lower in LS quail (0.068) than in the HS birds (0.232). The number of individuals having a combined TW less than 2.4 g was more than 3-fold greater ($P < 0.02$) in the HS than LS genotype.

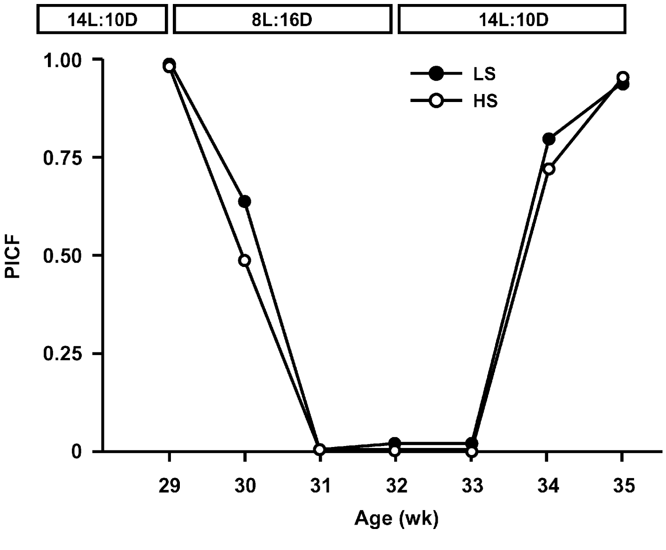

FIGURE 3. Proportion of individuals producing cloacal gland foam (PICF) in photostimulated low stress (LS) and high stress (HS) Japanese quail subjected to short day lengths followed by long days.

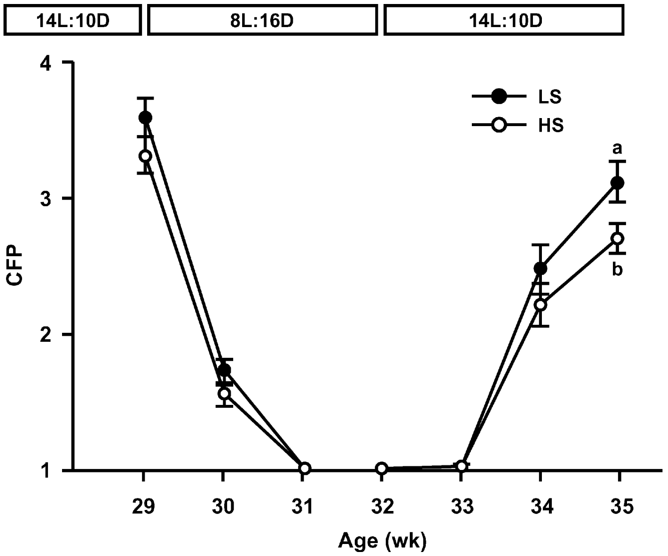

FIGURE 4. Mean (± SE) cloacal gland foam production (CFP) in photostimulated low stress (LS) and high stress (HS) Japanese quail subjected to short day lengths followed by long days.

DISCUSSION

When subjected to a wide variety of stressors, such as cold, crating, feed and water deprivation, manual restraint, or social tension, LS quail show a reduction in adrenal stress responsiveness when compared with HS ones (Satterlee and Johnson, 1988; Jones et al., 1994; Jones, 1996). Thus, the lines appear to exhibit differences in plasma corticosterone responses that are nonspecific in terms of stressor application. In the present study, the quail were not intentionally stressed. Nevertheless, because of the apparent nonspecific nature of stress responsiveness in the lines, we would expect differential adrenocortical responses consistent with each line's genetic background to have occurred often during the course of the study. Specific husbandry procedures (primarily animal-caretaker interactions) used in hatching, bird identification, brooding, and rearing quail to adulthood routinely used in our laboratory that would be potentially stressful and that were also used herein are described in detail elsewhere (Satterlee et al., 2002; Marin and Satterlee, 2004). In addition, during the period of data collection in the present study, LS and HS quail likely had contrasting adrenocortical responses to bird capture and handling for the purposes of making cloacal gland size measurements and to the procedures employed for manual expression of foam.

Held on Long Day Lengths

Male Coturnix held on long day lengths have fully developed gonads and cloacal glands that actively secrete foam (Tanaka et al., 1965; Sachs, 1967; Siopes and Wilson, 1975; Chaturvedi et al., 1992, 1993). In contrast, growth under, or transfer of sexually developed birds to short days is associated with regression of the testes and cloacal gland (Delville et al., 1985; Chaturvedi et al., 1992, 1993). Transfer of sexually regressed males back to long days results in regrowth and restored function of the testes and cloacal gland (Domjan, 1987; Chaturvedi et al., 1993). Thus, in the present study, the observed involution and subsequent revitalization of cloacal gland size and function in LS and HS quail were expected consequences of the light treatments used. However, observation of these phenomena was not independent of stress line genotype. For example, following the initial, long term, and up to that point lifelong, exposure to long day lengths, LS quail showed significant enhancements in both CAREA and CVOL. By using these indicators of sexual development, we have shown that not only is puberty accelerated in LS males (Satterlee et al. 2002; Marin and Satterlee, 2004), but they also have larger cloacal glands than HS males as young adults (at 16 wk of age; Marin and Satterlee, 2004). The present line differences in CAREA and CVOL suggest that selection for reduced adrenocortical responsiveness has been accompanied by augmented cloacal gland size in middle-aged (29-wk-old) adult males as well.

Although essentially all quail, regardless of genotype, were producing foam at the start of the experiment (i.e., following previous, lifelong exposure to long d), the amount of CFP by LS quail tended to be more ($P < 0.07$) than that being secreted by HS birds. The latter observation supports the line differences detected in the cloacal gland size measures made at this time (see above).

Exposure to Short Day Lengths

THe LS and HS quail also differed in response to treatment with short days in that 3 wk of exposure was required for the line differences (LS > HS) in CAREA and CVOL to dissipate. However, close examination of CAREA and CVOL data revealed that during the first 2 wk of enhanced scotophase treatment the weekly rates of decline in the cloacal gland size measures relative to each line's starting points were markedly similar between the lines. Using CVOL only as an example, declines in size from 29 to 30 wk and from 29 to 31 wk in the LS line were 30.4 and 18.2%, respectively; the HS line lost cloacal gland mass at the similar rates of 28.8 and 18.5%, respectively. Thus, arguably, maintenance of a greater cloacal gland size in LS males during the first 2 wk of short days may simply reflect the fact that LS males started at a higher point of cloacal gland development before light reduction. Indeed, despite the loss of stress line differences in CVOL by the end of the third week of light reduction (i.e., at 32 wk of age) wherein CVOL further declined to its lowest level observed during short days, reductions in CVOL as a percentage of each line's fully developed sizes at 29 wk remained remarkably similar (LS = 81.8%, HS = 81.5%).

By the end of 3 wk of light reduction, it was difficult to accurately measure cloacal gland size as the gland barely protruded in most birds. Therefore, we chose not to continue short day treatment and cloacal gland measures beyond this time. In addition, the degree of gland involution observed in both lines at this time (slightly more than 18% of original size) was similar to that previously observed in sexually immature quail at 4 wk of age when compared with the same birds at 16 wk of age and following 12 wk of photostimulation (LS = 16%, and HS = 18%; Marin and Satterlee, 2004). It should also be noted that other workers (Siopes and Wilson, 1975; Domjan, 1987; Chaturvedi et al., 1993), who have switched both moderately and fully photostimulated (range: 13 to 16 h L) quail to reduced daily light treatments ranging from 2 to 8 h (for 2.5 to 4 wk; also study-dependent), have reported similar, maximally reduced CAREA or CVOL measurements as those found herein at the end of 3 wk on 6 h of light.

The negative effects of short day treatment on the cloacal gland foam parameters (PICF and CFP) were dramatic. After only 1 wk of daily light reduction (at 30 wk of age), less than half (49%) of the HS males and approximately 64% of the LS males that were previously producing foam were still in foam production. Thus, approximately 15% more LS quail remained in foam production at this time, a line difference that, although only of marginal statistical relevance ($P < 0.07$), supports the cloacal gland size reductions noted at 30 wk. On the other hand, the amount of CFP by those individuals still in foam production following 1 wk of short days was not different by line. The inability to detect CFP line differences after 1 wk of light reduction is not surprising when one considers that 1) foam production had ceased in well over 50% of the overall population resulting in assignment of a value of "1" (zero foam production) to the majority of CFP observations (which resulted in censored data even with data transformation), and 2) individuals assigned CFP values of ≥3 were exceedingly rare, resulting in further data censure. By the end of the second week of treatment with short days essentially no LS or HS quail were in foam production, an effect that remained evident for an additional 2 wk, the last of which followed a full week of relighting. Interestingly, continued cloacal gland involution occurred during all 3 wk of light reduction. Thus, collectively, these findings suggest that under short day lengths CFP ceases well before the cloacal gland achieves maximum involution.

Unfortunately, plasma testosterone was not measured and testes were not sampled until the end of the present study. However, it is reasonable to expect that, because cloacal gland hypertrophy and foam production are androgen dependent and highly positively correlated with testes size and function (Coil and Wetherbee, 1959; McFarland et al., 1968; Sachs, 1969; Siopes and Wilson, 1975; Oishi and Konishi, 1983; Delville et al., 1984), testes weight, daily sperm production, and circulating levels of testosterone were likely significantly compromised during exposure to short days herein. Furthermore, consistent with our CAREA, CVOL, and CFP line differences (LS > HS), similar line differences in TW, sperm production, and blood levels of testosterone would be expected to have persisted for at least 1 wk of short day treatment.

Relighting to Long Days

Unlike the immediate and dramatic negative effects of exposure to short days on cloacal gland involution, essentially no regrowth in CAREA or CVOL was evident by the end of 1 wk of relighting, and essentially all quail, regardless of line, remained out of foam production (PICF of zero and CFP of 1) at this time. Moreover, by the end of 3 wk of return to long days, CVOL had similarly returned to only about two-thirds of each line's original CVOL (LS = 64.7%, HS = 63.9%). Nevertheless, following the second and third week of relighting, CAREA and CVOL were higher in LS than in HS males, and by 3 wk, CFP was significantly higher (by 13%) in LS males. In addition, at 35 wk of age (end of 3 wk of relighting), TW and RTW were markedly higher in LS than HS quail, and there were significantly fewer LS quail having a combined testes weight less than 2.4 g. The finding of a line difference in RTW suggests that the enhanced testes weight found in middle-aged LS adults is likely a direct function of an altered endocrinology (see below) and independent of changes in BW that was found to be similar between the lines at 35 wk. The TW finding (LS > HS) also supports our previous observation that TW is greater in developing and young adult LS males (Marin and Satterlee, 2004).

Collectively, the above data support a conclusion that, following treatment with short day lengths, rephotostimulation results in greater short-term sexual development in LS quail. However, without further measurements, it is uncertain whether there would have been further cloacal gland regrowth and return to higher foam production and whether the CAREA, CVOL, CFP, RTW, and PTW < 2.4 line differences (LS > HS) observed would persist beyond 3 wk of relighting. Two studies that used the same paradigm of light shifting from long to short and back to long days address the first question. First, although Domjan (1987) found 15 d of relighting (16L:8D) immediately following 18 d of extremely short-day cycles (2L:22D) only restored CAREA to approximately 71% of original size, Siopes and Wilson (1975) found complete restoration of CAREA when birds were switched from 3 wk of 6L:18D to 16L:8D after 14 to 21 d. Thus, our use of 3 wk of relighting (a longer period than that used by Domjan and equal to that used by Siopes and Wilson to achieve 100% recovery) should have been sufficient to achieve maximum recovery in cloacal gland size. However, differences in the postrelighting magnitude of cloacal gland recovery between our study and these reports may reflect differences in ages and genetic backgrounds of study animals, sampling techniques (i.e., use of repeated measures on same males vs. measurement of different males at different times), treatment day lengths or treatment durations, and studying males in isolation vs. in the presence of females. Rearing males paired with females on long days resulted in larger cloacal glands and testes than when males were reared in isolation (Delville et al., 1984).

In the present study, the question remains, what are the underlying mechanisms producing the observed line differences in sexual development during photomanipulation? Endocrine links between stimulation of the hypothalamic-pituitary adrenal axis (heightened adrenocortical activity) and depression of the HPT axis (as evidenced by depression of gonadal and cloacal gland physiology) have been established (see Introduction) and we have also detailed the potential involvement of the gonadotrophic hormones in these links (Marin and Satterlee, 2004). Thus, for purposes of brevity, gonadotropin involvement will only be summarized here without specific reference to the well-known endocrinology that underlies the relationship between photoperiod and the HPT axis.

Long days stimulate hypothalamic gonadotropin-releasing hormone (GnRH) release that, in turn, causes release of pituitary gonadotrophins (follicle-stimulating hormone, FSH; luteinizing hormone, LH). Follicle-stimulating hormone and LH then travel to the testes where they co-orchestrate the production and development of spermatozoa. Luteinizing hormone also stimulates the testes to release testosterone that feeds back on the hypothalamus to decrease GnRH release and thereby decrease release of FSH and LH. In photostimulated birds, FSH helps insure maximum testicular growth and LH markedly increases testicular growth in birds held on short d.

In reptiles, exogenous corticosterone (Yajurvedi and Niagal, 2000) was found to inhibit FSH-induced testicular recrudescence, an effect likely brought about by suppression of testosterone secretion. Interestingly, these same workers (Ganesh and Yajurvedi, 2002) also showed that stressors, such as handling, chasing, and noise, prevent seasonal and FSH-induced ovarian recrudescence. In ovariectomized monkeys, i.v. infusion of corticotropin-releasing hormone also inhibits FSH and LH secretions (Xiao et al., 1989). In broiler breeders, Vizcarra et al. (2000) found circulating FSH to be highly correlated to testis weight and development of the seminiferous tubules and testis weight to be highly correlated with daily sperm production. In addition, acute and chronic stress states have been associated with reductions in blood levels of LH and testosterone (Deviche, 1983) and preincubation of quail adenohypophyseal cells with corticosterone inhibits GnRH-induced LH secretion (Connoly and Callard, 1987). Thus, it is reasonable to conclude that the elevations in cloacal gland size, CFP, TW, and RTW as well as the enhanced PTW < 2.4 observed in LS males may reflect this line's reduced adrenocortical responsiveness as such endocrine activity would relate to providing for a greater pool of the gonadotrophic hormones available for stimulation of the testes and cloacal gland.

In conclusion, selection for reduced adrenocortical responsiveness in Coturnix has resulted in an enhancement of cloacal gland size and foam production in middle-aged, adult males maintained on stimulatory photoperiods. Furthermore, when LS and HS quail previously held on long day lengths were subjected to short days, the LS advantage in cloacal gland size persisted during the first 2 wk of photocastration. Finally, rephotostimulation following short days showed that LS males regrew their cloacal glands to a larger size by 2 wk and restored foam production to a greater amount by 3 wk. At the end of 3 wk of relighting, LS males also showed greater TW and RTW and fewer individuals with testes weights in the lowest 15% of the overall population.

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