An Anomalous Northern Saw-whet Owl (*Aegolius acadicus*) Egg

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An anomalously large and coloured egg was found within a clutch of the Northern Saw-whet Owl (*Aegolius acadicus*) in Nova Scotia; all other eggs of the clutch were within the normal size and colour range for the species. Analysis of three mitochondrial genes suggests all eggs in the clutch were laid by Northern Saw-whet Owl(s) with similar genetic make-up. This is the first report of an anomalous egg from this species, and a rare example of added pigment.

Key Words: Northern Saw-whet Owl, *Aegolius acadicus,* egg, Nova Scotia.

For most birds species, some variation in size and pigmentation of eggs laid by an individual female is common and expected (Williams 1994; Takagi 2003). Such variation may result from natural variations in food availability (Hakkarainen and Korpimäki 1994; Aparicio 1999) or food supplementation (Wiebe and Bortolotti 1995). However, unusual eggs can sometimes be found in a bird's clutch, either as a result of interspecific brood parasitism (Lowther 1993) or intraspecific brood parasitism ("egg-dumping"), e.g., in ducks (Semel et al. 1988; Yom-Tov 2001; Evans et al. 2002).

Reports of anomalous eggs contained within a clutch include that of a Mallard (*Anas platyrhynchos*) laying an egg in the nest of a Short-eared Owl (*Asio flammeus*) (Wiggins et al. 2006) and a Hooded Merganser (*Lophodytes cucullatus*) laying an egg in the nest of a Northern Flicker (*Colaptes auratus*) (Wiebe 2000). These latter cases probably do not involve brood parasitism, but rather competition for nest sites, or simply misplaced laying by the female.

Within clutches laid by a single female, unusually small or large eggs may appear which are outside the typical range for the species (e.g., Sharp 1904; Kendeigh et al. 1956; Rothstein 1973; Jenkins 1984; Petty and Anderson 1989). Unusually large eggs may have two yolks or embryos (e.g., Petty and Anderson 1989), whereas small eggs may be missing a yolk (e.g., Ricklefs 1975). Such small "runt" eggs are very common among some woodpeckers and may represent an adaptive breeding strategy (Koenig 1980). Frequently, these unusually sized eggs have poor hatching success, but occasionally they are fertile (hatchings have been documented from Western Bluebird (*Sialia mexicana*) (Hayes 1985), as well as the hybrids of Carrion Crow (*Corvus corone corone*) and Hooded Crow (*C. c. cornix*) (Saino and Villa 1992).

In addition to differences in size, oddly coloured eggs have been extensively reported. Almost all of these reports are of pale or achromatic eggs (e.g., Sprunt 1926; Hayes 1985; Radke and Radke 1988; Saino and Villa 1992), or eggs lacking their characteristic markings (Rowan et al. 1919). Such size and colour differences may be the result of developmental anomalies (Sprunt 1926; Jenkins 1984; Hayes 1985; Rhymer 1988; Saino and Villa 1992).

Here we describe an egg, anomalous in both size and colour, from a Northern Saw-whet Owl (*Aegolius acadicus*) nest in Nova Scotia.

Methods

Study site and general methods

Near the community of Bay Road Valley (46°58'N, 60°28'W), on Cape Breton Island, Nova Scotia, we have placed 17 nest boxes (Korpimäki 1985) for owls. The box in which the unusual egg was laid was erected in February 2008, and it was found occupied on 3 May 2008; only the adult female Saw-whet Owl was seen in the box. The box was not opened to inspect the contents until 13 June, when a clutch of six eggs was found abandoned. The clutch, including the anomalous egg (Figure 1), was brought to the laboratory and held at 4°C. Maximum length and diameter of all the eggs were measured using Marathon digital Vernier callipers. Because the size and shape of the anomalous egg did not match the eggs of other cavity-nesters on our study site, the clutch of six eggs underwent genetic analysis.

DNA sequencing

To identify the egg, two genes in the mitochondrial genome were amplified and sequenced: NADH dehydrogenase 2 (ND2) and cytochrome *b* (cyt *b*). Genomic DNA was isolated from egg membranes and egg contents using a modified Chelex extraction (Walsh et al. 1991; Burg and Croxall 2001).

Portions of the ND2 (1.5 kb) and cytochrome *b* (150 bp) genes were amplified with 5 pmol of each primer (L5215 5'-TATCGGGCCCATACCCCGAATAT-3'

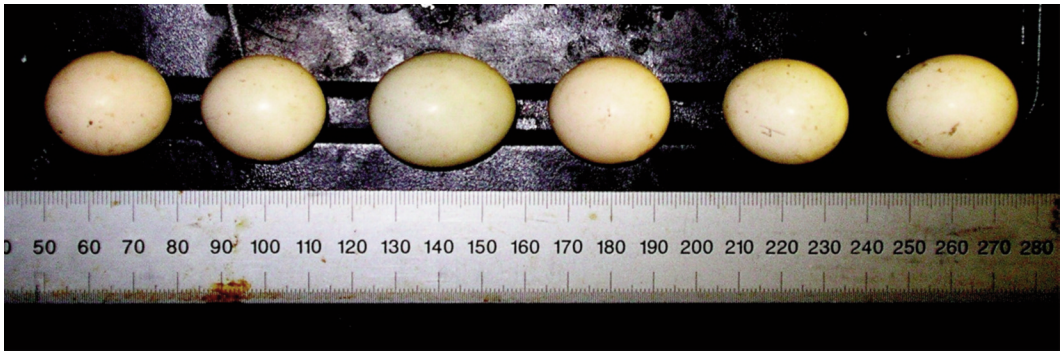

FIGURE 1. The clutch of Northern Saw-whet Owl eggs. The anomalous egg is third from the left. Photo: R. F. Lauff.

(Hackett 1996) and HTrp 5'-CGGACTTTAGCAGA AACTAAGAG-3' (Eberhard and Bermingham 2004) for ND2 and L15560 5'-GYGAYAARATCCCATTC CACCC-3' (Marthinsen et al. 2009) and H15646 5'-GGGGTGAAGTTTTCTGGGTCTCC-3' (Sorenson et al. 1999) for cyt *b*) in 2.5 mM $MgCl_2$, 1 unit (U) *Taq* polymerase, 200 µM dNTP, and Promega Flexi Taq buffer (Promega Corporation, Madison, Wisconsin) in a 25 µL reaction. Both loci were amplified using one cycle for 2 minutes at 94°C, 45 seconds at 54°C, and 60 seconds at 72°C; 37 cycles of 30 seconds at 94°C, 45 seconds at 54°C, and 60 seconds at 72°C; and one final cycle of 5 minutes at 72°C. Samples were sent for sequencing to Génome Québec, Montreal, Quebec. The samples were compared with sequences in GenBank using blastx (National Center for Biotechnology Information, National Library of Medicine, Bethesda, Maryland).

A third gene, the control region, was also sequenced. Typically the control region mutates at a higher rate than either ND2 or cyt *b* and is often used to examine differences within a population (e.g., Friesen et al. 2002; Steeves et al. 2005). By examining the control region, we sought to determine whether the egg was the result of intraspecific brood parasitism by an unrelated female. A portion of the control region, approximately 2 kb in length, was amplified using N1 (5'-AACATTGGTCTTGTAAGCCAA-3') and D16 (5'-AGTGCATCAGTGTCTAGGTGATTC-3') primers from Barrowclough et al. (1999) using the same polymerase chain reaction (PCR) conditions as above except with a final extension step at 72°C for 10 minutes.

Results

Egg size and colour

The anomalous egg measured 33.9 mm in length × 25.9 mm in diameter. The mean length (± standard error) of the other five eggs was 29.0 mm (± 0.41) and the mean diameter was 24.0 mm (± 0.06). Therefore, the anomalous egg was 17% longer but only 8% wider than the mean of the remaining eggs, i.e., it was not just larger than the other eggs but it also had a different shape. All the eggs had only one embryo ($n = 4$) or no visible embryo ($n = 2$, including the anomalous egg). In coloration, five of the eggs were not different from typical Northern Saw-whet Owl eggs—dull and off-white. The anomalous egg was pale blue with brown flecking.

DNA analysis

A 133 bp fragment of cyt *b* and a 407 bp fragment of ND2 were obtained from the contents and shell membranes for each of the six eggs. The sequences from all six samples were identical (Table 1). The cyt *b* sequence showed a 93% match to *Aegolius acadicus* sequences from British Columbia and Alaska (Table 2). The ND2 sequence was a 99% match to the same species. None of the other sequences in GenBank had as high a match as the Northern Saw-whet Owl sequences.

The 1584 bp fragment of the control region was identical for the four eggs from which we were able to obtain a sequence (eggs 1, 4, 5, and 6), with egg 6 being the anomalous egg. All four sequences were identical.

Discussion

Genetic analysis

The high degree of similarity between the sequences from the study eggs and known Northern Saw-whet Owl sequences, combined with visual observation of a female Northern Saw-whet Owl incubating the eggs, suggests that all the eggs were of that species. The fact that all sequences for the three mitochondrial genes were identical does not rule out the possibility that a second female from the same mitochondrial lineage laid the anomalous egg. The nest box was erected the previous winter, so the possibility that the anomalous egg was laid the previous year can be eliminated.

Our DNA results rule out interspecific brood parasitism because the minor differences (1–3 bp) between published Northern Saw-whet Owl sequences and those we obtained are typical of intrapopulation variation. For the cyt *b* data, Topp and Winker (2008) found two variable sites within a 971 bp fragment from 30 North-

ern Saw-whet Owls from western North America. Similarly, Proudfoot et al. (2006) found up to 1% sequence difference in Northern Pygmy Owls (*Glaucidium gnoma, n* = 103).

Egg size and colour

Within a bird species, variation among clutches laid by different females is typically greater than variation within clutches laid by a single female (Christians 2002), and size differences in eggs have been used to identify intraspecific nest parasitism, or "dumped eggs" from other females in the same population (Pöysä 2006). We are unable to distinguish between two likely explanations for the anomalous owl egg. It may have been a dumped egg laid by a conspecific with the same matrilineal lineage. However, the extreme length of the anomalous egg (longer than the mean sizes reported for Saw-whet Owls) (Rasmussen et al. 2008) and the fact that it appeared infertile could also mean it was a developmentally abnormal egg laid by the same female.

The eggs of all North American owls are normally white (Baicich and Harrison 1997), which may represent the ancestral condition in birds (Kilner 2006). Oniki (1985) suggested that only cavity-nesting birds should lay unspotted white eggs because cryptic or heat-absorbing coloration is not needed in a cavity nest. The anomalous egg in this study was pale blue, more typical of birds using thick cup nests in isolated bushes (Oniki 1985).

Whatever the explanation for normal variation in spotting and ground colour in some species, anomalous eggs, such as the one reported here, stand out from others in the same clutch as well as from the species' standard. Most reported cases of miscoloured eggs involve the complete or partial loss of pigment, i.e., the anomalous eggs are typically white (e.g., Hayes 1985; Radke and Radke 1988). Gross (1968) summarized the occurrence of albinistic eggs and found 18 species in only three orders (Falconiformes, Charadriiformes, and Passeriniformes) that laid these pigment-free eggs, sometimes as one anomalous egg among the clutch, sometimes as a whole clutch.

The egg we found had additional pigment, both as ground colour and as spotting. An extensive review of the literature revealed no other case in which a species which normally lays an immaculate egg of one ground colour has laid a spotted egg with a different ground colour. Biliverdin is responsible for the blue in the eggshells of many species, and is likely synthesized in the shell gland (Zhao et al. 2006). White eggs, including those of owls, are not necessarily devoid of these pigments; they may be present in minute quantities serving structural roles (Kennedy and Vevers 1976; Mikšík et al. 1994). For unknown reasons, the large egg in the study nest had much more pigment added to it than normal; whether this was related to the egg also being over-sized or to some general developmental problem is not known.

TABLE 1. Mitochondrial ND2 sequences for egg contents (E) and shell membrane (S) from eggs from the Northern Saw-whet Owl nest. Dots indicate matches with Northern Saw-whet (*Aegolius acadicus*, GenBank EU601051) and Asian Barred Owlet (*Glaucidium cuculoides*, GenBank EU601047); upper-case letters indicate mismatches and dashes indicate missing sequence. Variable sites are listed along the top.

	11111111	1111111111	1111222222	2222222222	2233333333	3333333344 4
	4455556889	9900011122	2333444556	7899011223	3344455577	5555677800 0
	7923891785	7834906835	7068269571	0737658460	3815901728	3977903681 0468519906 7
E1	TGTGTCGGCT	TTCGTGTTGG	TGTAATCGTA	ATACTGTGGT	CGCTGTATCG	TTGTGGATAT TATGACCTTC T
S1	....	....	....	....	....	....
E3	....	....	....	....	....	....
S3	....	....	....	....	....	....
E4	....	....	....	....	....	....
S4	....	....	....	....	....	....
E5	....	....	....	....	.....-----	---------- ---------- -
E6	....	....	....	....	....	...------- ---------- -
S6	....	....	....	....	....	....
Aegolius acadicus	....	....	....	....	.C........	....
Glaucidium cuculoides	CACACTTATG	AGTACAGGTT	CAGGGGTAGT	GCGTGAGTAG	GTTGACGGTT	A.ACATGCGA GGGAGGTGGG G

TABLE 2. Cytochrome *b* alignment for shell membranes (S) from eggs from the Northern Saw-whet Owl nest. Highest sequence matches were with Northern Saw-whet Owl (*Aegolius acadicus*, GenBank EU75412; *A. a. acadicus* EU348959, *A. a. brooksi* Y15686), Boreal Owl (*A. funereus*, GenBank AJ004061), Rufous-legged Owl (*Strix rufipes*, GenBank AJ004353), and Spectacled Owl (*Pulsatrix perspicillata*, GenBank AJ004044). Numbers along the top refer to positions of variable sites.

	122335666 7358012014	1 6667777990 5673578182	111111111 000112233 469284701
S1	GTCTTAGGGT	GTTAAATGAT	TATGTC?AC
S3	....	....	......?..
S4	....	....	......?..
S5	....	....	......G..
S6	....	....	......??.
Aegolius acadicus	....	....	...AC.A..
A. a. brooksi	.........G	....	...AC.A..
A. a. Acadicus	.........G	....	...AC.A..
A. funereus	.....GT.AG	..GTGGCA.A	.G....AG.
Strix rufipes	ACACGG....	AGGCGGC..A	AGG..TG..
Pulsatrix perspicillata	..ACG..A..	AGGTGGC.GA	AGG..TGGG

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