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Maternal investment influences development of behavioural syndrome in swordtail fish, *Xiphophorus multilineatus*

Danny M. D'Amore [a, *], Oscar Rios-Cardenas [b, 1], Molly R. Morris [a, 2]

[a] *Department of Biological Sciences, Ohio University, Athens, OH, U.S.A.*
[b] *Instituto de Ecología A.C., Red de Biología Evolutiva, El Haya, ASD, Veracruz, Mexico*

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Despite a rising interest in behavioural syndromes (correlations between different behaviours across context and time), the development of these syndromes is not well understood. Using the swordtail fish *Xiphophorus multilineatus*, we looked at the effects of maternal investment and social environment during development on the formation of a behavioural syndrome. A previous study demonstrated that the diet on which mothers were reared, controlling for their size, influenced the growth rate and size of their sons: females reared on high-quality diets had sons that grew faster and were larger at sexual maturity. In addition, males that were exposed to an adult male during development grew faster. We examined the behaviours of these same males across two contexts: aggression towards a mirror image and boldness under threat of predation. We detected an influence of maternal investment but not of social environment on the formation of a behavioural syndrome. The sons of mothers reared on high-quality diets exhibited a positive correlation between boldness and aggression, while there was no correlation among the sons of mothers reared on low-quality diets. Maternal investment influenced the formation of the syndrome by producing changes in only one of the behaviours, the behaviour most closely linked to energy acquisition (boldness under risk of predation) rather than energy expenditure (aggression). Our results suggest that energy balances could help predict the circumstances that would favour the formation of behavioural syndromes. Our results also support the hypothesis that behaviours involved in energy acquisition as compared to energy use will be more sensitive to fluctuations in variation in resources.

Behavioural syndromes are consistent correlations of behaviours across contexts and time within a population (Sih, Bell, & Johnson, 2004), and understanding their development will not only provide valuable insights into the adaptive costs and benefits of behavioural plasticity, but are important for designing the studies to examine their causation and evolution (Stamps & Groothuis, 2010). Behaviours that are not independent across varying situations can persist if the errors of individual behavioural types cost less than remaining behaviourally plastic, assuming the information required to behave optimally is available in a given context (Bell, 2007; Huntingford, 2004; Sih et al., 2004). However, if the development of syndromes is plastic, the benefits of correlated behaviours could be achieved in some environments without the costs associated with syndromes in other environments (Stamps, 2007). Despite growing interest in behavioural syndromes across taxa, our understanding of their development is limited (Stamps & Groothuis, 2010). In this study, we examined the influence of maternal investment and early social environment on the development of a behavioural syndrome in the swordtail fish *Xiphophorus multilineatus* to gain further insights into the factors influencing its development.

Mothers across taxa produce inherited environmental effects through a variety of mechanisms, such as adjusting the sex ratio within a brood, manipulating brood size, and adjusting hormonal and nutritional resources partitioned to individual offspring (Mousseau & Fox, 1998). Variation in resource allocations to eggs influences metabolic and growth rates in brown trout, *Salmo trutta* (Regnier, Bolliet, Labonne, & Gaudin, 2010) and swordtails (*X. multilineatus*: Murphy, Goedert, & Morris, 2014; Rios-Cardenas, Brewer, & Morris, 2013), and growth rates have been suggested to be associated with the development of behavioural types and syndromes in fish (Huntingford, 2004; Regnier et al., 2010). These

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* Correspondence: D. M. D'Amore, Department of Biological Sciences, Life Sciences Building 245, Ohio University, Athens, OH 45701, U.S.A.
E-mail address: da045312@ohio.edu (D. M. D'Amore).
[1] *E-mail address*: oscar.rios@inecol.mx (O. Rios-Cardenas).
[2] *E-mail address*: morrism@ohio.edu (M. R. Morris).

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maternal effects are not limited to fish. Variation in prenatal hormonal investment influences the responses of adult zebra finches, *Taeniopygia guttata*, towards novel stimuli (Tobler & Sandell, 2007), the reproductive behaviour of adult house sparrows, *Passer domesticus* (Partecke & Schwabl, 2008), as well as growth rates and escape behaviours in garter snakes, *Thamnophis elegans* (Robert, Vleck, & Bronikowski, 2009). In addition, the juvenile social environment has been hypothesized to influence the development of a behavioural syndrome (Huntingford, 2004) and has been shown to influence individual behaviours such as aggression (e.g. green swordtail, *Xiphophorus helleri*: Hannes & Franck, 1983; mangrove killifish, *Kryptolebias marmoratus*: Edenbrow & Croft, 2012) and social dominance (e.g. blue tits, *Cyanistes caeruleus*, and great tits, *Parus major*: Hansen & Slagsvold, 2004) in adult animals. However, the extent to which maternal effects and social environment influence the development of behavioural syndromes has not been previously examined.

We tested the behaviours of males of the swordtail *X. multilineatus* to investigate the extent to which maternal investment and social environment influence correlations between behaviours across two contexts: aggression towards a mirror image and boldness under risk of predation. Murphy et al. (2014) found that both maternal investment and early social environment influenced growth rates in these males. Controlling for mother's size, mothers reared on high-quality diets produced fry that were larger, grew faster and were larger at sexual maturity (Murphy et al., 2014). In addition, male offspring exposed to an adult male during early development grew faster than control male offspring. Given the hypothesis that consistent differences in growth rates can lead to the development of behavioural syndromes (Huntingford et al., 2010; Stamps, 2007), we hypothesized that variation in maternal investment and/or social experience as juveniles could influence the formation of a behavioural syndrome.

One of the interesting differences between the two behaviours we examined is their relationship to an individual's energy balance. Aggression is often associated with energy expenditure, whereas boldness is often associated with energy acquisition (Biro & Stamps, 2010). This is very likely to be the case in swordtails as well. Male aggression towards conspecifics is primarily a behaviour that uses energy; in nature, males defend access to groups of feeding females (Morris, Batra, & Ryan, 1992). In contrast, boldness under risk of predation is a behaviour associated with energy acquisition, as being bold around a predator increases time available to forage. Alterations in food intake are predicted to have stronger effects on energy balance than alterations in expenditure (Prentice, 2005). Therefore, we examined whether variation in maternal investment and early rearing environment would influence the behaviours from the two contexts independently, to determine whether variation in these two factors influences changes in both behaviours. We predicted that the behaviour involved in energy acquisition would be more sensitive to environmental changes.

METHODS

The *X. multilineatus* males we tested ($N = 60$) were reared to sexual maturity by Murphy et al. (2014) to examine the influences of maternal investment on growth rate. The mothers of the males we examined were laboratory reared to sexual maturity by Lyons, Goedert, and Morris (2013) on two diets (high quality versus low quality) that differed primarily in protein content. The high-quality food treatment, Tetra-min Tropical Flakes (Tetra®, United Pet Group, Blacksburg, VA, U.S.A.), had 47% protein content and was supplemented by bloodworms three times a week; the low-quality food treatment, Nishikoi Wheat Germ Koi Food (Nishikoi®, Whitehall, Wethersfield, Essex, U.K.), had 20% protein content and was not supplemented. Protein content influences female reproductive performance in swordtail fish (Chong, Ishak, Osman, & Hashim, 2004). Once females reached sexual maturity, they were all bred to males from the large size class (genotype Y-L), which controlled for differences in maternal allocation based on male alternative reproductive tactic (Rios-Cardenas et al., 2013). Offspring of each brood were individually isolated at 2 weeks of age in separate 5-gallon (18.9-litre) ‘home’ aquaria and maintained on the low-quality diet treatment. At 70 days of age, half of the fry in each brood were presented with an adult courter male in a fry box (a clear plastic box that allows water exchange without physical contact) once daily for 5 min, for 2 weeks; the other half of the fry were presented with an empty fry box for the same amount of time. Twenty-eight courter males measuring 32–47 mm standard length (SL) were used for the exposure treatment. Growth rates were measured by subtracting size (measured as SL) at 70 days (beginning of social treatment) from size at 130 days (prior to sexual maturity) and dividing by 60 days (see Murphy et al., 2014). We tested males for aggression (mirror test) and boldness (predator inspection test) 14–17 weeks after sexual maturity, with 24–28 h between the two tests. We tested fish for aggression prior to testing for boldness to decrease the possibility of carryover effects (Bell, 2013) and because fish exposed to predators take longer to recover than fish exposed to a conspecific or mirror image (Bell et al., 2007).

Mirror Test (Aggression)

Fish were left in their individual tanks, their established ‘home territory’, for the aggression test. One hour before testing, we removed all refugia from the tank. Gravel substrate was left undisturbed. Then, the focal fish was presented with a mirror on the outside of its tank lengthwise and observed for 300 s. We recorded time until first approach (latency, scored as negative numbers, with lower numbers being more aggressive), number of displays, number of bites and number of approaches (following Moretz & Morris, 2003). After testing, the mirror was removed and all refugia were replaced.

Predator Inspection Test (Boldness)

The predator *Herichthys cyanoguttatus*, which is similar to predators of *X. multilineatus*, was placed in one of two side compartments of the test tank (50-gallon (189.3-litre); each side compartment, 47 × 32 × 43 cm; central compartment, 73 × 32 × 43 cm), separated by glass. This allowed visual recognition of the predator without water exchange or physical contact. The focal fish was then placed in an opaque tube at the centre of the test tank. After 60 s of acclimation, we released the fish by lifting the tube out of tank (via a string from behind a viewing screen). The focal fish was observed for 300 s. We recorded the time spent in the third of the tank closest to the predator, the time until first approach and the number of approaches (following Bell & Stamps, 2004).

Analysis

We used a principal component analysis to determine which of the behaviours measured in each context best explained variation in behaviour (i.e. heaviest loading and most consistent across treatments). We used latency to approach the mirror as our measure of aggression, and number of predator approaches as our measure of boldness (see Table 1).

Determining whether the maternal diet and early social environment affected the behaviours in one or both contexts required