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Social preferences in chickens–effects of domestication and tameness

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It has been suggested that evolution of domesticated phenotypes may have evolved as a result of correlated selection responses to reduced fear, a prominent feature in early domestication. To investigate whether domestication changes in social preferences can be attributed to increased tameness, we studied two lines of Red Junglefowl, ancestors of domesticated chickens, bidirectionally selected during 12 generations for high (HF) or low (LF) fear of humans and compared the differences between these lines to those between unselected Red Junglefowl (RJF) and domesticated White Leghorn egg layers (WL). One bird at a time was observed on its own for 12 min in an arena with one adjacent pen behind netting on each side. One of the adjacent pens contained familiar birds, and the other contained unfamiliar birds. Towards the end of the observation period, a sudden stressful stimulus was displayed, and we compared the reactions of the breeds to this as well. Male RJF spent more time close to the unfamiliar birds than WL, and performed more agonistic behaviour, but this was not mirrored in the selected birds. For females, it was WL that performed the most agonistic behaviour. Both LF and WL males showed more non-agonistic social exploration than HF and RJF respectively. Male LF and WL emitted more food calls than HF and RJF respectively. We conclude that several differences between RJF and WL were mirrored in the selection lines, with LF behaving more like domesticated WL. This is in line with the hypothesis that selection for tameness may have driven domestication related changes in social behaviour against conspecifics.

KEYWORDS

domestication, Red Junglefowl (*Gallus gallus*), social preference, chickens, tameness, social behaviour, White Leghorn

1 Introduction

Domestication has been defined as the process whereby animals change genetically and phenotypically in response to selection pressures associated with a life under human supervision (Jensen and Wright, 2022). One of the most important aspects of this selection is a reduction in fear of humans (Agnvall et al., 2018) and during the initial stages of domestication, at least passive tameness was likely required to tolerate human presence without causing stress that could impair growth and fertility. Tameness, defined as the innate propensity to be tamed, hinges on the motivation to approach humans and engage in calm interactions devoid of fear or aggression (Price, 2002). Tameness comprises two critical components: active tameness, reflecting a willingness to approach humans without fear or aggression, and passive tameness, indicating a reluctance to avoid human interaction and higher levels of active tameness in mice are associated with increased motivation to seek positive social interactions with conspecifics (Venkatachalam et al., 2024). In chickens, it is known that domestication has modified social behaviour in general (Väisänen et al., 2005; Väisänen and Jensen, 2003), and selection for increased tameness appears to have similar effects, corroborating the importance of reduced fear of humans as a possible driver of the evolution of domesticated phenotypes (Gjøen and Jensen, 2021).

Chickens were domesticated from the South-East Asian Red Junglefowl about 8 000 years ago (Tixier-Boichard et al., 2011) (although some authors suggest a later time-point for the onset of domestication; see Best et al., 2022). In the wild, Red Junglefowl live in stable family groups and defend a small territory against intruders (Collias and Collias, 1996), whereas domesticated chickens have throughout history mostly been kept in large and unstable groups which could possible lead to a high level of social tolerance against unfamiliar individuals (D'Eath and Keeling, 2003). However, previous studies on domestication effects on sociality have shown that social preferences and reactions to strangers in young White Leghorns and Red Junglefowl differed from what could be expected, since young domesticated White Leghorns showed a preference for familiar chicks and avoidance of unfamiliar, which was not observed in Red Junglefowl chicks (Väisänen et al., 2005; Väisänen and Jensen, 2003). Hence, there is a need for further studies of social preferences in relation to chicken domestication, incorporating adult animals in addition to the previous studies of young chicks.

A previous study found that White Leghorn chicks were less agitated and fed more following a stressful stimulus compared to Red Junglefowl (Gjøen et al., 2023b). The same study also found that presence of a partner had a stronger effect on Red Junglefowl chicks. Hence, before sexual maturity, White Leghorns have a higher feed motivation and lower effect of a social buffer than ancestral birds.

As mentioned, tameness may be a factor driving early evolution of domesticated phenotypes. This insight was prompted by studies by the Russian geneticist Belyaev on captive silver foxes, selected for reduced fear of humans only (Trut et al., 2009). The selected animals evolved a range of domestication related phenotypic traits, both morphological (loss of pigmentation, curled tails, etc.) and behavioural, e.g., increased playfulness. This suggests that tameness may have affected social behaviour as a correlated side-effect already during the early phases of animal domestication. In an experiment inspired by the Belyaev studies, we selected ancestral Red Junglefowl for reduced fear of humans over several generations, and similar to what was found in the fox studies, tameness had a relatively high heritability (Agnvall et al., 2012) and caused the development of several domestication related phenotypical changes in chickens, such as increased growth, reduced stress sensitivity and modifications to some aspects of social behaviour (Agnvall et al., 2018; Gjøen and Jensen, 2021).

A prominent aspect of the domesticated phenotype is an overall reduction in relative brain size (Wright et al., 2020). However, whereas the brains of domesticated chickens are about 25-35% smaller relative to body size compared to Red Junglefowl, the cerebellum is actually relatively larger in domesticates (Henriksen et al., 2016). This suggests that cerebellum affects traits that have been important during domestication. While this part of the brain has traditionally been associated mainly with motor control, recent research clearly shows that it is involved in a wide range of cognitive functions, such as memory consolidation, emotional regulation and social cognition (Katajamaa et al., 2021; Heleven et al., 2019; Adamaszek et al., 2017; Prati et al., 2024). Interestingly, selection for reduced fear of humans in Red Junglefowl caused a similar reduction in overall relative brain size, but an increase in relative cerebellum size, as well as altered neuron density in the cerebellum (Gjøen et al., 2023a), suggesting that tameness drives also neurobiological adaptations to domestication. Again, this underlines the importance of increasing our general understanding of how social behaviour is affected by tameness and domestication.

To investigate how domestication and tameness affect social behaviour and social tolerance in chickens, we studied four different lines of birds: Ancestral Red Junglefowl (RJF), domesticated White Leghorn (WL) egg-layers, and Red Junglefowl selected for either high (HF) or low (LF) fear of humans. The LF and HF lines are thought to represent the early stages of domestication, where tameness was most likely a major selection criterion. Based on the above-mentioned selection history of the chicken, we hypothesised that the LF and WL lines would show more positive social behaviour and less agonistic behaviour when confronted with unfamiliar birds than the RJF and HF lines. We also hypothesised that all birds would seek social support mainly from familiar individuals following a brief, stressful experience, and that this tendency would be less pronounced in LF and WL. Additionally, we also hypothesised sex difference, as the male and female phenotypes in both Red Junglefowl and modern chickens differ significantly in various traits (Gjøen and Jensen, 2021; Oscarsson and Jensen, 2024). These inherent differences and the fact that our animals were sexually mature made it reasonable to expect the results being strongly influenced by the sex of the individual.

2 Method

2.1 Ethical note

The experiments were approved by the Linköping Animal Ethics Committee under license no. 14916-2018, with all procedures conducted accordingly.

2.2 Animals and rearing

We used a total of 84 birds (both males and females) from four lines: Red Junglefowl (RJF) from an unselected line (N=25, females

= 13, males = 12), domesticated White Leghorn layers (WL) (N=18, females = 11, males = 7), and RJF selected for high fear of humans (HF; N=24, females = 14, males = 10), or low fear of humans (LF; N=17, females = 5, males = 12). The unselected RJF were originally from a captive zoo population that has been kept in our lab, maintaining as much genetic diversity as possible, for about 20 years. The WL were birds from a non-commercial, outbred population originally bred at the Swedish University of Agricultural Sciences, and in the last 20 years kept in our lab with occasional outbreeding from unrelated birds of the same line, in order to maintain as much genetic diversity as possible. More information about the background of these birds can be found in (Schütz and Forkman, 2001).

The selected RJF were from generation 12 of a selection program started by outbreeding two different zoo populations for two generations, followed by 12 generations of selection according to a standardised fear-of-human test applied when the birds were 12 weeks old. For details about the birds and the selection scheme, see (Agnvall et al., 2012).

All birds were hatched at Linköping University, Sweden. They were kept in floor-pens (0.9 x 1.8 m; expanded to 0.9 x 3.6 m when the chicks were two weeks old) with 30-40 chicks per pen in the hatchery unit. At six weeks of age they were transferred to the breeding facility of the university, where the same groups were kept in aviary pens (3 x 3 x 3 m) with three levels, nests, perches and feed and water ad lib. All birds except the WL males also had free access to an outdoor area of similar size. Chicks were vaccinated against Marek's disease on day one after hatch, and they had ad lib access to commercial chicken feed and water throughout their lives.

The behavioural tests were performed when the selected lines of birds were 48-52 weeks of age, while RJF and WL were two years old, i.e., all birds were sexually mature.

2.3 Test arena and procedure

The experimental setup consisted of an arena (80 cm x 285 x 190) that was monitored by a video camera (GoPro Hero10) placed at one of the short ends. A drinker, as well as a perch (80 cm wide, 80 cm above the ground) were situated in the far end of the arena from the camera position. Feed (pellets and sweet corn) was available along each side of the arena, situated in the middle of the arena. One animal was placed in this arena at the time, and instantly behavioural recording through video commenced.

On each side of the arena, detached by wire mesh, there were two similar sized pens. One contained a pair of unfamiliar same-sex birds as the test bird, and the other contained a familiar pair that was from the same group as the test bird. The adjacent enclosures were covered in saw dust and the birds in them had access to food and water ad lib, as well as perches (80 cm above ground, 85 cm wide) and a hay ball for enrichment. Each pair of stimulus birds remained in the test arenas for a maximum of three consecutive days.

On the day of testing, a maximum of seven birds at a time were collected in the morning from their home pen and transferred to a holding area (3 x 2 m, equipped with a perch and food and water ad lib.). Each test bird was collected from the holding pen, placed in the middle of the arena and its behaviour was recorded for 12 minutes. During tests, the test birds could see, hear, and smell the stimulus birds, but not touch them through the wire mesh. After 25 minutes following placement in the arena, a stressful stimulus was suddenly exposed in order to startle the test bird. The stimulus was only visible to the test bird, and not to those in the adjacent pens. The stimulus consisted of a dark-blue working overall, manually dropped by a hidden person from the roof where it had been out of sight for the birds. After the stimulus was dropped, it would dangle on a cloth hanger in full view of the test animal for 5 minutes, 1.50 meters away from the arenas' short side, creating an impression of a suddenly appearing, potentially stressful, human figure. The behaviour of the test bird was recorded for the entire 5 min following the stress stimulus.

The test sessions were video-recorded and later analysed according to the ethogram in Table 1. Behaviour was coded using Solomon coder (Solomon Coder beta 19.18.02). Only one observer (the first author) analysed the videos in this study, so an inter-observer index was not calculated. The ethogram included clear, detailed descriptions, making the observations relatively straightforward and leaving little room for conflicting interpretations.

2.4 Statistics

We first calculated the average values of each of the recorded variables (% of time for the duration variables, nrs of crows per minute for the crowings) separately before and after the exposure of the stress stimulus. We then calculated the difference in the value of each variable after stress exposure. Generalised Linear Models (GLMz) was used to analyse the effects of group and sex, as well as the interaction between these two, comparing HF vs LF and WL vs RJF in separate models. The reason for using separate models was that we specifically hypothesised similar differences between HF and LF as between WL and RJF. After visual examining P-P plots, the models were fitted with the appropriate link function (normal or

TABLE 1 The behaviours coded during the behavioural tests, their definitions and the scoring method used.

Behaviour	Definition	Scoring Method
Time spent on unknown side	The Proportion Of Time Spent On The Half Of The Arena Close To The Unknown Birds	Duration
Agonistic behaviour	Threatening Or Attacking The Stimulus Birds Through The Wire Mesh	Duration
Social exploration	Showing Interest Or Affiliation Towards The Stimulus Birds, Such As Standing Or Following Them With A Neutral Or Exploratory Posture	Duration
Non-social exploration	Exploring The Arena, Perching, Feeding, Or Drinking	Duration
Food calling	Repeated, Short Clucks Emitted By Males Or Females When Finding Food	Duration
Crowing	Male Territorial Call	Frequency

Gaussian). We considered a P-value of less than 0.05 as significant, and when 0.1>P>0.05, we interpreted it as a tendency. The statistical analyses were carried out using R studios statistical software (RStudio Team,4.3.0, 2020) and SPSS (IBM Corp., 2017).

3 Results

The results of the statistical analyses, which are referred to below, are summarised in Tables 2 and 3.

3.1 Preference of familiar vs unfamiliar birds

There was no effect of either group or sex on the time spent on the side of the arena close to the unfamiliar birds when comparing HF vs LF (Table 2), neither before nor after the stress exposure. However, in the comparison between WL and RJF (Table 3) there was a tendency for a group effect before stress, as well as a tendency for an interaction between group and sex. Following stress, there was a significant interaction effect of group and sex in this respect (Table 3). As seen in Figure 1, male RJF spent more time on the side with unfamiliar birds than WL males, and decreased the time on this side after stress, whereas the other groups only changed their behaviour marginally following stress. For females there were no clear effects before stress, but both LF and WL tended to decrease their time with unfamiliar birds following stress compared to HF and RJF respectively (Figure 2).

3.2 Agonistic behaviour

Considering agonistic behaviour, there were no effects of either group or sex when comparing HF vs LF (Table 2), neither before nor after stress. However, there was a tendency for an effect of group when comparing WL and RJF, as well as a significant interaction between group and sex (Table 3). As seen in Figure 1, in males, RJF showed clearly more agonistic behaviour than any of the other groups, while for females, it was the WL that showed most agonistic behaviour (Figure 2). In both cases (male RJF, female WL), the frequencies of agonistic behaviour decreased after stress, while it remained unaffected in the other groups.

3.3 Social exploration

Regarding social exploration, i.e., non-agonistic social interactions, there was a significant effect of group when comparing HF vs LF before stress, but not after (Table 2). Comparing WL and RJF, there was a significant interaction of group and sex before stress (Table 3). As seen in Figure 1, LF and WL males tended to perform more social exploration before stress than HF and RJF respectively, and the numerical tendency was the same after stress, although this effect was not significant. For females, there was no similar trend (Figure 2).

TABLE 2 Outcome of the statistical analysis (Generalised Linear Models) of the behavioural variables when comparing Red Junglefowl selected for high vs low fear of humans (Group = selection line).

Behaviour	Predictor(s)	Wald Chi-Square	*P*-Value
Time unknown side	Group	0.28	0.60
	Sex	1.34	0.25
	Group: Sex(Interaction)	0.077	0.78
Time unknown side change	Group	1.03	0.31
	Sex	1.66	0.20
	Group: Sex(Interaction)	.45	0.50
Agonistic behaviour	Group	1.59	0.21
	Sex	0.75	0.39
	Group: Sex(Interaction)	0.45	0.50
Agonistic Behaviour change	Group	2.15	0.14
	Sex	0.26	0.61
	Group: Sex(Interaction)	1.14	0.29
Social Exploration	Group	11.28	<.001
	Sex	1.96	0.16
	Group: Sex(Interaction)	0.46	0.50
Social Exploration Change	Group	0.77	0.38
	Sex	0.05	0.83
	Group: Sex(Interaction)	0.003	0.96
Non-Social Exploration	Group	5.65	0.017
	Sex	4.31	0.034
	Group: Sex(Interaction)	0.13	0.73
Non-Social Exploration Change	Group	2.33	0.13
	Sex	1.19	0.28
	Group: Sex(Interaction)	0.68	0.41

3.4 Non-social exploration

Considering non-social exploration (i.e., exploring the arena, foraging, perching), there were significant effects of both group and sex when comparing HF vs LF before stress, but no effects after stress (Table 2). In the comparison between WL and RJF, there was a significant effect of group, but not of sex before stress, and a tendency for an interaction between group and sex after stress (Table 3). As seen in Figure 1, HF and RJF males performed more

TABLE 3 Outcome of the statistical analysis (Generalised Linear Models) of the changes in behavioural variables when comparing Red Junglefowl and domesticated White Leghorns (Group = breed).

Behaviour	Predictor(s)	Wald Chi-Square	*P*-Value
Time unknown side	Group	3.635	0.057
	Sex	1.157	0.28
	Interaction	2.716	0.10
Time unknown side change	Group	1.474	0.23
	Sex	.108	0.74
	Interaction	12.176	<.001
Agonistic behaviour	Group	3.156	0.076
	Sex	.199	0.66
	Interaction	40.493	<.001
Agnostic behaviour change	Group	1.680	0.20
	Sex	0.004	0.95
	Interaction	13.725	<.001
Social Exploration	Group	1.490	0.22
	Sex	0.879	0.35
	Interaction	9.719	<0.01
Social exploration change	Group	0.081	0.78
	Sex	2.884	0.09
	Interaction	6.013	0.014
Non-Social Exploration	Group	6.562	0.010
	Sex	0.157	0.69
	Interaction	0.104	0.75
Non-social Exploration Change	Group	0.417	0.52
	Sex	0.385	0.54
	Interaction	2.756	0.10

non-social exploration before stress than LF and WL respectively. In females, HF and LF performed similar amounts of non-social exploration, whereas WL performed less than RJF (Figure 3). After stress, both LF and WL females performed less exploration than HF and RJF respectively.

3.5 Male vocalisations

Male LF and WL emitted significantly more food calls than HF and RJF respectively before stress (LF vs HF: $W\chi^2$ = 32.8, P<0.001; WL vs RJF: $W\chi^2$ = 17.8, P<001). After stress, both LF and WL reduced their food calling significantly compared to HF and RJF respectively (LF vs HF: $W\chi^2$ = 33.9, P<0.001; WL vs RJF: $W\chi^2$ = 18.0, P<0.001) (Figure 3).

Before stress, male LF emitted more crows than HF ($W\chi^2$ = 6.4, P=0.012) (Figure 3) but there was no significant difference between RJF and WL ($W\chi^2$ = 0.18, P=0.67). After stress, WL tended to decrease the crowing more than RJF ($W\chi^2$ = 3.1, P<0.08), whereas there was no such effect for HF compared to LF ($W\chi^2$ = 0.91, P=0.34), although the numerical tendency was that LF decreased crowing slightly more than HF (Figure 3).

4 Discussion

We studied the effects of selection for reduced fear of humans, so called passive tameness (Venkatachalam et al., 2024), on social preferences in the ancestor of domestic chickens, the Red Junglefowl. The rationale behind this was the assumption that selection for passive tameness must have been a prerequisite for early domestication and could possibly have been a driver of many other traits typically associated with domesticated animals through correlated responses (Agnvall et al., 2018). We would therefore expect selection for tameness to affect social behaviour and social preferences in line with differences that can be seen in present-day domesticated chickens compared to ancestral Red Junglefowl (RJF). Therefore, we compared the difference between two lines of RJF bidirectionally selected for tameness (High Fear, HF and Low Fear, LF) with the differences between a representative breed of modern laying hens, the White Leghorn (WL) and unselected RJF. We found several aspects where the differences between the selected RJF-lines mirrored the differences between RJF and WL, indicating that selection for tameness can indeed cause modifications in social behaviour in line with that caused by domestication. This corroborates earlier suggestions that tameness may be an important driver of domesticated phenotypes.

We also included a brief stress experience in the test, assuming that this would reveal both possible differences between lines and breeds with respect to their immediate reactions, as well as in their tendency to seek social support. This is a well-known phenomenon, where animals (and humans) recover better from a stressful event if they are close to social partners (Rault and Rault, 2012). Given that RJF in the wild form stable, long-lasting family groups with tight relationships we expected birds in our experiment to seek out familiar companions following stress, and that this tendency would be stronger in RJF and in HF compared to WL and LF respectively.

As expected, male RJF spent more time close to unfamiliar birds before stress but more with familiar after stress, whereas LF and WL females, opposite to our predictions, tended to affiliate more with familiar birds after stress compared to HF and RJF females. Hence, in the selected lines, females tended not to follow the expected pattern, whereas the results for males were ambiguous. The sex differences may be related to the highly sexually dimorphic traits displayed in this species. Males are brightly coloured, about 50% larger than females and compete within the group for matings as well as being responsible for territory defence in the wild (Desta, 2019; Collias and Collias, 1996). Hence, males may react with more intense territorial behaviour as a consequence of being close to unfamiliar birds, but still seek social support following stress. Even thought there was a similar numerical trend when comparing HF

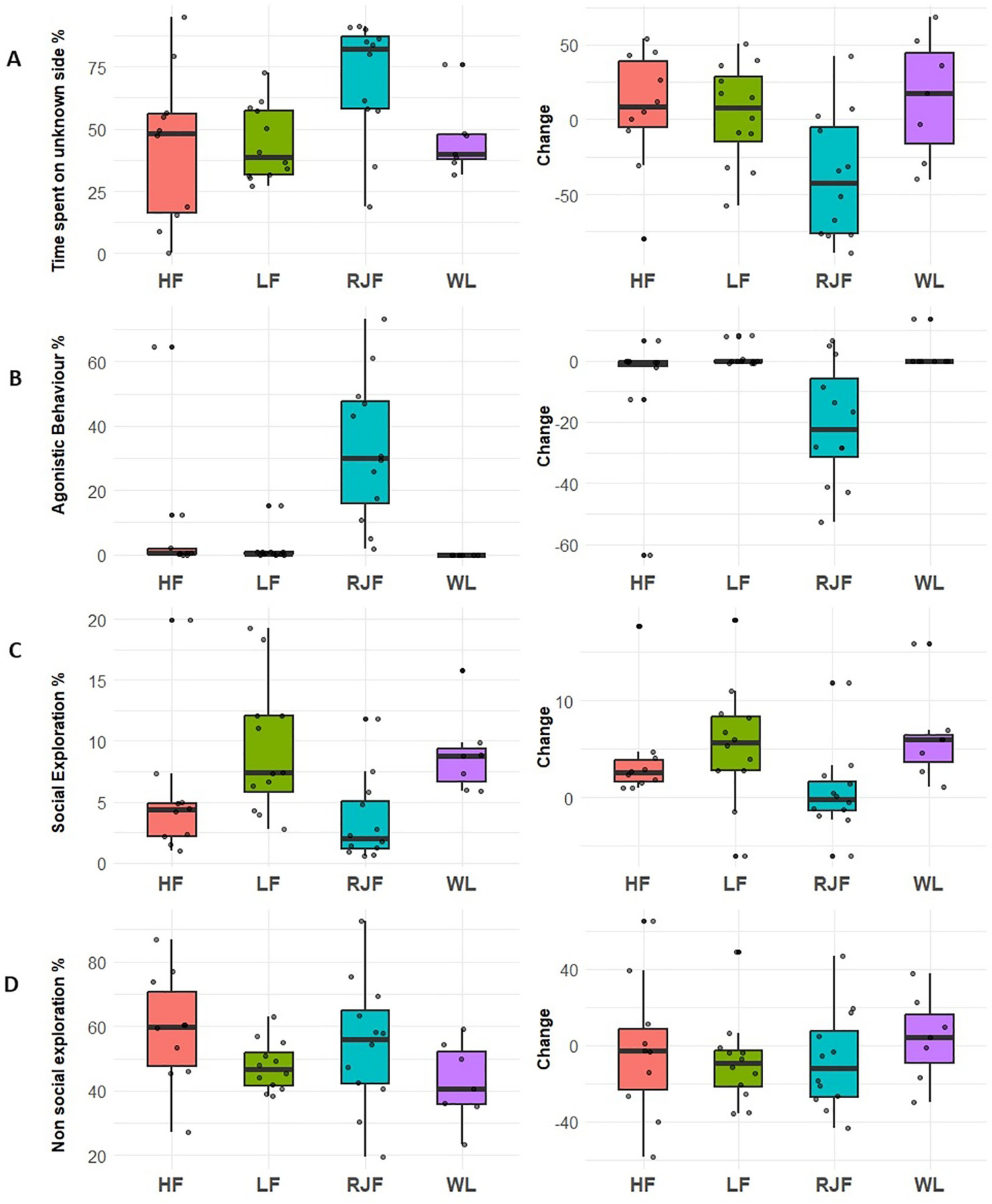

FIGURE 1
Occurrence of different behaviour categories **(A-D)** before stress stimulus occurrence (left column), and the change in behaviour following stress stimulus (right column) in male Red Junglefowl selected for low (LF) or high (HF) fear of humans, as well as in unselected Red Junglefowl (RJF) and domesticated White Leghorns (WL). The graphs are box- and dot-plots displaying individual values (dots), medians (horizontal line), upper and lower interquartile range (box) and min and max values (whiskers).

and LF males, this was not significant, indicating that tameness has not affected territoriality as measured here.

The fact that males are more territorial than females may explain the more frequent agonistic behaviour in RJF males compared to all other groups. Here, we did not find any effect of selection, again indicating that selection for tameness has not caused any direct effects on intra-specific aggression and territoriality. However, it is noteworthy that both selection lines showed very low frequencies of aggression, both before and after stress. However, for females, it was the WL that performed most aggression, and just like in RJF males, this was clearly reduced after stress. We expected domesticated chickens to have adapted to a more socially unstable group life which would be typical for a life in captivity and under production conditions, but our observations were not in line with this for the WL females. Some previous research has shown a connection between selection for high production in egg laying chickens and modifications in the functions of serotonin, catecholamines and corticosterone that are all coupled to a possible increase in aggression (Cheng and Muir, 2007). Although the LF birds have previously been shown to grow

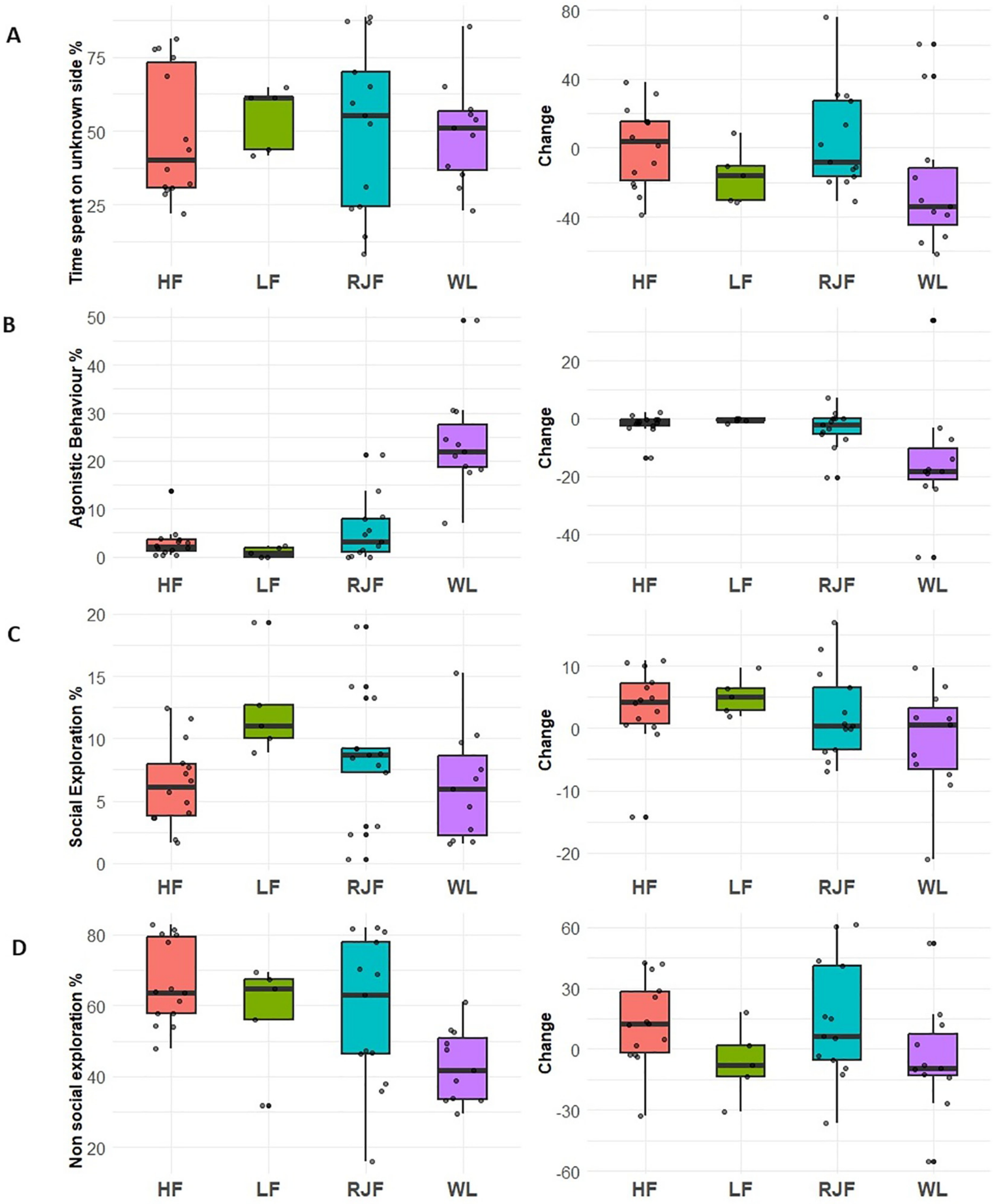

FIGURE 2
Occurrence of different behaviour categories **(A-D)** before stress stimulus occurrence (left column), and the change in behaviour following stress stimulus (right column) in female Red Junglefowl selected for low (LF) or high (HF) fear of humans, as well as in unselected Red Junglefowl (RJF) and domesticated White Leghorns (WL). The graphs are box- and dot-plots displaying individual values (dots), medians (horizontal line), upper and lower interquartile range (box) and min and max values (whiskers).

larger and lay bigger eggs (Agnvall et al., 2014), no specific selection pressure has been imposed on production in this line, which may explain the lack of increased aggression in female LF compared to HF. It is also possible that WL females, which have been bred for increased growth and high egg-production have a higher food motivation, and this may have caused a higher tendency for resource defence.

In line with the observed less aggression in LF and WL males, they also performed more non-agonistic social explorations, i.e., interacting with both familiar and unfamiliar birds in a non-aggressive manner. This was not the case in females, so the results may suggest that tameness has driven a generally reduced aggression in males, but not in females. This is in line with previous findings, showing that there is more fighting amongst RJF than WL following regrouping with un-familiar individuals (Väisänen et al., 2005). HF and RJF males were also generally more explorative than LF and WL males, perhaps as an aspect of territoriality, although this is a speculative suggestion.

Food calling is a typical male behaviour (Collias and Collias, 1996; Nicol, 2015), emitted by cockerels when they encounter edible

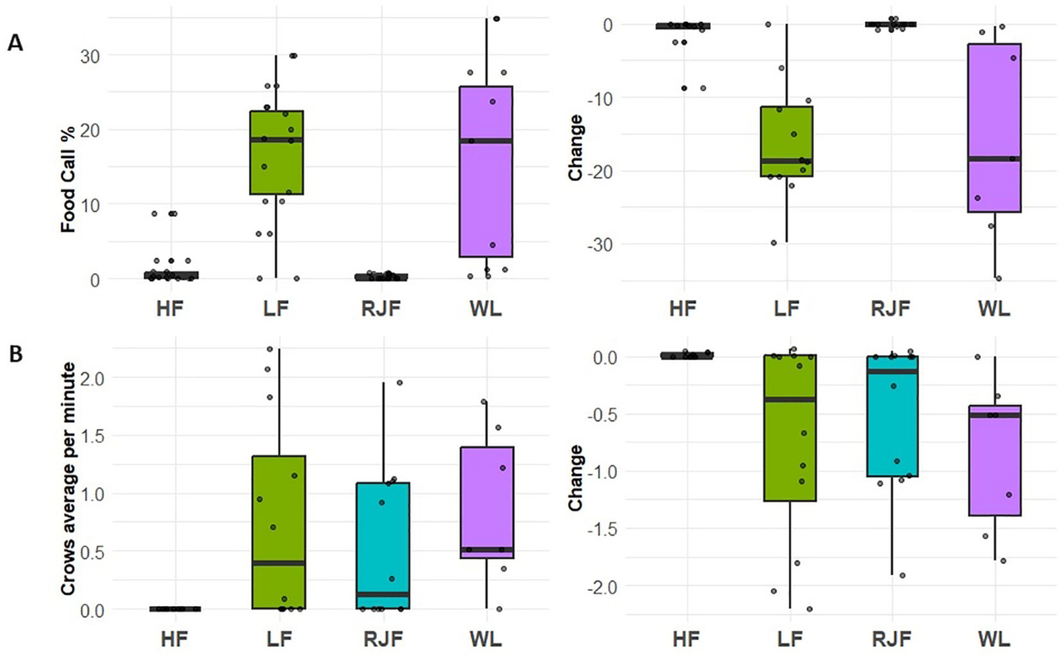

FIGURE 3
Male vocalisations **(A)** Food call; **(B)** Crowing before stimulus occurrence (left column), and the change following stress stimulus (right column) in Red Junglefowl selected for low (LF) or high (HF) fear of humans, as well as in unselected Red Junglefowl (RJF) and domesticated White Leghorns (WL). The graphs are box- and dot-plots displaying individual values (dots), medians (horizontal line), upper and lower interquartile range (box) and min and max values (whiskers).

objects, being highly effective in attracting other (mainly female) members of the flock to the food. In this sense, it is a kind of prosocial behaviour, i.e., providing benefits for other conspecifics (Rault, 2019), and as such can be expected to increase as aggression is reduced. In line with this, both LF and WL males emitted more food calls than HF and RJF respectively. This was significantly reduced following stress in both breeds. Crowing by males, on the other hand, is a territorial call that also signals social dominance (Johnsen et al., 2001). Here, LF crowed more than HF, and the numerical trend was the same when comparing WL and RJF. Both LF and WL reduced crowing following stress compared to HF and RJF respectively, although this was weakly supported by the statistical analysis. Hence, there was a trend that selection for reduced fear of humans affects crowing in a way that mirrors that seen during domestication.

Our results show that at least some aspects of social preference are affected by selection on tameness, possibly driving the phenotype towards that seen during domestication. This suggests that selection for tameness may have been an important causal factor for several aspects of the so-called domestication syndrome (Price, 2002). This term is used to describe the recurrent set of phenotypes that tend to develop in most domesticated species, including morphological, physiological and behavioural traits. The classic studies by Belyaev on farmed foxes suggest that large parts of this syndrome can occur as unintentional, correlated side-effects related to the reduction in fear of humans that is necessary for successful domestication (Trut et al., 2009). Previous studies in our group have corroborated this in the case of chicken domestication (Agnvall et al., 2018), and the present study lends further support to the hypothesis. A recent study on mice selected for active tameness observed similar responses as those reported here, i.e., tame mice indulging more in non-aggressive behaviour towards conspecifics (Venkatachalam et al., 2024).

The mechanisms underlying such correlated selection responses remain speculative. It has been suggested that the domestication syndrome can be explained by selected mutations affecting neural crest cells, a type of cells that migrate to the brain and many parts of the body in vertebrates during embryonal development (Rubio and Summers, 2022; Wilkins et al., 2014) and that therefore in theory can affect a range of phenotypes. However, this remains to be empirically verified, and some authors also question both this theory and the importance of tameness in the evolution of domesticated phenotypes (Gleeson and Wilson, 2023).

Today, there are hundreds of chicken breeds, each developed through centuries of selective breeding. With more than 1600 recognised breeds worldwide (Nicol, 2015), our study focuses on phenotypic changes from early domestication as well as one breed selected for production in Sweden. It does not capture the full repertoire of behaviours, nor the genetic diversity found in domestic chickens. The RJF and the HF lines represent the wildtype *Gallus gallus*, while the LF and the WL lines illustrate domestication at two divergent stages. The SLU White Leghorn (WL), for example, has been selectively bred for high egg production under Swedish conditions as primary objective, rather than traits such as sociality, maternal instincts, social tolerance, meat production, or resilience. While WL chickens are just one outcome of chicken domestication, chicken breeds vary greatly in activity, alertness, size, and even aesthetic traits, such as those in game breeds. Although our selection lines cannot represent all these variations, they highlight the role of tameness as a fundamental trait enabling chickens to adapt to living in close proximity to humans.

We have previously suggested that cerebellum may be a particularly interesting part of the brain in relation to possible mechanisms underlying behaviour responses to domestication, given that its size is largely preserved and even expanded in domesticated chickens unlike the rest of the brain, and that selection for increased tameness in RJF causes similar enlargement of the cerebellum (Gjøen et al., 2023a). Cerebellum is involved in a wide array of cognitive processes, including social cognition (Prati et al., 2024; Katajamaa et al., 2021; Heleven et al., 2019; Van Overwalle et al., 2015), and it remains an interesting hypothesis that this part of the brain may be involved in adaptive modifications of social behaviour during domestication.

Some caution is needed in the interpretation of our results. Firstly, we only recorded the first 12 min of each test, and five min following stress exposure. This was done since we expected that the initial exposure to the test situation would reveal the clearest differences between the breeds, and that the most intense reactions to a sudden stress event occur during the first few minutes following exposure (Ericsson et al., 2014). Furthermore, there was a significant age difference between the LF and HF birds on one hand, and WL and RJF on the other, which could potentially bias the comparisons. However, we still think that the comparisons are valid, since all birds were sexually mature, LF and HF were the same age, and also the WL and RJF were the same age. Generally, after sexual maturity, social behaviour and relationships stabilise in chickens (Favati et al., 2016). We consider the P-values <0.1 as tendency or trends, however, it's important to emphasise that one should be cautious with such interpretations. These values can point to issues like a small sample size or high variation in the data.

Previously, we have found several physiological differences between the selection lines, for example with respect to plasma levels of serotonin and possibly corticosterone reactivity (Agnvall et al., 2015). To minimise stress on each animal, we therefore chose to not include physiological measurements or stressful behaviour tests like tonic immobility, to support our findings. While our original ethogram included a range of behaviours to comprehensively assess the emotional states of the individuals, our conclusion relies solely on these behavioural observations. This limitation should be considered when interpreting our results.

5 Conclusion

In conclusion, although not all observations followed our predictions, we found a number of traits related to social preferences where selection for increased tameness in Red Junglefowl (RJF) has caused birds from the tamer selection line to behave similar to domesticated White Leghorns (WL). Some of the effects were sex-specific. Food calls were more frequent in Low Fear (LF) males compared to High Fear (HF), mirroring the fact that it was also more common in WL than in RJF. Differences in non-agonistic social exploration as well as non-social exploration was similarly mirrored in male HF vs LF as compared to WL vs RJF. Also some of the behavioural reactions to a sudden stressful stimulus were similar in the comparison between HF and LF as in that between RJF and WL. A striking exception to our expectations was the fact that there was more agonistic behaviour in WL females than in any other females, an observation that needs further studies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Linköping Animal Ethics Committee under license no. 14916-2018. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

PJ: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. JG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2024.1487688/full#supplementary-material

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