Effect of the density of conspecifics on runway social reinstatement behavior of male Japanese quail genetically selected for contrasting adrenocortical responsiveness to stress[1]

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ABSTRACT Runway tests are considered indicative of underlying sociality in birds and their ability to make social discriminations and establish interactions among conspecifics. Herein, social reinstatement behavior in male juvenile Japanese quail selected for a reduced (LS, low stress) or exaggerated (HS, high stress) adrenocortical response to brief mechanical restraint was evaluated. Individual males were given the choice to reinstate with either 2 (low density, LD) or 8 (high density, HD) unfamiliar randombred conspecifics placed in goal boxes at opposite ends of a two-choice runway (TCRW). Then, the same males were individually retested in a single goal box runway wherein they were exposed to a goal box containing either a LD or a HD of males. In the TCRW, a higher ($P < 0.01$) number of HS males started their ambulation toward the goal box containing the HD as opposed to LD of conspecifics. The HS males also spent more ($P < 0.01$) time in close proximity (within a 10-cm close zone; CZ) to the HD (218 s) rather than LD (57 s) of conspecifics. In contrast, the LS males did not differ in their initial direction of travel and they spent similar average amounts of times in the CZ of their stimulus LD (141 s) and HD (124 s) conspecifics. Similar to the TCRW results, in the single goal box runway, HS males spent more ($P < 0.01$) time in the CZ of HD rather than LD conspecifics, whereas LS quail spent similar amounts of time in the CZ of LD and HD males. Considering that runways are novel (and therefore frightening) environments, our findings suggest that HS quail may find better shelter (i.e., more comfort) in close proximity to a larger rather than smaller group of conspecifics, whereas LS birds find groups of varying conspecific density equally attractive. The results suggest that LS quail possess favorable social adaptive qualities because they appear to be better suited to cope with situations where the density of conspecifics is variable.

Key words: runway behavior, sociality, corticosterone, Japanese quail

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INTRODUCTION

Genetic selection has been proposed as a reliable method of eliminating harmful traits and promoting desirable ones in farm animals, thereby improving their welfare and productivity (Mench, 1992; Craig and Swanson, 1994; Jones, 1996; Faure and Mills, 1998; Jones and Hocking, 1999). Lines of Japanese quail have been genetically selected at the Louisiana State University Agricultural Center for either reduced (low stress, **LS**) or exaggerated (high stress, **HS**) plasma corticosterone response to brief mechanical restraint (Satterlee and Johnson, 1988). Selection for low stress has been accompanied by several intuitively desirable changes. For example, quail of the LS line show a lower fearfulness, a nonspecific reduction in stress responsiveness, and less developmental instability (Jones and Satterlee, 1996; Jones et al., 1992, 1994, 1999b, 2000; Satterlee et al., 2000; Satterlee and Marin, 2006; Davis et al., 2008; Kembro et al., 2008) than do quail of the HS line. Compared with quail of the HS line, LS quail also exhibit less pronounced reductions in BW after exposure to multiple sequential stressors (Satterlee and Johnson, 1985) and accelerated puberty and enhanced reproductive performance in both males (Satterlee et al., 2002,

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2007; Marin and Satterlee, 2004; Satterlee and Marin, 2004) and females (Marin et al., 2002b; Satterlee et al., 2008). Of particular relevance to the present study, LS quail possess a greater sociality compared with their HS counterparts as seen in very young birds (less than 15 d of age) in both home cage social proximity and a single test of social affiliation (Jones et al., 2002) as well as in adult male:female social proximity and copulatory behaviors (Marin and Satterlee, 2003).

The main benefits for animals that live in groups would be reduced predation risk (Hamilton, 1971), enhanced foraging opportunities (Clark and Mangel 1986), or increased positive social interactions, or all of these benefits. However, modern farming practices can impose several deviations from what might be considered in a natural situation. For example, crowding, alteration of group membership, and large groups whose size exceeds social recognition capacity often disturb or completely prevent natural social relationships between birds within flocks, which can potentially cause harmful effects (Jones et al., 1996; Hughes et al., 1997). In addition, under certain circumstances, underlying sociality (motivation to be near conspecifics) has been linked to stress responsiveness and fearfulness in domestic chicks (Jones, 1996; Marin et al., 2001; Guzmán and Marin, 2008). For example, depending on the test conditions, previously stressed birds have shown either an enhanced or a reduced social reinstatement behavior when placed in an unfamiliar environment (Marin et al., 2001; Guzmán and Marin, 2008). In children, shyness and social withdrawal have been positively linked with cortisol levels and general fearfulness (Kagan et al., 1988; Schmidt and Fox, 1998). Inappropriate levels of sociality could also exert undesirable effects on all aspects of social interaction in birds, including affiliation, aggression, dispersal, and mating, as well as their ability to cope with social disruption, such as isolation, exposure to strangers, or crowding (Vallortigara, 1992; Jones, 1996; Jones and Mills, 1999; Jones et al., 1999a). Furthermore, a mismatch between underlying sociality of a bird and its social environment could elicit either a series of acute stress responses or chronic social distress with associated negative effects on performance (Mills et al., 1993; Jones and Hocking, 1999; Jones and Mills, 1999). Thus, low-sociality birds may be poorly suited for housing in very large or in very confined groups, which makes the continued study of sociality behavior in poultry of important fundamental and practical relevance.

Although we commonly consider the behavioral dynamics of entire groups (Estevez et al., 2002), it is the decisions of each individual that ultimately shape the collective behavior of a social group (Gueron et al., 1996). For example, individuals may prefer to be closer to their conspecifics to reduce predation risk or they may maintain greater distances to minimize competition (Leone and Estevez, 2008). Because increased stocking density (crowding) is known to increase stress and thereby negatively affect animal productivity, health, and welfare in modern poultry production systems, it is important to improve our understanding of the influence of conspecific density on social behaviors that may reflect fear or distress, or both. Indeed, birds that are genetically predisposed toward divergent nonspecific adrenocortical stress responsiveness (HS > LS), and who also show differences in productivity (LS > HS), fearfulness (HS > LS), and sociality (LS > HS) as correlated traits (see above) serve as good models for such study. Thus, the present study evaluated whether males of the LS and HS quail lines differ in their social reinstatement behaviors when individually exposed to low or high densities of age- and sex-matched unfamiliar randombred (**RB**) conspecifics. First, males were given the choice to reinstate with either 2 (low density, **LD**) or 8 (high density, **HD**) unfamiliar conspecifics placed in goal boxes at opposite ends of a two-choice runway (**TCRW**). Second, the same males were individually retested in a single runway (**SRW**) wherein they were exposed to either a goal box containing a LD or HD of unfamiliar conspecific males. Runway tests have been widely used to measure social reinstatement responses; they are considered to be indicative of underlying sociality in birds as well as of their ability to make social discriminations and establish social interactions (Suarez and Gallup, 1983; Vallortigara et al., 1990; Mills et al., 1995; Carmichael et al., 1998; Jones et al., 1999a; Marin et al., 2001; Väisänen and Jensen, 2004; Guzmán and Marin, 2008). For example, both domestic chicks and Japanese quail approach conspecifics more readily than they approach an empty goal box or one containing members of different avian or mammalian species (Suarez and Gallup, 1983; Mills et al., 1995; Jones and Mills, 1999). Also, birds in variable group sizes may present different movement patterns and different amounts and kinds of vocalizations (i.e., variable stimuli) that could be perceived differently by test birds. The effects of variable bird density on runway sociality behaviors have yet to be determined in LS and HS quail.

MATERIALS AND METHODS

Genetic Stocks and Bird Husbandry

Sex differences in stress susceptibility (Marin et al., 2002a) and in sociality (Vallortigara, 1992) have been reported. Thus, to minimize interindividual variation due to sex, only male Japanese quail from generation 39 of the LS and HS lines of Satterlee and Johnson (1988) were used. The more recent genetic history of the lines, up to generation 34, is discussed in detail elsewhere (Satterlee et al., 2000; Marin and Satterlee, 2004; Satterlee et al., 2006). Although line differences in levels of plasma corticosterone were not measured in the present study, recent findings in the stress lines attest to the maintenance of divergent adrenocortical responsiveness to a variety of nonspecific systemic stressors. Indeed, Satterlee et al. (2008) have offered explanations as to why the gene(s) that control the adrenocortical responsiveness trait in these lines may have become fixed.

The study quail were taken from a larger population of an approximately 300 mixed-sex bird hatch. Egg incubation, chick brooding, feeding, and lighting procedures were similar to those described by Jones and Satterlee (1996) with the exception that, at hatch, chicks were leg-banded and housed in mixed-line groups of 56 birds (28 LS + 28 HS) per compartment in each of 3 compartments (102 × 64 × 20 cm; length × width × height) of a model 2S-D, 6-deck Petersime (Petersime Incubator Co., Gettysburg, OH) brooder battery modified for quail. Sixty-six straight-run RB birds were also maintained in 2 brooder compartments of the same battery and were visually isolated from LS and HS birds. These RB quail were used as the unfamiliar conspecifics during runway testing (see below). To maintain the line identity of each bird, leg bands were replaced with permanent wing bands at 21 d of age.

At 4 wk of age, birds were sexed by plumage coloration, and 24 males from each line were randomly cage-housed in groups of 4 same-line birds on 1 tier of a 4-tier cage battery (Alternative Design Manufacturing and Supply Inc., Siloam Springs, AR) that contained 48 cages (12 cages/tier). Forty RB males were also randomly cage-housed in groups of 4 in 10 cages of another tier. Cages measured 50.8 × 15.2 × 26.7 cm (length × width × height). Coincident with placement into their cages, birds were switched to a quail breeder ration (21% CP; 2,750 kcal of ME/kg) and feed and water were continued ad libitum. The birds were also subjected to a daily cycle of 14 h of light (280 to 300 lx):10 h of dark at this time. Once housed into cages, the birds then remained undisturbed, apart from routine maintenance, until testing began (see below). During runway testing, temperature and illumination were maintained at similar levels to those in the rearing room. The testing was conducted between 0800 and 1600 h. During each test day, males from the LS and HS lines were tested in an alternating stress line order to minimize any effect that testing order may have had on variable outcomes.

TCRW

At 38 d of age, 48 male quail (24 LS and 24 HS) were tested individually and once only in a runway with either 2 (LD) or 8 (HD) unfamiliar RB conspecifics placed in goal boxes at opposite ends of a TCRW (Figure 1a). The apparatus consisted of an unpainted wooden runway measuring 30 × 200 × 30 cm (width × length × height) that was divided into 3 main compartments by Plexiglas partitions (Regal-Champion Plastics, Baton Rouge, LA; Figure 1a). The 2 compartments (each 20 cm long) situated at opposite ends of the runway were designated as LD and HD goal boxes. The compartment situated between the 2 goal boxes was considered to be the runway and it measured 160 cm in length. Another removable compartment was constructed in the middle of the runway (a 20 × 30 cm start box area). It was used solely to place and briefly hold a test bird before beginning a test (see below). The start box compartment allowed a test bird enough time to see the stimulus birds in the LD and HD goal boxes of the TCRW through Plexiglas partitions before being released. It consisted of 2 sliding Plexiglas partition walls that could be completely removed to the outside of the test apparatus when a test began. To allow removal of excreta during testing, the runway apparatus was fitted with a wire-mesh floor (1-cm grid) that was raised approximately 2 cm above the surface upon which the apparatus resided. A transparent lid was used to cover the runway and prevent bird escape by flight and a video camera was suspended about 1.5 m above the TCRW to record behavioral activity during the experiment. The LD and HD unfamiliar RB conspecifics were placed in each of the goal boxes (see below) before testing began. New stimulus birds (n = 10; 2 LD + 8 HD) were used 4 times during the test day; stimulus bird changes were made each time that a total of 12 stress line birds (6 LS + 6 HS) had been tested. During TCRW testing, the position of the RB stimulus birds (right or left side relative to stocking density) was also alternated every 2 tests to control for any potential effects of side of the test apparatus. Runway tests were also performed in an experimental room isolated from the rearing room.

At test, a male from either the LS or HS line was placed into the centrally located start box compartment of the TCRW wherein it could see the birds in the goal boxes through the Plexiglas partitions of both the start and goal boxes. After a 1-min period in the start compartment, the wall partitions keeping a test male in the start compartment of the TCRW were removed, and the locomotor behavior of the test male was recorded onto videotape during an 8-min test period.

During subsequent video replay, the screen floor image of the runway portion of the TCRW was divided into 2 halves, each relating to either the HD or LD goal boxes located on opposite sides of the runway (Figure 1a). The 10-cm zones nearest to each of the goal boxes were also referred to as the close zone (**CZ**) areas (i.e., areas within 10 cm of a goal box). Because of a technical problem during video recording, results from 6 birds (3 birds from each line) were not able to be included in the data analyses.

SRW

At 39 d of age, the same males tested in the TCRW were reevaluated individually and only once in a SRW apparatus that had a single stimulus bird goal box (Figure 1b). The SRW consisted of an unpainted wooden apparatus measuring 30 × 110 × 30 cm (width × length × height) that was subdivided into 3 compartments by Plexiglas partitions. The goal box compartment (that

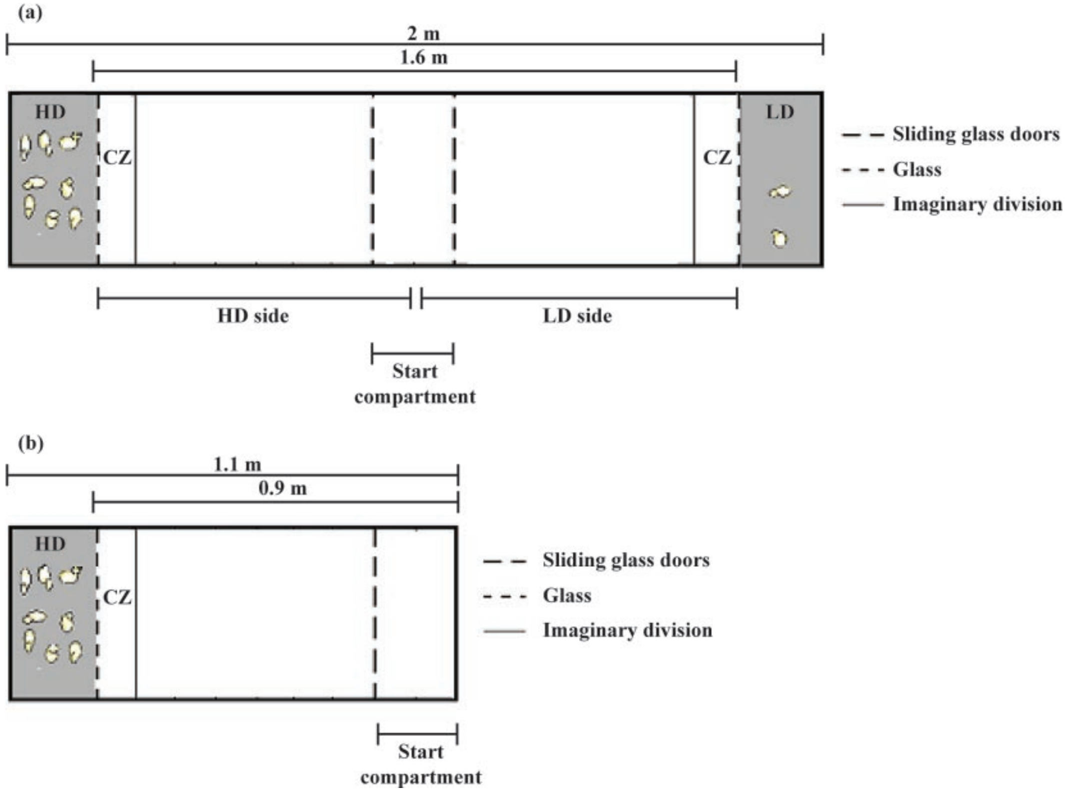

Figure 1. Diagram of the runway apparatus. (a) Two-choice runway with 2 goal boxes. Numbers of goal box randombred conspecifics were 8 (high density, HD) and 2 (low density, LD). Placement of the HD and LD conspecifics on the left and right side of the runway, respectively, is depicted only as an example. Close zone (CZ) = a distance within 10 cm of conspecifics. (b) Single runway with a single goal box. Numbers of goal box conspecifics were either 8 (HD) or 2 (LD). Close zone (CZ) = a distance within 10 cm of conspecifics. Color version available in the online PDF.

was used to house either 2 or 8 RB males, LD or HD stimulus birds, respectively) and the start box compartment (which had a sliding Plexiglas partition wall that could be completely removed when a test began) were situated at opposite ends of the SRW. Each of these end box areas were 20 cm long. The actual runway length, including the removable start compartment floor length of 20 cm, measured a total of 90 cm. The LD or HD unfamiliar RB conspecifics were placed in the SRW goal box (see below) before testing began. Similarly to the TCRW testing, test order throughout the day in the SRW also alternated between male representatives of the LS and HS lines and between the stimulus bird condition (LD or HD) to minimize any effects that testing order or stimulus bird changing, or both, may have had on variable outcomes. During the test day, the same groups of stimulus birds (either LD or HD) were used 2 times (while testing 1 LS and 1 HS quail) before being replaced with a new group of stimulus birds. To allow the passage of excreta during testing, the SWR apparatus was fitted with a wire-mesh floor (1-cm grid) that was raised approximately 2 cm above the surface upon which the apparatus resided. A transparent Plexiglas lid was used to cover the runway and prevent bird escape by flight and a video camera was suspended about 1.5 m above the SRW to record locomotor behavioral activity during the experiment. The camera used for both TCRW and SRW tests was a HP Elite Autofocus Webcam camera (Hewlett-Packard Co., Palo Alto, CA). As was done for TCRW tests, SRW tests were performed in a separate room that was completely isolated from the rearing room.

At test, a male from a given quail line was removed from its home cage, transported to the test room, and placed into the start compartment of the SRW. From the start compartment, a test bird could see the birds in the goal box through the Plexiglas partitions that separated the test male from its stimulus males (either LD or HD). After a 1-min holding period in the start compartment, the sliding glass walls that initially confined the male to his start box were removed, and behavior of the bird was recorded onto videotape during a 6-min test period. Similar to the TCRW, the 10-cm zone nearest to the goal box was also referred to as the CZ area in the SRW.

Video Recording Analyses

We used digitized pixel images to analyze the ambulation of the birds in the runway as described by Kembro et al. (2009). During the playback of a video recording, the X, Y coordinates of the center of the bird were noted at 0.5-s time intervals. The distance ambulated was defined as the distance that a test bird moved between 2 successive images (a time interval of 0.5 s). If the distance ambulated in a given time interval did not exceed a threshold distance of 1 cm, then the bird was considered nonambulatory. In such an instance, a 0 distance value for ambulation during that interval was recorded. The following variables were quantified:

- Latency to ambulate (s): time from the start of a TCRW or SRW test until the test bird took a step in any direction (i.e., the first time interval that showed more than 1 cm of distance ambulated).
- Latency to leave the start compartment (s): time from the start of a TCRW or SRW test until the test bird left the zone corresponding to the start compartment.
- Distance ambulated (cm): the total (cumulative) distance ambulated by a test bird during the respective test periods used in the TCRW and SRW tests.
- Latency to enter CZ (s): time from the start of a TCRW or SRW test until the test bird entered the 10-cm CZ nearest a goal box.
- Time spent in CZ (s): total time spent by a test bird in a CZ during either TCRW or SRW testing.
- First direction of travel (only measured in TCRW tests): the direction the test bird took when leaving the start compartment area (i.e., either toward the HD or LD goal box).

Statistical Analyses

A 1-way ANOVA was used to determine differences between LS and HS quail in their latencies to ambulate and leave the start compartment as well as their ambulation distances in the TCRW. The latency to enter the close zones and the time spent in each of the close zones within the TCRW were evaluated using a split-plot ANOVA, with line (LS vs. HS) as the between-subject variable and side of the runway (LD vs. HD) as the within-subject variable. Analysis of variance assumptions were verified. A Fisher's test was used for post hoc mean comparisons. To evaluate differences between LS and HS quail in their first direction of travel in the TCRW, a χ^2 goodness-of-fit test was used.

A 2-way ANOVA was used to determine the effects of line (LS vs. HS), density of conspecifics placed in the goal box (LD vs. HD), and their interaction on the latency to ambulate, the latency to leave the start compartment, the latency to enter the close zone, the time spent in the close zone, and the distance ambulated of the SRW tested quail. The latency to ambulate and the latency to leave the start box were transformed to ranks (Shirley, 1987) to fit ANOVA assumptions. A Fisher's test was used for post hoc mean comparisons. A P-value of less than or equal to 0.05 was considered to represent significant differences in both TCRW and SRW statistical tests.

RESULTS

Mean ± SEM ambulatory behavior results during TCRW testing are given in Table 1. There were no significant differences between male quail of the divergent adrenocortical stress response lines in their latencies to ambulate and to leave the start box of the TCRW and the distances that the LS and HS males ambulated within the runway area of the TCRW were also similar. However, although LS males showed complete indifference in ambulating toward the RB conspecifics housed in the HD or LD goal boxes ($\chi^2 = 0.00$, $P = 1$; identical first directions of travel), a significantly higher ($\chi^2 = 5.76$, $P < 0.01$) number of HS quail started ambulating toward the RB conspecifics in the HD than LD goal box (Table 2). When the stocking density runway side effect was included in the ANOVA of TCRW data, a significant main effect of side of the runway was found for the latency to enter a CZ (HD < LD; $F_{1,40} = 6.58$, $P < 0.014$) and for the time spent in each of the CZ (HD > LD; $F_{1,40} = 4.77$, $P < 0.035$; Table 3). No significant main effects of quail line were detected for the latency to enter the CZ or for the time spent in each CZ of the TCRW. Indeed, for both variables, the LS and HS overall means were very similar (data not shown). However, although quail line and side of the TCRW did not interact in affecting the latencies to enter the CZ of the TCRW, a significant interaction was found between quail line and side of the TCRW for the time spent in each CZ ($F_{1,40} = 4.10$, $P < 0.049$; Table 3). Fisher's least significant difference tests showed that LS males had similar latencies to enter the CZ near RB conspecifics placed in the LD or HD boxes and LS males spent similar times in close proximity to the RB quail in either the LD or HD goal boxes. In contrast, HS males arrived sooner ($P < 0.01$) to, and spent more ($P < 0.001$) time in, the CZ near the HD than LD goal boxes.

Neither test male quail line nor the density of RB conspecifics in the goal box of the SRW affected the latency of a test male to leave the start compartment, the latency to enter the CZ, and the distance ambulated within the SRW (Table 4). However, a significant main effect of the density of RB conspecifics was detected for the time spent in CZ (HD > LD; $F_{1,\,44} = 4.12$, $P < 0.048$) of the SRW. Moreover, a significant interaction ($F_{1,\,44} = 4.14$, $P < 0.047$) between the line of a test male quail and the density of RB conspecifics in the goal box was also detected for the time spent in the CZ of the SRW. Similar to the TCRW results, in the SRW, HS males spent more ($P < 0.01$) time in the CZ of HD rather than LD conspecifics, whereas LS quail spent similar amounts of time in the CZ of both LD and HD stimulus males (Table 4).

DISCUSSION

Runway tests have been widely used to measure social reinstatement responses that are considered to be

Table 1. Ambulation behaviors of male quail from lines selected for reduced (LS, low stress) or exaggerated (HS, high stress) adrenocortical response to brief mechanical restraint tested in a two-choice runway with the goal boxes containing 2 (low density) and 8 (high density) unfamiliar randombred conspecifics

	Line		Probability	
Behavioral measure[1]	LS	HS	$F_{1,41}$	P-value
Latency to ambulate (s)	31.7 ± 12.3	56.7 ± 21.6	0.98	0.32
Latency to leave start box (s)	59.8 ± 20.6	79.12 ± 24.8	0.35	0.55
Distance ambulated (cm)	1,685.1 ± 431.4	1,511.2 ± 301.9	0.11	0.74

[1]The values represent means ± SEM. Latency to ambulate = time from the start of the test until the bird takes its first step; latency to leave the start box = time from the start of the test until the bird leaves the zone corresponding to the centrally located start compartment; distance ambulated = distance ambulated in the runway area during the duration of the test (8 min).

indicative of underlying sociality in birds as well as of their ability to make social discriminations and establish social interactions (Suarez and Gallup, 1983; Vallortigara et al., 1990; Carmichael et al., 1998; Faure and Mills 1998; Jones et al., 1999a; Marin et al., 2001; Väisänen and Jensen, 2004; Guzmán and Marin, 2008). In the present study, differences in certain social reinstatement responses between LS and HS quails were evident in 2 different runway tests, the TCRW and SRW. First, when individual males were given the choice to reinstate with either a LD or a HD of unfamiliar RB stimulus bird conspecifics placed in the goal boxes at opposite ends of the TCRW, a higher number of HS males started their ambulation toward the goal box containing a HD as opposed to LD of conspecifics. The HS males also spent more time in close proximity to a HD rather than LD of conspecifics. In contrast, LS males did not differ in their initial direction of travel and they spent similar amounts of times in close proximity to their stimulus LD and HD conspecifics. Second, when tested in a SRW, HS males again spent more time in the CZ of HD rather than LD RB conspecifics, whereas LS quail spent similar amounts of time in the CZ of LD and HD of stimulus birds. Very young quail (<15 d of age) of the LS line are known to show greater sociality in comparison to their HS counterparts as seen in both home cage social proximity tests and in SRW social affiliation trials, both of which used fixed densities of stimulus conspecifics (Jones et al., 2002). Certain adult male:female social proximity and copulatory behaviors are heightened in LS quail as well (Marin and Satterlee, 2003). The present findings extend this knowledge base of social behavior differences between the quail stress lines in that we now know that although selection for a reduced adrenocortical responsiveness to an acute stressor has not altered male quail social reinstatement responses to different numbers of conspecifics (bird densities), selection in the HS line has (i.e., more HS males prefer to spend more time closer to HD rather than LD quail groups).

Social reinstatement evaluations in runways involve manual removal of a bird from its home environment, placing it into a novel one, and then measuring responses of the bird to a small group of conspecifics held in 1 or 2 goal boxes (e.g., the time it takes a test bird to approach the stimulus birds and how much time a test bird spends near them; Vallortigara, 1992; Jones and Mills, 1999). Therefore, these tests clearly incorporate many different potentially stress-inducing elements (e.g., bird capture by the experimenter, who the bird likely perceives as a potential predator; transport and exposure to a novel environment; and transient separation from home-cage companions or isolation distress). In broilers, exposure to a stressor before SRW testing affects social reinstatement responses by reducing the time the test birds take to approach conspecifics and increasing the time that focal birds spend near their stimulus birds (Marin et al., 2001). In contrast, in a TCRW with the same density of broiler conspecifics in each of the goal boxes, exposure to a similar stressor was associated with a significant reduction in the time spent near conspecifics and such treatment altered the

Table 2. Number of male quail from lines selected for reduced (LS, low stress) or exaggerated (HS, high stress) adrenocortical response to brief mechanical restraint that started their ambulation in a two-choice runway toward the goal box containing either 2 (low density, LD) or 8 (high density, HD) unfamiliar randombred conspecifics

	First direction of travel			Probability[1]	
Line	HD	LD	No preference	χ^2	P-value
LS	10	10	1	0.00	1.00
HS	16	5	0	5.76	0.01

[1]Because only 1 bird did not show a travel direction (no preference), its data was not considered in the χ^2 statistical test.

Table 3. Ambulation behaviors of male quail from lines selected for reduced (LS, low stress) or exaggerated (HS, high stress) adrenocortical response to brief mechanical restraint tested in a two-choice runway with the goal box sides of the runway containing either 2 (low density, LD) or 8 (high density, HD) unfamiliar randombred conspecifics

	LS line		HS line		*P*-value[2]		
Behavioral measurement[1]	HD side	LD side	HD side	LD side	L	D	L × D
Latency to enter close zones (s)	228.7 ± 48.9[ab]	264.7 ± 45.1[ab]	168.9 ± 38.9[a]	339.9 ± 36.8[b]	0.508	0.014	0.136
Time spent in close zones (s)	141.4 ± 39.7[ab]	123.5 ± 33.8[ab]	218.4 ± 36.2[a]	57.3 ± 23.3[b]	0.295	0.035	0.049

[a,b]Means within a row with no common letter differ significantly ($P < 0.01$).

[1]The values represent means ± SEM.

[2]*P*-value = probability value calculated with a 2-way ANOVA that analyzed line (L), goal box bird density (D), and the L × D interaction.

spatial distribution of locomotion behavior by reducing the ambulatory activity in the CZ (Guzmán and Marin, 2008). Thus, the way a bird will react in a runway test apparently depends upon the characteristics of the test situation and the most recent prior life experiences of the test bird. It has been suggested that the presence of conspecifics in an otherwise unfamiliar situation serves to reduce fear in domestic fowl (Jones and Merry, 1988). Moreover, it has been proposed that large flocks offer an individual greater natural protection (Bertram, 1978; Caraco, 1981; Clark and Mangel, 1984; Pulliam and Caraco, 1984). It is also thought that flocking individuals gain enhanced protection from predators even in a captive environment (Newberry et al., 2001); thus, the HD side of our present TCRW should have been, theoretically, more attractive than the LD side. In that context, although our birds were not intentionally stressed before runway testing, the behavioral differences presently observed during runway trials between males of the LS and HS lines (both in the TCRW and SRW) may be the consequences of the well-known line differences in their underlying fearfulness (HS > LS; Jones et al., 1992, 1994; Jones and Satterlee, 1996; Satterlee et al., 2000; Kembro et al., 2008) or adrenocortical stress responsiveness (HS > LS; Satterlee and Johnson, 1988; Satterlee and Marin, 2006; Satterlee et al., 2007), or both. In other words, even without intentional stress treatments, considering that runways are novel (and therefore presumably stressful and frightening) environments (see above), it is logical to suspect that the HS quail were likely experiencing greater fearfulness and adrenocortical responsiveness than the LS quail when placed in the runways and therefore more attracted to approach a goal box that offers better shelter (i.e., a bigger flock). On the other hand, it can be argued that because of the absence of differences between lines in the latency to ambulate or to leave the start compartment, no clear indicators of differences in fearfulness have been shown in the present study. However, it should also be recognized that ambulation latencies for the quail stress lines placed in novel environments are typically reported to be less than 2 min (Satterlee and Marin, 2006; Kembro et al., 2008). Moreover, in novel environments, the presence of conspecifics can serve to reduce fear and it has been shown that the latencies to approach them are significantly reduced when birds have been previously stressed (Marin et al., 2001). Thus, presently, it would be logical to expect HS quail to show a reduced latency to start their ambulation when compared with their LS counterparts. However, in both the TCRW and SRW tests, because birds were kept in the start compartments of the runways for a 1-min period before release, most of the birds would have started ambulation activity even before the sliding Plexiglass partitions of the start compartments were removed, thus reducing the chances of finding line differences in the latencies to ambulate or to leave the start compartments once the tests had started. The fact that no differences were observed between the lines in the distance ambulated is also consistent with the findings of Kembro et al. (2008), who showed that, in a novel environment test, once ambulation begins, birds of both

Table 4. Ambulation of male quail from lines selected for reduced (LS, low stress) or exaggerated (HS, high stress) adrenocortical response to brief mechanical restraint tested in a single runway with a goal box containing either 2 (low density, LD) or 8 (high density, HD) unfamiliar randombred conspecifics

	LS line		HS line		*P*-value[2]		
Behavioral measurement[1]	HD	LD	HD	LD	L	D	L × D
Latency to ambulate (s)	22.4 ± 14.8	6.7 ± 2.6	33.5 ± 20.2	42.7 ± 24.1	0.874	0.468	0.728
Latency to leave start box (s)	31.8 ± 16.4	14.6 ± 4.8	42.2 ± 22.2	47.8 ± 25.7	0.999	0.929	0.698
Latency to enter the close zone (s)	87.2 ± 33.4	75.7 ± 31.8	61.7 ± 29.6	112.6 ± 35.7	0.863	0.550	0.345
Time spent in close zone (s)	183.8 ± 32.0[ab]	185.6 ± 29.8[ab]	225.2 ± 30.4[a]	109.9 ± 20.4[b]	0.567	0.048	0.047
Distance ambulated (cm)	597.3 ± 99.2	509.7 ± 94.6	513.2 ± 191.9	981.8 ± 327.3	0.341	0.350	0.175

[a,b]Means with no common letter differ significantly ($P < 0.01$).

[1]The values represent means ± SEM.

[2]*P*-values: probability value calculated with a 2-way ANOVA that analyzed line (L), goal box bird density (D), and the L × D interaction.

the LS and HS lines have similar ambulatory organization. In the TCRW, most birds visited both CZ and, once they arrived in a CZ, the most frequent behavioral pattern observed was back-and-forth ambulation along the front of the Plexiglas partition wall that separated them from the stimulus conspecifics. This nonquantified behavior most likely represented a desire of the test subjects to enter the goal box and make closer contact with the conspecifics in an otherwise unfamiliar surrounding (Jones, 1996; Jones et al., 1996).

Because both the test and stimulus birds were not able to physically interact in the present study (i.e., they were always separated by at least 1 Plexiglas partition), direct (bird-to-bird) sexual or aggressive, or both, behavior measurements could not be made. Thus, whether or not these behaviors affected the outcome of HS quail preferring more to approach the HD than LD bird compartment is unknown. And, although indirect sexual or agonistic, or both, behavioral measurements in the CZ (e.g., pecking at the Plexiglas partition) were also not systematically made, our clear impression was that neither of these types of behaviors were evident in the present study. The lack of such behaviors is most likely due to the early age of the quail used, an age at which sexual development is in a very early stage.

Regardless of the etiology of the present line differences in TCRW and SRW social behaviors, our findings show that HS quail find better shelter (i.e., more comfort) in close proximity to a larger rather than smaller group of conspecifics, whereas LS birds find groups of varying conspecific density equally attractive. Therefore, we suggest that LS quail possess yet another favorable social adaptive quality: they appear to be better suited to cope with situations in which the density of conspecifics is variable. This social flexibility showed by the LS line could be regarded as an additional advantage of the selection program for reduced adrenocortical responsiveness to an acute stressor, particularly if the phenomenon generalizes to include the more commercially important avian species that are often housed at high stocking densities, like chickens or turkeys.

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