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Short communication

Parthenogenesis in a passerine bird, the Zebra Finch *Taeniopygia guttata*

E. SCHUT,† N. HEMMINGS & T. R. BIRKHEAD*
Department of Animal & Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK

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Parthenogenesis is defined as cell division in a female gamete without any genetic contribution from the male (Beatty 1967, Rougier & Werb 2001). When such cell division results in the development of an embryo parthenogenesis is known as 'virgin birth'. Parthenogenesis occurs naturally in certain plants and animals and in the latter is most frequent among invertebrates (e.g. aphids and *Daphnia*). Among vertebrates parthenogenetic development occurs in a small number of fish and reptile species (Soumalainen 1948, Watts *et al*. 2006) and has been recorded occasionally in mammals, including humans (Rougier & Werb 2001, Kono *et al*. 2004).

Parthenogenetic development in birds was first reported in the domestic fowl *Gallus domesticus* (Oellacher 1872), but was also discovered in the domestic pigeon *Columba livia* (Bartelmez & Riddle 1924) and the domestic turkey *Meleagris gallopavo* (Olsen & Marsden 1954). In all three bird species parthenogenetic development was disorganised and almost always abortive. In the chicken, parthenogenetic development was relatively infrequent (< 5% of eggs) with only a single record of a parthenogenetic adult fowl (Sarvella 1973). Parthenogenesis was more common in the turkey (up to 20% of eggs; Olsen & Marsden 1954, Savage & Harper 1986) and by selective breeding an entire strain of parthenogenetic birds, all of which were male and some of which produced fertile spermatozoa, was produced (Olsen 1975).

Parthenogenetic development has not been previously reported in any passerine birds (Johnson 2000). Here we report the occurrence of parthenogenetic development in the Zebra Finch *Taeniopygia guttata*.

*Corresponding author.
Email: T.R.Birkhead@sheffield.ac.uk
†Present address: Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

CRITERIA FOR DETERMINING PARTHENOGENETIC DEVELOPMENT

Parthenogenetic development in the fowl, turkey and pigeon was established in virgin females whose eggs exhibited some embryo development in the form of cell nuclei in the germinal disc or unorganised embryonic tissue development (see Olsen 1975 for review; see also Savage 1995). The most important criteria for establishing parthenogenetic development are: (i) the complete absence of sperm associated with the ovum, (ii) a smaller number of cell nuclei than expected for the stage of development in the germinal disc (GD), and (iii) GD cell nuclei that are typically aggregated and with an irregular form (Kosin 1945, Kosin & Nagra 1956).

METHODS

During the course of a study to establish criteria for distinguishing between unfertilised eggs and those that had undergone early embryo mortality in a passerine bird (the Zebra Finch), in which we examined the ova of hundreds of eggs (T.R. Birkhead unpubl. obs.), we discovered several instances of apparent parthenogenesis. The birds were part of a population of captive, domesticated Zebra Finches maintained at the University of Sheffield since 1985. Levels of inbreeding in this population are low and almost identical to those in a wild population (see Birkhead *et al*. 2006). In the present study birds were maintained in cages (63 × 48 × 39 cm high) on a 10:14 light:dark cycle, with a regular ad lib finch mixed seed diet, supplemented with minced hard-boiled egg, and ad lib water. Eggs exhibiting apparent parthenogenetic development came from females that had been separated from any male for a minimum of six months. Since the maximum recorded duration of sperm storage in the Zebra Finch is about 13 days (Birkhead *et al*. 1989), these females could not lay fertile eggs. To induce egg laying in these females they were 'paired' to males but behind a wire screen that prevented any physical access. The eggs of these birds were examined after 0, 24 or 48 h of artificial incubation.

We used the following criteria in combination to identify parthenogenetic eggs: (1) complete absence of sperm associated with the perivitelline layers of the egg, and (2) the presence of irregular cell nuclei in the germinal disc (that is, nuclei whose surface was not smooth and rounded – see Fig. 1). The absence (or presence) of sperm associated with the outer perivitelline layer and holes (made by sperm) on the inner perivitelline layer of the entire ovum, but in particular over the germinal disc was established using methods described by Gupta and Bakst (1993; see also Bakst & Cecil 1997). Briefly this consisted of removing and separating the inner and outer perivitelline layers from the ovum and staining the entire outer perivitelline layer with the fluorescent live-dead stain dye (LIVE/DEAD cytotoxicology assay, Molecular Probes, Invitrogen, Carlsbad,

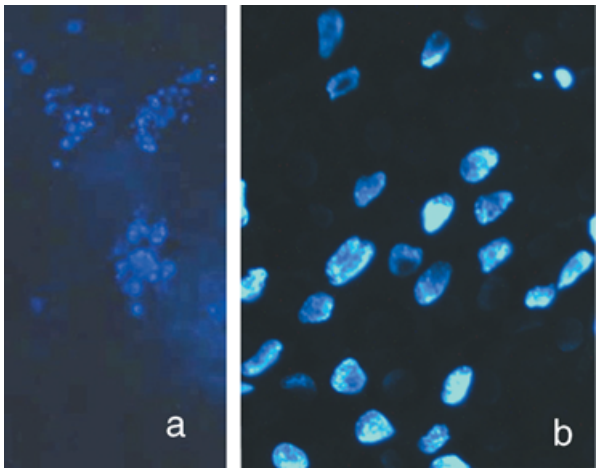

Figure 1. (a) Irregularly shaped nuclei from an infertile egg exhibiting parthenogenetic development stained with Hoechst dye 33342, and (b) regularly shaped nuclei from a fertile, developing egg stained with Hoechst dye 33342.

CA, USA), incubating the tissue in the dark for 5 min and then examining it with a microscope (at × 40). The entire inner perivitelline layer was examined under dark field optics (at × 20) to visualise any holes. The presence (or absence) of cell nuclei in the germinal disc (GD) was established using the method described by Gupta and Bakst (1993), which was briefly as follows: material from the GD was dispersed in a pipette in phosphate buffered saline (PBS) and placed on a microscope slide, 10 µl of Hoechst 33342 (0.05 mg/ml) added, incubated in the dark for 5 min and then examined with a microscope (at × 20). Any cell nuclei present stained bright blue. The number of nuclei was estimated either by counting fields of known area and extrapolating, or in cases when few nuclei were present by systematically scanning the entire slide. The shape of the nuclei was recorded as either regular (i.e. indicative of normal development) or irregular (indicative of parthenogenetic development: Kosin & Nagra 1956) and photographed for later examination and comparison with reference material from fertile eggs.

RESULTS

We detected seven instances of parthenogenetic development out of 34 eggs (21%) laid by three females (in several successive clutches). Six of these eggs were unincubated and one was incubated for 24 h when they were examined. No instances of parthenogenesis were detected among a further 11 eggs examined after 48 h of incubation [including one egg from a female, all of whose eggs (examined at < 48 h incubation) exhibited parthenogenesis]. This latter observation is consistent with the fact that in poultry parthenogenetic nuclei often disintegrate within a few days of the onset of incubation (Kosin 1944, Romanoff 1960).

DISCUSSION

Our finding of parthenogenetic development in Zebra Finch eggs appears to be the first report of natural parthenogenesis in a passerine bird. Since parthenogenetic development has apparently not previously been looked for in other passerines, it is possible that it also occurs in other species. In other taxa parthenogenetic development has been shown to arise through several different routes (Rougier & Werb 2001); in the present study we did not attempt to identify the underlying mechanism resulting in parthenogenetic development.

We have one further observation of parthenogenesis in another species: in a single egg from a captive female domestic Canary *Serinus canaria* paired to a male Eurasian Bullfinch *Pyrrhula pyrrhula* (in an attempt to establish whether interspecific fertilisation occurs – see Birkhead & van Balen 2007), we found no sperm or holes on the perivitelline layers, but 77 cell nuclei in the germinal disc. In total we examined 51 Canary eggs from eight different Bullfinch–Canary pairs (some of which laid more than one clutch), without sperm or holes on the perivitelline layers, but this was the only case of apparent parthenogenesis.

We also detected what appeared to be parthenogenetic development in the eggs of Zebra Finches that had been paired and then, after laying the first egg, separated by a wire screen to prevent any further copulations. Of 185 eggs from such pairs we recorded 18 (9.7%) cases of apparent parthenogenesis where there were no sperm and holes present on the perivitelline layers and no fertilisation had apparently occurred, but there were cell nuclei in the germinal disc. We are cautious about claiming these to be cases of parthenogenesis because given that the birds were paired we cannot be absolutely certain that a single sperm had not penetrated the germinal disc. On the other hand, most of these cases involved pairs that had been separated by a wire divider for at least 13 days (the maximum duration of sperm storage) and the nuclei in these cases were similar in their irregular appearance to those cases (above) where females had been separated from males for several months.

Knowing that parthenogenesis can occur in non-domesticated birds may be important in helping to resolve the cause(s) of hatching failure. Hatching failure is common in birds (Koenig 1982) and ornithologists often refer to unhatched eggs as 'infertile' even though hatching failure may be the result of either a failure of the ovum to be fertilized, or embryo mortality. If embryo mortality occurs within two or three days of oviposition, before any macroscopic signs of development, it is easily confused with an unfertilised egg (Gupta & Bakst 1993, T.R. Birkhead pers. obs.). As we have shown here, unfertilised eggs may sometimes exhibit a degree of parthenogenetic development, and may therefore be mistaken for a fertile egg exhibiting early embryo mortality. We discuss this further elsewhere (T.R. Birkhead, N. Hemmings, J. Hall & E. Schut in prep.).

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