

An individually fitted physical barrier device as a tool to restrict the birds' spatial access: can their use alter behavioral responses?

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ABSTRACT Social interactions have been extensively studied in poultry in a variety of environmental situations. Many studies allow full social contacts between birds, but there are others in which the interactions are tested through barriers (wire mesh or glass). Thus a situation where, according to their needs, some birds can get access to physical contact with conspecifics while others cannot, would be useful to expand the testing options for social interaction studies. We developed an individual physical barrier device (IPB) that is fitted on the birds to delimit their ambulation areas by preventing them from passing across metal mesh boundaries that IPB free counterparts can easily overcome. The prototypes showing greater efficacy consisted of a small metal bar placed in the bird's back perpendicular to the sagittal plane that slightly exceeds body width, held with a harness fitted by 2 elastic fabric

bands around the wings' base. To be useful, the IPB should allow natural movements and not affect the expression of behaviors (non-invasive). This study assessed whether the IPB may alter adult Japanese quail behavioral responses using 4 classical test situations: Open-Field, Runway, Time Budget in Home Box, and Mating Interactions. Open-field ambulatory behaviors were affected 1 h, but not 7 d, after IPB was fitted, suggesting that 7 d (or less) are required to habituate to the device. After that time period, IPB fitted birds showed no differences in any of the behaviors registered in the other 3 tests situations when compared to non IPB fitted birds. Findings suggest that after habituation, the IPB does not affect main behaviors in adult quail. Its application could be expanded if an IPB device is also found suitable for other poultry species.

Key words: social interaction, Japanese quail, behavioral tests

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INTRODUCTION

The ancestors of the most common domesticated birds (e.g., turkeys, red jungle fowl, and Japanese quail) are social species living in small family groups or in larger mixed groups with an established social structure (Mench and Keeling, 2001). Relationships between individuals play a fundamental role in flock cohesion. In domestic birds, motivation to seek close proximity with conspecifics, defined as social motivation, influences the type of social contact that an individual seeks (e.g., mating, affiliation, dominance) and its behavioral and physiological responses to these specific social interactions (Schweitzer and Arnould, 2010). Spatial proximity might play a large role in determining patterns of social interaction as animals are presumably more likely

to interact with individuals that are physically close by (Sih et al., 2009).

Social interactions in poultry have been extensively studied (Mench and Keeling, 2001; Rodenburg et al., 2010; Alcock, 2013). There are many studies that allow full social interaction between animals, for example the intruder-resident test (Rutkowska et al., 2011; Guzman et al., 2013). There are also a number of studies where social interactions are tested through environmental barriers, such as wire mesh or glasses (Schlinger et al., 1987; Jones et al., 1999; Jones et al., 2002; Peartree et al., 2012). Birds that are not able to physically interact with their conspecifics may behave differently from those that have the opportunity to do so. Considering the theoretical framework presented, a situation where some birds can freely ambulate according to their needs, gain access to certain environmental areas, and physically interact with conspecifics while other group mates are environmentally confined, would be useful to expand the testing options for social interaction studies. A device called the individual physical barrier (IPB) was developed in our laboratory and works in

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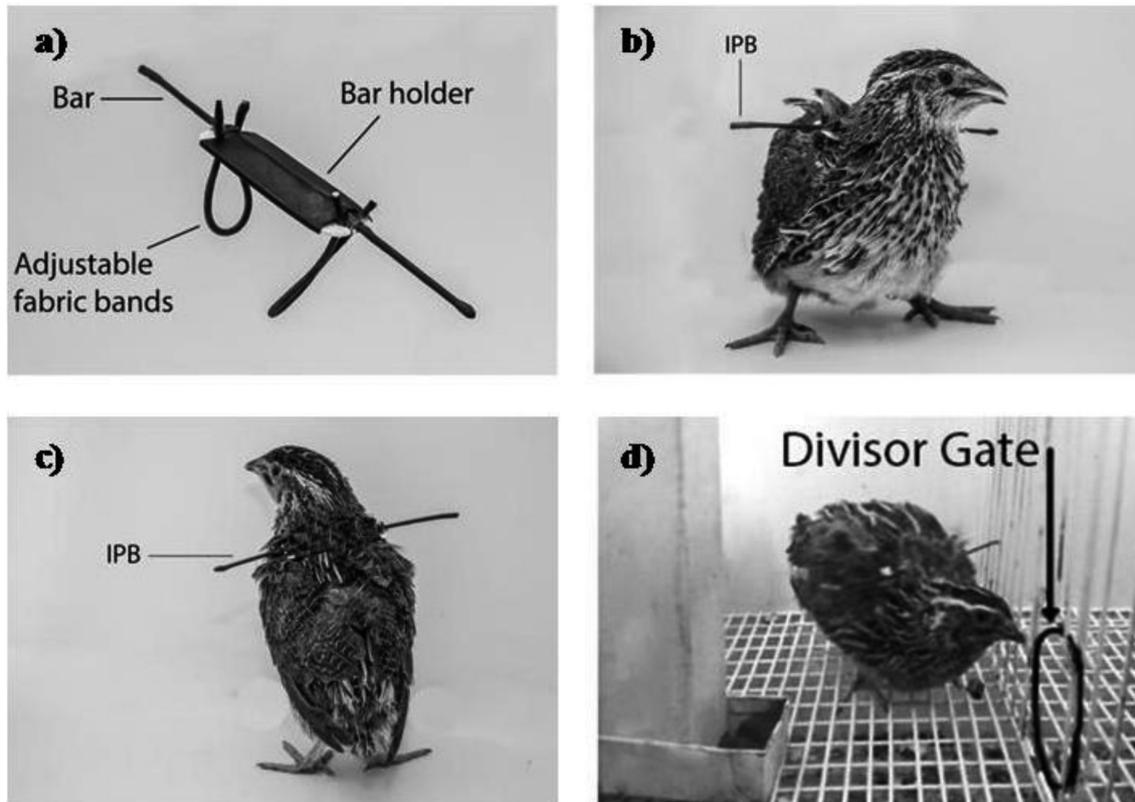


Figure 1. a) Individual physical barrier device (IPB); b) IPB fitted on a quail (front view); c) IPB fitted on quail (back view); d) A circular shaped gate located at least 3 cm apart from either side of the environmental divisor is used to preclude IPB birds' passage.

combination with an environmental divisor containing a gate (Figure 1d). Thus, this device allows delimiting the ambulation area of the IPB fitted birds within a larger experimental box area. The IPB has been tested with males and females interacting and passing through the divisor gates for 7 d showing a 94% of efficacy (remaining positioned on the quail's back and therefore delimiting their ambulation areas) (Quinteros, 2014).

The IPB device appears to be a useful tool to expand the testing options for social interaction studies. However, before encouraging their use, we must prove that the fitting of birds with the IPB device still allows common natural movements, and does not directly or indirectly affect the expression of behaviors. For example, the IPB on the bird's back could be sensed by the birds that carry them, probably inducing some physical discomfort and an anxiety/fear like state that however, with time, would be expected to wane. It is also possible that the IPB could affect individuals' discriminations affecting affiliative interactions. Therefore, the aim of this study was to assess whether the IPB may alter adult Japanese quail main behaviors. We used 4 classical test situations that cover a wide range of behavioral responses: Open-field, Runway, Time Budget in Home Box and Sexual Interactions. Open-field has been commonly used to assess fear and anxiety reactions in a novel environment (Gallup and Suarez, 1980; Faure et al., 1983; Jones, 1996; Kembro et al., 2008). Runway tests have been widely used to study social reinstatement responses;

these are considered to be indicative of underlying sociality in birds as well as their ability to make social discriminations and establish social interactions (Suarez and Gallup Jr., 1983; Mills et al., 1995; Carmichael et al., 1998; Jones et al., 1999; Marin et al., 2001; Vaisanen and Jensen, 2004; Guzman and Marin, 2008). Because the use of the IPB can affect general birds' activity and/or locomotor ability, 10 selected behaviors while in home boxes were also evaluated (Schmid and Wechsler, 1997; Alvino et al., 2009). Finally, because the positioning of the IPB on the birds' body could interfere in social interactions requiring direct physical contact, main behaviors involved in a mating encounter were also determined (Marin and Satterlee, 2003; Labaque et al., 2008; Correa et al., 2011).

MATERIALS AND METHODS

Animals and Husbandry

Adult Japanese quail were used in the current study. Briefly, after hatching, 360 birds were placed in wooden boxes (90 cm long × 90 cm wide × 60 cm high) until 4 wk of age. Each box had 2 feeders covering the front part and 16 automatic nipple drinkers (8 on each side). A wire-mesh floor (1 cm grid) was raised 5 cm to allow the passage of excreta and a lid prevented the birds from escaping. Brooding temperature was 37.5°C during the first week of life, with a weekly decline of 3.0°C until room temperature (24 to 27°C) was achieved. At

4 wk of age, birds were sexed by plumage coloration, and wing-banded to ensure further identification. Then, 320 quail were randomly housed in pairs (1 female and 1 male) in cage batteries each measuring 45 cm long \times 20 cm wide \times 25 cm high. At this time, birds were switched to a laying feed (21.5% crude protein and 2,750 kcal ME/kg), with plain water provision ad libitum. Quail were subjected to a daily cycle of 14L:10D (240 to 280 lux) during the study, with light on at 0600 h. Temperature was kept at $25 \pm 2^\circ\text{C}$ during the rest of the study. Daily maintenance and feeding chores were performed at the same time each day (1600 h).

All experiments were carried out in accordance with local Argentinean laws and following the National Institute of Health Guide for the care and use of laboratory animals (NIH Publications No. 13, revised 2011).

Individual Physical Barrier (IPB) and Test Groups

The IPB device (Figure 1a) consisted of a small 12 cm long and 1 mm diameter metal bar attached to a plastic harness that was fitted on the bird's back (perpendicular to the sagittal plane and exceeding body width) and held in place with 2 adjustable elastic fabric bands around the base of the wings (Figure 1b,c). Following Bernardo et al. (2011) recommendations, the IPB weighed about 3.2 g ($<2\%$ of the birds' body weight). Between 45 and 84 d of age, depending on the randomly assigned test situation, the quail pair within each cage were either fitted with an IPB or remained with no IPB and were used as controls (CON). To avoid potential confounding effects due to manipulation, control birds have been handled in a similar way as the IPB birds. There were 4 test groups (Female-CON, Female-IPB, Male-CON, and Male-IPB) for open-field, runway and home-box testing. For runways, these 4 test groups were tested when the goal box contained either stimulus quail with no IPB, or with IPB (Stimulus-CON vs. Stimulus-IPB). Four pair combination groups (Female-CON and Male-CON, Female-IPB and Male-CON, Female-CON and Male-IPB, and Female-IPB and Male-IPB) were used for mating behavior testing.

Open Field Test

Open-field was first studied to evaluate short (1 h) and mediate (7 d) IPB effects. At 45 d of age, 32 quail (16 females and 16 males) were tested in an open-field apparatus one hour after half of them (8 females and 8 males) received an IPB. The other half remained with no IPB and were used as controls. Each bird was tested individually and once only, and its behavior was video recorded. Testing was conducted between 0800 and 1600 h and the order of individuals was randomized over the 4 treatment groups during the testing day. Similarly, a different set of 32 quail were also tested in an open-field 7 d after half of them received an IPB

(52 d of age). We did not test the same quail twice in the open-field to avoid potential confounding effects of habituation to the IPB with habituation to the test apparatus.

A white wooden box measuring $60 \times 60 \times 60$ cm (width \times length \times height) was used following the procedure described by Kembro et al. (2008). Briefly, to begin a test, each bird was placed near the midpoint of the open-field floor, and its behavior was recorded on videotape for 10 min by using a closed-circuit television system with a video camera suspended approximately 1.8 m directly above the open-field. This arrangement made certain that the experimenter was completely hidden from the bird's view during testing. We used Any-maze (ANY-maze, 2009) to analyze the ambulation of the birds in the open-field apparatus at 0.5 s intervals. The following variables were registered: (1) latency to ambulate (s): time from the start of the test until the animal showed its first ambulatory event (first time interval that showed more than 1 cm of distance ambulated); (2) time spent ambulating (%); (3) distance ambulated (cm): the total (cumulative) distance ambulated by the animal during the test period.

After preliminary open-field analysis, no differences were detected in open-field behaviors after 7 d of the IPB fitting. Thus, runway, home box, and mating behaviors were all evaluated with birds that were allowed 7 d to habituate to the IPB.

Runway Test

At 53 d of age, 32 females and 32 males were tested individually and once only in a runway apparatus where the goal box contained 2 age-matched conspecifics. Because different characteristics of conspecifics in the goal box can clearly influence behavior of the experimental birds (Vallortigara, 1992; Guzman and Marin, 2008), experimental birds were evaluated when the goal box contained either a pair (female and male) fitted with an IPB, or a pair with no IPB (Stimulus-CON vs. Stimulus-IPB, respectively). Half of the quail received the IPB 7 d before runway testing and the other half remained with no IPB and were used as controls. Testing was conducted between 0800 and 1600 h and the order of individuals was randomized over the 4 treatment groups during the testing day.

The runway apparatus and main procedures are fully described elsewhere (Guzman and Marin, 2008). Briefly, we used a runway measuring $160 \times 40 \times 40$ cm (length \times width \times height), divided into three compartments by removable wire-mesh partitions. The compartments situated at the opposite ends were each 20 cm long and comprised the start box and the goal box, respectively. A female and a male stimulus birds were placed in the goal box and allowed to acclimatize before testing began. The assignment of the CON or IPB pair of conspecifics to the goal box was maintained along four consecutive tests and then swapped. Thus, in

total, 16 pairs of quail were used as stimulus during runway testing. At test, a quail was placed in the start box and allowed 1-min to acclimatize; it could see the stimulus birds at this time. The door was then raised and during 5-min the following measures were taken: (1) latency to emerge (s): time elapsed from beginning of the test till the quail leave the start box; (2) latency to enter close zone (s): time from the beginning of the test till the quail enters a 12 cm “close” zone (**CZ**) nearest to the goal box; (3) total distance ambulated (cm): the total (cumulative) distance ambulated by the animal during the test period; (4) distance in CZ (cm): the total distance ambulated by the animal in the CZ; (5) time in CZ (s): accumulated time spent near conspecifics in goal box during the 5-min test period; (6) entries in CZ (N°): total number of entries registered to the CZ.

Behavioral Time Budget in a Home Box

At 70 d of age, 24 quail pairs were re-housed in white wooden boxes measuring 45 × 40 × 40 cm (length × width × height) with feed and water provision continuing ad libitum. These home boxes contained a 15 × 15 cm nest plastic area. Birds were given 72 h to acclimate to this “new” home box ($n = 24$ boxes). Seven days prior home cage testing, half of the pairs received an IPB and the other half remained with no IPB. Tests were conducted between 0800 and 1600 h.

Using a closed-circuit system with a video camera suspended approximately 1.8 m directly above the home boxes and for the 8 testing hours, the behavior of each pair was video-recorded every hour during 5 min sessions. There were 10 defined behaviors that were registered: walking, stand up, preening, feeding, drinking, resting in a nest area, resting out of the nest area, pecking at the environment, pecking at the ground, and mating. The percentage of time that each individual was showing each of the 10 selected behaviors was calculated at intervals of 1 s following a scan sampling technique (Schmid and Wechsler, 1997; Marin and Satterlee, 2003).

Mating Behaviors

At 77 d of age, 48 females and 48 males were re-housed individually for one week before being tested for IPB potential interference on mating behaviors. At the same time of re-housing, half of quail received an IPB and the other half remained with no IPB and were used as controls. One wk later, mating behaviors were video recorded during 5 min intervals while males remained resident in their home-cages and received a visit from a non-familiar female. Test order was randomized over the 4 mentioned pair combinations during the testing day. Tests were conducted between 0800 and 1600 h.

The mating sequence consists on a male grabbing the feathers of a female’s head or neck, mounting, and

reaching back with the wings spread. Initiating mating is defined as head grabbing, and head grab latency is the time from the beginning of the mating opportunity to the first head grab. Cloacal contact events are defined as the number of time intervals that a male positions its cloaca in close proximity to the cloaca of a female partner. Completing mating was defined as cloacal contact followed immediately by cessation of the mating attempt (Adkins-Regan, 2014). It is important to note that not all mating attempts result in a completed mating. We registered: latency to the first grab (s); number of grabs, mounts, cloacal contact events; and completed matings.

Statistical Analyses

Open-field and home-box data were evaluated using a 2-way ANOVA that assessed the effects of IPB (CON and IPB), gender (female and male) as well as their interaction. Runway data were evaluated using 3-way ANOVAs that assessed the effects of IPB fitted on the experimental birds (CON and IPB), gender (female and male), and goal box stimulus birds IPB (Stimulus-CON vs. Stimulus-IPB), as well as their interactions. Mating behaviors were evaluated also using 2-way ANOVAs that assessed the effects of females with or without IPB (Female-CON and Female-IPB), males with or without IPB (Male-CON and Male-IPB), and their interaction. In all cases ANOVA assumptions were verified. If significant effects were detected, Fisher LSD test was used for pairwise comparisons. Data on latency to ambulate (open-field test), latency to enter close zone (runway test), and latency to first grab (in mating behavior test) were evaluated using Kruskal Wallis non parametric analysis (Di Rienzo et al., 2014). A P -value of less than or equal to 0.05 was considered to represent significant differences.

RESULTS

Open-field results are summarized in Table 1. Analysis showed that one hour after IPB was fitted on the birds’ back, IPB quail reduced the percentage of time spent ambulating ($P = 0.02$) and the distance ambulated ($P = 0.01$) in comparison to their control IPB free counterparts. No particular gender effects, and no interaction between IPB treatment and gender were detected. No main effects were detected on the latency to ambulate.

No effects on open-field behaviors were detected 7 d after the IPB was fitted on the birds (Table 1).

Variables measured in runway, time budget in home box, and mating behaviors were all registered 7 d after quail were fitted with the IPB. No IPB effects or interactions between IPB and gender were detected in any of variables registered. Runway, time budget in home box, and mating behaviors results are summarized in Tables 2, 3, and 4.

Table 1. Open-field behavioral responses (mean \pm SEM) one h or 7 d after adult Japanese quail were fitted with an individual physical barrier device (IPB). CON = Controls with no IPB.

One h after IPB fitting				
Behavioral measure	Treatment			
	Female-CON	Female-IPB	Male-CON	Male-IPB
Latency to ambulate (s)	34.1 \pm 17.8	5.8 \pm 2.3	6.4 \pm 2.8	30.5 \pm 12.9
Time spent ambulating (%)	27.2 \pm 6.5 ^a	12.39 \pm 3.5 ^b	20.84 \pm 4.6 ^a	11.32 \pm 6.1 ^b
Distance ambulated (cm)	19.2 \pm 4.9 ^a	6.73 \pm 1.9 ^b	12.96 \pm 3.7 ^a	6.13 \pm 3.2 ^b
Seven d after IPB fitting				
Behavioral measure	Treatment			
	Female-CON	Female-IPB	Male-CON	Male-IPB
Latency to ambulate (s)	5.4 \pm 1.7	5.6 \pm 2.9	11.9 \pm 4.8	6.79 \pm 3.2
Time spent ambulating (%)	25.6 \pm 5.1	16.4 \pm 5.4	21.8 \pm 5.3	34.05 \pm 9.5
Distance ambulated (cm)	14.5 \pm 3.6	6.7 \pm 1.8	12.4 \pm 2.7	16.93 \pm 5.1

^{a,b}Groups with no common letters differ significantly ($P < 0.05$).

Table 2. Runway responses (mean \pm SEM) 7 d after adult Japanese quail were fitted with an individual physical barrier device (IPB). CON = Controls with no IPB.

Behavioral measure	Stimulus-CON				Stimulus-IPB			
	Female-CON	Female-IPB	Male-CON	Male-IPB	Female-CON	Female-IPB	Male-CON	Male-IPB
Latency to emerge (s)	41.0 \pm 31.1	56.3 \pm 18.1	100.6 \pm 40.8	46.9 \pm 22.5	49.2 \pm 25.4	40.6 \pm 16.7	60.0 \pm 17.7	57.1 \pm 21.2
Latency to enter close zone (s)	50.2 \pm 39.5	10.0 \pm 5.8	11.6 \pm 7.5	20.9 \pm 12.8	13.3 \pm 10.9	9.8 \pm 5.7	40.0 \pm 29.8	65.5 \pm 35.6
Total distance ambulated (m)	1.1 \pm 0.7	1.3 \pm 1.0	1.4 \pm 0.6	2.0 \pm 1.0	1.0 \pm 0.4	1.1 \pm 0.4	1.2 \pm 0.7	1.2 \pm 0.2
Distance in close zone (m)	0.7 \pm 0.3	0.6 \pm 0.3	0.3 \pm 0.1	0.7 \pm 0.2	0.6 \pm 0.2	0.5 \pm 0.2	0.4 \pm 0.1	0.5 \pm 0.2
Time in CZ (s)	191.4 \pm 48.4	257.4 \pm 16.5	247.0 \pm 18.6	199.7 \pm 27.5	230.3 \pm 87.1	259.4 \pm 98.0	226.9 \pm 80.2	211.4 \pm 74.7
Entries in CZ (N°)	5.7 \pm 1.7	7.3 \pm 2.1	2.98 \pm 0.7	7.7 \pm 2.3	5.9 \pm 1.3	3.8 \pm 1.0	4.0 \pm 0.4	5.2 \pm 1.7

Table 3. Percentage of time (mean \pm SEM) that each individual was showing each of the 10 selected behaviors in a home box 7 d after adult Japanese quail were fitted with an individual physical barrier device (IPB). CON = Controls with no IPB.

Behavioral measure	Treatment			
	Female-CON	Female-IPB	Male-CON	Male-IPB
Walking	25.5 \pm 3.1	23.1 \pm 3.2	26.2 \pm 2.9	24.9 \pm 3.4
Being alert	0.75 \pm 0.3	0.3 \pm 0.2	1.58 \pm 0.8	2.4 \pm 1.2
Preening	5.7 \pm 1.6	6.7 \pm 1.1	7.2 \pm 1.5	9.5 \pm 0.8
Feeding	12.8 \pm 2.9	7.7 \pm 1.5	10.0 \pm 2.4	6.4 \pm 1.5
Drinking	5.0 \pm 1.0	5.9 \pm 1.5	2.2 \pm 0.5	3.0 \pm 0.6
Resting in the nest area	15.3 \pm 2.5	17.4 \pm 5.0	15.2 \pm 3.3	17.2 \pm 3.9
Mating	0.08 \pm 0.1	0.01 \pm 0.0	0.08 \pm 0.1	0.10 \pm 0.1
Resting out of the nest area	23.5 \pm 3.6	30.6 \pm 4.6	27.0 \pm 2.5	27.4 \pm 2.7
Pecking at the ground	4.1 \pm 0.9	2.3 \pm 0.4	2.8 \pm 0.7	2.1 \pm 0.5
Pecking at the environment	1.9 \pm 0.7	1.9 \pm 0.8	2.5 \pm 0.6	1.5 \pm 0.5

DISCUSSION

In the present study, female and male quail that were fitted with an IPB one hour before open-field test showed a reduced percentage of time spent ambulating and also a reduced distance ambulated in comparison to their IPB free counterparts. The overall increased IPB immobility during open-field testing suggest that the novel device induced an increased anxiety/fearfulness state in the birds that were recently fitted with it on their backs. The increased fear state could be explained by the novelty component of the recently fitted unknown device and/or by an increased discomfort induced by the attachment of the harness around

their wings. However, it should also be expected that with time, the novel component of the IPB wane down and/or the potentially induced discomfort disappears (if it is not irritating the wings' base).

When open-field testing was performed in non open-field experienced female and male quail 7 d after fitting them with an IPB, no differences were found in the 3 ambulation variables registered. Interestingly, after 7 d of IPB placement on the quail, no effects were detected either during runway, time budget in home box, nor mating behaviors. These findings suggest that an habituation process to the IPB has taken place sometime between 1 h and 7 d after its placement on the quail. The term habituation usually implies that the individual

Table 4. Mating behaviors (mean \pm SEM) in adult Japanese quail 7 d after they were fitted with an individual physical barrier device (IPB). CON = Controls with no IPB.

Behavioral measure	Treatment			
	Female-CON Male-CON	Female-IPB Male-CON	Female-CON Male-IPB	Female-IPB Male-IPB
Latency to first grab (s)	5.33 \pm 1.7	7.58 \pm 2.9	6.50 \pm 1.5	6.08 \pm 1.3
Grabs (N $^\circ$)	2.58 \pm 0.6	2.92 \pm 0.8	2.92 \pm 0.9	2.67 \pm 0.6
Mounts (N $^\circ$)	2.42 \pm 0.5	2.67 \pm 0.7	3.17 \pm 0.9	2.42 \pm 0.6
Cloacal contact events (N $^\circ$)	2.08 \pm 0.5	1.75 \pm 0.5	2.17 \pm 0.6	2.00 \pm 0.4
Completed matings (N $^\circ$)	0.17 \pm 0.1	0.17 \pm 0.1	0.33 \pm 0.1	0.13 \pm 0.1

became familiar with the stimulus and thus no longer perceived that stimulus as stressful or a threat. However, habituation can also be used to describe a situation where an individual learns to ignore innocuous stimuli (Thompson and Spencer, 1966; Cyr and Romero, 2009). Whether the IPB quail stop perceiving the device as a potential threat or it become just an ignored innocuous stimuli we do not know, nevertheless, regardless of the underlying physiological and/or psychological mechanism, it is clear that in one week or less, birds appear behaviorally habituated to the use of the IPB.

As mentioned, no differences in runway responses were found in the experimental groups with or without an IPB. Moreover, no differences were detected either when those groups were tested in a runway containing in the goal box a quail pair fitted with an IPB, or a control quail with no IPB. Thus, taken together, runway findings suggest that IPB did not affect either underlying social motivation or discriminations between birds as conspecifics.

In this study, the time budget of behaviors was not influenced by the use of the IPB, suggesting that the device does not affect adult female or male quail behavioral repertoires along the day. Just a few behaviors were found different between females and males, however, those differences were not related to the IPB and are explained by natural gender differences related to some behavioral responses (Mills et al., 1997).

The use of the device could have interfered with the mating display due to a direct physical impediment or through affecting recognition or preference for a social partner. However, the results of our study clearly suggest that, at least 7 d after the quail have been fitted with the device, the use of the IPB do not affect any of the components of adult quail mating sequence.

Several studies have evaluated the use of devices such as harnesses or radio collars in different vertebrate species with no reported concerns about those elements affecting behaviors (Nussberger and Ingold, 2006; Golabek et al., 2008; Daigle et al., 2012; Hobbs-Chell et al., 2012). Thus our results, showing no signs of affecting any of the behavioral variables measured in the 4 tests performed after 7 d of IPB placement, are in line with those studies. Taken together, the findings suggest that IPB does not induce fear or anxiety, does not limit the ambulation or affects mating behaviors. Finally, it

is important to note that after all the experiments were conducted, a direct visual inspection was performed on all birds and no injuries or deaths induced by the IPB device were observed. Findings suggest that giving the birds some days to habituate, the device can be used in behavioral studies without expecting major interferences.

The IPB device appears useful for laboratory studies to improve knowledge on diverse aspects of the biology of birds. For example, their use would be adequate to assess social interactions where IPB fitted birds are confined to certain areas while their IPB free counterparts can freely ambulate, get access and potentially interact with them. It could also be useful for studies of sexual selection (choice of potential pairs) and aggressive interaction, among others. IPB applications could be expanded if a simile-quail IPB device is also found suitable for other poultry species.

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