Behavioural plasticity across social contexts is regulated by the directionality of inter-individual differences

Olivia L Guayasamin [a], Iain D Couzin [a,b,c], Noam Y Miller [a,d]*

[a] Department of Ecology and Evolutionary Biology, 106A Guyot Hall, Princeton University, Princeton, NJ, 08544, U.S.A.
[b] Department of Collective Behaviour, Max Planck Institute for Ornithology, Konstanz, Germany.
[c] Chair of Biodiversity and Collective Behaviour, Department of Biology, University of Konstanz, Konstanz, Germany.
[d] Department of Psychology, Wilfrid Laurier University, 75 University Ave. West, Waterloo, Ontario, N2L 3C5, Canada

* Corresponding author. E-mail: nmiller@wlu.ca.

ABSTRACT

An individual's behavioural phenotype is a combination of its unique behavioural propensities and its responsiveness to environmental variation, also known as behavioural plasticity. In social species, we must not only explore how individuals respond to variations in the physical environment but also how they react to changes in their social environment. A growing body of work has demonstrated that the behavioural heterogeneity of a group can alter its responsiveness, decision making, and fitness. Whether an individual is more or less extreme than a partner – what we term its 'relative personality' – may also alter individual behavioural responses. We determined exploratory tendencies of individual zebrafish (Danio rerio) and then constructed pairs with varying differences in 'relative personality' to determine the effect of differences between partners on behavioural plasticity. We find that relative personality, but not the magnitude of the difference between partners, is the most important determinant of behavioural plasticity across social treatments. Despite this overall effect, pairs of fish exhibited no predictable leader-follower interactions, suggesting that details of the experimental paradigm may be important in shaping social dynamics.

1. INTRODUCTION

It is well established that individuals of many species exhibit consistent individual differences in behaviour and that individuals also vary in the degree to which they can modify their behaviour in response to their physical environment (Biro and Adriaenssens, 2013; Brommer, 2013; Dingemanse and Wolf, 2013; Dingemanse et al., 2010a; McElreath et al., 2007; Nussey et al., 2007; Sih and Bell, 2008; Sih and Del Giudice, 2012; Sih et al., 2012, 2004a; Wolf and Weissing, 2010), often referred to as 'personality' and 'plasticity', respectively. Much of the work to date on animal personality has utilized social species as model organisms, yet has been restricted to studying the behaviours of isolated individuals across a range of physical environments. Given that isolated individuals of social species have been shown to behave differently from their behaviour in group settings (Aplin et al., 2014, 2013, 2012; Magnhagen and Bunnefeld, 2009; Magnhagen and Staffan, 2005; Magnhagen, 2007; Schuett and Dall, 2009; van Oers et al., 2005; Webster et al., 2007) and that individual differences and social structure may be expected to coevolve in social species (Dingemanse and Wolf, 2013; Dyer et al., 2008; Laskowski and Pruitt, 2014; Tanner and Jackson, 2012), it is important to consider inter-individual differences of members of social species within and across different social contexts.

Current methods of studying behavioural phenotypes typically involve comparing the responses of individuals to a range of physical environments (Briffa et al., 2008; Dosmann and Mateo, 2014; Ord et al., 2010) and/or within a single environmental gradient (Beckmann and Biro, 2013; Dingemanse et al., 2010b; Kluen and Brommer, 2013; Nussey et al., 2007; Quinn et al., 2012; Teyssier et al., 2014), such as different levels of predation risk (Quinn et al., 2012). However, observing how individuals respond to changes in their physical environment is not sufficient for understanding the ecological and evolutionary significance of a particular behaviour in a social species. When the behavioural traits of individuals in groups influence how they perceive, process, and respond to their environment, the specific composition of a group may have fitness consequences for all or some of its members (Cote et al., 2008; Dingemanse and Wolf, 2013; Dyer et al., 2008; Laskowski and Pruitt, 2014; Webster and Ward, 2011). For example, social context has the potential to substantially affect an individual's fitness by influencing the interaction between that individual and its physical environment (Magnhagen and Staffan, 2005; Magnhagen, 2007; Schuett and Dall, 2009; Webster et al., 2007), either through influencing an individual's knowledge of the environment by providing access to social information (Aplin et al., 2012; Brown and Laland, 2003; Krause and Ruxton, 2002; Laland and Williams, 1997; Magnhagen and Staffan, 2003), or through alteration (by other individuals) of the environment itself (Laskowski and Bell, 2013; Watters and Sih, 2005). The selection pressures that act to maintain inter-individual variation may also depend on collective behavioural phenomena, such that the adaptiveness of any phenotype depends on the ensemble of phenotypes in a particular group (Dingemanse and Wolf, 2013, 2010; Wolf and Krause, 2014; Wolf et al., 2008, 2007).

A growing number of studies have begun to address these effects by comparing an individual's asocial behaviour to their behaviour in a social setting (Aplin et al., 2014, 2013, 2013, 2012; Favreau et al., 2014; Herbert-Read et al., 2013; Kurvers et al., 2010, 2009; Magnhagen and Bunnefeld, 2009; Magnhagen and Staffan, 2005; Nakayama et al., 2012; Webster et al., 2007). Individuals of many species tend to conform their behaviour to that of partners or group members (Pike and Laland, 2010) but individual behavioural

differences may still be expressed to some degree under social conditions (Aplin et al., 2013; Herbert-Read et al., 2013; King et al., 2015; Kurvers et al., 2011, 2009; Laskowski and Bell, 2014; Magnhagen and Bunnefeld, 2009; Magnhagen and Staffan, 2005; Nomakuchi et al., 2009; Schuett and Dall, 2009; van Oers et al., 2005). For example, "shy" sticklebacks (*Gasterosteus aculeatus*) will become bolder when placed with a bolder partner, but their asocial behaviour remains a significant predictor of their social behaviour (Jolles et al., 2014). Distinct behavioural phenotypes also appear to demonstrate unique patterns of plasticity. For example, individuals that are boldest, most exploratory, and most aggressive when alone show the smallest change in behaviour upon being placed in a group, and vice versa (Coppens et al., 2010; Harcourt et al., 2009; Herbert-Read et al., 2013; Hulthén et al., 2014; King et al., 2015; Koolhaas et al., 1999a, 1999b; Kurvers et al., 2011; Magnhagen and Bunnefeld, 2009; Magnhagen and Staffan, 2005; Øverli et al., 2007; van Oers et al., 2005; Webster and Ward, 2011). Similar conformity effects have been found in studies of social behaviour in human groups (Bikhchandani et al., 1998; Bond and Smith, 1996).

Given that the assessment of inter-individual differences is an inherently relative process – relying on comparisons between behavioural measures within a test population – it is likely that the most informative measure of social effects on individual behaviour will be what we term the 'relative personality': the difference in behaviour between the members of a group. For example, a "shy" individual may respond to a "bold" individual by behaving more boldly (Jolles et al., 2014), but what will the behavioural consequences be if this "shy" individual is paired with an *even shyer* member of the population, making the "shy" individual the relatively bolder partner? Furthermore, within groups there are frequently changing individual-level interactions among behaviourally heterogeneous group members (Bell and Stamps, 2004; Couzin et al., 2002; Stamps and Groothuis, 2010; Sumpter, 2006). An individual's 'relative personality' will depend on the composition of its current neighbors, and the same individual may behave differently depending on its immediate social context. Some researchers have begun to address this question by testing the same individual in more than one social environment (Cornwallis and Birkhead, 2008; Favati et al., 2014; Jolles et al., 2014; King et al., 2015; Laskowski and Bell, 2013), finding that social status, group membership, personalities of partners, and individual traits can all influence behavioural plasticity.

Previous studies, however, have used randomly constructed groups, in which the relative personality composition of the group could not be experimentally manipulated. It is likely that the particular mix of behavioural phenotypes in a group will have a large effect on most aspects of the group's behaviour, from its cohesiveness to the fitness benefits each individual gains from being part of it (e.g., Pruitt & Reichert, 2011). In species that form fission-fusion groups – where group composition changes on quite short timescales (Croft et al., 2003) – behavioural plasticity will play a large role in determining the success of each individual as well as of the various groups they participate in. Thus, being able to experimentally manipulate the set of behavioral phenotypes comprising a group would allow us to explore the mechanisms by which behavioural differences between group members drive the dynamics of collective behavior.

Using a well-known model organism, the zebrafish (*Danio rerio*), we systematically manipulated the 'relative personalities' (as defined above) of pairs of individuals to determine their influence on the

plasticity of exploratory behaviour. Pairs of fish were tested in the same environment that was used to establish individual exploratory tendencies, so that the effects of changing social context could be isolated and quantified.

We recorded zebrafish's exploratory behaviour when tested in isolation and when they were with a partner. All trials measured behaviour using the same assay, an open field with a shelter that has been widely used to measure exploratory and boldness tendencies in fish (Harcourt et al., 2010, 2009; Ioannou et al., 2008; King et al., 2013; Maximino et al., 2010b; Nakayama et al., 2012). Each fish participated in two pair trials and we assigned pairings such that each subject was the more exploratory partner (ME) during one condition and the less exploratory partner (LE) during the other, based on scores from their asocial trials, and systematically varied the magnitude of the difference in exploratory tendency between the partners.

2. METHODS

2.1 Subjects

Subjects were 96 (63F; 33M) adult zebrafish (*Danio rerio*) of the WIK strain, bred in the Burdine lab at Princeton University. To identify individual fish for the duration of the study, each fish was injected dorsally at two separate locations with Visible Implant Elastomer tags (VIE; Northwest Marine Technology Inc., Washington, USA; Webster and Laland, 2009). Subjects in the experiment were randomly assigned to 4 groups of 24 uniquely marked fish each. Each group was housed in a single tank. Fish were allowed to recover from the tagging procedure for at least 4 days before experiments began (Doupe et al., 2003). All procedures were reviewed and approved by the Princeton University, NJ, Institutional Animal Care and Use Committee (IACUC; Protocol Number: 1890).

2.2 Housing and Care

Fish were housed in an environmentally controlled high-density housing rack (Pentair Aquatic Habitats, FL). Lights were on a 12:12 cycle (light:dark); salinity was held between 900-1200 micro-Siemens and the ambient temperature was maintained between 20-24°C. Fish were acclimated to the housing tanks for 4 weeks before experiments began. Once experiments began, fish were fed flake food (TetraMin Tropical Flakes) *ad lib.* daily after the completion of experimental trials.

2.3 Experimental Apparatus

The testing apparatus for both the asocial and pair trials was a 60 x 45 cm rectangular white PVC enclosure (Figure 1). One narrow end of the enclosure held a 7.6 cm wide plastic overhang just above the water surface with plastic aquarium plants attached to its underside, to serve as a shelter, and all the interior walls of the enclosure were lined with textured transparencies. This design was inspired by prior work with zebrafish demonstrating that open field tasks establish an internal conflict between a preference for dark, protected areas and a drive to explore novel environments (Maximino et al., 2010a, 2010b; Serra et al., 1999; Stephenson et al., 2011). All trials were filmed with an overhead camera (Sony

EX1) at 1920 x 1080 pixels and at 30 frames per second. Pair trials were additionally photographed every 2 seconds (Nikon D7000 DSLR) to aid in the accurate identification of each individual.

Four identical enclosures, as described above, were placed in a 210 x 120 x 15 cm white acrylic tank surrounded by floor-to-ceiling white curtains (Figure 1). Water depth was maintained at 7-8cm so that the movement of the fish was mostly constrained to 2D. The tank was filled with 'system' water identical to that used in the housing tanks. When fish were not present in the testing tank, bubblers and filters maintained water quality. The arena was lit by four fluorescent tube lights and four Chauvet LED PAR 56-24UVB Blacklights (www.chauvetlighting.com) to enhance the visibility of the elastomer tags.
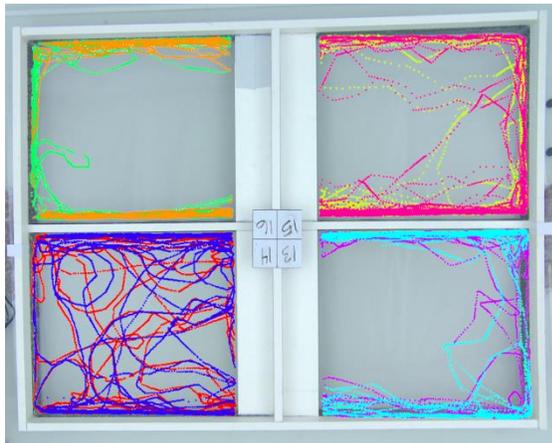

Figure 1: Testing enclosure and sample trajectories. The photograph is a still image from a data video showing the testing arena used for both asocial and social trials, containing 4 identical enclosures, each with a shelter along one edge (white areas in the image center). Trajectories from a sample set of pair trials are overlaid on the picture. Each color represents the entire trajectory of a single fish over the course of a 10 min trial.

2.4 Procedure

Each fish first completed three asocial trials, in which it was alone in the enclosure. Asocial trials were spaced 48 hours apart. Fish were netted from their home tanks and placed into beakers containing 300 mL of system water. A single fish was gently placed into each of the four enclosures, allowing four individual trials to be run simultaneously. After 2 min of acclimation time, the movement of each isolated fish was video-recorded for 9 minutes. All fish were then assigned exploratory scores (see below) on the basis of which pairings were determined. 24 hours after the end of the last asocial trial, each fish participated in two pair trials – in which two fish were placed into each enclosure- spaced 24 hours apart, with a different assigned partner for each trail. Pair trials were otherwise identical to the asocial trials. At the end of the experiment, all fish were sexed (estimated from body dimensions and coloration) and measured (body length: distance from snout to tip of tail fin; body depth: distance from front of dorsal fin to belly).

2.5 Determining Exploratory Tendency

Individual exploratory tendencies were quantified using the trajectory data from all three trials, extracted from the videos using custom software designed in-house (Rosenthal et al., 2015). From these trajectories we extracted the five following behaviours: total distance travelled during the trial, median distance from the closest enclosure wall, median distance from the shelter, total time spent out of the shelter, and median duration of each 'visit' to the shelter. We used median values for distances from the

walls and shelter and for shelter visit durations because the distributions of these measures were all highly skewed. All measures were normalized by dividing all recorded values by the maximal possible value of each measure (and so are dimensionless). All raw measures were entered into a Principal Components Analysis (PCA) using *Mathematica* (v.7, Wolfram Research) to give uncorrelated final scores representing the exploratory tendency of each fish.

Only the first component (PC1) resulting from the PCA was found to be significant and it described 57.7% of the variance (Supplementary Materials, Table S.1)**.** The raw measurement loadings on PC1 confirmed our intuition that this component successfully captured exploratory tendency (Table S.2; Dahlbom et al., 2011a; Moretz et al., 2007; Toms and Echevarria, 2014; Toms et al., 2010). For each of the three asocial trials completed by each fish, we multiplied all five raw measurements by the eigenvector of this first principal component, resulting in three exploration tendency scores for each fish (one for each asocial trial). These three scores were then averaged to arrive at a single asocial exploratory tendency score for each fish, denoted A_i for individual i. This procedure for assigning exploratory scores was established and validated with a separate population of zebrafish, whose behaviours yielded almost identical PCA results (see Appendix A for details).

To allow for direct comparisons of behaviour across both social treatments, individual exploratory behaviour during the pair trials was measured exactly as in the asocial trials, i.e., for this analysis the movement of each member of the pair was quantified independently, as if it were alone. If individual identities were unclear at any point in the video, we combined visual analysis of the higher-resolution DSLR images with a custom MATLAB (v. R2012B, Mathworks) script and manually assigned identities to each trajectory fragment. The same five measures as above were extracted from the trajectories of each member of the pair separately and the data were transformed using the PCA dimensions determined for the asocial data, ensuring that individual's scores on pair trials are directly comparable to their asocial scores. The first principal component of the resulting score was assigned as the new, social, exploratory tendency score for that individual on that trial, S_i. Every individual therefore received two new exploratory scores (in addition to their asocial score), one for each of their two social treatments.

For pair trials, we additionally analyzed the coordinated movement of members of each pair during their excursions out from the shelter. We quantified how often each partner initiated the excursion (left the shelter first) or returned to the shelter first. We also measured the mean durations of excursions and the mean distance between the two fish. Finally, we also counted any solo excursions – when a single partner left and returned to the shelter while the other fish remained under the shelter.

2.6 Pairing Fish

Each individual completed two social treatments: one in which they were the more exploratory (ME) partner and one when they were the less exploratory (LE) partner, a determination based on their asocial exploratory tendency scores (A_i). In addition, we systematically varied the magnitude of the difference in exploratory tendency between the members of a pair, a measure we call the Intra-Pair Exploratory Difference (IPED). The order of the ME and LE social treatments was randomized between

fish. The three least (most) exploratory fish in each tank completed both pairings with more (less) exploratory individuals.

2.7 Measuring Plasticity

Individual plasticity was defined as the difference between asocial and social exploration scores (S_i - A_i), and was calculated for each individual for both its ME and LE social trials. This change in behaviour is a known proxy for behavioural plasticity (Sih et al., 2004b). A negative plasticity value indicates that an individual became less exploratory when in a pair relative to when alone, while a positive value indicates an increase in exploratory behaviour. Most importantly, the trajectories for each member of the pair were analyzed independently, as if that fish were alone. In this way, pair trial behaviour could be directly compared to behaviour during the solo trials.

2.8 Data analysis

All statistical analyses were conducted in R (v. 3.0.2. R Development Core Team) using the *psych, quant psych, car, lme4,* and *ppcor* packages. Results with P < 0.01 are reported as significant due to correcting for multiple statistical tests (Bonferonni correction α = 0.01). We tested for individual behavioural change within and across the LE and ME social treatments using Wilcoxon Signed Rank tests. To determine if this change was significantly different from zero, Mann-Whitney U tests were used. To see if partners behaviourally conformed to each other, we used Wilcoxon Signed Rank tests to compare asocial exploratory score differences (A_i - A_j) between partners, to their differences in exploratory score during the two social treatments (IPED = S_i - S_j). We further compared the change in score differences between the asocial and social treatments [we denote this $\Delta IPED = (S_i - S_j) - (A_i - A_j)$]. We constructed 100 lists of shuffled (randomized) pairings of all fish and calculated $\Delta IPED$ for these random pairs. Real and shuffled distributions were compared using a 2-sample KS test. We examined individual plasticity for the LE and ME social treatments using separate linear mixed models (LMMs). Because pairings were within home tank and occurred over two days, we fitted home tank number and experiment day as random effects. For both models we entered A_i and IPED as continuous fixed effects. The fixed effects for the LE fish model had a variance inflation factor (VIF) of 4.039, and the fixed effects for the ME fish had a VIF of 1.059. Step-wise model selection was used to determine the best fitting models. In all cases we present the best fitting models as determined by the Akaike Information Criterion (AIC) and F-tests, as the significance of all terms are unchanged compared to the full models. Finally, correlation analysis with Kendall's τ was used to determine whether individuals showed similar plasticity in exploratory tendency across the ME and LE social treatments. Because this analysis explored intra-individual plasticity across social treatments, only individuals that participated in both treatments (ME and LE; N=72) were included. To determine whether the correlation in plasticity across social treatments could be explained by A_i, partial and semi-partial correlation analyses were employed (Aron et al., 2012).

3. RESULTS

There was no effect of sex (t(94) = 0.60, P = 0.53), body length (r = 0.03, t(94) = 0.25, P = 0.80), or body depth (r = 0.08, t(94) = 0.81, P = 0.42) on asocial exploratory tendency.

3.1 Asocial exploration is a poor predictor of social behaviour, but accounts for correlated plasticity across social treatments

Asocial exploratory tendency (A_i) was a poor predictor of exploratory behaviour during the LE or ME social treatments (S_i). Though asocial exploratory tendency was correlated with exploration scores during the LE treatment ($\tau = 0.268$, P = 0.008), it was not correlated with exploration scores in the ME treatment ($\tau = 0.099$, P = 0.34). There was also no correlation between an individual's social exploration across social treatments ($\tau = 0.139$, P = 0.18). In addition, an individual's score during pair trials (in both the LE and ME conditions) was not predicted by their partner's asocial exploration score (all Adj. $R^2 <$ 0.10, all $p > 0.10$).

Plasticity was defined as the change in an individual's exploratory behaviour between the asocial and social trials (i.e., $S_i - A_i$) and calculated separately for trials in which the focal individual was the more (ME) or less (LE) exploratory partner. A bivariate correlation conducted with Kendall's τ revealed a significant correlation in individual plasticity between the LE and ME social treatments (Figure 2), such that individuals that demonstrated a large increase in exploratory behaviour during one social treatment tended to also do so in the other ($\tau = 0.245$, P = 0.007). However, there were also significant bivariate correlations between asocial exploratory score and individual plasticity for both the LE ($\tau = -0.302$, P = 0.004) and ME ($\tau = -0.268$, P = 0.007) conditions. Thus, to determine whether asocial exploratory score (A_i) could account for this relationship, we used partial correlation analysis and re-calculated the correlation in plasticity between the LE and ME conditions while controlling for the effects of asocial exploratory score. This caused the observed correlation in plasticity between the LE and ME treatments to lose significance (partial $\tau = 0.178$, P = 0.03), demonstrating that an individual's plasticity across different social treatments is partially explained by their asocial exploratory tendency (A_i), such that less exploratory individuals are more plastic.
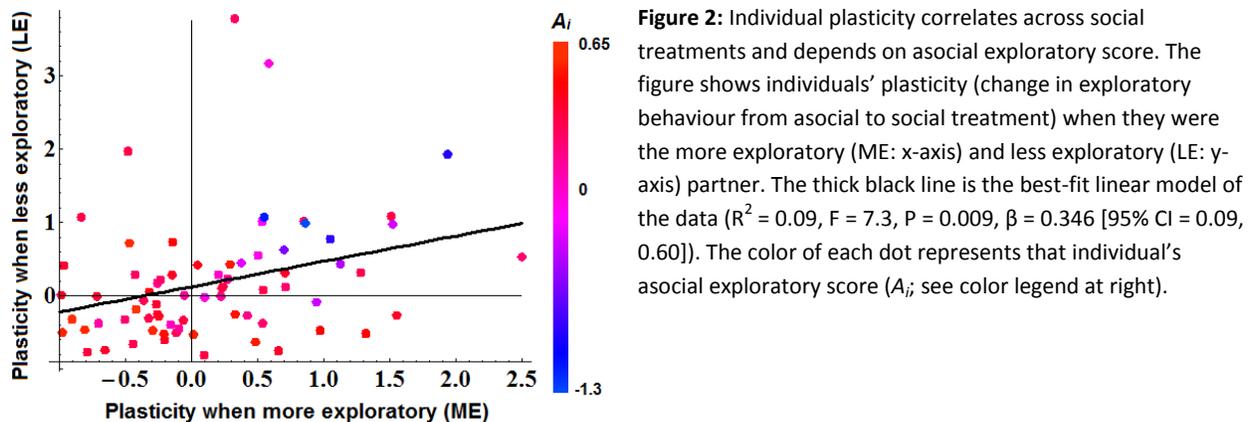

Figure 2: Individual plasticity correlates across social treatments and depends on asocial exploratory score. The figure shows individuals' plasticity (change in exploratory behaviour from asocial to social treatment) when they were the more exploratory (ME: x-axis) and less exploratory (LE: y-axis) partner. The thick black line is the best-fit linear model of the data ($R^2 = 0.09$, F = 7.3, P = 0.009, $\beta = 0.346$ [95% CI = 0.09, 0.60]). The color of each dot represents that individual's asocial exploratory score (A_i; see color legend at right).

3.2 Individual plasticity is largely determined by relative social condition, and leads to behavioural convergence between partners

To determine whether partners became more alike in exploratory behaviour during their interaction, we compared the difference in partners' asocial scores to their IPED, the difference between their scores

during the social trials (Figure 3A). The difference between partners' scores decreased from asocial to social trials (median asocial difference = 0.307; median IPED = 0.045; Wilcoxon signed-rank test, W = 3669, P < 0.001). This change in distance between individual scores of partners from the asocial to the social condition, which we denote ΔIPED, was significantly negative, indicating that partners' exploratory behaviour converged (Figure 3B; median change = -0.38; KS test, compared to randomized data, D = 0.26, P < 0.00001). The convergence in partners' behaviour was largely driven by increased exploration by the less exploratory (LE) individual. In nearly half of all social trials (45 of 96), the individual that was identified as less exploratory (LE) – based on asocial scores (A_i) – exhibited more exploratory behaviour during the social trials than the more exploratory (ME) individual (Figure 3A, the part of the social distribution that is < 0). Furthermore, during the LE social treatment, individuals displayed increased exploratory behaviour (compared to their exploratory behaviour in isolation), but this effect was not seen during the ME social treatment (Figure 3C). When individuals we relatively less exploratory than their partner (LE social treatment), the increase in exploratory behaviour was significantly greater than zero (U = 3497, P < 0.001; median change in score = 0.299). However, when individuals were the more exploratory partner (ME social treatment), they did not exhibit significant plasticity (U = 2315, P = 0.96; median change in score = -0.075). Comparing plasticity across social treatments confirmed that individuals expressed significantly different patterns of plasticity depending on whether they were the relatively less exploratory or more exploratory member of a pair (Figure 3C; KS test, D = 0.25, P = 0.005).
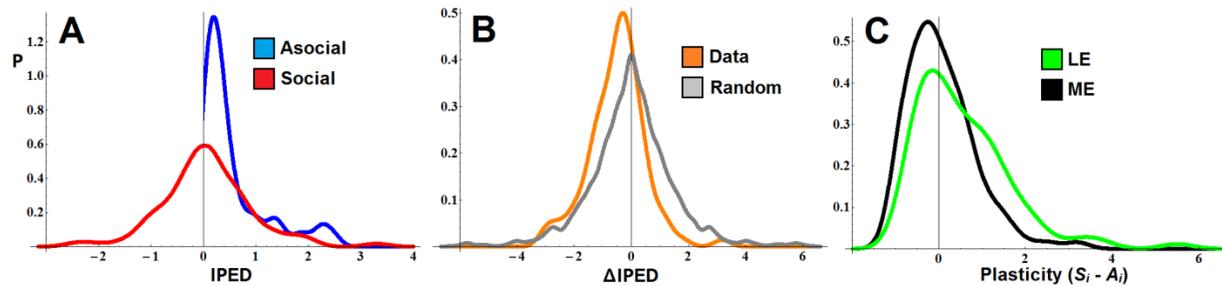

Figure 3: Distributions of differences in partner exploratory behaviour and plasticity. A. Density distributions of differences (between paired individuals) in asocial (blue) and social (red) exploratory scores (IPED). Only positive asocial values are shown (i.e., for each pair, the score for the less exploratory (LE) individual is subtracted from that of the more exploratory (ME) individual), and the same comparisons are used for the social distribution. B. The change in differences between partners from the asocial to the social trials (ΔIPED). The figure shows density distributions of ΔIPED for real (orange) and randomized (grey) pairings; see text for details. C. Density distributions of plasticity (change in exploratory tendency between the asocial and social treatments; S_i - A_i) when individuals were the more exploratory (ME; black) and less exploratory (LE; green) partner. See text for statistical comparisons. All distributions were created using the *SmoothHistogram* function in *Mathematica* (v.9, Wolfram Research).

3.3 Asocial exploratory tendencies do not predict the coordination of movement between individuals during pair trials

The more exploratory (ME) individual was not more likely to initiate excursions from the shelter (t(190) = 0.176, P = 0.392), be the first to return to the shelter (t(190) = 0.011, P = 0.398), or perform more solo excursions out from the shelter (t(190) = 0.009, P = 0.398). Higher asocial scores (A_i) were also not significantly correlated with initiating excursions (r = 0.137, t(94) = 1.336, P = 0.092), being the first to return to the shelter (r = 0.021, t(94) = 0.203, P = 0.420), or performing more solo excursions (r = 0.124,

t(94) = 1.216, P = 0.114). The difference in asocial scores between partners (IPED) did not correlate with mean excursion duration (r = 0.180, t(94) = 1.771, P = 0.080) or the mean distance between the fish (r < -8.6 X 10^{-18}, t(94) < -8.4 X 10^{-17}, P = 1). In summary, we did not find evidence that differences in asocial exploratory tendency directly determine the coordination of movement between partners.

3.4 Asocial exploratory tendency predicts the magnitude and direction of behavioural change

While relative exploratory tendency – whether an individual was more or less exploratory – determined whether individuals exhibited significant plasticity during the social trials, we attempted to understand what factors predicted individual plasticity in exploratory behaviour *within* each social treatment.

For both social treatments, the best fitting linear model of exploratory score had only one significant predictor variable: asocial exploratory score (A_i; Table 1, bottom). Individuals with the lowest asocial exploratory scores demonstrated the greatest plasticity: a large increase in exploratory score from asocial to social trials However, the best fitting model did not include the magnitude of inter-individual differences in exploratory tendency between partners as a predictor variable. . In other words, in our data, the primary determinant of plasticity was individual exploratory scores. The magnitude of the differences in exploratory tendency between partners did not significantly predict plasticity.

Table 1. Multiple regression analysis of individual plasticity for both social treatments. Different models were run for each treatment (ME and LE). In both treatments, individuals with lower asocial exploration scores (A_i) demonstrated greater plasticity in the direction of increasing exploratory behaviour.

	B	SE B	β	t	P
More exploratory treatment *(Adj. R^2 = 0.23, vs. null model F(2,93) = 15.26, P < 0.001)*					
Constant	0.145	0.111		1.305	0.195
A_i	-0.740	0.165	-0.416	-4.497	<0.001
Less exploratory treatment *(Adj. R^2 = 0.50, vs. null model F(1,94) = 97.82, P < 0.001)*					
Constant	0.308	0.083		3.72	<0.001
A_i	-0.911	0.092	-0.714	-9.89	<0.001

Note: N=96.

4. DISCUSSION

We examined the exploratory behaviour of zebrafish both alone and in pairs specifically constructed to explore the effects of differences in exploratory tendencies. By measuring exploratory behaviour in the exact same environment during both the asocial and social trials, (i.e., by analyzing individual movement during pair trials as if the focal fish were alone), we were able to isolate and quantify the effects of changing social treatment on individual behaviour. We found that the degree of plasticity in exploratory behaviour an individual displays is primarily determined by their relative exploratory tendency. Individuals only displayed significant plasticity – defined as the change in their behaviour from the asocial to the social treatments -- when they were the less exploratory partner (LE treatment). This plasticity was in the direction of increasing exploratory behaviour, such that in almost half of our pairings, the less exploratory (LE) individuals actually displayed stronger exploratory tendencies during the pair trials than their more exploratory (ME) condition partners. Within each social treatment, the best predictor of plasticity was an individual's asocial exploratory tendency (A_i), with less exploratory

individuals exhibiting greater plasticity, in line with predictions from the literature (Coppens et al., 2010; Harcourt et al., 2009; Herbert-Read et al., 2013; Hulthén et al., 2014; Jolles et al., 2015, 2014; King et al., 2015; Koolhaas et al., 1999a, 1999b; Kurvers et al., 2011; Magnhagen and Bunnefeld, 2009; Magnhagen and Staffan, 2005; Øverli et al., 2007; Ruiz-Gomez et al., 2008; Sneddon, 2003; van Oers et al., 2005; Ward et al., 2004; Webster and Ward, 2011).

The increase in exploratory behaviour by the less exploratory individuals resulted in a convergence of exploratory behaviours between partners during the pair trials, an effect that is in line with the 'conformity hypothesis' (King et al., 2015; Magnhagen, 2007; Webster and Ward, 2011). Results from previous work suggest a mechanism for this conformity effect: less exploratory individuals are known to be more responsive to their social environment and to pay more attention to social cues (Coppens et al., 2010; Harcourt et al., 2009; Jolles et al., 2015, 2014; Koolhaas, 2008; Koolhaas et al., 1999a; Kurvers et al., 2010; Magnhagen and Bunnefeld, 2009; Magnhagen and Staffan, 2005; Ruiz-Gomez et al., 2008; Sneddon, 2003; van Oers et al., 2005; Ward et al., 2004). By contrast, more exploratory individuals do not attend or react as strongly to their social situation. Conformity effects are therefore the result of less exploratory individuals adapting their behaviour to match that of their more exploratory partners.

Although the least exploratory individuals were the most plastic across social treatments, our analysis contradicts this hypothesized mechanism for conformity. We find that the amount of increased exploration displayed by the less exploratory partner – their 'adjustment' to the behaviour of their more exploratory partner – does not depend on the magnitude of the difference in exploratory tendency between the partners. In our data, the appearance of a conformity effect was driven by the least exploratory individuals showing the largest response to being in a social situation, but the magnitude of this response was not determined by the exploratory tendency of their partner. If conformity were being driven by the less exploratory individual in a pair attempting to match the behaviour of the more exploratory partner, we would have expected to see greater adjustments where the disparity between the partners was greater.

Unlike many published findings (Harcourt et al., 2010, 2009; Jolles et al., 2015, 2014; Leblond and Reebs, 2006; Nakayama et al., 2012), differences in asocial exploratory tendency was not found to directly determine the coordination of movement or leadership behaviours between partners in our experiment. Several studies of personality in a social context have shown that bolder individuals (which may share traits with our more exploratory individuals) become 'leaders' when paired with a shyer partner: they are more likely to leave a shelter first and to lead the pair's excursion out from the shelter (Harcourt et al., 2010, 2009; Jolles et al., 2015, 2014; Leblond and Reebs, 2006; Nakayama et al., 2012). We observed no such effects, and speculate that there are several potential reasons why. First, this study used zebrafish as a model organism, and it is possible that zebrafish coordinated movement is different from that of species used in prior work (primarily *Gasterosteus aculeatus*). Second, we used a purely exploratory task during the asocial and social trials. Unlike in other published studies, our exploratory task was not combined with a foraging task (i.e., there was no food in the enclosure and the fish were not food-deprived).

Perhaps most importantly, the setup of our pair trials differs in a key respect from that of most prior work. Pairs in our study were able to interact freely throughout their pair sessions, whereas in most previous work a barrier was kept between the fish to allow for individual identification (Harcourt et al., 2010, 2009; Jolles et al., 2016, 2015, 2014; Nakayama et al., 2012). The presence of a barrier during social interactions may confound social interactions with wall following behaviour, which both the current work and others (Dahlbom et al., 2011b; Ferrari et al., 2014; Jolles et al., 2015, 2014; Maximino et al., 2010b) have shown to be greater in less exploratory individuals. The fact that we did not observe any effects of exploratory tendency on leadership in our free-swimming pairs suggests that the presence of a barrier during social trials may fundamentally alter the dynamics of social interactions. Despite the lack of clear leader-follower dynamics, our data do indicate that individual behaviour was affected by the presence of a partner.

5. CONCLUSIONS

Consistent inter-individual differences, often termed 'personality', limit an individual's flexibility in reacting to changing environmental conditions – both physical and social. But the degree of plasticity across conditions that an individual displays can itself be considered a personality trait. Our results demonstrate that plasticity across social contexts in zebrafish varies between individuals in a consistent manner, is correlated to their asocial exploratory tendencies, and depends on the sign – but not the magnitude – of the relative personality of their partner. Plasticity is likely to also depend on many other factors that remain to be explored such as group size, the personality composition of the group, and the types of environmental challenges the group faces.

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Supplemental Materials:

Experiment Summary

Introduction

This analysis provides justification for the personality assignment methods used in the main experiment. The purpose of this experiment was to demonstrate that the asocial trials (as used in the main experiment) expose consistent individual differences and to determine how many days of asocial trials were necessary to capture consistent individual differences in exploratory personality. In addition, this experiment validates our methods for assigning exploratory personality scores by replicating our main experiment PCA results in a different population and strain of zebrafish.

General Methods

This validation experiment followed the same methods, apparatus, and procedures used in the main experiment for conducting the asocial trials and calculating individual PC1 scores with the exception that fish participated in 8 asocial trials instead of 3. Subjects were 34 adult unsexed zebrafish (*Danio rerio*) of the AB strain, bred and raised in the Burdine lab at Princeton University.

Comparing PCA Results from Main and Supplementary Experiments

Correlation Matrices

For both experiments we quantified the five following measurements from our individual movement trajectories: total distance travelled, average distance from the closest compartment wall, average distance from the shelter, total time spent out of the shelter, and average shelter visit duration. Covariance matrices of these five measurements for the main and supplementary experiments) demonstrated that during each experiment the five measures were all moderately correlated with one another, indicating that they each contributed separately to a fish's observed behaviour

Eigenvalues and Cumulative Variance Explained

We see further similarities between the two experiments in terms the eigenvalues and variance explained (Table S.1). One exception is that the second factor for the supplementary experiment was significant. Although during main experiment the second factor was close to significance (0.958) we, we ultimately decided to only use the first component to determine exploratory personality scores due to the consistency of the first factor loadings across experiments. More detailed analysis and reasoning for this decision are given in the next section.

Loadings of Measurements onto Factors

The measure correlations and first 2 PCA factors' measure loadings for the main and supplementary experiments are given in Table S.2. The first two factors of the PCA derived from the supplementary experiment had eigenvalues above 1: 2.67 and 1.11, explaining 53% and 22% of the variance, respectively. While the first factor loads neatly onto our intuitive concept of exploratory behaviour, the

second factor of the PCA has rather confusing loadings and an eigenvalue only slightly above 1. In the main experiment, only the first factor of the PCA achieved significance, (eigenvalues: 2.89, 0.96, explaining 58% and 19% of the variance), though the second factor came close. The loadings on the PC1 are very similar to the previous experiment and again confirm our intuition that it measures what we would call exploratory tendency. The pattern of loadings of the PC2 here are quite different from those in the supplementary experiment, and appear to represent 'thigmotaxis': excursions out from the shelter than remain very close to the walls of the enclosure.

That these data give an almost identical rotations onto the PC1 dimension but very different rotations onto PC2, led us to conclude that the variance captured by PC1 is structural and the variance captured by PC2 is either noise or perhaps due to strain specific factors. Therefore, PC2 was ignored when creating exploration scores during both experiments

Table S.1: *Main and Supplementary experiment eigenvalues and percentages of variance explained associated with each component resulting from Principle Components Analysis. The eigenvalues and percentage of variance explained for all components are remarkably similar between experiments.*

Component	Eigenvalue		Percentage of explained variance		Accumulated percentage of explained variance	
	Main	Supplementary	Main	Supplementary	Main	Supplementary
1	2.887	2.647	57.7	53.5	57.7	53.5
2	0.958	1.109	19.2	22.2	76.9	75.7
3	0.587	0.613	11.7	12.2	88.6	89.9
4	0.303	0.360	6.1	7.2	94.7	95.1
5	0.265	0.245	5.3	4.9	100.0	100.0

Table S.2: *Main and Supplementary experiment loadings of measurements on the principle and second components. The measure loadings for the first component are very similar between experiments and correspond well with established parameters of exploratory behaviour. The measure loadings on the second component are dissimilar between experiments, and not easily interpretable.*

Measures	Component 1		Component 2	
	Main	Supplementary	Main	Supplementary
Time Out	0.757	0.727	0.522	0.525
Distance from Wall	0.539	0.499	-0.780	0.769
Distance from Shelter	0.856	0.731	0.049	0.153
Total Distance	0.758	0.832	0.193	-0.390
Mean Latency in Shelter	-0.846	-0.818	0.194	-0.202

Repeatability Analysis

One of the main questions we wanted to answer with this experiment was: how many days of individual testing are necessary to establish a reliable estimate of exploratory personality? We decided to answer this question using repeatability analysis. Repeatability is a generalization of the intra-class correlation coefficient (ICC); a statistic widely used in psychology, and is often used in the field of animal behaviour as a measure of consistency across repeated measurements of a trait. Repeatability (*r*) is a measure of how much of the total variance in a sample is due to the between individual variance and is often represented as $\sigma_\alpha^2/(\sigma_\alpha^2 + \sigma_\varepsilon^2)$, where, the between individual variance is denoted σ_α^2 and the within individual variance is denoted by σ_ε^2. To determine how many days of individual testing were necessary to establish a reliable estimate of exploratory personality, we calculated repeatability of the PC1 scores for the first *n* days. The results of this cumulative trial repeatability analyses are summarized in Table S.3.

Table S.3: *Repeatability of cumulative days' PC1 scores from the supplementary experiment. Number of measures, within and between individual variance, repeatability estimate, lower and upper 95% confidence intervals estimates, and p values for each repeatability calculation are given. With three days included in the analysis, the PC1 repeatability estimate reaches significance. Including additional days in our repeatability estimate did not meaningfully change the repeatability estimate or the associated values. Note: p<0.001* indicates significance following Bonferonni correction of α = 0.0038.*

Days	# Measures	σ_ε^2	σ_α^2	ICC(r)	Lower CI	Upper CI	p
D1-D2	2	0.7	0.2811	0.2865	-0.03	0.58	0.036
D1-D3	3	0.5573	0.4158	0.4273	0.23	0.64	<0.001*
D1-D4	4	0.6401	0.3526	0.3552	0.22	0.58	<0.001*
D1-D5	5	0.641	0.3612	0.3604	0.26	0.59	<0.001*
D1-D6	6	0.6687	0.3134	0.3191	0.22	0.53	<0.001*
D1-D7	7	0.6512	0.3009	0.3161	0.22	0.52	<0.001*
D1-D8	8	0.6399	0.293	0.3141	0.2	0.5	<0.001*

We decided to could conduct additional repeatability analysis to determine if the between and/or within individual behavioural variance in our sample changed as a function of time (repeat trials) and affected the repeatability estimates. Carter et al., 2013 raised the possibility that individual behavioural variations only emerge when information is unreliable, and in most cases information is unreliable because an animal has entered a novel environment. This theory predicts that individual behavioural variations should be large in the first recorded behavioural measures, but decrease over time as individuals become more familiar with the testing apparatus (via repeat trials). This raises the possibility that changes in within and between individual variance could occur over time and potentially affect repeatability estimates. If large changes in sample variances occur in the early trials, it may be prudent to base exploratory personality assignments on data collected from later trials. To test for this, we conducted a "sliding window analysis" of repeatability by dividing the PC1 data into three days windows, incrementing each window one day at a time. Analysis windows were chosen to be three days long based on the results from the cumulative trial repeatability analysis (Table S.4). If Carter et al.'s hypothesis holds for our sample, then we expect analysis conducted on a subset of later days from our sample (Days 6-8) should show both decreased between individual variance (as all individuals behave more similarly) and decreased within individual variance (as individuals behave more consistently) compared to a subset of earlier days (Days 1-3). The results of our sliding window repeatability analyses are summarized in Table S.4.

Table S.4: *Sliding window repeatability analysis PC1 scores from the supplementary experiment. Number of measures, within and between individual variance, repeatability estimate, lower and upper 95% confidence intervals estimates, and p values for each repeatability calculation are given. The estimates of within individual variance, between individual variance, and repeatability did not meaningfully change over time. Note: p<0.001* indicates significance following Bonferonni correction of α = 0.0038.*

Days	# Measures	σ_ϵ^2	σ_α^2	ICC(r)	Lower CI	Upper CI	p
D1-D3	3	0.5573	0.4158	0.4273	0.23	0.64	<0.001*
D2-D4	3	0.5226	0.3898	0.4272	0.32	0.7	<0.001*
D3-D5	3	0.5746	0.3606	0.3856	0.22	0.63	<0.001*
D4-D6	3	0.5969	0.2619	0.305	0.088	0.52	<0.001*
D5-D7	3	0.4494	0.378	0.4569	0.25	0.65	<0.001*
D6-D8	3	0.5014	0.3006	0.3748	0.15	0.58	<0.001*

Results of Repeatability Analysis

Both the cumulative trial repeatability analysis and the sliding window repeatability analysis strongly support using the first three repeated measures of asocial trial data to assign exploratory personality scores based on PC1 data. The cumulative trial analysis demonstrated that only two days of data were not significant after Bonferonni correction, and that after three days of cumulative data the between and within individual variance and repeatability value remained consistent with the addition of each subsequent day of data (Table S.4).

This experiment was also used to explore how many days of solo trials were necessary before the behaviour of the fish. Looking at the sliding window analysis of PC1 repeatability in Table S5, we see no evidence of within and/or between individual variance following any trend over time. Based on the results of the sliding window repeatability analysis, we conclude that individuals are neither becoming more similar, nor more consistent over time, so there is no reason to use any three days other than the first three.

We find these results to form a strong argument for using data from the three initial repeated measures of our solo behavioural assay to assign exploratory scores. Using the first three repeated measures to derive exploratory scores balances erring on the side of statistical caution with spending the least amount of valuable time gathering unnecessary repeated measures.