

Hybro chicks outperform Ross308 in a numerical-ordinal task. Cognitive and behavioral comparisons between 2 broiler strains of newborn domestic chicks (*Gallus gallus*)

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ABSTRACT Domestic chickens (*Gallus gallus*) are among those species subject to intensive selection for production. Among the most widely used broiler strains are the Ross308 and the Hybro. From the perspective of animal production, Ross308 were superior to Hybro in weight gain, final body mass, and feed conversion. Intensive selection is thought to also cause behavioral changes and to negatively affect cognitive abilities. Up to date, though, no evidence has been provided on broiler breeds. The aim of this study was to explore cognitive differences among Hybro and Ross308 chickens by assessing their ordinal-numerical abilities.

Chicks learned to find a food reward in the 4th container in a series of 10 identical and sagittally aligned containers. We designed a standard training procedure ensuring that all chicks received the same amount of

training. The chicks underwent 2 tests: a sagittal and a fronto-parallel one. In the former test, the series was identical to that experienced during training. In the fronto-parallel test, the series was rotated by 90°, thus left-to-right oriented, to assess the capability of transferring the learnt rule with a novel spatial orientation.

In the sagittal test, both chicken hybrids selected the 4th item above chance; interestingly the Hybro outperformed the Ross308 chicks. In the fronto-parallel test, both strains selected the 4th left and the 4th right container above chance; nevertheless, the Hybro chicks were more accurate.

Our results support the hypothesis that intense selection for production can influence animal cognition and behavior, with implications on animal husbandry and welfare.

Key words: broiler breed, chicken, cognitive differences, selection for production, animal welfare

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INTRODUCTION

Nonhuman animals master various nonsymbolic numerical tasks: proto-numerical estimations that are performed without mathematical symbols or words. To date, most of the evidence comes from adult subjects, barring to appreciate the amount of experience needed to grasp numerical tasks and how early in life numerosity starts to be processed (Rugani, 2018; Vallortigara, 2018). The availability of numerical knowledge shortly after birth can hardly be investigated in altricial species, whereas it can be addressed more effectively in precocial species. The domestic chicken (*Gallus gallus*) is extremely precocial in terms of sensory-motor

development, allowing for precise control over the sensory experience, pre- and posthatching. Behavioral analyses can be conducted shortly after hatching, making the domestic chick a golden animal model to pull nature and nurture apart (Rugani and Regolin, 2022).

Day-old chicks, after being reared in standardized conditions to control for the role of experiential factors, master a wide range of numerical skills. They discriminate between different numbers of artificial social companions (Rugani et al., 2010b; Lemaire et al., 2021), compute simple arithmetic sums and subtractions (Rugani et al., 2009, 2017), and process the ratios of sets of green and red dots (Rugani et al., 2015, 2016a). The chicks to find a food reward also manifest ordinal competence: these can be defined as the capability to identify a target item in a series of identical items based on its numerical/ordinal position in the series (Rugani et al., 2007, 2010a; Rugani and Regolin, 2020). In the seminal study (Rugani et al., 2007), different groups of chicks were trained to select (to obtain a food reward) the 3rd, the 4th, or the 6th container in a series of 10 identical

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ones maintained in a fixed position during training and testing (Rugani et al., 2007). Chicks consistently selected the container that was associated with food during training, but they may have relied on either numerical or spatial information. To rule out the possibility that chicks relied on cues other than ordinal, in subsequent studies birds were trained and tested for their ability to select the 4th container (Rugani et al., 2011; 2016b; Rugani and Regolin, 2020, 2021).

Regardless of changes in the location of the series, the position of the containers, and the distance between them from trial to trial, day-old chicks succeeded (Rugani et al., 2007, 2011, 2016b; Rugani and Regolin, 2020, 2021). The secondary role of the spatial cue in performing an ordinal task has also been proved when ordinal and spatial cues were pulled apart. The chicks learnt to find the food reward in the 4th container in a series of 10 identical containers that were kept in a constant position during training. The 4th container was thus identifiable based on its specific position in the apparatus; for example, it was always at the same distance from the beginning or from the end of the series. At the test, the overall number of the containers was halved: the chicks faced a series of 5 containers, which were more spaced apart with respect to the training. In the test series, the 2nd container was in the location previously occupied by the 4th while the 4th was farther away. The birds selected the 4th container and neglected the 2nd one, demonstrating that even if during training they could have learnt to find the reward based on spatial cues, they had also extracted the ordinal/numerical information and transferred the learnt rule to a new series. Soon after hatching, despite their limited experience, young chicks naturally encode and use ordinal information. A fascinating observation was made in an experiment featuring a reorientation of the spatial arrangement of the containers between training and test. The animals were trained to choose the 4th container out of 10 identical containers aligned sagittally. During the test, the containers were rotated by 90°, resulting in a left-to-right displacement in front of the animals. This change in orientation revealed the existence of a preference for one side. Given their symmetrical distribution on the left and right space, the 4th left and right containers should have been selected equally often. Instead, only the left one was selected above chance (Rugani et al., 2010a, 2011; Rugani and Regolin, 2021). The result implies that the search for an ordinal position from left to right exists without immersion in a culture that reads and writes from left to right, which has long been a major explanation of the human spatialization of numbers (Shaki et al., 2009), as in the Mental Number Line where small numbers are typically on the left and larger ones on the right (Galton, 1880; Dehaene et al., 1993; Dehaene, 2011). Despite their scientific relevance regarding the identification of the precocious ability of avian species to encode and use ordinal cues, these studies imply methods that have been focused on a group-level analysis of performance. Moreover, the subjects could advance to test after they have reached an

arbitrary and preestablished learning criterion of at least 8 correct responses across 20 valid trials (Rugani et al., 2007, 2010a, 2011, 2016b; Rugani and Regolin, 2020, 2021). Thus, each bird may have received a different amount of training before testing, canceling individual differences in the acquisition.

Since the second half of the twentieth century, intensive selection has been performed on animal species with the primary objective to obtain higher energy use efficiency and faster growth rates at minimized production costs, particularly feed expenses (Zuidhof et al., 2014). Ross308 and Hybro are 2 hybrid broiler chicken strains selected for meat production (Sarker et al., 2001; Bjedov et al., 2011) and commonly bred in Italy; due to their commercial distribution and availability. In this study, we focused on these breeds. Previous research has shown that the Ross308 demonstrates superior weight gain, final body weight, and feed conversion compared to Hybro, but Ross308 consumed more food. The 2 strains have shown diverse responses to feeding programs, with Ross308 showing higher weight gain, especially in the early “skip-a-day” feeding schedule—feeding on alternate days during an early period (7–21 d), followed by ad libitum feeding during a successive phase (21–35 d of age; (Benyi et al., 2009). Moreover, the Ross308 chickens develop a significantly higher proportion of breast meat than Hybro chickens (Lippens et al., 2000). Further research has studied the impact of low temperatures. Hybro broilers exhibited better adaptation to low temperatures in terms of energy and oxygen requirements for maintaining homeothermy compared to Ross broilers (Kranen et al., 1996). Although the physical effects of this intensive selection have been investigated, studies on the effects of intensive selection on cognition and behavior are sparse (Dudde et al., 2018; Ferreira et al., 2021). Santolin et al. (2020) found that hybrid chicken strains interacted with sex in a statistical learning task. Both male and female Hybro chicks showed a preference for familiar sequences, while the Ross308 displayed a sex difference, with a familiar preference emerging only in females (Santolin et al., 2020). Behavioral and cognitive difference is possibly explained by brain modification that occurs alongside artificial selection for physical traits, as artificial selection affects brain size and composition (for a review see Mehlhorn and Caspers, 2020). A comparison between the body and brain mass of red junglefowl and that of broiler chickens revealed that broilers have larger body masses but smaller brains than red junglefowl (Jackson and Diamond, 1996). Such brain size difference could selectively affect performance on some tasks. Red junglefowl has shown to be more efficient at acquiring spatial information about the location of 2 food sources, discerning the one that provided high-gain food over the low-gain alternative, compared to White Leghorn layers (Lindqvist et al., 2002). On the other hand, other studies did not report any differences caused by artificial selection. A study that investigated domesticated White Leghorn chickens and their wild ancestor, the red junglefowl, did not report any differences in a spatial orientation task, which requires identifying a

target cup in an array of 8 cups. Both domesticated and wild breeds showed a preference for local cues (the color of the cup) over distal ones (the color of the walls; (Ferreira et al., 2022)). Moreover, another study revealed an absence of difference when comparing domestic White Leghorn, red junglefowl, red junglefowl selected for high fear of humans, and red junglefowl selected for low fear of humans, in response to chicken alarm calls and contentment calls. All breeds/lines showed similar increases in vigilance behavior for both types of calls, suggesting no significant discrimination between them (Bessa Ferreira et al., 2022; for further evidence on the lack of domestication effects—i.e., in wild boars and domestic pigs—see Albiach-Serrano et al. (2012)). In a discrimination task, that requires hens to tell apart a green and a red bar to obtain food reward, high-production laying hens (300 eggs/yr) outperformed moderate-production hens (200 eggs/yr). Such superior learning abilities in highly productive hens may be a result of selection pressures to maximize productivity. High-production hens may have developed more advanced cognitive skills to optimize energy intake (Dudde et al., 2018).

Understanding chicken cognition is essential for improving their living conditions and promoting more ethical practices in poultry farming (Ferreira et al., 2021). For example, feather pecking is a typical stress-associated behavior, expressed by commercial laying hens who peck at and pull out feathers of their mates (El-Lethey et al., 2000). This problematic behavior has both economic and welfare impacts. Research has mainly focused on practical solutions, while ignoring the underlying causes that induce birds to repeat this behavior. Cognitive and behavioral research, which allows for a better understanding of cognitive and behavioral needs, can provide solutions that address the root causes of behavior while promoting welfare (Fijn et al., 2020). Remarkably, stress effects seem to also influence offspring. Red junglefowl and domesticated White Leghorns who lived in a stressful environment perform worse in a spatial learning task than birds that were not exposed to any stressor. The effect of parental stress is transmitted to the offspring of White Leghorns, but not red junglefowl, raised without parental contact. White Leghorns offspring showed reduced spatial learning ability in a similar test to that used for their parents, as well as more competitive behaviors and faster growth than offspring of nonstressed animals (Lindqvist et al., 2007).

Nawroth et al. (2019) pointed out the lack of research on most farm animal species specifically for what concerns critical cognitive abilities, such as numerosity discrimination and object permanence. Our better understanding of such abilities, which allow animals to interact with their physical and social environment, can lead us to promote better conditions by acting on positive welfare indicators to improvements in housing, management conditions, and ethical treatment of animals during production (Nawroth et al., 2019, 2022).

The present study aims to investigate if intensive selection for meat production can negatively affect cognitive performance in 2 broiler strains of newborn

domestic chicks (*Gallus gallus*). To this aim, we explored individual differences in learning serial ordinal information as well as spatial-numerical association in the Hybro and the Ross308 strains. According to the resource allocation theory (Beilharz et al., 1993), we expect higher cognitive performance by Hybro chicks. The resource allocation theory posits that animals proportionally allocate their energetic resources between production and fitness. Evolutionary adaptation leads to an optimal distribution of resources for self-preservation and reproductive traits to enhance fitness. However, under additional selection pressure to increase production performance in broilers we should expect a reallocation of resources from behavioral and possibly cognitive traits, thus their decline (Beilharz et al., 1993; Mignon-Grasteau et al., 2005; for contrasting evidence indicating that cognitive and behavioral traits are unaffected, see Albiach-Serrano et al., 2012; Bessa Ferreira et al., 2022; Ferreira et al., 2022).

We adapted a spatial-ordinal task used by Rugani et al. (2007), in which young chicks were trained to choose the 4th container among 10 identical and sagittally aligned containers. After a fixed and preestablished number of training trials, subjects underwent 2 tests. A sagittal test, in which the containers were sagittally aligned as they were during training, was aimed at exploring the memorization of the ordinal rule. A fronto-parallel test, in which the series of containers was rotated by 90° and thus fronto-parallel oriented with respect to the chicks' starting point, allowed to explore whether the birds could transfer the learned rule to a new oriented series and to investigate any side bias, left or right. Differently from the previous procedure, which required training chicks until they all met the same learning criteria, in the present study, we established strict training standards to ensure that each chick underwent the same number of training trials before testing. This approach minimized the potential impact of differential training and allowed us to better capture individual differences in ordinal-numerical comprehension. Furthermore, by measuring individual left or right asymmetrical responses during frontal-parallel tests, we could highlight various patterns of spatial-numerical association.

MATERIALS AND METHODS

Ethical Statement

The experiments comply with all applicable national and European laws concerning the use of animals in research and were approved by the Italian Ministry of Health (permit number: 32662 granted on 19/07/2011 and 306/2019-PR granted on 15/4/2019). All procedures used in the experiments included in this study were examined and approved by the Ethical Committee of the University of Padova (Comitato Etico di Ateneo per la Sperimentazione Animale – CEASA, or by the Organismo Preposto al Benessere Animale – OPBA) and by the Italian National Institute of Health (NIH).

Subjects

Subjects were 17 Hybro and 19 Ross308 male domestic chicks (*Gallus gallus*). Two chicks (1 for each group) were removed because they did not complete all tests. The final sample sizes were 16 Hybro and 18 Ross308 chicks. Male chicks were preferred for the study as the experimental paradigm requires subjects motivated in searching food; indeed, males perform better than females under food reinforcement (Vallortigara et al., 1990; Regolin et al., 2005). On Monday morning, the chicks were obtained from a local commercial hatchery (Agricola Berica, Montegalda, Vicenza, Italy for the Hybro strain and La Pellegrina, S. Pietro in Gu, Padova, Italy for the Ross308 strain). Chicks reached the laboratory at around 9:00 am, when they were a few hours old. Upon arrival, the chicks were paired in metal cages (28 × 40 × 32 cm; width, depth, height) in a rearing room at a controlled humidity of 68% and temperature of 28°C to 31°C. Water and food were available ad libitum in glass jars (5 cm in diameter, 5 cm high). From the morning of the first day to the morning of the third day (Monday through Wednesday around 10:00 am), the chicks received 2 mealworms (*Tenebrio molitor larvae*) a day, which were later used as reinforcement during training and testing. On Wednesday morning, chicks were single-caged. At 8:00 am on Thursday mornings (2 h before the start of training), the food was removed from the cages to be returned only once the training was completed. At 8:00 am on Friday morning, 2 h before the retraining and testing session, the food was removed again. Once all tests were over, the chicks were caged in pairs with food and water ad libitum, and on Friday afternoon they were donated to local farmers.

Stimuli and Apparatus

The subjects were trained and tested in an experimental room near the rearing room. The apparatus consisted of a square arena (100 × 100 × 40 cm) with wood shavings covering the floor. A starting box (15.5 × 15.5 × 10 cm) was attached outside one of the arena walls in correspondence to a central opening (7 × 11 cm). A removable partition (10 × 17 cm) covered such opening, and once lifted the chick could enter the arena to initiate a training or a testing trial.

In front of the opening on the floor of the arena, 10 identical items (plastic bottle caps, 3 cm in diameter and 0.8 cm in height) were homogeneously aligned (4.5 cm between the center of 2 consecutive caps), comprising a total length of 43.5 cm. Each item was filled with wood shavings so as to look identical to any other item at the beginning of each trial. The spatial displacement of the series of items depended on the experimental phase. During training and the sagittal test, the items were sagittally aligned with respect to the opening (Figure 1A). In the sagittal arrangement, the array was aligned along a midline of the arena. The end of the array closer to the starting box was 38 cm from it; the other end was 18.5 cm from the opposite wall. In the fronto-parallel

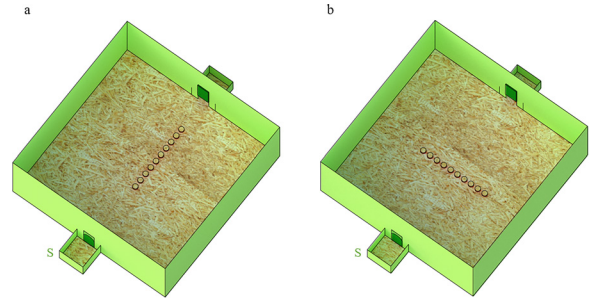


Figure 1. Top view illustration of the apparatus as in the training and sagittal test (A) and in fronto-parallel test (B). “S” indicates the starting box, where the chick was at the beginning of a trial.

test, the series was rotated by 90° thus horizontally oriented from left to right with respect to the starting box and 48.5 cm from it; the distance from both side walls was 28.25 cm (Figure 1B).

Procedure

We designed a standard training procedure to ensure that all chicks received the same amount of training. On Thursday (d 4,) each chick underwent familiarization, pretraining, and training; all conducted with the sagittal arrangement. On Friday (d 5), chicks took part again in pretraining and training and then in the sagittal test. Then the chicks were placed back in their rearing cages to rest for 2 h. Thereafter they underwent pretraining and, as soon as this was completed, the frontal-parallel test.

Familiarization In the morning (at about 10:00 am) of d 4, each chick individually underwent familiarization. A mealworm was always visible over the 4th item. Initially, the chick was placed in the arena, between the starting box and the sagittal array, and it was allowed to freely explore the apparatus for about 2 min. After it got acquainted with the new environment, the procedure started. In the first trials, the chick was placed directly in the arena near the starting box. In subsequent trials, the chick was always placed in the starting box before a trial. The experimenter lifted the partition and let the chick enter the arena. If the chick could not find the reward in about 30 s, a metal stick (about 35 cm long) was used to indicate the mealworm on the 4th item. The stick touched the item and moved slightly upward and downward again, to signal the presence of food (Daisley et al., 2009). Trials in which the experimenter used the stick to help the chicks to identify the target item were never considered as correct trials. In this as well as in the subsequent experimental phases, as soon as the chick pecked at any one of the items, the trial was ended, and the chick was gently placed back in the starting box. Thus, in each trial, only a single peck was allowed, and the chick could gain the reward only by selecting the 4th item. If 60 s elapsed without a response, the chick was placed back into the starting box, and the trial was considered null. The familiarization ended when the chick

had spontaneously pecked at the 4th item in 10 separate trials, even if they were not consecutive, without any help provided by the metal stick. The session lasted about 15 min for each bird. Only chicks that successfully passed the familiarization phase could access the pretraining. The chick was placed back in its rearing cage with only water available for at least 1 h and at most 2 h prior to the pretraining.

In the subsequent sessions, the protocol for administering a trial was identical to that in familiarization, except for the frequency of the stick use. In this, as well as in the following training and testing sessions from trial to trial, i) the arena was randomly rotated, to prevent chicks from using external cues possibly available in the experimental room; ii) the position of each item in the series was randomly rearranged, and the wood shavings were mixed and scattered, to prevent the chicks from using possible internal cues.

Pretraining In pretraining, the procedure was similar to that used in the familiarization, the only difference being that the mealworm was gradually hidden beneath the wood shavings. This way, the mealworm was clearly visible in the first trials and then progressively buried and concealed, to induce the chicks to memorize its fixed location in the 4th item. If the chick did not perform a valid choice in about 50 s, the stick was used to indicate the correct item. The pretraining ended when 3 correct responses (pecks at the 4th item without receiving any cue) were observed in 3 consecutive trials. Only chicks that completed the pretraining accessed the training. This session lasted about 5 min for each bird.

Training Immediately after the end of the pretraining, the training was administered. This consisted of 25 trials. In the first 5 trials, the mealworm was visible in the 4th item, but it was gradually buried within the wood shavings. In the following 20 trials, the mealworm was totally buried and thus invisible so that the 10 items looked identical. The experimenter pointed to the correct item with the stick if the chick did not respond within 50 s. The session lasted about 20 min. Afterward, the chick was placed back into the cage with water and food available. Only chicks that completed the training could enter the testing phase.

Sagittal Test On d 5, at approximately 10:00 am, the chick underwent a retraining. This comprised 3 trials similar to those in the pretraining, with the mealworm always visible in the 4th container, followed by 25 trials identical to the training phase, then the sagittal test took place. The sagittal test consisted of 20 trials, in which the experimenter did not provide any cue with the stick. On each trial, the chick was allowed 1 peck; once an item had been pecked, the trial was considered over. Food rewards following correct responses were administered only in pre-established trials (i.e., trial 4, 5, 7, 10, 13, 14, 16, and 19). In all testing trials (rewarded and unrewarded), the chicks kept searching for food because all items looked identical and this task was associated with food during training (Rugani et al., 2011, 2016b; Rugani and Regolin, 2020, 2021). This variable reward schedule has been designed to maintain

motivation for food throughout the test, preventing response extinction over multiple unrewarded trials (Rugani et al., 2016b; Rugani and Regolin, 2020). The use of this rewarding schedule does not seem to induce additional learning during testing, as chicks do not perform better in the last trials (Rugani et al., 2016a; Rugani and Regolin, 2020). If the chick did not respond to any items within 60 s, the trial was terminated and considered null. At the end of each trial, the chick was gently placed back in the starting box and after approximately 5 s, a new trial began. The sagittal test lasted about 20 min, then the chick was placed back in its rearing cage, where only water was left available, for about 2 h of rest before undergoing the fronto-parallel test. During the test, the experimenter watched online the behavior of the chicks from a monitor connected to a video camera. Chicks' behavior at test was also video-recorded for off-line scoring.

Frontal-Parallel Test Immediately before the frontal-parallel test, the chicks underwent a short retraining. The experimental procedure was identical to that used during training. The criterion to pass the fronto-parallel test was set at 3 consecutive correct responses. All chicks successfully completed this phase within 5 to 10 min. The frontal-parallel test consisted of 20 trials and was carried out with the fronto-parallel arrangement of the items. Correct choices on either the 4th left or right item were rewarded in preestablished trials (Trial 4, 5, 7, 10, 13, 14, and 19). The session lasted about 20 min. The experimenter could observe online the behavior of the chicks from a monitor connected to a video camera. The whole test was also video-recorded for off-line scoring.

Scoring The scoring procedure was identical for the sagittal and for the fronto-parallel test. Video recordings were scored by a second experimenter, blind to the hypothesis being tested. For each of the 20 testing trials (both rewarded and unrewarded), the first peck emitted by each chick at any of the 10 items was recorded. The choices for each item were further summed up to calculate the percentage of choices on each item for each subject (percentages of choices for one item = number of pecks at that item/total valid trials \times 100). Data analysis was performed with R (Version 4.0.3) with BayesFactor, rcompanion, pwr packages and JASP (Version 0.14.1). Exact binomial/Bayesian binomial tests as well as proportion/Bayesian A/B tests were conducted to examine individual performance. Wilcoxon/Bayesian t tests were conducted to examine group performance. We used the evidence categories suggested by (Lee and Wagenmakers, 2014) to interpret the Bayes factor (BF).

RESULTS

Sagittal Test

Individual Performance The number of correct choices was counted for each subject (see Table 1). Exact binomial and Bayesian binomial tests were carried out on the number of successful trials (pecks at the 4th

Table 1.

Test	Strain	Target	Subject	n	N	Percent on target	P	Cohen's h	BF			
Sagittal test	Hybro	4	H1	16	19	84.211	<0.001	1.681	>10000			
			H2	7	19	36.842	0.002	0.661	39.022			
			H3	1	20	5.000	0.878	-0.192	0.071			
			H4	6	19	31.579	0.009	0.550	8.036			
			H5	10	20	50.000	<0.001	0.927	8213.23			
			H6	6	19	31.579	0.009	0.550	8.036			
			H7	8	18	44.444	<0.001	0.816	383.271			
			H8	8	18	44.444	<0.001	0.816	383.271			
			H9	6	17	35.294	0.005	0.629	15.876			
			H10	9	19	47.368	<0.001	0.875	1724.768			
			H11	9	20	45.000	<0.001	0.827	1003.828			
			H12	9	18	50.000	<0.001	0.927	3104.593			
			H13	5	19	26.316	0.035	0.434	2.065			
			H14	6	18	33.333	0.006	0.587	11.135			
			H15	8	19	42.105	<0.001	0.769	234.215			
			H16	9	19	47.368	<0.001	0.875	1724.768			
Sagittal test	Ross308	4	R1	7	20	35.000	0.002	0.623	26.835			
			R2	4	20	20.000	0.133	0.284	0.559			
			R3	1	20	5.000	0.878	-0.192	0.071			
			R4	4	20	20.000	0.133	0.284	0.559			
			R5	6	19	31.579	0.009	0.550	8.036			
			R6	3	20	15.000	0.323	0.152	0.236			
			R7	9	20	45.000	<0.001	0.827	1003.828			
			R8	4	19	21.053	0.115	0.310	0.666			
			R9	7	20	35.000	0.002	0.623	26.835			
			R10	7	20	35.000	0.002	0.623	26.835			
			R11	5	18	27.778	0.028	0.467	2.662			
			R12	3	12	25.000	0.111	0.404	0.969			
			R13	4	19	21.053	0.115	0.310	0.666			
			R14	2	20	10.000	0.608	0.000	0.12			
			R15	5	20	25.000	0.043	0.404	1.634			
			R16	3	20	15.000	0.323	0.152	0.236			
			R17	5	20	25.000	0.043	0.404	1.634			
			R18	2	19	10.526	0.580	0.017	0.132			
Frontal-parallel test	Hybro	4th (4L and 4R)	H1	5	16	31.250	0.202	0.259	0.548			
			H2	13	20	65.000	<0.001	0.948	4469.461			
			H3	12	20	60.000	<0.001	0.845	687.59			
			H4	8	19	42.105	0.023	0.485	3.723			
			H5	11	20	55.000	0.001	0.744	128.902			
			H6	10	20	50.000	0.003	0.644	29.273			
			H7	5	18	27.778	0.284	0.183	0.365			
			H8	7	20	35.000	0.087	0.339	1.044			
			H9	8	18	44.444	0.016	0.532	5.433			
			H10	9	20	45.000	0.010	0.543	8.025			
			H11	11	20	55.000	0.001	0.744	128.902			
			H12	5	17	29.412	0.242	0.219	0.443			
			H13	6	19	31.579	0.163	0.266	0.598			
			H14	8	20	40.000	0.032	0.442	2.647			
			H15	9	19	47.368	0.007	0.591	12.275			
			H16	10	19	52.632	0.002	0.696	49.199			
			Frontal-parallel test	Ross308	4th (4L and 4R)	R1	9	20	45.000	0.010	0.543	8.025
						R2	8	19	42.105	0.023	0.485	3.723
R3	6	20				30.000	0.196	0.232	0.486			
R4	12	20				60.000	<0.001	0.845	687.59			
R5	8	20				40.000	0.032	0.442	2.647			
R6	5	20				25.000	0.370	0.120	0.262			
R7	7	19				36.842	0.068	0.377	1.365			
R8	7	20				35.000	0.087	0.339	1.044			
R9	9	20				45.000	0.010	0.543	8.025			
R10	7	20				35.000	0.087	0.339	1.044			
R11	5	19				26.316	0.327	0.150	0.307			
R12	4	18				22.222	0.499	0.054	0.206			
R13	4	20				20.000	0.589	0.000	0.16			
R14	3	20				15.000	0.794	-0.132	0.107			
R15	5	20				25.000	0.370	0.120	0.262			
R16	4	20				20.000	0.589	0.000	0.16			
R17	7	19				36.842	0.068	0.377	1.365			
R18	4	17				23.529	0.451	0.086	0.238			
Frontal-parallel test	Hybro	4L	H1	2	16	12.500	0.485	0.079	0.181			
			H2	11	20	55.000	<0.001	1.027	8131.073			
			H3	10	20	50.000	<0.001	0.927	8213.23			
			H4	6	19	31.579	0.009	0.550	8.036			
			H5	3	20	15.000	0.323	0.152	0.236			

(continued)

Table 1 (Continued)

Test	Strain	Target	Subject	n	N	Percent on target	<i>P</i>	Cohen's <i>h</i>	BF
Frontal-parallel test	Ross308	4L	H6	5	20	25.000	0.043	0.404	1.634
			H7	1	18	5.556	0.850	-0.168	0.082
			H8	6	20	30.000	0.011	0.516	5.948
			H9	7	18	38.889	0.001	0.703	58.541
			H10	5	20	25.000	0.043	0.404	1.634
			H11	5	20	25.000	0.043	0.404	1.634
			H12	2	17	11.765	0.518	0.057	0.162
			H13	6	19	31.579	0.009	0.550	8.036
			H14	2	20	10.000	0.608	0.000	0.12
			H15	4	19	21.053	0.115	0.310	0.666
			H16	3	19	15.789	0.295	0.174	0.268
			R1	6	20	30.000	0.011	0.516	5.948
			R2	6	19	31.579	0.009	0.550	8.036
			R3	4	20	20.000	0.133	0.284	0.559
			R4	4	20	20.000	0.133	0.284	0.559
			R5	6	20	30.000	0.011	0.516	5.948
			R6	3	20	15.000	0.323	0.152	0.236
			R7	4	19	21.053	0.115	0.310	0.666
R8	5	20	25.000	0.043	0.404	1.634			
R9	4	20	20.000	0.133	0.284	0.559			
R10	3	20	15.000	0.323	0.152	0.236			
R11	2	19	10.526	0.580	0.017	0.132			
R12	3	18	16.667	0.266	0.198	0.308			
R13	3	20	15.000	0.323	0.152	0.236			
R14	2	20	10.000	0.608	0.000	0.12			
R15	5	20	25.000	0.043	0.404	1.634			
R16	1	20	5.000	0.878	-0.192	0.071			
R17	4	19	21.053	0.115	0.310	0.666			
R18	2	17	11.765	0.518	0.057	0.162			
Frontal-parallel test	Hybro	4R	H1	3	16	18.750	0.211	0.252	0.421
			H2	2	20	10.000	0.608	0.000	0.12
			H3	2	20	10.000	0.608	0.000	0.12
			H4	2	19	10.526	0.580	0.017	0.132
			H5	8	20	40.000	<0.001	0.726	148.703
			H6	5	20	25.000	0.043	0.404	1.634
			H7	4	18	22.222	0.098	0.338	0.806
			H8	1	20	5.000	0.878	-0.192	0.071
			H9	1	18	5.556	0.850	-0.168	0.082
			H10	4	20	20.000	0.133	0.284	0.559
			H11	6	20	30.000	0.011	0.516	5.948
			H12	3	17	17.647	0.238	0.224	0.358
			H13	0	19	0.000	1	-0.644	0.05
			H14	6	20	30.000	0.011	0.516	5.948
			H15	5	19	26.316	0.035	0.434	2.065
			H16	7	19	36.842	0.002	0.661	39.022
			R1	3	20	15.000	0.323	0.152	0.236
			R2	2	19	10.526	0.580	0.017	0.132
R3	2	20	10.000	0.608	0.000	0.12			
R4	8	20	40.000	<0.001	0.726	148.703			
R5	2	20	10.000	0.608	0.000	0.12			
R6	2	20	10.000	0.608	0.000	0.12			
R7	3	19	15.789	0.295	0.174	0.268			
R8	2	20	10.000	0.608	0.000	0.12			
R9	5	20	25.000	0.043	0.404	1.634			
R10	4	20	20.000	0.133	0.284	0.559			
R11	3	19	15.789	0.295	0.174	0.268			
R12	1	18	5.556	0.85	-0.168	0.082			
R13	1	20	5.000	0.878	-0.192	0.071			
R14	1	20	5.000	0.878	-0.192	0.071			
R15	0	20	0.000	1	-0.644	0.048			
R16	3	20	15.000	0.323	0.152	0.236			
R17	3	19	15.789	0.295	0.174	0.268			
R18	2	17	11.765	0.518	0.057	0.162			

Abbreviations: n, number of correct trials; N, number of valid trials.

item) out of valid trials (maximum 20) against a chance of 10% for each chick. Fifteen out of 16 (93.75%) Hybro chicks remembered the 4th item significantly above chance (*Ps* = [<0.001 , 0.035], Cohen's *hs* = [0.434, 1.681]), which was also confirmed by the Bayesian binomial test (1 chick showed anecdotal evidence

(BF = 2.065), 2 chicks showed moderate evidence (BF = 8.036), 2 chicks strong evidence (BFs = [11.135, 15.876]), 1 chick very strong evidence (BF = 39.022), and 9 chicks extreme evidence (BFs = [234, >10,000])). Eight out of 18 (44.44%) Ross308 chicks performed successfully (*Ps* = [<0.001 , 0.043], Cohen's *hs* = [0.404,

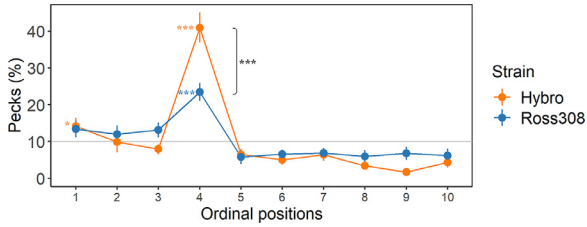


Figure 2. The percentage of choosing each item in the sagittal test by the 2 strains (the Hybro and the Ross308). The error bars indicate standard errors. The gray line represents the chance level ($y = 10$); *** indicates $P < 0.001$. Both groups selected the 4th item above the chance level ($P_s < 0.001$). The Hybro chicks performed better than the Ross308 chicks ($P < 0.001$).

0.827]), which was confirmed by the Bayesian binomial test: 3 chicks showed anecdotal evidence (BFs = [1.634, 2.662]), 1 moderate evidence (BF = 8.036), 3 strong evidence (BFs = 26.835), and 1 extreme evidence (BF = 1003.828). A proportion test showed that the Hybro chicks had a higher probability of success than the Ross308 chicks (proportion of successful, Hybro = 93.75%, Ross308 = 44.44%, $\chi^2 = 7.291$, $df = 1$, $P = 0.007$, Cohen's $h = 1.177$), confirmed by strong evidence in the Bayesian A/B test (BF = 27.551).

Group-Level Performance The group-level performance was calculated by averaging the percentage of choices on each item of each subject (number of pecks at an item / total valid trials $\times 100$; Figure 2). Because the data were not normally distributed (Shapiro-Wilk test of normality, $P < 0.001$), 1-sample Wilcoxon tests, as well as Bayesian 1-sample tests, were carried out on their percentage of choices against the chance level (10%).

In the sagittal test, both the Hybro and the Ross308 chicks chose the 4th item significantly above chance (Hybro: Mean = 40.930, SE = 4.056, $V = 135$, $P < 0.001$, $r_{rb} = 0.985$; Ross308: Mean = 23.444, SE = 2.441, $V = 150$, $P < 0.001$, $r_{rb} = 0.961$). The Bayesian 1-sample tests produced extreme evidence (Hybro: BF = 23768; Ross308: BF = 1,322) in favor of the successful recognition of the 4th item, indicating that both strains could remember the target item. The 1st item was selected above chance by Hybro chicks (Mean = 14.126, SE = 2.356, $V = 101$, $P = 0.046$, $r_{rb} = 0.485$), even if the Bayesian evidence was anecdotal (BF = 1.665); but not by Ross308 chicks (Mean = 13.374, SE = 2.288, $V = 103.5$, $P = 0.104$, $r_{rb} = 0.353$; BF = 1.110). No other significant results were observed ($P_s > 0.265$, BFs < 0.51; Figure 2, see also Table 2; also the selection for the 3rd item by the Ross308 chicks was not significant, Mean = 13.122, SE = 2.033, $V = 98$, $P = 0.063$, $r_{rb} = 0.441$, BF = 1.205).

To investigate whether 1 strain overperformed the other, the Wilcoxon signed rank test was conducted on the accuracy (percentage of choosing the 4th item) of the 2 groups. A significant difference was observed between the Hybro and the Ross308 chicks ($W = 246$, $P < 0.001$, $r_{rb} = 0.708$). The Bayesian t-test confirmed the result by yielding very strong evidence that the Hybro chicks selected the 4th item more than the Ross308 chicks (BF = 45.318).

To assess whether the overall performance was affected by learning taking place during testing—and since the fourth was the first rewarded trial in all tests—we restricted the analysis to the initial 4 trials. In these first 4 trials, both the Hybro and the Ross308 chicks chose the 4th item significantly above chance (Hybro: Mean = 40.625, SE = 6.583, $V = 130$, $P > 0.001$, $r_{rb} = 0.912$, BF = 209.595; Ross308: Mean = 22.222, SE = 4.905, $V = 143$, $P = 0.006$, $r_{rb} = 0.673$, BF = 5.199). Moreover, Hybro outperformed the Ross308 chicks ($W = 203$, $P = 0.036$; BF = 2.244). Additionally, for each strain, a comparison of the mean accuracy in the first 4 trials with the performance in the last 4 trials did not reveal any statistical difference (Hybro: First 4 trials: Mean = 40.625, SE = 6.583; Last 4 trials: Mean = 33.854, SE = 7.315; $V = 45.5$, $P = 0.636$; BF = 0.335; Ross308: First 4 trials: Mean = 22.222, SE = 4.905; Last 4 trials: Mean = 17.592, SE = 5.385; $V = 35$, $P = 0.458$; BF = 0.318).

Fronto-Parallel Test

Individual Performance First, we explored the capability of each chick in transferring the ordinal rule learned during sagittal training to a differently oriented—fronto-parallel—series, thus selecting either target: the 4th on the left, 4L, or the 4th on the right, 4R. Individual performance was computed, for each chick, by summing up the number of successful trials (choices on 4L and 4R) over valid trials (maximum 20) against chance expectation (20%). Data were analyzed separately for each chick, by using exact binomial and Bayesian binomial tests. Eleven out of 16 (68.75%) Hybro chicks remembered the 4th items significantly above chance ($P_s = [<0.001, 0.032]$, Cohen's $h_s = [0.442, 0.948]$). Bayesian binomial tests showed anecdotal evidence for 2 chicks (BFs = [1.044, 2.647]), moderate evidence for 3 chicks (BFs = [3.723, 8.025]), strong evidence for other 2 chicks (BFs = [12.275, 29.273]), and extreme evidence for 4 chicks (BF = [128.902, 4,469.461]). Five out of 18 (27.78%) Ross308 chicks succeeded in the task ($P_s = [<0.001, 0.032]$, Cohen's $h_s = [0.442, 0.845]$). Bayesian binomial tests showed that 5 chicks showed anecdotal evidence (BFs = [1.044, 2.647]), 2 chicks moderate evidence (BFs = [3.723, 8.025]), and 1 chick extreme evidence (BF = 687.590). A proportion test showed that the Hybro strain had a higher probability of success than the Ross308 (proportion of Hybro = 68.75%, proportion of Ross308 = 27.78%, $\chi^2 = 4.182$, $df = 1$, $P = 0.041$, Cohen's $h = 0.845$); Bayesian A/B test provided moderate evidence (BF = 8.376).

Then we analyzed the presence of lateral bias. We computed separately the number of pecks at 4L or 4R, out of valid trials, and we performed additional binomial tests against a chance of 10% (see Table 1). For what concerns the left side, 9 out of 16 (56.25%) Hybro chicks significantly chose 4L ($P_s = [<0.001, 0.043]$, Cohen's $h_s = [0.404, 1.027]$), the Bayesian binomial test revealed

Table 2.

Test	Strain	Op	Pecks% Mean	Pecks% SE	N	V	<i>P</i>	<i>P</i> _{adjust}	<i>r</i> _{rb}	BF
Sagittal	Hybro	1	14.126	2.357	16	101	0.046	0.462	0.485	1.665
		2	9.867	2.864	16	61	0.651	1.000	-0.103	0.247
		3	7.908	1.345	16	42.5	0.847	1.000	-0.292	0.115
		4	40.930	4.056	16	135	0.000	0.003	0.985	23768.195
		5	6.459	1.544	16	33	0.967	1.000	-0.515	0.093
		6	4.978	1.289	16	20	0.994	1.000	-0.706	0.073
		7	6.382	1.615	16	32	0.971	1.000	-0.529	0.094
		8	3.367	0.958	16	3	1.000	1.000	-0.956	0.021
		9	1.685	0.647	16	0	1.000	1.000	-1.000	0.010
		10	4.298	1.371	16	13	0.997	1.000	-0.783	0.071
Sagittal	Ross308	1	13.374	2.288	18	103.5	0.104	1.000	0.353	1.110
		2	11.938	2.336	18	71.5	0.265	1.000	0.192	0.512
		3	13.122	2.033	18	98	0.063	0.629	0.441	1.205
		4	23.444	2.441	18	150	0.000	0.003	0.961	1321.564
		5	5.767	1.891	18	37	0.919	1.000	-0.383	0.088
		6	6.585	1.242	18	30	0.978	1.000	-0.559	0.079
		7	6.863	1.322	18	26	0.976	1.000	-0.567	0.086
		8	5.950	1.664	18	33	0.970	1.000	-0.515	0.085
		9	6.756	1.772	18	28.5	0.942	1.000	-0.457	0.099
		10	6.200	1.795	18	37.5	0.972	1.000	-0.510	0.091
Frontal-parallel	Hybro	1L	5.575	2.005	16	15	0.992	1.000	-0.714	0.095
		2L	7.829	2.075	16	49.5	0.839	1.000	-0.272	0.139
		3L	5.713	1.643	16	14	0.988	1.000	-0.692	0.087
		4L	25.232	3.514	16	117	0.001	0.007	0.950	120.765
		5L	7.503	1.737	16	36	0.919	1.000	-0.400	0.119
		5R	10.952	1.978	16	61	0.307	1.000	0.162	0.380
		4R	19.241	2.940	16	93.5	0.005	0.055	0.781	15.163
		3R	8.877	1.903	16	47.5	0.772	1.000	-0.208	0.175
		2R	4.982	1.578	16	22	0.987	1.000	-0.633	0.079
		1R	4.095	1.661	16	19	0.996	1.000	-0.721	0.076
Frontal-parallel	Ross308	1L	9.997	1.790	18	55.5	0.437	1.000	0.057	0.243
		2L	7.421	1.185	18	17	0.962	1.000	-0.564	0.090
		3L	8.637	1.886	18	59	0.690	1.000	-0.132	0.154
		4L	19.036	1.751	18	148.5	0.000	0.003	0.941	699.171
		5L	8.653	1.623	18	38	0.830	1.000	-0.276	0.146
		5R	9.062	1.964	18	60	0.671	1.000	-0.118	0.177
		4R	13.345	2.098	18	79.5	0.047	0.475	0.514	1.305
		3R	7.669	1.233	18	37	0.951	1.000	-0.456	0.097
		2R	7.047	1.599	18	43	0.910	1.000	-0.368	0.098
		1R	9.133	1.859	18	66.5	0.692	1.000	-0.131	0.178

Bold typefaces indicate target elements.

Abbreviation: Op, ordinal position.

that 3 chicks showed anecdotal evidence (BFs = 1.634), 3 moderate evidence (BFs = [5.948, 8.036]), 1 strong evidence (BF = 58.541), and 2 extreme evidence (BFs = [8,131.073, 8,213.230]). Five out of 18 (27.78%) Ross308 chicks selected 4L above chance (*P*s = [0.009, 0.043], Cohen's *h*s = [0.404, 0.55]; 2 chicks showed anecdotal evidence (BFs = 1.634) and 3 showed moderate evidence (BFs = [5.948, 8.036]) in Bayesian binomial tests). For what concerns the right side (4R), 6 out of 16 (37.50%) Hybro chicks performed above chance (*P*s = [<0.001 , 0.043], Cohen's *h*s = [0.404, 0.726]; 2 chicks showed anecdotal (BFs = [1.634, 2.065]), 2 showed moderate (BFs = 5.948), one showed very strong (BF = 39.022) and one showed extreme evidence (BF = 148.703) in Bayesian binomial tests), while 2 out of 18 (11.11%) Ross308 chicks selected 4R above chance (*P*s = [<0.001 , 0.043], Cohen's *h*s = [0.404, 0.726]; 1 chick showed anecdotal (BF = 1.634) and one showed extreme evidence (BF = 148.703) in Bayesian binomial tests).

Group-Level Performance To assess the capability of each strain in transferring the learned ordinal rule to a differently oriented series, we scored the number of

correct trials: the selection of the 4th left, 4L or the 4th right item, 4R. The group-level performance was calculated by averaging the individual percentages of choices on each item, which were computed using the following formula:

$$\frac{\text{the number of choices at 4R} + \text{the number of choices at 4L}}{\text{total valid trials}} \times 100$$

Because the data were not normally distributed (Shapiro-Wilk test of normality, $P < 0.001$), the 1-sample Wilcoxon tests and Bayesian 1-sample tests were carried out on their percentage of choices against chance level (20%; having 2 possible correct items in an array of 10 items the probability to select a correct response by chance is 20%).

The result of the 1-sample Wilcoxon test against a chance level of 20% indicated that both strains performed successfully in the frontal-parallel test (Hybro: Mean accuracy = 44.473, SE = 2.852, $V = 136$, $P < 0.001$, $r_{rb} = 1.000$; Ross308: Mean accuracy = 32.381, SE = 2.699, $V = 132$, $P < 0.001$, $r_{rb} = 0.941$). The Bayesian 1-sample tests provided extreme evidence that

both strains were able to transfer the learned rule to a frontal-parallel arrangement (Hybro: BF = 89,438; Ross308: BF = 241).

The Wilcoxon sign rank test and Bayesian t-test showed that the Hybro chicks outperformed the Ross308 chicks in frontal-parallel test ($W = 223.5$, $P = 0.006$, $r_{rb} = 0.552$, BF = 9.763).

To assess whether the overall performance depended on learning, we restricted the analysis to the initial 4 trials. From the very first test trials, both the Hybro and the Ross308 chicks chose the 4th items significantly above chance (Hybro: Mean = 48.958, SE = 5.886, $V = 131$, $P < 0.001$, $r_{rb} = 0.926$, BF = 331.645; Ross308: Mean = 37.037, SE = 5.996, $V = 144$, $P = 0.005$, $r_{rb} = 0.684$, BF = 9.489), no difference was found in the performance of the 2 strains ($W = 184$, $P = 0.161$; BF = 0.705). Additionally, for each strain, a comparison of the mean accuracy in the first 4 trials with the performance in the last 4 trials did not reveal any statistical difference (Hybro: First 4 trials: Mean = 48.958, SE = 5.886; Last 4 trials: Mean = 35.000, SE = 5.587; $V = 63.5$, $P = 0.057$; BF = 1.457; Ross308: First 4 trials: Mean = 37.037, SE = 5.996; Last 4 trials: Mean = 33.796, SE = 4.940; $V = 49$, $P = 0.832$; BF = 0.258).

We then looked at each item to understand whether their performance differed in selecting the left and the right target, both strains chose 4L and 4R above chance (Hybro; 4L: Mean = 25.232, SE = 3.514, $V = 117$, $P = 0.001$, $r_{rb} = 0.950$, BF = 121; 4R: Mean = 19.241, SE = 2.940, $V = 93.5$, $P = 0.005$, $r_{rb} = 0.781$, BF = 15; Ross308; 4L: Mean = 19.036, SE = 1.751, $V = 148.5$, $P < 0.001$, $r_{rb} = 0.941$, BF = 699; 4R: Mean = 13.345, SE = 2.098, $V = 79.5$, $P = 0.047$, $r_{rb} = 0.514$, BF = 1.305). No other significant results were observed ($P_s > 0.3$; BF < 0.38; Figure 3 and Table 2).

The Wilcoxon sign rank test and Bayesian t-test showed that the Ross308 chicks chose 4L more than 4R in frontal-parallel test ($W = 119.5$, $P = 0.045$, $r_{rb} = 0.562$, BF = 2.346) while the Hybro chicks did not show any difference ($W = 75$, $P = 0.410$, $r_{rb} = 0.250$, BF = 0.412).

Lateralality Index To investigate lateral asymmetries in selecting the correct items, we calculated 2 laterality indexes for each strain. We first computed the

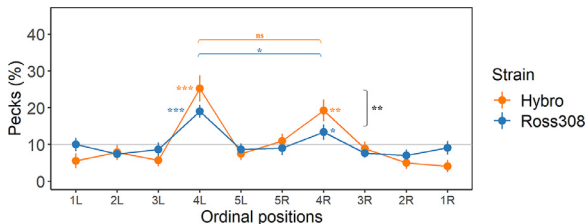


Figure 3. The percentage of choosing each item in frontal-parallel test by the 2 strains: Hybro and Ross308. Error bars indicate standard errors. The gray line represents the chance level ($y = 10$); * indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$. The Hybro chicks performed better than the Ross308 chicks when combining 4L and 4R ($P < 0.01$). Both strains chose 4L and 4R above chance level ($P_s < 0.05$).

asymmetry for each chick and then we averaged individual values to assess whether the strains showed any group-level asymmetry (Figure 4).

We first assessed whether each strain showed an unspecific lateral bias, which reflected a lateral asymmetry, by using the following formula:

$$\text{Unspecific laterality index (ULI)} = \frac{|\text{the number of choices at 4R} - \text{the number of choices at 4L}|}{\text{the number of choices at 4R} + \text{the number of choices at 4L}} \times 100$$

Values computed by using this formula can range from 0 to 100: 0 indicates an absence of any lateral bias, while 100 indicates an extreme lateral bias either toward the left or the right (Figure 4A). While this index can assess the existence of a lateral bias, it is unable to assess the direction of the bias (Versace et al., 2020). ULI provides indications about any overall preference toward either side, without specifying if the bias is left or right oriented, thus allowing us to assess the presence of a bias independent of its specific direction.

To assess the side of the asymmetry—to disentangle between left vs. right bias—we used a second formula, which allowed us to determine the direction of the bias:

$$\text{Side-specific laterality index (SLI)} = \frac{\text{the number of choices at 4R} - \text{the number of choices at 4L}}{\text{the number of choices at 4R} + \text{the number of choices at 4L}} \times 100$$

Values computed by using this formula range from -100 to 100. Zero indicates an absence of any lateral bias; 100 indicates an absolute right bias; -100 indicates an absolute left bias (Figure 4B). Thus, SLI reflects the direction of the bias (Versace et al., 2020).

Two-sided 1-sample Wilcoxon and Bayesian tests were performed on both indexes against 0. ULI revealed that both strains showed a significant asymmetry (Hybro: Mean = 43.693, SE = 7.307, $V = 120$, $P < 0.001$, $r_{rb} = 1.000$; Ross308: Mean = 34.453, SE = 5.408, $V = 153$, $P < 0.001$, $r_{rb} = 1.000$). Bayesian analyses provided extreme evidence for the presence of an unspecific asymmetry in both strains (Hybro: BF = 967; Ross308: BF = 3,061).

Whenever we analyzed the direction of the side bias, by using the SLI index, the Ross308 chicks showed a significant left asymmetry (Ross308: Mean = -20.150, SE = 8.671, $V = 121$, $P = 0.036$, $r_{rb} = 0.582$), supported by anecdotal evidence by the Bayesian statistic (BF = 2.011). The Hybro chicks did not show any lateral bias (Hybro: Mean = -11.736, SE = 13.095, $V = 76$, $P = 0.378$, $r_{rb} = 0.267$); Bayesian analyses provided no evidence for sided-lateral bias (BF = 0.362). Proportion test and Bayesian A/B test showed that more chicks in the Ross308 group showed left bias than right bias (left bias (SLI < 0) = 66.667%, right bias (SLI > 0) = 27.778%, $\chi^2 = 4.012$, $df = 1$, $P = 0.045$, Cohen's $h = 0.800$, BF = 7.739) while in the Hybro group, there was no difference (left bias = 43.75%, right bias = 50%, $\chi^2 = 0$, $df = 1$, $P = 1$, Cohen's $h = 0.125$, BF = 0.757).

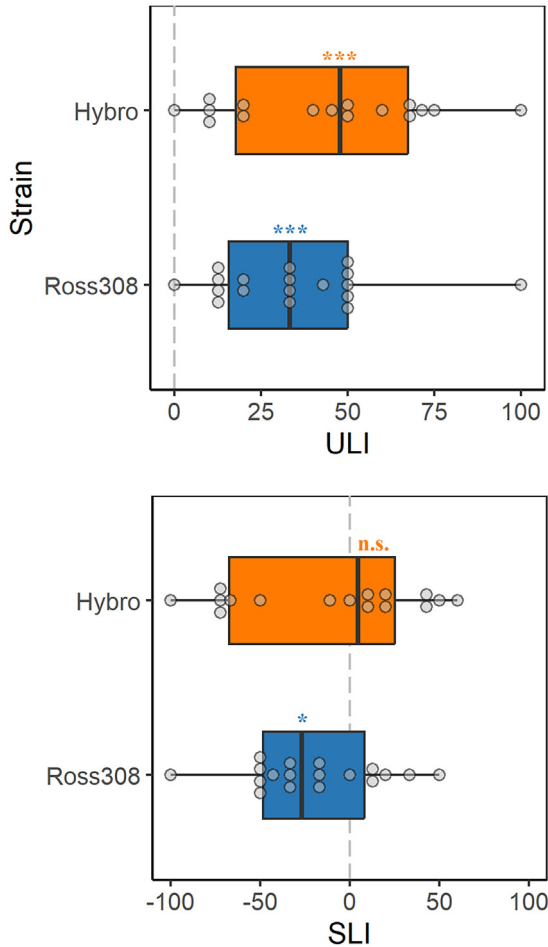


Figure 4. Laterality indexes. (A) Unspecific laterality index (ULI). Departure from zero indicates progressively stronger asymmetrical choice in either 4L or 4R in frontal-parallel test. (B) Side-specific laterality index (SLI). Values below zero indicates a left preference (4L) while values above zero indicates a right preference (4R). Each dot represents an individual chick. In both figures, A and B, * indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$.

Together, these indexes provide comprehensive evidence of lateralized behavior. While an unspecific side bias was shown by ULI in both strains, the SLI index allowed to better investigate bias direction and to ascertain a left-sided bias only in the Ross308 chicks.

DISCUSSION

In the present study, we investigated numerical cognitive abilities in 2 chicken hybrids strain: the Hybro and the Ross308. Despite the similar appearance, these strains differ in feed conversion ratio, weight gain, and final body mass (Benyi et al., 2009). The intense selection for production traits, such as weight gain and egg laying, may be linked to undesirable side effects and changes in animal cognition and behavior (Dudde et al., 2018), but these are up to now poorly examined and understood (Ferreira et al., 2021). In particular, we explored whether these strains could reach the same level of performance in dealing with an ordinal task and whether they showed different behaviors in spatial

responses whenever the task required transferring a learned rule to a differently oriented series.

We first trained all chicks to find a food reward in the 4th container in a series of 10 identical and sagittally aligned containers, which were maintained in fixed positions throughout training. We selected this kind of training because it has been extensively used to test ordinal abilities in animals (rats (Davis and Bradford, 1986; Suzuki and Kobayashi, 2000), day-old domestic chicks (Rugani et al., 2007, 2010a, 2011, 2016b; Rugani and Regolin, 2020, 2021), adult Clark’s nutcrackers (Rugani et al., 2010a), fish (Petrazzini et al., 2015; Potrich et al., 2019), rhesus monkeys (Drucker and Brannon, 2014)) and it was also adapted to test ordinal abilities in children (West and McCrink, 2021; Rugani et al., 2022). Whereas in previous versions this task required subjects to reach a pre-established performance at training to enter the test, here we controlled that all subjects received the same amount of training. This difference in the procedure allowed us to assess individuals’ cognitive and behavioral differences. Chicks then underwent a sagittal test aimed at establishing the learning of the ordinal/numerical rule. The fronto-parallel test had a 2-fold objective. It allowed to examine the possibility of transferring the learnt rule to a new oriented series, thus not providing any cues that birds could use during training to solve the task (during training, e.g., the 4th container was always in the same position in the experimental apparatus). Moreover, the fronto-parallel test allows testing spatial bias. Differently from training, where the correct option was only 1 (the 4th), there were 2 correct options - the 4th item from the left and the 4th from the right - allowing to assess lateralized behaviors.

Our main finding was that the Hybro chicks outperformed the Ross308 chicks. In the sagittal test, although both strains selected the 4th item above chance, the Hybro chicks scored a higher percentage of correct responses. In the fronto-parallel test, despite both strains selected the 4th left and right containers above chance, the Hybro chicks selected the correct items with greater accuracy than Ross308. Both broiler strains succeeded in learning the ordinal task and in transferring the learnt rule to a series with a novel orientation. For what concerns the assessment of the lateral bias, both strains showed a significant spatial asymmetry according to the Unspecific Laterality Index, ULI. When analyzing the direction of the lateral bias using the side-specific laterality index, SLI, we observed that Ross308 chicks exhibited a significant left asymmetry; Hybro showed a similar asymmetry, although not statistically significant. This suggests that genetic factors may play a role in side bias in chicks. Further research could investigate the underlying genetic mechanisms and potential implications for poultry breeding and management. Additionally, this research may have broader implications for our understanding of lateral bias in other species, including humans, and its relationship to genetics and behavior. The difference in cognitive performance though, suggests a possible role of artificial selection. These results prompt more investigation aimed at better

understanding whether and how intensified artificial selection affects cognitive capabilities. For example, future studies could investigate whether these differences between strains are already present during the learning process by analyzing the learning curves during training. This of course may lead to implications for animal welfare, though at present we have no reasons to assume that the lower performance of Ross308 correlates with lower sentience (Marino, 2017a,b; Vallortigara, 2017).

Our findings might be compatible with the domestication hypothesis. This hypothesis suggests that domestication has led to cognitive and behavioral adaptations, which allow domesticated species to perform better in human-controlled environments (Lewejohann et al., 2010; Albiach-Serrano et al., 2012). It should though be pointed out that broilers underwent artificial selection more than a domestication process. Thus, our results are more in line with the hypothesis that intense selection for production traits, such as weight gain, can generate changes in cognition and behavior (Dudde et al., 2018). The resource allocation theory (Beilharz et al., 1993) can explain the mechanism that causes these side effects. This theory claims that, under selection within a specific environment, animals optimally and proportionally allocate their available energetic resources between production and fitness. Evolutionary adaptation should result in an optimal energetic distribution among self-preservation and reproductive traits to enhance evolutionary fitness in wild animals, like the red junglefowl. This implies that any additional selection which increases production performance and requires an increase in dedicated resources, will cause a remodulation of the resources assigned to fitness, resulting in a decline (Beilharz et al., 1993; Mignon-Grasteau et al., 2005). Differences in feeding behaviors have been reported in 3 different poultry breeds, representative of increased degrees of domestication: the red junglefowl (*Gallus gallus*); the Swedish bantam, which is a domestic breed that has not undergone selection for production; and the Hy-Line, a White Leghorn laying hybrid, selected mainly for food conversion efficiency. Junglefowl and bantam preferred to feed in the feeding site where food was mixed with wood shavings, while Hy-Line showed a preference for the site where food was freely available and unmixed (Schutz and Jensen, 2001). Junglefowl and bantam thus showed feeding behaviors requiring higher energetic costs, while Hy-Line preferred options that maximized energy intake and reduced energetic costs. Moreover, Hy-Line was less active and involved in social interactions. Selection for high production seems therefore to modify behavioral strategies, supporting the idea that it induces variations in the behavioral repertoire of artificially-selected hybrids to sustain energetic costs needed for higher production (Schutz and Jensen, 2001). For a more detailed discussion of the effects of artificial selection and domestication on behavioral and cognitive traits, see the Introduction section (e.g., Lindqvist et al., 2002; Albiach-Serrano et al., 2012; Dudde et al., 2018; Ferreira et al., 2021, 2022; Bessa Ferreira et al., 2022). On the contrary,

in a discrimination learning task that allowed to gain a food reward under the selection of the correct option, highly productive laying hens (300 eggs/yr) performed better than moderate productive laying hens (200 eggs/yr). Better learning strategies were then mastered by high productive than by moderate productive laying hens. This higher learning achievement might be a response to constraints imposed by selections that maximize the pressure on productivity. To cover such high energetic costs, hens could have developed more sophisticated cognitive abilities to optimize their energy intake (Dudde et al., 2018). Considering that egg production may be regarded as an integral aspect of fitness, as it increases offspring, these results do not necessarily contradict the resource allocation theory. Integrating this perspective with our findings calls for a deeper understanding of the implications for behavior and cognition in species selected for high egg laying and meat production purposes, and their underlying biological (genetic) bases. Independently of the direction—an increase or a decrease—in cognitive abilities, research demonstrated that selection aimed at enhancing productivity traits also affects animal behavior and cognition. These side effects are up to now poorly examined and understood. Recently, it has been emphasized the need for a deeper understanding of hens' cognitive abilities (Ferreira et al., 2021). A better comprehension of the relationship between chicken cognition and common welfare issues (e.g., feather pecking) in chicken production systems is crucial for creating appropriate environmental conditions to enhance animal welfare (Nawroth et al., 2019; Ferreira et al., 2021). Thus, knowledge of chicken cognition, as well as that of the other farm animals, plays a pivotal role in improving their living conditions and promoting more humane practices in poultry farming (Fijn et al., 2020; Ferreira et al., 2021).

Understanding how genetic manipulations aimed at increasing meat commercial production may affect cognition and behavior can have important implications on animals' husbandry and consequently on their welfare (Nicol, 1996; Abeyesinghe et al., 2005; Smith and Johnson, 2012; Dudde et al., 2018; Ferreira et al., 2021).

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DISCLOSURES

The authors declared no conflicts of interest.

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