Citation:

Nelson XJ, Wilson DR, Evans CS (2008) Behavioral syndromes in stable social groups: an artifact of external constraints? *Ethology*, 114: 1154–1165. doi: 10.1111/j.1439-0310.2008.01568.x

Behavioral syndromes in stable social groups: an artifact of external constraints?

Running title: Social hierarchy and behavioral syndromes

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Words: 6039

Abstract

Individuals of many species differ consistently in their behavioral reactions toward different stimuli, such as predators, rivals, and potential mates. These typical reactions, described as ‘behavioral syndromes’ or ‘personalities’, appear to be heritable and therefore subject to selection. We studied behavioral syndromes in 36 male fowl living in 12 social groups and found that individuals behaved consistently over time. Furthermore, responses to different contexts (anti-predator, foraging, and territorial) were inter-correlated, suggesting that males exhibited comparable behavioral traits in these functionally distinct situations. We subsequently isolated the same roosters and conducted tests in a ‘virtual environment’, using high-resolution digital video sequences to simulate the anti-predator, foraging, and territorial contexts that they had experienced outdoors. Under these controlled conditions, repeatability persisted but individual responses to the three classes of stimuli failed to predict one another. These were instead context-specific. In particular, production of each type of vocal signal was independent, implying that calls in the repertoire are controlled by distinct mechanisms. Our results show that extrinsic factors, such as social position, can be responsible for the appearance of traits that could readily be mistaken for the product of endogenous characters.

Introduction

Animals are typically faced with the challenge of acquiring resources, such as mates or food, while minimizing associated costs, such as exposure to predators or rivals (Lima & Dill 1990). An individual's assessment of this trade-off can be captured using behavioral axes, the most frequently used of which is the shy-bold continuum (Wilson et al. 1994; Wilson 1998; López et al. 2005). Boldness is often thought to span across functional contexts, such that an individual's boldness rank in the population will be similar in situations as distinct as foraging, mating, anti-predator behavior, and the challenge of novel situations (functional behavioral categories, *sensu* Sih et al. 2003, 2004a,b; Johnson & Sih 2005; López et al. 2005). These typical reactions, described as behavioral syndromes, temperaments, personalities or coping strategies (Boissy 1995; van Oers et al. 2005a; Bell 2007), appear to have a genetic basis (van Oers et al. 2004, 2005a) and should therefore be subject to selection. Central to this model is the idea that behavior should be consistent over time, or repeatable (Dall et al. 2004).

One of the implications of behavioral syndromes is that behavioral responses may not be fully flexible across different contexts. This model is thus a powerful hypothesis for explaining apparently 'suboptimal', or maladaptive behavior (Bell 2007), such as boldness when foraging in the presence of predators. This does not mean that expression of a particular trait need be absolutely constant across contexts, just that the relative levels (for example, of boldness) of individuals in the population will remain consistent with one another (Sih et al. 2003; Johnson & Sih 2005). An important implication of such constrained responses is that they might reflect heritable variation, such that correlated traits are selected together, thus informing our thinking regarding the evolution of behavior.

Another view, suggested by Wilson et al. (1994), is that individual differences, while repeatable, may have to be context-dependant because any challenge that influences survival and reproduction will require an adaptive response. Context-sensitivity occurs when an individual’s relative expression level of a given behavioral trait changes depending on the context. Such a pattern implies that behavioral traits may be free to evolve independently (Wilson 1998; Réale et al. 2000). Context-specific individual differences are predicted by frequency-dependent selection models, and there is considerable evidence, from a wide range of taxa, that responses can be expressed in this way (e.g., fish; Coleman & Wilson 1998, mammals; Réale et al. 2000, cephalopods; Sinn & Moltschaniwskyj 2005 and birds; van Oers et al. 2005b).

At a phenotypic level, bold behavior is influenced by many factors, including hormone levels (reviewed in Boissy 1995), food availability (Martín et al. 2003), predation risk (Martín & López 1999; López et al. 2005), age (Brodie 1993; Sinn & Moltschaniwskyj 2005), sex (reviewed in Boissy 1995), experience (Frost et al. 2007) and environmental conditions (Brodie & Russell 1999). Social factors clearly exert a pervasive influence on human behavior and the study of such phenomena has a long history (e.g., Mischel 1968). Analogous studies of animals have increased markedly in recent years (reviewed by Galef & Giraldeau 2001). Evidence that social constraints interact with individual characteristics is now permeating the field of behavioural syndromes (Dall et al. 2004, Sih & Watters 2005). For example, the exploratory behavior of great tits is related to risk-taking behavior (van Oers et al. 2004) and dominance within an experimental dyad (Dingemanse & de Goede 2004). Similarly, the dispersal rates of lizards are affected by ‘sociability’, or social personality (Cote & Clobert 2007) and exploratory behavior has been related to subsequent social rank in great tits (Verbeek et al. 1999).

However, the stability of individual differences across a variety of contexts has not previously been studied in hierarchy-forming social animals. In addition, despite the central role of communication in social behavior of all kinds, hypotheses concerning animal personalities have yet to integrate signaling as a trait.

Fowl, *Gallus gallus*, are ideal for investigating the relationship between behavioural syndromes, particularly as reflected by signaling behavior, and social position. They live in hierarchical, mixed-sex, social groups that are relatively stable throughout the year (Collias & Collias 1967, 1996). Food calls and alarm calls of fowl are referential signals (Evans, 1997) produced in response to specific stimuli (discovery of food; approach of a particular type of predator) and sufficient to evoke appropriate reactions from conspecifics, even without other cues. Recent work suggests that referential signaling may be relatively widespread (e.g., tufted capuchins; Di Bietti 2003, lemurs, Macedonia 1990, suricates; Manser 2001; Manser et al. 2001, ravens; Bugnyar et al. 2001). We now have quite a detailed understanding of the cognitive mechanisms underpinning some of these systems, but much less is known about how they evolved.

In the present study, we explored the effect of social factors on the expression levels of several behavioral traits by contrasting behavior in a semi-natural environment with that in a highly controlled ‘virtual environment’. Our particular interest is in communication, an aspect of behavior that has been relatively neglected in previous work in this area. Here we focused on calls associated with three functionally distinct contexts (foraging, anti-predator, and territorial) and measured individual variation in production under both naturalistic and controlled conditions, assessing whether there are phenotypic correlations in signaling behavior. Specifically, we measured the propensity of roosters to crow (a territorial call produced exclusively by males; Collias & Collias 1967), aerial alarm call (a signal that warns group members of approaching avian predators, produced by males when they have a conspecific audience; Evans et al. 1993a) and food call (a signal produced predominantly by males, advertising the discovery of food to females; Evans & Marler 1994; Evans & Evans 1999). These three vocalizations allowed us to test whether a rooster's vocal behavior was correlated across contexts.

After observing focal males in naturalistic groups housed in large aviaries, we isolated them for one month to remove social constraints (Parker & Ligon 2002) and then re-tested them individually, using high-definition digital video stimuli simulating the three contexts. If expression of behavioral traits is socially constrained by the dominance hierarchy, then such an effect should be clearly apparent in comparisons between these two settings. Our objectives were to assess: 1) the repeatability of male behavior under semi-natural and controlled conditions, 2) whether signaling behavior across contexts is intercorrelated or whether it is expressed in a context-specific manner, and 3) the effect of social factors.

Methods

Outdoor observations

Subjects were 36 male and 36 female golden Sebright bantam chickens (*Gallus gallus domesticus*), a strain behaviorally similar to the ancestral red jungle fowl (Evans & Marler 1995). Individuals were divided into 12 groups of 3 males and 3 females each, a composition matching that described for red jungle fowl in the wild (Collias & Collias 1967), and observed under semi-natural conditions during the 2005/2006 and 2006/2007 breeding seasons. Groups were formed by simultaneously releasing the 6 birds into 1 of 4 large (10 m x 20 m) outdoor aviaries. We observed three cohorts of four groups each. Observation times were standardized within each aviary for a given cohort to control diel variation in behaviour, but randomized across cohorts.

Aviaries provided birds with an unobstructed view of their surrounding environment. Each contained a coop fitted with perches for roosting, *ad libitum* food and water, areas of fresh wood mulch to encourage foraging, native plants for cover, and an awning affording shelter from the sun (see electronic supplementary material, Figure 1). Overt aggression between males was invariably brief (less than 3 min) and terminated when one bird signaled subordinate status by turning away. Birds were given 1 week prior to observation to establish stable social structure, acclimate to the surroundings, and habituate to humans. All groups formed linear hierarchies comprising an alpha, beta, and gamma male.

We observed each group for 40 min per day over a 12-day period. Observations used continuous recording of a focal animal and were conducted between 0705 and 1005 h or between 1620 and 1920 h, to correspond with the periods of greatest activity. During each group's daily session, 2 of the 3 males were observed simultaneously. Selection of focal males and assignment of observer (XJN or DRW) were both randomized. Observers sat on either side of one end of the aviary, and scored behavior using JWatcher™ 1.0 (Blumstein et al. 2000) on a Macintosh laptop computer. We recorded the number of crows, representing a territorial context, aerial alarm calls, representing an antipredator context, and food calling bouts, representing a foraging context. Vocalizations separated by more than 5 s were scored as separate bouts. Each male was observed on 8 occasions (total 320 min).

Data were analyzed in SPSS v. 11 (SPPS Inc). For each behavior, we assessed repeatability by examining intra-individual correlations (Spearman's Rho, 2-tailed) across the 8 days of observation and summarized repeatability using Cronbach's alpha estimate of reliability (Cronbach 1951) across the 8 days of observation. This approach was adopted because our data were not normally distributed, precluding the use of parametric statistics traditionally used to assess repeatability (Lessells & Boag 1987). We then calculated individual average call rates and tested for correlations among them using $\log_{10}+0.5$ transformed data.

Laboratory experiment

Housing

Indoor housing and feeding conditions were as in Evans and Evans (1999). Briefly, roosters were housed singly in metal cages (l x w x h: 1.0 x 1.0 x 0.5 m) fitted with wooden perches, with a deep layer of bedding material (shredded paper) on the floor to facilitate the expression of natural behavior. Food and water were continuously available. At the end of the laboratory experiment, the birds were returned to outdoor aviaries.

Context tests

We isolated males for 1 month to remove social constraints (Parker & Ligon 2002) and then tested them in a 'virtual environment', using high-definition digital video stimuli to reproduce each of the three functional contexts observed outside. We adopted a repeated-measures randomized-block design, in which each male experienced the contexts in a random order at 24 h intervals. Males were given a second trial for all contexts exactly 1 week after the first, making a total of 6 trials per male.

Each trial began with 5 min in which we measured baseline behavior. In the anti-predator and foraging contexts, we presented a high-definition video female after 1 min because food calling and aerial alarm calling both have pronounced audience effects (Marler et al. 1986; Evans & Marler 1991, 1992, 1994). In the territorial context, no audience was necessary, so the test male was shown a video of an empty cage.

Stimuli were presented at the end of the baseline period. A computer-generated soaring hawk (4 s) simulated the antipredator context (see Evans et al. 1993a; electronic supplementary material, Figure 2). Live mealworms delivered from a concealed hopper provided the foraging context. To evoke territorial behavior we presented 5 min of high-definition video showing a rival male engaged in aggressive behavior.

Test males had 2 min following stimulus presentation. In the anti-predator and foraging contexts, the audience hen remained present, while in the territorial context the stimulus male disappeared, leaving the empty cage on the screen (see below for details). For the foraging and anti-predator contexts 'before' was defined as the 4 min in which an audience hen was present prior to stimulus onset. In the foraging context 'during' was defined as the 2 min following stimulus onset and in the anti-predator context 'during' was defined as the 30 s time bin beginning with the stimulus onset. For the territorial context, 'before' was defined as the 5 min of empty cage preceding stimulus onset and 'during' as the 5 min in which the stimulus male was present on the screen. To avoid habituation, test males experienced a different audience hen, hawk, and male opponent in each trial.

The only behaviors common to all three contexts were crowing and walking. In addition to these spontaneous behaviors, we scored specific responses evoked by the stimuli. In the anti-predator context, we measured level of crouching on a scale from 0 to 5 (see Evans et al. 1993a for details) and number of aerial alarm calls. In the foraging context, we scored total time tidbitting (a stereotyped head and neck movement associated with food calling; Evans & Evans 1999), number of food call pulses, latency to begin food calling and latency to tidbit. To count food calls, we digitized test session videorecordings using a Canopus ADVC-110 (sound track 44.1 kHz, 16 bits), generated a normalized oscillogram with Peak Pro 5.2 (Bias Inc.) and then scrolled through the sound waveform while simultaneously watching a time-locked image of the corresponding video frame. In the territorial context, we measured crow rate, latency to crow and latency to aggressive display (defined as head bobbing with hackle feathers raised), as well as total time spent engaged in aggressive behavior.

Behavior was scored using JWatcher™ 1.0 (Blumstein et al. 2000) for each time period (before and during) and then converted to rates to facilitate comparisons across periods of unequal duration. Data were tested for normality using Kolmogorov-Smirnov tests and analyzed in SPSS v.11. We used ANOVA to test whether previous rank in outdoor social groups affected behavior in individual laboratory tests. To determine repeatability in the laboratory setting we used Lessells & Boag's (1987) equation on baseline behavior (locomotion and crowing) across all 6 trials. We also used Spearman correlations to examine the consistency of stimulus-specific behavior across stimulus presentations. Response variables for each context were subsequently simplified using PCA, the factors from which were used in correlation analyses comparing behavior between functional contexts and between the laboratory and outdoor settings. We used an alpha level of 0.05 throughout.

Playback stimuli

Our experimental design required high-quality audio and video recordings of fowl engaged in natural behavior. Footage of hens was used to provide a 'virtual' audience to potentiate male calling in the foraging and anti-predator contexts, while that of males was used to provide a simulated opponent in the territorial context. This approach allowed uniquely sensitive tests for predictive relations between different types of signaling behavior by controlling variation in audience behavior.

Four females (one for each of two trials in the foraging and anti-predator contexts) and two males (one for each trial in the territorial context) were recorded in a sound-attenuating chamber (see Evans & Evans 1999 for details) for approximately one hour apiece. Roosters were confined within a wire cage (l x w x h: 0.6 x 0.5 x 0.8 m) with a glass front and a wood floor covered by an artificial grass mat. Territorial behavior (threat posture with raised hackles and crowing) was elicited by the male's own reflection in the glass. This had the advantage of being frontally-directed, so that during playback the male was apparently confronting the test male. Females were recorded in the same cage as males. Since we wished to obtain relaxed behavior (standing with occasional contact calls), we substituted a front panel of black-painted open wire mesh. The cage was lit by two broad-spectrum incandescent lamps (Aspherics®, model DLH4) placed 1.5 m apart and 1.0 m from the front.

We used a Sony HDV high-definition 3-CCD camcorder (model HVR-Z1E) mounted on a tripod at bird eye-height. Focal length was adjusted so that the image appeared life-sized on the plasma screen subsequently used for playback. To avoid motor noise, we used a Sennheiser microphone (model MKH 40) placed away from the camcorder, 0.3 m to one side of the cage. The frequency response of this system was flat (±1 dB) over the avian hearing range. High-definition digital video is a new standard which provides substantially improved resolution compared with previous studies (1080 lines; c.f. 576 lines for PAL DV and 240 lines for VHS). Spatial detail, including the facial features known to mediate individual recognition in fowl (Guhl & Ortman 1953) was hence much better reproduced than has been possible in the past.

Audio and video signals were transferred digitally using an IEE1394 'firewire' interface and edited with Final Cut Pro (version 4.5) on a Macintosh computer. In assembling playback sequences, we minimized artifacts by applying a 4-frame cross-dissolve transition between successive clips. Similarly, we used a 1 s cross-dissolve to avoid a startle response when the image of a conspecific replaced that of an empty cage, and again at the end of the stimulus sequence. Completed high-definition sequences were exported to tape for playback.

Male stimuli were edited such that the simulated opponent was engaged in non-aggressive behavior for 2 min, followed by 3 min of escalating aggression. These sequences were comprised of 5 min of footage of the male's empty cage, followed by a 5 min sequence of the stimulus male and 2 min of empty cage, for a total duration of 12 min.

Audience hen sequences were designed so that hens were walking about and apparently peering toward the subject male. These comprised 1 min of the empty cage, followed by a 6 min sequence in which the hen was present, to create a total duration of 7 min.

In the foraging context, the stimulus was four mealworms. These were delivered by a remote-controlled hopper (see Marler et al. 1986 for details) 4 min following the appearance of the audience hen. The hen then remained on screen for a further 2 min, ensuring that males experienced food under conditions known to potentiate calling and tidbitting (Evans & Marler 1994).

Predator trials were identical to foraging trials except that the stimulus was a computer-generated raptor presented on a monitor (Lowe, model 8672 2P, 100 Hz refresh rate) mounted overhead (see Evans et al. 1993a for details). This consisted of a black silhouette that made four alternating passes across a white background at a speed of 8.8 body lengths per second. Four different versions of the stimulus were used in random order to minimize subject habituation. Stimuli subtended 4.5° at the subject’s eye (see Evans et al. 1993a for details) and varied with respect to the corner of the monitor from which they originated (top left, bottom left, top right, bottom right).

Video Playbacks

We tested three cohorts of 12 males each between October and December 2006. To minimize diel variation in responsiveness, each male experienced all three simulated contexts at the same time of day, between 0730 – 1230. Playbacks were conducted in a sound chamber (see Evans & Evans 1999 for details). Males were placed into a wire cage (l x w x h: 1.1 x 0.5 x 0.8 m) with a wood floor covered by an artificial grass mat. As in numerous previous studies (e.g., Evans & Marler 1991, 1992, 1994; Evans et al. 1993a,b), trials began once the subject bird recovered from handling and resumed normal relaxed behavior such as preening, walking and crowing. The cage was lit by two incandescent lights placed 1.6 m apart and 1.0 m in front. Responses were recorded onto VHS tape using a Panasonic video camera (model WV-CL320) and a Sennheiser microphone (model MKH 40) connected to a Panasonic videocassette recorder (model AG-7750).

High-definition sequences of audience hens and stimulus males were played directly from a Sony HDV 1080i tape deck to a Sony flat panel plasma display (model PFM - 42X1; 105.8 cm measured diagonally), which was placed facing the subject's cage 30 cm from one end. We selected this distance based upon characteristics of the fowl visual system (Dawkins & Woodington 1997); it allowed males to approach as they would a conspecific, but not close enough to resolve individual pixels, which would likely have compromised the realism of the simulation. Like the TFT panels used in recent successful mate-choice experiments with quail (*Coturnix japonica*; Ophir and Galef 2003), plasma displays offer clear advantages over the CRT monitors used in the first video playback experiments with birds (Evans & Marler 1991). Principal among these is the relative absence of flicker, as only the pixels that change from one video field to the next are selectively refreshed. The video soundtrack was broadcast from a Nagra DSM loudspeaker-monitor concealed beneath the centre of the display. Hawk animations were controlled by Final Cut Pro 3, running on a Macintosh G3 computer, and were converted to an analogue signal using a Canopus ADVC110.

Results

Outdoor observations

Rooster behavior was repeatable across the 8 days of observations, especially for aerial alarm calling ($\alpha = 0.878$) and crowing ($\alpha = 0.829$) (Table 1). Food calling was also repeatable ($\alpha = 0.611$), although appreciably less than the other two vocalizations (Table 2).

Rooster behavior also correlated across contexts (Figure 1a-c). Aerial alarm calling was strongly correlated with both crowing (Pearson correlation: $r = 0.706$, $P < 0.0001$) and food calling ($r = 0.460$, $P = 0.005$), though the latter were not correlated with each other ($r = 0.232$, $P = 0.173$).

Laboratory studies

Roosters responded to 96.3% of stimulus presentations and in all cases exhibited behavior typical of that toward a real (non-video) stimulus. Our virtual environment was hence successful in simulating natural events.

Baseline behavior consisted primarily of crowing and walking. The repeatability of these behaviors was high (crowing = 0.866, $F_{1,215}$ = 6.593; walking = 0.930, $F_{1,215}$ = 3.730). Previously held rank (in the outdoor groups) had no effect on the food call rate in the food context (one way ANOVA: $F_{1,35}$ = 0.497, p = 0.613), the aerial alarm call rate in the anti-predator context ($F_{1,35}$ = 0.081, p = 0.922), or crowing rate in the territorial context ($F_{1,35}$ = 1.341, p = 0.276). However, Spearman correlations for stimulus-specific behaviors during presentations were poor (crowing, r = 0.169, p = 0.33; alarm calling, r = -0.225, p = 0.19), with the exception of food calling (r = 0.379, p = 0.02).

We ran PCA on the stimulus-specific behaviors evoked in each context to reduce the data to a single factor (Table 3). Factors were then compared with each other and with their equivalent behavior from the outdoor observations to evaluate the consistency of behavior across contexts in a social and a non-social situation.

Remarkably, behavior in each functional context had no predictive utility for that in any of the others (Fig. 1d-f). This finding is in striking contrast with the patterns apparent when the males were in social groups.

Comparisons between equivalent behavior in groups and in individual tests similarly revealed no significant correlations. There was no relation between the territorial factor and crow rate (Fig. 2a), between the foraging factor and food calling (Figure 2b), or between the anti-predator factor and aerial alarm calling (Figure 2c).

Finally, we compared vocalization rates in the indoor and outdoor settings directly. There were no correlations between the indoor and outdoor settings for crowing (Pearson correlation: r = -0.016, P = 0.925, Fig. 2d), food calling (r = 0.079, P = 0.649, Fig. 2e) and aerial alarm calling (r = 0.160, P = 0.352, Fig. 2f).

Discussion

Our results suggest that signaling of male fowl is not inter-correlated between functional contexts, but is instead context-specific. In the outdoor groups, calling rates had predictive utility for other contexts, but this relationship vanished when we assessed the same males under controlled conditions in the absence of a dominance hierarchy. Furthermore, comparisons of individual propensity to express territorial, anti-predator and food-related behavior in the outdoor and laboratory settings failed to reveal any correlations. Taken together, these results suggest that vocal behavior apparent under naturalistic conditions likely reflects the operation of social constraints, rather than that of endogenous factors.

In laboratory tests, we found that individual behavior prior to stimulus presentations was highly repeatable, demonstrating that behavioral consistency was detectable when present, although this broke down during stimulus presentation, possibly due to undersampling, as the duration of these events was necessarily brief. In addition, biologically relevant responses were evoked in almost every trial, verifying the effectiveness of video stimuli for simulating both social companions and predators in this system (Evans & Marler 1991; Evans et al. 1993b; c.f. D’Eath 1998). Nevertheless, we were unable to detect any relation between signaling and associated behavior across different functional contexts. This pattern was in complete contrast with that obtained when males were in social groups.

We conclude that our results provide support for the cautionary notion that "even highly reversible 'states' can appear as 'traits' in environments that reinforce individual differences" (Wilson et al. 1994). In this case, the crucial environmental factor was likely dominance, which has long been known to have pervasive effects on the behavior of fowl (Schjelderup-Ebbe 1935; Bayly et al. 2006).

As originally conceived, the term behavioral syndrome referred simply to a suite of correlated behaviors, either within a context, or between contexts (Sih et al. 2004a). Despite this, current usage often implicitly assumes the broader meaning of between contexts, and hence is regularly referred to as 'personality' (e.g., Dall 2004; Dall et al. 2004). In the latter sense, behavior in one context can have the power to predict that exhibited in another, thus providing a mechanism to explain why responses can sometimes appear suboptimal (see Introduction). The exciting evolutionary implications that have stimulated much recent work explicitly require that the constraints responsible for between-context predictive relations be heritable in nature. We suggest that this usage, that of behaviors having powerful constraining effects on other behaviors such that they reduce behavioral plasticity between contexts, be referred to as behavioral syndromes in the *strong* sense.

In contrast if we restrict ourselves to the identification of correlative relations among measured behaviors, remaining neutral about causality, then we are doing little more than a data reduction exercise. While this may be useful, it is at best an incremental advance, offering benefits similar to those afforded by a host of wellestablished techniques such as PCA, Factor Analysis or Multidimensional Scaling. In particular, a purely descriptive approach offers few insights into the evolution of behavior. We suggest that this usage be referred to as behavioral syndromes in the *weak* sense.

A recent review of the literature on behavioral syndromes urges researchers to move away from the study of model organisms under controlled conditions, the better to evaluate the distribution and functional significance of this phenomenon (Sih et al. 2004b). While this will clearly be an important step, the present study demonstrates that individual differences documented in natural social groups can be challenging to interpret.

Our results also have implications for the evolution of signaling behavior. The food calls and alarm calls of fowl are referential signals (Evans 1997); they are produced in response to specific stimuli (discovery of food; approach of a particular type of predator) and are sufficient to evoke from companions the full suite of appropriate responses (food search, Evans & Evans 1999, 2007; adaptive escape behavior, Evans et al. 1993a). This type of call system was first described in vervet monkeys (Struhsaker 1967; Seyfarth at al. 1980) 30 years ago. More recent work suggests that it may be relatively widespread. For example, referential signaling is also present in other cercopithecines (Zuberbühler 2000, 2001), tufted capuchins (Di Bietti 2003), lemurs (Macedonia 1990), at least one non-primate mammal (suricates; Manser 2001; Manser et al. 2001) and several other species of birds including ravens (Bugnyar et al. 2001), yellow warblers (Gill and Sealy 2004) and black-capped chickadees (Templeton et al. 2005). Advances have been made in characterizing the cognitive mechanisms underpinning these systems, but remarkably little is known about the selective regimes that have produced them.

Studies of audience effects and sensitivity to androgen levels reveal that the referential signals of fowl each have distinct properties. Food calling is potentiated by hens and inhibited by a rival male (Marler et al. 1986), while any conspecific is an adequate audience for aerial alarm calls (Evans & Marler 1991, 1992); ground alarm calling has no audience effect at all (Evans 1997). Similarly, aerial alarm calling is testosterone-dependent, while food-calling is not (Gyger et al. 1988). Our finding that the calling responses of males to functionally important events are not correlated, once social constraints are removed, is wholly consistent with these previous reports. The emerging pattern thus suggests that signaling has not been selected as a global trait, but rather that signals are decoupled across contexts. Comparative studies will be necessary to identify the specific social and ecological factors responsible for each call system.

Acknowledgements

Housing and experimental procedures were approved by the Macquarie University Animal Care and Ethics Committee (Protocol No. 2003/033). We thank Robbie Miller and Wayne McTegg for bird care and the Australian Research Council for funding.

Literature Cited

Bayly, K. L., Evans C. S. & Taylor, A. 2006: Measuring social structure: a comparison of eight dominance indices. Behav. Process. **73,** 1-12.

Bell, A. M. 2007: Future directions in behavioural syndromes research. Proc. Roy. Soc. Lond. B **274,** 755-761.

Blumstein, D. T., Evans, C. S. & Daniel, J. C. 2000: JWatcher Alpha. http://galliform/bhs.mq.edu.au/jwatcher/.

Boissy, A. 1995: Fear and fearfulness in animals. Q. Rev. Biol. **70,** 165-191.

Brodie, E. D. 1993: Consistency of individual-differences in antipredator behavior and color pattern in the Garter Snake, *Thamnophis ordinoides*. Anim. Behav. **45,** 851-861.

Brodie, E. D. & Russell, N. H. 1999: The consistency of individual differences in behaviour: temperature effects on antipredator behaviour in garter snakes. Anim. Behav. **57,** 445-451.

Bugnyar, T., Kijne, M. & Kotrschal, K. 2001: Food calling in ravens: are yells referential signals? Anim. Behav. **61,** 949-958.

Collias, N. E. & Collias, E. C. 1967: A field study of red jungle fowl in North-Central India. Condor **69,** 360-386.

Collias, N. E. & Collias, E. C. 1996: Social organization of a red junglefowl, *Gallus gallus*, population related to evolution theory. Anim. Behav. **51,** 1337-1354.

Coleman, K. & Wilson, D. S. 1998: Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. Anim. Behav. **56,** 927-936.

Cote, J. & Clobert, J. 2007: Social personalities influence natal dispersal in a lizard. Proc. Roy. Soc. Lond. B **274,** 383-390.

Cronbach, L. J. 1951: Coefficient alpha and the internal structure of tests. Psychometrika **16,** 297-334.

D'Eath, R. B. 1988: Can video images imitate real stimuli in animal behaviour experiments? Biol. Rev. **71,** 267-292.

Dall, S. R. X., Houston A. I. & McNamara J. M. 2004: The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecol. Lett. **7,** 734-739.

Dawkins, M. S. & Woodington, A. 1997: Distance and the presentation of visual stimuli to birds. Anim. Behav. **54,** 1019-1025.

Di Bitetti, M. S. 2003: Food-associated calls of tufted capuchin monkeys (*Cebus apella nigritus*) are functionally referential signals. Behaviour **140,** 565-592.

Dingemanse, N. J. & de Goede, P. 2004: The relation between dominance and exploratory behavior is context-dependent in wild great tits. Behav. Ecol. **15,** 1023-1030.

Evans, C. S. 1997: Referential signals. In: Perspectives in ethology (Owings, D., Beecher, M. D. & Thompson, N. S., eds. Vol. 12. New York (USA): Plenum Press, New York, pp. 99–143.

Evans, C. S. & Evans, L. 1999: Chicken food calls are functionally referential. Anim. Behav. **58,** 307-319.

Evans, C. S. & Evans, L. 2007: Representational signalling in birds. Biol. Lett. **3,** 8-11.

Evans, C. S. & Marler, P. 1991: On the use of video images as social stimuli in birds: audience effects on alarm calling. Anim. Behav. **41,** 17-26.

Evans, C. S. & Marler, P. 1992: Female appearance as a factor in the responsiveness of male chickens during antipredator behaviour and courtship. Anim. Behav. **43,** 137-145.

Evans, C. S. & Marler, P. 1994: Food calling and audience effects in male chickens, *Gallus gallus*: their relationship to food availability, courtship and social facilitation. Anim. Behav. **47,** 1159-1170.

Evans, C. S. & Marler, P. 1995: Language and animal communication: parallels and contrasts. In: Comparative Approaches to Cognitive Science (Roitblat, H. & Arcady-Meyer, J., eds.) MIT Press, Cambridge, Massachusetts, pp. 341-382.

Evans, C. S., Macedonia, J. M. & Marler, P. 1993a: Effects of apparent size and speed on the response of chickens, *Gallus gallus*, to computer-generated simulations of aerial predators. Anim. Behav. **46,** 1-11.

Evans, C. S., Evans, L. & Marler, P. 1993b: On the meaning of alarm calls - functional reference in an avian vocal system. Anim. Behav. **46,** 23-38.

Frost, A. J., Winrow-Giffen, A., Ashley, P. J. & Sneddon, L. U. 2007: Plasticity in animal personality traits: does prior experience alter the degree of boldness? Proc. Roy. Soc. Lond. B **274,** 333-339.

Galef, Jr. B. G. & Giraldeau, L. A. 2001: Social influences on foraging in vertebrates: causal mechanisms and adaptive functions. Anim. Behav. **61,** 3-15.

Gill, S. A. & Sealy, S. G. 2004: Functional reference in an alarm signal given during nest defence: seet calls of yellow warblers denote brood-parasitic brown-headed cowbirds. Behav. Ecol. Sociobiol. **56,** 71-80.

Guhl, A. M. & Ortman, L. L. 1953: Visual patterns in the recognition of individuals among chickens. Condor: **55,** 287-298.

Gyger, M., Karakashian, S. J., Dufty, A. & Marler, P. 1988: Alarm signals in birds: the role of testosterone. Horm. Behav. **22,** 305-314.

Johnson, J. C. & Sih, A. 2005: Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. Behav. Ecol. Sociobiol. **58,** 390-396.

Lessells, C. M. & Boag, P. T. 1987: Unrepeatable repeatabilities - a common mistake. Auk **104,** 116-121.

Lima, S. L. & Dill, L. M. 1990: Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. **68,** 619-640.

López, P., Hawlena, D., Polo, V., Amo, L. & Martín, J. 2005: Sources of individual shy-bold variations in antipredator behaviour of male Iberian rock lizards. Anim. Behav. **69,** 1-9.

Macedonia, J. M. 1990: What is communicated in the antipredator calls of lemurs: evidence from playback experiments with ringtailed and ruffed lemurs. Ethology **86,** 177-190.

Manser, M. B. 2001: The acoustic structure of suricates' alarm calls varies with predator type and the level of response urgency. Proc. Roy. Soc. Lond B **268,** 2315-2324.

Manser, M. B., Bell, M. B. & Fletcher, L. B. 2001: The information that receivers extract from alarm calls in suricates. Proc. Roy. Soc. Lond B **268,** 2485-2491.

Marler, P., Dufty, A. & Pickert, R. 1986: Vocal communication in the domestic chicken: I. Does a sender communicate information about the quality of a food referent to a receiver? Anim. Behav. **34,** 188-193.

Martín, J. & López, P. 1999: When to come out from a refuge: risk-sensitive and state-dependant decisions in an alpine lizard. Behav. Ecol. **10,** 487-492.

Martín, J., López, P. & Cooper, W. E. 2003: When to come out from a refuge: balancing predation risk and foraging opportunities in an alpine lizard. Ethology **109,** 77-87.

Mischel, W. 1968. Personality and assessment. New York: Wiley.

Ophir, A. G. & Galef, Jr. B. G. 2003: Female Japanese quail affiliate with live males that they have seen mate on video. Anim. Behav. **66,** 369-375.

Parker, T. H. & Ligon, J. D. 2002: Dominant male red junglefowl (*Gallus gallus*) test the dominance status of other males. Behav. Ecol. Sociobiol. **53,** 20-24.

Réale, D., Gallant, B. Y., Leblanc, M. & Festa-Bianchet, M. 2000: Consistency of temperament in bighorn ewes and correlates with behaviour and life history. Anim. Behav. **60,** 589-597.

Schjelderup-Ebbe, T. 1935: Social behaviour of birds. In: A Handbook of Social Psychology. Vol. 2. (Murchison, C., ed.). Russell and Russell, New York, pp. 947-972.

Seyfarth, R. M, Cheney, D. L. & Marler, P. 1980: Monkey responses to different alarm calls: evidence of predator classification and semantic communication. Science **210,** 801-803.

Sih, A. & Watters, J. V. 2005: The mix matters: behavioural types and group dynamics in water striders. Behaviour **142,** 1417-1431.

Sih, A., Kats, L. B. & Maurer, E. F. 2003: Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system. Anim. Behav. **65,** 29-44.

Sih, A., Bell, A. M. & Johnson, J. C. 2004b. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol. Evol. **19,** 372-378.

Sih, A., Bell, A. M., Johnson, J. C. & Ziemba, R. E. 2004a: Behavioral syndromes: an integrative overview. Q. Rev. Biol. **79,** 241-277.

Sinn, D. L. & Moltschaniwskyj, N. A. 2005: Personality traits in dumpling squid (*Euprymna tasmanica*): context-specific traits and their correlation with biological characteristics. J. Comp. Psychol. **119,** 99-110.

Struhsaker, T. T. 1967: Auditory communication among vervet monkeys (*Cercopithecus aethiops*). In: Social communication among primates. (Altmann, S. A., ed.) University of Chicago Press, Chicago, pp. 281-324.

Templeton, C. N., Greene, E. & Davis K. 2005: Allometry of alarm calls: Black-capped chickadees encode information about predator size. Science **308,** 1934-1937.

van Oers, K., Drent, P. J., de Jong, G. & van Noordwijk, A. J. 2004: Additive and nonadditive genetic variation in avian personality traits. Heredity **93,** 496-503.

van Oers, K., de Jong, G., van Noordwijk, A. J., Kempenaers, B. & Drent, P. J. 2005a: Contribution of genetics to the study of animal personalities: a review of case studies. Behaviour **142,** 1185-1206.

van Oers, K., Klunder, M. & Drent, P. J. 2005b: Context dependence of personalities: risk-taking behavior in a social and a nonsocial situation. Behav. Ecol. **16,** 716-723.

Verbeek, M. E. M., de Goede, P., Drent, P. J. & Wiepkema P. R. 1999: Individual behavioural characteristics and dominance in aviary groups of Great tits. Behaviour **136,** 23-48.

Wilson, D. S. 1998: Adaptive individual differences within single populations. Phil. Trans. Roy. Soc. B **353,** 199-205.

Wilson, D. S., Clark, A. B., Coleman, K. & Dearstyne, T. 1994: Shyness and boldness in humans and other animals. Trends Ecol. Evol. **9,** 442-446.

Zuberbühler, K. 2000: Referential labelling in Diana monkeys. Anim. Behav. **59,** 917-927.

Zuberbühler, K. 2001: Predator-specific alarm calls in Campbell's monkeys, *Cercopithecus campbelli*. Behav. Ecol. Sociobiol. **50,** 414-422.

Table 1: Spearman correlations for crowing (below diagonal) and alarm calling (above diagonal) across 8 days in outdoor social groups.

Day	1	2	3	4	5	6	7	8
1		0.310	0.512**	0.449**	0.126	0.567**	0.491**	0.252
2	0.346*		0.265	0.333*	0.447**	0.398*	0.229	0.252
3	0.540**	0.329*		0.577**	0.390*	0.545**	0.427**	0.682**
4	0.520**	0.244	0.613**		0.676**	0.488**	0.652**	0.438**
5	0.214	0.366*	0.521**	0.168		0.466**	0.304	0.431**
6	0.330*	0.381*	0.399*	0.291	0.331*		0.522**	0.393*
7	0.595**	0.240	0.508**	0.428**	0.467**	0.381*		0.422*
8	0.769**	0.402*	0.498**	0.572**	0.127	0.283	0.462**	

*$p < 0.05$, ** $p < 0.01$ (2-tailed).

Table 2: Spearman correlations for food calling across 8 days in outdoor social groups.

Day	1	2	3	4	5	6	7	8
1	1.0	-	-	-	-	-	-	-
2	0.156	1.0	-	-	-	-	-	-
3	0.195	0.165	1.0	-	-	-	-	-
4	0.102	0.190	0.326	1.0	-	-	-	-
5	0.421*	0.146	0.237	0.201	1.0	-	-	-
6	0.447**	0.150	0.219	0.343*	0.336*	1.0	-	-
7	0.220	-0.107	0.100	0.133	0.222	0.120	1.0	-
8	-0.037	0.166	0.046	0.181	0.238	0.321	0.080	1.0

* $P < 0.05$, ** $P < 0.01$ (2-tailed).

Table 3: Unrotated PCA factor loading scores for behaviors seen in the laboratory during each of three contexts.

Behavior	Territorial	Behavior	Foraging	Behavior	Anti-predator
Crow rate	0.831	Food call rate	-0.848	Aerial alarm call rate	0.723
Aggression (s)	-0.740	Tidbit (s)	-0.770	Crouch level	0.723
Crow latency	-0.730	Tidbit latency	0.962		
Aggression latency	0.843	Food call latency	0.843		
Eigenvalue	2.480	Eigenvalue	2.948	Eigenvalue	1.046
Variance explained (%)	62.01	Variance explained (%)	73.71	Variance explained (%)	52.32

Figures

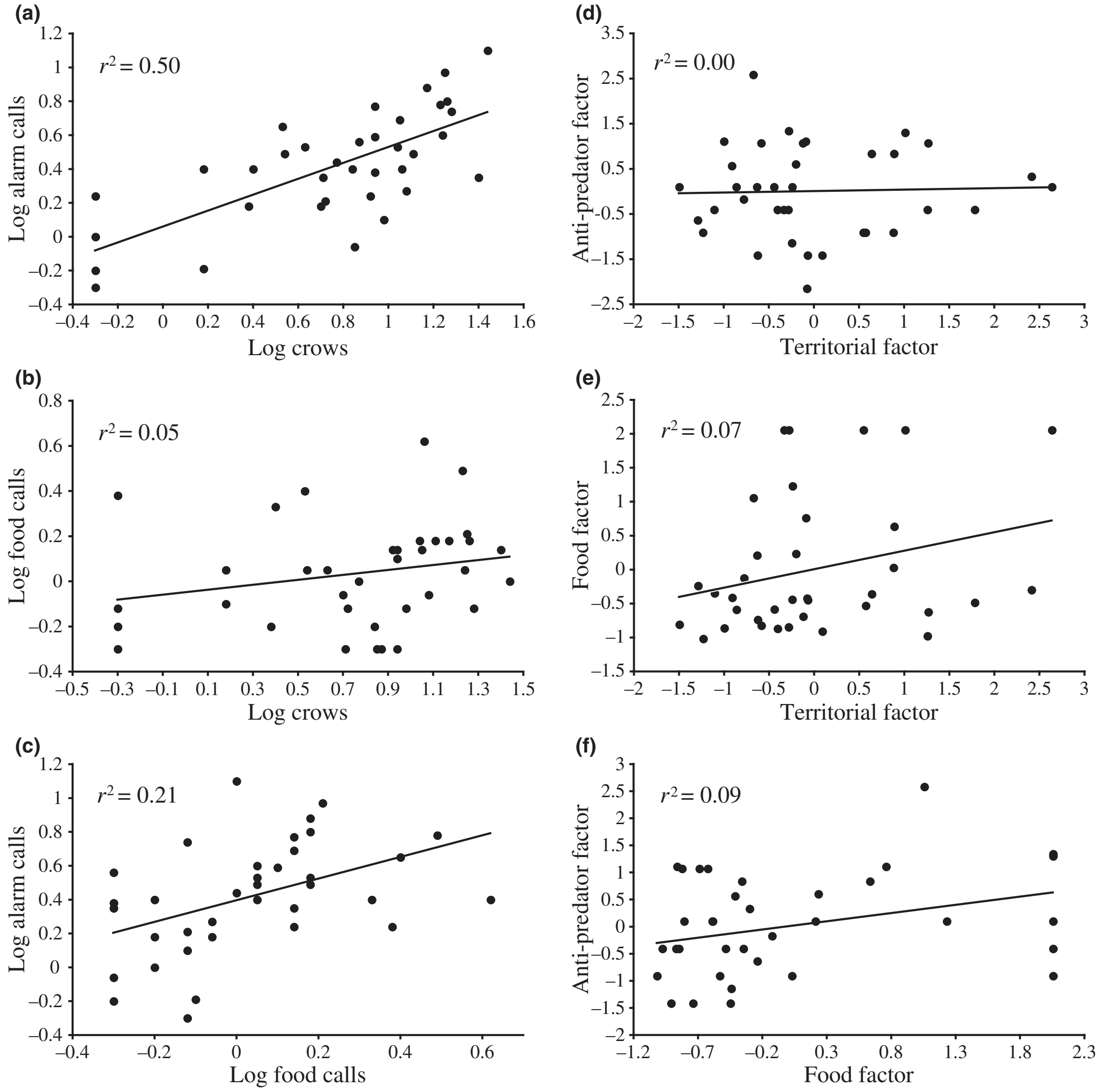

Fig. 1: Regression plots of behavior in the anti-predator, foraging, and territorial contexts in outdoor social groups (a-c) and for the same males tested individually under controlled conditions (d-f).

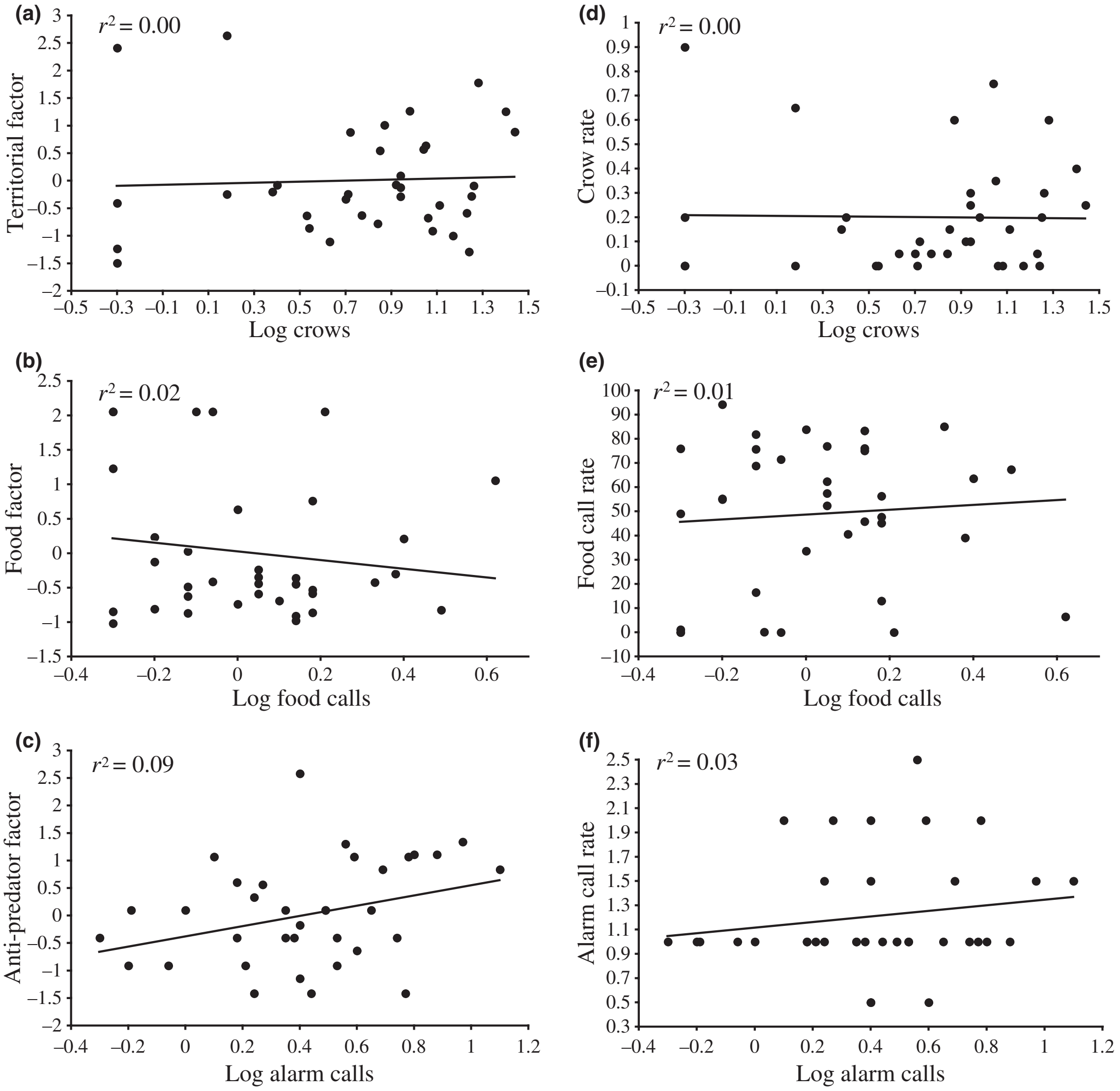

Fig. 2: Regression plots comparing behavior in the territorial context (a, d), foraging context (b, e) and anti-predator context (c, f) in outdoor social groups (abscissa) and when tested individually (ordinate). Comparisons use factors from Principal Components Analysis summarizing all behavioral responses (left) and each call type (right).

Electronic Supplementary Material Figures

Fig. 1: Outdoor setting. Four large aviaries, each containing a group of three male and three female *Gallus gallus*. Aviaries were fitted with a coop, plants for cover, and an awning affording shelter from the sun.

Fig. 2: Virtual environment. Setup used for individual tests in the anti-predator context. High-definition video audience hen is presented to subject male inside cage (not shown) while a raptor crosses a monitor overhead.