Heritable variation underlies behavioural types in the mating context in male bluefin killifish

Katie E. McGhee [a,*], Joseph Travis [b]

[a] School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, IL, U.S.A.
[b] Department of Biological Science, Florida State University, Tallahassee, FL, U.S.A.

In many species, consistent behavioural differences among individuals are linked to fitness variation. Determining the environmental and genetic factors that mould these behavioural types is crucial to understanding how behaviours might respond to selection. Male bluefin killifish, *Lucania goodei*, show extensive consistent behavioural variation in their levels of courtship, male-directed aggression and female-directed aggression, resulting in a range of fitness-related behavioural types coexisting within a population. To determine whether the behavioural components underlying a male's stable behavioural type in the mating context are heritable and genetically correlated, we performed paternal half-sib crosses. Using animal models, we found that all three of these mating behaviours were moderately heritable ($h^2 = 0.17-0.29$) and courtship behaviour was also heritable as a binomial trait (court yes/no: $h^2 = 0.50$). Including effects of dam identity/common rearing environment experienced by full sibs decreased model fit, suggesting that early social interactions might contribute to behavioural types. In addition, we found evidence consistent with the possibility that the positive phenotypic correlations among mating behaviours are underlain by positive genetic correlations. Thus, it is possible that the seemingly maladaptive aggression that males direct towards females during social interactions persist due to genetic constraints and direct selection on male-directed aggression and courtship behaviour.

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Unlike the case with their morphological and life history traits, individuals can often alter their behaviour in seconds in response to changing conditions. However, it is increasingly recognized that this behavioural plasticity can be limited. For example, some individuals tend to be consistently more aggressive, bold or exploratory than other individuals and these consistent behavioural types, or personalities, can extend across entirely different functional contexts (Sih et al. 2004a, c). That suites of behaviours are stably correlated with one another (i.e. behavioural syndromes) opens the possibility that behavioural reactions to different stimuli are not independent of one another and thus that any individual's range of behaviours might be constrained (Sih et al. 2004a, c; Sih & Bell 2008).

Discerning how much of the phenotypic variation reflects underlying genetic variation is important to our inferences about how behavioural types might respond to selection (Dochtermann & Roff 2010; Dochtermann 2011). While a number of studies have found evidence for genetic variation in behavioural types (Dingemanse et al. 2009; Réale et al. 2009; Schielzeth et al. 2011; reviewed in: van Oers et al. 2005; Réale et al. 2007), stable behavioural tendencies can also be influenced by environmental conditions (Stamps & Groothuis 2010a, b). For example, behavioural types can be affected by abiotic environmental factors early in development (e.g. Carere et al. 2005; Arnold et al. 2007; Chapman et al. 2010) as well as by contemporary environmental conditions (e.g. Bolhuis et al. 2006; Biro et al. 2010; Pruitt et al. 2011). Similarly, there is accumulating evidence that behavioural types can be influenced by social experiences during development and in the recent past (e.g. Frost et al. 2007; McGhee & Travis 2011; Naguib et al. 2011) as well as by the current social environment (e.g. Travis & Woodward 1989; Schuett & Dall 2009; Piyapong et al. 2010; Morand-Ferron et al. 2011). The social environment in particular could exert powerful but subtle effects on behavioural types via dominance interactions and winner–loser effects (Hsu et al. 2006), information transmission (Earley 2010) and position within a social network (Krause et al. 2010).

The freshwater bluefin killifish, *Lucania goodei*, offers a striking example of fitness-related behavioural types and a system in which

* Correspondence: K. E. McGhee, School of Integrative Biology, University of Illinois at Urbana-Champaign, 433 Morrill Hall, 505 S. Goodwin Ave., Urbana, IL 61801, U.S.A.
   E-mail addresses: kemcghee@illinois.edu, katieemcghee@gmail.com (K. E. McGhee).

their origin is especially interesting. During the breeding season, males defend areas of aquatic vegetation from other males and spawning with females occurs in these territories (Foster 1967; Fuller 2001), but there is no evidence of parental care (Fuller & Travis 2001). Females are highly iteroparous and mate with multiple males daily throughout the breeding season (McGhee et al. 2007; K. E. McGhee, personal observation). Males show extensive behavioural variation in a competitive mating context, ranging from males that show intense aggressive and courtship behaviour to males that rarely show aggression and courtship in the presence of a rival male. These differences among males in their aggression and courtship levels are highly repeatable ($r = 0.61$–0.72) and lead to stable outcomes in dominance interactions (McGhee & Travis 2010). Most importantly, males that show high levels of aggression towards males and females, as well as high levels of courtship, have increased spawning success in the laboratory (McGhee et al. 2007) and in the field (Fuller 2001).

In this study we examined whether the behavioural components that contribute to a male's behavioural type within the mating context are significantly heritable and thus, might respond to selection. We also examined whether the strong phenotypic correlations among these behaviours might be underlain by similarly strong genetic correlations. To measure behaviours within the mating context, we assessed a male's response to both a female and a rival male presented simultaneously. Our aim was to create an ecologically relevant behavioural assay that captures the conflicting stimuli (i.e. potential mates versus rivals) that a male must deal with during the breeding season.

METHODS

Half-sib Breeding Design

We collected adult fish from the Upper Bridge site on the Wakulla River, FL, U.S.A., in March 2007 by dipnet and seine. We chose sires randomly with respect to colour pattern and size and paired each male with two randomly chosen dams (note: one sire was paired with three dams). Each dam was housed in a large 114-litre tank filled with well-water at a greenhouse with natural light. Each tank had an external activated carbon bio-filter and two yarn mops, each one consisting of a floating yarn mop attached to a yarn mop on a sunken piece of PVC. We assessed the water quality in the tanks monthly and adjusted it as needed. We covered the sides of the tanks with sheets of aluminium foil to prevent behavioural interactions between tanks. We fed adults frozen *Artemia* and chironomids daily. We switched males between their two dam tanks every other day and collected eggs from the yarn mops daily. We reared 33 complete half-sib families ($N_{sires} = 33$, $N_{dams per sire} = 2$ except for one sire who was mated to three dams thus $N_{dams total/full-sib families} = 67$).

We put fertilized eggs in small plastic buckets with fresh well-water and several drops of methylene blue to prevent fungal growth. After hatching (10–14 days postfertilization), larvae were reared in small buckets and fed hatched *Artemia* nauplii for ~2 weeks. We put approximately 30 fry per cross (one sire X one dam) into fresh 114-litre tanks covered with aluminium foil on the sides. Fry tanks were randomly distributed with respect to family across tables throughout the greenhouse. In March 2008 we moved a subset of the largest fully mature fish (approximately six males and six females per rearing tank/full-sib family) to the laboratory for behavioural trials. This was to ensure that the fish for the behavioural assays were sexually mature and F1 not F2 offspring. In doing this, there is the possibility that we biased our sample to behavioural types with fast growth (Stamps 2007; Biro & Stamps 2008). Although offspring within a rearing tank differed by less than 7 mm in length on average (range of sizes across all rearing tanks = 19–40 mm), further examination of whether growth rates are associated with behavioural traits in this species are needed.

Offspring Behavioural Trials

While housed in the laboratory, we kept fish in brother–sister pairs (due to space limitations) in 19-litre tanks with a yarn mop and sponge filter. Opaque black plastic covered the walls to prevent behavioural interactions between neighbouring tanks. We fed fish frozen *Artemia* daily and maintained them at thermal (22 °C) and light (14:10 h light:dark cycle) regimes typical of the natural breeding season. For each behavioural trial, we tested three sons per full-sib family on average (range 2–4 sons, $N_{total individuals} = 192$), and thus we obtained behavioural measures on 4–8 sons per half-sib family. Males were tested randomly with respect to family, and thus siblings were not tested more closely in time than unrelated individuals.

We measured each male's behavioural reaction to a live female and a live male introduced simultaneously into the focal male's tank. Our aim in this assay was to simulate a potential mating opportunity in the presence of a nearby rival male similar to what occurs in nature when females swim through neighbouring male territories. We recorded the focal male's aggressive and courtship behaviour towards the female as well as their aggressive behaviour towards the rival male. We conducted these trials in a focal male's home tank but removed the female (sister) for >24 h before the trial so that focal males were alone in their tank until the addition of a new female. In the afternoon on the day before the trial, we removed the filter and the yarn mop and put an unfamiliar gravid field-caught female in a transparent, porous container overnight in the focal male's tank. We began the trial the next morning. We released the female and added a field-caught stock male that was physically distinguishable from the focal male. Field-caught males were kept in a large stock tank and were likely reused across trials but they were not reused within 48 h.

Once the focal male encountered the female or rival male, we recorded all of the focal male's aggressive behaviours (fin flares, sigmoids, chases and attacks) and courtship behaviours (head-flicks and courting circles) for 10 min (600 s). Fin flares consist of a male spreading both dorsal and anal fins at another individual. Sigmoids often precede an attack and consist of a male moving sideways towards another individual and curving its body in a sigmoid shape. Chases consist of a male chasing another male with a brief burst of speed. Attacks consist of a male rapidly lunging at another individual and striking the individual with its head. Headflicks consist of a male rapidly flicking his head side to side under the abdomen of the female or directly in front of the female. Courting circles consist of a male slowly swimming in a looping circle either around, to the side or in front of the female. To reduce the number of behavioural variables, we calculated (1) the total number of aggressive behaviours directed towards the other male in the trial (sum of fin flares + sigmoids + chases + attacks), (2) the total number of aggressive behaviours directed towards the female (sum of fin flares + sigmoids + attacks), and (3) the total number of courtship behaviours directed towards the female (sum of headflicks + courting circles). We measured standard length of both the focal male and the rival male immediately after the assay.

Estimation of Variance Components

To estimate all variance components, we used the 'animal model' as implemented in the MCMCglmm R package (Hadfield 2010), which uses Bayesian statistics with Markov chain Monte Carlo (MCMC) simulations. Prior to analysis, behavioural data were mean-