INTRODUCTION

Chickens were domesticated from jungle fowls (mainly red jungle-fowl) around areas of South and Southeast Asia (Liu et al., 2006; Peters, Lebrasseur, Deng, & Larson, 2016). After domestication, chickens have been bred worldwide and diversified greatly. There are several hundred chicken breeds in the world, and approximately 50 breeds of chickens in Japan (Tsudzuki, 2003). Chickens provide...
us food resources such as meat and eggs as well as opportunities for enjoyment of ornamental and behavioral characteristics, which include: long tail feathers, crowing, and aggressive and tameness behaviors (Ekarius, 2007). Productivity of egg and meat has improved markedly in layer and broiler industries, respectively. Of layers, the number of eggs per hen per year improved from 170 eggs in 1925 to as many as 325 eggs in 2006 (Besbes, Tixier-Boichard, Hoffmann, & Jain, 2007). Havenstein, Ferket, and Qureshi (2003) reported that genetics, nutrition, and other management changes over the last 44 years (from 1957 to 2001) have resulted in broilers that required approximately one-third time (32 vs. 101 days) and over a threefold decrease in the amount of feed consumed (feed conversion of 1.47 vs. 4.42) to produce an 1.815 g broiler, which means 85%–90% of the increase in broiler growth is contributed by genetic selection. Because the diverse indigenous breeds, layer, and broiler show a positive correlation with the increase in broiler growth, the Jidori brand is supported by opened free-range floor rearing systems (Inoue-Murayama, Nakamura, Nagao, & Ito, 2008). In order to prevent economic loss by the accidents, the chickens are required to be more gentle, tame, and imperturbable toward humans’ handling and environmental stimuli in behavioral traits.

Animal tameness has been investigated using experimentally domesticated animal resources that are foxes, rats, mice, and red junglefowls (Albert et al., 2011; Katajamaa, Larsson, Lundberg, Sorensen, & Jensen, 2018; Matsumoto et al., 2017; Wang et al., 2018). Behavioral tests have been established for measuring fox response to a human observer using a standard four-step test in their home cages (Nelson et al., 2017), rat response of tameness/aggressiveness when confronted with a gloved human hand in a test cage (Albert et al., 2008), and bird fear response toward humans in an arena measuring 100 × 300 × 210 cm (Agnvall, Jongren, Strandberg, & Jensen, 2012). Because tameness is defined as increased interaction of animals with humans and Price (2002) stated that tameness has two behavioral components which are “a measure of the extent to which an individual is reluctant to avoid or motivated to approach humans,” we established tameness tests for mice to measure active approaches to humans (active tameness) and a reduction in the avoidance of humans (passive tameness) separately (Goto, Tanave, Moriwaki, Shiroishi, & Koide, 2013; Nagayama et al., 2018). Since there is no example of a behavioral test to measure these kinds of tame behaviors using adult chickens in individual cages, we will introduce a handling test for tameness of adult chickens in this study. Breeding of Jidori often utilizes grand-parental and parental stocks in individual cages in the National Livestock Center and Prefectural Livestock Farms in Japan. If a suitable method to test tame behaviors of adult chickens is developed, breeding toward tameness of Jidori can be applied in the future. Since there is still no clear correlation between reactions to humans (tameness) and to environmental stimuli, the birds selected for tameness may show not only reduction in anxiety to humans but also imperturbable toward environmental stimuli in opened free-range floor rearing systems. Moreover, revealing the genetic basis of tame behavior is crucial to understanding future application to the livestock industry and the history of animal domestication (Goto, Matsumoto, Tanave, & Koide, 2015).

In this study, we try to establish a handling test for tame behavior of adult chickens in their home-cage. The objective of this study is to investigate breed and sex differences in tame behaviors by the handling test. This will be a first step in evaluating and understanding tameness in adult chickens.

2 | MATERIALS AND METHODS

2.1 | Animals

Five breeds and one F₁ hybrid of chickens were used in the Animal Research Center, Agricultural Research Department, Hokkaido Research Organization, Japan. A total of 100 birds from each sex of Shamo (SHA; n = 10), Rhode Island Red (RIR; n = 10), Nagoya (NGY; n = 10), Australorp (AUS; n = 10), and Ukokkei (UKO; n = 10) were investigated. In addition, we analyzed only hens of F₁ hybrid (F₁; n = 10) based on a cross between SHA males and RIR females. The chickens were reared under the photoperiod cycle of 16-hr light and
8-hr dark with free access to diets and water in the individual cages. Management was performed according to the rules of Standards Related to the Care and Management of Experimental Animals (Prime Ministers’ Office, Japan, 1980) and the Guide for the Use of Experimental Animals in Universities (The Ministry of Education, Science, Sports, and Culture, Japan, 1987).

2.2 | Tameness test

Based on the behavioral tests for tameness in foxes (Nelson et al., 2017), rats (Albert et al., 2008), and mice (Goto et al., 2013; Nagayama et al., 2018), we designed a handling test that measured active approaches to humans (active tameness) and a reduction in the avoidance of humans (passive tameness) in adult chickens. The handling test was performed using adult chickens (>23 weeks of age) in their home cages. The handling test was set for 60 s in total. There were two phases, which are the active tameness phase (first 40 s) and the passive tameness phase (last 20 s).

At the active tameness phase of the handling test, the experimenter’s hand stayed in the cage in order to evaluate active tameness of the chicken. The active tameness phase consisted of two steps. The first step was to keep the experimenter’s hand near the entrance of the cage for 20 s (Figure 1a). After that, the experimenter’s hand moved to the far end of the cage and stayed for 20 s, as the second step (Figure 1b). Immediately after the active tameness phase, the passive tameness phase was started. At the passive tameness phase of the handling test, the experimenter stroked the backs of the chickens in order to evaluate passive tameness of the chickens (Figure 1c). The frequency of stroking was set at approximately once per 2 s. After 20 s of passive tameness phase, the experimenter removed his hand from the cage.

We focused on direction and frequency of moving away in reactive behaviors during the handling test. If the chicken shows some interest and/or positive reaction toward the experimenter’s hand, the number of times heading toward the hand is expected to decrease along with being high in explorative behavior. Conversely, if the chicken expresses less interest and/or negative reactions toward the experimenter’s hand, the number of times heading toward the hand is expected to increase along with being high in active avoidance. Therefore, we measured both the number of times a chicken heads toward the experimenter’s hand (shown as heading) and the number of times a chicken avoids in other directions (shown as avoiding), as well as number of steps taken (shown as step) for locomotive activity. Behavioral analysis was performed in real time by two observers. An observer counted numbers heading and avoiding in active and passive tameness phases separately. The other observer counted number of steps during the handling test. Behaviors were recorded by a video camera (iVIS HF M41, Canon, Japan) for the purpose of confirmation.

2.3 | Statistics

In order to test effects according to breed and sex, we used 100 chickens in total in RIR, SHA, NGY, UKO, and AUS (n = 10 in each breed and sex). Main effects by breed and sex and their interaction effect were tested by two-way analysis of variance (ANOVA). If there is significant difference (p < .05), a post hoc test was performed by Tukey’s HSD test. In addition, in order to test the heterosis effect on behavioral traits, one-way ANOVA was carried out with the data of F₁ hybrid hens derived from a cross between SHA and RIR (n = 10), and hens of SHA and RIR (n = 10 in each breed). Pearson’s correlations among behavioral traits were calculated. Data were shown in mean ± standard deviation. Statistical analyses were conducted by R software (R Core Team, 2018).

3 | RESULTS

3.1 | Active tameness phase of the handling test

In the number heading at the active tameness phase (Figure 2a), two-way ANOVA revealed no main effects by breed (F₄,₉₀ = 1.239, p = .300) or sex (F₁,₉₀ = 0.001, p = .982), but a significant showing of interaction effects by breed × sex (F₄,₉₀ = 2.880, p = .027). Although SHA and NGY showed that females were lower than males, the
opposite relationship was seen in RIR, AUS, and UKO. This meant the combination of breed and sex influenced phenotypic variations in the heading trait at the active tameness phase. Mean values in all groups were low (from 0.9 to 5.8), which implying that the chickens showed low levels in active approaches to humans (active tameness).

In terms of avoiding at the active tameness phase (Figure 2b), there were significant main effects by breed ($F_{4,90} = 10.563, p = 4.62E^{-07}$) and sex ($F_{1,90} = 78.795, p = 6.22E^{-14}$) and their interaction effect by breed × sex ($F_{4,90} = 5.907, p = .0003$). This avoiding trait was thought to indicate a chicken’s tendency to avoid the mild stimulus, the presence of a human hand. Males exhibited a higher number avoiding in other directions from a human hand than the females. RIR and SHA were the highest and lowest mean values in males, respectively, whereas UKO and NGY were the highest and lowest of those in females. Since NGY females were especially lower than the others, NGY females showed the lowest level of avoidance of humans (passive tameness) from mild stimuli by human hands.

3.2 | Passive tameness phase of the handling test

Two-way ANOVA indicated no significant main effects by breed ($F_{4,90} = 1.792, p = .1373$) or sex ($F_{1,90} = 0.392, p = .5328$), but a significant interaction effect by breed × sex ($F_{4,90} = 3.152, p = .0179$) in the number heading at the passive tameness phase (Figure 3a). The heading trait was thought to be active tameness under the severer stimulus, forced handling by a human. Mean values ranged from 0 to 1.6, and all chickens in the six groups indicated zero value. This implied that the chickens do not actively approach a human hand under the condition of forced handling by a human.

In terms of avoiding at the passive tameness phase (Figure 3b), two-way ANOVA revealed significant main effects by breed ($F_{4,90} = 3.097, p = .0194$) and sex ($F_{1,90} = 41.478, p = 5.71E^{-09}$), but not by their interaction effect ($F_{4,90} = 2.343, p = .0608$). The avoiding trait was thought to show a chicken’s tendency to avoid forced handling by a human. Males showed higher number avoiding than females. NGY and AUS indicated lower mean values than the others, especially in females, which implying these birds were thought to have a high level of passive tameness.

3.3 | Locomotive activity in handling test

In number of steps taken during the handling test (Figure 4), there were significant main effects by breed ($F_{4,90} = 5.530, p = .0005$)
and sex ($F_{1,90} = 67.106, p = 1.64E-12$) and their interaction effect ($F_{2,90} = 7.142, p = 4.85E-05$). The step trait indicated total amount of locomotive activity. Males basically showed higher activity than females. Females in RIR, NGY, and AUS indicated lower activity, whereas males in NGY and AUS showed higher activity. On the other hand, UKO indicated comparable levels of activity between males and females.

### 3.4 | Phenotypic correlations among behavioral traits

In this study, five behavioral traits were evaluated with males and females from five breeds ($n = 100$). In three traits, significant sex effects were detected. Therefore, we calculated phenotypic correlations in each sex in order to compare the relationships between the different behavioral traits. Pearson’s correlation coefficients among the five traits in males and females were shown in Figures 5 and 6. There were positive phenotypic correlations between avoiding at active tameness phase and avoiding at passive tameness phase ($r = .30$ and $.48$ in male and female, respectively) and between avoiding at active tameness phase and number of steps during handling test ($r = .32$ and $.63$ in male and female, respectively). In males, there was a negative correlation ($r = -.63$) between heading and avoiding at the active tameness phase. On the other hand, in females, a positive correlation ($r = .47$) was seen between avoiding at the passive tameness phase and number of steps counted during the handling test. In the remaining trait combinations, correlation coefficients were statistically comparable to zero ($p > .05$). These indicated there is a similar tendency of phenotypic correlations in males and females. In the case of high value in avoiding, locomotive activity and active approach behavior will show increases and decreases, respectively.

#### 3.5 | Heterosis effect

In order to evaluate the heterosis effects on behavioral traits, we analyzed female data from SHA, RIR, and their F1 hybrid. In Figure 7, one-way ANOVA revealed significant effects by breed in avoiding at active tameness phase ($F_{2,27} = 8.624, p = .0013$) and step ($F_{2,27} = 11.290, p = .0003$). Tukey’s HSD tests indicated there were significant differences between F1 hybrid and their parental breeds ($p < .05$), but no difference among parental breeds ($p > .05$). The F1 hybrid had higher avoidance and locomotive behaviors than those of the parental breeds.

### 4 | DISCUSSION

The present study aimed to make a new handling test to quantify animal tameness in adult chickens reared in individual cages. Male chickens indicated higher avoidance behavior from a human hand than females. NGY female had the lowest level of avoidance behavior, which implies passive tameness. In terms of active tameness, a breed × sex interaction effect was observed, which indicated a variety of phenotypes can be obtained in different combinations of breed and sex. There was a type of individual that showed active tame behavior toward humans, whereas no individual expressed aggressive (nontame) behavior toward humans in this study. These results suggested that the handling test has a potential to reveal characteristics of animal tameness, such as active tameness ("motivation to approach humans") and passive tameness ("reluctant to avoid humans") behaviors, even though the duration of the handling test is only one minute per individual. Thus, the handling test will be one of the optimal methods for rapid screening of individual differences in chickens’ tame behavior toward humans.

Regarding tameness, our previous genetic correlation analyses with 17 wild-derived and laboratory strains of mice revealed evidence that when a higher level of passive tame behavior is observed, active tame behavior tends to be high (Goto et al., 2013). Furthermore, comparative analyses between wild and domestic mice revealed that the heritable behavioral characteristics, reluctance to avoid humans but not motivation to approach humans, will be the key for domestication of mice (Goto et al., 2013). There is massive evidence that long-term selection of tameness significantly increased the tame behavior in foxes, rats, and mice (Matsumoto et al., 2017; Trut, 1999). Agnvall et al. (2012) have estimated 17% of heritability for fear-related behavior in the fear of humans test with chickens’ wild ancestor, red junglefowl (RJF). Given that animal tameness is defined some extent to increase interaction of animals with humans, tameness is involved with many behavioral characters including anxiety, fear, and novelty seeking. There are some hypotheses that (a) animals with higher tameness show low levels of anxiety and fearfulness toward not only humans but also other external stimuli and (b) high-tameness animals show a high level of novelty seeking toward humans and the other stimuli. In the Jidori brands, there are sometimes severe crowding
accidents where hundreds of birds in the flock die due to environmental stimuli, such as thunder. There are proactive and reactive coping styles in animals. Proactive animals show a more sympathetic stress activation (flight/fight), although reactive animals often respond to a stressful situation by a higher parasympathetic stress activation (withdrawal reaction; Favati, Leimar, & Løvlie, 2014). Proactive/reactive animals tend to show higher/lower in aggressive and active avoidance, and lower/higher in HPA axis reactivity, respectively. Moreover, reactive animals are more flexible and react to environmental stimuli (Koolhaas et al., 1999). Further studies are needed to understand the relationship between reactions to humans (tameness) and the other behavioral and physiological traits, including reactivity to environmental stimuli, as well as for productivity of eggs and meat.
Natt, Agnvall, and Jensen (2014) have reported substantial sex differences in chicken behavior with many RJF, using a battery of behavioral tests for phenotyping different aspects of fear, exploration, and sociability. In open field tests and foraging and exploration tests, males displayed less fear and less exploration than females. On the other hand, in predator tests and tonic immobility tests, females were more fearful than males. They concluded that females were more explorative and prone to forage, but more fearful in their reactions to various stressful stimuli in RJF (Natt et al., 2014). Nakasai et al. (2013) also indicated sexual differences in fear response via tonic immobility in Tosa-Jidori chicks, which indicated females were more fearful than males. Conversely, our handling test revealed that females exhibited more passive tameness than males. This evidence may imply that female chickens in this study accepted handling (passive tameness) with some levels of fear-related behavior, because life-threatening events tend to induce the animals to both fight or flight and freezing (Peres, Goncalves, & Peres, 2009).

Regarding this, male chickens in this study showed higher levels of fearfulness to human and higher basal level of corticosterone compared to the parent genotypes. This evidence indicated that there will be a possible common mechanism underlying heterosis of response to humans in not only avian but also other livestock animals. Since it is very likely that the presence or the absence of molecular heterosis can also depend on the genetic background of stock animals. Since it is very likely that the presence or the absence of molecular heterosis can also depend on the genetic background of stock animals.

In domestic chickens, there was an effect on fear response by breed using tonic immobility test for chicks of NGY and White Leghorn (WL), which indicated WL showed relatively lower fear levels than NGY (Abe, Nagao, Nakamura, & Inoue-Muraiyama, 2013). Tomonaga et al. (2007) investigated stress responses in chicks using both meat-type and layer-type NGY, which are selected according to two directions over a few decades and found that meat-type chicks indicated bolder behavior under isolation-induced stress than the layer-type of NGY. In this study, we analyzed meat-type NGY, which is a different strain from the previous ones (Tomonaga et al., 2007), and found that NGY females are less likely to avoid handling. These results imply that behaviors in chicks will be a good indicator to estimate those behaviors in the adult stage. Favati, Zidar, Thorpe, Jensen, and Lovlie (2016) have investigated ontogeny of personality traits in RJF from chick (4 weeks of age) to adult (40 weeks of age). They performed three different personality tests, which are as follows: the novel arena test, the tonic immobility test, and the predator test; and found that the adult personality can only to some extent be predicted early in life (Favati et al., 2016). Although there are some studies using chicks in Japanese breeds, there may be low correlations among them. In the future, behavioral relationships between chicks and adult chickens should be analyzed more using many Japanese breeds of chickens. For breeding tameness, it may be effective to use behavioral phenotypes at several stages.

As Price (2002) indicated that tameness is defined “reluctant to avoid or motivated to approach humans,” a low level of fearfulness will be common behavioral characteristics in domestic animals. Actually, Campler, Jongren, and Jensen (2009) have reported that the fear response of WL is less than chicken's wild ancestor, RJF in four different behavioral tests including fear of humans test, which suggested selection for low fearfulness has been an important element of domestication. Agnvall, Katajamaa, Altimiras, and Jensen (2015) have selected RJF for high or low fear of humans during six generations and revealed that low-fear birds showed
higher basal metabolic rate, feed efficiency, and boldness in novel object test. Selection of tameness brought many of phenotypic changes in tissue size (adrenal gland and spleen), serum and brain biochemical components (corticosterone, glucose, serotonin, and taurine) in rats, and physical changes (coat color, floppy ears, and rolled tails) in foxes, along with the Belyaev’s hypothesis (Albert et al., 2008; Trut, 1999). There are massive efforts to understand the genetic basis of animal domestication using comparative population genomics with chickens, dogs, rabbits, cats, and their wild ancestors (Axelsson et al., 2013; Carneiro et al., 2014; Montague et al., 2014; Rubin et al., 2010). Since animal tameness is a key feature for domestication, further researches are required to reveal the genetic basis regulating the phenotypic variation in tameness.

In this study, our efficient handling test can reveal phenotypic differences by breed and sex in tameness of adult chickens. Osman et al. (2006) revealed a wide variety of genetic backgrounds among SHA, NGY, UKO, and RIR. In the future, we would like to analyze the tameness with cross experiments to reveal whether behavioral changes among breeds are due to overall genomic differences or some specific genetic differences related to tameness. Combining both the behavioral screening and the population genomics will find types of evidence about the mechanism of tameness and domestication in animals.

In conclusion, we established a new handling test to evaluate tame behavior of adult chickens and revealed breed and sex differences and heterosis effects. The Japanese Jidori industry, which aimed to increase value-added meat production should pay attention to behavioral responses toward humans in the future. This study is a first step to conduct genetic study of tame behavior in chickens.

REFERENCES

Abe, H., Nagao, K., Nakamura, A., & Inoue-Murayama, M. (2013). Differences in responses to repeated fear-relevant stimuli between Nagoya and White Leghorn chicks. Behavioural Processes, 99, 95–99. https://doi.org/10.1016/j.beproc.2013.07.004

Agnvall, B., Jongren, M., Strandberg, E., & Jensen, P. (2012). Heritability and genetic correlations of fear-related behavior in Red Junglefowl-possible implications for early domestication. PLoS ONE, 7, e35162.

Agnvall, B., Katajamaa, R., Altimiras, J., & Jensen, P. (2015). Is domestication driven by reduced fear of humans? Boldness, metabolism and serotonin levels in divergently selected red junglefowl (Gallus gallus). Biology Letters, 11, 20150509.

Albert, F. W., Hodges, E., Jensen, J. D., Besnier, F., Xuan, Z., Rooks, M., ... Pääbo, S. (2011). Targeted resequencing of a genomic region influencing tameness and aggression reveals multiple signals of positive selection. Heredity, 107, 205–214. https://doi.org/10.1038/hdy.2011.4

Albert, F. W., Shchepina, O., Winter, C., Römpler, H., Teupser, D., Palme, R., ... Pääbo, S. (2008). Phenotypic differences in behavior, physiology and neurochemistry between rats selected for tameness and for defensive aggression towards humans. Hormones and Behavior, 53, 413–421. https://doi.org/10.1016/j.yhbeh.2007.11.010

Arnaud, I., Mignon-Graustein, S., Larzul, C., Guy, G., Faure, J.-M., & Guémené, D. (2008). Behavioural and physiological fear responses in ducks: Genetic cross effects. Animal, 2, 1518–1525. https://doi.org/10.1017/S1751711008002784

Axelsson, E., Ratnakumar, A., Arendt, M.-L., Maqbool, K., Webster, M. T., Perloski, M., ... Lindblad-Toh, K. (2013). The genomic signature of dog domestication reveals adaptation to a starch-rich diet. Nature, 495, 360–364. https://doi.org/10.1038/nature11837

Besbes, B., Tixier-Boichard, M., Hoffmann, I., & Jain, G. L. (2007). Future trends for poultry genetic resources. In Proceedings of the International Conference of Poultry in the 21st Century: Avian Influenza and Beyond, Bankok, 5–7 November 2007. Retrieved from http://www.fao.org/waicent/FAOINFO/Agricult/gainfo/home/events/bangkok2007/docs/part1/1_8.pdf

Campler, M., Jongren, M., & Jensen, P. (2009). Fearfulness in red junglefowl and domesticated White Leghorn chickens. Behavioural Processes, 81, 39–43. https://doi.org/10.1016/j.beproc.2008.12.018

Carneiro, M., Rubin, C.-J., Di Palma, F., Albert, F. W., Alfoldi, J., Barrio, A. M., ... Andersson, L. (2014). Rabbit genome analysis reveals a polygenic basis for phenotypic change during domestication. Science, 345, 1074–1079. https://doi.org/10.1126/science.1253714

Comings, D. E., & MacMurray, J. P. (2000). Molecular heterosis: A review. Molecular Genetics and Metabolism, 71, 19–31. https://doi.org/10.1006/mgme.2000.3015

Ekarius, C. (2007). Storey’s illustrated guide to poultry breeds. North Adams, MA: Storey Publishing.

Faure, J. M., Val-Laillet, D., Guy, G., Bernadet, M. D., & Guemene, D. (2003). Fear and stress reactions in two species of duck and their hybrid. Hormones and Behavior, 43, 568–572. https://doi.org/10.1016/S0018-506X(03)00062-X

Favati, A., Leimar, O., & Levlie, H. (2014). Personality predicts social dominance in male domestic fowl. PLoS ONE, 9, e103535. https://doi.org/10.1371/journal.pone.0103535

Favati, A., Zidar, J., Thorpe, H., Jensen, P., & Levlie, H. (2016). The ontogeny of personality traits in the red junglefowl, Gallus gallus. Behavioural Ecology, 27, 484–493.

Goto, T., Matsumoto, Y., Tanave, A., & Koide, T. (2015). Genetic analyses for tame behavior in animals: Exploration of genetic loci affecting animal domestication. Journal of Animal Genetics, 43, 3–11 (In Japanese). https://doi.org/10.5924/abgri.43.3

ACKNOWLEDGMENTS

We appreciate all staff members in Animal Research Center, Agricultural Research Department, Hokkaido Research Organization for rearing the chickens and all members of Animal Breeding Research Group in the Obihiro University of Agriculture and Veterinary Medicine for continuous supports. We thank especially Kanna Takahashi for her drawing the pictures in Figure 1.

CONFICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Tatsuhiko Goto https://orcid.org/0000-0002-1787-7533
Goto, T., Tanave, A., Moriwaki, K., Shirioishi, T., & Koide, T. (2013). Selection for reluctance to avoid humans during the domestication of mice. Genes, Brain and Behavior, 12, 760–770.

Goto, T., & Tsudzuki, M. (2017). Genetic mapping of quantitative trait loci for egg production and egg quality traits in chickens: A review. Journal of Poultry Science, 54, 1–12. https://doi.org/10.2141/jpsa.0160121

Havenstein, G. B., Ferket, P. R., & Qureshi, M. A. (2003). Growth, liveness, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science, 82, 1500–1508. https://doi.org/10.1093/ps/82.10.1509

Havenstein, G. B., Ferket, P. R., & Qureshi, M. A. (2003). Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science, 82, 1509–1518. https://doi.org/10.1093/ps/82.10.1509

Hong, K. W., Inoue-Murayama, M., Nakamura, A., Nagao, K., & Ito, S. (2008). Characterization of two microsatellites in chicken monoamine oxidase A. Animal Science Journal, 79, 641–643. https://doi.org/10.1111/j.1740-0929.2008.00575.x

Katajamaa, R., Larsson, L. H., Lundberg, P., Sorensen, I., & Jensen, P. (2018). Activity, social and sexual behaviour in Red Junglefowl selected for divergent levels of fear of humans. PLoS ONE, 13, e0204303. https://doi.org/10.1371/journal.pone.0204303

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., ... Blokhuis, H. J. (1999). Coping styles in animals: Current status in behavior and stress-physiology. Neuroscience and Biobehavioral Reviews, 23, 925–935. https://doi.org/10.1016/S0149-7634(99)00026-3

Liu, Y.-P., Wu, G.-S., Yao, Y.-G., Miao, Y.-W., Luikart, G., Baig, M., ... Zhang, Y.-P. (2006). Multiple maternal origins of chickens: Out of the Asian jungles. Molecular Phylogenetics and Evolution, 38, 12–19. https://doi.org/10.1016/j.ympev.2005.09.014

Matsumoto, Y., Goto, T., Nishino, J. O., Nakaoka, H., Tanave, A., Takano- Shimizu, T., ... Koide, T. (2017). Selective breeding and selection mapping using a novel wild-derived heterogeneous stock of mice revealed two closely-linked loci for tameness. Scientific Reports, 7, 4607. https://doi.org/10.1038/s41598-017-04869-1

Montague, M. J., Li, G., Gandolfi, B., Khan, R., Aken, B. L., Searle, S. M. J., ... Warren, W. C. (2014). Comparative analysis of the domestic cat genome reveals genetic signatures underlying feline biology and domestication. Proceedings of the National Academy of Sciences of the United States of America, 111(48), 17230–17235. https://doi.org/10.1073/pnas.140083111

Nagayama, H., Matsumoto, Y., Tanave, A., Nihei, M., Goto, T., & Koide, T. (2018). Measuring active and passive tameness separately in mice. Journal of Visualized Experiments, 138, e58048. https://doi.org/10.3791/58048

Nakasei, E., Tanizawa, H., Takawaki, M., Yanagita, K., Kawakami, S.-I., Oka, T., ... Bungo, T. (2013). Age-dependent change of tonic immobilization response in chicks of a native Japanese chicken breed, Tosa-Jidori. Journal of Poultry Science, 50, 321–325. https://doi.org/10.2141/jpsa.0130018

Natt, D., Agnwall, B., & Jensen, P. (2014). Large sex differences in chicken behavior and brain gene expression coincide with few differences in promoter DNA-methylation. PloS ONE, 9, e96376. https://doi.org/10.1371/journal.pone.0096376

Nelson, R. M., Temnykh, S. V., Johnson, J. L., Khramanova, A. V., Vladimirova, A. V., Gulevich, R. G., ... Kukekova, A. V. (2017). Genetics of interactive behavior in silver foxes (Vulpes vulpes). Behavior Genetics, 47, 88–101. https://doi.org/10.1007/s10519-016-9815-1

Osman, S. A. M., Sekino, M., Nishihata, A., Kobayashi, Y., Takenaka, W., Kinoshita, K., ... Tsudzuki, M. (2006). The genetic variability and relationships of Japanese and foreign chickens assessed by microsatellite DNA profiling. Asian-Australasian Journal of Animal Science, 19, 1369–1378. https://doi.org/10.5713/ajas.2006.1369

Peres, J. F. P., Goncalves, A. L., & Peres, M. F. P. (2009). Psychological trauma in chronic pain: Implications of PTSD for fibromyalgia and headache disorders. Current Pain and Headache Reports, 13, 350–357. https://doi.org/10.1007/s11916-009-0057-2

Peters, J., Lebrasseur, O., Deng, H., & Larson, G. (2016). Holocene cultural history of Red jungle fowl (Gallus gallus) and its domestic descendant in East Asia. Quaternary Science Reviews, 142, 102–119. https://doi.org/10.1016/j.quascirev.2016.04.004

Price, E. O. (2002). Animal domestication and behavior. New York, NY: CABI Publishing.

R Core Team (2018). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rikimaru, K., & Takahashi, H. (2010). Evaluation of the meat from Hinaijidori chickens and broilers: Analysis of general biochemical components, free amino acids, inosine 5’-monophosphate, and fatty acids. The Journal of Applied Poultry Research, 19, 327–333. https://doi.org/10.3382/japr.2010-00157

Rubin, C. J., Zody, M. C., Eriksson, J., Meadows, J. R. S., Sherwood, E., Webster, M. T., ... Andersson, L. (2010). Whole-genome resequencing reveals loci underlying selection during chicken domestication. Nature, 464, 587–591. https://doi.org/10.1038/nature08832

Sasaki, K., Motoyama, M., Tagawa, Y., Akama, K., Hayashi, T., Narita, T., & Chikuni, K. (2017). Qualitative and quantitative comparisons of texture characteristics between broilers and Jidori-niku, Japanese indigenous chicken meat, assessed by a trained panel. Journal of Poultry Science, 54, 87–96.

Tomonaga, S., Noda, K., Suenaga, R., Asechi, M., Adachi, N., Kino, K., ... Furuse, M. (2007). Stress responses in neonatal meat and layer Nagoya chicks. Animal Science Journal, 78, 541–545. https://doi.org/10.1111/j.1740-0929.2007.00474.x

Trut, L. N. (1999). Early canid domestication: The farm-fox experiment. American Scientist, 87, 160–169. https://doi.org/10.1511/1999.2.160

Tsudzuki, M. (2003). Japanese native chickens. In H. L. Chang & Y. C. Tsudzuki, M. (2003). Japanese native chickens. In H. L. Chang & Y. C. Chang, M. (Eds.), The relationship between indigenous animals and humans in APEC region (pp. 91–116). Tainan, Taiwan: CSAS.

Wang, X., Pipes, L., Trut, L. N., Herbeck, Y., Vladimirova, A. V., Gulevich, R. G., ... Clark, A. G. (2018). Genomic responses to selection for tame/aggressive behaviors in the silver fox (Vulpes vulpes). Proceedings of the National Academy of Science of the United States of America, 115, 10398–10403.

How to cite this article: Moroi S, Nishimura K, Imai N, Kunishige K, Sato S, Goto T. Rapid behavioral assay using handling test provides breed and sex differences in tameness of chickens. Brain Behav. 2019;9:e01394. https://doi.org/10.1002/brb3.1394