Divergent cloacal gland photo-responsiveness in male Japanese quail exposed to short days and associated differences in social interactions and reproduction

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ABSTRACT Quail under short d (SD) reduce their gonadal development, and consequently their cloacal gland (CG) size, aggressiveness, sexual behaviors, and reproductive performance. However, some quail appear nonresponsive to SD inhibition. When male quail were arbitrarily classified according to their CG involution during maximum photoinhibition (5 wk after SD exposure) as either nonresponsive (NR-SD) or responsive (R-SD), NR-SD quail showed intermediate CG volume between R-SD quail and the control quail kept on long d (C-LD). Herein, we evaluate whether NR-SD and R-SD male Japanese quail differ in their social interactions and reproductive performance while under SD; C-LD males were used as fully reproductive control. First, we assessed over 4 consecutive d, brief (5-min) home cage encounters between individually housed C-LD, NR-SD, or R-SD males and an unfamiliar C-LD male visitor. To determine male reproductive capacity, the following wk, males received the visit (10-min) of a mature female over 3 consecutive days. C-LD, NR-SD, and R-SD resident males showed higher aggressiveness than their photostimulated male visitors, respectively, in 100, 64, and 0% of the studied cases and were also able to, respectively, fertilize 100, 100, and 15% of their female visitors. Second, male-female encounters were again repeated 4 wk later to further assess reproductive performance and behavior. Naive C-LD, NR-SD, and R-SD males also were evaluated to assess potential consequences of the previous male-male interactions on the later performance. The number of males performing aggressive pecks towards females showed a C-LD>NR-SD>R-SD pattern. While mounts, CG contacts, and reproductive capacity showed a C-LD = NR-SD>R-SD pattern, copulatory efficiency exhibited an NR-SD>C-LD = R-SD pattern. Sexual behaviors were not modulated by previous male-male brief social interactions. Findings suggest that NR-SD males are able to breed similarly to their C-LD counterparts, which could be considered an indicator of a reduced reproductive dependence on seasonal photoperiod cues. Differences in the expression of aggressions between NR-SD and C-LD males may underlie the observed NR-SD improved copulatory efficiency when interacting with females.

INTRODUCTION

The Japanese quail (*Coturnix japonica*) is an important avian species reared worldwide in conditions that range from highly intensive production systems to backyard poultry owners. They are mainly reared for commercial (meat and egg production) and research purposes (Caron and Minvielle, 1990; Baumgartner, 1994; Jones, 1996; Minvielle, 2004). Quail bred for meat production are often kept on floor pens whereas breeding stock and layers are usually kept in battery cages. A ratio of one male per 2 to 3 females is recommended for breeding stock purposes (Schmid and Wechsler, 1997). However, for pedigree purposes, males and females also can be reared individually or in pairs of one male and one female. During quail husbandry, one of the main welfare related issues is male aggressiveness that ultimately affects bird performance and productivity (Schmid and Wechsler, 1997). Thus, a continued search for knowledge aimed to help reduce aggressiveness is highly relevant both from a welfare and productive point of view.

Quail also have proven to be an excellent model to study how variation in photoperiod regulates reproduction and how variation in gonadal steroid hormones controls sexual behavior (Ball and Balthazart, 2010). In males, long d (**LD**) associated with the start

of the breeding season strongly stimulate testicular activity increasing testosterone plasma levels, cloacal gland (**CG**) hypertrophy and foam production, testicular weight, and the frequency of sexual and aggressive behaviors (Farner and Follett, 1966; Sachs, 1969; Follett and Maung, 1978; Oishi and Konishi, 1983; Biswas et al., 2007). These behaviors are testosterone-dependent since castration completely abolishes them and exogenous testosterone administration fully restores these activities (Adkins and Adler, 1972; Balthazart et al., 1979; Charlier et al., 2013). Aggressiveness also can be restored by the aid of other exogenous androgens (Tsutsui and Ishii, 1981; Tsutsui and Ishii, 1981; Schlinger and Callard, 1989)(Schlinger and Callard, 1989). According to Balthazart et al. (1979) castrated male quail, or those exposed to short d (**SD**), which induce testicular regression, would be non-aggressive.

Quail CG volume (**Cvol**) and foam production are positively correlated with the level of plasma testosterone and male fertility (Oishi and Konishi, 1983; Biswas et al., 2007). Thus, visual CG examination can provide a valuable non-invasive tool for predicting the fertilizing ability and testosterone levels. Indeed, previous studies showed that after 5 wk of SD exposure (8L:16D photoperiod), males significantly reduced their Cvol, plasma testosterone, and androgen metabolites in comparison with photo-stimulated males exposed to LD (14L:10D photoperiod) (Oishi and Konishi, 1983; Delville et al., 1984; Busso et al., 2013; Dominchin et al., 2014). Interestingly, some males were apparently non-responsive to the SD inhibition (**NR-SD** males) and continued exhibiting a Cvol comparable to the volume observed in fully reproductive controls that are kept under LD (**C-LD**) (Busso et al., 2013; Dominchin et al., 2014). The Cvol observed in those non-responsive birds, suggest a priori that they also would be reproductively active, with testes highly developed, and therefore able to perform behaviors that are typically observed during the breeding season. However, testosterone metabolite concentrations in those NR-SD quail were found to be intermediate between the levels observed in males that responded to the SD inhibition (**R-SD** males) and the C-LD males that remained photostimulated (Dominchin et al., 2014). Thus, differences in aggressive and reproductive performance between those groups also would be expected, which could have productive and welfare livestock breeding implications.

This study aims to determine whether male quail showing either a pronounced or a minor reduction in the Cvol during maximum photoinhibition differ in their social behavior when interacting in their home cage with fully developed and sexually active unfamiliar C-LD male visitors. While still under SD, we also aimed to assess males reproductive performance when interacting with a fully developed female partner.

Although little is known about the specific effect of social interactions as non-photoperiodic cues over the seasonal timing of breeding in birds, the "challenge hypothesis" (Wingfield et al., 1990) predicts that testosterone concentrations are most likely elevated during times of social instability, i.e., when territory boundaries are being disputed or dominance hierarchies established (Hau et al., 2008). Conversely, the level of aggression declines during socially stable periods and when territories have been established (Wingfield et al., 1990). Thus, non-photoperiodic cues such as adult male social interaction experiences between unknown birds and/or with a different reproductive stage also could interact with the photoperiodic signals modulating their later hormonal and therefore reproductive activity. In this study behaviors during male-female interactions were therefore also assessed 4 wk after previous male-male encounters were finished. Male residents that were not exposed to previous male-male encounters were used as controls.

MATERIAL AND METHODS

Birds, Husbandry and Photoperiodic Treatment

The study animals were Japanese quail (*Coturnix japonica*). Egg incubation, chick brooding, and lighting procedures from one day to 4 wk of age were similar to those described elsewhere (Nazar and Marin, 2011). Briefly, all birds had ad libitum access to a commercial starter diet (28% CP; 2,800 kcal of ME/kg) and water. Birds were kept under LD (long photoperiod; 14L:10D; lights on at 6:00 AM) and controlled temperature (brooding temperature was 37.5°C during the first wk of life, with a weekly decline of 3°C until room temperature (24 ± 2°C) was achieved). From 4 wk onward, birds were switched to a commercial breeder ration (21% CP; 2,750 kcal of ME/kg). At that moment, birds were sexed by plumage coloration and 107 males and 71 females were randomly and individually housed in cages measuring 45 × 20 × 25 cm (length × width × height). Birds were not visually or acoustically isolated. Rooms also continued with automatic temperature (24 ± 2°C) and photoperiod control.

From 10 wk of age onwards (after all birds have reached full reproductive development), indicators of CG development (Cvol and foam production) were registered every one or 2 wk until the end of the study (20 wk of age; please see further details bellow). At 10 wk of age and during 3 consecutive d sessions, all male quail received 10-min visits by a randomly assigned female partner to ensure the criteria that all birds included in the study were both sexually experienced and fertile. Fertility was assessed through embryo visual inspection in eggs incubated during 10 d, and if eggs laid from a given female were found to be infertile, both the female and its male partner were withdrawn from the study. Immediately after the aforementioned social interactions, 54 males were switched to the SD photoperiod (8L:16D; lights on at 10:00 AM) to induce

photoinhibition and to classify them (5 wk later) according to their Cvol and foam photoperiodic responses (see below). The remaining males and all females were kept under LD to maintain their full reproductive capacity. They were used as either controls (C-LD males), or as for male-male or male-female encounters (C-LD visitors) (see below).

Male Classification According to Divergent Cloacal Gland Responses to Short Photoperiod Exposure

Cloacal gland length (mm) and width (mm) were measured using a digital caliper. Cloacal gland volume was calculated from these measurements according to the formula proposed by Chaturvedi et al., ([1993](#)): Cvol $= (4/3^*3.5414^*a^*b^2$, where a $= 0.5^*$long axis and b $= 0.5^*$short axis). In addition, the expression of CG foam production was evaluated after its ejection upon manual squeezing of the gland.

Similarly to the procedure described by Busso et al. ([2013](#)), after 5 wk of SD exposure (when on average birds reach the peak of photoinhibition), quail that showed a reduction in the Cvol below 1,000 mm^3 and that did not expressed foam production were classified as responsive to SD (R-SD; n = 33). The remaining males under SD were classified as non-responsive (NR-SD; n = 21; Cvol > 1,000 mm^3 and at least some CG foam expression). The mentioned classification criteria were based on 2 findings, 1) a report by Oishi and Konishi ([1983](#)) in which different individual responses to changes in photoperiod length were reported, and 2) studies showing that CG development, foam production, or a combination of both measurements have potential to predict the fertilizing ability of a male quail (Follett and Maung, [1978](#); Ottinger and Brinkley, [1979](#); Mohan et al., [2002](#); Henare et al., [2012](#)). As mentioned above, some males (n = 17) were kept under LD and were used as controls. Thus, according to their photoperiod treatment (LD and SD) and/or photoperiod responses to short d (NR-SD and R-SD) 3 male resident groups were evaluated in this study: C-LD, NR-SD, and R-SD.

Male-male Social Interactions

One d after male classification, male-male social interactions were assessed during 5 min when individually caged C-LD, NR-SD, and R-SD residents received the visit of a similar-weight, unfamiliar, photostimulated male (Guzman et al., [2013](#)). Experimental birds were used as residents to favor the expression of their aggressive/defensive behaviors (Koolhaas et al., [2013](#)). A short experimental period of 5 min was chosen, taking into account previous experiences in which agonistic interactions between different males started immediately after receiving the visit of an unknown conspecific (Ramenofsky, [1984](#); Schlinger et al., [1987](#)). If during the encounter a quail received more than 5 consecutive pecks and/or showed a clear escaping (retrieval) behavior, and/or showed any sign of physical damage, the encounter was immediately interrupted. Birds' behaviors were assessed through direct observation of video images obtained with the aid of camcorders that were placed 0.5 m in front the cages 10 min prior to assessments. The latency to the first attack (time to the first aggressive peck or grab), the number of aggressive pecks, and aggressive pecking events (repeated pecks and grabs directed at the other male within 3 s were considered part of the same aggressive event) were registered. Grabs and mount behaviors from a male to the other male also were assessed. After completing the 5-min visits, the visitor male was returned to its home cage. Male-male encounters were repeated during the next 3 consecutive d in a random order. During these 4-d male visits, the visitors were always unfamiliar to the resident male.

In order to summarize the results obtained from the 4 d of male-male interactions, aggressiveness performance was determined for each experimental male based on the most observed behaviors during the encounter. Considering that grabs and mounts between males has been previously considered as aggressive behaviors (Schlinger and Callard, [1990](#)), first, for each male-male interaction, we added the total number of aggressive pecks, grabs, and mounts displayed by each quail (Adkins and Adler, [1972](#)). Male residents showing higher totals than their visitor counterparts in less than 2 of the encounters were termed "low aggressive males" (**LAP**), while if totals were higher in more than 3 encounters, they were termed as "high aggressive males" (**HAP**). In the cases in which resident males showed higher aggressions than their visitors in 2 out of the 4 encounters, they were arbitrarily classified as LAP or HAP depending on whether they showed, respectively, a lower or higher total in the last encounter.

It is important to note that half of the residents within each photoperiodic group were not exposed to male-male encounters (naive C-LD, NR-SD, and R-SD male residents) and were used as controls during the following male-female interactions (see below). The individual housing and the 4-d visit schemes used were chosen because the animals of the present study were also part of a larger project in which endocrine testicular and adrenal potential modulations was being evaluated by noninvasive hormonal monitoring through individual droppings samples from each male.

Male-female Social Interactions

Twenty-four h after male-male encounters were finished, all C-LD, NR-SD, and R-SD males received 10-min visits from the same randomly chosen female partner during 3 consecutive days. This test was aimed to evaluate the reproductive potential by determining whether males with divergent SD responses were still

able to fertilize a female even after reaching peak of photoinhibition. All eggs laid by each female were individually collected and labeled for further identification. Eggs were collected daily starting 48 h before the male-female interactions began and continued for 10 d after the last encounter. Fertility was again assessed through visual inspection of embryo development in eggs incubated during 10 days. The egg collection range was chosen in order to include a period in which no fertilized eggs were expected and a period in which at least some eggs would be fertile if the experimental males kept on SD were still able to maintain an active reproductive status. Eggs with presence of a developed embryo were determined as "fertile" and those males whose female partner lay at least one fertile egg were deemed as "fertile males." The proportion of fertile eggs laid per female also was recorded.

At 20 wk of age, aggressive and mating behaviors from resident males toward the females were assessed on 3 consecutive days. C-LD, NR-SD, and R-SD male residents that were not exposed to previous male-male interactions (naive males) were used as controls. As previously, the same photostimulated and sexually mature females were partnered with their randomly assigned male. During each of the 3 d, sexual behaviors were assessed through direct observation of video recordings obtained with the aid of a camcorder placed 0.5 m in front of each cage. The latency to the first grab and the number of grabs, mounts, and CG contacts from resident males toward the females were registered. As described by Marin and Satterlee, (2003), a copulatory efficiency parameter was estimated each d by dividing the number of CG contacts by the number of grabs. In addition, whether males were performing aggressive pecks towards the females was determined. After finishing each encounter, females were returned to their home cages. Daily encounters were performed in a random order. During 10 d, eggs were again collected daily starting 24 h after the last male-female encounter. The proportion of fertile eggs laid per female, and male reproductive potential were assessed as described above.

Statistical Analyses

Cloacal gland volume data from 15 to 20 wk of age were subjected to a repeated-measures ANOVA to assess the effects of photoperiod responses (C-LD, NR-SD, and R-SD), the exposure to male social interactions (with or without visits), the time (wk of age; 15, 16, 18, and 20, the repeated factor), and their interactions on Cvol development. This time range encompassed Cvol measurements made when males were classified (basal measurement) and after male interactions.

The latency to the first attack, the number of aggressive pecks, and the number of aggressive events, grabs, and mounts from test birds during male-male encounters were evaluated by separated repeated-measures one-way ANOVAs that examined the effects of pho-

toperiod responses (C-LD, SDNR, and R-SD), the time (d of the male visits 1, 2, 3, and 4; the repeated factor) and their interaction.

The copulatory efficiency, the latency to the first grab, and the number of grabs, mounts, and CG contacts from resident males toward the females were evaluated by a repeated-measures two-way ANOVA that examined the effects of photoperiodic responses (C-LD, NR-SD, and R-SD), the exposure to male social interactions (with or without visits), the time (d of the female visits 1, 2, and 3; the repeated factor), and their interactions. The latencies to the first attack and first grab were transformed to ranks to fit the ANOVA assumptions (Schirley, 1987).

After classifying males according to their aggressive performance (LAP or HAP), 100% of C-LD quail and 0% of R-SD quail were classified as HAP and LAP, respectively. Only the NR-SD males showed HAP and LAP representatives. Thus, potential differences between those males in the mentioned male-female behaviors were evaluated using repeated-measures one-way ANOVA examining the effects of aggressive performance (HAP and LAP), the time (female visit d 1, 2, or 3; a repeated factor), and their interactions. Whenever repeated-measures ANOVA reached significance ($P < 0.05$), LSD post hoc tests were performed.

A proportion test was used to assess differences among the C-LD, NR-SD, and R-SD groups in the number of males that were fertile, in the number of males that were classified as HAP, and in the number of males that performed at least one aggressive peck towards their female partners. The proportion of fertile eggs that were laid after females visited C-LD, NR-SD, and R-SD males also was compared. Statistical analyses were performed using Infostat (Di Rienzo et al., 2008).

RESULTS

Figure 1 depicts C-LD, NR-SD, and R-SD male Cvol changes from 15 to 20 wk of age. A significant interaction between the photoperiodic responses and the time (wk of age) was found on Cvol ($F_{6,260} = 3.86$; $P < 0.001$). Cvol was assessed in all males, including those exposed to male visits (male-male interactions) as well as in the naive control males. Because no changes on Cvol were induced by the male visits ($F_{1,260} = 0.004$; $P = 0.949$) nor interactions between male visits and photoperiod responses, and/or time, were detected ($P > 0.63$ in all cases), data from all males from each photoperiodic response group were pooled per week. Comparisons showed that previous to male-male encounters (15 wk of age) and about 5 wk after the encounters (20 wk of age), Cvol in NR-SD quail were found to be intermediate ($P < 0.05$) between values observed in their C-LD and R-SD counterparts (Figure 1). The R-SD Cvol was found significantly lower than in NR-SD and C-LD males throughout the study.
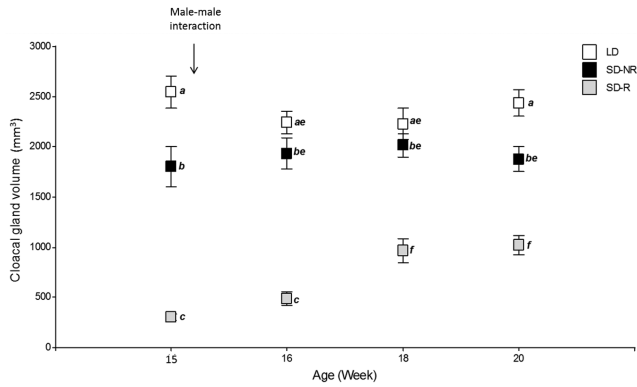
Figure 1. Cloacal gland volume (Cvol) (mean ± SEM) in male Japanese quail classified at 15 weeks of age according to their cloacal gland photoresponsiveness as either nonresponsive (SD-NR, n = 21) or responsive (SD-R, n = 33) to short photoperiod. A group of male quail kept on long days was used as control (LD, n = 17). The arrow points to when 4 consecutive day male-male interactions started. Because neither male-male social interactions effects, nor interactions between male-male interactions and photoperiodic responses or time were found on Cvol data, values from males that receive other male visits and from control males that were not visited are shown pooled. [a-c]Means with no common letter differ significantly at $P < 0.05$.

Table 1 summarizes the results of aggressive behaviors performed by C-LD, NR-SD, and R-SD males in their home cages along 4 d at 15 wk of age while visited by photo-stimulated male counterparts. ANOVAs revealed a significant main effect of photoperiod responses on the latency to first attack ($F_{2,132} = 64.95$; $P < 0.001$), the number of aggressive events ($F_{2,132} =$ 87.73; $P < 0.001$), aggressive pecks ($F_{2,132} = 39.51$; $P < 0.001$), grabs ($F_{2,132} = 74.07$; $P < 0.001$), and mounts ($F_{2,132} = 65.39$; $P < 0.001$). No main effects of the day of male visits, nor interaction between photoperiodic responses and d of visits, were evidenced in any of the aggressive behaviors evaluated ($P > 0.05$ in all cases). Post-hoc test showed the same C-LD > NR-SD > R-SD pattern for all behaviors, but for the latency to first attack that showed the reverse sequence.

The proportion of males determined as HAP also followed the pattern C-LD > NR-SD > R-SD ($P < 0.05$; Table 1). Noteworthy, all C-LD males were classified as HAP and all R-SD males were classified as LAP. Only the NR-SD group showed representatives in both categories (7 HAP males and 4 LAP males).

The week after male-male encounters, C-LD and NR-SD males reached maximum fertilization rate (100%); meanwhile, only 15% of the R-SD males (5 out of 33) were able to fertilize a female. Tests showed a C-LD = NR-SD > SDR pattern ($P < 0.05$). Females visiting those males showed the same pattern (C-LD = NR-SD > R-SD) on the proportions of fertile eggs they laid (0.47, 0.60 and 0.06, respectively).

Table 2 summarizes the results of behaviors performed by C-LD, NR-SD, and R-SD resident males when visited by females at 20 wk of age. ANOVAs revealed significant main effects of photoperiod responses on the latency to first grab ($F_{2,195} = 8.63$; $P < 0.001$), the number of grabs ($F_{2,195} = 14.92$; $P < 0.001$), mounts ($F_{2,195} = 7.64$; $P < 0.001$),

Table 1. Behaviors during male-male encounters in adult male Japanese quail showing divergent cloacal gland responses to a short photoperiod during maximum photoinhibition (15 wk of age).

Variables	C-LD	NR-SD	R-SD
Latency of first attack (s)	15.09 ± 9.01[a]	33.09 ± 9.02[b]	179.5 ± 15.15[c]
Aggressive pecking events (n°)	22.09 ± 1.28[a]	15.11 ± 1.13[b]	2.36 ± 0.92[c]
Aggressive pecks (n°)	17.94 ± 1.41[a]	10.09 ± 1.24[b]	2.56 ± 0.51[c]
Grabs (n°)	18.15 ± 1.25[a]	12.75 ± 1.10[b]	0.8 ± 0.24[c]
Mounts (n°)	4.35 ± 0.42[a]	2.77 ± 0.38[b]	0.14 ± 0.06[c]
High aggressive males (%)	100[a]	64[b]	0[c]

C-LD = control males under long days. NR-SD and R-SD = nonresponsive and responsive males to short d exposure, respectively. Data are either expressed as means ± SEM of the 4 test d considering that no effect of test d was observed for any of the variables, or as a percentage of the high aggressive males (i.e., male test birds showing higher overall aggressions than their visitor counterparts) observed within each group.
[a-c]Within each behavior, values with no common letter are significantly different at $P < 0.05$.

Table 2. Behaviors during male-female encounters, and male fertility assessment at 20 wk of age in adult male Japanese quail showing divergent cloacal gland responses to a short photoperiod.

Variables	C-LD	NR-SD	R-SD
Latency to first grabs (s)	2.64 ± 0.28[a]	2.19 ± 0.2[a]	11.59 ± 3.03[b]
Grabs (n°)	18.63 ± 1.35[a]	13.84 ± 1.21[b]	9.97 ± 0.96[c]
Mounts (n°)	6.36 ± 0.58[a]	7.5 ± 0.52[a]	4.95 ± 0.41[b]
Cloacal gland contact (n°)	1.73 ± 0.17[a]	1.73 ± 0.16[a]	0.97 ± 0.12[b]
Copulatory Efficiency	0.16 ± 0.03[b]	0.25 ± 0.03[a]	0.16 ± 0.02[b]
Fertile males (%)	88[a]	76[a]	36[b]
Males performing aggressive pecks towards females (%)	82[a]	42[b]	38[b]

C-LD = control males under long days. NR-SD and R-SD = nonresponsive and responsive males to short d exposure, respectively. Data are expressed as percentages or means ± SEM of 4 test d considering that no effect of test d was observed for any of the variables.
[a-c]Within each behavior, values with no common letter are significantly different at $P < 0.05$.

cloacal contacts ($F_{2,195} = 9.87$; $P < 0.01$), and copulatory efficiency ($F_{2,195} = 3.17$; $P < 0.05$). No effects of the d of female visit or the previous male-male encounters (with or without visits) or the interaction among d, male-male encounters, and the photoperiodic response were evidenced for any of the reproductive behaviors evaluated ($P > 0.05$ in all cases). Post-hoc test showed that C-LD and NR-SD males were similarly fast to grab a female and showed similarly higher number of mounts and cloacal contacts than their R-SD counterparts ($P < 0.05$ in all cases). The NR-SD and R-SD males performed a lower ($P < 0.05$) number of grabs. The copulatory efficiency was found to be higher in the NR-SD group than in the C-LD and R-SD groups ($P < 0.05$). A significantly higher proportion of C-LD males ($P < 0.01$) were found performing aggressive pecks toward their female compared to their NR-SD and R-SD male counterparts. A similarly higher proportion of C-LD and NR-SD males was able to fertilize the females compared to their R-SD male counterparts. Females visiting those males also showed the same pattern (C-LD = NR-SD > R-SD) on the proportion of fertile eggs they laid (0.56, 0.54 and 0.27, respectively). The ANOVA also examined the effects of previous male-male interactions outcomes on reproductive behaviors and showed no differences between the LAP and HAP- NR-SD males on the number of grabs, mounts, and CG contacts (data not shown). However the ANOVA revealed a significant main effect of their aggressive category on the copulatory efficiency ($F_{1,27} = 5.55$; $P < 0.05$) with HAP- NR-SD males exhibiting a higher copulatory efficiency than their LAP- NR-SD male counterparts (0.28 ± 0.05 and 0.08 ± 0.07, respectively).

DISCUSSION

Japanese quail exposed to a short photoperiod exhibited significant differences in the development of the Cvol among individuals, a phenomenon that has already been reported in our laboratory population (Busso et al., 2013; Dominchin et al., 2014) and in other laboratory populations (Oishi and Konishi, 1983; Delville et al., 1984; Mills, 1997; Ball and Balthazart, 2010). In general our results show that males that do not fully respond to a short photoperiod (NR-SD) showed intermediate aggressiveness between their responsive (R-SD) and C-LD control counterparts, while showing fertility and most reproductive behaviors equal to C-LD males and the highest copulatory efficiency.

As expected, after 5 wk of SD exposure, maximum divergence between males in CG responses to SD inhibition was observed, with NR-SD exhibiting intermediate Cvol values between their responsive (R-SD) and C-LD counterparts. Similar to the Cvol results, differences in aggressiveness among the 3 male experimental groups also were observed. Reductions in Cvol and androgen hormones, with consequences on the aggressiveness of quail, are expected when they are exposed to short d

(Mills, 1997). In our study, after short photoperiod exposure, both NR-SD and R-SD quail were found to be less aggressive than C-LD males. Moreover, R-SD males showed even less aggressive behaviors than their NR-SD counterparts. Considering that CG development is positively correlated with the level of testosterone in plasma (Balthazart et al., 1979; Biswas et al., 2007), our results on aggressiveness were consistent with those that indicated that testosterone is profoundly involved in the regulation of aggressive and agonistic behavior (Schlinger and Callard, 1990; Charlier et al., 2013). Indeed, R-SD male aggressive behavior was suppressed to a level observed in castrated males (Balthazart et al., 1979; Tsutsui and Ishii, 1981). Interestingly, the NR-SD male Cvol and their subsequent aggressive behavior were not fully inhibited despite being exposed to a typically inhibitory environmental signal.

Similar to aggressiveness, our results showed that the likelihood of being classified as HAP or LAP was associated with the CG responses to photoperiod. Indeed, 100% of C-LD quail were classified as HAP, and 100% of the R-SD quail were classified as LAP. Only the NR-SD males showed both HAP and LAP representatives. These results clearly suggest that extreme Cvol developments (males with fully developed or fully inhibited CG) are useful predictors of the outcome of a male encounter when the test birds are evaluated in their home cages. Consistently, it already has been proposed that fully developed resident males in their home cages tend to be more aggressive than an unknown male visitor or members of a stable group (Edens, 1987). Moreover, when male-male encounters were performed between a NR-SD male resident (with intermediated Cvol measurements) and a photostimlutated male visitor, the NR-SD male showed a higher aggressive performance in 64% of their encounters, which further emphasizes the intermediate aggressiveness state of the individuals within group.

The wk following male-male encounters and while birds were still at their maximum photoinhibition, males received the visit of females reared on LD and therefore were expected to be sexually receptive. As anticipated, results showed that all C-LD males were able to fertilize their female partners and no R-SD male was able to do the same. Interestingly, all NR-SD males (100%) also were able to fertilize their female partners, suggesting that despite their intermediate Cvol values during this photoinhibitory stage, their testosterone levels were enough to maintain an active reproductive behavior and a full capacity to fertilize a female.

In the wild, environmental external cues such as photoperiod, temperature, food availability, humidity, and social interactions are used by birds to adjust different life history stages (Dawson, 2008). Because a change in photoperiod is entirely predictable at any particular latitude, it is considered as the fundamental cue to time the physiological preparations for the breading season (Dawson et al., 2001). However, non-photoperiodic cues, such as social interactions, also could potentially

modulate the exact time to breeding (Dawson, 2008). Under captivity, where breeding conditions are highly controlled, it is conceivable that environmental cues such as social interactions or stressors could became powerful signals modeling the reproductive behavior adjustment over the photoperiod. In our study neither a brief male-male encounter nor the outcome of that encounter (performing a high or low aggressive response) was able to influence their later sexual behaviors. Thus, despite the history of domestication of Japanese quail and the stability of resources in the rearing environment, photoperiod still appears to be a major cue modeling expression of sexual behaviors.

At the end of the study, when a slight CG recovery was evident in R-SD males, sexual behavior in NR-SD quail was found to be similar to those observed in C-LD males. As observed during the maximum photoinhibition, fertility was again found to be similar between C-LD and NR-SD males suggesting again that even though their Cvol and, presumably, their testosterone levels are statistically lower than those in C-LD males, they are above the threshold needed to maintain an active reproductive behavior and the capacity to fertilize a female. Noteworthy, NR-SD showed a copulatory efficiency significantly higher than C-LD males. The copulatory efficiency is a variable that estimates the number of times that a copulatory sequence is completed once it has been initiated (Burns et al., 1998; Marin and Satterlee, 2003). Thus, our results clearly suggest that males from the NR-SD group were more effective than C-LD males in completing a copulatory event once the reproductive sequence was initiated.

Sexual behavior of Japanese quail has many components that are involved in a successful mating (Burns et al., 1998; Jones and Mills, 1999). Potential sexual partners have to be attracted to, and approach one another. The tendency to approach must be stronger than the tendency to withdraw. Therefore, higher levels of sociality, time on sexual withdrawal, and success in previous copulatory encounters may predict an enhanced tendency to approach the partner. Withdrawal may occur because of an enhanced fearfulness elicited by the other animal through the expression of negative behaviors like aggressiveness (Ophir et al., 2005), which may lead to perception of a potential sexual partner as a rival or a predator. Successful copulatory encounters also would be related with the mating preferences of the female as well. As females generally invest more in their offspring than males, females typically should be more selective than males when deciding whether to engage in reproductive activities with a potential partner (Ophir et al., 2005). In our study, a significantly higher number of C-LD males were found performing aggressive pecks towards their female compared to their NR-SD and R-SD male counterparts. Thus, considering also that the NR-SD males showed higher copulatory efficiency, it is conceivable that copulations with those males were on average facilitated by a more sympathetic male approach. However, in some

species like Japanese quail, females mate choices appear subverted by males forcing copulations (Persaud and Galef, 2004) and therefore, female choice would influence the probability of fertilization and not frequency of cloacal contacts during copulation. The probability of fertilization would be controlled by females during or after copulation (Persaud and Galef, 2005), probably by expelling sperm from her cloaca immediately after a male has inseminated her (Adkins-Regan, 2005). In this study, although female copulations with NR-SD males appeared facilitated (higher copulatory efficiency), both C-LD and NR-SD males ended up consummating a similar number of cloacal contacts, suggesting that C-LD copulations were at least in some cases forced. The potentially different C-LD and NR-SD male approach behavior towards females may have important welfare implications when birds are reared in small confined enclosures where females would not be able to avoid a social interaction with a male partner. Because a similar proportion of fertile eggs was laid by females that were visited by C-LD or NR-SD males, the proposal of female control over their ability to control the probability of eggs being fertilized is not supported.

Finally, the fact that NR-SD males showed a smaller Cvol and less aggressiveness than their C-LD male counterparts while showing a more efficient copulatory behavior and had been able to equally fertilize the females makes them an interesting group for studies in poultry production, behavior, and endocrinology. Recently, a domestication related mutation affecting photoperiodic response by reducing dependence of seasonal reproduction has been reported in chicken (Karlsson et al., 2016). Therefore, the lack of NR-SD male responses to a strong inhibitory environmental signal as a short photoperiod could be a trait that has been selected during quail domestication since a prolonged reproduction season is clearly beneficial for production in livestock breeding. Studies aimed to evaluate whether female quail also show different degrees of dependence to photoperiod inhibition would be worthy to assess whether or not this is only a male related phenomenon.

Taken together these studies about effect of light quality (defined by length of d) on physiological and behavioral responses, and a recent report of Yadav and Chaturvedi (2015) about reproductive response in Japanese quail to different light colors and intensity (other characteristics of light quality), we consider that high reproductive performances may still be achieved in poultry production using new ways of photoperiod manipulation.

In conclusion, results suggest that NR-SD males are able to breed similarly to their C-LD counterparts regardless of the outcome of previous social encounters. Potential differences in the expression of aggression between NR-SD and C-LD males may underlie the observed differences in copulatory efficiency. Finally, we consider that males under SD that do not fully respond to a photoperiodic inhibition while still being able to fertilize a female could be evaluated in a breeding pro-

gram aimed to obtain fully productive and less aggressive birds with a reduced dependence from seasonal reproduction.

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