Heritabilities and co-variation among cognitive traits in red junglefowl

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Abstract

Natural selection can act on between-individual variation in cognitive abilities, yet evolutionary responses depend on the presence of underlying genetic variation. It is therefore crucial to determine the relative extent of genetic vs. environmental control of these among-individual differences in cognitive traits to understand their causes and evolutionary potential. We investigated heritability of associative learning performance and of a cognitive judgement bias (optimism), as well as their covariation, in a captive pedigree-bred population of red junglefowl (*Gallus gallus*, n>300 chicks over 5 years). We analysed performance in discriminative and reversal learning (two facets of associative learning), and cognitive judgement bias, by conducting animal models to disentangle genetic from environmental contributions. We demonstrate moderate heritability for reversal learning, and weak to no heritability for optimism and discriminative learning, respectively. The two facets of associative learning were weakly negatively correlated, consistent with hypothesised trade-offs underpinning individual cognitive styles. Reversal, but not discriminative learning performance, was associated with judgement bias; less optimistic individuals reversed a previously learnt association faster. Together these results indicate that genetic and environmental contributions differ among traits. Whilst modular models of cognitive abilities predict a lack of common genetic control for different cognitive traits, further investigation is required to fully ascertain the degree of covariation between a broader range of cognitive traits and the extent of any shared genetic control.

Introduction

Cognition (i.e., how individuals perceive, process, store and act on environmental information [1]), is a defining feature of complex animals, and has been the focus of much psychological, neurobiological and ethological research. Traditionally, cognitive abilities are investigated at a species level (e.g., comparative studies [2,3]), with between-individual variation being mainly disregarded as statistical noise [4]. More recently, however, individual cognitive abilities have come under focus [4], paralleling burgeoning interest in animal personality [5]. Importantly, if among-individual variation in cognitive abilities is associated with differences in fitness, cognitive traits will be under selection and may thus evolve given the presence of additive genetic variation and associated heritability [6,7].

Quantifying the heritability of cognitive traits thus represents a fundamental step for understanding the causes of individual variation in cognitive abilities, and for assessing their evolutionary potential [8,9]. Despite this, the number of studies investigating the genetics of cognitive traits is still limited,

partly due to difficulty to meet the demands for substantial sampling effort and the genetic information required (e.g., known relatedness). Moreover, since most research has used humans or a few laboratory strains of animals (reviewed in [6]), current understanding may be limited by a narrow taxonomic focus and biased towards study populations potentially suffering from founder effects, inbreeding and artificial selection. With this in mind, available estimates indicate moderate to high heritabilities within most cognitive domains (e.g., learning, memory, attention, [6,8,10]). The highest values are typically provided by human studies of general cognitive ability ('g'), which represents the main dimension of covariation between cognitive traits ([11,12], but see [13–15]). However, whether other animals possess a general cognitive ability remains debated [4,16–19].

Evidence for the alternative view, that different cognitive domains are governed by distinct developmental processes and genetic mechanisms, and thereby may evolve independently under diverse selection pressures, has been found in non-human primates and birds (e.g., [16,19]). Thus, given the uncertainty still surrounding the genetic architecture of cognitive traits, a statistically robust approach entailing multivariate genetic analysis [20,21] is conducive to evaluating these two hypotheses. Notably, multivariate animal models allow estimation of additive genetic components, and associated heritabilities, for each cognitive trait, and also permit partitioning of pairwise phenotypic correlations into genetic and environmental components [11,22].

Learning has traditionally held a central place in cognition research due to its widespread taxonomic occurrence and its involvement in behavioural flexibility under variable environmental conditions [1]. Particularly, associative learning may have far-reaching fitness consequences, as it mediates adaptive individual responses to environmental contingencies [23]. Nonetheless, research on the heritability of associative learning has been largely limited to a few model species (e.g., honeybees, [24], fruit flies, [25], reviewed by [23]). Importantly, associative learning includes distinct facets such as discriminative learning (i.e., the process by which animals learn to respond differently to different stimuli) and reversal learning (i.e., the extinction of a previously learnt association and the formation of a novel one [1]). Reversal learning is tightly linked to behavioural flexibility and typically associated with behavioural inhibition (i.e., impulse control [26]). Because discriminative and reversal learning may depend on different neural processes involving different brain regions [27–29], individual abilities in these facets of associative learning may not be positively correlated. Empirical research has, so far, provided mixed results. Some studies show a positive association between discriminative and reversal learning, consistent with a general underlying cognitive ability (e.g., [28,30–32]). Other

studies indicate a lack of (e.g., [33]), or negative association between the two (e.g., [34,35]). The extent to which these disparate findings are due to different evolutionary history of species, or methodological differences between studies, is unresolved. While limited statistical power could explain a lack of association, evidence for a negative association between discriminative and reversal learning agrees with theoretical models predicting speed-accuracy trade-offs in information gathering and decision making [36,37]. Speed-accuracy trade-offs may occur within-individuals (e.g., due to changes in cost of errors, [36]) and among-individuals (e.g., [38]). In the latter case, individuals are predicted to exhibit different cognitive styles, associated with different behavioural types [35,37,39]. While empirical evidence provides some support for the existence of cognitive styles [40–42], studies investigating the extent of genetics vs. environment in their control are, to our knowledge, lacking.

The interplay between learning and other cognitive traits may also involve trade-offs, which may be genetically mediated. Although this would have important evolutionary consequences, available evidence is limited [10]. Past research has mainly considered links between learning abilities, memory formation and problem solving (e.g., [43,44]), while relationships with other cognitive domains have remained largely unexplored. Among these, judgement biases have received increasing attention over the past decade, particularly within the field of applied ethology and animal welfare [45,46]. Cognitive judgement biases are consistent deviations from an accurate judgement of situations [47] typically implied to reflect individual affective state (i.e., emotions or mood, [45]). Optimism and pessimism are examples of judgement biases; optimistic individuals overestimate the chances that they will benefit from a situation, pessimistic individuals overestimate that the situation will have adverse consequences [46]. Judgement biases may arise from long-lasting effects of early life conditions [48], and be associated with personality traits (e.g., [49–51]). Theoretical models predict that judgement biases may constitute stable individual traits [47,49], with a heritable component, and therefore may respond to natural selection [47]. Interestingly, theory predicts that varying selection pressures associated with spatio-temporal environmental heterogeneity may lead to genetically-based individual differences in both judgement biases and learning abilities [47,52]. Unpredictable environmental variation may select for either optimism or pessimism, depending on extent of ecological variability and movements between habitat patches [52], and at the same time favour behavioural flexibility [53]. Thus, we may expect co-variation between these cognitive domains. At a proximate level, variation in the monoaminergic systems (e.g., dopamine and serotonin), is associated with both learning performance [54,55] and judgement bias [56]. For

instance, dopaminergic function is implicated in the establishment of stimulus-reward associations during learning and is positively associated with optimism in mammals [57,58], birds [59] and insects [56,60]. Nonetheless, inter-relationships between learning abilities and judgement biases are still largely unexplored (but see [61]). In particular, how reversal learning abilities may map onto among-individual differences in judgment, and if these traits may be under shared genetic control, is unclear.

Here we explore the inter-relationships between different cognitive traits and assess their underlying genetic components, using as a captive population of red junglefowl (*Gallus gallus*), the wild ancestor of the domestic chicken [62]. Specifically, we investigated: (i) the associations between individual performance across a discriminative learning-, a reversal learning-, and judgement bias-test; and (ii) narrow-sense heritabilities of these three cognitive traits.

Methods

Study Population

We tested chicks (n > 300, 2013-2017) from a captive population of red junglefowl housed at Linköping University, pedigree bred since 2011 and spanning six generations (see ESM S1). To reduce the expected influence of maternal effects, all eggs were artificially incubated. To minimise environmental contribution to between-individual differences, all chicks were raised in a laboratory environment (for details, see [63–65]). Chicks were individually tagged, kept on a 12:12 h light: dark cycle (7-19 local time), and observations were carried out 8-18.

Associative learning

Learning tests followed earlier described work using the same population [63,64]. In short, all birds were tested alone, in arenas (46 x 36 x 18 cm, L x W x H). Cues consisted of coloured bowls (5 x 3 cm, Ø x H), and laminated cards (9 cm^2) of the same colour (2013: blue and green, 2014-2017: black and white, [63,64]). Before testing, chicks were familiarised with being alone in the arena [63,64]. Initially, chicks were encouraged to approach the cues by the observer. A chick was regarded to have made a choice if it moved towards a cue without help and had its head within 2 cm of it. Correct choices were rewarded with 1/3 of a mealworm placed inside the bowl. In 2013, chicks were allowed to eat the reward even if the unrewarded cue was chosen, while for 2014-2017 the set up was refined and the chick was collected immediately after choosing the unrewarded cue. We statistically controlled for effects of these methodological differences (see statistical analysis section below). In addition, sub-analyses specific to each of the two study setups provided similar heritability estimates.

A new 'trial' started immediately after a choice had been made. A test 'session' lasted for a maximum of 15 minutes and was terminated earlier if the chick had lost motivation, with ≥ 1 hour between test sessions [64].

Discriminative learning

At 3-6 days old, chicks were trained to discriminate between a rewarded and an unrewarded cue (2013: half of the birds were rewarded on blue and half on green; 2014: half of the birds were rewarded on black and half on white; 2015-2017: all were rewarded on white). In 2013, the side of the rewarded cue alternated between subsequent trials, while for 2014-2017 the test was refined and the side the reward was presented on varied according to a predetermined, pseudorandom schedule. Chicks were categorised as having learnt the discrimination once they chose the rewarded cue five (for 2013) or six (for 2014-2017) consecutive times. Even with the less stringent criterion of five correct choices, the chance of putative learners being false positives is low (ESM S3). 'Learning speed' was measured as the total number of trials needed to reach learning criterion. Ten birds did not learn to discriminate between the two cues due to lack of motivation to engage in the test (e.g., trying to escape the test arena). These individuals were therefore removed from the sample and not analysed further.

Reversal learning

After passing the discriminative learning test, chicks took part in a reversal learning test at around 5-7 days of age. If > 7 hours had passed since the final discriminative learning session, the chick was exposed to a "refresh" session in which it had to again reach the learning criterion, before continuing to the reversal learning test. This was done to ensure that the association between the previously learned cue and the reward was still salient before performing reversal learning. In the reversal learning test, the previously rewarded cue was unrewarded, while the previously unrewarded cue was rewarded [64]. For this test, birds were not helped by the observer. Learning criterion and learning speed were measured as described for discriminative learning (above). Twenty-five birds did not pass this test, due to lack of motivation to engage in the test, and so were removed from the sample.

Cognitive judgement bias

In 2014-2017, at 12-13 days old, chicks were exposed to a judgement bias test (for further details, see [66]). Briefly, individuals were first exposed to a refresh of the reversal learning test, to ascertain

that the previously learnt association had not been extinguished. Immediately following the refresh, chicks were then presented with five different colour cues, one at the time and in a pre-determined, pseudorandom order. The cues were the previously learnt white ('positive', i.e., rewarded) and black ('negative', i.e., unrewarded) cues, and three novel, unrewarded, grey cues ('ambiguous'), intermediate in colour between the black and white cues (25%white/75%black, 50%white/50%black, 75%white/25%black). Chicks that were more likely to approach ambiguous cues and had a shorter latency to do so were considered optimistic. Individuals were exposed to each type of ambiguous cue three times in 2014 and 2017 (i.e., nine ambiguous cues interspersed between 24 positive and negative cues), and twice in 2015-2016 (i.e., six ambiguous cues interspersed between 16 positive and negative cues), due to other studies. Whether the chick approached the cue (yes/no) and the latency to approach (in sec), were recorded. Max time per trial was set to 30 sec.

Statistical analyses

All analyses were conducted in RStudio (version 1.1.383).

We analysed factors affecting learning speed in discriminative and reversal learning, two measures of judgement bias (i.e., probability of, and latency to approach ambiguous cues), and their associations, using univariate and multivariate mixed models implemented in the statistical software ASREML-R [67]. Additive genetic variances and corresponding heritabilities were estimated using a standard animal model approach by including individual genetic merit as a random effect and utilising the inverse of the pedigree-derived additive genetic relatedness matrix (see e.g., [22]; ESM S2 gives a brief overview of this approach and its advantages over classical techniques). For measures with repeated individual observations (i.e., judgement bias), we fitted a random permanent environment effect ('pe') as well as the additive genetic merit ('G'). Significance of heritability estimates was assessed via likelihood ratio tests ('LRT'). Fixed effects for each trait (described below) were selected based on the results of previous studies on the same population (e.g., [63–65]). Categorical factors were numerically coded by n-1 (n = number of levels of the factor) dummy (0/1) variables. To aid model interpretation and numerical convergence, all predictors were centred by subtracting population mean values, and continuous variables were standardised by dividing centred values by twice their standard deviation. Correlation between individual learning speed in the discriminative and reversal tests was evaluated by calculation of Spearman's rank order correlation coefficient. Pairwise associations between each learning speed and individual judgement bias were estimated from bivariate mixed models (see below).

Discriminative and reversal learning

Learning speed in the discriminative and reversal tests were analysed separately, following log-transformation to achieve normality, by conducting animal models (Gaussian distribution and identity link function; see ESM S2-M1,2 for model syntax) to allow estimation of heritabilities ('$h^{2\prime}$'). 'Sex' (male, female), and the colour of the rewarded cue ('cue type') were included as fixed effects. Because cue type was associated with year (i.e., 2013: green/blue, 2014-2017: black/white) inclusion of cue type (four-level factor) as a fixed effect allowed us to control for the effect of methodological differences between the first and subsequent years. Excluding data from the first study year yielded virtually identical heritability estimates (data not shown).

Cognitive judgement bias

Since, in many trials, chicks did not approach within the given 30 second period, approach latencies constituted a censored variable with a neat bimodal distribution. We therefore analysed two measures of individuals' responses in the judgement bias test: (i) approach probability, and (ii) approach latency to cues, if an approach had occurred.

We first considered responses to all the five cues (i.e., positive, negative, and each of the three intermediate, ambiguous cues) and fitted models with cue-specific individual random effects (i.e., "5-cues models" with a 5 x 5 covariance matrix for individual identity, to calculate repeatabilities for each cue type, and correlations of individual responses across cue types; see below). For approach probability, we specified univariate models including cue type as a fixed effect and its interactions with other predictors (ESM S2-M3). For approach latencies, we conducted multivariate models (5 response variables, one for each cue; ESM S2-M4) to allow cue-specific residual variances (i.e., 5 x 5 diagonal error matrix to model heteroscedasticity of error terms across cues). This approach allowed assessment of judgement bias at the population level (mean level effects; see below), calculation of cue-specific repeatabilities ('R', adjusted for fixed effects, [68]) and evaluation of individual consistency of responses across the five cue types (pairwise correlations between individual responses to each cue type: 'r_{bw}').

We analysed the probability of approaching cues using binomial (bernoulli) mixed effects models (employing the Penalized Quasi-Likelihood algorithm), with a binary response variable (1/0 for approaching vs. not) and a logit link function. 'Cue type' ('POS', 'NEG', 'NearNEG', 'MID', 'NearPOS')

was predictor in all models, allowing to quantify how approach probability differed between positive, negative and the three ambiguous cues. 'Sex' was included as a fixed effect term. In addition, to assess whether approach probability may have been affected by repeated exposure to ambiguous cues, and by changes in emotional state (i.e., following recent access to a reward), we considered 'Trial number' (1-33), and whether the previous cue was rewarded or not (i.e., 'Previous cue rewarded') as additional predictors. To further evaluate if 'Sex', 'Trial number', or 'Previous cue rewarded' may have affected approach responses differently according to cue type, all two-way interactions involving cue type were considered. Approach latency was analysed including only trials in which the focal individual approached a cue within the trial max duration (30 sec) and following log-transformation to achieve normality. Fixed effects included 'Sex', 'Trial number', 'Previous cue rewarded', and two-way pairwise interactions, as for previous modelling on approach probability (see ESM S4 for the results of mean level effects).

Having verified the similarity of repeatabilities of responses to ambiguous cues, and a strong consistency in individual response across the three types of ambiguous cues (see results), we subsequently re-ran models on ambiguous cue only, to estimate overall random effects on pooled ambiguous cues ("ambiguous cues models", ESM S2). By doing so, we obtained repeatability estimates ('$R_{ambiguous}$') for responses to ambiguous cues (one for approach probability and one for latency; see ESM S2-M5,7 for detailed model formulation), and corresponding heritability estimates ('$h^2_{ambiguous}$'), as well as the proportion of repeatability explained by permanent environmental effects ('$e^2_{ambiguous}$'; ESM S2-M6,8). Note that significance values are reported only for approach latencies, since LRT tests are not applicable to binomial mixed effects models. For the latter, significance can be approximately inferred from confidence intervals (i.e., whether 0 is included in ± 2SE, 22]). We then analysed the association between individual approach probability and approach latency to ambiguous cues, to assess whether individuals that were more likely to approach a cue, were also on average faster to do so. We specified a bivariate mixed model, with approach probability and approach latency as the two dependent variables, 'Individual identity' as a random term, and previously fitted predictors as added fixed effects (i.e., 'Cue type', 'Trial number' and 'Previous cue rewarded'). Correlations between individual approach probabilities and latencies were estimated based on model variance-covariance matrixes [22]. This analysis was restricted to the phenotypic level, since sample size did not yield the power necessary for calculation of a genetic correlation.

Relationship between learning speeds and judgement bias

To investigate associations between individual learning speed (in discriminative and reversal tests) and degree of optimism towards ambiguous cues, we fitted a series of bivariate Gaussian mixed models, with one dependent variable being either discriminative or reversal learning speed (log-transformed values), and the other either approach probability (binomial variable: 0/1) or approach latency (log-transformed). Fixed effects were specified as in previous models for learning speed and judgement bias. In all models, individual identity was included as random term in a 2 x 2 covariance matrix, allowing us to calculate correlations (± SE) from estimated variances and covariances. As models with approach probability assumed an underlying Gaussian error distribution, corresponding uncertainty estimates (SE) of correlations are approximate. Likewise, since likelihood ratio test assumptions are not met with binomial variables, corresponding P-values should be treated with caution and considered as indicative only. By doing so, we evaluated associations between task-specific individual learning performances and individual optimism. Hence, covariation was evaluated on the four combinations between measures of learning speed (discriminative and reversal tests) and cognitive judgement bias (approach probability and latency).

Results

Associative learning

Individual consistency across learning tests

Individuals were not consistent in their learning speed across tests; to the contrary, learning speed in the discriminative learning test was weakly, but significantly, negatively correlated with learning speed in the reversal test (r_s = - 0.22, P < 0.001, N = 317; figure 1).

Discriminative learning

The number of trials that individuals needed to reach the set learning criterion for discrimination between two colour cues (learning speed) averaged 23.4 ± 11.1 (SD) (range = 8-70). Learning speed did not differ between the sexes (males = 23.1 ± 0.8 (SE); females = 23.5 ± 0.9; table S3a) but varied according to the colour cue associated with the reward (2013 colour cues: blue = 26.5 ± 1.9, green = 34.9 ± 2.6; 2014 - 2017 colour cues: black = 21.2 ± 0.6, white = 28.1 ± 2.2; table S3a). There was no evidence for heritability of learning speed in the discriminative test (h^2 = 0.00 ± 0.06, P = 0.49; figure 2a). Given the absence of detectable additive genetic variance for discriminative learning we did not attempt to estimate a genetic correlation between this and reversal learning (see below).

Reversal learning

Learning speed in the reversal learning test averaged 46.2 ± 21.7 (SD) (range = 9-158), did not differ between male and female chicks (males = 47.2 ± 1.8 (SE); females = 45.1 ± 1.7; table S3b), and varied according to colour cue/year (blue = 28.9 ± 1.8; green: = 33.9 ± 2.4; black = 45.7 ± 4.9; white = 49.9 ± 1.4; table S3b). Contrary to the discriminative test, there was significant heritable variation in reversal learning speed (h^2 = 0.26 ± 0.11, P <0.01; figure 2b). Restricting the analysis to the years 2014-2017, to match the sample available for the judgement bias (see below) and remove methodological differences between years, yielded virtually the same heritability estimate (h^2 = 0.25 ± 0.12).

Cognitive judgement bias

Repeatabilities, individual consistency across cue types, and heritabilities

Individuals differed in their probability of approaching cues across the entire range of cue types (i.e., repeatabilities: median = 0.44, range = 0.36 - 0.58; table 1, diagonal). Further, there was a high individual consistency in approach probability across cue types (i.e., between-individual correlations: $r_{bw;}$ all > 0.77; table 1). We therefore pooled ambiguous cues, to increase power and accuracy of estimates. Overall, repeatability of probability of approach to ambiguous cues was moderate ($R_{ambiguous}$ = 0.34 ± 0.03). Between-individual variation in probability of approaching ambiguous cues was driven by environmental effects (e^2 = 0.26 ± 0.07), while the heritable component was low (h^2 = 0.09 ± 0.07).

Individual repeatabilities in approach latency were similar across all cue types, apart from the negative cue for which repeatability was lowest (table 1, diagonal). Across cue types, there was an overall high individual consistency in approach latency, particularly between contiguous cues (POS-NearPOS, NearPOS-MID, MID-NearNEG, NearNEG-NEG: all r_{bw} > 0.70; table 3). Overall, repeatability of approach latency to ambiguous cues was moderate ($R_{ambiguous}$ = 0.25 ± 0.03). Similar to approach probability, the repeatability was mainly driven by environmental effects (e^2 = 0.16 ± 0.05, P <0.01), while the heritable component was again low (h^2 = 0.10 ± 0.06, P = 0.04). Finally, individuals that were more likely to approach ambiguous cues were also faster in doing so (r_{bw} = -0.59 ± ~0.09, P <0.01).

Association between learning speed and individual judgement bias

Learning speed in the discriminative learning test was neither associated with individual approach probability, nor individual latency to approach ambiguous cues in the judgement bias test (approach probability: r = -0.02 ± ∼0.08, P ∼0.80; latency to approach: r = - 0.07 ± 0.08, P = 0.39; figure 3a-b). However, there was an association between learning speed in the reversal test and both approach probability and latency to approach ambiguous cues (approach probability: r = 0.28 ± ∼0.07, P <0.01; latency to approach: r = -0.24 ± 0.08, P <0.01; figure 3c-d). Individuals that were less likely, and slower, to approach ambiguous cues (i.e., less optimistic) tended to learn the reversal test faster than more optimistic chicks.

Discussion

We examined associations between performance across cognitive tests, and their heritabilities, in the red junglefowl. Our analysis revealed weak covariation between measured cognitive traits. Heritability estimates of performance across tests ranged from virtually null to moderate. Reversal learning yielded the highest heritability, while discriminative learning performance was not heritable. Individual optimism, inferred from responses to ambiguous cues, showed low heritability and was predominantly governed by environmental effects. Less optimistic chicks learnt the reversal, but not the discriminative test, faster. Finally, performance did not differ between the sexes in any cognitive test, matching the absence of sexual dimorphism in young junglefowl.

Discriminative vs reversal learning

Individual performance was not consistent across the two learning contexts we assayed (discriminative and reversal associative learning). To the contrary, we demonstrated a weak negative association between learning speed in the discriminative - and in the reversal test, suggestive of speed-accuracy trade-offs and resulting individual cognitive styles [37]. The proximate control of these putative cognitive styles is presently unclear. A possible mechanism could entail among-individual differences in strength of instantiation of initial associations between cues and rewards. Strong instantiation may lead to fast learning of novel associations, which would, presumably, be mostly adaptive under stable environments. Strong instantiation could also be expected to increase the threshold for extinguishing previously learnt responses, should environmental conditions change (as required by reversal learning). Such a trade-off between rapid learning and behavioural flexibility has been demonstrated in invertebrates [29] and may also underlie speed-accuracy trade-offs found across vertebrate species.

Irrespective of mechanism, the lack of heritable variation underpinning individual differences in discriminative learning performance does not seem to support a genetically-encoded trade-off. Notably, despite the absence of heritable variation in discriminative learning, we have previously found, in the same population, a high degree of temporal consistency in individual performance (from chick-stage to sexual maturity, repeatability: $R > 0.4$, [69]). Thus, long-lasting between-individual differences in discriminative learning performance may arise through environmental effects acting during development, and/or parental effects mediated by the gametes. Disentangling the pathways leading to these individual differences will require experimental manipulations of the environment experienced by young individuals and their parents. Regardless, the lack of heritable variation implies that selection on individual discriminative learning performance would not lead to an evolutionary change. Further, the lack of additive genetic variation does not seem to support that discriminative learning ability is part of a general intelligence ('g'), since the latter is typically explained by a common genetic underpinning (i.e., high heritability of 'g', [11]). Yet, the presence of 'g' cannot be presently ruled out in the junglefowl and its assessment will require further testing using a battery of cognitive assays encompassing a wide range of cognitive abilities and domains (e.g., mice studies, [70,71]).

Conversely, we demonstrated a moderate heritability for performance in the reversal learning test, of similar strength as estimates available from other species (e.g., bees, [24,72], mice [73]). While the lack of test repeats precludes direct calculation of between-individual variation, heritability sets a lower bound for repeatability [74]. Accordingly, we can infer moderate to high between-individual differences in reversal learning abilities, with a substantial genetic component. Therefore, contrary to discriminative learning, between-individual differences in reversal learning abilities show the potential for microevolutionary responses to changing selection forces. Why performance in reversal, but not discriminative learning was heritable, is unclear. A possible explanation is that reversal learning performance is affected by individual differences in inhibitory control [26], a trait under genetic control in humans [75,76] and other animals (e.g., mice, [77–79]). Then if, for example, spatially or temporally varying selection maintains genetic variation in inhibitory control, among-individual differences in reversal learning performance may be indirectly selected for (or vice versa if reversal learning is under selection).

Generally, the degree to which different cognitive abilities are heritable and genetically correlated to other cognitive and non-cognitive traits, has important implications for their evolvability [80]. For example, strong positive genetic covariation between cognitive traits, as in the case of general intelligence, implies that selection on a single cognitive trait may cause evolutionary changes in other cognitive traits, even if these are not strongly associated with fitness. On the other hand, negative genetic correlations may place constraints on evolvability of certain cognitive traits, for example, if these are traded-off with other cognitive abilities under strong positive selection [81]. Finally, if different cognitive traits are underpinned by largely independent genetic control, evolutionary trajectories are most likely to differ, leading to individual and population differences in the association between cognitive abilities (such as modular cognitive structure and mosaic evolution, [82]).

Cognitive judgement bias

Overall, red junglefowl chicks appeared to behave optimistically and inspected ambiguous cues in > 60% of test trials. This high approach probability was most likely a consequence of no cost (i.e., no punishment) of sampling non-positive/unrewarded cues, aside from the negligible energetic expenditure of approaching the cue [50,83]. Chicks differed in their probability of approaching ambiguous cues, and across individuals, approach probabilities to different cue types were strongly correlated. Similar results were obtained using latencies. Together these findings suggest that approach probability and latency similarly captured individual differences in judgement.

Heritability estimates for approach probability and latency were similarly low, with estimates of additive genetic variation two-three times lower than environmental variance. Therefore, between-individual differences in judgement of ambiguous cues seemed to be driven by environmental effects. Importantly, because individual consistency in judgement was assessed over a single testing session (duration up to 15 minutes), these environmental effects could have been the result of transient between individual differences in affective state (e.g., mood, [66]). Alternatively, between-individual differences in judgement may have resulted from long-lasting effects of developmental conditions or maternal effects, and thus underpin stable between-individual differences in judgement, possibly associated with personality [49,51]. The low heritability of judgement bias we observe here is compatible with both scenarios, provided that any long-term stability of individual optimism is driven by permanent environmental effects. However, the relatively limited number of individuals tested to date resulted in substantial uncertainty for our heritability estimates, with 95%

CIs ranging 0-0.2. Thus, at one extreme there may have been minor heritable variation underlying between-individual differences in judgment, while at the other extreme individual differences in optimism may have been associated with low to moderate heritability. Since heritable variation is a prerequisite for the occurrence of evolutionary responses to selection [6,9,22], distinguishing between these two alternatives should represent a priority for future research.

Covariation between learning performance and judgement bias

Individual judgement bias was weakly associated with learning performance in the reversal test: less optimistic individuals were faster in reversing the association between colour cue and reward. There was no association between judgement biases and discriminative learning performance.

Why individual optimism may correlate with one facet of associative learning, but not another is an unanswered question. To date, only a few studies have examined co-variation between performance in discriminative learning and judgement biases [61,84] and have mostly reported no association between these two cognitive traits, similar to our results. However, to the best of our knowledge, links between judgement biases and reversal learning have not previously been empirically investigated.

To understand interplays between learning and judgement biases, it is useful to evaluate different causal pathways that may give rise to associations between learning performance and judgement. First, common traits may be causally linked to both performance in reversal learning and individual optimism. For example, speed-accuracy trade-offs underlying different cognitive styles, and typically associated with personality types (e.g., coping styles, [37]), may also underpin associations between learning performance and responses to ambiguous cues. Optimism may, thus, represent an individual cognitive trait, likely with genetic underpinnings. Yet, the lack of association between discriminative learning speed and optimism in the junglefowl is not easily reconciled with a speed-accuracy trade-off framework, which predicts that fast/proactive individuals should learn discriminative tests faster [37] and be at the same time more prone to impulsively approach ambiguous cues. Nevertheless, the negative association between optimism and performance in the reversal learning tests is compatible with individual differences in cognitive and coping styles. This is because reactive/slow types are considered to be both more competent in reversal learning and more susceptible to stress [26,85]. In turn, both acute and chronic stress have been linked to negative affective states, and, thereby, pessimistic-like behaviour [56,86–88]. Another possible

explanation for our findings may entail between-individual differences in persistence underlying both reversal learning [89] and optimistic response to ambiguous cues [90]. Under this hypothesis, more persistent individuals are expected to continue responding during extinction (i.e., when presented with unrewarded cues) for longer and are therefore predicted to be both slower in reversing previously learnt associations and more persistent in approaching when exposed to ambiguous or negative cues. Individual differences in extinction, associated with personality and emotional traits, have been demonstrated in human infants and mice [91].

Finally, an alternative mechanism may involve a direct causal relationship, with individual affective state modulating learning performance. The affect-as-information hypothesis (AAI, [92,93]), posits that negative mood suppresses impulsive behaviour conducive to negative fitness consequences under challenging conditions, and favours instead inhibitory control [92]. Since inhibition is also implicated in reversal learning, it follows that individuals in a negative affective state (i.e., less optimistic) may show enhanced performance in a reversal learning test.

Fully distinguishing between these hypotheses will require appraisal of temporal consistency of individual optimism, interplays with personality traits, and experimental manipulations of mood to evaluate resulting changes in cognitive performance. Primarily, more data is required to ascertain the extent to which the phenotypic correlation between individual optimism and reversal learning may arise from shared genetic control (i.e., pleiotropy or genetic linkage).

General conclusion

To summarise, we have demonstrated genetic variation underlying individual differences in reversal learning performance, and a lack of genetic effects for discriminative learning. Between-individual variation in judgement of ambiguous cues was mainly driven by environmental effects and showed low heritability. Thus, the examined cognitive traits do not seem to have a shared genetic control. Importantly, our findings suggest that in the junglefowl, reversal but not discriminative learning abilities may evolve in response to selection. The proximate mechanisms behind differences in the genetic control of these two facets of associative learning are unclear. Additive genetic variation in individual inhibitory control provides a possible explanation to this conundrum. Understanding what maintains heritable individual differences in reversal learning will require linking performance in reversal learning with fitness [94]. Further work should also aim at elucidating the extent to which

optimism may be heritable, and what mechanisms are driving covariation between learning abilities and judgement biases.

Ethical note

The study followed ethical requirements in Sweden and the study was approved by Linköping ethical committee (permit numbers 122-10 and 50-13).

Data accessibility

Data are available online (ESM).

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Authors' contributions

HL coordinated and funded the study; ES carried out statistical analyses with input from AW; ES, LG, JZ, HL collected behavioural data; ES & HL drafted the manuscript with input from JZ, LG, AW. All authors gave final approval for publication.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1. Relationship between learning speed in discriminative and reversal learning tests in red junglefowl chicks. Learning speed is measured as trials until criterion reached (please see main text for further details). Each point represents an individual bird. * symbolises significant values.

Figure 2. Variance components and heritability for performance of red junglefowl chicks in cognitive tasks. a) learning speed in a discriminative learning test, b) learning speed in a reversal test, c) approach probability to ambiguous cues, d) approach latency to ambiguous cues. Stacked bars show, from bottom to top: residual variance (white bars), permanent environmental effects variance (light grey bars; limited to judgement bias), additive genetic variance (h^2, dark grey bars). Estimates for approach probability are on the latent scale (logit). * symbolises significant values.

Figure 3. Relationship between performance of red junglefowl chicks in various cognitive tests. Associations between: a) learning speed in a discriminative learning test and approach probability to ambiguous cues, b) learning speed in a discriminative learning test and approach latency to ambiguous cues, c) learning speed in a reversal task and approach probability to ambiguous cues, d) learning speed in a reversal task and approach latency to ambiguous cues. Points represent individual BLUPs estimates from bivariate mixed models. Correlations were calculated from model 2 x 2 covariance matrixes of individual random effects. Significance (*) was assessed via likelihood ratio tests.

Table 1. Cue-specific repeatabilities and individual behavioural consistency across cue types for red junglefowl chicks, in a judgement bias test. Repeatabilities ('R' by cue type, grey cells) and pairwise individual-level correlations ('r_{bw}' between cue types, white cells) for: (i) 'Approach probability' (i.e., probability of approaching a cue) above the diagonal line. (ii) 'Approach latency' (i.e., latency to approach a cue) below the diagonal line.

Approach probability

Approach latency	**POS**	**NearPOS**	**MID**	**NearNEG**	**NEG**
POS	**0.58 (0.04)**	**0.99 (0.08)**	**0.88 (0.08)**	**0.81 (0.10)**	**0.77 (0.07)**
NearPOS	**0.97 (0.08)**	**0.55 (0.07)**	**0.93 (0.13)**	**0.87 (0.15)**	**0.81 (0.11)**
MID	**0.87(0.05)**	**0.80 (0.08)**	**0.44 (0.05)**	**0.96 (0.11)**	**0.86 (0.08)**
NearNEG	**0.65 (0.09)**	**0.48 (0.12)**	**0.80 (0.11)**	**0.35 (0.04)**	**0.96 (0.07)**
NEG	**0.54 (0.08)**	**0.48 (0.10)**	**0.71 (0.09)**	**0.74 (0.10)**	**0.36 (0.03)**

0.41 (0.03) **0.37 (0.05)** **0.41 (0.05)** **0.37 (0.07)** **0.24 (0.03)**

Repeatabilites and individual-level correlations were calculated from model variance-covariance estimates. '**POS**' = positive, i.e., familiar rewarded cue, '**NearPOS**' = ambiguous unfamiliar and unrewarded cue, most similar to the positive cue; '**MID**' = ambiguous unfamiliar and unrewarded cue, intermediate between positive and negative cues; '**NearNEG'** = ambiguous, unfamiliar and unrewarded cue, most similar to the negative cue; '**NEG'** = negative, i.e., familiar unrewarded cue. Estimate standard errors are provided in parenthesis.

Figure 1

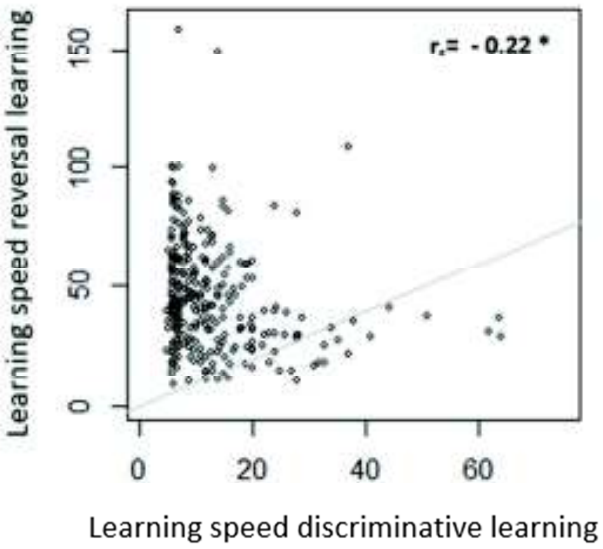


Figure 2

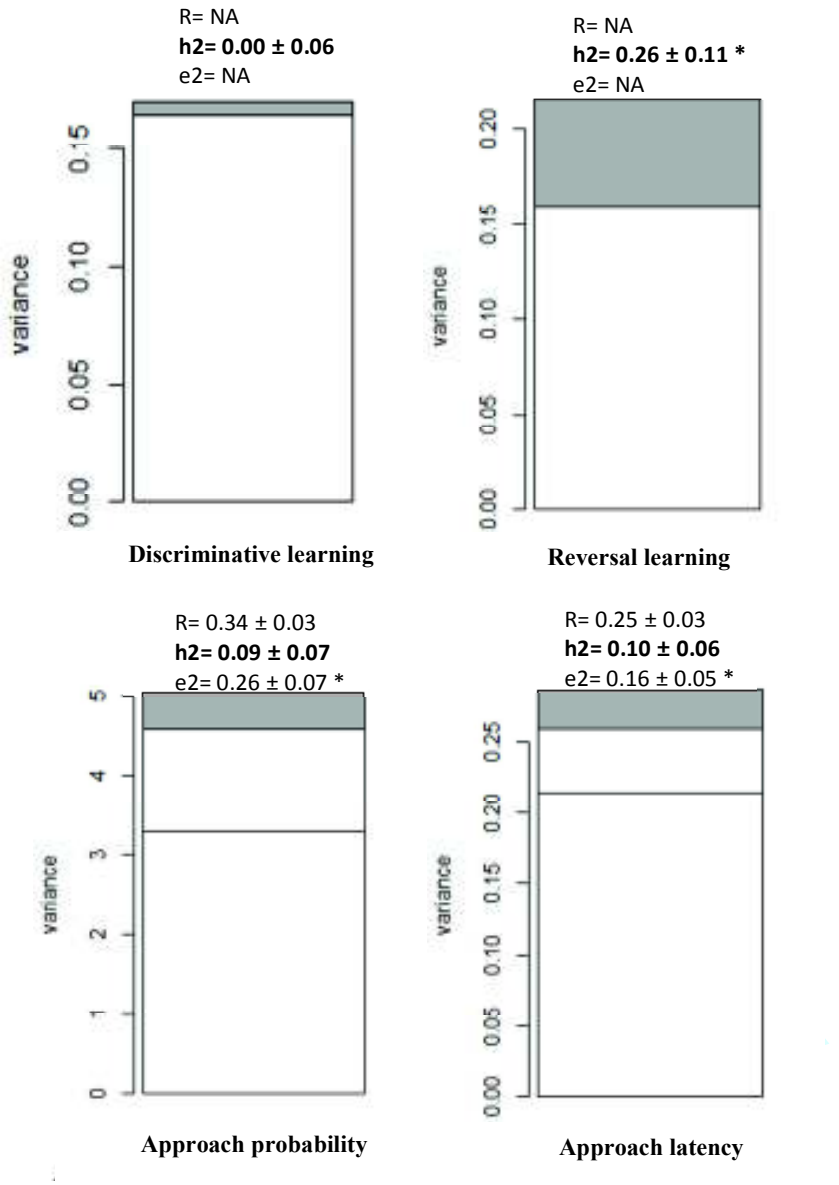
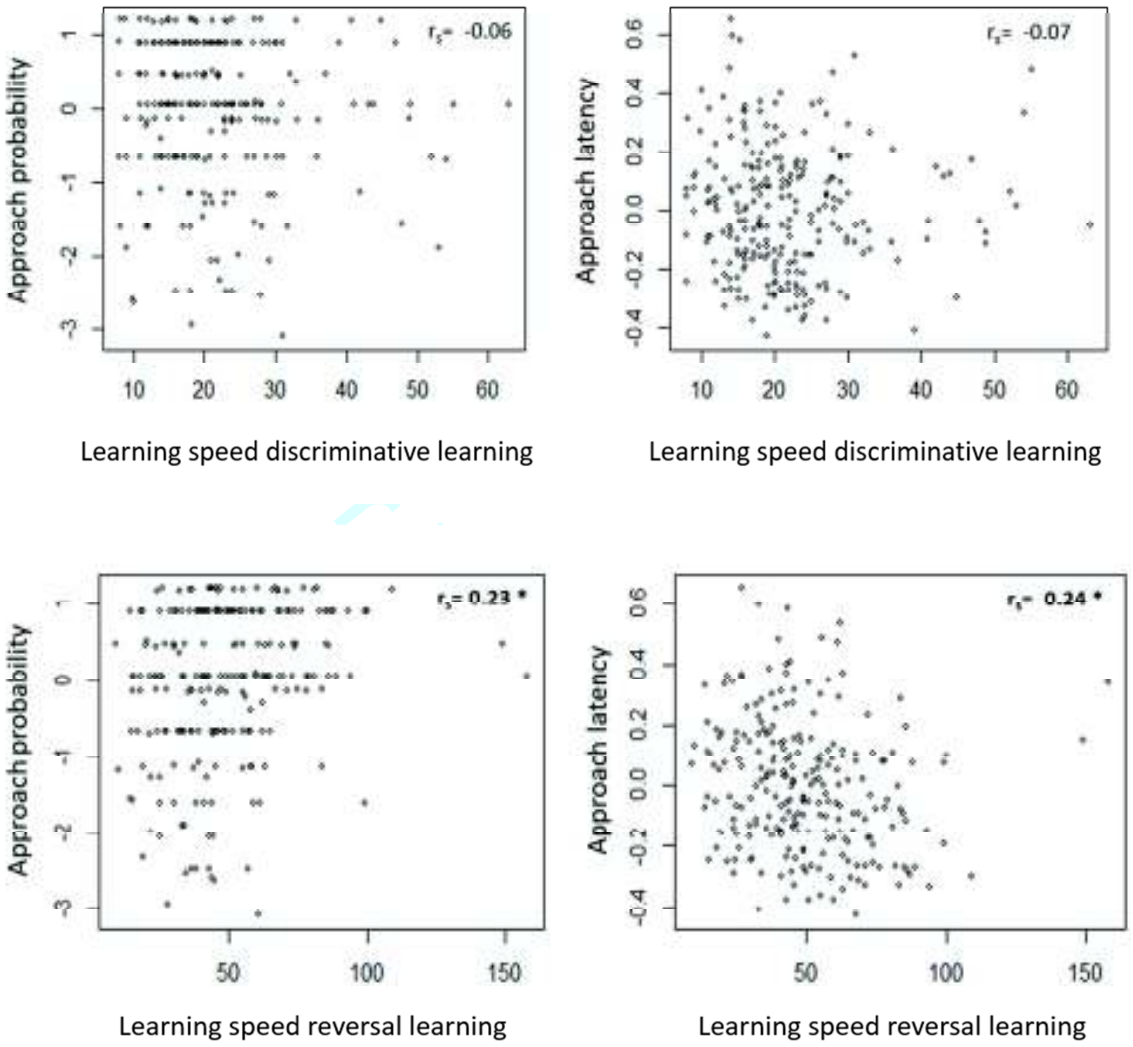

R= NA
h2= 0.00 ± 0.06
e2= NA
variance
0.00
0.05
0.10
0.15
Discriminative learning
R= NA
h2= 0.26 ± 0.11 *
e2= NA
variance
0.00
0.05
0.10
0.15
0.20
Reversal learning
R= 0.34 ± 0.03
h2= 0.09 ± 0.07
e2= 0.26 ± 0.07 *
variance
0
1
2
3
4
5
Approach probability
R= 0.25 ± 0.03
h2= 0.10 ± 0.06
e2= 0.16 ± 0.05 *
variance
0.00
0.05
0.10
0.15
0.20
0.25
Approach latency

Figure 3

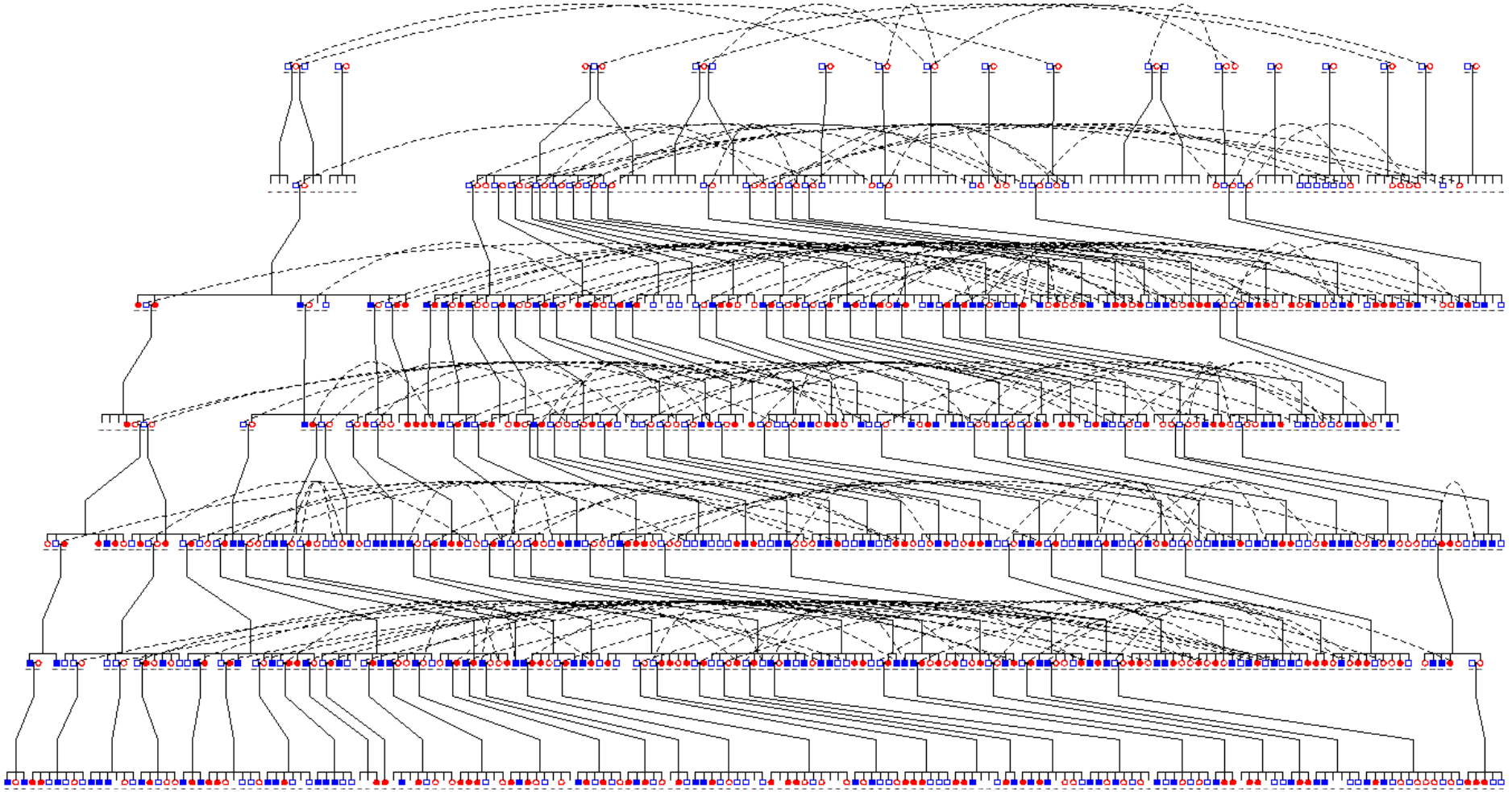

Approach probability
r_s= -0.06
Learning speed discriminative learning
Approach latency
r_s= -0.07
Learning speed discriminative learning
Approach probability
r_s= 0.23 *
Learning speed reversal learning
Approach latency
r_s= 0.24 *
Learning speed reversal learning

Electronic supplementary material for Sorato E, Zidar J, Garnham L, Wilson A, Løvlie H: Heritabilities and co-variation among cognitive traits in red junglefowl.

The supplementary material contains:

S1) Pedigree

Figure S1. Pedigree of the study population.



Continuous lines connect parent-offspring (vertical/diagonal lines) and siblings (horizontal lines), dotted lines join breeding pairs. Squares (blue) indicate males, circles (red) females. Filled symbols mark individuals that were assayed in cognitive tests (n = 340).

Table S1. Pedigree statistics.

No. of individuals	503
Maternities	471
Paternities	470
No. of full sibling dyads	763
No. of maternal sibling dyads	776
No. of paternal sibling dyads	837
No. of maternal half-sibling dyads	13
No. of paternal half-sibling dyads	74
No. of maternal grandmothers	409
No. of maternal grandfathers	412
No. of paternal grandmothers	412
No. of paternal grandfathers	404
Maximum pedigree depth	6
No. of founders	29
Mean maternal siblingship size	3.5
Mean paternal siblingship size	3.7
Mean pairwise relatedness	0.08
% Dyads with pairwise relatedness ≥ 0.125	24%
% Dyads with pairwise relatedness ≥ 0.25	5%
% Dyads with pairwise relatedness ≥ 0.5	1%

Statistics were obtained using the R package Pedantics (Morrissey & Wilson 2010). “Maternities” and “paternities” refer to the number of individuals with known sires and dams respectively. “Maximum pedigree depth” indicates the number of generations in the pedigree.

S2) Simple overview of the animal model analytical approach

Animal models are a type of mixed effects model, originally developed within the field of animal breeding and more recently applied in evolutionary and behavioural ecology studies. Here we provide a brief overview for the unfamiliar reader with the goal of highlighting the possible value of this method for animal cognition research. For more in-depth information, we refer readers to the introductory review by Wilson and co-workers (2010) that assumes little prior knowledge of quantitative genetics.

Compared to older methods that estimate heritability using only a subset of possible relationship types (e.g., parent-offspring regression, ANOVA using data on siblingship), animal models have three main advantages. Firstly, because the approach uses pairwise relationships among a set of individuals, it maximises statistical power, especially when multigenerational pedigrees are analysed. Secondly, it is easy to incorporate other (possible) sources of resemblance between relatives into the model (e.g., common environmental effects) to produce less biased heritability estimates (as compared to classical approaches). Thirdly, the mixed model framework is much better able to accommodate missing data, unbalanced pedigrees (e.g., different family sizes) and other complexities of real world datasets, compared to alternative approaches.

In simple terms, animal models utilise the matrix of pairwise relatedness between all possible pairs of individuals (which can be inferred from a supplied pedigree structure or using molecular marker data). This, coupled with phenotypic data, allows inclusion of the individual 'breeding value' or 'genetic merit', i.e., the additive effect of an individual's genotype relative to the population average phenotype, as a random effect. Variance explained by breeding values is then estimated as the additive genetic variance (commonly denoted 'V_A'). Unexplained residual variance 'V_R' is normally interpreted as arising from environmental sources. Thus, in its simplest form, an animal model of trait 'y' expressed by individual 'i' may be written as:

$$y_i = \mu + a_i + e_i$$

where 'μ' is the population mean phenotype, 'a' is the breeding value and 'e' is a residual term. Breeding values are assumed to be normally distributed with a mean of zero and variance 'V_A' (the additive genetic variance) and correlated between individuals in a manner that depends on the

degree of relatedness (which is what allows estimation of 'V_A'). Residuals are assumed to be normally distributed with a mean of zero and variance 'V_R', but also to be uncorrelated across individuals. Importantly however, by utilising a very general linear mixed model formulation, 'V_A' can be estimated conditional on other fixed and/or random effects. For instance, inclusion of parental identity, year, or habitat patch as random effects results in additional partitions of phenotypic variance.

In studies that have repeated observations on individuals, it is also possible to partition variance into between-individual and within individual components. While 'V_A' contributes to among-individual variance, it is generally expected that environmental effects will as well. To avoid upward bias of 'V_A' it then becomes necessary to include a 'permanent environment' effect ('pe' with variance 'V_{PE}') in the model to account for non-genetic sources of individual repeatability. In such case, the model would be (for an observation of individual 'i' on occasion 'j'):

$$y_{ij=} \mu + a_i + pe_i + e_{ij}$$

After fitting an animal model to the data, the narrow sense heritability ('h^2'), which represents the proportional trait variation due to additive genetic effects, is calculated from the variance estimates, such that in the above case with repeated measures:

$$h^2 = V_A/V_P = V_A /(V_A+V_{PE}+V_R)$$

Where 'V_P' is the total phenotypic variance (conditional on any fixed effects in the model) and other terms are as defined above (but note that with repeated measures 'V_R' is now interpreted as within-individual variance attributable to short-term environmental effects).

Animal models are readily extended beyond the univariate case, to include more than one response variable (i.e., bivariate or multivariate animal models). Multivariate models allow partitioning of covariance, and so estimation of correlations, across multiple levels. This is exactly analogous to the univariate partitioning of variance and means that correlations between traits can be dissected into, for example, genetic and environmental signals. With repeated observations, within- and between-individual sources of environmental covariance among traits

can also be partitioned. For a set of 'n' traits, multivariate animal models are often used to estimate the additive genetic variance-covariance matrixes (denoted 'G') which in turn allows evaluation of phenotypic evolvability, constraint and genetic integration. More specifically genetic covariances among traits can both constrain or facilitate responses to selection, depending on their sign and magnitude, and on the nature of selection (whether natural or artificial). For instance, a trait under positive selection may fail to evolve (detectably) despite a moderate, or even high heritability if it is negatively genetically correlated with one or more other trait also under positive selection. This is the familiar idea of a trade-off acting as an evolutionary constraint.

Embracing a multi-trait approach is therefore pivotal in evolutionary studies because traits do not evolve in isolation from each other (Walsh & Blows 2009), and multivariate animal models constitute a powerful tool in this respect. While this applies to multivariate cognitive phenotypes just as much as to life histories or morphology (Thornton & Wilson 2015), we also recognise the challenges and demands of applying these data-hungry techniques in studies of animal cognition. We nevertheless urge researchers in the field of cognitive psychology and ecology to conceive and conduct further studies amenable to the application of animal models. Organisms with short generation times that can be readily bred in captivity are ideal candidates for quantitative genetic studies as large volumes of data can be accumulated under controlled conditions. Nonetheless, long-term studies of organisms in the wild (e.g., birds breeding in artificial nests) have been widely used to investigate the evolutionary genetics of other trait types (Kruuk et al 2014) and may prove useful for cognitive studies too. Even though assessing cognition in the wild over a large sample of individuals and across different tasks is clearly demanding, it can nevertheless be achieved (e.g., Quinn et al 2016). By combining multi-trait measures of cognition, with fitness estimates and relatedness data, such studies would be instrumental in understanding how among-individual differences in cognition are maintained in the face of selection acting in the wild.

References

Kruuk LEB, Charmantier A, Garant D. 2014 Quantitative Genetics in the Wild. (doi:10.1093/acprof:oso/9780199674237.001.0001)

Morrissey MB, Wilson AJ. 2010 Pedantics: An r package for pedigree-based genetic simulation and pedigree manipulation, characterization and viewing. *Mol. Ecol. Resour.* **10**, 711–719. (doi:10.1111/j.1755-0998.2009.02817.x)

Quinn JL, Cole EF, Reed TE, Morand-Ferron J. 2016 Environmental and genetic determinants of innovativeness in a natural population of birds. *Philos. Trans. R. Soc. B Biol. Sci.* **371**, 20150184. (doi:10.1098/rstb.2015.0184)

Thornton A, Wilson AJ. 2015 In search of the Darwinian Holy Trinity in cognitive evolution: A comment on Croston et al. *Behav. Ecol.* **26**, 1460–1461. (doi:10.1093/beheco/arv119)

Walsh B, Blows MW. 2009 Abundant Genetic Variation + Strong Selection = Multivariate Genetic Constraints: A Geometric View of Adaptation. *Annu. Rev. Ecol. Evol. Syst.* **40**, 41–59. (doi:10.1146/annurev.ecolsys.110308.120232)

Wilson AJ, Réale D, Clements MN, Morrissey MM, Postma E, Walling CA, Kruuk LEB, Nussey DH. 2010 An ecologist's guide to the animal model. *J. Anim. Ecol.* **79**, 13–26. (doi:10.1111/j.1365-2656.2009.01639.x)

Asreml-R model syntax

Syntax is provided for fitted models (numbered as described in the main text) using the R package Asreml-R. As written below, the first line specifies the dependent variable(s), with subsequent lines detailing fixed effects, followed by random effects, and the assumed distribution of residuals and link function. The final line of each model also contains a job qualifier ('maxiter') to set the maximum number of iterations allowed to reach convergence and the name of the data file.

In the random effect specification, ID indicates a random effect of individual identity. In the multivariate context (e.g., 'M4', see models specified below), ID:us is used to specify a fully unstructured variance-covariance matrix for the random effects of individuals across traits. Note that in 'M4', where residual covariances between traits are not statistically identifiable, the corresponding residual structure specified by rcov= ~units:idh fits a diagonal matrix (with heterogenous residual variance across levels of the specified factor or trait (i.e. to allow heteroscedasticity). Models M1, M2, M6, M7 are animal models, and the term ped(ID, var=T), ginverse=list(ID=ainv)is used to specify a random effect of individual genetic merit (using the identity of each individual indexed to a supplied pedigree structure). This allows the estimation of the additive genetic variance. For animal models fitted to data with repeat observations on individuals, the additional random term ide(ID,var=T) specifies a permanent environmental effect (i.e., non-additive genetic component of among-individual variance).

ASSOCIATIVE LEARNING TESTS

```
# Discrimination learning
M1 <- asreml(log(N.runs.discriminant)~
             1 + sex + cue.color,
             random = ~ped(ID, var=T), ginverse=list(ID=ainv),
             family = asreml.gaussian (link='identity'), maxiter = 100,
             data = data.learning.discr)

# Reversal learning
M2 <- asreml(log(N.runs.reversal)  ~
              1 + sex + cue.color,
              random = ~ped(ID, var=T), ginverse=list(ID=ainv),
              family = asreml.gaussian (link='identity'), maxiter = 100,
              data = data.learning.rev)
```

COGNITIVE JUDGMENT BIAS TEST

- **5-cues models**

Probability of cue approach

Phenotypic level only

```
M3 <- asreml(Approached  ~
               1 + NP*trial.number + M*trial.number + NN*trial.number + Ne*trial.number
               + prev.cue.rewarded,
               random = ~ ID:us(cuetype.factor),  rcov= ~ units,
               family = asreml.binomial(link='logit'), maxiter = 100, data = data.CJB.5cues)
```

Latency to cue approach

Phenotypic level only

```
M4 <-asreml(cbind(log(Latency.Po),log(Latency.NP),log(Latency.M),log(Latency.NN),log(Latency.Ne)~
trait +trait:trial.number +at(trait,1):prev.cue.rewarded.Po+at(trait,2):prev.cue.rewarded.NP+
at(trait,3):prev.cue.rewarded.M+at(trait,4):prev.cue.rewarded.NN+at(trait,5):prev.cue.rewarded.Ne,
           random= ~ ID:us(trait),  rcov= ~ units:idh(trait),
           family = asreml.gaussian (link='identity'), maxiter = 100,
           data = data.CJB.5cues_approached)
```

- **Ambiguous(3-)cues models**

Probability of cue approach

Phenotypic level => Individual Repeatability

```
M5 <-asreml (Approached ~
              1 + M + NN + trial.number + prev.cue.rewarded,
              Random = ~ ID,    rcov= ~ units,
              family = asreml.binomial(link='logit'), maxiter = 100,
              Data = data.CJB.3ambiguouscues)
```

Genetic level => heritability

```
M6 <-asreml(Approached ~
              1 + M + NN + trial.number + prev.cue.rewarded ,
              random = ~  ped (ID, var=T) +ide (ID, var=T), ginverse=list(ID=ainv),
              family = asreml.binomial(link='logit'),  maxiter = 100,
              data = data.CJB.3ambiguouscues)
```

Latency to cue approach

Phenotypic level => Individual Repeatability

```
M7 <- asreml(Latency_tr ~
               1 + M + NN  + trial.number + prev.cue.rewarded,
              random= ~ ID, rcov= ~ units,
              family = asreml.gaussian (link='identity'), maxiter = 100,
              data = data.CJB.3ambiguouscues_approached)
```

Genetic level => heritabiliy

```
M8 <- asreml(Latency_tr ~
               1 + M + NN + trial.number + prev.cue.rewarded,
              random= ~  ped (ID, var=T) +ide (ID, var=T), ginverse=list(ID=ainv),
              family = asreml.gaussian (link='identity'), maxiter = 100,
              data = data.CJB.3ambiguouscues_approached)
```

Cue types: '**Po**' = near positive, '**NP**' = near positive, '**M**' = middle, '**NN**' =n ear negative, '**Ne**' = negative.
'**prev.cue.rewarded**': whether the previously presented cue was rewarded (i.e., positive cue) or not.

S3) Associative learning

Simulations of false positive learners during the 1st study year (learning criterion = 5 consecutive correct choices).

Given a null hypothesis of random choice, there is the possibility that individuals will reach a fixed learning criterion of n correct choices in a row by chance (i.e., representing false positives, or false learners). Such probability will increase with the number of runs (i.e., opportunities to make a choice) available. With just 5 runs, there is a probability $P = 0.5^5 = 0.03$ than individuals will meet the criterion by change, i.e., 3 individuals out of 100 would be on average false positives. While this would represent an acceptable rate of false positives, things get worse as the number of runs increases, and with enough choices (on the order of 100s) almost all individuals would with time eventually meet the criterion simply by chance.

We have therefore run simulations to further evaluate the extent of the problem, trying to match as close as possible our experimental procedure and sample size (see below). We simulated a series of experimental runs conducted on a sample of 66 individuals (66 individuals took part in the discriminative learning test in 2013, when the learning criterion was 5 correct choices in a row) and repeated this simulation 10000 times (R script and data is available in separate files upon request). We started with a simulation in which individuals had a max of 64 binary choices available to reach our learning criterion (64 was our max number of runs/trials). On average 65% of individuals were solvers simply by chance (see histogram below showing the frequency distribution of number of solvers across simulations, Figure S3-I).

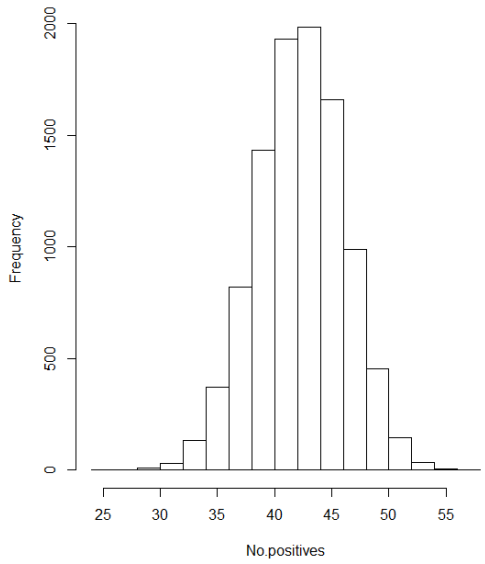


Figure S3-I. Number of false positives (5 correct choices in a row) over a sample of 66 individuals with a max of 64 choices available (10000 simulations).

However, this scenario is a very conservative one, since the average number of runs in our tests was much less than 64. We have therefore run a simulation closely matching our sample's number of runs (individual average=24; Figure S3-II), that is, each simulated individual was given the same number of runs that the actual bird was exposed to in our experiment. We again conducted 10000 simulations and found that the average number of individuals meeting our learning criterion by chance was 18 out of 66 (28%; the conservative upper 95% CI value gives 25 false positives, i.e. 38% of individuals). Therefore, the average number of false positives is considerable lower than in the previous simulation. Yet, ca 30% of random solvers would arguably still represent an unacceptably high rate of false positives.

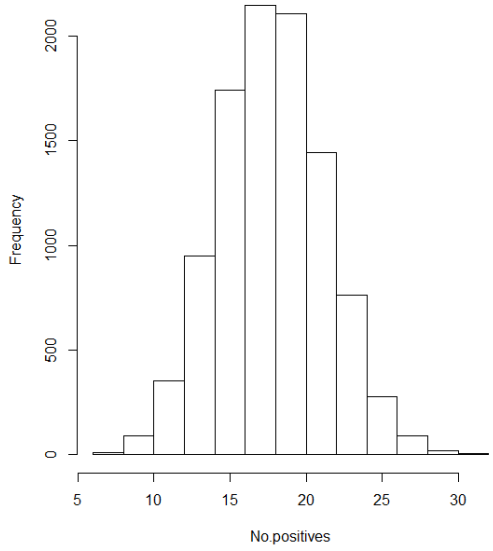


Figure S3-II. Number of false positives (5 correct choices in a row) over a sample of 66 individuals with number of available choices matching the experimental sample (10000 simulations).

Fortunately, we have a sample of chicks for which we can examine whether individuals could have met a stricter criterion (from 6 up to 10 correct choices in a row). This sample is available because, when individuals reached the learning criterion of 5 correct choices in a row late in the afternoon, they could not be tested further during that same day (lights in the chicks' facility automatically switched off at a set time). These individuals were tested again early the next morning with a 'refresh session', aimed at checking that they were still meeting the test criterion (e.g., they did not forget the association they had learnt the previous day; remember these were only a few days old chicks and we wanted to be conservative in case of reduced memory). Thirty-six individuals, representing a random sample of subjects, were therefore exposed to a 'refresh' trial the next day. Here, chicks needed to make again 5 correct choices in a row, before being exposed to the

next test (reversal learning). Because the test on the previous day was ended when the chick had met the 5-in-a -row criterion, and on the refresh day they were exposed to a minimum of 5 further runs, this means that we can also consider which proportion of putative 'learners' met stricter criteria of 6, 7, 8, 9, 10 correct choices in a row. We found that 78% of individuals chose the correct colour in the first trial the following day (i.e., in their 6^{th} choice). Further, when considering the proportion of correct choices made during the entire refresh session (until criterion of 5 correct choices was reached again), 72% of individuals made ≥80% correct choices, which is clearly much higher than what we would expect if the individuals were making random choices. In fact, all 36 individuals where above chance level of 50%. Furthermore, twelve individuals (a third of the sample of refresh birds) made zero errors the following day, in other words they reached 10 correct choices in a row.

To further assess the likelihood of false positive given the observed number of consecutive correct choices in our refresh sample, we have run another set of simulations, aimed at estimating the probabilities of getting individuals making 5, 6,…, 10 correct choices in a row given a null hypothesis of random choice and the experimental sample sizes; we then used these probabilities to estimate how many of the individuals that in our experimental tests reached a certain number of consecutive correct choices, may have done so by chance. We estimated this by multiplying, for each category x (x = number of correct consecutive choices, 5-10): the 'Probability of reaching a learning criterion' (> = 5 consecutive choices; P = ca 0.3, see previous simulation) * the 'Probability of x consecutive' choices (5-10; Figure S3-III) * 'Number of individuals that in our experiment made x consecutive choices'. According to these calculations, on average 1 individual out of 35 could have been a false positive. When extrapolating to the total sample of birds assayed in the associative learning test, we get ca 2 individuals out of 66 (3%). Taking the much more conservative upper 95% CI estimates of P of reaching criteria under H0, we get 6 out of 66 (9%).

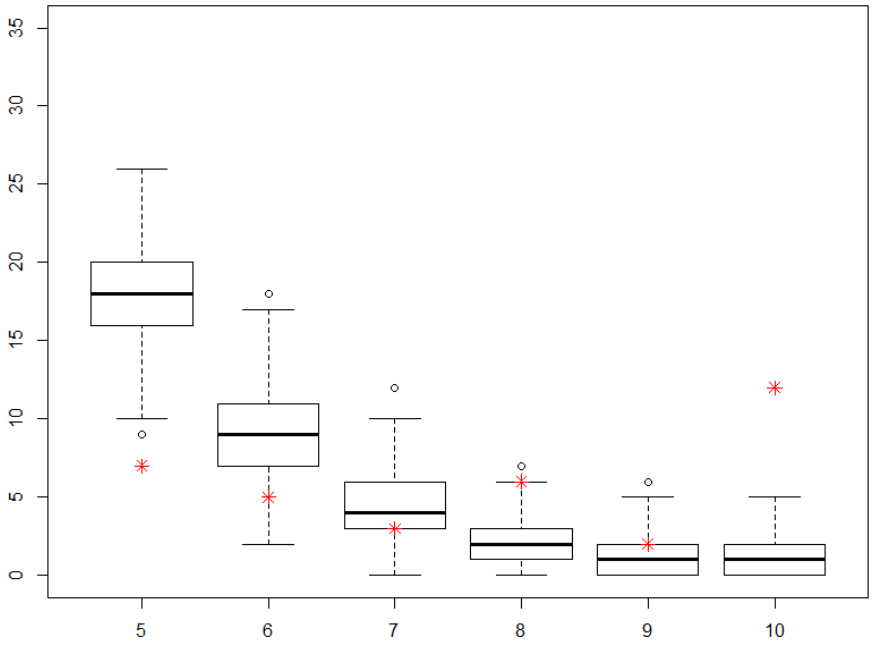


Figure S3-III. Boxplots showing number of individuals (out of 36 putative learners; 10000 simulations) expected to reach a given number of consecutive correct choices (5-10) by chance. Red stars indicate observed number of individuals in our test experiments.

We therefore believe that our criteria of 5 consecutive correct choices was successfully identifying "true learners" in the vast majority of cases. Given all the evidence, the noisiness in the speed of learning variables does not appear nearly as high as it may have seemed at first.

Table S3. Mean-level effects on learning speed in red junglefowl chicks.

(a) Discriminative learning (N = 340), and (b) Reversal learning (N = 317).

	Estimate	SE	z ratio
a) Discriminative learning			
intercept	**3.06**	**0.02**	**141**
Sex (female)	-0.02	0.04	-0.5
Blue rewarded	-0.08	0.11	-0.7
Green rewarded	0.19	0.11	1.8
Black rewarded	**-0.30**	**0.09**	**-3.5**
b) Reversal learning			
intercept	**3.7**	**0.02**	**144**
Sex (female)	-0.02	0.04	-0.4
Blue rewarded	**-0.51**	**0.09**	**-5.4**
Green rewarded	**-0.37**	**0.09**	**-4.4**
Black rewarded	-0.11	0.10	-1.1

'Blue rewarded', 'Green rewarded', 'Black rewarded' refers to the colour of the cue that was rewarded. Significant values are highlighted in **bold.**

S4) Additional results from the cognitive judgement bias test

Mean-level effects

The probability of approaching a cue decreased from positive (P_{POS} = 0.98 ± 0.00 (SE), N_{trials} = 2679) to negative cues (P_{NEG} = 0.41 ± 0.02, N_{trials} = 2003), and was intermediate for ambiguous cues ($P_{NearPOS}$ = 0.94 ± 0.01, N_{trials} = 638; P_{MID} 0.84 ± 0.02, N_{trials} = 558; $P_{NearNEG}$ = 0.41 ± 0.02, N_{trials} = 632, table S4a, figure S4-I). The probability of approaching negative and near-negative cues decreased as the test progressed (i.e., showed a 'Trial number' effect), with approaches during last test trials (i.e., trial number 33) being six and two times less likely, compared to first trials, respectively (table S4a, figure S4-II). Furthermore, if the previous cue had been rewarded (i.e., positive) increased the probability of approaching the following cue by 10% on average compared to cues that followed unrewarded ones (table S4a, figure S4-III).

Excluding the censored data and considering only observations where an approach was made, mean latency to approach a cue increased from positive to negative cues (Lat_{POS} = 2.5 ± 0.07 (SE) sec, N_{trials} = 2545; Lat_{NEG} = 6.7 ± 0.24 sec, N_{trials} = 1175), and was intermediate for ambiguous ones ($Lat_{NearPOS}$ = 2.5 ± 0.08 sec, N_{trials} = 602; Lat_{MID} = 3.5 ± 0.14 sec, N_{trials} = 530; $Lat_{NearNEG}$ = 5.1 ± 0.25 sec, N_{trials} = 370; table S4b, figure S4-I). There was a slight decrease in approach latency during later trials for positive cues (initial trials = 2.7 sec; last trials = 2.3 sec; table S4b), no significant change for near-positive cues (table S4b), whereas latency to approach middle to negative cue increased, with test progression (middle cues: initial trials = 3.1 sec, last trials = 4.3 sec; near-negative cues: initial trials = 4.6 sec, last trials = 6.1 sec; negative cues: initial trials = 5.2 sec; last trials = 10.0 sec; table S4b, figure S4-II). Finally, there was an effect of previous cue type (positive vs. not) on approach latency, but limited to positive and negative cues, where latencies decreased by 29 and 19%, respectively when the preceding cue was rewarded compared to when it was not (table S4b, figure S4-III).

There were no sex-differences in either approach probability (across all cues: males = 0.75 ± 0.04 (SE); females = 0.72 ± 0.04; table S4a) or approach latency (males = 4.4 ± 0.4; females = 4.4 ± 0.4; table S4b).

Table S4. Mean-level effects on behavioural responses by red junglefowl chicks (N = 251) in a cognitive judgement bias test. (a) 'Approach probability' (i.e. probability of approaching a cue), and (b) 'Approach latency' (i.e. latency to approaching a cue).

	estimate	SE	z ratio
a) Approach probability			
intercept	**2.19**	**0.12**	**17.5**
Near POS	**-0.68**	**0.24**	**-2.8**
MID	**-2.58**	**0.20**	**-12.6**
Near NEG	**-4.06**	**0.20**	**-20.3**
Trial Number	**-1.06**	**0.11**	**-10**
Trial Number: Near POS	0.01	0.47	0.0
Trial Number: MID	0.67	0.37	1.8
Trial Number: Near NEG	-0.04	0.32	-0.1
Trial Number: NEG	**-0.57**	**0.24**	**-2.4**
Previous rewarded	**0.65**	**0.08**	**8.0**
(b) Approach Latency			
POS	**1.26**	**0.02**	**64.8**
Near POS	**1.25**	**0.02**	**55.2**
MID	**1.51**	**0.03**	**51.1**
Near NEG	**1.81**	**0.04**	**45.2**
NEG	**2.04**	**0.03**	**66.7**
Trial Number _ POS	**-0.06**	**0.01**	**-4.7**
Trial Number _ Near POS	-0.01	0.04	-0.2
Trial Number MID	**0.14**	**0.05**	**2.8**
Trial Number _ Near NEG	0.13	0.08	1.6
Trial Number _ NEG	**0.32**	**0.04**	**8.1**
Previous rewarded_ POS	**-0.16**	**0.06**	**-2.7**
Previous rewarded_ Near POS	**-0.07**	**0.03**	**-2.1**
Previous rewarded_ MID	-0.06	0.04	-1.6
Previous rewarded_ Near Neg	-0.06	0.06	-0.9
Previous rewarded_ NEG	**-0.14**	**0.04**	**-3.7**

Estimates for approach latency are from log-transformed values. '**POS**' = positive, i.e. familiar rewarded cue, '**NearPOS**' = ambiguous unfamiliar and unrewarded cue, most similar to the positive cue; '**MID**' = ambiguous unfamiliar and unrewarded cue, intermediate between positive and negative cues; '**NearNEG'** = ambiguous, unfamiliar and unrewarded cue, most similar to the negative cue; '**NEG'** = negative, i.e. familiar unrewarded cue. 'Trial number' refers to the order a cue was presented in the test session. 'Previously rewarded' refers to whether the cue previously presented was rewarded, or not. Significant values are highlighted in **bold.**

Figure S4-I. Responses of red junglefowl in a cognitive judgement bias test dependent of cue type. (A) Probability of approaching, and (B) latency to approach, a cue. Cue types: Po = Positive, NP = Near Positive, M = Middle, NN = Near negative, Ne = Negative.

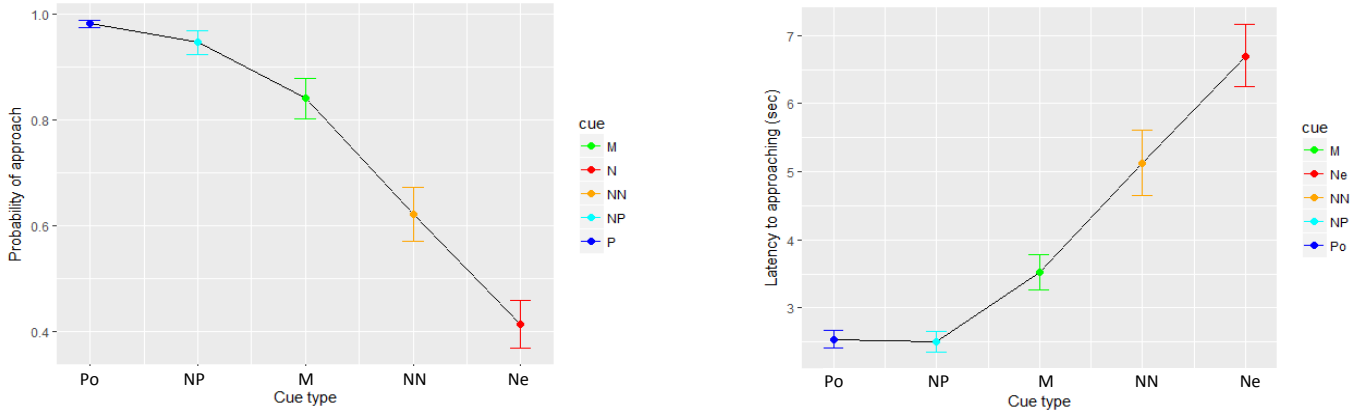


Figure S4-II. Responses of red junglefowl to a cognitive judgement bias test dependent on trial number in a test session. (A) Probability of approaching, and (B) latency to approach, a cue. Cue types: Po = Positive, NP = Near Positive, M = Middle, NN = Near negative, Ne = Negative.

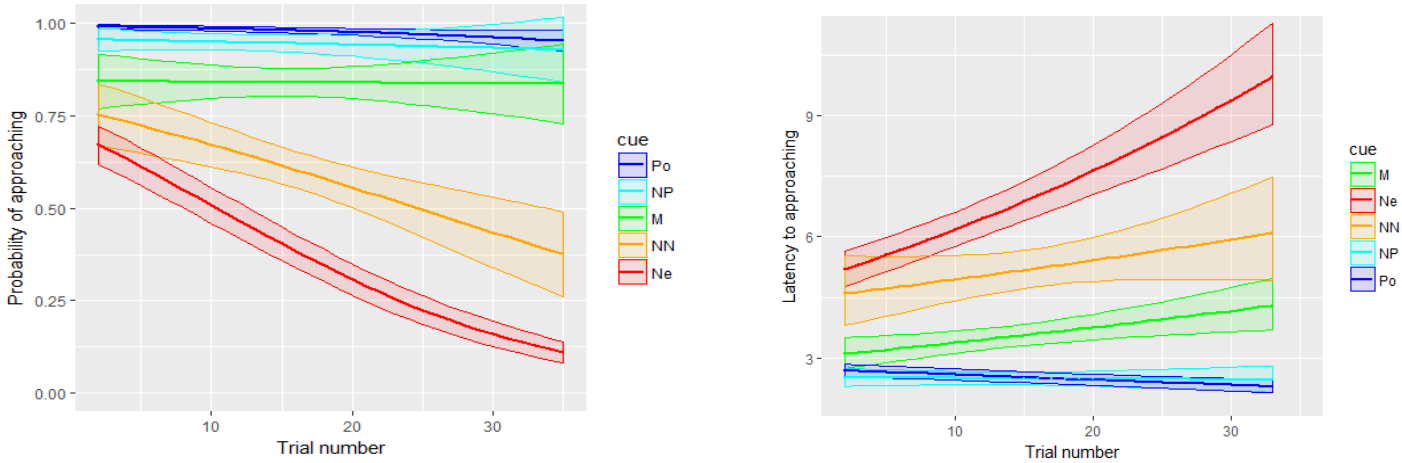


Figure S4-III. Responses of red junglefowl to a cognitive judgement bias test dependent on whether the previous cue was rewarded or not. (A) Probability of approaching, and (B) latency to approach, a cue. Rewarded = previously cue was rewarded, Unrewarded = previously cue was not rewarded. Cue types: Po = Positive, NP = Near Positive, M = Middle, NN = Near negative, Ne = Negative.