

# Japanese quail classified by their permanence in proximity to a high or low density of conspecifics: a search for underpinning variables

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**ABSTRACT** Test of sociality in poultry is mainly based on the bird's individual ability to make quick social discriminations. In recent years, a density-related permanence (DRP) test has been developed that enables us to classify young birds (while in groups) according to their individual permanence in proximity to either a high or low density of conspecifics (HD or LD, respectively). Thus, the result of the classification depends not only on the bird's individual response but also on the outcome of the social interactions within the whole group. The birds' performance in DRP was associated with underlying differences in social responses of their individuals. Quails in homogeneous groups of LD residents responded with less compact groups and higher levels of agonistic interactions to the presence of an intruder and showed higher levels of agonistic interactions among cage-mates than the homogeneous groups of HD birds. An acute stressor also induced a

higher corticosterone response in the LD birds than in their HD counterparts. The present study addressed the question of whether contrasting DRP performance by Japanese quail can also reflect underlying differences in fearfulness and social reinstatement responses. Thus, LD and HD categorized juvenile birds underwent one of the following tests: tonic immobility (TI), open-field (OF), or a one-way runway. Results showed that HD birds required more inductions and developed shorter responses ( $P \leq 0.05$ ) in the TI test and walked more, faster, and greater distances in the OF ( $P \leq 0.05$ ) than their LD counterparts. No differences between groups were found in short social reinstatement responses. The present findings suggest that underlying fearfulness is lower in the HD than in the LD birds. A reduced fearfulness could be regarded as an additional favorable trait of the HD-classified quail to cope with environmental challenging situations.

**Key words:** stocking density test, fearfulness, social interactions, open-field, runway

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## INTRODUCTION

A group is defined as a set of organisms, belonging to the same species that remain together for a period of time interacting with one another to a distinctly greater degree than with other conspecifics (Wilson, 1980). The maintenance and successful functioning of the groups are certainly influenced by the social behavior of each of

their individuals. A common characteristic of farm animals is that when given a choice, they choose to be in groups. However, spontaneously conformed free-living groups generally would differ from the ones observed during intensive farm rearing, where inappropriate groupings can generate abnormal social interactions that are not usually observed in natural environments or extensive rearing (Blackshaw et al., 1997; Špinková, 2006; Gygas and Hillmann, 2018).

Many aspects of social interactions within a group, such as individuals' association, aggression, dispersion, social and breeding structure, as well as behavioral and physiological avian responses to social disruption (Guzmán et al., 2013; Carvalho et al., 2018), are deeply influenced by the natural individual's social and

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emotional characteristics (Koolhaas and Van Reenen, 2016; Seebacher and Krause, 2017; Pellegrini et al., 2019). Clearly, a mismatch between the individual social characteristics and their social environment could elicit either a series of acute stress responses or chronic social distress (Mills and Faure, 1990; Carmichael et al., 1998; Spencer, 2017). For instance, highly social birds could encounter social isolation from conspecifics particularly stressful or frightening (Mills et al., 1993), whereas less sociable birds could be inappropriate for rearing at high stocking densities or in large groups (Jones, 1996). In this context, social mismatch could not only contribute to the development of depression and social withdrawal but also it could seriously damage the birds' health and decrease productivity (Duncan, 1981; Mills and Faure, 1990; Marin et al., 1999; Elfving et al., 2015; Ericsson et al., 2016).

In recent years, a density-related permanence (DRP) test has been developed (Guzman et al., 2013), where 11-day-old chicks are classified according to whether they are consistently found in 1 of 2 visually isolated sections of an apparatus containing either high-density or low-density of conspecifics (**HD** or **LD**, respectively). The main feature of this test is that within the social environment, individual social responses are evaluated along almost a full day (from 8:00 to 17:00 hrs). Thus, the result of the classification depends not only on the bird's individual response but also on the outcome of the social interactions of the whole group. Interestingly, when the social interaction and aggressive responses of DRP-classified birds were tested as juveniles at 38 d of age, HD and LD individuals showed clear polarized differences both while undisturbed in their home cages or when disturbed by the presence of an unknown intruder counterpart (Guzman et al., 2013). Specifically, HD showed a lower level of aggressiveness in general and toward intruders compared with LD birds. Moreover, evaluation of plasma corticosterone showed no changes in the intruders that were introduced in an unfamiliar group of HD conspecifics. However, intruders (both HD and LD) that were visiting the LD residents showed an increased corticosterone response compared with their controls (Guzman et al., 2018). In a separate experiment, plasma corticosterone responses were individually assessed after submitting DRP categorized birds to a brief partial restraint. This experiment showed that basal corticosterone levels were similar in all groups and that partial mechanical restraint induced an increase in plasma corticosterone concentration also in all groups. However, the increase induced by the stressor was higher in the LD birds than in their HD counterparts (Guzman et al., 2018). Thus, the early individual social permanence in the DRP test does not seem to be an isolated behavioral response (context-dependent) to one particular experimental condition but rather could be part of a different adaptability strategy for group living. Thus, DRP performance might represent an interesting tool for the study of social interactions. In addition, it could be used as a

selection criterion for future reproductive programs aimed to obtain birds that would be better suited for rearing in high-density breeding conditions.

When using DRP or any other physiological or behavioral response for classification, it is important to take into consideration that variation in certain characters might also be associated with divergences in other important welfare/performance related traits (Siegel, 1979; Jones and Hocking, 1999; Marin et al., 2003; Hazard et al., 2008; Bolhuis et al., 2009; Rodenburg et al., 2009a; Rodenburg et al., 2009b). Thus, complementary studies covering potential correlated factors are of strategic as well as fundamental interest. It is considered that fear response could be an influential factor affecting DRP performance. This is relevant considering that testing involves (i) capture, (ii) brief manual restraint, and (iii) sudden temporal allocation in the center of the test apparatus, before the group is allowed to freely ambulate again. Thus, all of these events are potential stressors that are likely to elicit fear/stress responses that could influence the outcome of DRP social interactions (Jones, 1996; Marin et al., 2001).

The objective of the study is to address whether contrasting DRP performance by Japanese quail can also reflect underlying differences in fearfulness and quick social reinstatement responses in a novel environment. First, birds were evaluated in a classic tonic immobility (**TI**) test (Experiment 1) which is an antipredator unlearned reaction highly regarded as an indicator of underlying fearfulness (Gallup, 1979; Jones, 1986; Jones et al., 2005; Forkman et al., 2007). Second, HD and LD quail were evaluated in an open-field (**OF**) test (Experiment 2) which is a commonly used method to assess fear and anxiety reactions to a novel environment (Forkman et al., 2007). This test in poultry has also been proposed to represent a compromise between opposing tendencies to minimize detection in the face of possible predation and to reinstate contact with conspecifics (Gallup and Suarez, 1980). These opposing tendencies can be associated with 2 phases within the OF test. A first phase reflecting the inhibition of all behavioral patterns by fear, and a second phase representing the waning of fear and the consequent expression of socially motivated behaviors (Faure et al., 1983). In the OF data analysis, we not only studied the traditional variables, latency to ambulate and mean ambulation, indicators of the 2 phases of the test, respectively, but also incorporated the use of fractal analysis tools (detrended fluctuation analysis, **DFA**, see below). This last analysis allowed the evaluation of temporal behavioral dynamics (Goldberger et al., 2002) during testing. Specifically, the self-similarity parameter estimated with DFA has been proposed as an indicator of stress responses in poultry (Rutherford et al., 2003; María et al., 2004; Alcalá et al., 2019). Finally, quail were evaluated in a classic short-duration trial measuring social reinstatement responses of individually tested quail in a runway. Specifically, the goal box of the runway contained unfamiliar stimulus conspecifics at either a high or low density. Runways are novel environments where the behavior

of the test bird is regarded to be indicative of its motivation to reinstate social contact as well as of their ability to make quick social discriminations (Suarez and Gallup Jr., 1983; Vallortigara et al., 1990; Mills et al., 1995; Jones et al., 1999). For example, both domestic chicks and Japanese quail approached conspecifics more readily than an empty goal box or one containing members of different avian or mammalian species (Suarez and Gallup Jr., 1983; Mills et al., 1995; Jones and Mills, 1999). Taken together with the DRP previous findings, this study enabled us to address 2 main questions. First, can the study of DRP performance in juvenile quail be associated with additional traits that would be favorable for group living in intensive rearing conditions (i.e., HD quail showing reduced fearfulness)? Second, does the strong individual social preferences shown by HD and LD birds remain evident when birds are induced to make quick decisions regarding social interactions in a novel environment?

## MATERIALS AND METHODS

All the procedures were in compliance with the Guide for the Care and Use of Laboratory Animals issued by the National Institute of Health (NIH Publications, Eighth Edition). Experimental protocol was approved by the Institutional Council for the Care of Laboratory Animals (CICUAL, Comité Institucional de Cuidado de Animales de Laboratorio) of the Instituto de Investigaciones Biológicas y Tecnológicas (IIByT, CONICET—Universidad Nacional de Córdoba), Acta N°6.

### **Animal Considerations and General Rearing Conditions**

It is already known that the exposure to fearful experiences facilitates (and increases) the occurrence of fear responses in subsequent conflict situations (i.e., TI, OF) (Jones, 1996; Martijena et al., 1997). It is also known that fear can affect all other motivational systems, and its elicitation is likely to impair the ability of birds to interact successfully with other birds (i.e., in other socially motivated tests such as a runway) and can also affect how birds utilize new resources (Jones, 1996). Thus, to minimize confounding factors, birds were tested in only 1 experimental situation (see details below).

This study was conducted with a total of 904 Japanese quail (*Coturnix japonica*) obtained from 3 incubation batches (343, 245, and 316, respectively). Birds from each batch were evaluated only once in either TI, OF, or runway (see descriptions in corresponding subsections). It is important to note that all eggs came from the same parental flock, and birds from this study were also part of a large selective breeding program aimed to evaluate whether it is possible to apply the DRP test “social density preference test” as selection criteria to obtain divergent lines of HD and LD quail. Because a large number of chicks are needed to initiate that program, after finishing this study, all categorized quail (see

corresponding subsection) were added to the program to conform the parental generation.

At hatch, chicks were leg-banded to maintain individual identification and housed in mixed-sex groups of 60 to 62 birds, in white wooden rearing boxes (90 × 90 × 60 cm, length × width × height, respectively). Each box had a feeder covering the entire front of the box, 16 automatic nipple drinkers, and a lid to prevent birds from escaping and heat loss. Each box was also provided with a heating system that allowed maintaining brooding temperature at 37.5°C during the first week of life, with a weekly decline of 3.0°C until final room temperature (25 ± 1°C) was reached. Quail were subjected to a daily cycle of 14 h light (300 to 320 lx):10 h darkness during the study. Lights were turned on at 06:00 h and turned off at 20:00 h. Leg bands were replaced with permanent wing bands at 15 d of age. A quail starter diet (28% CP; 2,800 kcal ME/kg) and water were provided *ad libitum* throughout the study. All tests were performed as either chicks or juveniles (<28 d of age); therefore, sex differences in plumage coloration were not evident at testing time. Nevertheless, according with a previous study, equal proportions of male and female birds are assumed to be present in each treatment group (Guzmán, 2011).

### **Classification of Quail in the Density-Related Permanence Test**

Classification of quail in the DRP test was conducted when birds were 11 d of age, which is before either TI, OF, or runway testing (see below). The DRP test is described in full detail in Guzman et al. (2013). Briefly, the classification apparatus consisted of 2 boxes interconnected by a central region delimited by 2 sliding doors that holds 34 experimental birds. Each box also contained at its distal end either 12 or 3 conspecifics confined behind a glass (a total of 15 stimulus birds). After over 18 h of habituation to the experimental setup, starting at 8:00 am and every 1h, the sliding doors were closed, and 34 experimental birds were identified and released back in the central region of the device. According to where they were found (box containing high-density, low-density or in the central region), each bird received a 1, -1, or a 0 score, respectively. The procedure was repeated 9 times, and the scores summed. Birds with final values of ≥3 or ≤ -3 were respectively categorized as HD or LD. Most HD and LD categorized birds scored >3 or <-3, meaning they were consistently found either in one or the other side (HD or LD) of the apparatus. Birds with intermediate scores were categorized as showing no preference. After the classification process, all birds (except those used as stimulus birds) were housed back in their initial home boxes. Stimulus birds were not further used in the study.

### **Tonic Immobility Test**

After DRP test classification of the 238 experimental quail, a total of 45 birds were categorized as HD, and

84 birds were categorized as LD. Between 21 and 23 d of age, the TI reactions of all classified birds were registered. Each bird was tested individually and once only. The TI was induced by restraining the chick on a table top for 15 s; it was held on its left side facing away from the experimenter with one hand on its sternum and one lightly cupping its head (Forkman et al., 2007; Marin et al., 2001). We measured the number of inductions (15 s periods of restraint) necessary to attain TI lasting at least 20 s and the duration of TI, that is the latency until the bird righted itself. If the bird failed to meet the 20 s immobility criterion, the induction procedure was repeated immediately. If TI could not be induced by the 5th attempt, the chick was deemed to be nonsusceptible, and a TI duration of 0 s was given. A test ceiling of 10 min was set, and any chick that failed to right itself before this time elapsed was given a maximum score of 600 s (Marin et al., 2001). All testing was carried out between 9.00 and 15.00 h.

### Open-Field Test

After DRP test classification of 170 experimental quail, a total of 25 birds were categorized as HD, and 61 birds were categorized as LD. Specifically, in this experiment, 50 of the previously classified birds (25 HD and 25 LD) were randomly chosen and tested at 20 d of age in an OF apparatus consisting of a white wooden box measuring  $60 \times 60 \times 130$  cm (width  $\times$  length  $\times$  height) with two 25W light bulbs. The OF testing was conducted between 8 am and 4 pm, where 8 identical OF apparatuses were used. Each bird was tested individually and once only. The testing order of all individuals was also randomized. To begin a test, each bird was placed near the midpoint of the OF floor using a side door, and its behavior was recorded on videotape for 32 min by using a closed-circuit television system with a video camera fixed to the ceiling of the OF. The interior of the OF was maintained at an ambient temperature similar to that in the room wherein the birds' home cages were located. Following completion of that test, the floor was wiped clean before reuse for the next test.

We used the ANY-MAZE (Stoelting, Co., Wood Dale, IL) computer program to analyze the locomotion of the birds in the OF apparatus at 0.5 s intervals. At any given interval, if the bird was ambulating, a number 1 was recorded, and if the bird was immobile, a 0 was recorded (see technical validation of methodology in Guzman et al. (2016)). Thus, a time series of locomotion during the 30 min test period (i.e., 3600 time intervals) was constructed for each bird. The following variables were also measured.

1. Latency to initiate ambulation (s): time from the start of the test until a bird ambulated at least 2 consecutive seconds.
2. Distance ambulated (m): the total (cumulative) distance ambulated by the animal during the test period.
3. Ambulation speed (m/s): distance ambulated divided by the time spent ambulating
4. Locomotor event (s): interval of time ( $>1$  s) in which the animal was continuously ambulating.
5. Number of locomotor events: Number of time intervals ( $>1$  s) in which the animal was continuously ambulating.
6. Total time spent ambulating (s): the total (cumulative) time spent ambulating by the animal during the test period.
7. Self-similarity parameter,  $\alpha$ , estimated with DFA. This method to analyze the ambulation pattern of the birds was introduced by Peng et al. (1994) and has been described in detail by Kembro et al. (2013; 2008). Following Kembro et al. (2008), we applied this method to the time series that corresponded to the active period of OF ambulation behavior (wherein each test subject's initial period of nonambulation was eliminated considering the latency to initiate ambulation). The self-similarity parameter ( $\alpha$ ) provides information regarding the temporal structure of locomotor behavior and relates to the autocorrelation structure of the time series. If  $\alpha = 0.5$ , then the series is uncorrelated (random) or has short-range correlations (i.e., the correlations decay exponentially), whereas the situation of  $0.5 < \alpha < 1$  indicates that long-range autocorrelations (i.e. showing long-term memory with correlations decaying as a power-law) exist, meaning that ongoing behavior is influenced by what has occurred in the past (Kantelhardt et al., 2001).
8. Probability distribution of spatial use of quail within the OF apparatus was assessed by pooling the spatial x,y coordinates (standardized in a scale from 0-1) of all the birds tested belonging to the same classification (HD or LD) and estimating the probability of finding a bird in a given zone using the kernel density function *ksdensity* in MATLAB (2018) and a grid [0:0.05:1] for estimation.

### One-Way Runway Test

After DRP test classification of the 136 experimental quail, a total of 21 birds were categorized as HD, and 39 birds were categorized as LD. Specifically, to assess the birds' social reinstatement response, in this experiment, 40 (randomly chosen) of these previously classified birds (20 HD and 20 LD) were individually tested in a runway with both 2 (low density) and 10 (high density) unfamiliar conspecifics stimulus placed in the goal box at the end of a runway. The first runway test was performed when the birds were 26 d old, and half of the birds in each category (randomly chosen) were evaluated with high density and the other half with low density of conspecifics placed in the goal box. The next day, all birds were tested again with the other density of conspecifics in the goal box.

Two runways were used at a time so that 2 quail could be individually tested simultaneously by 2 experimenters. Each test apparatus consisted of a white



wooden corridor measuring  $120 \times 40 \times 40$  cm (length  $\times$  width  $\times$  height) divided into 3 compartments using removable glass partitions. The compartments situated at opposite ends of the runway were each 20 cm long and comprised the start box and the goal box, respectively. Each runway was situated in a separate experimental room, and the temperature and illumination were maintained at similar levels to those in the room in which the chicks were reared with the addition of a 60-W light bulb suspended above the goal box. Age-matched quail, housed in separate home boxes and without visual contact with the experimental birds, were used as stimulus birds in the goal boxes. It was considered likely that the stimulus birds would be more attractive if they were active or standing than if they were immobile or asleep so stimulus birds were changed after each block of 4 tests. The chicks could not see the experimenters, who assessed the quail's behavior through the observation of the images provided by video cameras (placed 1.5 m above the runway) on a screen.

Each experimental quail was placed in the start box of one of the runways and allowed 2 min to acclimatize to the testing apparatus; from this position, the bird could see the stimulus birds. The glass partition was then removed, and we measured the latency for the experimental bird to reach a 20 cm "close" zone (CZ) nearest the goal box, the accumulated time spent in the CZ as well as the accumulated number of entries to this CZ over a 5 min test period. Testing began at 8:00 h and was completed by 16:00 h on both of testing days. Each consecutive block of 4 tests comprised representatives from each of the 2 treatment groups (HD and LD).

### Statistical Analysis

Generalized linear mixed models were used to evaluate the effects of the bird's category (HD and LD) on the registered variables in both TI and OF tests. Category was included as a fixed effect and brooding box as random effect. Normal distribution was used for all variables except for latency to ambulate and number of TI inductions that used a Gamma and Poisson distribution, respectively. Owing to an injury (bleeding due to a broken claw) of 1 of the HD birds during OF testing, data from that bird were not considered in the OF analysis.

Time spend in and number of entries to the CZ were also analyzed with generalized linear mixed models. Category (HD vs. LD) and goal box density (goal box with 10 or 2 birds) were included as fixed effects, and brooding box and testing day were included as random effects. Normal distribution was used for time spend in CZ and Poisson distribution for number of entries. Because the latencies to reach the CZ were on average very low (under 3 s in all groups), that variable was not statistically analyzed.

All statistical analyses were performed in R version 3.4.0, using the user-friendly interface InfoStat 2017 (Di Rienzo, 2017). A  $P$ -value of  $\leq 0.05$  was considered

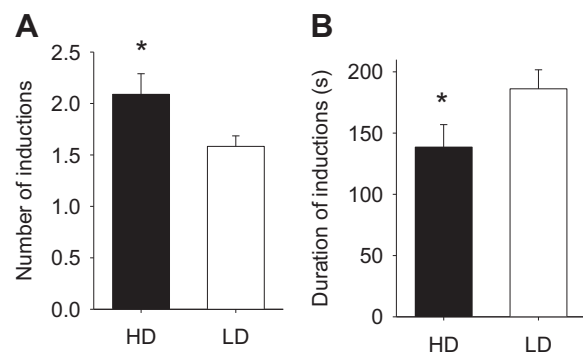
to represent significant differences in all tests. The best fitting models were selected on the basis of the lowest Akaike information criterion and Bayesian information criterion values calculated in Infostat. Model fitness was evaluated visually through diagnostic plots in R using the *fitdistrplus* package (Delignette-Muller and Dutang, 2015), whereas the statistical power of the tests was calculated using *pwr* (Cohen, 1988). A power of  $>82\%$  was found for all tests.

## RESULTS

Tonic immobility testing showed a higher number of inductions ( $F_{1, 127} = 4.23$ ;  $P = 0.04$ ) and a shorter duration of TI ( $F_{1, 127} = 6.46$ ;  $P = 0.01$ ) in the HD in comparison to the LD birds (Figures 1A and 1B, respectively).

Open-field test performances are shown in Table 1 and Figure 2. In this test, main differences were also found between birds classified as HD and LD, where HD birds showed higher values than their LD counterparts in the distance ambulated, the ambulation speed, the number of locomotor events, and the total time spent ambulating during the test (Table 1). As shown in Figures 2A and 2B, birds from both groups, as expected, showed a nonhomogeneous spatial use of the open field. Birds predominantly used corners (red–yellow tones in heat map) and avoided the center of the apparatus (dark blue). No difference between latency to ambulate was observed between groups (Table 1) nor in the  $\alpha$ -value estimated with DFA, showing similar long-range correlations in both groups once ambulation begun ( $\alpha = 0.780 \pm 0.011$  and  $0.779 \pm 0.009$ , in HD and LD birds, respectively). Latency to ambulate was short, lower than 30s, in about 75% of the tested birds (27/49, Figure 2C) and only 5 birds, representing 10% of the population, showed long latencies above 100s. Four of those 5 birds belonged to the LD group (Figure 2C) representing a weak tendency (Proportion test,  $P = 0.11$ ) to be larger than the 1 out of 5 observed in HD birds.

Results of the social reinstatement responses of HD and LD birds tested with low or high density of unfamiliar conspecifics placed at the end of a runway are depicted in Table 2. No significant effects or interactions



**Figure 1.** (A) Number of inductions and (B) duration of tonic immobility in juvenile Japanese quail. (Mean  $\pm$  SEM). \* $P < 0.05$ .  $N = 129$  (45 HD and 84 LD birds). Abbreviations: HD, high density of confined conspecifics; LD, low density of confined conspecifics.

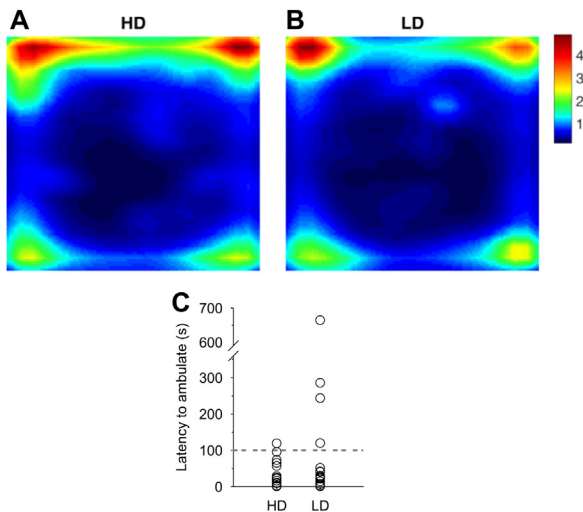
**Table 1.** Behavioral responses (Mean  $\pm$  SEM) in an open-field test of Japanese quail categorized by their permanence in proximity to either a high or a low density of confined conspecifics (HD or LD, respectively).

Behavioral measurements	HD birds (n = 24)	LD birds (n = 25)	$F_{1, 47}$ -values	$P$ -values
Latency to initiate ambulation (s)	26.3 $\pm$ 6.6	64.6 $\pm$ 28.9	3.44	0.07
Distance ambulated (m)	75.1 $\pm$ 7.2	49.2 $\pm$ 5.7	7.99	$\leq 0.01$
Speed (cm/s)	3.47 $\pm$ 0.38	2.21 $\pm$ 0.30	8.33	$\leq 0.01$
Number of locomotor events	445.6 $\pm$ 21.3	372.5 $\pm$ 27.8	4.31	0.04
Total time spent ambulating (s)	400.7 $\pm$ 35.6	280.7 $\pm$ 35.8	5.5	0.02

between bird category and density of stimulus birds were detected in the number of entries to the CZ or in the time spent in the CZ. However, in regard to the number of entries into the CZ, a strong tendency ( $P = 0.054$ ) toward an interaction between factors was observed that could represent that HD quail transitioned less out of the runway's high-density CZ than out of the low-density CZ. Latencies to reach the CZ were on average less than 3 s in all groups (data not shown).

## DISCUSSION

This study shows that HD Japanese quail required more inductions and developed shorter responses in the TI test and walked more, faster and greater distances in the OF than their LD counterparts. These findings suggest that classification of chicks based on the individuals' permanence in proximity to either a HD or LD is associated with divergent fear response as juveniles.



**Figure 2.** Open-field test of Japanese quail categorized by their permanence in proximity to either a high or a low density of confined conspecifics (HD or LD, respectively). (A,B) Probability distribution of spatial use within an open-field. The color bar in the top right corner shows the probability (expressed as a percentage) of a bird being localized in this area, red-yellow colors represent a high probability of permanence while blue represents a low probability. Note that birds from both categories prefer corners of the boxes. The top border represents the side of box used when placing birds into the box. (C) Latencies to ambulate of each individual within a category (mean values are shown in Table 1). Gray line mark (100s) highlights that only 5 birds (1/23 HD and 4/24 LD birds studied) showed latencies above this arbitrary threshold. Abbreviations: HD, high density of confined conspecifics; LD, low density of confined conspecifics.

Specifically, birds classified as HD were less fearful than their LD quail counterparts. Results are also consistent with the lower adrenocortical response to mechanical restraint observed previously in HD in comparison to the LD counterparts (Guzman et al., 2018). Nonetheless, both HD and LD juveniles showed strong motivations to remain close to stimulus conspecifics when tested in a novel runway environment. Both categories spent approximately 83% (250s out of 300s) of the test time in the close zone to conspecifics.

Both TI and OF tests are commonly used methods to assess fear and anxiety reactions to a novel environment (Jones, 1996; Forkman et al., 2007). As stated previously, while TI is mainly considered an antipredator response (Jones, 1996), OF behavior can be considered to parallel the adaptive response to danger with 2 distinct phases (Faure et al., 1983). The first phase may include either panic running or, more commonly, immobility and silence, and this phase probably reflects the inhibition of all behavioral patterns by fear. The second phase is an active phase that includes ambulation, jumps, and distress vocalizations. This latter phase may represent a waning of fear allowing also the expression of socially motivated behaviors (Faure et al., 1983). In this context, given that HD birds were less fearful than LD birds in the TI test (i.e., showed more inductions needed to induce TI and shorter TI once induced), we would have expected that latency to ambulate in the OF should have also been shorter in HD birds; however, this was not case. On the other hand, longer latencies and the low activity in an OF test are considered to be associated with high novelty in the test arena (Jones and Faure, 1982). Thus, given the high similarity in size, color, and materials between the breeding box and the OF apparatus used in the present study, it could also be considered that the test apparatus represents a stimulus of low novelty for our quail. Ultimately, this could have led to the reduced latencies observed in both groups. The different responses observed for the other variables evaluated (distance ambulated, speed, and time spent ambulating) could also be interpreted as socially motivated rather fear/anxiety motivated behaviors (Gallup and Suarez, 1980; Jones and Merry, 1988). This interpretation may be also supported by the lack of differences between HD and LD quail in the fractal structure of their ambulation once birds started to move within the apparatus (a similar autosimilarity  $\alpha$  parameter and long-range correlations in both HD and LD quail). It is also important to take into

**Table 2.** Behavioral responses (Means  $\pm$  SEM) of Japanese quail categorized by their permanence in proximity to either a high or a low density of confined conspecifics (HD or LD, respectively), tested in a single runway with a goal box containing either 10 (high density) or 2 (low density) unfamiliar conspecifics.

Behavioral measurements	HD birds (n = 20)		LD birds (n = 20)		P-values		
	10 goal box birds	2 goal box birds	10 goal box birds	2 goal box birds	Category	Goal box	Interaction
Number of entries to the CZ <sup>1</sup>	1.55 $\pm$ 0.3 <sup>a</sup>	2.75 $\pm$ 0.7 <sup>b</sup>	2.40 $\pm$ 0.4 <sup>a,b</sup>	2.35 $\pm$ 0.3 <sup>a,b</sup>	0.34	0.07	0.054
Time spend in the CZ (s)	271 $\pm$ 14	253 $\pm$ 19	254 $\pm$ 13	244 $\pm$ 16	0.45	0.16	0.70

<sup>a-b</sup>Groups that do not share letters tend to be different ( $P = 0.054$ ).

<sup>1</sup>Close zone (CZ) = the 20 cm zone nearest the goal box.

consideration that birds did not move homogeneously within the OF apparatus but rather predominately remained in sides and corners. This movement pattern could be related with escaping behaviors that are frequently associated with attempts to run away from predators present in the environment. However, in highly social species and in the absence of a predator, the movements in sides and corners could also be strongly motivated by the need to regain social reinstatement with conspecifics (Gallup and Suarez, 1980).

Motivation for social reinstatement behavior was specifically assessed in a 5 min runway test. Under the experimental conditions applied (short testing time and a novel environment), a high motivation to establish social contact with conspecifics was observed in both HD and LD birds. On average, all birds reached the CZ near conspecifics in less than 3 s and spent about 83% of the testing time in that area. However, it can also be considered that once birds approached their conspecifics, the HD group tended to transition less out of the CZ when the stimulus birds were in a high density rather than a low density, suggesting a higher motivation to remain in close proximity to a large group of conspecifics. The LD quail did not show differences while near a high-density or low-density of stimulus birds. These findings are in line with previous observations within home boxes reporting HD and LD differences in underlying sociability (Guzman et al., 2013). In that study, HD quail showed highly compact groups and low levels of aggressiveness toward conspecifics than their LD counterparts. This is not the first time that some differences between short social reinstatement behaviors and the underlying sociability are described within birds of a single species. For example, Jungle fowl seemed to affiliate more tightly with social stimuli in a short (5 min) runway test than in behavioral tests of a longer duration (Väisänen and Jensen, 2003). Moreover, Leghorns were less prone to move out of sight from conspecifics when placed for 10 min in a novel environment. They showed stronger affiliations to group members when given a considerable amount of time (30 min) to roam freely in a novel pen (Väisänen and Jensen, 2003). We consider that even giving the birds a short (2 min) period of adaptation before runway testing, the apparatus still represents a novel situation, and because the experimental bird has just one option to find social protection in close proximity to conspecifics (Petit and Bon, 2010), the expression of social reinstatement can be highly exacerbated (Marin et al.,

2001). Future experiments with a 2-choice runway (Guzman and Marin, 2008; Guzman et al., 2009), where both densities of stimulus birds are presented simultaneously during the test, could provide additional evidence to enrich our understanding of the observed phenomenon.

Differences in fearfulness between HD and LD quail could also be explained by associated personality traits and/or specific coping strategies. Differences in sociality and fear responses in our DRP-classified quail are comparable to responses in lines of Japanese quail genetically selected by different traits. For example, a selection program based on high and low levels of social reinstatement behavior toward conspecifics (HSR and LSR line, respectively) (Faure and Mills, 1998) lead not only to profound changes in the underlying sociability of the birds (Launay et al., 1991; Mills et al., 1993; Burns et al., 1998; François et al., 1998; 2000) but also to differences in fearfulness (i.e., HSR quail walked longer than LSR quail in an OF test) (Launay, 1993; Formanek et al., 2008). Another quail selection program was based on low rather than high-stress plasma corticosterone response to a brief mechanical restraint (stress divergent lines) (Satterlee and Johnson, 1988). These quail stress lines also showed strong differences both in fear responses in a variety of tests, including TI and OF (Jones et al., 1992a; Jones et al., 1992b; Satterlee and Marin, 2006; Kembro et al., 2008), and in social reinstatement behaviors (Jones et al., 2002; Guzman et al., 2009). Interestingly, in the stress lines, as well as in this study, no differences between groups were found in the self-similarity parameter estimated with DFA once ambulation began (Kembro et al., 2008). Thus, it appears that differences in sociability and fear could be considered as part of associated personality traits. Furthermore, the level of the behavioral response of these traits that are co-expressed could also be representative of a proactive or reactive strategy to cope with challenging situations (Koolhaas et al., 1999; Cockrem 2007; Carere et al., 2010).

It should be noted that all DRP tested quail were reared during the study at an intermediate fixed stocking density of 72 birds/m<sup>2</sup> (Shanaway, 1994). However, a mismatch between the individual social characteristic and the imposed social rearing environment could have also occurred affecting in a greater manner the LD birds. If because of the social preferences the LD birds were sensitive to the used stocking density; then, the social



environment during rearing could also have been perceived as more stressful for LD than for HD birds (El-Tarabany, 2016). Two important pieces of information should be considered in this regard: (1) a repetitive exposure to low intensity stressors can lead to an habituation to that particular stressor but also to a sensitization to other types of stressors (Ladewig, 2000; Grissom and Bhatnagar, 2009) and (2) a chronic exposure to a stressor has been shown to affect other birds' challenging responses (Altan et al., 2003; Yalcin et al., 2003; Cockrem, 2007). Thus, a mismatch between the social needs of the LD birds and the rearing environment could have acted as a stressor and so modulated the underlying fear/stress response during the IT and OF testing. This contention is important from a welfare perspective given that welfare guidelines are often drafted considering an "average" bird and not subgroup particularities. However, it is clear that even after decades of targeted selection, strong diversity in sociability and/or associated fear/responses can still be found within bird populations (Jones and Hocking, 1999; Guzman et al., 2013).

It is known that maternal environmental conditions could impact offspring development and phenotype (Groothuis and Von Engelhardt, 2005; Della Costa et al., 2019). Therefore, a potential limitation of this work is that each test was performed on a different batch of birds. However, because the 3 experiments (1 for each test) were performed with chicks that were the offspring of the exact same parental flock, using the same incubator/hatcher and were reared under standardized procedures and husbandry management, we considered that potential maternal effects were minimized in our study.

In all, our results further support our previous studies highlighting that early individual social permanence in the DRP test would not be an isolated behavioral response (context-dependent) to one particular experimental conditions but rather be part of an adaptability strategy of the birds for living in groups. A complex interaction between individual traits and behavioral responses to the social environment could be involved in modulating fear response in birds classified in the DRP test. Because of this complexity, the variability between birds should be considered when recommending guidelines for welfare standards. Whether the higher OF activity (walking more, faster and greater distances) shown by the HD quail was mainly driven by a reduced fear, by an increased social reinstatement motivation, and/or a proactive copying style, they are all considered advantageous characteristics for birds in intensive rearing systems. Moreover, if fearfulness is one of the main variables influencing DRP behavior, it is also conceivable that HD birds would also be better prepared to be raised in enriched environments (i.e., less fearful birds respond better in more challenging and stimulating environments) which is highly relevant from a welfare point of view. Thus, DRP performance might represent an interesting tool not only for the study of social interactions but also as an attractive selection criterion for future reproductive programs.

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## DISCLOSURES

The authors declare no conflict of interest in this manuscript.

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