Hatching asynchrony and offspring sex influence the subsequent exploratory behaviour of zebra finches

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The ecological and evolutionary consequences of variation in behaviour between individuals are well studied, yet the causes of such variation remain poorly understood. Parents can generally exert a great deal of influence on the early life of their offspring, so one possibility is that the mode of parental investment determines subsequent offspring behaviours. In birds, hatching asynchrony occurs when females begin incubation prior to clutch completion, and the subsequent hatching regime creates phenotypic disparities between early and late-hatched offspring. By experimentally manipulating hatching patterns in zebra finches, *Taeniopygia guttata*, we examined the influence of hatching asynchrony on subsequent exploratory behaviour during adulthood. Late-hatched birds from asynchronous broods explored the novel environment more than their siblings which hatched earlier or came from synchronous broods, although there were no differences in exploratory behaviour towards a novel object. Additionally, females were bolder towards a novel object than were males, independently of hatching regime, but there were no sex differences in exploration of the novel environment. Our results support the idea that both hatching asynchrony and sex have previously underappreciated influences on offspring behaviours, and highlight the importance of variation in parental investment in generating and maintaining behavioural variation.

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Consistent variation in behaviours between individuals is a taxonomically widespread phenomenon (Dall 2010). Behavioural variation occurs in relation to a wide range of social, antipredator and sexual activities and is under strong selection pressures as such variation has important consequences for the fitness of individuals (Dall 2010; Krause & Naguib 2011). Individuals often exhibit consistent differences in suites of correlated behaviours, such as aggression, fearfulness, risk taking, exploration and reactivity that generally vary along a shy–bold continuum (Wilson et al. 1994; Sih et al. 2004; Bell 2007; Réale et al. 2007), and are often considered analogous to human personality traits (Wilson et al. 1994).

Variation in exploratory behaviours between individuals is widespread, presumably because, being essential components of finding foraging and breeding sites and avoiding predators (Dall 2010; Krause & Naguib 2011), they are under strong natural selection pressure. Furthermore, exploratory behaviour is a well-established proxy for a personality trait in birds (Bell 2007; Réale et al. 2007), which may have important ecological and evolutionary consequences because they can influence fitness (Smith & Blumstein 2008). Additionally, because the sexes are subject to different and sometimes conflicting selection pressures, behaviours are often expressed differently between males and females (Krause & Ruxton 2002; van Oers et al. 2005; Schuett & Dall 2009; Piyapong et al. 2010; Lendvai et al. 2011). We should, therefore, expect sex differences in many behaviours, and previous studies have shown sex differences in exploratory behaviour in great tits, *Parus major* (van Oers et al. 2005), and zebra finches, *Taeniopygia guttata* (Schuett & Dall 2009; Mainwaring et al. 2011a). While the ecological and evolutionary consequences of behaviours are important for a wide range of traits linked to fitness (Réale et al. 2007; Smith & Blumstein 2008), the causes of variation in behaviour remain poorly understood.

Rearing conditions have an impact upon many aspects of an animal's life history, so it is possible that they also contribute to the variation in a range of behaviours (Lindström 1999; Metcalfe & Monaghan 2001), including exploratory behaviours (Stamps 2007; Stamps & Groothuis 2010; Reddon 2012). Furthermore, it has been suggested that parents may adaptively influence offspring behaviours to suit future environmental conditions (Marshall & Uller 2007; Uller 2008). For example, one study showed that offspring raised under poor nutritional conditions showed compensatory growth and subsequently explored less than offspring raised under control conditions (Krause & Naguib 2011). An important aspect of parental care in birds is parentally controlled hatching asynchrony (Magrath

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1990; Forbes 2011). This can create phenotypic size variation within a brood, which has a direct impact upon sibling competition for resources, such as parentally provisioned food (Royle et al. 2002; Forbes 2011). Within the brood hierarchy, early and late-hatched nestlings face different selection pressures, which can influence traits such as growth (Mainwaring et al. 2010a; Forbes 2011) and subsequent sexual attractiveness (Lindström 1999; Metcalfe & Monaghan 2001; Mainwaring et al. 2012), but also, it has been suggested, variation in subsequent behaviour as an adult (Reddon 2012). Therefore, it is highly likely that poor conditions experienced during early life may well affect basic behavioural traits such as exploratory behaviour and food searching (Krause & Naguib 2011).

This study aimed to examine the influence of hatching asynchrony on the subsequent exploratory behaviour of zebra finch offspring, a species in which parental care has been extensively studied (Zann 1996; Griffith & Buchanan 2010). Domestic zebra finches lay one egg per day and both parents begin to incubate the clutch as soon as the first egg is laid, so that broods hatch asynchronously over approximately 4 days (Mainwaring et al. 2010b). There is a decline in the deposition of egg constituents (Royle et al. 2003; Blount et al. 2006) and an increase in the egg mass/volume (Royle et al. 2003; Rutkowska & Cichoñ 2005; Mainwaring et al. 2012) with laying order. Meanwhile, previous studies have shown sex-specific laying order patterns in zebra finches with males (Clotfelter 1996) and females (Kilner 1998) hatching from eggs laid early in the laying sequence. Parents respond to competitive sibling interactions, so early-hatched nestlings are provisioned preferentially (Mainwaring, et al. 2011b) and remain larger throughout the nestling period (Mainwaring et al. 2010b; Gilby et al. 2011). Developmental conditions are known to influence exploratory behaviour during adulthood (Krause & Naguib 2011) and there are also sex differences in exploratory behaviour (Schuett & Dall 2009; Mainwaring et al. 2011a). Consequently, we would predict that hatching asynchrony would influence the subsequent exploratory behaviour during adulthood, and that the effect may differ between the sexes.

METHODS

General Methods

Zebra finches were housed at Lancaster University in a temperature-controlled room at 20 °C, under full light spectrum, artificial light (Bennett et al. 1996) and on a 16:8 h light:dark regime. All birds were ringed and, prior to breeding, birds lived in single-sex aviaries where the other aviary was visible but no contact was possible. When in the aviaries, birds were provided daily with ad libitum seed, cuttlebone, grit and drinking water in containers and weekly with a vitamin supplement (vitamins A and E) added to the drinking water, charcoal and bathing water (Mainwaring et al. 2010b, 2012).

Birds that were randomly chosen as breeding birds were, for a period of 7 days, introduced to breeding cages (120 × 45 cm and 40 cm high) with another bird of the same sex, and were given a diet of rearing food and seed. Then, each of the 15 pairs was assigned a breeding cage, with access to an externally attached nestbox lined with hay and fresh nesting material. Birds were provided with the same food, drinking water and associated vitamins and bathing water as those birds in aviaries (see details above). Furthermore, tray liners were exchanged for new ones on a weekly basis, and all cages were thoroughly cleaned on a monthly basis (Mainwaring et al. 2010b, 2012).

Manipulation of Hatching Patterns

For breeding, we used birds that had previously reared at least one brood. Pairs were randomly assigned to the asynchronous (control) or synchronous (experimental) hatching regime and, after the first breeding event, they were immediately allowed to have a second brood with the alternative regime. Consequently, genetic effects on offspring behaviour were controlled as much as possible. In experimental broods, eggs were removed on the day of laying and replaced with an artificial egg and returned on the day following clutch completion, in order to establish hatching synchrony. Experimental eggs were kept in small, tissue-lined bowls designed to match closely the natural nest conditions and all experimental eggs were turned daily to prevent the yolk from settling. Temporary storage in this way does not affect embryonic or nestling development in a range of bird species (Sachdev et al. 1988; Siikamäki 1998) including zebra finches (Rutkowska & Cichoñ 2005; Mainwaring et al. 2010b). The manipulation of hatching patterns had no effect on the hatching success, as defined by the proportion of eggs that hatched, of synchronous and asynchronous clutches (Mainwaring et al. 2010b, 2012). In all nests, brood sizes were maintained at four, either by the addition of extra foster nestlings from other broods, or through fostering nestlings to other broods as necessary (Mainwaring et al. 2010b). Note that the eight extra foster nestlings hatched from the corresponding eggs, so that, for example, a brood of three had a nestling added that was from a fourth-laid egg. The experimental manipulation of hatching intervals was successful as all of the synchronous broods hatched within 24 h and the maximum difference in hatching weight within those broods never exceeded 0.5 g (Mainwaring et al. 2010b, 2012; Gilby et al. 2011). Within asynchronous broods, early-hatched nestlings were defined as those hatching from the first- and second-laid eggs and late-hatched nestlings were defined as those hatching from the third- and fourth-laid eggs. When the oldest nestling was 10 days old, the mean masses of early-hatched asynchronous (9.75 ± 0.31 g) and synchronous (9.43 ± 0.25 g) nestlings were greater than late-hatched asynchronous (7.65 ± 0.32 g) nestlings (Mainwaring et al. 2010b, 2012). Nestlings remained with their parents until day 35, and were then transferred to mixed-sex cages until day 50, when the sexes were separated into single-sex cages before being moved to single-sex aviaries at day 65. Note that both aviaries were identical to each other and that there were similar numbers of birds in each aviary, meaning that sex-specific behaviours were not determined by any differences between the aviaries.

Novelty Tests

Two different tests of exploratory behaviour in response to novelty were performed: exploration of a novel environment and behaviour towards a novel object (Martins et al. 2007; Mainwaring et al. 2011a). Tests were carried out when the birds were 300−500 days old, by which time the mean masses of early-hatched asynchronous (14.48 ± 0.21 g), late-hatched asynchronous (14.21 ± 0.18 g) and synchronous (14.75 ± 0.16 g) nestlings were all comparable to each other (Mainwaring et al. 2010b, 2012). All tests were carried out in a room isolated from the flight cages, and as no ambient sounds were broadcast, then tests were also carried out in acoustic isolation. Before the tests, none of the birds were exposed to the novel environment or object. All tests were carried out between 1000 and 1600 hours and there was a 15 min interval between tests.

Novel Environment

The first experiment tested the extent of exploratory behaviour in a novel environment. Prior to the trials, birds were deprived of food, but not water, for 1 h to standardize hunger levels. The test cage contained five empty white feeding trays (60 × 40 mm and 40 mm high), which were familiar to the birds and were consistently positioned in the same place within the cage for all trials. Focal birds