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White Leghorn and Red Junglefowl female chicks use distal and local cues similarly, but differ in persistency behaviors, during a spatial orientation task

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ABSTRACT

Although there is evidence to suggest that animal domestication acts as a modulator of spatial orientation, little is known on how domesticated animals, compared to their wild counterparts, orientate themselves when confronted to different environmental cues. Here, using domesticated White Leghorn chicks, and their ancestor, the Red Junglefowl (*Gallus gallus*), our main objective was to investigate how bird domestication influences the use of distal and local cues, during an orientation task. We also investigated the memory retention of these cues over time, and how persistent/flexible individuals from both breeds were at pecking at unreachable mealworms. Our results showed that the breeds did not differ in their use of distal or local cues, with both showing a marked preference for the use of local cues over distal ones. Over time, individual performance declined, but this was not influenced by the type of cue present during the tests, nor by the breed. Domesticated chicks showed greater signs of persistency compared to their wild conspecifics. In conclusion, domestication did not seem to alter how birds orientate spatially, but may have caused more subtle changes, such as an increase in behavioral persistency, a feature that may be adaptive in human-controlled and homogenous environments.

1. Introduction

Animals, both in the wild and in captive settings, are constantly relying on surrounding, environmental cues to adapt their behavior accordingly. While some environmental cues allow the animals to perceive daily/seasonal changes over time (e.g., light/dark cycles, temperature fluctuations), other cues enable the individuals to orientate themselves in their spatial environment and more rapidly find the necessary resources to survive and reproduce. Over the last decades, research on animal spatial orientation showed that animals can rely on different sources of environmental information, from simpler to more complex, to reach a particular goal (Cheng and Newcombe, 2005; Vallortigara, 2009). One of the simplest ways for an individual to orientate in an environment is to rely on a stimulus-response association between its goal and the local cues near to it, such as color, shape, and odors (Cheng et al., 2013; Morandi-Raikova et al., 2020). For example, if a conspicuous green tree is near a food patch (goal), the featural characteristics of this object (the color and the shape of the tree) can be treated

individually (and primarily) from other cues in the environment. The orientation based on cues far from the goal, i.e., distal cues, may also be an available option, but it is usually considered to be more complex than the use of local cues. The use of distal cues considers the creation of a cognitive map, i.e., a mental representation of how the goal and the different objects in the environment are spatially arranged (Lormant et al., 2020a; Packard and Goodman, 2013; White and McDonald, 2002).

When confronted with different types of cues at the same time, individuals may differ on how they favor one type of cue over another (Poldrack and Packard, 2003). In mammals and birds, there is evidence that stress and highly negative emotions reduce the abilities of the animals to use distal cues and induce a preference for the use of local cues (Lormant et al., 2020b; Packard, 2009). Inter-individual differences on various behavioral traits, such as fearfulness and boldness, may also influence which preferred cue the individuals will rely on. For example, in an orientation task where quail (*Coturnix coturnix*) selected for divergent levels of emotionality were trained to reach a goal either based on distal cues or local cues, quail selected for high emotionality

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relied preferentially on distal cues, while less emotional birds relied more on local cues (Lormant et al., 2018). Variation in exploratory tendencies may also impact how well animals use different environmental cues: in free-range systems, broiler chickens (*Gallus gallus domesticus*) that used the outdoor range less performed better when requested to use distal cues compared to chickens that used the outdoor range more. However, no group differences were found for the use of local cues (Ferreira et al., 2019, 2020a).

Another less studied factor that may impact how individuals orientate in space is the process of animal domestication. When domesticated animals and their wild counterparts are compared, beyond the physical and zootechnical changes (e.g., increased growth and improved feed conversion), it is also known they may differ on various neurocognitive and behavioral parameters (e.g., reduced brain size and fearfulness, and increased boldness) (Agnvall et al., 2018, 2017; Campler et al., 2009; Katajamaa and Jensen, 2020). However, when it concerns spatial orientation, there is little (mostly focused on mammals) and contrasting evidence for the domestication impacts on this cognitive ability. On the one hand, wild and domesticated rat (*Rattus norvegicus*) strains performed similarly in the Morris water maze task, a task requiring the use of distal cues to find a hidden platform (Troy Harker and Whishaw, 2002). On the other hand, domesticated guinea pigs (*Cavia porcellus*) had superior performance compared to wild cavies (*Cavia aperea*) in the same task (Lewejohann et al., 2010). Although these results come from orientation tests where only the use of distal cues was investigated, one can hypothesize that, for some species, domestication may impact the way individuals (wild vs. domesticated) use distal and local cues. However, to further confirm this hypothesis more research is required, with a particular need to include other taxa of domesticated species, such as domesticated birds, for a greater understanding of the phenomenon.

In the present work, our main aim was to further investigate whether and how domestication influences the spatial orientation of captive domestic and wild birds, focusing on the use of distal and local cues. On this purpose, we used the domesticated White Leghorn chickens, and its ancestor, the Red Junglefowl (*Gallus gallus*), as the animal models. Individual birds were trained and tested on an orientation task that was previously validated on domestic birds, such as quail and free-range broiler chickens (Ferreira et al., 2019, 2020a; Lormant et al., 2020b, 2020a, 2021). Firstly, birds were trained to find a food reward when both distal and local cues were available. After training, individuals were tested in two different test settings. During the first test, the local cue was absent. Therefore, the animals could rely solely on the use of distal cues. During the second test, the local cue was displaced. Individuals could then either follow the local cue to its new position, or again rely on the distal cues to find their goal.

In addition to our main objective, we also investigated the memory retention of these cues, and how they fade over time. For this, the animals were retested for a second time, one week later, without retraining between tests. Finally, at the end of second test day, and to verify whether possible performance differences could arise from other cognitive/behavioral factors between breeds (e.g., behavioral flexibility), in a third test, a persistency test, we measured how persistent individuals were at pecking at unreachable mealworms.

Based on previous results on the spatial orientation of domestic birds (Ferreira et al., 2020a; Lindqvist and Jensen, 2009; Lormant et al., 2018, 2020b; Morandi-Raikova et al., 2020), we predicted that White Leghorn, due to the selection for improved adaptation to a more homogeneous and less complex environment, would perform less well than Red Junglefowl in the use of distal cues, while both breeds would prefer the local cue when in a conflicting situation (local vs. distal cue). Concerning the memory retention of the cues, we predicted that, due to its greater complexity, the memory of distal cues would fade more quickly compared to that of local cues (Lormant et al., 2020a; Packard and Goodman, 2013; White and McDonald, 2002). Finally, since these two breeds were shown to present different foraging strategies (Andersson

et al., 2001; Lindqvist et al., 2002; Lindqvist and Jensen, 2008), we expected White Leghorns to be more persistent than Red Junglefowl.

2. Methods

2.1. Ethical statement

This study was conducted at the University of Linkoping, Sweden, in November 2021. All applicable international, national, and/or institutional guidelines for the care and the use of animals followed the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Linköping Council for Ethical Licensing of Animal Experiments, license number 14916-2018.

2.2. Animals, housing, and previous experiences

Birds from two different breeds were used in this experiment: the domesticated laying hybrid breed White Leghorn (WL), and the wild breed Red Junglefowl (RJF). While WL eggs were obtained from a commercial hatchery, RJF eggs originated from a RJF parental population bred in our research facilities for about 20 generations (for further details concerning this captive wild population, see Schutz and Jensen, (2001)). Eggs from both breeds were incubated and hatched under the same conditions. From day 1 to day 17 of incubation, the incubator ambient settings were of 37.8 °C, and 55% of relative humidity. On day 18, and until hatching, eggs were placed in a hatcher with the following settings: 37.5 °C and 65% relative humidity.

On the day after hatching (D2), the chicks were taken out of the incubator, weighed, and identified with leg rigs. 40 WL and 40 RJF mixed-sex chicks were separated (per breed) and housed in solid floor cages ($0.7 \times 0.68 \times 0.57$ m), in four groups of 20 individuals/cage. All cages were supplied with sawdust, a heating roof, a feeder, and a water bell. On D23, following chick growth, all individuals were moved to two large pens (0.7×2.1 m), with 40 individuals/pen (separated per breed). On D42, the pens were once again enlarged (0.7×2.8 m). The cages/pens were cleaned once a week, and both had a 12-h light/dark schedule.

Over their first seven weeks of life, and twice a week, from D8 to D53, all individuals participated in behavioral tests aimed at measuring their play behavior (Oscarsson et al., in prep.). Hence, the birds were accustomed to human handling. No other behavioral or cognitive tests were performed on these animals.

2.3. Orientation task

Since females are known to be more food motivated than males (Zidar et al., 2018), at the end of the play experiment (D53), we selected only females from both breeds to participate in our orientation task. On D57, 18 females from each breed (WL and RJF) were moved from the pens back to the cages (8–10 individuals/cage) and stayed there for the whole/part of the experiment (see 2.3.1 for more information). No changes to husbandry procedures occurred in this period.

The orientation task lasted over three weeks, from D57 to D78, and comprised different task phases: habituation, training, tests, and retests. Half of the individuals were tested in the morning (8–12 h30), while the other half was tested in the afternoon (12h30–17 h). One hour before the beginning of each half day of testing, food was removed from the cages, to increase individual motivation for the food reward (mealworms) in the arena. Birds were transported from the cages to the test arena in a cardboard box (transport time less than 30 s), placed in the arena with their heads facing the arena wall, and released back into the cages after the trial was finished. At the end of each day period (morning or afternoon), individuals were given access to food ad libitum. The testing order of individuals was balanced between breeds and kept similar throughout the experiment.

The following procedures and the arena used for the orientation tests

were previously used and validated on domestic birds (Ferreira et al., 2019, 2020a; Lormant et al., 2018, 2020b, 2020a, 2021). Briefly, the arena was a cardboard octagonal structure (1.45 × 1.45 × 0.9 m), with a brown rubber mat floor, lighted by a central lamp (40 lux), and surrounded by a white curtain, to prevent birds from escaping. Black visual cues of different shapes (rectangles, triangles, circles, and stars) were fixated on four walls of the arena, allowing the individual to orientate itself during the task (Fig. 1). Eight equidistant cups were placed in this arena. The cups were 13,5 cm high and 5 cm deep, which required individuals to be near the cups to see whether it contained or not a food reward (mealworms). All behaviors were observed and recorded directly by the same experimenter (VHBF) outside of the view of the tested animal, using a digital video camera recorder connected to a monitor.

2.3.1. Habituation

To habituate individuals to the arena and its components (cups, mealworms), the habituation phase was carried out in three steps: in groups, in pairs, and, finally, individually. Firstly, individuals from the same breed were placed once a day in groups of four/five individuals in the arena and allowed to explore it for 10 min, for 2 consecutive days (from D57 to D58). During group habituation, all eight cups (four black cups and four white cups) contained a mealworm. Every day, the arrangement of these cups was changed randomly. The starting point for introduction of individuals into the arena also changed each day.

Habituation in pairs and individual habituation were carried over 3 days each (from D59 and D61, and from D63 and D66, respectively), and followed the same principles of group habituation. However, during individual habituation, we recorded the number of cups visited (number of times the bird approached the cups and ate the mealworm) over 10 min, or until all the mealworms were eaten, for each daily trail.

By the end of individual habituation, and due to time constraints, half of the individuals (n = 9 per breed) were selected to proceed to training and testing. Only birds that visited (and ate the mealworms) the most cups were selected ($\bar{X}_{\text{white Leghorns}}$ = 8 cups visited, $\bar{X}_{\text{Red Junglefowl}}$ = 7,73 cups visited). Unselected birds were excluded from the next phases of the experiments, and removed from the cages (4–5 five individuals/cage, after selection).

2.3.2. Training

Training was carried out from D69 to D71, with two training trials per day (spaced ca. one hour apart). The duration of the training phase (in days) was decided based on a daily assessment of the birds' behavior, with the goal that, by the end of training, both groups would learn the task similarly and consistently.

During this phase, only one cup, the target cup, was food rewarded (three mealworms). The target cup was of black color, amongst the other seven white cups. The positioning of the cups was the same for all birds, and the target cup was always placed at the corners. The trial was considered to be over when the tested individual reached the target cup, or after the total time has elapsed (5 min/trial). Three possible starting points were chosen, and changed from trial to trial, to prevent individuals solving the task based on the position of their bodies (use of egocentric cues).

The variable recorded was the number of cups visited before reaching the target cup and eating the mealworm (from 0 to 7) (i.e., number of times the tested individual approached and inspected the cups, either by placing its head above the cup, or stretching its neck ca. 10 cm from the cup, to see its contents). If the bird did not reach the target cup, it was scored the maximum number of cups visited (seven cups).

One RJF was excluded from further testing, due to multiple escapes from the arena and signs of distress (e.g., vocalizations, freezing). The total number of birds for the next phase was 17, $n_{\text{white Leghorns}}$ = 9, $n_{\text{Red Junglefowl}}$ = 8.

2.3.3. Tests

At the end of training, birds were tested individually in two (on day 72) and three (on day 79) testing situations. Each test was performed only once during a testing day, in the same arena and in the same room, for all birds. No retraining was performed between the two testing days. The position of the cups was kept the same for all three tests, and both testing days. To prevent any odor cues, no mealworms were available in the cups or the arena.

The first test, the distal test, aimed to investigate how birds used the distal cues. Therefore, all cups within the arena were white (Fig. 1). To reach the target cup, animals could orientate themselves based solely on the distal cues (black patterns fixated on the arena walls). The distal test
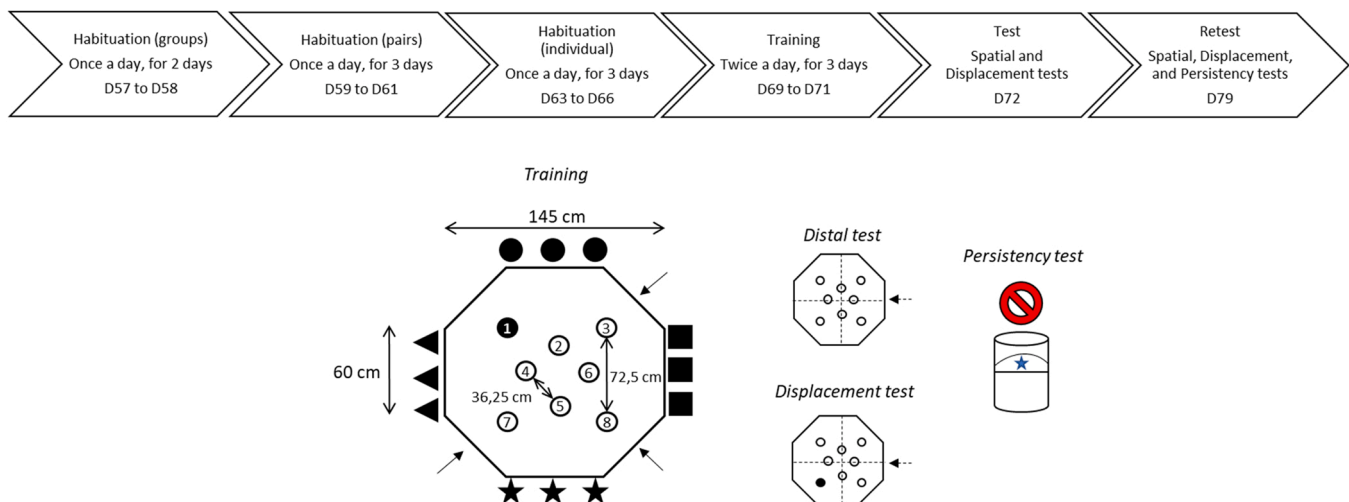

Fig. 1. Schedule of the experiment and schematic representation of the apparatus used for training and testing. At 8 weeks of age, White Leghorn (WL) and Red Junglefowl (RJF) chicks started the habituation (in groups, in pairs, and individually) to our orientation task arena and its components (cups and mealworms). After the end of habituation phase, individuals were trained that a black cup, the target cup, which was always at the same position, was the only cup rewarded in the apparatus (three mealworms), among seven white non-rewarded cups. To reach the target cup, birds could orientate themselves based on black patterns located on the walls of the arena (distal cues) and/or use the color of the cup as a cue (local cue). The birds were then tested in a distal test, where all cups were white, and a displacement test, where the black cup was relocated to a new position. Birds had three different starting points (dark arrows) during training, but only one during testing. The dotted lines represent the division of apparatus into four different quadrants. Birds were retested, one week later, on both distal and displacement tests, and also in a persistency test, where birds could peck at unreachable mealworms (blue star), in a black cup covered by a transparent glass.

was systematically followed by a second test, the displacement test, on both testing days.

During the displacement test, the target black cup was relocated to another position within the arena. Animals could either rely on the distal cues and go to previous position of the target cup (as during training), or use preferentially the local cue, and follow the target cup to its new position.

Finally, during the second testing day, following the displacement test, we measured how persistent individuals were during a third test, the persistency test. During this test, the birds could peck at five unreachable mealworms, covered by a transparent glass.

All tests lasted 2 min. During the distal and displacement tests, birds were introduced in the arena from the same starting point (different from those provided during training). While for the persistency test, birds were placed with their heads close to the target cup, so they could see the mealworms.

For the distal and displacement test, the variables recorded were: 1) the latency to reach the target cup (in seconds) (i.e., time elapsed between the start of the test until the individual approached the target cup), 2) the number of cups visited before reaching the target cup, and 3) the number of revisits of the target cup (i.e., number of times the tested individual moved away from the target cup and came back to inspect it). We also virtually divided the arena in four equal quadrants, and recorded the time spent in the quadrant of the target cup (i.e., the tested individual has both feet in the quadrant and has its head oriented towards the target cup). If a bird did not visit the target cup, it was given the maximum number of cups visited (seven cups), and the maximum latency (120 s) to reach the target cup.

For the persistency test, the variable recorded was the time spent pecking at the glass covering the unreachable mealworms.

3. Statistics

All statistical analyses were performed using IBM SPSS 21 and R version 3.6.1. Variables recorded during the distal and displacement tests were analyzed either through linear mixed model (LMM, 'lmerTest', R package, Kuznetsova et al., 2017) or generalized linear mixed-effects models (GLMM, 'lme4', R package, Bates et al., 2015) fitted for Poisson or Negative Binomial error distribution. Normality of residuals and overdispersion were verified and corrected if necessary. The time spent pecking at the unreachable mealworms, during the persistency test, was compared between breeds using Mann–Whitney *U* tests.

Fixed factors, and their interactions, included in the models were, for the training: breed (RJF vs. WL), and training day (D69 to D71, mean values for both training trials within days), and for testing: breed, test situation (distal vs. displacement), and testing day (first testing fay, D72 vs. second testing day, D79). Visits before reaching the target cup during training, and latency to reach the target cup during tests were square-root-, and log-transformed, respectively, to meet normality assumptions. Non-significant interactions were excluded from the final models. The individual ID nested in 'Cage' was considered as a random factor in all models to account for repeated observations. When interactions were significant, post hoc ANOVA comparisons of estimated marginal means ('emmeans', R package, Lenth et al., 2019) were carried out with Tukey adjustment for multiple comparisons.

Semi-partial r-squared values (i.e., the proportion of variance explained by each fixed effect adjusted for the other predictors in the model) of the final models were obtained using the r2beta function ('r2glmm', R package, Jaeger et al., 2017). Eta-squared values were also calculated to estimate the effect sizes of the non-parametric analyses (Fritz et al., 2012; Tomczak and Tomczak, 2014).

Within the displacement test, only few birds went to the previous position of the target cup, which would suggest a use of distal cues (2 WL birds during D72, and 1 WL bird during D79); thus, a statistical analysis of the preferential use of distal or local cues during the displacement test

was not possible.

Statistical significance was accepted at $p \leq 0.05$. Results are presented as raw means $\pm$ SE.

4. Results

4.1. Training

The number of cups visited before reaching the target cup decreased over training days in the same way for both breeds, but, overall, WL chicks visited more cups than RJF chicks (LMM, breed: $F_{1,15} = 12.290$, $p = 0.003$; training day: $F_{2,32} = 42.48$, $p < 0.001$, r^2sp (model) = 0.63; r^2sp (D2) = 0.49; r^2sp (D3) = 0.54; r^2sp (breed) = 0.24, Fig. 2). Follow-up analyses, separated by breed, revealed that the number of cups visited decreased similarly for both breeds, from day 1 to day 2, while it was constant between day 2 and 3 (WL: training day: $F_{2,16} = 24.17$, $p < 0.001$, r^2sp (model) = 0.59; r^2sp (D2) = 0.46; r^2sp (D3) = 0.56; RJF: training day: $F_{2,14} = 20.78$, $p < 0.001$, $r^2m = 0.63$; r^2sp (D2) = 0.58; r^2sp (D3) = 0.55).

4.2. Distal and displacement tests

There was a significant interaction between the breed and test situation for the latency to reach the target cup. Post-hoc analyses revealed that RJF chicks took longer to reach the target cup during the distal test compared to the displacement test ($p < 0.001$). Separately, the main effects breed, test situation, and testing day were also significant. Overall, RJF had a greater latency than WL, and both breeds had a longer latency to reach the target cup during the distal test compared to the displacement test. Also, the latency to reach the target cup increased between the first and second testing days (LMM, breed*test situation: $F_{1,48.002} = 6.32$, $p = 0.01$; breed: $F_{1,24.016} = 5.15$, $p = 0.03$; test situation: $F_{1,48.002} = 22.82$, $p < 0.001$; testing day: $F_{1,48.002} = 6.43$, $p = 0.01$, r^2sp (model) = 0.31; r^2sp (breed*test situation) = 0.07; r^2sp (breed) = 0.07; r^2sp (test situation) = 0.03; r^2sp (testing day) = 0.07, Figs. 3A and 4A).

There was a significant main effect of test situation and testing day, but not breed, on the number of cup visits before reaching the target cup. Birds made more visits before reaching the target cup during the distal test than the displacement test, and these cup visits increased between testing days (GLMM, breed: Z-value = −0.25, $p = 0.79$; test situation: Z-value = −4.51, $p < 0.001$; testing day: Z-value = 1.98, $p = 0.04$, r^2sp
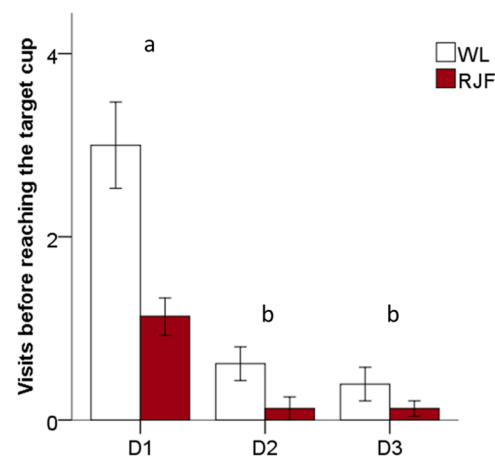
Fig. 2. Performance of White Leghorn (WL) and Red Junglefowl (RJF) female chicks during the training phase of an orientation task. Cup visits before reaching the target cup over three days of training. During the training phase, only one black cup was rewarded with three mealworms, the other cups were white and non-rewarded. Different letters indicate significant differences between days. Mean $\pm$ SE are given ($n_{white\ Leghorns} = 9$, $n_{Red\ Junglefowl} = 8$).
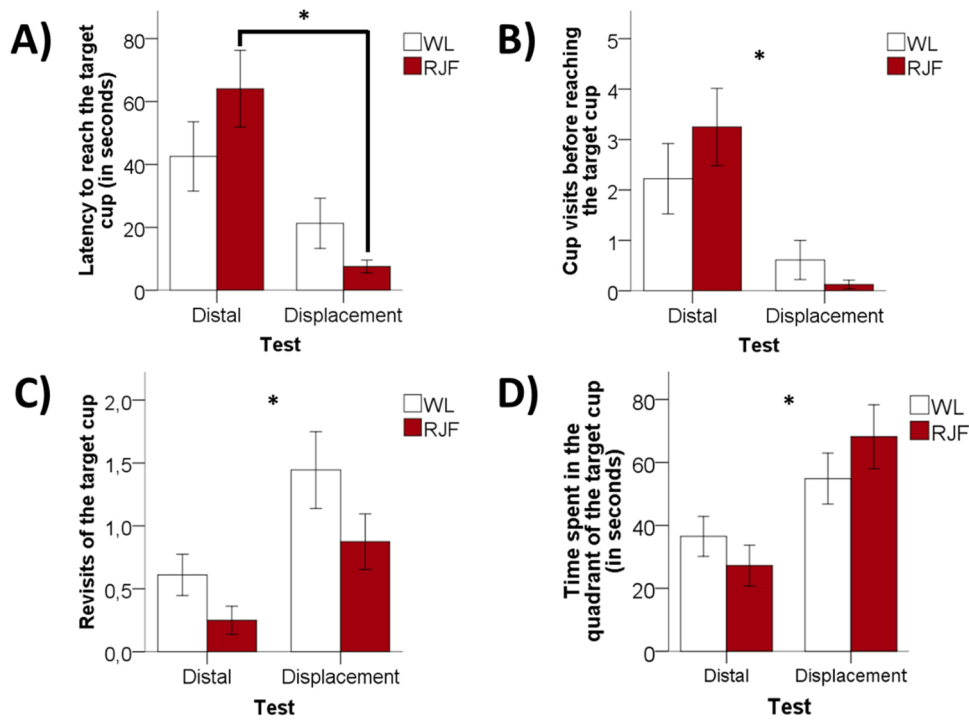
Fig. 3. Performance of White Leghorn (WL) and Red Junglefowl (RJF) female chicks during the distal and displacement tests. A) Latency to reach the target cup (in seconds), B) Number of cup visits before reaching the target cup, C) Revisits of the target cup, and D) Time spent in the quadrant of the target cup (in seconds). *$p < 0.05$. Mean ± SE are given. ($n_{white\ Leghorns} = 9$, $n_{Red\ Junglefowl} = 8$).
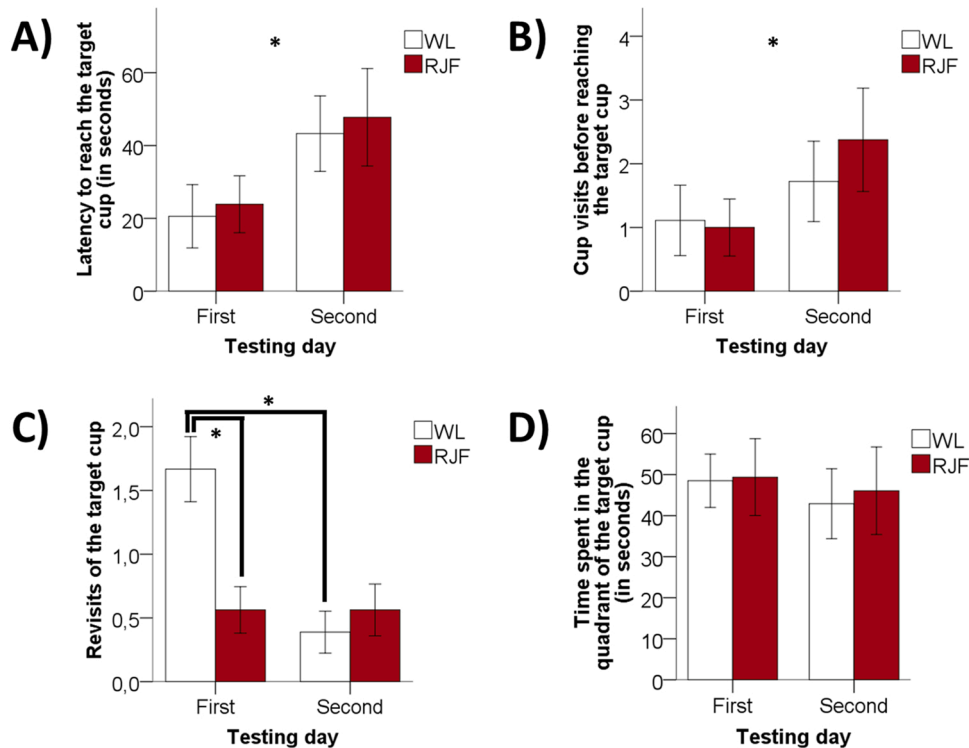

Fig. 4. Performance of White Leghorn (WL) and Red Junglefowl (RJF) female chicks during the first and second testing days of distal and displacement tests (combined). A) Latency to reach the target cup (in seconds), B) Number of cup visits before reaching the target cup, C) Revisits of the target cup, and D) Time spent near the target cup (in seconds). *$p < 0.05$. Mean ± SE are given. ($n_{white\ Leghorns} = 9$, $n_{Red\ Junglefowl} = 8$).

(model) $= 0.25$; r^2sp (breed) $= 0$; r^2sp (test situation) $= 0.21$; r^2sp (testing day) $= 0.05$, Figs. 3B and 4B).

The revisits were significantly influenced by the interaction breed and testing day, and the main effects: breed, test situation, and testing day. WL chicks revisited more the target cup than RJF. However, post-hoc analyses showed this difference occurred mainly during the first

testing day. Between the first and second testing days, the revisits of WL chicks declined, reaching RJF revisit levels. Revisits of the target cup were more frequent during the displacement test than the distal test, and also more frequent during the first testing day than the second testing day (GLMM, breed*testing day: Z-value = 2.3, p = 0.02; breed: Z-value = −2.78, p = 0.005; test situation: Z-value = 3.24, p = 0.001; testing day: Z-value = −3.46, p < 0.001, r^2sp (model) = 0.24; r^2sp (breed*-testing day) = 0.07; r^2sp (breed) = 0.08; r^2sp (test situation) = 0.12; r^2sp (testing day) = 0.14, Figs. 3C and 4C).

Only the testing situation, but not breed nor testing day, influenced significantly the time spent near the target cup. Animals spent more time near the target cup during the displacement test rather than during the distal test (LMM, breed: $F_{1,2.076}$ = 0.06, p = 0.81; test situation: $F_{1,62.142}$ = 13.68, p < 0.001; testing day: $F_{1,62.142}$ = 0.33, p = 0.56, r^2sp (model) = 0.16; r^2sp (breed) = 0; r^2sp (test situation) = 0.16; r^2sp (testing day) = 0, Figs. 3D and 4D).

4.3. Persistency test

Our last test showed that WL chicks spent significantly more time pecking at the glass, trying to reach the mealworms than RJF chicks (White Leghorn: 103.77 s ± 4.90 s, Red Junglefowl: 77.75 s ± 7.61 s, U = 8.50, p = 0.008, η^2 (breed): 0.41, Fig. 5). Exploratory non-parametric Spearman correlations showed that the time spent pecking at the glass during the persistency tests was positively and significantly correlated with the total number of visits during training (three days of training combined, r_s = 0.6, p = 0.01). There was also a positive and significative correlation between time spent pecking at the glass and the total number of revisits of the target cup (distal and displacement tests, two days combined, r_s = 0.54, p = 0.025), but not with the total latency to reach the target cup (distal and displacement tests, two days combined, r_s = −0.03, p = 0.9).

5. Discussion

In the present work, we investigated whether and how domestication influenced the use of distal and local cues by domesticated and wild birds, during an orientation task. We also investigated the influences of time on the memory retention of these cues, and if other cognitive/behavioral propensities, such as persistency, could be linked to individual performances during the task. Contrary to our expectations, both WL and RJF birds performed similarly during the two orientation tests, and presented a marked preference for the use of the local cue over the distal cues. We also observed a decline in the performance of individuals between the first and the second testing day that was independent of the test situation (distal vs. displacement test). Although we did not find differences in spatial orientation strategies between the breeds tested, WL and RJF birds did differ on how their learn and how persistent they

Fig. 5. Time spent pecking at unreachable mealworms during a persistency test, for White Leghorn (WL) and Red Junglefowl (RJF) female chicks. *p < 0.05. Mean ± SE are given. ($n_{\text{white Leghorns}}$ = 9, $n_{\text{Red Junglefowl}}$ = 8).

were: compared to RJF, WL visited more empty cups before reaching the target cup during training, they did more revisits of the target cup during the orientation tests, and they spent more time pecking at unreachable mealworms during the persistency test. Combined, these results suggest that similarities between these domesticated and wild birds still persist, despite years of artificial selection.

Previous studies aiming to investigate the relationship between domestication and spatial learning suggested that RJF had better spatial performances than their WL conspecifics (Lindqvist et al., 2007, 2002; Lindqvist and Jensen, 2009), in contradiction with the results presented in the current work. However, beyond this apparent contradiction, these different results may complement each other, and offer a better picture of the existent differences between the two breeds. In Lindqvist and colleagues' works, birds were tested in mazes varying from two to four arms, the goal was not always visible to the animal, and to solve the task, individuals could rely not only on distal cues, but also on egocentric cues (i.e., based on the body position (Packard and Goodman, 2013)). As these cues were not dissociated from each other, one can hypothesize that the difference between breeds was based on the use of egocentric cues, but not necessarily on the use of distal cues, as investigated here. Furthermore, the fact that the goal was always visible during the present study, but not during previous studies, may also be an important factor impacting the results. RJF are known to be more active and prone to search and work for food (i.e., contrafreeload) than WL (Jensen, 2006; Lindqvist et al., 2002; Lindqvist and Jensen, 2008). Therefore, the need to explore their environment and memorize non-visible food patches is likely greater for RJF, since they evolved in a more heterogeneous environment, than WL. Further studies, using different mazes/arenas and comparing how birds use different types of available cues, are needed to shed greater light on these apparent contradictions.

While the disagreement between studies is understandable due to variation on the methodologies used to investigate the phenomenon, the lack of significative differences between breeds in the use of distal and local cues can be more or less easily explained.

With regard to the use of distal cues, multiple studies on birds and mammals showed that inherent levels of fearfulness/anxiety can impact the way, either positively or negatively, individuals use and rely on this type of cue (Herrero et al., 2006; Lormant et al., 2020b, 2018; Packard, 2009). Since fearfulness is known to be reduced in domesticated animals compared to their wild counterparts (Campler et al., 2009; Jensen, 2006), we hypothesized that RJF would differ from WL on their use of distal cues. RJF did differ from WL in the latency to reach the target cup during tests, but this was mainly due to RJF taking a longer time during the distal test compared to the displacement test. A possible reason behind this particular result is that RJF may have become more hesitant, due to their increased fearfulness, with the disappearance of the local cue (i.e., the black cup was no longer present in the arena) during the distal test, while the conditions during the displacement test were more similar to that of habituation and training (i.e., black and white cups in the arena). No other differences were found between the breeds during the distal test, suggesting that, similar to domesticated and wild rats (Troy Harker and Whishaw, 2002), chicken domestication did not change the way animals use distal cues.

One can imagine that, even though reared in less complex environments, WL are still required to use distal cues to easily locate their preferred spots, resources, and companions. Therefore, the use of distal cues could have been maintained over the course of WL domestication to similar levels of that of their ancestor, the RJF. However, there are at least three non-mutually exclusive alternative explanations that should be taken into account when interpreting our results. The first one concerns the early environment/experience of our tested animals. It is known that early adaptation to laboratory conditions may have an impact on animal cognition: domesticated and captive-born wild Mongalian gerbils (*Meriones unguiculatus*) had similar and better performance during a sound discrimination task, compared to individuals born and raised in the wild. The authors state that domesticated and captive-
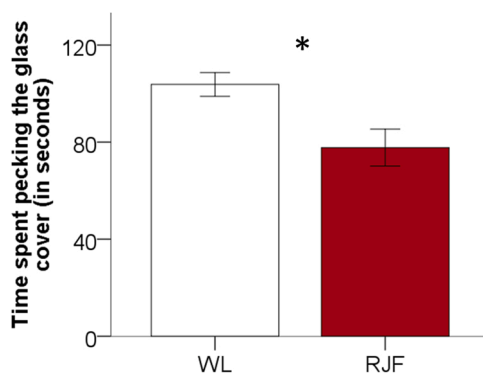
born wild individuals converged cognitively due to the same conditions they faced during their early lives (Stuermer and Wetzel, 2006). The same may have happened to our WL and RJF birds. Since they were kept in similar conditions, and in frequent contact with humans, for the whole duration of the experiment, we can expect their cognitive abilities to be shaped in similar ways. Testing WL and RJF raised in the wild, or that experienced wild-like environments during their early life, would allow us to better dissociate the influence of the early environment from that of domestication.

The second alternative explanation to the lack of breed differences in the use of distal cues concerns a possible involuntary domestication of our RJF population over generations. In a previous study aimed to investigate how the captive environment alter behavioral propensities on two populations of captive RJF, it was concluded that, over only four generations, these populations tended to become similar in their anti-predatory behavior, even though there was no intentional selection to modify this particular behavior (Håkansson and Jensen, 2008). There-fore, we cannot exclude an increase in cognitive similarities between RJF and WL that occurred involuntarily over generations. However, although we cannot say our RJF population fully represents the pure, wild genotype, we still consider our RJF captive population to be more similar to their wild counterparts than to WL domesticated birds. Our recent studies showed consistent differences between the tested breeds, at behavioral, physiological, and genetic levels (Ericsson et al., 2014; Ericsson and Jensen, 2016; Fallahsharoudi et al., 2015; Løtvedt et al., 2017).

Finally, the third alternative explanation for our results relates to our own methodologic choices during the orientation task. By the end of the habituation to the task, and due to time constraints, we selected only the best individuals to undergo training (i.e., individuals that were visiting all cups in the arena). By doing this, we may have caused a sampling bias, selecting, on both breeds, only animals that were fearless, less socially motivated, and that habituated quickly to the task, which in turn may have influenced their performance during the task (Carter et al., 2012; Michelangeli et al., 2016; van Horik et al., 2017). To reduce the sampling bias and allow for a better representation of the animals, it would be interesting for upcoming experiments to adapt our methodology, by, for example, increasing habituation and training time, and avoiding the exclusion of a subsample of individuals, in order to allow for the full panel of behavioral profiles (e.g., more and less fearful animals, fast and slow learners) within the WL and RJF populations to be tested.

We could also apply the previous alternative explanations to interpret the results obtained during the displacement test. However, the fact that almost all RJF and WL followed the local cue to its new position during the displacement test, instead of relying on the distal cues available, corroborates previous research on domestic chickens showing the same pattern (Ferreira et al., 2020a; Morandi-Raikova et al., 2020; Vallortigara and Zanforlin, 1989). Overall, the results from both tests add to the scientific literature suggesting that, despite years of selection for increased production traits (i.e., increased growth and egg production), domestication did not altered how chickens orientate spatially. WL and RJF seem to have, to a certain extent, a similar spatial perception of their environment.

Another aim of the current study was to investigate the memory retention of the distal and local cues, and how they faded (i.e., become extinct) over time. For that, animals were tested over two days, spaced of one week, without any additional retraining in between testing days. Since the use of distal cues is considered to be more complex than that of local cues (Gold et al., 2013; Lormant et al., 2020a; White and McDonald, 2002), we expected the former to fade quicker than the latter. Our results showed that the performance of the animals declined between the first and the second testing day, but this decline did not differ between test situation, nor between our tested breeds. It is possible that only two testing days were not sufficient to allow a differential memory extinction between cues, and more testing days would have been

necessary to confirm our initial hypothesis. Although declined, animals' performance was still below chance level during the second testing day (i.e., they visited less than four cups before reaching the target cup and reached it in less than one minute), suggesting that birds still remembered the cues, but lacked motivation to approach the empty target cup. In a previous study testing food conditioned place preference on free-range chickens, it was shown that multiple expositions of the birds to an empty arena, that was previously associated to food, made the animals less eager to move and explore (Ferreira et al., 2020b). Future studies aiming to study memory retention in these birds should ensure the animals are motivated to participate in the task before testing, so a dissociation between memory extinction and motivation is possible. Besides, the investigation of different time gaps between the last day of training and the day of testing would also be of great interest, since, to the best of our knowledge, there are no studies on how long this species can retain and remember a learned information.

The main differences between our breeds, found at different moments of training and tests, revealed that WL were slower to learn (more visits of non-target cup during training) and were more persistent (more revisits of the target cup during the first testing day and more time spent pecking the glass) than RJF. The time individuals spent pecking the glass during the persistency task was positively correlated with both the number cup visits (during training) and the number of revisits of the target cup (during tests), suggesting that persistency, a form of cognitive/behavioral flexibility, may have influenced individual performance during the orientation task. These results seem to corroborate our previous contrafreeloading studies (Lindqvist et al., 2002; Lindqvist and Jensen, 2009, 2008), and may reflect an adaptation of each breed to the environment they evolved in. Therefore, for RJF, being persistent/more flexible in an unpredictable environment, the wild, is probably a counterproductive strategy, while it may pay off for WL to be more persistent/inflexible in an extremely homogeneous environment, where food, water, and companions are present in a stable and predictable way. It is also important to note that while showing poorer initial performance during training and being more persistent, WL reduced their revisits of the target cup between testing days, reaching the same level of revisits as RJF. This indicates that WL do show some sort of flexibility and can adapt to the testing situation by reducing their persistence over time. Combined, these results allow us to hypothesize that, although WL and RJF did not differ in the way they orientate themselves, they may differ in the way they let go obsolete information and adapt to new ones (i.e., cognitive flexibility) or how they interpret ambiguous cues (i.e., cognitive bias). These hypotheses should be further tested in the future.

To summarize, our results showed that WL and RJF did not differ on how they orientate and use distal or local environmental cues, with both breeds orientating preferentially using local cues. Individual performance declined over time, but this decline is probably due to a lack of motivation, rather than a memory extinction of the learned cues. Although we could not confirm that the birds differ on their spatial orientation strategies, they did differ on how persistent/flexible they were, with domesticated birds showing greater signs of persistency during tests compared to their wild counterparts, a feature that may be adaptative in human-controlled and homogenous environments.

Due to the possibility to control their environment from the point of egg laying and their precociality, birds, such as WL and RJF, have become an essential model to diverse research domains (Freire and Hazel, 2017; Garnham and Løvlie, 2018; Jensen, 2006; Marino, 2017), and may serve an important role to better understand whether and how animal domestication impacted animal cognition, and how domestic animals perceive and adapt to artificial environments. Ultimately, this knowledge can help us to improve animal welfare, by better adapting the environment to the animal perceptions and abilities (Ferreira et al., 2021; Freire, 2020), and not the other way around, as it was commonly done in the previous decades.

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