Animal behaviour

Is domestication driven by reduced fear of humans? Boldness, metabolism and serotonin levels in divergently selected red junglefowl (Gallus gallus)

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Domesticated animals tend to develop a coherent set of phenotypic traits. Tameness could be a central underlying factor driving this, and we therefore selected red junglefowl, ancestors of all domestic chickens, for high or low fear of humans during six generations. We measured basal metabolic rate (BMR), feed efficiency, boldness in a novel object (NO) test, corticosterone reactivity and basal serotonin levels (related to fearfulness) in birds from the fifth and sixth generation of the high- and low-fear lines, respectively (44–48 individuals). Corticosterone response to physical restraint did not differ between selection lines. However, BMR was higher in low-fear birds, as was feed efficiency. Low-fear males had higher plasma levels of serotonin and both low-fear males and females were bolder in an NO test. The results show that many aspects of the domesticated phenotype may have developed as correlated responses to reduced fear of humans, an essential trait for successful domestication.

1. Background

Animal domestication changed human history and was used by Darwin as a proof of principle for evolution [1]. During domestication, animals adapt through genetic and learning mechanisms to living with humans, and simultaneously develop a suite of traits, the ‘domesticated phenotype’ [2]. This may include increased size and reproduction. Mechanisms underlying this phenotypic complex recurring in many species are largely unknown, but it has been suggested that they may partly be side effects of reduced fear of humans [3,4]. However, it is not clear if tameness is a unique trait or reflects generally increased boldness and reduced stress susceptibility.

Chickens were domesticated 8000 years ago and are now the most abundant food-producing animals [5]. In a previous experiment, ancestral red junglefowl selected only for reduced fear of humans developed correlated traits, e.g. higher food competitiveness, larger eggs and bigger offspring [6]. Hence, less fear of humans could be associated with higher basal metabolic rate (BMR) and more efficient food conversion [7], for example through linkage effects [8]. Changes in levels of serotonin (5-HT) may be involved, since high peripheral 5-HT levels are associated with less fear and anxiety in chickens [9].

We studied BMR, feed conversion and 5-HT levels, as well as boldness and stress responses in red junglefowl, selected during five or six generations for high or low fear of humans, respectively.

2. Material and methods

The complete data set is available as electronic supplementary material (table S1). We studied red junglefowl from the fifth (S5) and sixth (S6) generation of populations, divergently selected for low (L-birds) versus high (H-birds) fear of humans.
The breeding scheme and housing conditions have been described elsewhere [6,10]. Briefly, birds bred and maintained with identical experiences of humans were selected in each generation based on divergent scores in a fear-of-human test. Each generation was maintained at about 50 birds per selection line, from 5 to 10 families per generation and selection line. The birds were hatched and reared under standardized conditions in mixed groups (see below).

In S5, we measured BMR using open flow respirometry as previously described [11] in 48 birds (six females and nine males from the low-fear line; 15 females and 18 males from the high-fear line) at five to six weeks of age. Four birds were individually placed in 3.6 l opaque tight plastic containers (CurTec) in a thermostatically controlled chamber at 24°C (Rubarth GmbH type 3001) for overnight measurements in darkness (18:00–06:00 h, similar to their normal diurnal light pattern). Airflow through the chambers was set at 1000 ml min⁻¹. Flow was measured continuously in all chambers using a multi-channel flow meter (FlowBar8, Sable Systems Inc.). A computer controlled multiplexer (Sable Systems Inc.) allowed the cycling of oxygen measurements between all chambers and a baseline channel. Oxygen concentration in the sample channel was measured with a FoxBox oxygen meter (Sable Instruments Inc.). BMR was assessed from the lowest 90 min average oxygen consumption during the night, which corresponds to three consecutive measurements per chamber.

When 19 weeks old (135 days), birds were placed in individual battery cages (60 × 40, height 40 cm) with water, perch, nest box and dust bath, and we measured the feed intake during 7 days in eight females and 10 males from the low-fear line, and 14 females and 12 males from the high-fear line. Feed was offered ad libitum in a spill-safe food-container, and consumption was assessed by weighing feed remains daily. As a proxy for feed conversion (‘feed efficiency’), we divided the average weekly growth of each bird between days 112 and 200 by its total feed intake during these 7 days.

At 21 weeks of age, when the birds were still in the cages, we performed a novel object (NO) test in the same birds, as a measure of boldness. An egg was cracked in the feed trough (known to be attractive food), and when the bird started eating from it, an NO (33 cl Coca Cola can) was placed 10 cm from the egg. During the test, the observer was hidden behind a screen. We recorded latency to start feeding after the NO had been placed in the trough, with a max time of 180 s.

In S6 at 28 weeks of age, we assessed plasma corticosterone levels before and after a short period of physical restraint, using methods described in detail earlier [12]. Briefly, each bird (eight males and seven females from the low line; six males and eight females from the high line) was removed from its individual cage, and a blood sample was obtained. It was then stressed by being placed in a hanging fish net for 10 min, followed by a second blood sample, after which it was returned to its home cage. A third blood sample was collected 30 min after the first one. When the S6 birds were 47 weeks old, blood samples were obtained in undisturbed conditions during midday, from 19 males and 10 females from the low-fear line, and six males and 10 females from the high-fear line, for serotonin analysis. Blood was centrifuged at 900 g at room temperature for 20 min and the plasma was stored at –18°C until analysis.

Corticosterone was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, NY, USA) following the manufacturer’s instructions. All samples were tested in duplicate and the analytic range of the assay was 32–20,000 pg ml⁻¹. The concentration of serotonin in the plasma samples was determined in duplicates using a commercial serotonin ELISA kit (analytic range 6.25–200 ng ml⁻¹, MyBiosource, San Diego, CA, USA).

Data were analysed with generalized linear models (linear mixed models for repeated measures on corticosterone), using

3. Results

BMR was significantly higher in L-birds (figure 1a). There was also a significant sex effect, where females had lower BMR than males, but no interaction was found between sex and selection.

There was a tendency for higher feed efficiency in L-birds, but no sex effects (figure 1b). However, the interaction was significant, showing that the females principally drove the effect. L-birds weighed significantly more than H-birds at
Effects of selection:


References


Figure 2. Physiological responses to selection in the two lines (high and low fear of humans; estimated marginal means with SEM). (a) Plasma levels of corticosterone in males and females at baseline, after 10 min of physical restraint, and 30 min after first blood sample. Effects of selection: \( F_{1,41} = 0.17, p = 0.68 \); sex: \( F_{1,41} = 1.02, p = 0.32 \); selection \( \times \) sex: \( F_{1,41} = 0.27, p = 0.61 \). (b) Plasma levels of serotonin (5-HT) in males and females. Effects of selection: \( \chi^2_1 = 1.1, p = 0.29 \); sex: \( \chi^2_1 = 6.0, p = 0.014 \); selection \( \times \) sex: \( \chi^2_1 = 5.7, p = 0.017 \).

112 (825 ± 10.9 g versus 691 ± 12.7 g; \( \chi^2_1 = 64.2, p < 0.001 \)) and 200 days (1054 ± 16.0 g versus 845 ± 13.2 g; \( \chi^2_1 = 101.4, p < 0.001 \)).

In the NO test, L-birds (particularly females) were significantly bolder (figure 1c). There was a significant effect of time after restraint on corticosterone levels (\( F_{1,45} = 94.7, p < 0.001 \)) but no effects of selection or sex or its interactions (figure 2b). Serotonin levels were significantly higher in L-males, as shown by a significant selection \( \times \) sex interaction (figure 2b).


