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# A ‘pebble test of anxiety’ did not differentiate between Japanese quail divergently selected for stress and fear

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**Abstract**

It has been suggested that the time taken by an individually tested domestic chick to begin pecking at pebbles on the floor of a novel arena might be used as a test of fear and anxiety, with low latencies to peck indicating low fear and vice versa, and as a potential selection criterion ‘to choose fowls with the best performance later in life’ [Appl. Anim. Behav. Sci. 73 (2001) 102]. The present study tested the above hypotheses by comparing the responses of 1-day-old Japanese quail chicks from genetic lines known to show high (HS) or low (LS) levels of fearfulness when they were exposed individually to a similar test situation. Since social separation is a stressful event the quail were housed either individually (IND) or in groups (SOC) before test to establish whether the prior social environment influenced behaviour in the pebble test. The LS chicks walked sooner and more than HS ones but there were no line effects on pecking at the pebbles. Chicks that had been housed individually walked and pecked at the pebbles sooner than did those that were housed in a group prior to test, indicating that sudden isolation elicited greater fear in SOC than in IND quail. The higher levels of activity then shown by SOC than IND quail probably reflected greater social reinstatement motivation in socially housed birds. Although the inconsistency between the present results and those of Salvatierra and Arce [Appl. Anim. Behav. Sci. 73 (2001) 102] might simply reflect species differences, our findings sound a cautionary note and point to the need for further study before a pebble test could be confidently used to assess fearfulness.


*Keywords:* Anxiety; Fearfulness; Japanese quail; Pebble test; Pecking

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## 1. Introduction

Fearfulness can be defined as a predisposition to be easily frightened by exposure to real, immediate and recognisable events (Jones, 1987a, 1996; Boissy, 1995) whereas anxiety is considered to be a diffuse state of apprehension stemming from the anticipation of an unreal or imagined threat (Rowan, 1988; Toubas et al., 1990). Elevated levels of fearfulness and anxiety can seriously damage poultry welfare, productivity and product quality (Mills and Faure, 1990; Jones, 1996; Faure et al., 2003). For example, the expression of inappropriate fear reactions, like panic, can cause injury, pain or death, with the associated economic losses. Inescapable or prolonged fear and anxiety can lead to chronic distress that, in turn, adversely affects immunocompetence and general health, growth, food conversion efficiency, egg production, and egg and meat quality (Jones, 1996). The reduction of harmful states like, fear, anxiety and distress would greatly benefit the birds, the farmers and the consumers. The application of remedial strategies based on environmental enrichment (increased complexity and stimulation) and the incorporation of ‘desirable’ resources, such as nest boxes, dust baths and perches can help in this respect (Jones, 2002, 2004). However, selective breeding against these undesirable traits is also likely to yield rapid and robust improvements (Jones and Hocking, 1999; Faure et al., 2003).

Encouragingly, many of the behavioural traits apparent in poultry are sensitive to genetic selection (Siegel, 1993; Jones and Hocking, 1999). In this context, the identification and validation of reliable selection tests and measures are essential first steps in breeding programs intended to promote welfare-friendly characteristics and/or to eliminate harmful ones. Ideally, the selection tests should be non-invasive, inexpensive, rapid and suitable for carrying out on young chicks (Marin et al., 2003). Divergent selection of Japanese quail (a suitable and affordable model for the domestic fowl (Aggrey and Cheng, 1994; Faure et al., 2003)), for contrasting levels of fearfulness or stress responsiveness has already yielded valuable information. For example, quail selected for short rather than long durations of tonic immobility (TI) fear reactions (Mills and Faure, 1991) or for reduced (LS, low stress) rather than exaggerated (HS, high stress) adrenocortical responses to brief mechanical restraint (Satterlee and Johnson, 1988) show reduced fear responses to a wide variety of alarming events, including manual restraint, an approaching human being, and exposure to an unsheltered area or to novel places and objects (Jones et al., 1991, 1999; Jones and Satterlee, 1996; Faure and Mills, 1998; Faure et al., 2003).

It was recently proposed that the latencies for individually tested, 1-day-old domestic chicks to begin pecking at pebbles on the floor of a novel arena might be used to categorise them in terms of fear and anxiety (Salvatierra and Arce, 2001). Chicks categorised as showing low (LL) rather than high (HL) latencies to peck were thought to be less anxious or fearful because they subsequently showed shorter TI responses and less sensitivity to anxiolytic doses of diazepam in open field and TI tests. Salvatierra and Arce (2001) then suggested that the latency to peck “could be used as a test for fear and/or anxiety state, and useful to choose fowls with the best performance later in life”. This test satisfies many of the requirements of suitable selection measurements outlined above. It was exposed to rigorous examination in the present study by comparing the pecking (and other) responses of 1-day-old chicks from genetic lines of Japanese quail that have been selected for high or low stress responsiveness and that are known to show high and low levels of underlying fearfulness, respectively (Jones and Satterlee, 1996; Jones and Hocking, 1999; Faure et al., 2003). The sudden interruption of social contact is a frightening and stressful procedure for most members of gregarious species, including chickens and quail (Jones, 1987a; Jones et al., 1991, 1992). Whereas all the domestic chicks tested by Salvatierra and Arce (2001) were individually housed prior to test, the present study used quail housed either individually or in groups in order to determine whether potential line differences in the chicks' responses in the pebble test were sensitive to their prior social environment.

## 2. Materials and methods

### 2.1. Animals and their husbandry

Mixed-sex Japanese quail (*Coturnix japonica*) from the LS and HS lines selected by Satterlee and Johnson (1988) for low and high plasma corticosterone response to mechanical restraint were used. Examination of generations 13–24 (see Satterlee et al., 2000) confirmed maintenance of divergent adrenocortical responsiveness to the selection stressor (mechanical restraint). The lines then remained closed to outside blood and were maintained without selection pressure for $G_{25}$ and $G_{26}$. Selection pressure was re-imposed to produce G27 wherein mean (+S.E.) plasma corticosterone responses to restraint were $14.7 \pm 0.3$ and $6.3 \pm 0.2$ ng/ml in the HS and LS lines, respectively. The lines were reproduced for two more generations without selection until their use in the present study ($G_{29}$). The selection history is described in greater detail elsewhere (Satterlee et al., 2002).

Forty-eight newly hatched quail (24 birds per line) were housed individually (IND) and in visual isolation from each other in cardboard boxes measuring 18 cm × 20 cm × 20 cm. A further 96 birds were housed in two groups of 48 (24 chicks from each line) in each of two brooders measuring 102 cm × 64 cm × 20 cm (SOC). All the birds were housed in a quiet 300 cm × 400 cm room under dim red light (approximately 10 lx) and at an ambient temperature of $37.8 \pm 1.0\,^{\circ}$C. No food was provided until completion of the present experiment approximately 24 h later but water was freely available from bell drinkers. All the chicks were transferred to stock cages containing ad libitum food and water as soon as they had been tested (see below).

### 2.2. Tests and statistical analyses

At 1 day of age 96 chicks (the 48 individually caged birds as well as 24 LS and 24 HS quail from the group cages) were tested individually in an unfamiliar cage (18 cm × 20 cm × 20 cm) containing inedible 'aquarium' pebbles that were glued to the floor. Thus, there were four treatment groups: LS-IND, LS-SOC, HS-IND and HS-SOC. In order to approximate the conditions used by Salvatierra and Arce (2001), the pebbles covered the floor of the test cage, they were 2–4 mm in diameter, the shapes varied and the colours included beige, sand, yellow and white. A 60 W bulb was suspended directly over each test cage thereby affording a light intensity of approximately 100 lx at chick head height. Four chicks, representing all four treatments, were tested simultaneously (one in each of the four test cages), and their behavioural responses during the 3 min observation period were recorded onto videotape

Table 1
Behavioural responses of 1-day-old quail chicks of low and high stress lines that had been reared individually or in groups and tested individually in a novel environment containing pebbles on the floor (mean ± S.E.)

| | LS | | HS | | *P*-values | | |
|---|---|---|---|---|---|---|---|
| | IND | SOC | IND | SOC | Line (L) | Housing (H) | L × H |
| Lat. peck (s) | 54.79 ± 11.32 | 96.16 ± 15.49 | 61.12 ± 12.84 | 83.70 ± 16.32 | 0.829 | 0.026 | 0.508 |
| Pecks (no.) | 24.95 ± 4.45 | 5.91 ± 1.31 | 20.83 ± 4.04 | 11.91 ± 3.22 | 0.787 | 0.001 | 0.148 |
| Lat. ambulate (s) | 9.62 ± 3.24 | 31.33 ± 8.81 | 23.87 ± 3.57 | 47.54 ± 11.05 | 0.044 | 0.003 | 0.896 |
| Sections entered (no.) | 28.29 ± 3.74 | 58.95 ± 9.06 | 18.70 ± 3.00 | 24.58 ± 4.71 | 0.001 | 0.002 | 0.035 |

Lat. peck: latency to peck pebbles; pecks: numbers of pecks at pebbles; Lat. ambulate: latency to ambulate; sections entered: number of delineated areas of the test cage entered; s: seconds; no: number.

using cameras situated directly above each of the four test cages. Treatments were rotated across the four test cages from trial to trial to minimise any effects of location. Upon subsequent analysis of the videotapes an acetate sheet subdivided into 12 areas with indelible ink was placed over the monitor; this effectively delineated the test cage into 12 equal-sized (6 cm × 5 cm) sections. The following behaviours were then measured during the 3 min observation period: the latencies to peck at the pebbles and to ambulate and the numbers of pecks at pebbles and of sections entered. A chick was deemed to have entered a new square if it moved the major part of its body into that delineated area.

The untransformed data were analysed using a two-way analysis of variance with a 2 × 2 factorial arrangement of treatments. The factorial was made on the effects of line (LS versus HS), rearing condition (IND versus SOC) and their interaction.

## 3. Results

Quail chicks of the LS line ambulated sooner and entered more sections of the unfamiliar test cage than did the HS ones but there were no detectable line effects on the latency to peck at the pebbles or on the numbers of pecks that were directed at them (Table 1). Rearing condition was found to be markedly influential on all the measures. Thus, chicks that had been housed individually prior to test pecked at the pebbles sooner and more often than did those that had been caged in groups. The IND chicks also began to walk sooner than did the SOC ones, although the latter birds entered more sections of the test cage once they had begun ambulating than did the IND quail (Table 1). A significant effect of line × rearing interaction on the number of sections entered may have reflected the fact that rearing effects were more pronounced in LS than HS chicks.

## 4. Discussion

It has been proposed that 1-day-old domestic chicks that pecked at pebbles on the floor of an unfamiliar test arena soon after their introduction were less fearful (or anxious) than their slower counterparts because they subsequently showed shorter TI fear responses and less sensitivity to an anxiolytic agent (Salvatierra and Arce, 2001). These findings were taken to imply that the latency to peck at pebbles in this test situation might be a useful selection criterion for future breeding programmes intended to reduce fearfulness and/or anxiety in poultry. However, using a similar test situation, the present study revealed no line differences in the pecking responses of chicks from two well-established genetic lines of Japanese quail that are known to show contrasting levels of fear behaviour in a variety of intuitively alarming situations (Jones and Satterlee, 1996; Jones et al., 1992, 1999; Faure et al., 2003). The absence of line differences in the present study does not support the existence of a positive relationship between underlying fearfulness and the latency to peck at pebbles in a novel test arena.

It is conceivable that the apparent inconsistency between the present findings and those reported by Salvatierra and Arce (2001) may have simply reflected species differences (domestic chicks versus quail). On the other hand, there is generally good agreement between the responses of Japanese quail and domestic chicks in recognised tests of fear as well as in their reactions to known fear-inducing and fear-reducing stimuli (Jones, 1996; Mills and Faure, 1992; Faure et al., 2003). Regardless of the reason(s) underpinning the contrasting results, our findings inject a note of caution and identify the need for further investigation before the pebble test could be confidently used as an indicator of underlying fearfulness.

The quail chicks that had been group-housed prior to test took longer to ambulate and to peck at pebbles and showed fewer pecks than did those that had been housed in individual cages. Fear is thought to be positively related to behavioural inhibition (Archer, 1976; Faure et al., 1983; Jones, 1987a, 1996); simplistically, inactive birds are considered to be more frightened than those that ambulate, explore the environment or attempt to escape. Sudden social separation is a particularly stressful and frightening procedure (Wood-Gush, 1971; Mills and Faure, 1986; Jones and Merry, 1988; Vallortigara et al., 1990). Therefore, the SOC quail might be expected to have suffered greater separation distress at tests than the IND birds (Jones and Mills, 1999). Not surprisingly, the present findings suggest that the SOC quail were, indeed, more frightened of the test situation than IND ones. Unlike the line comparison, it might be argued that these findings provide some support for a positive link between fearfulness and pecking at pebbles. However, our finding that the SOC quail entered significantly more sections of the test arena (collective mean of 41.77 representing both LS and HS quail) than did the IND chicks (collective mean of 23.49) once they had started to ambulate probably reflects the higher levels of social reinstatement motivation (Vallortigara et al., 1990; Jones, 1996) in birds that had been socially reared prior to isolation at test. In other words, their activity was directed primarily towards attempts to reinstate contact with their companions; this may, in turn, have overcome any exploratory tendencies, such as pecking at pebbles. Conversely, since social reinstatement motivation should intuitively be less pronounced among birds that had been housed in visual and physical isolation, once fear had waned sufficiently in the IND quail the nature of their ambulation probably reflected cautious investigation of the unfamiliar pebbles as well as the general environment (Salzen, 1979; Jones, 1987a). This would be adaptive in allowing the bird to gain potentially useful information about a novel environment and its resources (pebbles).

Fear levels are thought to be very low in 1-day-old domestic chicks (Bateson, 1964; Salzen, 1979; Andrew and Brennan, 1984) and to thereafter increase via a series of peaks and troughs until high levels are reached at approximately 9 days of age. On the other hand, since fear takes precedence over and inhibits all other behaviours systems (Archer, 1976; Jones, 1996) the significant effects of genetic line (HS, LS) and of housing (social, individual) on the latencies to ambulate and to peck found in the present study suggest that fear is sufficiently well developed at 1 day of age in the Japanese quail to allow the expression of such differences.

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