Social dominance and personality in male fowl \((\textit{Gallus gallus domesticus})\)

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Social dominance and personality in male fowl (*Gallus gallus domesticus*)

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LIST OF PAPERS

This thesis is based on the following manuscripts, referred to by their roman numerals.

Paper I
Favati, A., Leimar, O., Radesäter, T. and Løvlie, H. Social ‘states’ and personality: Stability in social status and individual characteristics explain consistency in behavioural responses. Submitted manuscript

Paper II
Favati, A., Leimar, O. and Løvlie, H. Aggressiveness and exploration predicts social status in male domestic fowl (Gallus gallus domesticus). Manuscript
Social dominance and personality in male fowl (*Gallus gallus domesticus*)

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ABSTRACT

Individuals in social species commonly form dominance relationships among each other, and are often observed to differ in behaviour depending on their social status. However, whether such behavioural differences are a consequence of dominance position, or also a cause to it, remains unclear. In this thesis I therefore investigated two perspectives of the relationship between social dominance and personality in the domestic fowl (*Gallus gallus domesticus*), a social species that forms relatively stable dominance hierarchies. In paper I I investigated the influence of social status on the expression and consistency of behaviours by experimentally changing status between repeated personality assays. The level of vigilance, activity and exploration changed with social status, while boldness and territorial crows appeared as stable individual properties, independent of status. These results showed that social status contribute to both variation and consistency in behavioural responses. Social status should therefore be taken into account when investigating and interpreting variation in personality. In paper II I showed that behaviour in a novel arena test and during encounter with an opponent can predict social status, more specifically that fast exploration and aggressiveness predicted a dominant social position. Together, these results highlight the dynamics of the two-way relationship between social position and individual behaviour and indicate that individual behaviour can both be a cause and a consequence of social status.
INTRODUCTION

The take-off point of this licentiate thesis is the notion that not all individuals of a group behave the same. Individuals of group living species often form dominance relationship among each other, characterised by repeated outcome in favour of one participant of dyadic agonistic interactions (Chase 1980). Socially dominant individuals commonly enjoy increased access to resources, such as mating partners, which typically results in higher reproductive success (Andersson 1994). Beside differences in social behaviour, like who is more aggressive or perform more mating behaviours (e.g. Korzan et al. 2006, McGhee and Travis 2010), there are also a few examples of behavioural differences between dominant and subordinate individuals in non-social contexts (e.g. Colleter and Brown 2011, Dahlbom et al. 2011, David et al. 2011). For example, explorative great tits (Parus major) outcompete less explorative ones (Verbeek et al. 1996), and bold three-spined sticklebacks (Gasterosteus aculeatus) are more aggressive than shyer individuals (Huntingford 1976). However, there are also a couple of examples of negative correlations, and no correlations between explorative behaviour or boldness and dominance (Gomez-Laplaza 2002). In the mountain chickadee (Poecile gambeli) less explorative individuals have higher chances of becoming dominant, and boldness does not differ between dominant and subordinate individuals (Fox et al. 2009). Interestingly, variation in such behavioural differences as activity, exploration, boldness and aggression has been demonstrated to be consistent across time and context (Wilson et al. 1994, Dall et al. 2004, Sih et al. 2004b, Groothuis and Carere 2005). Consistent individual differences in behaviour have been described in a large number of species in multiple taxa (Gosling 2001), and have been referred to as ‘animal personality’ (Dall et al. 2004) or ‘temperaments’ (Reale et al. 2007). When multiple behavioural traits are correlated across time or context, the term ‘behavioural syndromes’ (Sih et al. 2004a) is used, and when focus lays on the relationship between behavioural consistency and stress responses, the related concept of ‘coping styles’ is often applied (Koolhaas et al. 1999). By scoring individuals in personality tests, they can be categorised along a continuous axis of specific personality traits, like boldness or exploration, and an individual is then said to have a 'behavioural type' or 'personality type' (e.g. more or less explorative, Sih et al. 2004b). The research field of animal personality is still young, although fast growing. Nevertheless, there are still major gaps in the understanding of why there is personality variation, including questions about the mechanisms behind stable behavioural responses and the evolution and
maintenance of behavioural polymorphism (Dingemanse and Wolf 2010, Reale et al. 2010). Current ideas of how variation is maintained include negative frequency dependence (Dall et al. 2004, Wolf and Weissing 2010), selection regimes that fluctuate over time (Dingemanse and de Goede 2004), and various trade-offs, for example between survival and reproductive success (Smith and Blumstein 2008), or between growth and mortality (Stamps 2007). Behavioural consistency within the individual has been suggested to be maintained by physiological limitations in plasticity (Sih et al. 2004b), or by stability in physiological or environmental states that changes slower than behaviour, and that affects the costs and benefits of the individual's actions (e.g. body size, territory size or stable social hierarchies; Houston and McNamara 1999, Dall et al. 2004, Dingemanse and Wolf 2010, Luttbeg and Sih 2010, Wolf and Weissing 2010). To learn more about the significance and evolution of animal personality, studies of both through survival and reproductive success of different personality types are requested (Dingemanse and Reale 2005, Smith and Blumstein 2008). Social dominance typically affects reproductive success positively (Andersson 1994), and as mentioned above, often seem to correlate to various personality traits. It would therefore be interesting to further study this relationship. Not least, we should also aim to investigate the causality behind this relationship, to reveal possible fitness consequences of various personality traits.

There are four partly nonexclusive scenarios that may describe the causality of the relationship between individual behaviour and social status. First, behavioural response may be a consequence of the current social position (Cornwallis and Birkhead 2008). If so, we expect a change in dominance status to lead to a change in behavioural response. This would also mean that social status may represent a state that gives rise to consistent differences in behaviour (Dall et al. 2004). Nevertheless, there are very few studies that have experimentally altered social status to study if a change in behavioural response follows (but see Cornwallis and Birkhead 2008). Second, social status and behavioural responses may share a common underlying cause such as stress reactivity (Øverli et al. 2007, Carere et al. 2010). In this case we expect behaviours to be correlated to social status, but showing less plasticity when status is changed. Third, and not exclusive from the second, the personality type may affect an individuals' possibility to obtain a dominant social position (e.g. Dahlbom et al. 2011). In this case, we expect to predict future social status by means of behavioural responses in non-social contexts. Phenotypic traits like body size or size of armaments etcetera have traditionally been investigated in this scenario and have been shown to predict
social status (Andersson 1994, Berglund et al. 1996). The potential link between behaviour and future status is on the other hand much less investigated and the role for behavioural differences to influence establishment of status is still poorly explored (but see e.g. Øverli et al. 2004, Korzan et al. 2006, Dahlbom et al. 2011). And last, there is the remaining possibility that variation in behavioural responses or personality traits is uncorrelated to variation in social status. In this case, we expect no difference in the behavioural responses between dominant and subordinate individuals, and behaviours to be unaffected by changes in social position. Revealing the causal relationship between behavioural differences and social status is essential for further understanding of how variation in personality types affect fitness, and ultimately for understanding of the evolution of personality types. Each scenario described above has been examined to some extent, but there is a lack of studies exploring more than one of the scenarios outlines above in the same animal model. In this thesis, I aim to study the causality of the relationship between behaviour and social status by an experimental approach, using the male domestic fowl (Gallus gallus domesticus) as my study species.

**Study species**

The domestic fowl (Gallus gallus domesticus) is, like its wild ancestor the red junglefowl (Gallus gallus; Fumihito et al. 1994), a group living species. Natural groups are typically constituted by a dominant male, a few subordinate males and several females, and group sizes ranges from pairs to around 10-15 individuals (McBride et al. 1969, Collias and Collias 1996). The sex ratio varies, but is generally equal or slightly female biased (e.g. 2:3-2:5, males to females), probably due to higher predation on subordinate males in the periphery of the group (McBride et al. 1969, Collias and Collias 1996). Both males and females form (most often linear) social hierarchies defined by threats, attacks and avoidance, and males always dominate over females (Banks 1956, Collias et al. 1994). The fowl is suitable for studies of the potential link between behaviour and social status for three main reasons. First, the status of an individual is rather stable and can persist for several years, but last on average approximately one breeding season in free-ranging flocks of red junglefowl (Collias and Collias 1996). This is a considerable part of the lifespan of males, which lives up to 5.5 years in semi-natural flocks exposed to predation (Collias and Collias 1996). However, changes in groups over shorter time spans can occur and cause changes in the status of individuals, for example if the dominant male is predated (McBride et al. 1969). Second, dominant males have higher reproductive success compared to subordinate males (Pizzari and Birkhead 2000, Wilson et al. 2008), thus the prerequisite for evolutionary consequences of a relationship
between social dominance and personality traits. Third, dominant and subordinate male fowl differ quantitatively in behaviour. Dominant males not only perform more courtship behaviour (Cheng and Burns 1988), but also crow more (a signal of territoriality and dominance; Leonard and Horn 1995), and spend more time being vigilant compared to subordinate males (Cornwallis and Birkhead 2008). However, the relationship between personality traits and social status is unclear.

METHODS

The study population is kept at Tovetorp research station (Björnlunda, Sweden) in six mixed-sex (sex ratio ~ 1:1), mixed-age (1-10 yrs) groups (15-20 individuals per group) in outdoor aviaries (approximately 6x10x2 m) with access to chicken houses. All aviaries are furnished with perches and dust baths, and the animals have ad libitum access to regular chicken food and water. The experiments were performed during the breeding season (may-september) in 2007 ($n$ males = 36), 2011 ($n$ = 48, of which 9 were reused from 2007) and 2012 ($n$ = 50).

Personality assays

There were no personality assays specifically developed for chickens when I started the work with this thesis. Therefore, I developed two novel arena tests to measure activity and explorative behaviour. The design of these were inspired by the open field test commonly used in applied ethology to measure variation in fear in domestic fowl (often a small barren arena, e.g. 1 m. in diameter; Jones et al. 1995) and by the novel arena tests commonly used to measure variation in exploration propensity in passerine birds (commonly a room furnished with five artificial trees; Verbeek et al. 1994). The arena used in paper I consisted of an outdoor roofed arena (3x6 m), with peat floor and five artificial trees (50 cm high). The trees were added to prevent immediate overview of the arena, encouraging the fowl to explore it (Fig. 1a). The arena was divided in 8 equal-sized subareas by drawing subtle lines in the substrate, and the number of subareas visited was used as a measure of exploration propensity. A personality test should optimally be designed to reflect variation in individual behaviour within a population (Reale et al. 2007). However, around half of the males retained the maximum exploration score of visiting 8 subareas in the novel arena. In paper II I therefore used a larger arena (~ 7x10 m oval, divided into 36 equal-sized subareas; Fig. 1b) placed in the forest, without a roof, in order to approve of more variation in explorative behaviour. The forest floor was naturally covered with leaves, herbs and bushes, as in the
natural habitat of Red junglefowl (Collias et al. 1964). A full description of the behavioural responses measured in the novel arena tests are given below.

Startle tests are commonly used to estimate variation in risk-taking behaviour within the research field of personality (van Oers et al. 2004, Bell 2005, Quinn and Cresswell 2005, Briffa et al. 2008, Rudin and Briffa 2012). In general a stressor (a sound, a predator model etc.) is presented, and thereafter the latency until the individual retain regular activities like feeding, is measured (e.g. van Oers et al. 2004). The fowl utter alarm calls (loud cackling vocalisations) in response to terrestrial predators, to which flock mates react on by vigilance (Evans et al. 1993). In paper II I therefore used a recorded male fowl alarm call as a startle. The latency until the male retained normal activities after the call was played, thus stopped being vigilant and resumed feeding, was used as a measure of how alert the male was, or in other words how risky the individual appreciated the environment to be.

Morphological measures

Male body size and comb size (a fleshy red ornament on the head) often correlate positively with social status in male fowl (Ligon et al. 1990; Zuk and Johnsen 2000; Parker et al. 2002), and are also shown to interfere with the establishment of social status (Parker et al. 2002). Males I used, were therefore matched for these morphological features in both studies (to the nearest 10%). Comb size, approximated by comb length and body size, approximated by tarsus length and body weight, were measured prior to the experiments, at the day of the pair setup (paper I), or at the day of isolation (paper II).
To study the influence of social status on the expression and consistency of behaviours, I experimentally changed status of male fowl between repeated personality assays. Pairs of matched males were set up, and the dominance order of the two males was determined. After acclimatising to the new social situation, a novel arena test was performed in order to investigate their boldness (latency to enter the arena), activity (no. of subarea transitions) and explorative behaviour (no. of subareas visited). The males’ vigilance (proportion of time a male spent being vigilant) was also registered, as was the number of crows (a vocal territorial display, Collias et al. 1964, Leonard and Horn 1995). Then pair members were experimentally changed so that the two previously dominant males formed a new pair, and the two subordinate males another pair, forcing one male in each pair to change social status, as there can only be one dominant male and one subordinate male in each pair (Fig. 2; see Cornwallis and Birkhead 2007, 2008 for previous use of this design). After two days of acclimation to the new social context, the novel arena test was performed once again in order to estimate the influence of change of social status on behaviour.

Figure 2. Schematic picture of the design used when manipulating social status of male fowl. Males were either A) dominant across both pair set-ups, B) increased status from subordinate to dominant, C) reduced status from dominant to subordinate, or D) were subordinate over both pair set-ups.
Paper II

To investigate the role for personality on establishment of status, I scored individual behaviour in a novel arena test and a startle test (play back alarm sound of a conspecific, see above). In paper II I added the latency to visit at least five subareas as a complementary measure of exploration propensity (Verbeek et al. 1996). Besides this additional measure, the behavioural measures were the same as in paper I. All males were isolated from same-sex competitors for five days prior to the behavioural tests in order to standardise their previous social experience. After personality was assessed, males were set up in duels resulting in one male becoming dominant and the other subordinate. The agonistic interaction between males started spontaneously they were placed in front of each other, and the initial reaction to the duel situation was used to score the males' aggressiveness from 1-4 (Fig. 3). The males aggressiveness was scored between 1 (least aggressive) and 4 (most aggressive) as follows: (1) fleeing away from the other male, (2) straight body posture, no avoidance or attraction to the other male, (3) crouched body posture and dropped wing or raised hackles (Collias 1943, Kruijt 1964) or (4) directly – within 2 seconds – approaching the other male (Fig. 3). All duels ended with one of the opponents retreating and no longer retaliating further attacks from the opponent. A minimum of five occasions of avoidance within an hour by the same male was used to define a male as subordinate, and the other male as dominant.

Although I only measured behavioural responses once, and thus did not explicitly evaluate their repeatability, similar responses have been found to be consistent within the individual in an earlier study of male fowl (paper I). Moreover, a number of studies in other species have shown that explorative behaviour and boldness typically show substantial repeatability over time (e.g. Verbeek et al. 1994, Dingemanse et al. 2002, Quinn and Cresswell 2005). I therefore consider the single measurement in this study as useful for determination of personality types.
SUMMARY OF RESULTS

Paper I

There were several behavioural differences observed between dominant and subordinate males. Dominant males were more vigilant and active and crowed more compared to subordinate males, but did not differ significantly in exploration and boldness in the first trial of the novel arena test (see Fig. 1 in paper I). The main result of this study was however that some of these responses (vigilance, activity and exploration, Fig. 4 b-d; Table 2 in paper I) were plastic and changed in frequency when social status was changed, while others were not (boldness and crowing Fig. 4a, 4e). At the same time, there was a significant relationship between behaviour in the first and the second trial for all behavioural responses. In other words, the individual behaviours we recorded were partly stable over time, and change of status.

Paper II

Among the behaviours observed in the novel arena test in paper II, exploration was the only response that could forecast social dominance. The quantitative difference in exploration between future dominant and subordinate males was fairly large; dominant males explored the novel arena were more than twice as fast as the subordinate males (dominant 243.92±57.16 s., subordinate 500±96 s., Fig. 5). In contrast, there were no significant
differences in activity, vigilance, alertness after being startled, or crowing between future dominant and subordinate males (Fig. 5). Aggressiveness in the first seconds of duels could also predict a dominant position (Fig. 6), and males that showed a higher aggressiveness more often won the duel. Exploration and aggressiveness were not significantly correlated, thus not forming a behavioural syndrome along a proactive-reactive axis (Table 1), but instead independently predicted a dominant social position.

Figure 4. Behavioural response of dominant and subordinate males during the first and second test trial of a repeated novel arena test. (a) Boldness (whether the males entered the novel arena within 30 s. or not) was stable within the individual across changes in social status. (b-d) Males that had a stable status across trials showed more stable behavioural responses of exploration, activity and vigilance compared to males that changed social status, irrespective of their social status. (e) The number of crows uttered in the second trial was significantly
affected by previous, but not current, social status, and no clear effects of the change in status were detected. Values presented are mean ± SE for (b-e), but for (a) the percentages of males that entered the arena within 30 seconds ± 95% CI, are given.

Figure 5. Difference in behavioural response in the novel arena test (a-e) and the startle test (f) between males that later became dominant (filled dots), or subordinate (empty dots), respectively. Future dominant males were more explorative compared to subordinate males. All other differences were non-significant. Mean values ± SE are given.
Figure 6. Difference in aggressiveness score between males that subsequently became dominant and subdominant. Males that later won the duel and obtained a dominant social position more often had a higher aggressiveness score compared to the later subordinate male. However, both males of a pair had equal aggressiveness score in nearly half of the cases.

To sum up the main results of both papers, dominant and subordinate males differed quantitatively in several behavioural aspects in non-social contexts. These differences were partly a result from the current social status (paper I), but also existed prior to the establishment of social status (paper I and paper II). Together the results reveal a two-way causality between social position and individual behaviour.

DISCUSSION

In this thesis, I investigated the causality of the relationship between individual behaviour and social status in male fowl. Dominant and subordinate males differed in several behavioural responses in a non-social context, and by an experimental approach I showed that the current social status primarily causes these differences (paper I). However, in paper II I showed that whether a male end up being dominant or subordinate is not random with respect to personality types, but influenced by males' personality.

Dominant males were more vigilant, explorative and active, and the occurrence of these behaviours changed after the experimental change in social status (Fig. 1b-d, Table 2 in paper I). This meant that individual males adjusted their level of expression of these behaviours according to their current social status, supporting the first scenario of the causality of the relationship between social dominance and behaviour. In a study of male fowl by Cornwallis and Birkhead (2008), vigilance was similarly found to be a plastic response which frequency changed when social status was changed. It has earlier been
demonstrated in male fowl that dominants suppress the behaviour of subordinates (Leonard and Horn 1995, Johnsen et al. 2001). The quick change in behaviour following a change in social status (paper I) indicates that subordinate males have an intrinsic capability of becoming dominant and behaving thereafter, but that such behaviour seem to be suppressed by the presence of a more dominant male (Wilson et al. 2009). In addition, all males behaved in a dominant manner already after a very short period of isolation from other males (personal observation, paper II; see also Pizzari et al 2007 for rapid changes in male reproductive physiology associated with alteration of status). This indicates that variation in responses to personality assays do not necessarily reflect fixed behavioural types, but may reflect the current social position of the individuals. This is in congruence with studies of male rats (Rattus norvegicus) brought up under various social regimes, showing that social position affects behaviour also in non-social test situations (Arakawa 2006). The strong effect of social status indicates that status may act like a ‘state’; a slow changing condition that infers stability also in behavioural response (Wilson et al. 1994, Dall et al. 2004). Despite the intuitiveness of the idea of stabilising (external) states causing consistent behaviour, there is a lack of empirical studies explicitly testing this idea. However, there is one example; a study investigating Siamese fighting fish (Betta splendens), which demonstrated that holding a territory stabilise individual behaviour (Dzieweczynski and Forrette 2013), a phenomenon closely related to social position. Part of the behaviour remained stable across the change in status in our study, which means that other states and/or heritability also play a role in shaping individual behaviour. In the study by Cornwallis and Birkhead (2008), no such behavioural consistency was detected across a change in social status. Behaviour was monitored in a social context in their study, and it therefore appears that social status has an even stronger effect in the presence of conspecifics, overriding individual differences. In another study, male vocalisations (Nelson et al. 2008) were shown to correlate across functional contexts only when the males were observed in their (stable) social groups, further strengthening this conclusion.

Dominant and subordinate males differed in crow rate during the first novel arena trial, and little plasticity was observed in this behaviour when social status was changed. Individual males seemed to vary in their underlying propensity to crow and this supports the second scenario outlined previously where social status and behavioural responses share a common underlying cause. A potential underlying factor for crowing and social dominance could be testosterone, which regulates development of crowing frequency in male fowl (Davis and
Domm 1943). Further, males fowl with a high testosterone level have shorter attack latencies and more often become dominant (Johnsen and Zuk 1995). Indeed, a high crow rate in the novel arena test was predictive of dominance, although the study in paper I was not specifically designed to test predictors of status. The latter result was not repeated in paper II, thus the predictive value of crow rate for social status is questioned. A potential explanation for this discrepancy could be that the social settings prior to the novel arena test affects crowing in the arena. In paper I, males were housed in pairs, which forces the subordinate male to reduce its crowing frequency, since dominant males punish subordinate males that crow (Leonard and Horn 1995). This might have reinforced the difference in crow rate between males that were high or intermediate in relative dominance ability (that were dominant in one or two pair setups) and males that were repeatedly found in subordinate positions (subordinate in both trials, Fig. 4). In contrast, males were individually caged for five days prior to the arena test in paper II, thus no males were suppressed in their crowing. In paper II I performed additional tests to investigate the possibility that behavioural type could predict future social status, thus testing the third causality scenario; that personality affects social status. Males that were either more explorative or aggressive had increased chances of obtaining a dominant position. The results indicate that pre-existing differences in behavioural type affected the outcome of duels, in other words that not all individuals had equal chances of obtaining a dominant position. These traits were uncorrelated and equally strong predictors of social status, suggesting that multiple factors are important for winning a fight. Scoring high in these traits is considered proactive on the proactive-reactive-continuum (Koolhaas et al. 1999, Sih et al. 2004b), and the results are therefore in congruence with studies of other species, which show a general pattern of more proactive individuals having higher chances of becoming dominant (e.g. Huntingford 1976, Verbeek et al. 1996, Colleter and Brown 2011, Dahlbom et al. 2011, David et al. 2011). However, there are a couple of examples of less bold or less explorative individuals having higher chances of obtaining dominant positions (Gomez-Laplaza 2002, Fox et al. 2009). In a study of great tits, the success of individuals differing on a proactive-reactive axis differed depending on the intensity of intraspecific competition over space and food (Dingemanse et al. 2004), indicating that fitness of different personality types might be context dependent. Fitness of different personality types under various environmental conditions, as well as the exact pathway through which these personality traits affect the outcome of duels warrants further investigations.
The remaining suggested scenario, that there is no causal relationship between social status and behavioural response, was observed for only one behavioural response: boldness. There was no difference in latency to enter the novel arena between males of different social status, and the change in social status did not alter the latency to enter the arena in the second trial. However, a recent study of sea anemones (*Actinia equina*; Rudin and Briffa 2012) showed that boldness (measured as response to a startle) decreased after the experience of losing a fight. This indicates that the social environment can affect also boldness in some cases.

The results of this thesis clearly show that social status must be taken into account during studies of individual behaviour in group-living animals. It is preferable that the social status of the individual, and the eventual changes of the same, is known during the course of the study. Other properties of the individual like quality of territory or partner, properties may potentially affect individual behaviour in a test context in a corresponding way in solitary species or species with a less clear hierarchical structure of the dominance relationships.

*Future prospects*

Under natural conditions, several aggressive interactions may follow after one another. In a socially hierarchical species like the fowl, individuals need to settle the dominance relationships to all other group members of the same sex, for example when an individual transfers from one flock to another (Collias and Collias 1996). The ability to recover from a loss, or the ‘boost’ obtained by a win, have proved to affect the outcome of subsequent duels, the so-called ‘winner- and loser effects’ (Chase *et al.* 1994), but it is unknown whether the magnitude of these effects depend on personality type. In future studies I therefore aim to investigate how several interactions after each other affect male fowl of different personality types to improve our understanding of how personality type affect fitness through social dominance.
CONCLUSION

In this thesis I aimed to disentangle the commonly observed relationship between variation in behaviour and social status. I conclude that behavioural differences between dominant and subordinate male fowl were partly plastic and changed with changes in social status, but also that the social position of an individual could be a consequence of pre-existing differences in behavioural style. Plasticity of behavioural responses according to the current social position have been previously shown in the domestic fowl (Cornwallis and Birkhead 2008), but it is the first time the effect of personality type on the establishment of social status is shown in the species. Together, the results of paper I and II reveal a two-way causality between social position and individual behaviour. This leads to the conclusions that social position should be taken into account when investigating and interpreting variation in personality, as it could otherwise act as a confounding factor, and that individual personality type should vice versa be considered in studies of the underlying reasons of variation in social status.
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Paper I
Social ‘states’ and personality: Stability in social status and individual characteristics explain consistency in behavioural responses

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ABSTRACT

Stability of ‘state’ has been suggested as an underlying factor explaining behavioural stability and animal personality (i.e., variation among, and consistency within individuals in behavioural responses), but the possibility that stable social relationships represent such states remains unexplored. Here we investigated the influence of social status on the expression and consistency of behaviours by experimentally changing social status between repeated personality assays. We used male domestic fowl (Gallus gallus domesticus), a social species that forms relatively stable dominance hierarchies, and showed that behavioural responses were strongly affected by social status, but also by individual characteristics. The level of vigilance, activity and exploration changed with social status, while boldness appeared as a stable individual property, independent of status. Further, variation in vocalisation predicted future social status, indicating that individual behaviours can both be a predictor and a consequence of social status, depending on the aspect in focus. Our results illustrate that social states contributed to both variation and stability in behavioural responses, and should therefore be taken into account when investigating and interpreting variation in personality.

Keywords: behavioural syndromes; intra-sexual selection; phenotypic plasticity; social dominance; chicken.
1. INTRODUCTION

Understanding variation in phenotypes is a key issue in evolutionary biology [1]. The topic includes the study of animal personality, in the form of consistent individual differences in behaviour, across time and/or situations [2], also referred to as temperaments [3] or coping styles [4]. The phenomenon has been described in a large number of species in multiple taxa [5], but there are still major gaps in our understanding of why there is personality variation, including unanswered questions about the mechanisms behind stable behavioural responses and the evolution and maintenance of behavioural polymorphism [6, 7]. Properties or circumstances of an individual that may affect the costs and benefits of its behaviours, such as size or energy reserves are sometimes referred to as ‘states’ [8]. Stability of such states is theoretically predicted to produce stability in behavioural responses which, in combination with between-individual variation in state, gives rise to variation in behavioural types, and thus personality [2, 6, 9]. More broadly, any state changing more slowly than behaviour per se is predicted to cause short-term stability in state-dependent behavioural responses [2]. Together with a positive feedback system, stability in state may also generate long-term stability of behavioural types [9]. Social relationships such as pair bonds or status hierarchies could be one, however not yet investigated, example of such states. If social positions or relationships constitute stable states, intra-individual stability of behavioural responses would follow as a consequence, whereas the behavioural responses should change when the social state of an individual is changed. Despite the intuitiveness of these predictions, the importance of social states for personality variation has not yet been empirically tested.

In social species, social relationships often take the form of dominance hierarchies, which in turn are based on repeated outcomes in favour of one participant of dyadic agonistic interactions [10]. Socially dominant individuals commonly enjoy increased access to resources, such as mating partners, which typically results in a positive relationship between social status and reproductive success [11]. Aggression and the ability to dominate conspecifics can correlate positively with boldness, exploration and active stress handling, thereby defining a ‘proactive’ behavioural style of the reactive-proactive coping style continuum [4, 12]. For example, explorative great tits (Parus major) win more fights compared to less explorative ones [13], and bold three-spined sticklebacks (Gasterosteus
aculeatus) are more aggressive than shyer individuals [14]. On the other hand, boldness and exploration have been found to have a negative, or no correlation, with social dominance [15, 16]. This indicates that the relationship between personality and social status can be species-specific, but also that there are currently limitations to our understanding of the relationship between them.

In principle, there are three possible scenarios for observed correlations between social dominance and personality traits. First, different social positions can be associated with different behavioural tendencies [17]. These differences can manifest themselves also outside the social group, and thus influence responses in personality assays [18]. In this scenario, it is expected that behavioural responses are flexible, adjusting to the current social position, at least to some extent. Second, differences in behaviour can directly influence the chance of obtaining a certain social position (e.g., aggression [19]). In this scenario, certain behavioural types are more likely to be found in specific social positions. However, and crucially, behavioural responses are independent of experimental change of social positions. Third (and partially overlapping with the previous), behaviour and social position can have a common underlying cause [12, 20]. In such a case, both personality traits and the underlying cause (e.g., hormonal state [20]) might predict social position, but behaviour is not necessarily altered when the social position is changed. Although there is some support for each of the three scenarios separately, they have not previously been tested simultaneously, leaving the causality between behaviour and social status unclear. Our aim here is therefore to investigate the issue by means of an experimental approach where social status of individual male domesticated fowl (Gallus gallus domesticus) is changed and behaviour scored in personality assays. If a change in dominance status leads to a change in behavioural response, we can conclude that social status may represent a state that gives rise to consistent differences in behaviour.

Male fowl are suitable for investigating the potential link between variation in personality and social status for two main reasons. First, dominance hierarchies are relatively stable; the status of an individual persists for approximately one breeding season in free-ranging flocks of fowl [21], but changes in groups over shorter time spans can occur and cause changes in the status of individuals (e.g., if the dominant male is predated [22]). Therefore, status can be considered as a slowly changing yet not permanent state. Second, dominant and
subordinate male fowl differ quantitatively in behaviour. For example, dominant males crow more, as a signal of territoriality, and spend more time being vigilant compared to subordinate males [17, 23]. Nevertheless, the causal relationship between these behavioural differences and status is untested.

2. METHODS

Animals and housing

The study took place during the breeding seasons (May - September) of 2007 and 2011 at Tovetorp Research station, Stockholm University, Sweden. We used 84 males (2007: n = 36; 2011: n = 48) of an old Swedish game breed of fowl (Gallus gallus domesticus, ‘Gammal svensk dvärghöna’) used to human handling and kept under semi-natural conditions in mixed-sex, mixed-age (1-9 years) groups. Data were collected during the hours of day when the birds are most active (0530-1100 and 1500-1900 hours local time, see [24] for further information on variation in daily behavioural patterns). Groups of four males ($n_{groups} = 21$) were matched for morphological measures (< 10% difference in comb and body size) to reduce the effect these variables can have on the establishment of status [19, 25, 26]. Comb size and body size were estimated as comb length and tarsus length respectively, measured with a digital calipeter to the nearest tenth of a millimetre. Body size was also estimated as body weight measured with a digital scale to the nearest gram. To generate males of different social positions, two pairs of males were randomly chosen from each of the matched groups of four males ($n_{pairs} = 42$). Pairs of males were housed in outdoor aviaries (approximately 3 x 3 meters) that were visually, but not vocally, isolated from other birds. This set up resulted in one of the males in each pair becoming dominant, and the other subordinate. A minimum of five observed successive submissive behaviours within 2 hours (avoiding the other male when he approached) by a male defined that male as subordinate [27]. Males within a pair had not been housed together for at least 2 weeks prior to the trials, reducing any effects earlier encounters may have had on the establishment of social status [28, 29]. All birds had ad libitum access to food and water, and all aviaries had dust baths and perches. Experiments were conducted according to ethical requirements in Sweden (Linköping Ethical committee, ethical permission number 60-10).

Behavioural assays and manipulations of social status
Two days after the pair was formed, which was enough time for males to establish a dominance relationship without reversals, each bird was exposed to its first ‘Novel arena’ test (referred to as trial 1) in a 3 x 6 meter large outdoor area connected to the aviary by a door. The order of testing of the males in a pair was random with respect to social dominance. The floor in the arena was covered with dark brown peat to create novelty (in comparison to the sandy floor of the birds’ home pens) and divided into 8 subareas by drawing subtle lines in the floor substrate to facilitate estimates of exploration (see below). The arena had five artificial bushes (green plastic spruce trees, approx. 70 x 50 cm) in order to obstruct direct view of the arena and thus encourage exploration (see S.I., figure S1). The cage mate of the focal male was removed from the aviary by herding him into an empty adjacent pen two minutes before the test started. The door between the aviary and the test arena was opened, and focal males that entered the arena within 30 seconds were considered bold, while those who did not were considered shy (i.e., a ‘boldness’ spectrum split into two categories, because of a bimodal distribution of the latencies). In cases when a male did not voluntarily enter the arena within 2 min, he was gently herded into it by the observer. The observer sat outside the arena, visible to the male during the test. Behaviours and vocalisations were recorded for 15 min, starting when the male entered the arena. Vigilance (head above shoulder height, eyes open) and behaviours categorised as non-vigilance (eating, preening, resting and dust bathing, behaviours that all occurred in lower frequencies) were recorded every 30s, and the percentage of recordings a male was vigilant during the test was calculated (hereafter named ‘vigilance’). The number of unique subareas a male visited (1-8) divided by the total number of possible subareas (8) was used as a measure of ‘exploration’ propensity. The number of times a male entered a subarea he had previously visited, was noted as a measure of ‘activity’. Number of crows were recorded ad libitum (‘crowing’). Crowing is a species-specific vocalisation related to territoriality in male fowl [30]. In addition to crowing, some males uttered alarm calls, which are loud and distinct warning vocalisations typically uttered when an individual is startled, for example when a predator appears [31], but these few vocalisations were not analysed further.

When the behaviour of all four males of a matched group had been scored in the first novel arena test, pair members were experimentally changed so that the two previously dominant males formed a new pair, and the two subordinate males another pair. The new pair setup
forced one male in each pair to change social status, as there can only be one dominant male and one subordinate male in each pair [see 18 for previous use of this design]. After two days of acclimation to the new social context, the novel arena test was performed a second time in order to estimate the influence of change of social status on behaviour [26]. We refer to this test as trial 2. For the second pair setup, the number of males was reduced to 82 due to the death of one male in 2007 and injury of one male in 2011. A. F. conducted all observations.

**Statistical analyses**

Our general approach to the statistical modeling of the behavioral responses was to first determine suitable Box-Cox transformations of the continuously distributed responses, in order to achieve homogeneous variances. The outcome of this procedure was that the measure vigilance did not need transformation, the transformation \((x + 1)^{0.25}\) was used for activity and crowing, and the transformation \(x^3\) for exploration. We applied these transformations in all analyses, including model selection and Bayesian MCMC modeling, but we show the untransformed variables in the figures. The binary measure of boldness was treated as a discrete variable. Based on our experimental design, with males being divided into matched groups, we used the group as a random effect in mixed models of the behavioral responses.

**Difference in behavioural responses of dominant and subordinate males in trial 1**

To investigate differences in behavioural response between dominant and subordinate males \((n = 84)\), we analysed the effect of social status on each response (boldness, vigilance, activity, exploration and crowing) in trial 1, by fitting mixed models with the trial 1 social status as a fixed effect and the group as a random effect (Bayesian Markov Chain Monte Carlo generalized linear mixed models, MCMCglmm, [32]).

**Individual characteristics versus the effect of social status on behavioural responses**

Behavioural response in the second trial can be dependent on behavioural response in the first trial \(i.e.,\) personality, current social status (thus behaviour is a plastic response) or the previous social status (suggesting a common underlying variable). In order to disentangle the effect of social status and personality on a behavioural response, we analysed the effect...
of both ‘current status’ (obtained in the second pair setup, ‘dominant’ vs. ‘subordinate’) and ‘previous status’ (obtained in the first pair setup, ‘dominant’ vs. ‘subordinate’), and behavioural response in the first trial on each of the responses in the second trial by Bayesian MCMCglmm \((n = 82)\). Random effect and error distributions were the same as in the previous analyses (above). To determine final models, we used bidirectional stepwise selection choosing the model with the lowest AIC value using Generalized linear mixed model [33]. The minimal model for each response in the second trial was set to include the corresponding response from the first trial, current status and previous status. The range of models used in stepwise search of the best fitted model included all response variables in the first trial, current status and previous status. We also estimated repeatability with and without taking social status into account (see S.I.).

Factors predicting social status

Comb size, body size (tarsus length, and body weight) and age are traits that may affect the establishment of status in male fowl [19]. We investigated whether the initial matching of males for these properties was successful in both pair setups by testing for the difference in comb size, body size, body weight and age between dominant and subordinate males in each pair by means of a two-sided, one-sample student’s t-test. The level of this analysis was thus the pair \((n_{\text{trial1}} = 42, n_{\text{trial2}} = 40)\). To investigate whether behaviour may predict social status, we tested if behavioural response in the first novel arena test could predict the outcome of the duel in the second pair setup. Difference between dominant and subordinate males in the binary response boldness was tested with Fisher’s exact test, while difference in vigilance, activity, exploration and crowing between dominant and subordinate male of each pair, were tested with a two-sided one-sample student’s t-test \((n_{\text{pairs}} = 41)\).

All statistics analyses were conducted using R version 2.15.1 [34].

3. RESULTS

Differences in behavioural responses of dominant and subordinate males

Dominant males were more vigilant and active, and crowed more compared to subordinate males, but did not differ significantly in boldness and exploration (table 1, figure 1).

Individual characteristics versus the effect of social status on behavioural responses
Analyses of factors affecting the observed behavioural responses of males in the second trial showed that all response variables investigated had a statistically significant positive relationship with the corresponding response in the first trial (table 2). At the same time, all responses but boldness and crowing, were affected by the current social position (table 2, illustrated by figure 2 showing variation in vigilance, for other variables see S.I. figure S2). Vigilance, activity and exploration were flexible behaviours and changed to a large extent with changed social status (table 2, figure 3b-d). This was especially pronounced for vigilance (figure 3b), while for example boldness did not follow changes in status (figure 3a). Males that had changed status clearly altered the time they spent being vigilant: males with reduced status from dominant to subordinate showed a decrease in vigilance, while an increase in vigilance was seen in males that improved their status from subordinate to dominant (figure 3a). Males with stable status across the two experimental pair setups (males that remained either dominant, or subordinate) showed a stable between-test response (figure 3a). The number of crows uttered by males was dependent on the previous social status: males that previously had been dominant crowed more in the second trial compared to other males, irrespective of their current social status (table 2, figure 3e).

There were additional statistically significant factors influencing the behavioural responses of males in the novel arena test of trial 2 (table 2). Part of variation in vigilance was explained by the number of crows a male uttered in the first trial, thus males that crowed more in the first trial were more vigilant in the second trial (table 2). In a similar way, activity was partly explained by vigilance in trial 1, and crowing was affected by activity in trial 1 (table 2).

Factors predicting social status

None of the measured morphological traits, or age, had a detectable effect on the establishment of social status in this experiment, indicating that the experimental matching for these features was successful (first pair setup: tarsus length, t_{41} = -0.080, p = 0.94; body weight, t_{41} = 0.36, p = 0.72; comb length, t_{41} = -1.03, p = 0.31; age, t_{41} = 0.49, p = 0.63, second pair setup: tarsus length, t_{40} = 0.76, p = 0.45; body weight, t_{40} = 0.0072, p = 0.99; comb length, t_{40} = 1.71, p = 0.09; age, t_{40} = -0.14, p = 0.89). On the other hand, the number of crows a male uttered in the first trial predicted future social status in the second pair
setup; males that crowed more had a higher probability of becoming dominant compared to males that crowed less ($t_{40} = 2.59, p = 0.01$). No other behavioural responses predicted future status (boldness, $p = 0.58$; vigilance, $t_{40} = 0.41, p = 0.68$; activity, $t_{40} = -0.73, p = 0.47$; exploration, $t_{40} = 0.78, p = 0.44$).

4. DISCUSSION

Our experiment showed that behavioural responses of male domestic fowl in behavioural assays are affected both by the social status of an individual and by its individual characteristics. Our results support the general interpretation of behaviours being plastic but also showing constraints in flexibility, in line with the few empirical studies where both possibilities have been investigated [35, 36]. How the two factors social position and individual characteristics explain variation and stability in a behavioural response depends on the behavioural response in focus, and will be discussed below.

We introduced three scenarios for how a relationship between social position and behavioural responses can come about. By exploring different aspects of behaviour of male fowl across changes in social position, we found experimental support for two of the three scenarios, namely that the social position in itself influences behaviour, and that social position and behaviour may share a common, underlying cause. According to the first of these, frequencies of behaviours are expected to change when social status changes, thereby putting a limit to the consistency of the individual responses. We found this to be the case for three of the recorded responses: activity, exploration and vigilance. Activity and exploration are commonly used behavioural dimensions in personality studies [3], but the effect of social status on their expression has previously not been investigated. In the fowl, dominant males guard territories [30]. The increased activity and exploration observed when a male becomes dominant may thus be explained by an increased propensity to patrol a territory and its surroundings. Our results support the general idea that individuals obtaining dominant social positions are more often observed to behave in a proactive way, rather than in a reactive manner [4, 13, 20]. They further suggest that an individual’s degree of activity and exploration is partly a consequence of the current social situation, and stays constant if social status is unchanged, but changes when social status changes. This is in line
with the reasoning of Dall et al. [2], who suggested that stability in various states can underlie consistency in behavioural responses.

Vigilance decreases predation risk by facilitating early detection of predators and can be considered a response to an individual’s assessment of risks in its environment [37]. Both in our study and as previously shown [17], dominant males were more vigilant, and the degree of vigilance changed dramatically when status changed, which can be interpreted as a risk-averse response to protect increased assets (e.g., access to females as sexual partners) [6]. Such a response follows the principle of asset protection, in that individuals should increase their cautiousness when assets increase [38, 39]. However, it has been argued that differences in state should converge over time, as cautiousness is assumed to lead to a decrease of assets [9]. We do, however, not expect increased access to females - as a consequence of high social status - to be negatively affected by an increased vigilance, thus the relationship between asset and behaviour in this case need not function as a negative feedback mechanism that causes a convergence of individual states. Our results indicate that social status could act as a stabilising condition, or ‘state’, generating at least short-term stability in behavioural responses [39, 40]. Whether long-term stability is achievable in a similar way remains to be studied. This could happen if, for example, early experiences of dominance positions lead to long-term individual differences in physiology or morphology that later in life affect the chances of obtaining a certain social position.

We also found support for the scenario that behaviour and social dominance share common underlying causes. The number of crows uttered by a male was correlated with both the previous social status and the behavioural type of an individual, indicating that individual males vary in their underlying propensity to crow and that this propensity can influence the chances of becoming dominant. The observation that dominant males crow more than subordinates has been reported previously in the species [17, 23], but to our knowledge our study is the first observation of an effect of social position on number of crows uttered outside of a social context. Also, we found that when two dominant, or two subordinate males, were paired up in preparation for the second trial, the male that had crowed more in the preceding novel arena test more often became dominant. Our observations thus suggest that crowing frequency partly reflects an underlying characteristic that influences the establishment of social status [20, 41, 42]. Note, however, that crowing is not a
commonly observed behaviour during the interactions that determine social status; it rather appears as a signal of the outcome [23, 43]. A potential underlying factor for crowing and social dominance could be testosterone, which regulates development of crowing frequency in male fowl [44]. A higher testosterone level during male-male interaction has been found to be associated to shorter attack latencies and an increase in the probability of obtaining high social status in male domestic fowl [45]. Further studies of the relationship between crowing, behaviour during duels and hormonal status could help elucidate why crowing in a non-social situation not only signals high social status, but also predicts it.

Our experimental design did not allow an explicit and thorough test of the remaining scenario; that behaviour per se leads to a certain social position, because males in our study had already obtained a social position prior to the first personality test. Behavioural responses from personality assays have sometimes proved to be predictive of future social status [4, 13], but that possibility is not mutually exclusive with the two other scenarios presented here, and thus remains to be investigated further.

Of the responses studied here, only the level of boldness was unaffected by changes in social status. Dominant males tended to be bolder than subordinate males in the first trial, but there was no change in boldness when status was changed. Compared to the other behaviours we investigated, boldness therefore appears to be less flexible, in being independent of changes in social status. However, a recent study of sea anemones (Actinia equina [46]) showed that the experience of a fight, and in particular for the losing part, decrease boldness (latency to retain regular activity after being startled). This indicates that the social environment can affect also boldness.

In addition to the sometimes striking effect of social status on behavioural responses in our study, there was always a remaining correlation between the responses of individuals across the two test occasions. This consistency in behaviour across social situations is an expression of individual characteristics that fall under the general heading of animal personality, but the precise nature and general significance of these characteristics is an open question. Studies on other species have shown that heritable components, together with early life experiences (e.g., maternal effects), can partly explain variation and stability of individual behaviour [47, 48]. Less attention has been directed to how intrinsic or
environmental factors later in life may shape personality. An recent experimental study on great tits (*Parus major*) showed that breeding effort is a potential example of a non-genetic cause of personality variation [49], in a broadly similar way as we found here for social positions. Our results emphasises the need for controlling for stability in the social context that is of importance for the species in question (*e.g.*, social status and group composition), and possibly stability also in other contexts or dynamic states, to further improve our understanding of causes and consequences of variation in personality.

**ACKNOWLEDGEMENTS**

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Tables

Table 1. Behavioural responses of dominant and subordinate male fowl in the first trial of a novel arena test (see main text for further details).

<table>
<thead>
<tr>
<th>response</th>
<th>dominant</th>
<th>subordinate</th>
<th>$P_{\text{MCMC}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>boldness</td>
<td>64%</td>
<td>45%</td>
<td>0.060</td>
</tr>
<tr>
<td>vigilance</td>
<td>0.79 ± 0.02</td>
<td>0.55 ± 0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>activity</td>
<td>23.00 ± 2.36</td>
<td>12.81 ± 1.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>exploration</td>
<td>7.26 ± 0.17</td>
<td>6.69 ± 0.24</td>
<td>0.066</td>
</tr>
<tr>
<td>crowing</td>
<td>14.79 ± 1.56</td>
<td>8.55 ± 1.52</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values presented are mean ± SE for all responses except boldness, where the percentages of bold males are shown. $P_{\text{MCMC}}$-values < 0.05 are highlighted in bold.

Table 2. Factors explaining variation in behavioural responses of male fowl in the second trial of a novel arena test (see main text for further details).

<table>
<thead>
<tr>
<th>response trial 2</th>
<th>response trial 1</th>
<th>status trial 1</th>
<th>status trial 2</th>
<th>additional variables included in model (from trial 1)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>boldness</td>
<td>2.11***</td>
<td>-0.45</td>
<td>-0.37</td>
<td>crowing, 0.08*</td>
</tr>
<tr>
<td>vigilance</td>
<td>0.27**</td>
<td>-0.05</td>
<td>0.18***</td>
<td>vigilance, -0.84**; boldness, -0.14</td>
</tr>
<tr>
<td>activity</td>
<td>0.42***</td>
<td>-0.06</td>
<td>0.23**</td>
<td>vigilance, -0.84**; boldness, -0.14</td>
</tr>
<tr>
<td>exploration</td>
<td>0.37**</td>
<td>-11.77</td>
<td>85.38*</td>
<td>boldness, 70.46</td>
</tr>
<tr>
<td>crowing</td>
<td>0.37***</td>
<td>0.33***</td>
<td>0.09</td>
<td>activity, 0.22*; boldness, -0.15</td>
</tr>
</tbody>
</table>

Values presented are posterior means of model parameters in the final MCMCglimm analyses, with effects of status given as dominant-subordinate differences. See main text for the variable transformations that were used in the model fitting. The star symbols indicate statistical significance: ‘*’ $P_{\text{MCMC}}$ < 0.05, ‘**’ $P_{\text{MCMC}}$<0.01, ‘***’ $P_{\text{MCMC}}$<0.001. *Inclusion of certain behavioural responses from trial 1 contributed to a better model fit.
Figure 1. Behavioural response of dominant and subordinate males in the first trial of a novel arena test. Dominant males ('dom', filled dots) (a) tended to be bolder (i.e., more often entered the arena within 30 seconds), (b) were more vigilant (i.e., had higher frequency of time spent being vigilant), (c) were more active (i.e., conducted more sub-area transitions), (d) tended to be more explorative (i.e., visited more sub-areas), and (e) crowed more often (i.e., number of crows) compared to subordinate males ('sub', open dots) (table 1). Values are given as mean ± SE, except for (a), where percentages of males that entered the arena within 30 seconds ± 95% CI, are given.
Figure 2. The effect of current social status, and previous behavioural response (in trial 1) on vigilance in a second novel arena test (trial 2). Males that were dominant (filled dots, solid line) spent more time being vigilant compared to males that were subdominant (open dots, dashed line). At the same time, vigilance was repeatable within individuals (demonstrated by a positive slope, table 2). Social status of males presented in the figure was based on social status obtained in trial 2 (see main text for further details). See S.I. for similar figures for other behaviours (figure S2).
Figure 3. Behavioural response of male fowl of different social status during the first (trial 1) and second test trial (trial 2) in a repeated novel arena test. (a) Males of different status did not differ in boldness (i.e., equal percentage of dominant, filled dots, and subordinate, open dots, males entered the arena within 30 seconds), and stable (solid lines) vs. changed (dashed lines) social status had no significant effect on variation in boldness. Dominant males were more (b) vigilant (frequency of time spent being vigilant), (c) active (i.e., conducted more sub-area transitions) and (d) exploratory (i.e., visited more sub-areas), compared to subordinate males. Males that had a stable status across trials showed more stable behavioural responses compared to males that changed social status, irrespective of their social status. (e) The number of crows uttered in the second trial was significantly affected by previous, but not current, social status. Values presented are mean ± SE, except for (a), where percentages of males that entered the arena within 30 seconds ± 95% CI, are given.
REFERENCES


Paper II
Aggressiveness and exploration predicts social dominance in male domestic fowl (Gallus gallus domesticus).

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ABSTRACT

Individuals in social species commonly form dominance relationships, where dominant individuals enjoy greater access to important resources compared to subordinates. Recent work on animal personality (i.e. consistent variation in individual behavioural responses) show that personality can covary with social status, thus suggest that besides morphological traits, also behavioural variation can play an important role in establishment of status. We investigated whether behavioural responses in non-social contexts could predict the outcome of duels of pairs of morphologically matched pairs of male fowl (*Gallus gallus domesticus*). Male fowl form social hierarchies only partly explained by variation in morphological traits, and with unknown influences of variation in behaviours. Several behaviours covary with social status in male fowl, however it is unclear whether such a relationship is a consequence of the dominance position, or a cause of it. We show that male fowl that more quickly explored a novel arena were more likely to obtain a dominant position. In addition, and independent of exploration propensity, males that were more aggressive at the start of a duel also had greater chances of becoming dominant. Our results therefore show that multiple, uncorrelated behavioural traits, can predict social status.

**Keywords**: behavioural syndromes; intra-sexual selection; social status; social hierarchy; chicken.
INTRODUCTION

In social species, relationships often take form of dominance hierarchies, commonly described as the repeated win of one individual over another (Chase 1980). Ornaments and other morphological features, like body size, typically contribute to the assessment of an antagonist, but do not fully predict social rank (Andersson 1994). Behavioural predictors of dominance have received much less attention, even though dominant and subordinate individuals commonly differ quantitatively in behaviour. For example, aggression often (but not always) shows a positive correlation with social status (Verbeek et al. 1996, Kim and Zuk 2000, McGhee and Travis 2010). Aggression often includes a heritable component, can have a positive effect on reproductive success and shows intra-individual stability (Smith and Blumstein 2008). Behaviours that are repeatable within the individual, but varies among individuals, are also known as personality traits describing an individual's personality type (Sih et al. 2004b). Dominance and aggression are sometimes included in the concept of coping styles, a composite personality classification typically describing individuals by their speed of exploration, boldness, and stress hormone profiles (Koolhaas et al. 1999). Individuals scoring high on these behaviours are said to have a 'proactive' coping style, while those scoring low are defined as having a 'reactive' coping style (Benus et al. 1987, Koolhaas et al. 1999, Schjolden and Winberg 2007). These traits are sometimes positively correlated within individuals (Verbeek et al. 1996, Øverli et al. 2004), but not always (Gomez-Laplaza 2002, Weiss et al. 2007, Fox et al. 2009, Weiss et al. 2009). Individual differences in coping with challenges experienced during behavioural assays may reflect variation in coping also with the challenge of meeting a same-sexed conspecific in a duel over social dominance. To date, there are only a few studies exploring the predictive power of variation in personality types for social status. Some of these studies have used lines selected for variation in proactivity or aggressiveness, and indicate that proactive and aggressive individuals have higher chances of obtaining a high social status (Benus et al. 1990, Pottinger and Carrick 2001). However, studies of the relationship between natural variation in personality and social status are still rare, and the results are inconclusive; either more proactive individuals or more reactive ones may become dominant (Verbeek et al. 1996, Øverli et al. 2004, Korzan et al. 2006, Fox et al. 2009, Dahlbom et al. 2011, David et al. 2011).
We have tested the predictive power of behavioural responses in a non-social context on the establishment of social status in male domestic fowl (*Gallus gallus domesticus*). The fowl is a group-living species that readily forms social hierarchies where individuals obtain clear social roles (Kruijt 1964). Despite the long history of studies on social hierarchies in the fowl, traits determining status are not fully understood. Ornaments (e.g. the comb, a fleshy red ornament on the head), and other morphological features (like body size) often contribute to assessment of the antagonist, but do not fully predict the social status of an individual (Ligon et al. 1990, Zuk and Johnsen 2000, Parker et al. 2002, Cornwallis and Birkhead 2007). Our hypothesis is that there is scope for also behavioural variation to explain variation in establishment of social status in the fowl. We therefore investigated if variation in behaviour in a novel arena test, and aggressive behaviour at the start of a duel, could predict the outcome of duels of morphologically matched males. Our expectation is that more proactive or aggressive males have a higher chance of becoming dominant.

**METHODS**

*Study population and housing*

The study took place during the breeding seasons (June - August) of 2011 and 2012 at Tovetorp Research station, Stockholm University, Sweden. The population of an old Swedish game breed of fowl (‘Gammalsvensk dvärghöna’, *Gallus gallus domesticus*) is kept in several mixed-sex, mixed-age (1-10 years) groups under semi-natural conditions, and are used to human handling. All males were marked with numbered leg rings to facilitate identification. In order to standardise the social position of experimental males, single males were housed together with one female in outdoor aviaries (3 x 3 meters) for 4-5 days before the first behavioural test. Aviaries were visually, but not vocally, isolated from other birds. This set-up resulted in that all males behaved like dominant birds prior to observations (Parker and Ligon 2002). The experiments followed ethical requirements for animal experimentation in Sweden (Linköping Ethical committee, ethical permission number 60-10).

*Personality assays*

To investigate variation in activity and explorative behaviour among males, 50 males were tested singly in a Novel Arena test (NA). NA is a commonly used personality test to measure
exploration and activity, and the fowl show repeatability in both traits in similar tests (Favati et al., unpublished (paper I)). A large oval shaped arena of a 10 x 7 m fenced area in a deciduous forest was divided into 36 even-sized subareas by a grid of thin wooden sticks. The male was placed in a cage of chicken wire connected to the arena by a wire door. The door to the start cage was immediately opened, after which the behaviour of the male was recorded for 20 minutes. The occurrence of ‘activity’ (i.e. movement of the legs of the bird, either by walking or scratching) was recorded every 15s. The number of subareas visited at least once by a male (between 1-36) was used as a measure of ‘area use’, and the latency to visit at least 5 subareas was used as measures of ‘exploration’ propensity. The limit of five subareas was chosen in order to obtain a continuously distributed variable, and a majority of the males visited five subareas within the time limit of the experiment. The observer sat outside the arena, visible to the male. The number of territorial crows uttered was also recorded (‘crowing’), as was the proportion of time a male spent being vigilant (‘vigilance’, head above shoulder height, recorded every 15s.). At the end of the NA, a recorded alarm call of a conspecific was played-back using an iPhone connected to a portable loudspeaker placed next to the arena (Philips Mighty Mini). Alarm calls are loud cackling vocalisations typically uttered in response to terrestrial predators, and which flock mates react on by vigilance (Evans et al. 1993). The latency until the male retained normal activities after the call was played, thus stopped being vigilant and resumed feeding after the startle, was used as a measure of how alert and wary the male was (‘alertness’). Males that did not resume feeding within 10 minutes received the maximum score of 10 minutes.

Aggressiveness and social status

To investigate whether variation in aggressiveness affects establishment of social dominance in the domestic fowl, aggressiveness was estimated during dyadic interactions not more than one day after the personality assays. Pairs of males \( n_{\text{pairs}} = 25 \) were simultaneously placed one meter from each other in an outdoor aviary (2 x 3 m) where none of the males had previously been housed. The males of each pair were matched for comb size and body size in order to minimise the influence of these traits on the establishment of social status (<10% difference). Body size and comb size were estimated by tarsus length and body weight, and comb length, respectively. Males within a pair had not been housed together for at least 2 weeks prior to trials, reducing any effects earlier encounters may have had on the
establishment of social status (McBride 1958, Cloutier et al. 1995). The duels were filmed using a digital camcorder (Sony DCR-VX1000E). The agonistic interaction between males started spontaneously when the two males of a pair were placed in front of each other. The intensity of fowl fights typically ranges from aggressive displays (raised hackles, dropped wing and ‘waltzing’ movements (Collias 1943, Kruijt 1964)) and surrender of one male without any physical attacks, to repeated attacks with the feet or the beak until one male retreats. The initial behaviour (aggressiveness) of each male was classified along a four grade scale as either 1: ‘surrendering’ (fleeing away from the other male), 2: ‘non-aggressive’ (straight body posture, no avoidance or attraction to the other male), 3: ‘aggressive’ (crouched body posture and dropped wing or raised hackles (Collias 1943)) or 4: ‘very aggressive’ (directly – within 2 seconds – approaching the other male). All duels ended with one of the opponents retreating and no longer retaliating further attacks from the opponent. We used a minimum of five occasions of avoidance within an hour by the same male to define a male as subordinate, and the other male as dominant. Overt aggression lasted not longer than a few minutes, and there were no duels involving injuries other than minor bleeding from the comb.

Statistical analyses

To investigate whether behaviour in the novel arena test could predict the outcome of the duels, we compared a generalized linear model with binary response containing all possible explanatory variables (activity, area use, exploration, crowing, vigilance, alertness) with a null model containing no explanatory variables (only the intercept), using log-likelihood $\chi^2$-statistics (deviance). To obtain only one measure per pair, we used the difference in response between the two males of each pair, and randomly altered between taking the dominant score minus the subordinate score and vice versa. The outcome of the duels (dominant or subordinate) was used as response variable and at the level of the pair (n = 25). To determine a final model, we used bidirectional stepwise model search, choosing the model with the lowest AIC using generalized linear modelling (Venables and Ripley 2002). The fit of the final model was tested using the log-likelihood $\chi^2$-statistic for the final model compared to the null model. To evaluate the predictive power of the model we used the Nagelkerke $r^2$ (Nagelkerke 1991). The strength of the models was further examined by computing classification tables using the data the models were built on. The cut-off value to
classify each males' social status was set to 0.5 based on the equal amount of dominant and subordinate males (Elliott and Woodward 2007). The same approach as described above was used in order to analyse whether behaviour in NA could predict aggressiveness during the first seconds of the duel, this time with aggressiveness as a binary response variable (0 = non-aggressive, score 1-2; 1 = aggressive, score 3-4). Whether aggressiveness could predict social dominance was analysed by a logistic regression with aggressiveness as explanatory factor and social dominance as response variable. Finally, pairwise correlations using Pearson’s product moment correlation were performed in order to investigate the relationship between all behavioural responses measured.

In order to achieve normally distributed variables, the transformation $\sqrt{x}$ was used for area use and crowing, log($x$) for exploration, and $x^{0.25}$ for alertness, based on visual inspection of histograms. These transformations were used for all analyses. R version 2.15.1 was used for all statistic analyses (R Development Core Team 2012).

RESULTS

A full model containing all behavioural responses of the novel arena test showed that the responses could forecast the social dominance in upcoming duels ($X^2 = 12.573, df = 6, p = 0.050$). We therefore proceeded with model selection which resulted in the following final model: social status $\sim$ exploration + vigilance + crowing. This model significantly predicted social status ($X^2 = 11.662, df = 3, p = 0.0086, r^2 = 0.50$), and the only significant term was exploration; males that more quickly explored the arena more often won the following duel (exploration, $X^2 = 5.50, df = 1, p = 0.019$; vigilance, $X^2 = 2.43, df = 1, p = 0.12$; crowing, $X^2 = 3.16, df = 1, p = 0.075$, Fig. 1). This model accurately classified the males in 84% of the cases (Table 2). Behaviour in NA could not predict aggressiveness at the start of the duel ($X^2 = 7.61, df = 6, p = 0.27$), and this was confirmed by pairwise correlations: no behaviour in NA had a significant correlation with aggressiveness (Table 1). However, aggressiveness predicted social dominance: more aggressive males had increased chances of becoming dominant ($X^2 = 8.21, df = 1, p = 0.0042, r^2 = 0.37$, Fig. 2). The logistic model of aggression correctly classified the status of males in 80% of the cases (Table 2).
DISCUSSION

Socially dominant individuals commonly enjoy increased access to resources, such as mating partners, which typically results in a positive relationship between social status and reproductive success and thereby individual fitness (Andersson 1994). We here showed that the behavioural type affected the chances of obtaining a high social status for individuals of the same apparent physical quality.

No strong correlations were observed between behavioural responses observed in the novel arena test, when exposed to a startle and during duels, other than the one between activity and exploration. In other words, we found no support for an overall behavioural syndrome categorising individuals along a proactive-reactive continuum, which is sometimes found in other species (Sih et al. 2004a). Therefore, we discuss each behavioural trait separately.

Dominant male fowl differ quantitatively from subordinate males in their behaviour, for example they crow more (Favati et al., Leonard and Horn 1995, Johnsen et al. 2001). Crowing is an auditory display, which signals dominance and territoriality, and dominant males often interfere with subordinate males that attempts to crow (Collias et al. 1964, Leonard and Horn 1995). Dominant males are also more vigilant (Pizzari 2003, Cornwallis and Birkhead 2008), perhaps due to mate guarding (Artiss and Martin 1995). However, we found no support of pre-existing differences between future dominant and subordinate individuals in neither how much a male crowed, nor how much time he spent being vigilant in NA. The differences observed in other studies (Favati et al., Leonard and Horn 1995, Johnsen et al. 2001, Pizzari 2003, Cornwallis and Birkhead 2008) therefore primarily seem to be an effect of the current social position rather than pre-existing differences in behavioural type (Favati et al., Cornwallis and Birkhead 2008). Among all behavioural traits observed in the novel arena test, including exposure to a startle, only exploration seemed to have any substantial relationship to future social status, as more explorative males more often became dominant. Exploration was strongly correlated to activity and the number of subareas visited, and the results suggest that this vigorousness brought advantages to the duel situation, although not through a direct relationship with aggressiveness. This adds to the general pattern of more proactive individuals being found in dominant positions, although only a few have investigated the relationship between exploration and social

That more aggressive individuals win more fights has been demonstrated in earlier studies of various species (e.g. (Verbeek et al. 1996)) including the domestic fowl, where individuals that initiate the first attack more often also win the fight (females, Ligon et al. 1990, males, Cloutier and Newberry 2000). Aggression is however sometimes considered a property of the interaction between individuals, in which case the individual aggressive response depends on the actions of the opponent (Francis 1988). For instance, whether an attack is initiated or not is not a purely individual decision, but also depends on the actions of the opponent. Alternatively, as adopted in the current study, one can attempt to estimate individual aggressiveness and distinguish it from social dominance. Thus, individual aggressiveness can be estimated by the very initial reaction of an individual when meeting an opponent. By basing our measure of aggressiveness on how the males react to the fighting situation during the very first seconds of the interaction, it seems unlikely that an evaluation of the opponent already has taken place. Using this approach, we found that an initial aggressive position forecasts the outcome of the duel in male fowl, which has previously been shown only in female fowl (Foreman and Allee 1959, Wilson 1974).

When males differed in aggressiveness within a pair, the male with higher aggressiveness scores almost always won the fight (Figure 2). However, in around half the cases both males initially adopted an aggressive body posture (i.e. received the same aggressiveness score), which decreases the strength of the overall predictability. Despite this, aggressiveness still appeared as a useful predictor of status, as it was used to correctly predict the outcome in 80 % of the duels. Explorative behaviour comes forth as an equally strong predictor of status (Table 2). Overall, our results show that when combatants were matched for morphological characters, behavioural traits did play an important role in the establishment of social status. We targeted aggressiveness and exploration as the prior attributes, or personality traits, most useful for prediction of a dominant social position. Over the last decade, reports on fitness effects of behavioural types have accumulated; not least studies showing that personality affects reproductive success (Dingemanse and Reale 2005, Smith and Blumstein 2008). Since dominance is important for sexual success in male fowl (McBride et al. 1969,
Collias and Collias 1996, Pizzari and Birkhead 2001), our results suggest that variation in personalities in male fowl have fitness consequences via the establishment of social status.

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Table 1. The relationship between behavioural responses in behavioural assays and initial aggressiveness in duels, of male fowl.

<table>
<thead>
<tr>
<th>Exploration</th>
<th>Area use</th>
<th>Activity</th>
<th>Crowing</th>
<th>Vigilance</th>
<th>Alertness</th>
<th>Aggressiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exploration</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area use</td>
<td>-0.70*</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>-0.51*</td>
<td>0.78*</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crowing</td>
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<td>0.14</td>
<td>-0.073</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vigilance</td>
<td>-0.34</td>
<td>0.25</td>
<td>0.10</td>
<td>0.24</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Alertness</td>
<td>-0.153</td>
<td>-0.14</td>
<td>-0.089</td>
<td>0.018</td>
<td>0.381</td>
<td>-</td>
</tr>
<tr>
<td>Aggressiveness</td>
<td>-0.038</td>
<td>-0.048</td>
<td>-0.021</td>
<td>-0.43</td>
<td>0.016</td>
<td>-0.055</td>
</tr>
</tbody>
</table>

Pearson's correlation coefficients (r) are shown. * denotes correlations that remained significant at the \( p < 0.05 \) level after sequential Bon Ferroni correction.
Table 2. Classification table comparing observed dominance status with dominance status predicted by final logistic models.

<table>
<thead>
<tr>
<th></th>
<th>Observed</th>
<th></th>
<th>Predicted</th>
<th></th>
<th>Percentage correct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub</td>
<td>Dom</td>
<td>Sub</td>
<td>Dom</td>
<td></td>
</tr>
<tr>
<td>Novel arena</td>
<td>Sub</td>
<td>10</td>
<td>2</td>
<td></td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Dom</td>
<td>2</td>
<td>11</td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>total</strong></td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Aggressiveness</td>
<td>Sub</td>
<td>7</td>
<td>5</td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Dom</td>
<td>0</td>
<td>13</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>total</strong></td>
<td>0.80</td>
<td></td>
</tr>
</tbody>
</table>

Dominance status of males (subordinate, 'Sub', and dominant, 'Dom') was successfully classified by the final logistic regression model of behavioural responses in the novel arena test in 84% of the cases, and in 80% of the cases by the logistic model of aggressiveness during the start of the duel (in bold).
Figure 1. Differences in behavioural response in behavioural assays between future dominant and subordinate male fowl. Males that in a later duel became dominant (filled dots) (a) were more explorative but (b) were not more active, (c) did not have a larger area use, (d) did not crow more, and (e) were not more vigilant or (f) more alert compared to subordinate males (open dots). Mean values ± SE are given. * = p < 0.05.
Figure 2. The distribution of male fowl duels with respect to the difference in aggressiveness between the contestants. The winning, dominant male either had a higher aggressiveness score compared to the losing, subordinate part (Dom>Sub, black column), a lower score (Sub<Dom, white column) or the same aggressiveness score (Dom=Sub, grey column). When threat score differed between the two males of a pair, the male with the higher aggressiveness score more often won (12 cases vs 1 case).
REFERENCES


