BRIEF COMMUNICATIONS

Consistent behavioural traits and behavioural syndromes in pairs of the false clown anemonefish *Amphiprion ocellaris*

M. Y. L. Wong*†, A. Medina‡, C. Uppaluri‡, S. Arnold§,
J. R. Seymour‡ and P. M. Buston‡

School of Biological Sciences, University of Wollongong, Northfields Avenue, NSW 2518, Australia, ‡Department of Biology, Boston University, 5 Cummington Street, Boston, MA, 02215, U.S.A. and §School of Life Sciences, PO Box 874501, Arizona State University, Tempe, AZ 85287, U.S.A.

Using the social clown anemonefish *Amphiprion ocellaris*, whether individuals exhibited consistency in activity levels, boldness and sociability in a paired context, and whether these three behavioural traits were positively correlated within a single behavioural syndrome, was investigated. The results highlight that consistent individual differences in behaviour are expressed in a social fish and suggest that consistent behavioural traits and behavioural syndromes could influence the structure and functioning of their societies.
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Ecologists have become increasingly concerned with understanding the causes and consequences of individual variation in behaviour (Gosling & Oliver, 1999; Sih *et al*., 2004; Réale *et al*., 2007). There are two main paradoxes. The first is why there is so much variation among individuals in their behavioural traits when natural selection may be expected to eliminate the poorest variants (Dall *et al*., 2004). The second is why individuals often exhibit remarkable consistency in behavioural traits over time and across different contexts, when natural selection may be expected to favour behavioural plasticity (Dingemanse & Wolf, 2010). Such consistent individual behavioural traits are often referred to as personality traits (Réale *et al*., 2007) and it is now widely accepted that animal models may be useful for understanding the evolution of personalities, including those exemplified by humans (Gosling 2001; Nettle & Penke, 2010).

Consistent behavioural traits can also be highly correlated with each other, giving rise to a behavioural syndrome (Sih *et al*., 2004). Just as consistent behavioural traits

†Author to whom correspondence should be addressed. Tel.: +61 2 4221 3574; email: marianw@uow.edu.au

are directly analogous to personality traits, a behavioural syndrome can be considered a major dimension of animal personality (Gosling & Oliver, 1999). Behavioural syndromes are challenging to understand, both in terms of the ultimate and proximate reasons for trait correlations (Sih *et al*., 2004), because it is not immediately apparent why behavioural traits, such as activity and boldness, should be correlated in the same way that morphological traits, such as length and mass, are correlated. Research on behavioural syndromes has focused largely on the shy-bold axis (Réale *et al*., 2007; Conrad *et al*., 2011). For example, individual consistency in boldness is often correlated with other consistent behavioural traits, including activity and sociability, in a wide range of species (Sih *et al*., 2004; Cote *et al*., 2010).

Research on the evolutionary causes and consequences of consistent behavioural traits and behavioural syndromes has largely focused on quantifying the behaviours of individuals in isolation (Bell, 2005; Dingemanse *et al*., 2007). For animals that naturally reside in groups throughout their entire lives, however, this method may not portray a reliable picture of an individual's personality because their behaviours have evolved and are maintained in the presence of other group members (Schuett *et al*., 2011; Webster & Ward, 2011; Magnhagen, 2012). As such, it is becoming apparent that investigations of consistent behavioural traits and behavioural syndromes in species exhibiting some degree of sociality should be performed within their natural social context to gain a better indication of behavioural variation and the associated fitness consequences (Seyfarth *et al*., 2012).

To increase understanding of behavioural variation in a natural social context, the consistency of individual behaviours and correlations between behaviours in the false clown anemonefish *Amphiprion ocellaris* Cuvier 1830 was quantified. This is a group-living coral-reef fish which lives in close association with sea anemones that provide individuals with essential protection from predators. Within an anemone, group sizes naturally range from two to six individuals, with only the largest female and male breeding with each other (Mitchell & Dill, 2005). While a considerable amount of background information on the behaviour and life history of *Amphiprion* spp. has been gathered (Buston, 2003; Mitchell, 2003; Mitchell & Dill, 2005; Buston & Elith, 2011), whether individuals exhibit consistency in their behaviours and whether consistent behavioural traits are correlated is currently unknown. If individuals display consistent behavioural traits and syndromes, it is predicted that there will be: (1) positive correlations between an individual's score for each behavioural trait at a given time and its score for the same behavioural trait at other times and (2) correlations between individual's scores for each behavioural trait.

The investigation was carried out at Boston University, MA, between the 13 and 17 June 2011. All *A. ocellaris* used for this investigation were F3 descendants of wild-caught *A. ocellaris* that had been maintained in captivity at Boston University for *c*. 6 years. *Amphiprion ocellaris* were housed and tested in pairs because this species does not naturally occur as singletons. Instead, individuals typically reside in pairs or groups of up to six individuals (Buston, 2003). As social context may modulate the personality traits and behavioural expression of individuals (Webster & Ward, 2011), this study focused on defining the personality traits of individuals in a social (paired) context only as this is more biologically relevant for this species. Importantly, individual *A. ocellaris* were always kept with their partners throughout the investigation, hence the potential confounds of modulating social context were avoided.

Each pair was maintained in an aquarium containing sand, rocks and colonies of the sea anemone *Entacmea quadricolor*. These elements created a semi-natural environment, which encouraged natural behaviour. Standard length (L_S) of males ranged from 3·28 to 6·23 cm, and females ranged from 5·44 to 6·59 cm; females were always larger than males within each pair. Throughout the investigation, all fish were maintained at a temperature of *c*. 28·5° C, salinity of *c*. 35 and at levels of dissolved oxygen > 95%. Other water quality variables, namely ammonia, nitrite and nitrate concentrations, were measured weekly.

Behaviours of six established pairs of *A. ocellaris* were recorded in total. To characterize individual behavioural traits, each pair was videotaped for 12 min using HD video recorders in the morning (0900–1200 hours) and afternoon (1300–1700 hours) on day 1, 3 and 5 (*i.e.* Monday, Wednesday and Friday). Video recordings of *A. ocellaris* were taken from the side of the tank (rather than from above) because a side-view provided clearer observations of individual behaviours. The behaviour of both partners was then scored, disregarding the first 2 min of each video to allow for acclimation, based on an ethogram of behaviours constructed during previous pilot observations of this species (see Table SI, Supporting information).

Three behavioural traits were measured in a social context: activity, boldness and social affiliation, as these are amongst the key personality traits so far identified in animals (Réale *et al*., 2007). Activity was defined as the total distance travelled by an individual during the observation period. A ruler taped to the bottom of the aquaria provided a length reference for subsequent distance quantification. On completion of each recording, videos were analysed using Tracker software (www.cabrillo.edu/~dbrown/tracker) that allows an object's co-ordinates to be determined and absolute distances calculated based on the length reference. Boldness was defined as the number of seconds an *A. ocellaris* spent outside the anemone during the observation period, and was measured using a stop-watch as soon as an individual was not in body contact with the anemone. Social affiliation was defined as the number of non-aggressive acts performed with its partner (specifically follows, meets and soft touches) (see Table SI, Supporting information), and was recorded by watching and scoring the videos. There were no significant differences between morning and afternoon behaviours for each of the behavioural traits on the 3 days (ANOVA; $P > 0.05$ for all) so morning and afternoon scores of each day were averaged prior to statistical analyses. All data conform to assumptions of the statistical methods used.

To investigate the existence of consistent behavioural traits, the repeatability of activity, boldness and sociability was estimated across the 3 days for individuals in a paired context. Following Nakagawa & Schielzeth (2010), linear mixed effects models and restricted maximum likelihood (REML) methods in JMP v.9 were used to estimate variance components and repeatability. 'Individual ID' was entered into the model as a random effect and the following factors as fixed effects: (1) partner's behaviour (to control for social context), (2) sex of focal individual and (3) body size of the focal individual. Factors were progressively eliminated using backward stepwise elimination if they were not significant ($P > 0.05$) to provide the simplest model. To detect a behavioural syndrome, principle components analysis (PCA) incorporating activity, boldness and sociability scores, was used to define possible personality trait dimensions.

TABLE I. Component loadings of each of the three behavioural traits (activity, boldness and sociability) and total variance explained by the first principle component (eigenvalue $= 2.24$)

	Principle component
Behaviour	PC1 (activity–boldness–sociability)
Activity	0.84
Boldness	0.89
Sociability	0.86
Total variance (%)	74.6

Adjusted repeatability (*i.e.* corrected for other factors) for activity (75.9%), boldness (30.4%) and sociability (50.2%) were high and differed significantly from zero for activity ($F_{11,23} = 9.9$, $P < 0.001$), boldness ($F_{11,23} = 2.2$, $P = 0.05$) and sociability ($F_{11,23} = 3.85$, $P < 0.01$). In addition, activity of an individual was significantly related to its sex ($F_{1,11} = 11.9$, $P < 0.01$) and the activity of its partner ($F_{1,32} = 9.8$, $P < 0.01$), but not related to an individual's L_S ($F_{1,9} = 0.56$, $P > 0.05$). Boldness of an individual was related to its sex ($F_{1,10} = 4.9$, $P = 0.05$) and the boldness of its partner ($F_{1,33} = 43.8$, $P < 0.001$), but not related to the individual's L_S ($F_{1,10} = 0.003$, $P > 0.05$). Sociability of an individual was related to the sociability of its partner ($F_{1,34} = 1437.9$, $P < 0.001$) but not related to an individual's L_S ($F_{1,10} = 0.1$, $P > 0.05$) or sex ($F_{1,10} = 1.9$, $P > 0.05$). Turning to consider the relationships between the behaviours, PCA revealed that one factor (PC1) explained 74.6% of the total variance, with strong component loadings for activity, boldness and sociability, suggesting the existence of a behavioural syndrome (Table I).

The results of this study demonstrate the existence of consistent and repeatable behavioural traits among individual *A. ocellaris* in a paired context. For boldness, activity and sociability, individuals were consistent in their behavioural scores over 5 days. Furthermore, activity, boldness and sociability loaded strongly onto the same component, which together suggests that these three behaviours form a behavioural syndrome. These findings, and observations of consistent behavioural traits and behavioural syndromes in other species, raise three distinct evolutionary questions: (1) why is there inter-individual variation in behavioural traits, (2) why is there within-individual consistency in behavioural traits and (3) why is there inter-specific variation in behavioural syndromes? Plausible explanations for each of these are now considered in turn.

In terms of the maintenance of inter-individual variation in behavioural traits, different levels of behavioural expression could simply be related to fundamental variables like size and sex (Edenbrow & Croft, 2012). Alternatively, frequency-dependent selection (Dall *et al.*, 2004) or context-dependent selection (Dingemanse *et al.*, 2004; Schuett *et al.*, 2011) could promote the coexistence of alternate behavioural types. For example, in great tits *Parus major* fast-exploring females survived better than slow-exploring females in low food years, because the aggressiveness of fast-exploring females enabled them to better compete for limited food patches. In contrast, fast-exploring males fared better than slow-exploring males in high food years because they could better compete over territories which were more limited when food was abundant (Dingemanse *et al.*, 2004). Hence, evaluating the fitness

consequences of different behavioural types and syndromes under varying contexts would be a crucial component of research in the future (Smith & Blumstein, 2008).

Within-individual consistency in behaviours, could potentially arise if being behaviourally predictable confers fitness benefits. In the social context of breeding pairs, behavioural consistency could be selectively advantageous if it provides a good indication of how much or what type of care an individual may provide and if other individuals use this information to make strategic partnering decisions (Both *et al*., 2005; Royle *et al*., 2010; Schuett *et al*., 2011). For example, Schuett *et al*. (2011) demonstrated that pairs of zebrafinches *Taeniopygia guttata* exhibiting similar personality traits fledged offspring in better condition than pairs of dissimilar parents, suggesting that consistency and similarity in a partner's personality could be an important cue of mate choice and affect fitness within a social context. Alternatively, such limited plasticity could arise because of high costs of behavioural plasticity, for example, if flexible responses are costly owing to errors made in behavioural adjustment to different conditions (Dall *et al*., 2004).

Finally, positive associations between consistent behavioural traits, like boldness and activity, have been reported in many species (Bell & Stamps, 2004; Webster *et al*., 2007), but other studies have revealed that they can also be negatively correlated (Ward *et al*., 2004; Croft *et al*., 2009). Hence, answering the third question, namely why a particular behavioural syndrome exists and why there are inter-specific differences in behavioural syndromes, would involve elucidating the ecological and social factors responsible for such variation. Comparative assessments of social structures and behavioural syndromes may provide a clue to such variation, since the form and function of social interactions can vary considerably depending on whether groups are open- or restricted-access, large or small or despotic or egalitarian (Keller & Reeve, 1994; Webster & Ward, 2011). This perspective therefore views behavioural syndromes not as evolutionary constraints, but as an adaptive combination of behaviours that are molded by natural selection in response to social and ecological factors.

In conclusion, individual *A. ocellaris* show consistent behavioural traits and behavioural syndromes with respect to activity, boldness and sociability, in a paired context. To increase understanding of behavioural variation in social contexts, future work should examine how finer-scale group characteristics, such as group composition, size and the formation of dominance hierarchies, affect behavioural consistency and correlations in social individuals (Colléter & Brown, 2011; Wilson *et al*., 2011; Magnhagen, 2012). This would not only enable determination of both how and why individual behavioural traits may vary, but the consequences of such behavioural variation at higher levels of the ecological hierarchy, including social groups and populations as a whole. Therefore, a deeper understanding of the causes and consequences of behavioural variation in a social context may ultimately prove useful for the conservation and management of social species (Conrad *et al*., 2011; Powell & Gartner, 2011; Wong, 2012).

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Supporting Information

Supporting Information may be found in the online version of this paper:
Table S1. Ethogram of behaviours exhibited by *Amphiprion ocellaris*.

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