Rare occurrence of embryonic twins in the Hihi (Stitchbird) Notiomystis cincta: an endangered passerine of New Zealand

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The Hihi (Stitchbird) Notiomystis cincta is a closely monitored, endangered, endemic passerine of New Zealand. The breeding success of one re-introduced island population, established on Tiritiri Matangi in 1995, has been a particular focus of detailed study by the New Zealand Department of Conservation and universities (e.g. Armstrong et al. 2002, Taylor et al. 2005). As part of this research, unhatched eggs during the last three seasons (2002/03, 2003/04 and 2004/05) were opened and the contents classified according to the stage of embryonic death (early death = no distinguishable features, mid death = eyes and body shape discernible, late death = fully formed embryo; the timing of these stages is not yet known) (Table 1). In total, 830 eggs from 204 nests were examined. In one clutch during the 2004/05 breeding season, the first-laid egg (from a clutch of four) was found to contain two intact ‘mid death’ embryos. The embryos did not differ in size, but, as in the few reported cases of twinning (Hollander & Levi 1940, Newman 1940), one embryo appeared to be deformed with a large protuberance on the anterior region of the head (Fig. 1). The cause of the deformity is not known.

Twinning can occur because of three circumstances: early embryonic cleavage resulting in identical monozygotic twins (Sturkie 1946), two blastoderms fertilized sharing a single yolk, or as two blastoderms on individual yolks encased within the same shell, with these last two scenarios resulting in non-identical embryos (Hollander & Levi 1940). The embryos were clearly connected by a shared yolk stalk, indicating that they must have originated from a single yolk. Unfortunately, the egg contents were too degraded to confirm this. In addition, as this was the first-laid egg in the clutch, we cannot infer the number of yolks from the presence or absence of a gap in laying. Without microsatellites, we are unable to determine the exact circumstance of twinning. However, if embryos result from early embryonic cleavage they would be the same sex. Although not definitive, it is interesting to examine the sex of the embryos, as differing sexes would allow embryonic cleavage to be ruled out. Using polymerase chain reaction-based sexing techniques, we identified both embryos as female. DNA was extracted from two separate samples of each Hihi (hht1 and hht2) as outlined in Huynen et al. (2003) and compared with DNA extracted from samples of known male and female Hihi. Samples were amplified in 10-µL reactions using CHD primers 2572F (5′-GTGGCAACAGAGTCTGATTTTCT-3′) and 2694R (5′-CCTCAATTCCCTTTTTATGAC-3′), which amplify one band (~500 bp) for males, and two (~500 and ~650 bp) for females. The mix was heated to 94 °C for 2 min, and then subjected to ten cycles of 94 °C for 20 s, 54 °C for 20 s and 72 °C for 20 s, before another 30 cycles of 94 °C for 20 s, 50 °C for 20 s and 72 °C for 20 s. Electrophoresis of amplified products was carried out in 1% LE/1% MS (low electroendosmosis molecular screening) agarose in tris borate EDTA (TBE) buffer. The products were then stained with ethidium bromide and viewed over UV light (Fig. 2). Given a shared yolk stalk and same sex, it seems unlikely that these twins were the result of individually fertilized yolks.

The incidence of twinning in birds is rare, but taxonomically diverse (e.g. domestic Chicken Gallus gallus, Nalbandov 1942; Mallard Anas platyrhynchos, Batt et al. 1975; Adélie Penguin Pygoscelis adeliae, Asheimer & Grau 1985; Emu Dromaius novaehollandiae, Bassett et al. 1999), and is generally identified incidentally during experiments or poultry farming. Occasionally, it is also observed in wild populations during routine ornithological monitoring (e.g. Cartwright 1939, Berger 1953, Griffith & Stewart 1998). No evidence of twinning has previously been detected for...
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Table 1. Reasons for hatching failure (number of eggs remaining/total eggs laid) of the total number of eggs laid by Hihi during three seasons on Tiritiri Matangi Island, New Zealand.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of eggs (no. of nests)</th>
<th>Infertile</th>
<th>Early death</th>
<th>Mid death</th>
<th>Late death</th>
<th>Unknown</th>
<th>Hatching failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004/05</td>
<td>356 (87)</td>
<td>36</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>34*</td>
<td>28.9</td>
</tr>
<tr>
<td>2003/04</td>
<td>283 (69)</td>
<td>9</td>
<td>15</td>
<td>4</td>
<td>9</td>
<td>19†</td>
<td>19.8</td>
</tr>
<tr>
<td>2002/03</td>
<td>191 (48)</td>
<td>4</td>
<td>17</td>
<td>5</td>
<td>3</td>
<td>20†</td>
<td>25.7</td>
</tr>
</tbody>
</table>

*Eggs abandoned before incubation.
†Eggs not opened, contents unknown.

Figure 2. Genetic sexing of Hihi twin embryos (hht1 and hht2). Amplified Hihi sex-specific DNAs are marked by arrowheads: a single DNA product indicates a male and two products a female. The female-specific product is amplified preferentially, so appears brighter. The negative control is indicated by nc. Known male (hhM) and female (hhF) Hihi were used as controls. DNA size markers were provided by a 1-kb+ DNA ladder (Gibco-BRL).

Hihi, despite the contents of unhatched eggs being inspected, where possible, for all monitored Hihi breeding attempts (R. Colleen, Mt Bruce Wildlife Centre; L. Huynen, Mokoia Island; this study). However, the likelihood of detecting twinning when embryonic death occurs early during incubation is very low. Our study provides further evidence of the potential for many species to produce twins, and in wild populations these may occur often but go unnoticed. We can only tentatively conclude that twinning is extremely rare in Hihi, and urge close examination of all unhatched eggs from this and other species to determine the rates of occurrence and potential causes in populations of wild birds.

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REFERENCES


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