Animal Behaviour 133 (2017) 153–160

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Developmental social experience of parents affects behaviour of offspring in zebrafish

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ARTICLE INFO

Article history:
Received 7 March 2017
Initial acceptance 10 May 2017
Final acceptance 11 August 2017

MS. number: 17-00218

Keywords:
activity
aggression
anxiety
behaviour
isolation
traits
transgenerational effects

Interactions between conspecifics early in life have the potential to shape phenotypic differences between individuals. These changes in phenotype may subsequently be passed to future offspring, something that has been studied in live-bearing mammals where there is often an element of parental care. The present study considers the transgenerational effects of social environment in zebrafish, *Danio rerio*, an egg-laying animal that shows no parental care, thus removing any influence of parental interaction and allowing the effects of conspecific interaction to be clearly determined. Zebrafish (F_0) were reared from fertilization to reproduction in three different social treatments: isolation, groups of 30 or groups of 100. At 28 days post fertilization, individuals were tested for anxiety and activity and at 3 months old for aggression. These F_0 fish were raised to sexual maturity and bred within their treatment group. The F_1 generation were then raised in groups of 30, irrespective of parental social environment and were assessed for behaviour in the same way as their parents. Social isolation increased anxiety and decreased aggression in the F_0 fish compared to those raised in social groups of 100. F_0 fish raised in social groups of 30 showed an intermediate response. Differences in anxiety were not passed to the F_1 generation; however, offspring of socially isolated fish were less aggressive than offspring of parents from social groups of 30 and 100. The social environment that an individual experienced from fertilization to reproduction affected their own behaviour and the behaviour of their offspring.

The early social environment experienced by an individual influences the development of behaviour as individuals in a social environment can learn from their conspecifics by observing them engaging in particular activities (Brown & Laland, 2001; Suboski & Templeton, 1989). Deprivation of social interaction early in development can, therefore, affect a variety of behaviours in a range of animals. For example, dairy calves housed individually immediately after birth were more reactive to environmental and social novelty than group-housed calves and calves housed with an older companion (Vieira, de Passille, & Weary, 2012). The majority of mammalian studies have considered the effects of social interactions after birth, although there is also evidence in rodents that interfetal communication can have a significant effect on behaviour later in life (vom Saal, 1989). In oviparous fish, which lay eggs into the external environment, exogenous cues such as the smell of predators or alarm cues from adults can alter developmental processes (Mirza, Chivers, & Godin, 2001; Mourabit, Rundle, Spicer, & Sloman, 2010). For example, in rainbow trout, *Oncorhynchus mykiss*, raised in different social group sizes from fertilization, presence of conspecifics affected both physiology and behaviour. Trout raised in isolation had lower oxygen consumption rates and were less aggressive towards their own mirror image than individuals raised in social groups (Sloman & Baron, 2010).

The developmental environment experienced by an organism may alter not only its own phenotype but also the behaviour of its offspring. Parental influence on offspring phenotype (both maternal and paternal) can occur by genetic and epigenetic mechanisms. Maternal effects are traditionally considered to be nongenomic, that is, they are not related to gene sequence, although there is variation in the way 'maternal effects' are defined (Wolf & Wade, 2009). Both paternal and maternal effects have been documented in the literature, although maternal effects have received the most attention and have been studied in a wide variety of animals (mammals: Inhasz Kiss, Woodside, Felicio, Anselmo-Franci, & Damasceno, 2012; birds: Guibert et al., 2011; Rubolini et al., 2005; reptiles: Robert, Vleck, & Bronikowski, 2009; Uller & Olsson, 2006; fish: Andersson, Silva, Steffensen, & Höglund, 2013; Eriksen et al., 2011; Sloman, 2010). For example, a study on the pea aphid, *Acyrthosiphon pisum*, showed that exposure of females to

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https://doi.org/10.1016/j.anbehav.2017.09.009
0003-3472/

the alarm pheromone (E)-β-farnesene prior to reproduction, a cue for predation risk, resulted in a change of feeding sites in offspring (Keiser & Mondor, 2013). Postnatal communal rearing in Balb/c mice, *Mus musculus*, induced transgenerational effects on emotional and reproductive behaviour of offspring (Curley, Davidson, Bateson, & Champagne, 2009). Communally raised females that received more postpartum maternal care exhibited lower anxiety, built higher quality nests and showed more postpartum care as adults than standard reared females. This behaviour was further carried through to F_2 mice that exhibited lower anxiety, larger litter size and increased nursing, suggesting the effect of postnatal social environment on the behaviour of offspring across generations (Curley et al., 2009).

Early in development the epigenome of an individual can be influenced by environmental and nutritional factors, such as transfer of hormones or nutrient provision by mothers to eggs or offspring (Nafee, Farrell, Carroll, Fryer, & Ismail, 2008). Parental conditions can alter the phenotype of offspring (Chen et al., 2013; Franzke & Reinhold, 2013; Krause & Naguib, 2014; Pittet, Le Bot, Houdelier, Richard-Yris, & Lumineau, 2014) and while some authors may conclude that these transgenerational effects occur via epigenetic mechanisms (Youngson & Whitelaw, 2008), others would argue that when considering environmentally-induced effects, an epigenetic basis can be inferred only if changes last over multiple generations (Grossniklaus, William, Ferguson-Smith, Pembreym, & Lindquist, 2013; see also Burggren, 2014). Mechanisms that allow transfer of information about the maternal environment to offspring are likely to be advantageous if they prepare the offspring for the environment they will be born into (Love, McGowan, & Sheriff, 2013; Sheriff & Love, 2013). Parental mechanisms that allow adjustments of offspring phenotype based on immediate parental environment may allow more flexibility than selection on genotypes (Crews, 2008; Wisenden, Sailer, Radenic, & Sutrisno, 2011).

A number of studies have looked at the effect of variations in the social environment during development. However, it is not known whether changes in phenotype induced by early social interactions can be passed across generations. Furthermore, previous studies have addressed transgenerational effects wherein the parents were exposed to the experimental manipulation only during a certain point of their own development and transferred to control conditions before reaching sexual maturity; any responses in offspring could thus be the result of differences in natal and adult environments (reviewed in Burton & Metcalfe, 2014). Therefore, the aim of the present study was, first, to examine the effects of different social environments maintained from fertilization to reproduction in F_0 zebrafish, *Danio rerio*, and, second, to investigate any transfer of behavioural effects to the subsequent F_1 generation. Social environments were varied by number of individuals rather than stocking density; it was hypothesized that zebrafish raised in different social environments would exhibit differences in behaviour later in life and these differences in behavioural phenotype may be transmitted to future generations.

METHODS

Adult zebrafish (AB, TL mixed strains) from an existing stock at the University of the West of Scotland were held on a recirculating system (27 ± 1 °C; pH 7.1 ± 0.4; dissolved oxygen 90 ± 5%; 14:10 h light:dark) and fed *Artemia* or Aquarian tropical flake twice daily. Fish were bred to produce embryos (F_0 generation) which were collected within 30 min of fertilization and placed into group sizes of 1 ($N = 36$), 30 ($N = 3$) and 100 ($N = 3$). The different group sizes were held in 50, 500 and 5000 ml containers with 10, 30 and 1000 ml of system water, respectively, and placed in a water bath at 28.5 °C. The walls of the containers were opaque to block any visual cues and a 30% water change was carried out daily. From 5 days post fertilization (dpf) larvae were fed Liquifry and ZM 00 daily. One week after hatching, fry were transferred in their group sizes into flow-through tanks with opaque sides on the main recirculating system. Tanks sizes were 1, 3 and 12 litres containing 0.94, 2.95 and 11.5 litres of water, respectively. Fry were fed ZM 100 from 11 dpf and ZM 200 and *Artemia* from 15 dpf. From 30 dpf they were fed twice daily with flake food and *Artemia*. Variations in tank sizes between the different treatments ensured that treatments represented a change in number of individuals within the social group, not differences in stocking density. An overview of the experimental design is shown in Fig. 1.

Breeding of F_0 Fish

When the F_0 fish were 3 months old, they were bred to produce the F_1 generation. To achieve this, F_0 fish raised in social isolation were combined within their treatment resulting in three replicate breeding tanks for each treatment. Thirty F_1 embryos from each replicate of each F_0 treatment (i.e. $N = 3$) were then raised in 1.5-litre containers held at 28.5 °C. Thus, F_1 offspring from all F_0 treatments were held at the same density and the only difference between F_1 treatments was the social environment experienced by their parents. Two weeks after hatching, fry were transferred into 3-litre flow-through tanks on the main recirculating system. The feeding regime was the same as for the F_0 generation.

Behavioural Testing of F_0 and F_1 Fry

At 28 dpf F_0 ($N = 27$) and F_1 ($N = 18$) fry were tested for their behaviour in the light and dark box test and the novel tank diving test. At 3 months old, fish ($N = 27$ F_0; $N = 18$ F_1) were tested for their response to their own mirror image as zebrafish show similar levels of aggression towards a mirror image as towards an opponent (Ariyomo & Watt, 2013).

Light and Dark Box Test

The experimental tank (16 × 10 cm and 10.5 cm high) was divided vertically in half and covered externally on the sides and bottom by white or black paper. The top was left completely open. The half of the tank covered externally with a white background represented the light compartment and the half with a black background was the dark compartment. An opaque cylinder was placed in the centre of the tank creating a compartment (5 cm diameter) for acclimating the fish. The tank was filled with system water which was replaced at the end of every trial (Blaser & Rosemberg, 2012; Maximino, de Brito, Colmanetti et al., 2010; Maximino, de Brito, Dias, Gouveia, & Morato, 2010). Fish were individually placed into the central compartment for 5 min, after which time the walls of the central compartment were removed, allowing the fish to freely explore the tank. Behaviour was recorded for 10 min with a video camera (Panasonic SD video camera, SDR-S50) placed above the arena. The tank was illuminated with a white daylight bulb (70 W) and the illumination kept constant between trials. The behavioural end points measured were the proportion of time the fish spent in the dark compartment (scototaxis), the time spent beside the walls (thigmotaxis) and the number of transitions between the light and dark compartment (activity) (Blaser & Rosemberg, 2012; Maximino, de Brito, Colmanetti et al., 2010; Maximino, de Brito, Dias et al., 2010).