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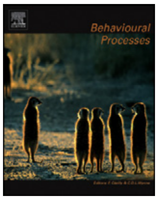

Sequential hermaphroditism and personality in a clonal vertebrate: The mangrove killifish

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ABSTRACT

Individuals are regularly documented to consistently differ in their behavioural types (BTs). For example, some individuals are bold whereas others are shy. Within the human personality literature, the big five personality dimensions are commonly documented to be sex-specific with testosterone suggested to underpin traits such as aggressiveness. In non-human animals recent research suggests sex-specific BT expression may be influenced by ecology, mating system and sexual selection. While most research on sex-specific personality has focused on dioecious species, we explore sex differences in BT expression in a sequential hermaphrodite the mangrove killifish. We replicate within 7 isogenic genotypes and investigate sex differences (hermaphrodite and secondary male) in three BTs (exploration, boldness and aggression). This approach allows us to investigate sex differences in BT expression whilst controlling for genetic variation. In this study we find that both secondary males and hermaphrodites are repeatable at the individual level yet there was no difference between the sexes in average BT scores. Furthermore, aggression scores differed between genotypes, and were repeatable at the genotype level, suggesting strong genetic control. Finally, male boldness was significantly more repeatable than hermaphrodites potentially supporting recent proposals relating to sexual selection. We document a behavioural syndrome in male fish with bolder individuals being more aggressive, this behavioural syndrome was not observed however in hermaphrodites. In contrast to a previous developmental study in this species exploration did not correlate with either aggression or boldness in either males or hermaphrodites.

1. Introduction

The study of animal personality is a rapidly growing subdiscipline of behavioural ecology, which focuses upon individual differences in behavioural types (BTs) (Bell, 2007; Reale et al., 2007; Sih et al., 2004b). Individuals are, for example, regularly documented to differ in boldness in the presence of predators, and aggressiveness towards conspecifics (Huntingford, 1976). Such behaviours tend to be stable over time i.e. repeatable, and have fitness consequences (Gosling, 2001; Sih et al., 2004a; Dingemanse and Reale, 2005; Smith and Blumstein, 2008). Behavioural types have been documented to be heritable (Drent et al., 2003; van Oers et al., 2004; Dingemanse et al., 2002; Sinn et al., 2006; Reale et al., 2009) and several neurotransmitter genes and associated single nucleotide polymorphisms, have been found to be important in personality expression (reviewed in Reif and Lesch, 2003; Ebstein, 2006; Inoue-Murayama et al., 2011). Multiple BTs such as boldness and exploration also often tend to co-vary in a variety of species, giving rise to what are commonly termed behavioural syndromes (Sih et al., 2004b). The presence of correlated suites of behaviours has been suggested to constrain independent BT expression; resulting from genetic correlations between traits (Sih et al., 2004b) and/or the same genes or hormones acting on several targets i.e. pleiotropy (Ketterson and Nolan, 1999).

A major factor that appears to be important in personality expression is sex. In humans for example the presence of sex differences in personality are regularly reported in the big 5 personality dimensions: openness, conscientiousness, extraversion, agreeableness, and neuroticism (McCrae and Costa, 1999). Males for example, generally take more risks (Byrnes et al., 1999) and exhibit greater assertiveness, while being less trusting and anxious (Feingold, 1994; Costa et al., 2001). Furthermore, males tend to be more aggressive than females (see Archer, 2006 for a discussion) which is thought to be driven by sex differences in testosterone and serotonin (see Nelson and Trainor, 2007 for reviews; Nelson and Chiavegatto, 2001; Archer, 1991). In addition to genetic and hormonal effects cultural and environmental influences during development are additional factors that may play an important role in the expression of human personality (Schmitt et al., 2008; Champoux et al., 2002).

Within the non-human animal personality literature research has documented that sex differences in average BT expression is by

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no means clear cut. For example while males tend to be bolder in some species (e.g. brown trout: Johnsson et al., 2001; and Trinidadian guppies: Dugatkin, 1988; Piyapong et al., 2010), no such sex differences are observed in others (e.g. Trinidadian killifish: Fraser et al., 2001; and great tits: Carere and van Oers, 2004; Dingemanse et al., 2002) suggesting evolutionary influences upon personality. In recent years, research has also begun to document that sex differences in personality traits are influenced by multiple ecological and social factors. For example sex-specific aggression and boldness have been found to be influenced by predation risk and motivation for access to mates or resources (Archard and Braithwaite, 2011; Brown et al., 2007). Moreover, sex differences in aggression have been documented to be mating system specific, with males tending to be more aggressive in poylgynous species (e.g. Johnsson et al., 2001; Bakker, 1986), whereas, females may be more aggressive in polyandrous or monogamous mating systems (e.g. Arnott and Elwood, 2009; Swenson, 1997; Goymann et al., 2004).

Recently, there has been growing interest into the mechanisms generating and maintaining consistent individual differences in behaviour (Dall et al., 2004; Schuett et al., 2010; Wolf et al., 2007; Stamps and Groothuis, 2010a,b; Stamps, 2007; Dingemanse and Wolf, 2010). In a recent review article, sexual selection was proposed as a likely mechanism generating sex differences in personality traits (see Schuett et al., 2010 for a review). Although personality has received minimal direct attention in relation to sexual selection, boldness has been shown to influence female mate-choice in guppies (*Poecilia reticualta*), with bolder males attaining higher reproductive success (Godin and Dugatikin, 1996). In addition behaviour repeatability, also termed consistency, has been proposed to be a sexually selected trait (Schuett et al., 2010). In this scenario, if a male exhibits consistent behaviour in one context and consistency predicts future behaviour expression, females may gain fitness benefits from choosing males based upon their repeatability. Male behavioural consistency may therefore be a reliable cue during mate choice from which females can predict a partner's behaviour (Nakagawa et al., 2007; Schuett et al., 2010; Schuett and Dall, 2009) or choose mates based upon behavioural or genetic compatibility (Schuett et al., 2011b). Sexual selection for personality is, however, by no means simple being likely to depend upon species-specific intra-sexual and inter-sexual selective pressures (Pruitt et al., 2011).

BTs have generally been studied within dioecious species; however, many organisms exhibit various forms of hermaphroditism (Ghiselin, 1969) with multiple examples within the teleost fishes alone (Sadovy de Mitcheson and Liu, 2008). Studying the expression of behavioural types in such species has the potential to provide new insight into the mechanism and functions underpinning the expression of personality traits in animals. Here we investigate commonly studied personality traits in *Kryptolebias marmoratus*, a self-fertilising, simultaneous hermaphrodite that outcrosses with functional males (primary and/or secondary). By using replicated isogenic genotypes that have both hermaphroditic and secondary male phenotypes we are able to control for genetic variation and tease apart the effect of genotype and sex on BT expression. In a previous developmental study (Edenbrow and Croft, 2011) we have shown that individual *K marmoratus* were not repeatable in boldness and exploration during the early stages of life (infant–juvenile–early adulthood). In addition, although individual level behavioural expression was not repeatable i.e. absence of personality *per se*, we have shown that these behaviours correlate at the genotype level, and these correlations emerged at 61 days post hatching and were maintained until day 151 of life (early adulthood) highlighting that maturation is an important driver of trait covariance. In this study, we expand upon this previous work and investigate whether size matched hermaphrodite and secondary male phenotypes differ in their behavioural repeatabilities, average BT expression, and individual level behavioural syndromes. Using 7 genotypes we investigate three commonly studied BTs; namely exploration of a novel maze, boldness following an aerial predation simulation, and aggression towards a mirror image. We predict that, if male personality is sexually selected in this species that males will exhibit significantly higher repeatability estimates in comparison to hermaphrodites for each of the three behavioural traits measured.

2. Methods

2.1. Study species

Within *K. marmoratus*, repeated self-fertilisation over successive generations has been documented to remove genetic diversity, generating homozygous, isogenic genotypes (Mackiewicz et al., 2006a). In addition, occasional outcross events between hermaphrodites and males has been shown to facilitate recombination of parental DNA following which, selfing returns a lineage to homozygosity (Lubinski et al., 1995; Mackiewicz et al., 2006a,b,c; Taylor et al., 2001). This interesting reproductive strategy therefore generates complex population genetic architectures, within which multiple genetically independent homozygous genotypes exist (Sato et al., 2002). In addition to self fertilisation, the mangrove killifish also exhibits sequential hermaphroditism i.e. sex change, where self-fertilising hermaphrodites lose female function, becoming secondary males (Harrington, 1971, 1968). These secondary males are also brightly coloured, compared to hermaphrodites, exhibiting orange caroteniod colouration. Males and hermaphrodites within a single genotype can therefore be classified as monozygotic twins that differ in their sexual and morphological phenotype. This species therefore presents an exciting model with which sex differences may be investigated while controlling for genetic variation.

2.2. General protocols

All fish were housed in 20 ppt synthetic salt water (Instant Ocean^{TM}) at 25 ± 0.5 °C in a 12 h light/12 h dark cycle and fed ad libitum with artemia naupili (*Artemia salinas*). 20 fish representing 20 homozygous genotypes were reared from hatching (15 day variation in hatching date) within stock tanks (394 mm × 250 mm × 140 mm) containing 3 l of salt water. All fish were maintained in genotype specific aquaria and all fish exhibited hermaphroditic phenotypes at ~3 months of age (mottled brown colouration and fertilised egg production). At approximately 600 days of age some hermaphrodites began to develop orange carotenoid colouration which has been documented to be a robust indicator of secondary male formation, during which female function ceases (Sakakura and Noakes, 2000). Stock tanks, containing hermaphrodites and secondary males, were maintained until 650 ± 15 days of age, at which point equal numbers of secondary males and hermaphrodites from each tank were selected. To control for size variation within each tank we selected pairs of hermaphrodites and secondary males that were size matched (±3 mm). Genotypes produced variable numbers of secondary males and thus the number of replicates per genotype were uneven (males per genotype (mean ± S.D.): 3.8 ± 1.4 (see Appendix Table A.1)) giving a total of 27 males and 27 hermaphrodites. Fish were then individually housed for 2 months in standard aquaria (28 cm × 17.5 cm × 16 cm) containing 2 l of salt water and a sparse layer of gravel, permitting us to further ensure phenotypic males laid no eggs prior to experimentation. After this 2-month isolation period, behavioural assays were completed for exploration, boldness and aggression.