Tonic Immobility and Open Field Responses in Nagoya, White Leghorn, and White Plymouth Rock Chicks

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Running title: Innate Fear Responses in Chicks

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Abstract

Innate fear responses to tonic immobility (TI) and open field (OF) were evaluated in newly hatched chicks of three breeds with distinct breed origin and genetic relationships. The breeds studied were Nagoya (NAG), a native Japanese breed; White Leghorn (WL), a representative of layers; and White Plymouth Rock (WPR), a parental breed of common broilers. The TI test revealed that WL was the most sensitive to extensive fear evoked by the TI test among the three breeds, followed in order by WPR, and NAG. In contrast, the OF test revealed that NAG was the most sensitive to mild fear evoked by the OF test, followed in order by WPR, and WL. The different fear responses between NAG and WL were supported by minimal phenotypic correlations between TI and OF traits in each breed. These results demonstrated that NAG and WL breeds exhibit extreme and opposite responses to TI and OF fears.

Keywords: Nagoya, open field, tonic immobility, White Leghorn, White Plymouth Rock
**Introduction**

During the process of animal domestication, fear-related behaviors have been reduced in frequency and intensity (Agnvall *et al*., 2012; Belyaev, 1979; Brlyaev *et al*., 1985; Price, 1999). In chickens, Campler *et al*. (2009) reported that Red Junglefowl, a wild ancestor of domesticated chickens, is more sensitive to fear than White Leghorn, a domesticated breed for egg production, which was derived from the Mediterranean type of chickens developed during the 19th century (Kerje, *et al*., 2003). Furthermore, previous studies have revealed differences in fear-related behaviors between and within chicken breeds developed for meat and egg production. For example, Abe *et al*. (2013) reported that White Leghorn is less sensitive to innate fear than Nagoya, a native Japanese breed with high-quality production of meat and eggs, which was established from the Nagoya Cochin breed in Aichi Prefecture, Japan in 1912–1926 (Tsudzuki, 2003). Nagoya is reported to be a cowardly chicken breed, and crowding accidents, often leading to the death of many birds in a flock, result from fear induced by environmental stimuli such as loud noises and intensive flashes (Kato *et al*., 1991). Innate fear was investigated in chicks of Tosa-Jidori, another native Japanese breed (Nakasai *et al*., 2013). In Japanese breeds, information on innate fear is limited to the above two reports on Nagoya and Tosa-Jidori. Although aggressive behavior, leading to a large animal welfare issue, has been reported in commercial male broilers (Millman *et al*., 2000; Li *et al*., 2016), little is known about fear-related behavior in meat-type breeds, such as White Plymouth Rock, which has been used worldwide as a parental breed of commercial broilers.

Several behavioral tests have been used to evaluate fear in chickens, and reviewed by Forkman *et al*. (2007). In the present study, we used tonic immobility (TI)
and open field (OF) tests, which are the most commonly used fear tests (Forkman et al., 2007). The TI test evokes intense fear in animals, through the application of light pressure from a human hand, while the OF test evokes mild fear in animals, indicating no immediate perceived threat, by placing them in a novel open field (Schütz et al., 2004).

In the present study, we evaluated innate fear responses to TI and OF in chicks of three chicken breeds, Nagoya (NAG), White Leghorn (WL), and White Plymouth Rock (WPR), which have distinct breed origin and genetic relationships among the breeds (Osman et al., 2006).

**Materials and Methods**

**Animals**

Hatching eggs for meat-type chickens of NAG (strain 87) and WPR (strain 981) were purchased from the Hyogo station, National Livestock Breeding Center, Hyogo, Japan, and those for egg-type chickens of WL (strain WL-G) were purchased from the Avian Bioscience Research Center, Graduate School of Biological Sciences, Nagoya University. Hatching eggs were incubated from 9:00 AM. Hatching was checked on the evening of the day prior to an expected hatching (3 weeks after incubation), and at 9:00 AM on an expected hatching day. Only chicks not hatched in the evening of the previous day but hatched by 9:00 AM on the expected day were used for behavioral tests. We could therefore assume that all chicks were almost the same age. All chicks were kept in an incubator at 32°C with 24 h lighting. Until the TI test was performed, chicks at 0 days of age were not provided food and water. After the TI test, chicks were provided water before the OF test. All chicks used in this study were
handled in accordance with the guidelines of the Animal Research Committee of Nagoya University.

**Behavioral Tests**

The TI test was performed in the afternoon of the hatching day and the OF test was performed in the morning of the next day using the same chicks. As handling of chicks was minimized as much as possible, the imprinting of chicks to the experimenter was assumed to be minimal. However, the chicks could imprint onto their companions in the same groups, because imprinting can occur within the first 4 days after hatching in chicks (Nakamori et al., 2013). Both tests were performed by the same person in a quiet, separate room at approximately 30°C under fluorescent lights with an average light intensity of approximately 300 lx.

For the TI test, a cradle comprising plywood (42 cm length, 23 cm width, and 2 cm thickness) fixed at a right angle to a V shape was used. Each chick was placed on its back on the V-shaped cradle covered with black felt (polyester 100%), and light pressure was placed on its breast for 5 s by a human hand. This trial was repeated three times consecutively. The duration of TI and the number of inductions were recorded for each chick using a fixed video camera (Handycam HDR-PJ675, Sony, Tokyo). In the present study, short and long durations of TI (hereafter referred to as S-duration and L-duration, respectively) were measured for each trial. The S-duration was defined as the time taken for the chick to move its head or peep loudly with eyes open. The L-duration was defined as the time taken for the chick to completely right itself. When chicks that righted within 5 s were not considered to have entered TI status, both S- and L-duration were scored as zero. Conversely, when the TI status lasted for 600 s and more, both S- and L-duration were scored as 600 s. The first S-duration recorded and the first L-
duration recorded were defined as the first S- and L-duration recorded among the three
consecutive trials, respectively. Similarly, short and long inductions (hereafter, S- and
L-induction, respectively) were recorded. The S-induction was the number of trials in
which a chick presented with the S-duration for the first time. The L-induction was the
number of trials in which a chick presented with the L-duration for the first time. The S-
and L-induction were scored as 4 if the TI status was not attained after three trials. In
total, 10 behavioral traits were recorded for the TI test (see Table 1). After the TI test,
the body weight of the chicks was measured.

An arena (54 cm length, 79 cm width, and 30 cm height) was used for the OF
test. The area 15 cm from the edge of the arena was defined as the periphery zone, and
the inside of the periphery zone was defined as the center zone (39 × 64 cm). Each
chick was placed at the lower left end of the periphery zone in darkness, and then the
OF test began when the light was turned on. Chick behavior was recorded for 10 min
from the top of the field with a video camera (Handycam HDR-PJ675). The record was
analyzed using the software program SMART v3.0 (Panlab Harvard Apparatus,
California). In total, 14 behavioral traits were measured during the OF test (see Table
3). Resting time was defined as the time when the moving speed of the chick was less
than 2.5 cm/s. Slow time was the time when the moving speed was 2.5–15 cm/s. Fast
time was the time when the moving speed was 15 cm/s and more. The parallel index
was used to indicate whether the animal progressed in a direction parallel to the
previous direction of progression. The value ranged from -1 to 1, and became close to 1
when the movement of animals was linear. However, it was close to -1 when the
movement of animals was not linear, showing a tendency toward exploration. The body
weight of chicks was measured after the OF test.
**Sexing**

Blood was taken from the chicks via the carotid artery. Genomic DNA was extracted from blood cells and animals were sexed by PCR amplification of the \textit{CHD} (chromo-helicase-DNA binding protein) gene on sex chromosomes, as described by Suzuki \textit{et al} (2019).

**Statistical Analysis**

Data on behavioral traits were analyzed with the JMP Pro software version 13.2.0 (SAS Institute Japan Ltd., Tokyo). Before breed differences were compared, the raw data were tested to determine whether the data were affected by environmental factors, such as sex and room temperature, using a linear model of JMP Pro. In the model, sex and the testing order of individuals measured on the same day were included as fixed effects. Room temperature and body weight were included as random effects. The effects, and their possible interaction effects that were significant at the nominal level of 5\% were used to adjust the raw data. Breed differences in adjusted data were tested by the Kruskal–Wallis test followed by the Steel–Dwass \textit{post hoc} test. Spearman's rank correlation coefficients were computed to measure phenotypic relationships among TI traits, among OF traits, and between TI and OF traits in each breed.

**Results**

Table 1 presents the results of the TI test in chicks of NAG, WL, and WPR breeds. There were no significant sex differences in any of the 10 behavioral traits examined at the nominal 5\% level. Data for four traits, S-duration in the 3rd trial, first S-duration recorded, L-duration in the 3rd trial, and first L-duration recorded, were
adjusted for room temperature. Data for S-induction were adjusted for testing order and body weight. Data for the remaining five traits were not adjusted for any environmental factor.

Significant differences among the three breeds were observed for five TI traits at the nominal 5% level, as shown in Table 1. Among them, four traits of S-duration in the 1st trial; S-duration in the 2nd trial; L-duration in the 1st trial; and L-induction exceeded the Bonferroni-corrected 5% level (0.05/10 = 0.005). Values for three traits of S- and L-durations in WL were significantly higher than those of NAG. In contrast, a value for L-induction in WL was significantly lower than that in NAG. Values for these four traits in WPR were approximately intermediate between NAG and WL. Therefore, the results demonstrated that WL was the most sensitive to fear evoked by the TI test among the three breeds; the second most sensitive was WPR; and the least sensitive was NAG, as chicks with higher values for TI durations and lower values for TI inductions are considered to be more fearful than those with lower values for TI durations and higher values for TI inductions (Schütz et al., 2004).

Table 2 presents the Spearman’s rank correlation coefficients among 10 TI traits in each of the three breeds. In the same trial, S-duration was positively correlated with L-duration at the Bonferroni-corrected 5% significance level (0.05/45= 0.0011) across three breeds. A positive correlation between the first S-duration recorded and the first L-duration recorded was observed at the Bonferroni-corrected 5% significance in all breeds. Likewise, a positive correlation between S-induction and L-induction was observed at the Bonferroni-corrected 5% significance level in NAG and WPR and at the nominal 5% level in WL. Conversely, the first S-duration recorded and the first L-duration recorded were positively correlated with S-duration in the 1st trial, and with L-
duration in the 1st trial, respectively, at the Bonferroni-corrected 5% significance level. Negative correlations between S-induction and S-duration in the 1st trial, and between L-induction and L-duration in the 1st trial were observed at the Bonferroni-corrected 5% significance level in NAG and WPR, and at the nominal 5% level in WL. Although only NAG and WL were positively correlated between the 1st and 2nd trials of S-duration at the nominal 5% level, no correlation was observed among trials of L-durations; however, the reasons for this are unknown. In addition to the result showing that only S- and L-durations were significant among the three breeds in the 1st trial (Table 1), only two TI traits (TI duration in the 1st trial and TI induction) for either short (S-) or long (L-) TI behavior were sufficient for evaluation of TI behavior in the three breeds.

Table 3 presents the results of the OF test in NAG, WL, and WPR chicks. None of 14 behavioral traits examined revealed a significant sex difference at the nominal 5% level. Seven traits, including latency of 1st entrance to the center zone, distance in the periphery zone, total distance, resting time, fast time, mean speed, and mean speed without resting were adjusted for room temperature. Two traits, maximum speed and parallel index, were adjusted for body weight. The remaining five traits were not adjusted for any environmental factor.

Significant breed differences were observed for 13 of 14 OF traits at the nominal 5% level (Table 3). Among them, eight traits (number of entries in the center zone, latency of the 1st entrance to the center zone, total time in the center zone, distance in the center zone, fast time, mean speed without resting, parallel index, and the number of excrements) exceeded the Bonferroni-corrected 5% level ($0.05/14 = 0.0036$). The value obtained for latency of the 1st entrance to the center zone in NAG
was significantly higher than that of WL. The values for the other seven traits in NAG were significantly lower than those in WL. WPR expressed fearfulness in the eight traits that was intermediate between that of NAG and WL. These results indicated that NAG was the most sensitive breed to fear evoked by the OF; the second most sensitive was WPR; and the least sensitive was WL, since chicks inactive were considered to be fearful (Schütz et al., 2004). Conversely, NAG and WL expressed opposing fear response to two traits, parallel index and the number of excrements, which differed in behavioral nature from the other traits. Interestingly, the lower parallel index of NAG meant that NAG had a higher tendency toward exploration than WPR in the OF arena.

Table 4 presents the Spearman’s rank correlation coefficients among 14 OF traits in each of the three breeds. Over three-quarters of 91 trait combinations were significantly correlated at the nominal 5% level in each of the three breeds. However, across breeds, 22 combinations remained significant at the Bonferroni-corrected 5% significance level (0.05/91 = 0.00055). Among these, we focused on correlations in OF traits with significant breed differences in Table 3, because describing all 22 combinations would be redundant. Latency of the 1st entrance to the center zone was negatively correlated with the number of entries in the center zone and the distance in the center zone, both of which were positively correlated with each other. Mean speed without resting was also positively correlated with fast time. However, neither parallel index nor the number of excrements was significantly correlated with any traits across breeds at the Bonferroni-corrected 5% significance level.

As NAG and WL displayed opposite responses to the TI and OF fear, Spearman’s rank correlation coefficients were estimated between 10 TI and 14 OF traits in each of the three breeds, and the results are summarized in Table 5. Among 140 trait
combinations compared, 19 were significant at the nominal 5% level in NAG. Only one and four combinations were significant in WPR and WL, respectively. However, no combination exceeded the Bonferroni-corrected 5% significance level (0.05/140 = 0.00036) in each of the three breeds.

Discussion

The social hierarchy of housed chicken groups is established during the first 5 weeks after hatching (Guhl, 1958). An ordered group structure is maintained for top-ranking chicks during the first 3 weeks of life (Rogers and Astiningsih, 1991). Hence, the difference in social order between young and adult age groups can affect TI and OF behaviors, as previously noted (Jones, 1986; Buitenhuis et al., 2004). In fact, Buitenhuis et al. (2004) revealed different quantitative trait loci (QTLs) for OF behavior between chickens at 5 and 29 weeks of age. In addition, Abe et al. (2013) quantified innate responses to TI fear in Nagoya and White Leghorn chicks at 1 and 2 days of age. Nakasai et al. (2013) reported age-dependent changes in innate TI responses in chicks of Tosa-Jidori, another native Japanese breed, at 2–15 days of age. Therefore, in the present study, newly hatched chicks were used before social order was established in order to measure innate responses to TI and OF fears.

Fear-related behavior is closely associated with a stress response that activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of glucocorticoids (mainly cortisol and corticosterone) from the adrenal glands of animals (Matteri et al., 2000). Ericsson and Jensen (2016) analyzed blood corticosterone levels and behavioral responses before and after physical restraint in Red Junglefowl and layer chicks at 1–23 days of age and revealed that the HPA axis responds to stress induced by physical
restraint in chicks at 1 day of age. By analyses of behavior, hormone levels, and production traits in layer chickens at hatching to 140 days of age, Hedlund et al. (2019) revealed that the stress of a commercial hatchery process (separation from shells, conveying, sex sorting, vaccination, etc.) during the first hours of life has short- and long-term effects on behavior and stress reactivity, and potentially egg production in later life. For example, chicks exposed to hatchery stress presented higher corticosterone levels at hatching and were more fearful to novelty at 1 day of age than control chicks at the same age not exposed to the stress. When the exposed chicks became adults at 140 days of age, the adults had more feather damage and injuries on their wattle and comb than control animals at the same age, suggesting that feather pecking and aggression occurred more often in the adults. Therefore, the results of those studies clearly demonstrated that the innate fear responses of chicks can be precisely measured even at hatching, and showed that stressful or fearful experiences at hatching and 1 day of age are linked to injurious pecking and other aggressive behaviors in later life, which are importantly related to poultry welfare.

We performed the TI test and then the OF test on the following day. Due to the order of these tests, it is possible that the first behavioral test affected the results of the second behavioral test. We considered this possibility to be minimal, because no phenotypic correlations between TI and OF behavioral traits were observed in each of our NAG, WL, and WPR breeds at Bonferroni-corrected 5% significance levels. The no phenotypic correlations were consistent with a previous report in which no phenotypic correlations were observed between TI and OF traits in other White Leghorn chickens (Heiblum et al., 1998). Ours and previous findings suggest that two kinds of fear evoked by the TI and OF tests differ in nature. Therefore, the opposite fear responses
observed in our NAG and WL breeds reflected differences in the nature of TI and OF fear.

Previously, Abe et al. (2013) reported that the TI duration of the Nagoya breed is longer than that of the White Leghorn breed. The findings of that previous report did not support our TI result with the NAG and WL breeds. This discrepancy may result from differences in genetic backgrounds reflecting differences in the breeding histories of Nagoya and White Leghorn used in our study and the previous report. The previous report used strains of Nagoya and White Leghorn, both of which were developed at the Aichi Agricultural Research Center, Aichi, Japan. Our WL was the WL-G line, which has been maintained as a closed breeding colony at Nagoya University since 1969, and our NAG was the 87 strain developed at the Hyogo station, National Livestock Breeding Center, Hyogo, Japan.

Several studies have reported QTLs for TI and OF fears in chickens. For example, Schütz et al. (2004) identified a QTL for TI duration on chromosome 1 in an F2 intercross population between White Leghorn chickens and Red Junglefowl. Fogelholm et al. (2019) reported 18 QTLs for TI behavior on chromosomes 1, 2, 4, 6, 7, 10, 12, 15, 20, and 24, and identified five candidate genes for behavior in an advanced intercross population between White Leghorn chickens and Red Junglefowl. For OF behavior, Buitenhuis et al. (2004) revealed 11 QTLs on chromosomes 1, 2, 4, 9, and 10 in an F2 hen population between two White Leghorn lines selected for egg production traits. Johnsson et al. (2016) reported 34 QTLs for OF behavior on chromosomes 1–4, 6–8, 10, 13, and 17, and identified 10 candidate genes for the behavior in an advanced intercross population between White Leghorn chickens and Red Junglefowl. In the present study, we demonstrated that NAG and WL breeds exhibit extreme and opposite
behavioral responses to TI and OF. It is thus suggested that the two breeds may be animal resources useful for identification of new QTLs governing the opposing TI and OF behaviors. Some causal genes for QTLs may be HPA-related genes (Matteri et al., 2000). Genetic selection for chicks with low fear genes may be beneficial for poultry production, because selected chicks can, for example, increase the ease of human handling and decrease the risk of injurious pecking (feather pecking and vent pecking), which often leads to cannibalism, all of which result in improved poultry welfare.

**Acknowledgments**

We thank Dr. Kenji Nagao, Livestock Industry Division, Agriculture, Forestry, and Fisheries Department, Aichi Prefecture for advice on the TI test.
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Table 1. **Means and standard errors of behavioral traits for a tonic immobility (TI) test in the Nagoya (NAG), White Leghorn (WL), and White Plymouth Rock (WPR) chicks**

| Trait                      | NAG       | WL (54) | WPR     | P value     | Fearfulness          |
|----------------------------|-----------|---------|---------|-------------|----------------------|
| No. of animals             | 70        | 71      | 47      |             |                      |
| S-duration in the 1st trial (s) | 51.0 ± 15.0<sup>a</sup> | 74.7 ± 13.9<sup>b</sup> | 91.4 ± 23.1<sup>ab</sup> | 6.1.E-04    | WL ≥ WPR ≥ NAG       |
| S-duration in the 2nd trial (s) | 52.1 ± 11.7<sup>a</sup> | 54.4 ± 7.3<sup>b</sup>  | 44.6 ± 14.0<sup>a</sup> | 0.0028      | WL > NAG ≥ WPR       |
| S-duration in the 3rd trial (s) | -539.4 ± 9.4 | -536.2 ± 6.2 | -536.2 ± 13.2 | 0.17        |                      |
| First S-duration recorded (s) | -408.8 ± 16.8 | -413.4 ± 13.7 | -371.8 ± 22.3 | 0.28        |                      |
| S-induction (no)           | 0.4 ± 0.1  | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.097       |                      |
| L-duration in the 1st trial (s) | 99.8 ± 21.2<sup>a</sup> | 173.4 ± 24.8<sup>b</sup> | 180.6 ± 31.1<sup>ab</sup> | 0.0011      | WL ≥ WPR ≥ NAG       |
| L-duration in the 2nd trial (s) | 71.9 ± 14.8  | 91.0 ± 16.5  | 79.3 ± 16.3 | 0.090       |                      |
| L-duration in the 3rd trial (s) | -637.3 ± 9.4 | -619.9 ± 10.6 | -636.9 ± 13.2 | 0.11        |                      |
| First L-duration recorded (s) | -462.5 ± 22.4<sup>a</sup> | -423.0 ± 24.9<sup>ab</sup> | -389.2 ± 28.9<sup>b</sup> | 0.041       | WPR ≥ WL ≥ NAG       |
| L-induction (no)           | 1.4 ± 0.1<sup>a</sup> | 1.1 ± 0.1<sup>b</sup> | 1.3 ± 0.1<sup>a</sup> | 4.3.E-04    | WL > WPR ≥ NAG       |

The S-trait denotes the short time from the trait until the chick either moved its head or peeped in a loud voice with eyes open, and the L-trait denotes the long time from the trait until the chick completely righted. The raw data were adjusted for random effects of environmental factors (see Material and Methods). The trait differences among the three breeds were tested by the Kruskal–Wallis test, and the P values
obtained were approximated by the chi-square value for the one-way test. The $P$ values in bold exceeded the Bonferroni-corrected 5% level.

1 71 and 54 animals were assessed for S- and L-traits, respectively, in WL.

a-c Means with different letters were significantly different between breeds at $P < 0.05$ by the Steel–Dwass post hoc test.

- = not applicable.
Table 2. **Spearman’s rank correlation coefficients (ρ)** between TI traits in the Nagoya (NAG), White Leghorn (WL), and White Plymouth Rock (WPR) chicks

| Trait 1                        | Trait 2                        | NAG  |       |       | WL   |       |       | WPR  |       |       |
|--------------------------------|--------------------------------|------|-------|-------|------|-------|-------|------|-------|-------|
| S-duration in the 2nd trial    | S-duration in the 1st trial    | 0.2784 | 0.017 |       | 0.2978 | 0.0069 |       | NS    | NS    |       |
| S-duration in the 3rd trial    | S-duration in the 1st trial    | NS    | NS    |       | NS    | NS    |       | NS    | NS    |       |
| S-duration in the 2nd trial    | S-duration in the 1st trial    | NS    | NS    |       | NS    | NS    |       | NS    | NS    |       |
| First S-duration recorded      | S-duration in the 1st trial    | 0.3834 | **0.00081** |       | 0.7262 | **1.7E-14** |       | 0.5913 | **1.2E-05** |       |
| S-duration in the 2nd trial    | S-duration in the 1st trial    | 0.3678 | 0.0014 |       | 0.3452 | 0.0016 |       | 0.445 | 0.0017 |       |
| S-duration in the 3rd trial    | S-duration in the 1st trial    | 0.5132 | **3.4E-06** |       | 0.2912 | 0.0084 |       | NS    | NS    |       |
| S-duration first recorded      | S-duration in the 1st trial    | -0.799 | **2.4E-17** |       | -0.3107 | 0.0048 |       | -0.702 | **3.8E-08** |       |
| S-induction                    | S-duration in the 1st trial    | -0.263 | 0.025 |       | NS    | NS    |       | NS    | NS    |       |
| S-duration in the 2nd trial    | S-duration in the 3rd trial    | NS    | NS    |       | -0.2921 | 0.0081 |       | NS    | NS    |       |
| S-duration first recorded      | S-duration in the 1st trial    | -0.255 | 0.029 |       | NS    | NS    |       | NS    | NS    |       |
| L-duration in the 1st trial    | S-duration in the 1st trial    | 0.8359 | **3.6E-20** |       | 0.6437 | **9.6E-09** |       | 0.8027 | **1.2E-11** |       |
| S-duration in the 2nd trial    | S-duration in the 3rd trial    | NS    | NS    |       | NS    | NS    |       | NS    | NS    |       |
| S-duration in the 3rd trial    | First S-duration recorded     | 0.2681 | 0.022 |       | 0.5293 | **6.9E-06** |       | 0.4589 | 0.0012 |       |
| S-induction                    | S-duration in the 1st trial    | -0.651 | **4.6E-10** |       | -0.2558 | 0.041 |       | -0.461 | 0.0011 |       |
| L-duration in the 2nd trial    | S-duration in the 1st trial    | NS    | NS    |       | NS    | NS    |       | NS    | NS    |       |
| S-duration in the 2nd trial    | S-duration in the 3rd trial    | 0.8418 | **1.1E-20** |       | 0.7524 | **7.7E-13** |       | 0.6616 | **4.1E-07** |       |
| S-duration in the 3rd trial    | S-duration first recorded      | 0.2408 | 0.040 |       | NS    | NS    |       | NS    | NS    |       |

**Note:** NS indicates non-significant correlation (p > 0.05).
|          | S-induction |          |          |          | L-duration in the 1st trial |          |          |          |          |          |          |          |          |          |
|----------|-------------|----------|----------|----------|----------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| L-duration in the 3rd trial |          |          |          |          | S-duration in the 1st trial |          |          |          |          |          |          |          |          |          |
|          | NS          | NS       | NS       | NS       | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | L-duration in the 3rd trial |          |          |          | S-duration in the 2nd trial |          |          |          |          |          |          |          |          |          |
|          | NS          | NS       | NS       | NS       | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | S-duration in the 3rd trial | 0.8627   | 1.0E-22  | 0.7691   | 1.2E-13                     | 0.8649   | 4.6E-15  |          |          |          |          |          |          |          |          |
| First S-duration recorded |          | 0.4018   | **0.00043** |          | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | S-induction |          |          |          | L-duration in the 2nd trial |          |          |          |          |          |          |          |          |          |
|          | NS          | NS       | NS       | NS       | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | L-duration in the 2nd trial |          |          |          | S-duration in the 3rd trial | 0.8649   | 4.6E-15  |          |          |          |          |          |          |          |          |
|          | NS          | NS       | NS       | NS       | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | NS          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          | L-duration in the 3rd trial |          |          |          | S-duration in the 2nd trial | 0.3315   | 0.0042   | 0.4327   | **0.00036** | 0.616   | **4.0E-06** |          |          |          |
| First L-duration recorded |          | S-duration in the 1st trial | 0.2436   | 0.038    | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | L-duration in the 1st trial | 0.416    | **0.00025** |          | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | S-duration in the 3rd trial | 0.611    | **9.4E-09** | 0.7001   | **1.2E-10**                | 0.6096   | **5.4E-06** |          |          |          |          |          |          |          |          |
| First S-duration recorded |          |          |          |          | S-induction                 |          |          |          |          |          |          |          |          |          |
|          | NS          | NS       | NS       | NS       | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | L-duration in the 1st trial | 0.4607   | **4.1E-05** | 0.8215   | **9.1E-17**                | 0.7603   | **5.7E-10** |          |          |          |          |          |          |          |          |
|          | L-duration in the 2nd trial | 0.3591   | 0.0018   | NS       | NS                          | 0.3022   | 0.039    |          |          |          |          |          |          |          |          |
|          | L-duration in the 3rd trial | 0.4326   | **0.00013** |          | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | L-induction |          |          |          | S-duration in the 1st trial | -0.678   | **4.4E-11** | -0.3483  | 0.0048   | -0.623   | **2.9E-06** |          |          |          |
|          | S-duration at 2nd trial |          |          |          | S-duration in the 3rd trial | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | S-duration in the 3rd trial |          |          |          | First S-duration recorded   |          |          |          |          |          |          |          |          |          |
|          | NS          | NS       | NS       | NS       | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
|          | S-induction |          |          |          | L-duration in the 1st trial | 0.6802   | **3.6E-11** | 0.3464   | 0.0051   | 0.5772   | **2.2E-05** |          |          |          |
|          | L-duration in the 1st trial | -0.786   | **1.9E-16** | -0.3662  | 0.0029                     | -0.735   | **4.2E-09** |          |          |          |          |          |          |          |          |
|          | L-duration in the 2nd trial |          |          |          |          | NS                          | NS       | NS       | NS       | NS       | NS       | NS       | NS       | NS       |
| L-duration in the 3rd trial | NS | NS | NS | NS | NS | NS | NS |
|----------------------------|----|----|----|----|----|----|----|
| First L-duration recorded  | NS | NS | NS | NS | NS | NS | NS |

The \( P \) values in bold exceeded the Bonferroni-corrected 5% level.

NS = not significant at the nominal 5% level.
Table 3. **Means and standard errors of behavioral traits for an open field (OF) test in the Nagoya (NAG), White Leghorn (WL), and White Plymouth Rock (WPR) chicks**

| Trait                                      | NAG         | WL          | WPR         | $P$ value | Fearfulness               |
|---------------------------------------------|-------------|-------------|-------------|-----------|---------------------------|
| No. of animals                              | 25          | 64          | 23          |           |                           |
| No. of entries in the center zone (no)      | $2.8 \pm 1.0^a$ | $6.4 \pm 0.8^b$ | $3.4 \pm 0.9^ab$ | **8.8.E-04** | NAG $\geq$ WPR $\geq$ WL |
| Latency of 1st entrance to the center zone (s) | $1190.6 \pm 41.4^a$ | $974.2 \pm 24.7^b$ | $1046.1 \pm 46.6^b$ | **3.2.E-04** | NAG $>$ WPR $\geq$ WL     |
| Total time in the center zone (%)           | $4.7 \pm 1.8^a$ | $11.9 \pm 2.0^b$ | $49.5 \pm 9.2^c$ | **3.4.E-05** | NAG $>$ WL $>$ WPR         |
| Distance in the periphery zone (cm)         | $-5964.5 \pm 122^a$ | $-5047.1 \pm 210.0^b$ | $-5625.8 \pm 115.3^ab$ | 0.018     | NAG $\geq$ WPR $\geq$ WL |
| Distance in the center zone (cm)            | $55.5 \pm 16.9^a$ | $143.4 \pm 18.4^b$ | $75.6 \pm 19.4^ab$ | **0.0021** | NAG $\geq$ WPR $\geq$ WL |
| Total distance (cm)                         | $-6240.3 \pm 135.4^a$ | $-5240.5 \pm 219.2^b$ | $-5870.8 \pm 124.0^ab$ | 0.012     | NAG $\geq$ WPR $\geq$ WL |
| Resting time (s)                            | $959.2 \pm 12.9^a$ | $899.2 \pm 13.9^b$ | $918.6 \pm 16.1^ab$ | 0.033     | NAG $\geq$ WPR $\geq$ WL |
| Slow time (s)                               | $54.7 \pm 9.6$ | $85.1 \pm 8.0$ | $81.5 \pm 15.0$ | 0.16      |                           |
| Fast time (s)                               | $-222.6 \pm 3.5^a$ | $-189.9 \pm 6.9^b$ | $-215.0 \pm 2.5^a$ | **0.0017** | NAG $\geq$ WPR $>WL$      |
| Mean speed (cm/s)                           | $-10.4 \pm 0.2^a$ | $-8.7 \pm 0.4^b$ | $-9.8 \pm 0.2^ab$ | 0.012     | NAG $\geq$ WPR $\geq$ WL |
| Mean speed without resting (cm/s)           | $-11.4 \pm 0.6^a$ | $-8.6 \pm 0.5^b$ | $-11.0 \pm 0.3^a$ | **1.7.E-04** | NAG $\geq$ WPR $>WL$      |
| Maximum speed (cm/s)                        | $66.4 \pm 4.6^b$ | $79.5 \pm 3.4^a$ | $64.6 \pm 2.7^b$ | 0.0087    | WPR $\geq$ NAG $\geq$ WL |
| Parallel index                              | $0.2 \pm 0.1^a$ | $0.8 \pm 0.0^b$ | $0.6 \pm 0.1^c$ | **1.0.E-07** | WL $>$ WPR $>$ NAG         |
| No. of excrements (no)                      | $0.3 \pm 0.1^a$ | $0.8 \pm 0.1^b$ | $0.3 \pm 0.1^a$ | **1.0.E-04** | WL $>$ NAG $=$ WPR         |
The raw data were adjusted for random effects of environmental factors (see Material and Methods). The trait differences among the three breeds were tested by the Kruskal-Wallis test, and the $P$ values obtained were approximated by the chi-square value for the one-way test. The $P$ values in bold exceeded the Bonferroni-corrected 5% level.

$^{a-c}$ Means with different letters were significantly different between breeds at $P < 0.05$ by the Steel–Dwass post hoc test.

- = not applicable.
Table 4. Spearman’s rank correlation coefficients (ρ) between OF traits in each of the Nagoya (NAG), White Leghorn (WL), and White Plymouth Rock (WPR) chicks

| Trait 1                                      | Trait 2                                      | NAG  | P value | WL  | P value | WPR  | P value |
|----------------------------------------------|----------------------------------------------|------|---------|-----|---------|------|---------|
| Latency of 1st entrance to the center zone   | No. of entries in the center zone            | -0.804 | 4.4E-07 | -0.5449 | 3.2E-06 | -0.7937 | 6.2E-06 |
| Total time in the center zone                | No. of entries in the center zone            | 0.9862 | 4.9E-21 | 0.6237 | 3.7E-08 | NS | NS |
|                                              | Latency of 1st entrance to the center zone   | -0.782 | 8.9E-07 | -0.4553 | 0.00016 | NS | NS |
|                                              | Total time in the center zone                | NS | NS | 0.5325 | 5.9E-06 | 0.4572 | 0.028 |
| Distance in the periphery zone               | No. of entries in the center zone            | NS | NS | 0.5325 | 5.9E-06 | 0.4572 | 0.028 |
|                                              | Latency of 1st entrance to the center zone   | -0.609 | 0.00058 | -0.3607 | 0.0034 | NS | NS |
|                                              | Total time in the center zone                | NS | NS | NS | NS | NS | NS |
| Distance in the center zone                  | No. of entries in the center zone            | 0.9875 | 1.4E-21 | 0.9583 | 1.9E-35 | 0.9356 | 5.8E-11 |
|                                              | Latency of 1st entrance to the center zone   | -0.807 | 2.1E-07 | -0.5392 | 4.3E-06 | -0.8196 | 1.7E-06 |
|                                              | Total time in the center zone                | 0.9804 | 7.0E-20 | 0.6191 | 4.9E-08 | NS | NS |
|                                              | Distance in the periphery zone               | 0.4509 | 0.016 | 0.5612 | 1.4E-06 | NS | NS |
|                                              | No. of entries in the center zone            | 0.4509 | 0.016 | 0.5612 | 1.4E-06 | NS | NS |
|                                              | Latency of 1st entrance to the center zone   | -0.694 | 4.3E-05 | -0.3843 | 0.0017 | NS | NS |
|                                              | Total time in the center zone                | 0.4429 | 0.018 | NS | NS | NS | NS |
|                          | Distance in the periphery zone | Distance in the center zone | No. of entries in the center zone | Latency of 1st entrance to the center zone | Total time in the center zone | Distance in the periphery zone | Distance in the center zone | Total distance                   |
|--------------------------|--------------------------------|------------------------------|-----------------------------------|-------------------------------------------|-------------------------------|--------------------------------|------------------------------|-------------------------------|
| **Resting time**         | 0.9896 **2.0E-23**             | 0.996 **1.2E-66**            | 0.9615                             | 3.0E-13                                   |                               |                                |                              |                               |
|                          | 0.5395                          | 0.6085 **9.6E-08**           | 0.5713                             | 0.044                                     |                               |                                |                              |                               |
|                          | -0.527                          | -0.6097 **8.9E-08**          | -0.6718                             | **0.00045**                              |                               |                                |                              |                               |
| **Slow time**            | 0.7199 **1.6E-05**              | 0.4555 **0.00016**           | 0.4486                             | 0.032                                     |                               |                                |                              |                               |
|                          | -0.526                          | 0.0040 **NS**                | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          | -0.941                          | **8.7E-14**                  | -0.8518                             | **2.5E-07**                              |                               |                                |                              |                               |
|                          | -0.613                          | **0.00052**                  | -0.6737                             | **0.00042**                              |                               |                                |                              |                               |
|                          | -0.969                          | **2.8E-17**                  | -0.9348                             | **6.6E-11**                              |                               |                                |                              |                               |
| **Fast time**            | 0.6063                          | 0.00080                      | 0.6145                             | **6.6E-08**                              | 0.6892 **0.00028**            |                                |                              |                               |
|                          | -0.617                          | **0.00048**                  | -0.4659                             | **0.00010**                              | -0.5006 **0.015**             |                                |                              |                               |
|                          | 0.6027                          | 0.00069                      | **NS**                              | **NS**                                   | **NS**                        |                                |                              |                               |
|                          | 0.7258                          | **1.2E-05**                  | 0.8371                             | **6.8E-18**                              | 0.8419 **4.8E-07**           |                                |                              |                               |
|                          | 0.6668                          | **0.00011**                  | 0.6542                             | **4.6E-09**                              | 0.7015 **0.00019**           |                                |                              |                               |
|                          | 0.7772                          | **1.1E-06**                  | 0.844                              | **2.0E-18**                              | 0.9308 **1.2E-10**           |                                |                              |                               |
|                          | -0.883                          | **4.8E-10**                  | -0.9287                            | **2.1E-28**                              | -0.996 **1.4E-23**           |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   | **NS**                        |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
|                          |                               |                              | **NS**                              | **NS**                                   |                               |                                |                              |                               |
| Parameter                                           | Mean speed | Mean speed without resting |
|-----------------------------------------------------|------------|-----------------------------|
| Total distance                                      | 0.9221     | 0.9999                      |
| Resting time                                        | -0.828     | -0.969                      |
| Slow time                                           | 0.5085     | 0.9222                      |
| Number of entries in the center zone                | 0.4359     | 0.6204                      |
| Latency of 1st entrance to the center zone          | -0.689     | -0.711                      |
| Total time in the center zone                       | 0.4391     | 0.6303                      |
| Distance in the periphery zone                      | 0.99       | 0.7592                      |
| Distance in the center zone                         | 0.5366     | 0.6965                      |
| Total distance                                      | 0.9999     | 0.7849                      |
| Resting time                                        | -0.969     | -0.767                      |
| Slow time                                           | 0.7776     | 0.6026                      |
| Fast time                                           | 0.9222     | 0.761                       |
| Number of entries in the center zone                | 0.4359     | 0.7845                      |
| Latency of 1st entrance to the center zone          | -0.689     | -0.711                      |
| Total time in the center zone                       | 0.4391     | 0.6303                      |
| Distance in the periphery zone                      | 0.99       | 0.7592                      |
| Distance in the center zone                         | 0.5366     | 0.6965                      |
| Total distance                                      | 0.9999     | 0.7849                      |
| Resting time                                        | -0.969     | -0.767                      |
| Slow time                                           | 0.7776     | 0.6026                      |
| Fast time                                           | 0.9222     | 0.761                       |
| Mean speed                                          | 0.4359     | 0.7845                      |
| Mean speed without resting                          | 0.6204     | 0.7845                      |

Note: The values are given in scientific notation.
|                           | Maximum speed                          | Parallel index                          |
|---------------------------|----------------------------------------|-----------------------------------------|
|                           | No. of entries in the center zone      | No. of entries in the center zone       |
|                           | 0.6426                                 | 0.4526                                  |
|                           | **0.00030**                            | **0.00018**                             |
|                           | 0.6179                                 | 0.5208                                  |
|                           | **5.3E-08**                            | **1.0E-05**                             |
|                           | 0.494                                  | 0.6452                                  |
|                           | 0.017                                  | 0.0089                                  |
| Latency of 1st entrance to| -0.607                                 | -0.534                                  |
| the center zone           | 0.00061                                | 0.0035                                  |
|                           | -0.4188                                | -0.3491                                 |
|                           | 0.00057                                | 0.0047                                  |
|                           | NS                                     | NS                                      |
|                           | NS                                     | NS                                      |
|                           | NS                                     | NS                                      |
| Total time in the center  | 0.6612                                 | NS                                      |
| zone                      | **0.00013**                            | NS                                      |
|                           | NS                                     | NS                                      |
|                           | 0.4781                                 | 0.8607                                  |
|                           | 0.021                                  | 0.0008                                  |
| Distance in the periphery | 0.4888                                 | NS                                      |
| zone                      | 0.0083                                 | NS                                      |
|                           | 0.7036                                 | 0.7318                                  |
|                           | **8.9E-11**                            | **7.2E-05**                             |
| Distance in the center    | 0.6894                                 | NS                                      |
| zone                      | **5.0E-05**                            | NS                                      |
|                           | 0.6355                                 | 0.8607                                  |
|                           | **1.7E-08**                            | **1.4E-07**                             |
|                           | 0.4274                                 | 0.8607                                  |
| Total distance            | 0.5457                                 | 0.4373                                  |
|                           | 0.0027                                 | 0.020                                   |
|                           | 0.7148                                 | 0.7562                                  |
|                           | **3.3E-11**                            | **5.0E-13**                             |
| Resting time              | -0.566                                 | -0.506                                  |
|                           | 0.0017                                 | 0.0060                                  |
|                           | -0.7245                                | -0.7979                                 |
|                           | **1.3E-11**                            | **2.9E-15**                             |
| Slow time                 | 0.5506                                 | 0.5282                                  |
|                           | 0.0024                                 | 0.0039                                  |
|                           | 0.7012                                 | 0.7684                                  |
|                           | **1.1E-10**                            | **1.2E-13**                             |
| Fast time                 | 0.4535                                 | 0.3743                                  |
|                           | 0.015                                  | 0.050                                   |
|                           | 0.6708                                 | 0.7208                                  |
|                           | **1.3E-09**                            | **1.9E-11**                             |
| Mean speed                | 0.5455                                 | 0.4377                                  |
|                           | 0.0027                                 | 0.020                                   |
|                           | 0.7141                                 | 0.7562                                  |
|                           | **3.5E-11**                            | **5.0E-13**                             |
| Mean speed without resting| 0.827                                  | NS                                      |
|                           | **5.8E-08**                            | NS                                      |
|                           | 0.8084                                 | 0.8607                                  |
|                           | **6.7E-16**                            | **1.4E-07**                             |
|                           | 0.7318                                 | 0.8607                                  |
|                           | 0.017                                  | 0.0089                                  |
| Latency of 1st entrance to| -0.534                                 | NS                                      |
| the center zone           | 0.0035                                 | NS                                      |
|                           | -0.3491                                | NS                                      |
|                           | 0.0047                                 | NS                                      |
|                           | -0.4905                                | NS                                      |
| Total time in the center  | NS                                     | NS                                      |
| zone                      | NS                                     | NS                                      |
|                           | NS                                     | NS                                      |
| Distance in the periphery | NS                                     | NS                                      |
| zone                      | NS                                     | NS                                      |
|                           | 0.7557                                 | NS                                      |
| Distance in the center    | 0.392                                  | 0.4373                                  |
| zone                      | 0.039                                  | 0.020                                   |
|                           | 0.5402                                 | 0.7562                                  |
|                           | **4.1E-06**                            | **5.0E-13**                             |
| Total distance            | 0.4373                                 | 0.5282                                  |
|                           | 0.020                                  | 0.0039                                  |
|                           | 0.7979                                 | 0.7684                                  |
|                           | **2.9E-15**                            | **1.2E-13**                             |
| Resting time              | -0.506                                 | 0.3743                                  |
|                           | 0.0060                                 | 0.050                                   |
|                           | -0.7979                                | 0.7208                                  |
| Slow time                 | 0.5282                                 | 0.4377                                  |
|                           | 0.0039                                 | 0.020                                   |
| Fast time                 | 0.3743                                 | 0.7562                                  |
|                           | 0.050                                  | 0.020                                   |
| Mean speed                | 0.4377                                 | 0.7562                                  |
| Mean speed without resting| NS                                     | NS                                      |
|                           | NS                                     | NS                                      |
|                           | NS                                     | NS                                      |
| Variable                                      | No. of excrements | No. of entries in the center zone | Latency of 1st entrance to the center zone | Total time in the center zone | Distance in the periphery zone | Distance in the center zone | Total distance | Resting time | Slow time | Fast time | Mean speed | Mean speed without resting | Maximum speed | Parallel index |
|-----------------------------------------------|-------------------|----------------------------------|---------------------------------------------|--------------------------------|--------------------------------|--------------------------------|----------------|-------------|-----------|-----------|-----------|-----------------|----------------|----------------|
| Maximum speed                                 | NS                | NS                               | 0.662                                       | 2.6E-09                        | NS                             | NS                             | 0.4031         | 0.037       | 0.2941    | 0.018     | NS        | NS               | NS              | NS            |
| No. of entries in the center zone             | 0.4031            | 0.037                            | 0.2941                                      | 0.018                          | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Latency of 1st entrance to the center zone   | NS                | NS                               | NS                                          | NS                             | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Total time in the center zone                | NS                | NS                               | NS                                          | NS                             | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Distance in the periphery zone               | 0.4747            | 0.011                            | NS                                          | NS                             | 0.4233                         | 0.044                          | 0.4747         | 0.011       | 0.2941    | 0.018     | NS        | NS               | NS              | NS            |
| Distance in the center zone                  | 0.3765            | 0.048                            | 0.3407                                      | 0.0059                         | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Total distance                                | 0.4695            | 0.012                            | NS                                          | NS                             | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Resting time                                  | -0.427            | 0.023                            | NS                                          | NS                             | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Slow time                                     | 0.4203            | 0.026                            | NS                                          | NS                             | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Fast time                                     | NS                | NS                               | NS                                          | NS                             | 0.4903                         | 0.018                          | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Mean speed                                    | 0.467             | 0.012                            | NS                                          | NS                             | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Mean speed without resting                    | 0.3919            | 0.039                            | NS                                          | NS                             | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Maximum speed                                 | NS                | NS                               | NS                                          | NS                             | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |
| Parallel index                                | NS                | NS                               | NS                                          | NS                             | NS                             | NS                             | NS             | NS          | NS        | NS        | NS        | NS               | NS              | NS            |

The $P$ values in bold exceeded the Bonferroni-corrected 5% level.

NS, not significant at the nominal 5% level.
Table 5. Spearman’s rank correlation coefficients (ρ) between traits for TI and OF tests in each of the Nagoya (NAG), White Leghorn (WL), and White Plymouth Rock (WPR) chicks

| TI trait                     | OF trait                          | NAG  |  | WL  |  | WPR |  |
|------------------------------|-----------------------------------|------|---|-----|---|-----|---|
|                              |                                   | ρ    | P value | ρ    | P value | ρ    | P value |
| S-duration in the 1st trial  | Mean speed without resting        | -0.50| 0.010  | NS  | NS  | NS  | NS  |
| S-duration in the 2nd trial  | No. of entries in the center zone | -0.50| 0.013  | NS  | NS  | NS  | NS  |
|                              | Latency of 1st entrance to center zone | 0.56 | 0.0036 | NS  | NS  | NS  | NS  |
|                              | Total time in the center zone     | -0.51| 0.010  | NS  | NS  | NS  | NS  |
|                              | Distance in the center zone       | -0.51| 0.010  | NS  | NS  | NS  | NS  |
|                              | Mean speed without resting        | -0.52| 0.0072 | NS  | NS  | NS  | NS  |
|                              | Maximum speed                     | -0.49| 0.012  | NS  | NS  | NS  | NS  |
|                              | Parallel index                    | -0.47| 0.018  | NS  | NS  | NS  | NS  |
| S-duration in the 3rd trial  | Distance in periphery zone        | 0.41 | 0.042  | NS  | NS  | NS  | NS  |
|                              | Fast time                         | 0.51 | 0.0095 | NS  | NS  | NS  | NS  |
|                              | Latency of 1st entrance to center zone | NS  | NS    | 0.29 | 0.036 | NS  | NS  |
| First S-duration recorded    | Distance in the periphery zone    | 0.43 | 0.033  | NS  | NS  | NS  | NS  |
|                              | Fast time                         | 0.50 | 0.010  | NS  | NS  | NS  | NS  |
| S-induction                  | Distance in the center zone       | NS  | NS    | -0.29| 0.030 | NS  | NS  |
|                              | Parallel index                    | NS  | NS    | -0.41| 0.0021 | NS  | NS  |
|                              | Total time in the center zone     | NS  | NS    | NS  | NS  | 0.42 | 0.044 |
| L-duration in the 2nd trial  | No. of entries in the center zone | -0.45| 0.026  | NS  | NS  | NS  | NS  |
|                              | Total time in the center zone     | -0.51| 0.0088 | NS  | NS  | NS  | NS  |
|                              | Distance in the center zone       | -0.48| 0.015  | NS  | NS  | NS  | NS  |
|                                | Value 1 | Value 2 | P-value | Level | Significance |
|--------------------------------|---------|---------|---------|-------|--------------|
| Mean speed without resting     | 0.40    | 0.045   | NS      | NS    | NS           |
| L-duration in the 3rd trial    | 0.46    | 0.022   | NS      | NS    | NS           |
| Mean speed without resting     | 0.47    | 0.017   | NS      | NS    | NS           |
| No. of excrements              | NS      | NS      | 0.34    | 0.012 | NS           |
| L-induction                    | Mean speed without resting | 0.40 | 0.045 | NS | NS |

No P value exceeded the Bonferroni-corrected 5% level.

NS = not significant at the nominal 5% level.