# Open-Field Temporal Pattern of Ambulation in Japanese Quail Genetically Selected for Contrasting Adrenocortical Responsiveness to Brief Manual Restraint[1]

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**ABSTRACT** Japanese quail selected for a low-stress (LS), rather than a high-stress (HS), plasma corticosterone response to brief restraint have been shown to possess lower fearfulness and a nonspecific reduction in stress responsiveness. Detrended fluctuation analysis provides information on the organization and complexity of temporal patterns of behavior. The present study evaluated the temporal pattern of ambulation of LS and HS quail in an open field that represented a novel environment. Time series of 4,200 data points were collected for each bird by registering the distance ambulated every 0.5 s during a 35-min test period. Consistent with their known reduced fearfulness, the LS quail initiated ambulation significantly sooner ($P <$ 0.02) and tended to ambulate more ($P <$ 0.09) than did their HS counterparts. Detrended fluctuation analyses showed a monofractal series (i.e., a series with similar complexity at different temporal scales) in 72% of the birds. These birds initiated their ambulatory activity in less than 600 s. Among these birds, a lower ($P <$ 0.03) autosimilarity coefficient ($\alpha$) was found in the LS quail than in their HS counterparts ($\alpha$ = 0.76 ± 0.03 and 0.87 ± 0.03, respectively), suggesting a more complex (less regular) ambulatory pattern in the LS quail. However, when the patterns of ambulation were reexamined by considering only the active period of the time series (i.e., after the birds had initiated their ambulatory activity), monofractal patterns were observed in 97% of the birds, and no differences were found between the lines. Collectively, the results suggest that during the active period of open-field testing, during which fear responses are likely less strong and other motivations are the driving forces of ambulation, the LS and HS lines have similar ambulatory organization.

**Key words:** detrended fluctuation analysis, fractal, open-field behavior, corticosterone, Japanese quail

## INTRODUCTION

Stressors are forces external to the body that tend to displace homeostasis and produce a state of stress (Siegel, 1995). Oftentimes, stress is accompanied by increased fearfulness and hyperadrenal activity. When fear or distress persists, animal welfare and production performance can be seriously compromised (Jones, 1996a). For example, energy wastage, feather damage, reduced growth, poor feed conversion, declines in egg production and eggshell quality, injury, pain, and

greater death rates have all been associated with fear and distress in poultry (Mills and Faure, 1990; Jones, 1996a,b, 1997; Jones and Hocking, 1999). Therefore, it has been suggested that genetic selection for a less pronounced fear response and decreased adrenocortical responsiveness may offer a rapid and effective method for reducing fear and distress and thereby enhance animal production performance and well-being (Jones, 1996b; Jones and Hocking, 1999; Jones et al., 2000). Indeed, Japanese quail genetically selected for low-stress (**LS**), rather than high-stress (**HS**), responsiveness based on the plasma corticosterone response to a brief mechanical restraint (Satterlee and Johnson, 1988) show 1) reduced fearfulness to restraint, novel places, objects, and human beings (Jones et al., 1992a,b, 1994a,b, 1999, 2000; Satterlee and Jones, 1995; Jones and Satterlee, 1996; Satterlee and Marin, 2006; Davis et al., 2008); 2) increased sociality (Jones et al., 2002);

3) lower adrenocortical responses to a wide range of stressors (handling, cold, cooping, social tension, human contact, and food and water deprivation; Satterlee and Johnson, 1988; Jones et al., 1992b, 1994b, 2000; Jones, 1996b), 4) less cortical bone porosity (Satterlee and Roberts, 1990); 5) less developmental instability (Satterlee et al., 2000, 2008); 6) less pronounced reductions in BW after exposure to multiple sequential stressors (Satterlee and Johnson, 1985); and 7) accelerated puberty and enhanced reproductive performance in both males (Satterlee et al., 2002, 2006, 2007; Marin and Satterlee, 2004; Satterlee and Marin, 2004) and females (Cadd et al., 2002; Marin et al., 2002b; Marin and Satterlee, 2006; Satterlee and Schmidt, 2008).

The open-field test is a commonly used method to assess fear and anxiety reactions to a novel environment (Jones, 1996b). Gallup and Suarez (1980) have proposed that open-field behavior in poultry represents a compromise between opposing tendencies to reinstate contact with conspecifics and to minimize detection in the face of possible predation. Assuming in gallinaceous birds that open-field behavior parallels the adaptive response to danger, Faure et al. (1983) classified the sequence of behavior patterns into 2 phases. 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 and the consequent expression of socially motivated behaviors (Faure et al., 1983). Second-phase ambulation and jumping behavioral patterns may reflect attempts to reinstate contact with conspecifics (Gallup and Suarez, 1980), which, in turn, may represent the primary motivation for open-field activity.

Some open-field responses of the LS and HS quail lines have already been studied (Jones et al., 1992b; Satterlee and Marin, 2006). Consistent with the hypothesis of reduced fear responsiveness to a novel environment during open-field tests, LS quail showed less freezing behavior and ambulated sooner (Jones et al., 1992b), and showed greater locomotor activity (Satterlee and Marin, 2006) than did their HS counterparts. However, both of these open-field studies included test durations of 10 min or less; therefore, such short durations of testing may have influenced the ambulation responses of the lines, because birds may have been in the first phase of the ambulation patterns suggested by Faure et al. (1983). Therefore, we have pondered herein whether the ambulatory pattern of the different quail stress lines may also diverge once the initial fear response to novelty has wound down. With this in mind, the present study focused on the open-field responses of previously undisturbed LS and HS quail during a longer testing time (35 min). In the data analyses, we also incorporated the use of a novel fractal analysis tool (detrended fluctuation analysis, **DFA**; see below) that allowed us to evaluate temporal behavioral complexity during both the total time of the trial and after the birds began their ambulation, that is, during the second phase of danger-induced adaptive ambulation behavior proposed by Faure et al. (1983).

The use of fractal analyses, such as DFA, has emerged as an effective tool to measure the temporal organizational complexity of a particular behavior (Ho et al., 1997; Peng et al., 2000). Detrended fluctuation analysis has also been proposed as an important and appropriate noninvasive tool to evaluate stress-related responses in poultry (Rutherford et al., 2003; María et al., 2004). Fractal analyses may even reveal hidden information (Peng et al., 2000) about the organization of behavior beyond that extracted by using conventional behavioral analyses, which are often limited to measures of the mean duration or frequency of particular behaviors (Rutherford et al., 2003). For example, Rutherford et al. (2003) observed that, in domestic chickens exposed to an acute stressor (restraint and blood sampling), the complexity of the vigilance behavioral pattern was increased and that it occurred independently of changes in the duration of this behavior, suggesting that DFA can reveal more subtle changes in behavioral organization during stress.

## MATERIALS AND METHODS

### Animals and Housing

Because sex differences in stress susceptibility (Marin et al., 2002a) and in open-field behavior have been reported (Jones, 1977; Jones, 1978; Jones and Faure, 1982), only female Japanese quail from generation 39 of the LS and HS lines of Satterlee and Johnson (1988) were used. The most 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 for 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 400 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 62 birds (31 LS + 31 HS) per compartment in each of 6 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. 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 color-ation, and 12 females from each line were randomly cage-housed in pairs consisting of one LS and one HS female in a 4-tier cage battery that contained 48 lay-ing cages (Alternative Design Manufacturing and Sup-ply Inc., Siloam Springs, AR). Cages measured 50.8 × 15.2 × 26.7 cm (length × width × height). Coincident with placement in their cages, birds were switched to a quail breeder ration (21% CP; 2,750 kcal of ME/kg) and water was continued ad libitum. The birds were also subjected to a daily cycle of 14L (280 to 300 lx):10D at this time, which continued until the end of the study (at 14 wk of age). Once housed in cages, the birds then remained undisturbed, apart from routine mainte-nance, until testing began. One HS bird escaped from its cage shortly after housing. To maintain the same experimental conditions in all tested animals, the HS bird that escaped was not replaced, and her remaining LS bird cagemate was not tested.

## General Procedure

At 32 d of age, open-field behavior (see below) of 11 quail from each of the 2 lines was evaluated. The open-field testing was conducted between 0800 and 1600 h. Each bird was tested individually and once only, and its behavior was videorecorded. The testing order of in-dividuals was randomized.

The open-field apparatus consisted of a wooden box measuring 60 × 90 × 60 cm (width × length × height). The walls of the open field were painted matte white. If defecation occurred during a test, after completion of that test, the floor was wiped clean before reuse for the next test. The test room was maintained at an ambient temperature and light intensity similar to that in the room where the birds' home cages were located.

To begin a test, each bird was placed near the mid-point of the open-field floor, and its behavior was re-corded on videotape for 35 min by using a closed-circuit television system with a video camera suspended ap-proximately 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 digitized pixel images to analyze the ambu-lation of the birds in the open-field apparatus at 0.5-s intervals. In each image the X, Y coordinates of the center of the animal were recorded. The distance am-bulated was defined by the distance the animal 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, and a 0 distance value for ambulation during that interval was recorded. Thus, a time series of the distance am-bulated during the 35-min test period (i.e., 4,200 time intervals) was constructed for each bird. The following variables were also measured:

- *Latency to ambulate* (s): time from the start of the test until a chick showed its first ambulatory event (first time interval that showed more than 1 cm of distance ambulated).
- *Percentage of time spent ambulating*:

$$t_\% = \frac{\Sigma t_i}{T} \times 100 \,,$$

where $t_i$ is the time interval (s) in which the ani-mal is ambulating and $T$ is the total duration of the test (s).
- *Distance ambulated* (cm): the total (cumulative) distance ambulated by the animal during the test period.
- *Ambulation speed* (cm/s): distance ambulated di-vided by the time spent ambulating.
- *Ambulation rate* (cm/s):

$$TA = \frac{D}{T - t_{lat}} \,,$$

where $D$ is the distance ambulated, $T$ is the total duration of the test, and $t_{lat}$ is the latency to am-bulate.

## Data Analyses

***Power Spectrum Analysis.*** To accurately use DFA (see below), a given time series must not present peri-odic oscillations. Thus, the presence of periodic oscil-lations was evaluated by using the power spectrum analysis tool from SigmaPlot (SPSS, 2001).

***DFA.*** A method to analyze the ambulation pattern of the birds was introduced by Peng et al. (1994), and this method has been described in detail by Kantelhardt et al. (2001). We applied this method to 2 time series of data ($x_i$): 1) the complete time series of the distance ambulated (all 4,200 data points), and 2) the time se-ries that corresponded to the second (active) period of open-field ambulation behavior (wherein each test sub-ject's first period of nonambulation was eliminated).

The general mean value of $x$ was calculated for each time series (equation [1]),

$$\langle x \rangle = \frac{1}{N} \sum_{i=1}^{N} x_i, \qquad [1]$$

where $N$ is the total number of data points. Each of the values of $x_i$ was then subtracted from the general mean $\langle x \rangle$, creating the time series, $\{y_i\}$ (equation [2]),

$$y_i = \left( x_i - \langle x \rangle \right). \qquad [2]$$

A cumulative time series ($Y_j$) was then generated by adding all data points found before $y_i$ in the time series (equation [3]),

$$Y_j = \sum_{i=1}^{j} y_i. \qquad [3]$$

This new time series $\{Y_j\}$ was then subdivided into $k$ nonoverlapping windows of equal length. The size of each window was represented by $n$ (number of data points included in each window) and it decreased from $N/4$ to a minimum of 10 data points per window. In each window, a polynomial function was fitted, which was called the local trend of the time series (see the detrending order below). Polynomials of the same degree were selected for all windows. The polynomial estimator of $Y_j$ in the $n$th window ($n = 1, \ldots, k$) was denoted by $P_{jn}$. Detrending occurred by subtracting the local trend in each window from the time series. Thus, the differences $Z_n(j) = Y_j - P_{jn}$ were calculated, where the index $j$ ran inside the selected window $n$.

In the next step, the variance inside each of the $k$ windows was calculated by

$$V_n(\nu) = \frac{1}{n} \sum_{j=1}^{n} \left\{ Z_\nu \left[ (\nu - 1)n + j \right] \right\}^2, \quad (\nu = 1, \ldots, k). \qquad [4]$$

Finally, for each size $n$, the DFA fluctuation function ($F$) was calculated as the square root of the average of the variances by

$$F(n) = \sqrt{\frac{1}{k} \sum_{\nu=1}^{k} V_n(\nu)}. \qquad [5]$$

The estimation of $F(n)$ was repeated for different window sizes, ($n$). Hence, once $F(n)$ at different window sizes was calculated, the fluctuation function [$F(n)$] was plotted against the window size ($n$) on a log-log scale. A linear relationship on this log-log plot indicates a power-law relationship between the fluctuations function and the window size. Thus,

$$F(n) = a\, n^a. \qquad [6]$$

The slope of the linear log-log plot equals the power-law exponent, α, which is the self-similarity parameter. The α-value relates to the autocorrelation structure of the time series. If α = 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 < α < 1 indicates that long-range autocorrelations (correlations decaying as a power-law) exist, meaning that ongoing behavior is influenced by what has occurred in the past (Kantelhardt et al., 2001). In DFA, the exponent α is inversely related to a typical fractal dimension, so in this case, the value increases with increasing regularity (or decreasing complexity) in the time series (Rutherford et al., 2003).

One of the common challenges is that the autocorrelation fit is not always constant, and crossovers often exist (i.e., a change of the scaling exponent, α, for different ranges of scales; Blesić et al., 2003; Ferri et al., 2005). A crossover usually can arise from actual changes in the correlation properties of the signal at different time or space scales (i.e., 2 levels of complexity), or from trends (a smooth and monotonous or slowly oscillating pattern caused by external effects) that were not correctly eliminated by the DFA (Hu et al., 2001; Kantelhardt et al., 2001). Thus, Kantelhardt et al. (2001) reported that trends within time-series data can lead to an artificial crossover in the slope of the log-log plot of $F(n)$ versus $n$; that is, the slope α is increased for large time scales. To determine the order of DFA that would eliminate these trends (and consequently the artificial crossovers), and thus be able to estimate the value of α reliably, DFA was calculated with different detrending orders. For this purpose, linear (DFA1), square (DFA2), cubic (DFA3), and higher order polynomials were used in the fitting procedure. Because the detrending of time-series data is performed by subtracting the fits from the data, these methods differ in their capability of eliminating trends in the data. In the $n$th-order DFA (DFA$n$), trends in the profile of order $n - 1$ are eliminated (Kantelhardt et al., 2001). In other words, the artificial crossover (more than one linear fit) disappears when the detrending order is larger than the order of the trend. If the crossovers did not disappear after using detrending orders of a magnitude up to 5, we concluded that the crossovers were not a consequence of the presence of trends in the data and that the series were not monofractal (see next paragraph). Therefore, after evaluating the fits of all DFA performed with different detrending orders, we selected the lowest detrending order that was able to eliminate trends in all monofractal data series, and we used this order for comparison of the ambulatory pattern of LS and HS lines.

When a pattern is homogeneous—that is, when it has the same scaling properties at different time scales (only one linear fit) and is therefore characterized by only one α-value—the pattern is termed monofractal (Stanley et al., 1999).

### Statistical Analyses

One-way ANOVA was used to determine differences between LS and HS quail in latency to ambulate, time spent ambulating, distance ambulated, ambulation speed, and ambulation rate. In all cases, the ANOVA assumptions were verified, except for the latency to ambulate data, which were transformed to ranks to better fit ANOVA assumptions (Shirley, 1987).
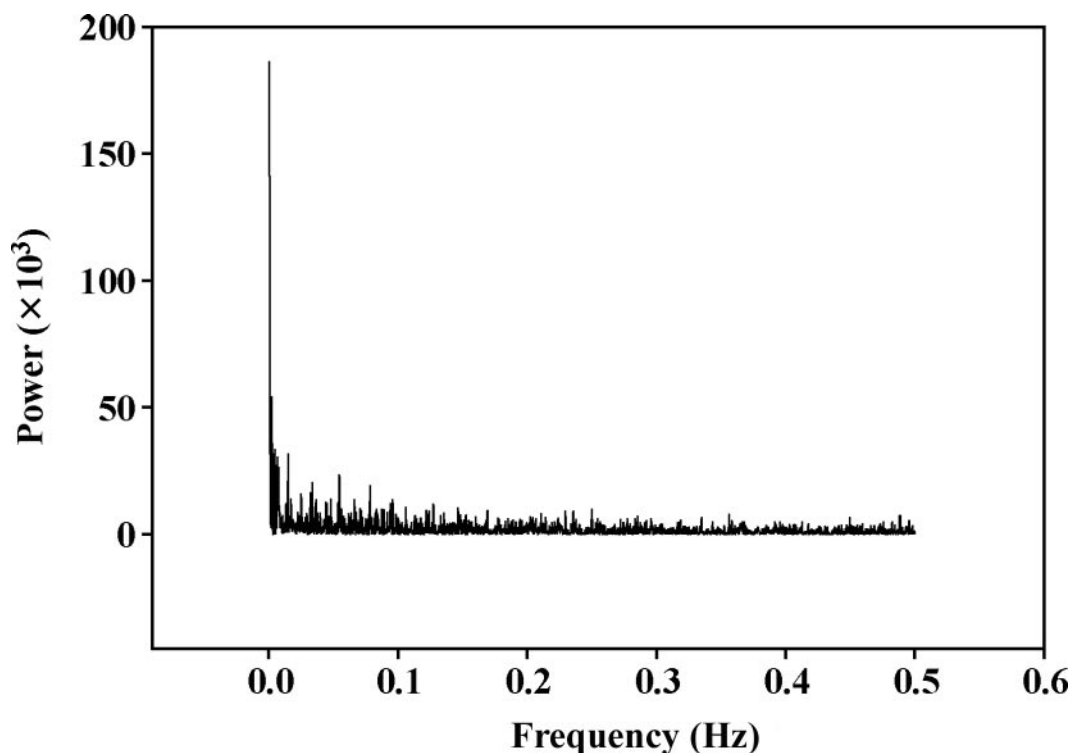
**Figure 1.** Example of a power spectrum analysis of a complete time series of distance ambulated. All individual quail data time series showed similar patterns.

To evaluate line differences in the proportion of monofractal time series, the 2-sample proportion test (Analytical Software, 2000) was used. A $P$-value of less than or equal to 0.05 was considered to represent significant differences.

## RESULTS

The latency to ambulate was significantly longer ($F_{1,20} = 6.66$, $P < 0.02$) and there was a tendency ($F_{1,20} = 3.12$, $P = 0.09$) toward a lower percentage of time spent ambulating in female quail of the HS line compared with those of the LS line (Table 1). No significant differences were found between the stress lines in the total distance ambulated ($F_{1,20} = 1.02$, $P > 0.05$), ambulation speed ($F_{1,20} = 0.15$, $P > 0.05$), or ambulation rate ($F_{1,20} = 0.03$, $P > 0.05$) in the open field.

Before DFA, power spectrum analyses were performed on all time series of the distances ambulated (see the materials and methods section). A large spike at the fundamental frequency was found in all the series, a characteristic of data series that are nonoscillatory (Figure 1). Trends within the distance ambulated time series were also studied systematically by applying consequently greater polynomial orders to the fitting procedure of DFA (see the materials and methods section). A DFA3 fit was selected for LS and HS line comparisons because it was the lowest detrending order that eliminated trends in all monofractal series (Figure 2).

**Table 1.** Behavioral analyses of the ambulatory activity in an open-field test of female Japanese quail selected for either a reduced (LS, low-stress) or exaggerated (HS, high-stress) plasma corticosterone response to a brief restraint[1]

| Behavioral measure[2] | Line | | $P$-value |
| --- | --- | --- | --- |
| | LS | HS | |
| Latency to ambulate (s) | $48.82 \pm 16.70$ | $424.45 \pm 159.5$ | 0.02 |
| Time spent ambulating (%) | $27.00 \pm 3.95$ | $17.59 \pm 3.56$ | 0.09 |
| Distance ambulated (cm) | $4,789.43 \pm 979.63$ | $3,360.00 \pm 1023.34$ | 0.32 |
| Ambulation speed (cm/s) | $2.03 \pm 0.12$ | $1.94 \pm 0.20$ | 0.70 |
| Ambulation rate (cm/s) | $2.32 \pm 0.47$ | $2.18 \pm 0.64$ | 0.86 |

[1]The values for each line represent means ± SEM.

[2]Distance ambulated (cm) = distance ambulated during the total duration of the test (35 min); ambulation speed (cm/s) = distance ambulated/time spent ambulating; ambulation rate (cm/s) = distance ambulated/(total duration of test − latency to ambulate).
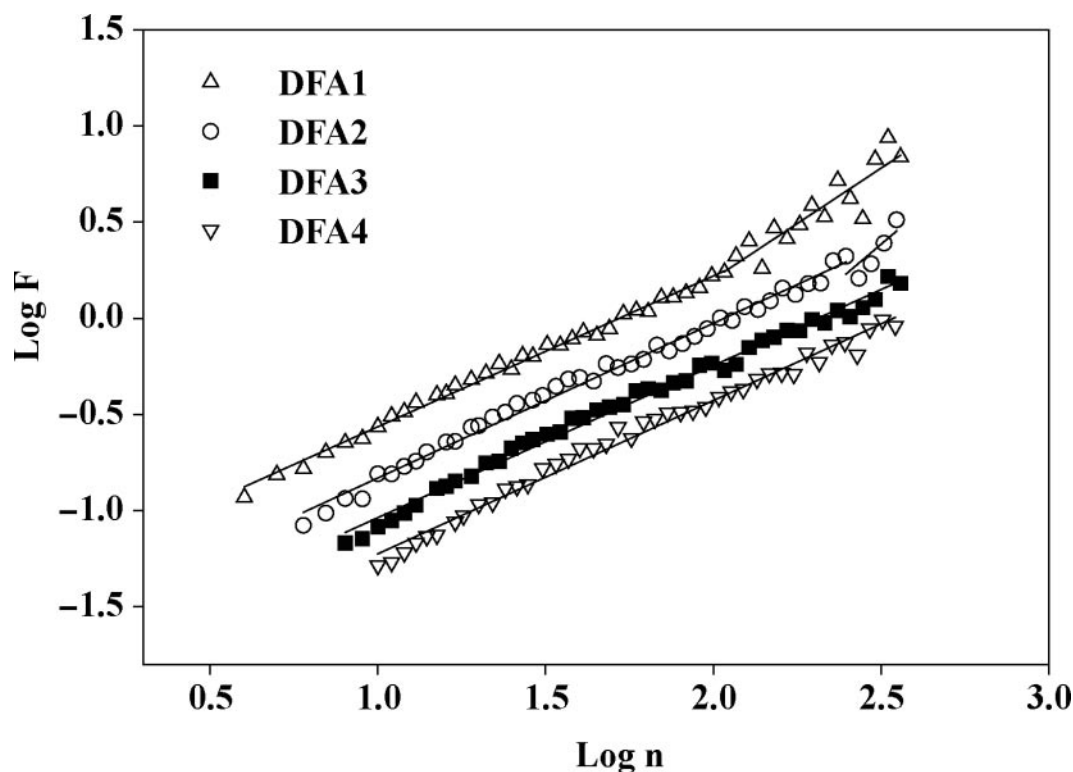
**Figure 2.** Example of the trend elimination capability of detrended fluctuation analysis (DFA) in time series using linear- (DFA1), quadratic- (DFA2), cubic- (DFA3), and quartic-order (DFA4) DFA data fits. Detrended fluctuation analysis 3 (■) was selected for group comparisons because third-order polynomials eliminated trends in the data. To improve visualization, the functions were shifted by multiples of 0.1.

When the complete data series (considering all 4,200 ambulation data points) was studied, a tendency ($P = 0.08$) toward a greater proportion of mononofractal series (series with similar complexity at different temporal scales) were found in LS quail (9/11; 82%) than in HS quail (7/11; 64%). All monofractal time series showed long-range correlations (i.e., α-values within 0.5 and 1; range: 0.65 to 0.97). Monofractal series were found in the birds that initiated their ambulatory activity in less than 600 s (72% of the birds). Among those birds, LS quail showed a lower ($F_{1,20} = 5.91$, $P < 0.03$) autosimilarity coefficient (α) than did their HS counterparts (Table 2). However, when the patterns of ambulation were reexamined by considering only the active period of the series (i.e., after the birds had initiated their ambulation activity), monofractal patterns were observed in all but one (97%) of the birds, and the differences between the LS and HS lines that were observed previously appeared to be lost ($F_{1,21} = 2.34$, $P = 0.14$; Table 2).

## DISCUSSION

In poultry, the open-field test has been used to assess fear and anxiety reactions, with inactivity behaviors interpreted as being characteristic of exaggerated fearfulness (Faure et al., 1983; Jones et al., 1992a; Moriarty, 1995; Jones, 1996b; Marin et al., 1997). In the present open-field study, quail selected for a high plasma corticosterone response to a brief immobilization stressor (HS birds) showed a longer latency to ambulate and a tendency toward a lower percentage of

**Table 2.** Values of the self-similarity parameter, α, of female Japanese quail selected for either a reduced (LS, low-stress) or exaggerated (HS, high-stress) plasma corticosterone response to a brief restraint on evaluation in an open-field test

| Time series of distance ambulated | Line[1] | | |
| --- | --- | --- | --- |
| | LS | HS | P-value |
| Complete[2] | 0.75 ± 0.03 (9) | 0.86 ± 0.04 (7) | 0.04 |
| Second period[3] | 0.75 ± 0.03 (11) | 0.83 ± 0.03 (10) | 0.14 |

[1]The values represent means ± SEM. The actual numbers of birds included in each analysis are denoted within parentheses. Only monofractal time series were used to estimate the means.

[2]Complete = the complete time series of distance ambulated (all 4,200 data points).

[3]Second period = the time series that corresponded to the second (active) period of open-field ambulation behavior (wherein each test subject's first period of nonambulation was eliminated).

time spent ambulating in comparison with quail from the line selected for a low adrenocortical response (LS birds). This is consistent with a previous study in these lines, in which LS chicks were found to show less freezing behavior and began to ambulate sooner than did their HS counterparts (Jones et al., 1992b). Satterlee and Marin (2006) also examined the effect of a 5-min exposure to an immobilization stressor applied immediately before assessing open-field behavior in the LS and HS lines. The LS control group in that study had a greater ambulation rate, and they entered more floor sectors and had a lower cumulative freezing time than did the stressed group of the LS line and both the control- and stressed-HS quail groups. Using the widely held belief that greater inactivity is an indicator of greater fearfulness, Satterlee and Marin (2006) concluded that these findings supported the contention of Jones (1996b) that selection for reduced adrenocortical responsiveness in the LS line has been accompanied by a concomitant decrease in underlying fearfulness. This is consistent with the findings of other fear assessment studies performed with the LS and HS lines as well, such as tonic immobility (Jones et al., 1992a; Satterlee et al., 1993), avoidance of novel objects (D. G. Satterlee and R. B. Jones, unpublished findings) and experimenters (Jones et al., 1994b), activity inhibition induced by immobilization (Jones and Satterlee, 1996), and timidity as assessed by "hole-in-the-wall" emergence box tests (Satterlee and Jones, 1995; Jones et al., 1999). However, it is important to take into account that all the studies mentioned above were limited to test durations of 10 min or less. Therefore, these studies may have been greatly influenced by fear-induced inhibition of all behavior patterns (e.g., silence, inactivity, and immobilization) as has been proposed by Faure et al. (1983) to occur during the initial (phase 1) adaptive response to perceived danger. Interestingly, in our 35-min-long open-field trial, no differences were found between quail of the LS and HS lines in the total distance ambulated, ambulation speed, and ambulation rate. Measurement of ambulation activities such as these are thought to be more important in examining behavioral repertoires that more closely reflect attempts at regrouping (i.e., socially motivated behaviors), which logically would be more evident when the fear level and novelty of a test situation are waning (Faure et al., 1983). Thus, the present results may also suggest that social reinstatement behavior is being experienced similarly in both lines once the initial shock of placement in the frightening open field begins to dissipate. That said, however, it should be noted that significant line (LS > HS) differences were reported by Jones et al. (2002) in 2 different tests of sociality (social proximity and reinstatement motivational tests, both of which measured the birds' desire to be near live conspecifics). Specifically, in same-line 4-d-old groups, undisturbed LS quail chicks stayed closer together than did HS chicks, and when naive,

individually tested chicks were put into a runway at 11 to 12 d of age, LS quail spent a longer time near a goal box containing cagemates than did the HS birds. Because the social responses of birds in a novel environment can be greatly influenced by the presence of other conspecifics or even the mirror reflection of the test birds (Gallup et al., 1972; Marin et al., 2001, 2003), the apparent inconsistency between the present study and the study of Jones et al. (2002) could be explained by differences in the test conditions of the 2 studies. In our study, birds were in complete social isolation in a novel environment, whereas in the studies by Jones et al. (2002), test birds were always able to establish visual and auditory contact with conspecifics placed either in the same home cage or in a nearby enclosure. Furthermore, no measurements of ambulation activity were made in the studies by Jones et al. (2002).

Detrended fluctuation analyses of the data series using a detrending of first and second order showed crossovers in several of the log-log plots of fluctuation versus window size that were eliminated by using higher detrending orders in the DFA procedure. These results indicate that the crossovers were not due to actual differences in the complexity of the behavior at different time scales, but to the presence of trends within the time series (a smooth and monotonous or slowly oscillating pattern caused by external effects, which can be eliminated by using a higher detrending order in the DFA procedure; Kantelhardt et al., 2001; see the materials and methods section). Detrended fluctuation analysis 3 was then selected for LS and HS line comparisons because DFA3 was the lowest detrending order that eliminated these trends in all monofractal series. Our results support the contention that, a priori, a DFA of first order should be used with caution because biological data are frequently affected by trends (Hu et al., 2001), which may lead to miscalculation of the α-value. The power spectrum analyses showed no periodic oscillations in the data series, suggesting that DFA was accurately applied to our data.

When the complete data series (considering all 4,200 ambulation data points) was studied, a tendency toward a greater proportion of mononofractal series (series with similar complexity at different temporal scales) was found in LS quail (82%) than in HS quail (64%). In the monofractal time series, long-range correlations were observed in all cases (α-values ranging from 0.65 to 0.97). This means that the ambulation behavior at one moment is statistically correlated with the ambulation behavior at a relatively remote time, and its influence decays in a scale-invariant, fractal manner. Long-range autocorrelations were also found in minnow reproductive behavior (Alados and Weber, 1999), chimpanzee social behavior (Alados and Huffman, 2000), domestic fowl walking and vigilant behavior (Rutherford et al., 2003; María et al., 2004), and quail home cage ambulatory behavior (Kembro et al., 2007). However, a reduced α-value was found in the LS

line when compared with the HS line, indicating that a more complex ambulatory behavior (a less regular activity pattern) was present in the LS quail.

Monofractal series were found only in the birds that initiated their ambulatory activity in less than 600 s (1/7 of the total duration of the trial), representing 72% of the birds. This result may suggest a priori that ambulation in birds showing long periods of inactivity (relative to the total duration of the test) are not monofractally organized; that is, their ambulation pattern cannot be described by only one self-similarity parameter, because its organization changes depending on the time scale being studied. However, it is possible that the long period of inactivity is affecting the calculation procedure, thus masking the actual temporal organization of the behavior. Interestingly, when the patterns of ambulation were reexamined by considering only the active period of the series, that is, after the birds had initiated their ambulation activity, monofractal patterns were observed in all but one (97%) of the birds, and the differences between quail of the LS and HS lines that were observed previously appeared to be lost. These results sound a cautionary note about the interpretation of fractal analysis results when, in proportion to the test duration, test animals show long periods of inactivity.

Taking into consideration the results obtained after analyzing the conventional behavioral measurements (distance ambulated, ambulation speed, and ambulation rate) and DFA, we propose that the differences found in the ambulatory pattern between quail of the LS and HS lines are primarily due to first-phase open-field behavioral responses, for which the immobility observed probably reflects the inhibition of all behavioral patterns by fear (Faure et al., 1983).

In conclusion, we submit that quail of the HS line showed exaggerated fear responses (heightened inactivity) in comparison with quail of the LS line during the first phase of open-field testing because the genomic differences in adrenocortical responsiveness were overwhelmingly in control of locomotor activity during this time. However, in the ensuing period of time, when the birds' fear responses to the novelty of the open field were likely waning and becoming increasingly less strong, and other motivations became the predominant behavioral driving forces, both lines showed similar ambulatory patterns because genomic controls on locomotor activity had abated.

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