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Triorchidism in a Hummingbird

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ABSTRACT.—We report a Great-billed Hermit (*Phaethornis malaris*) with three testes, a condition known as triorchidism. This is the first case to our knowledge of triorchidism in Neoaves, the clade that contains ~95% of avian species diversity. Triorchidism is inferred to be an exceptionally rare congenital abnormality in wild birds with developmental cause and evolutionary implications that are distinct from testicular asymmetry. *Received 16 November 2010. Accepted 21 March 2011.*

Triorchidism is the condition of having three testes. This and other forms of polyorchidism are associated with superfecundity, 'amorous propensities,' and 'generative faculties' in mythology (Gould and Pyle 1896). The true pathology is poorly known due to a paucity of cases and is dependent on the underlying developmental cause (Leung 1988). Several instances of triorchidism have been reported in Domestic Chickens (*Gallus gallus*) (Katiyar et al. 1986, Shrivastava et al. 1988, Hocking 1992) and in a captive-bred line of Japanese Quail (*Coturnix japonica*) (McFarland 1965), but the phenomenon of polyorchidism is otherwise scarcely known from non-human animals.

Triorchidism is typically thought to comprise a congenital developmental abnormality and is associated with normal histology and functional spermatogenesis in the third testis in more than half of human cases (Ozok et al. 1992, Spranger et al. 2002, Savas et al. 2009). It is distinct from testicular asymmetry, which is widespread in birds and has been found to correlate with age (Graves 2004) or secondary sexual characteristics (Møller 1994; but see Kimball et al. 1997). The phylogenetic distribution of triorchidism in vertebrates is poorly known due to a scarcity of reports. We describe a case of triorchidism in the Great-billed Hermit (*Phaethornis malaris*) from the humid lowland forests of northeastern Perú, and review previous reports of triorchidism in birds.

METHODS

We conducted a site inventory during June 2007 in the Rio Chipaota Valley, Department of San Martín, Perú, while concurrently collecting comparative data on bird physiology (Merkord et al. 2009). This work included collection of voucher specimens with detailed ancillary data for deposit in the collections of Centro de Ornitologia y Biodiversidad (CORBIDI, Lima, Perú) and the University of New Mexico, Museum of Southwestern Biology (MSB, Albuquerque, New Mexico, USA). We collected the following data for all specimens using standard methods: (1) body mass, (2) hemoglobin concentration (using Hemocue 201+ with correction for avian blood, following Simmons and Lill 2006), (3) hematocrit, (4) red blood cell concentration, (5) presence of hematozoa in a Giemsa-stained blood smear, (6) skull ossification, (7) presence and dimensions of the bursa of Fabricius, and (8) identity, condition, and dimensions of gonads.

OBSERVATIONS

We captured a specimen of Great-billed Hermit on 15 June 2007 that proved upon autopsy to have an anomalous third testis (Fig. 1). The left testis appeared to be divided into two similarly sized, spherical testes (left-most testis 2.87 mm diam, center testis 3.41 mm diam), connected to a single ductus deferens. The right testis was slightly smaller in size (2.65 mm diam) and appeared to be displaced posteriorly by the two left testes, which together occupied a substantial portion of the abdominal cavity. We lacked the ability to do histological examination in our remote field camp, but we suspect that all three testes were functional because they were similar in size and

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FIG. 1. Ventral view of the abdominal cavity of *Phaethornis malaris* (left = anterior). The digestive tract (bottom of image) has been lifted up out of the way to reveal left (top and center), and right (bottom) testes. Scaling is approximate, based on measurements of testis diameter taken in the field with digital calipers to the hundredth of a millimeter.

nearly indistinguishable in shape, color, firmness, and internal consistency and appearance.

The triorchid Great-billed Hermit was netted in mature forest interior habitat, at 374 m asl, 06° 38.660′ S, 76° 04.955′ W ±10 m. It lacked a bursa of Fabricius, and the skull was estimated to be 30% ossified, an exceptionally high ossification for a hummingbird and a strong indication that the bird was reproductively mature. The voucher specimen and frozen tissue are archived at MSB (Tissue catalog number NK162363, voucher number EBO247) with a duplicate tissue sample archived at CORBIDI. The bird was physiologically normal based on comparison to male conspecifics for six criteria: (1) body mass, 5.9 g; (2) hemoglobin concentration, 17.1 g/dl, typical of *Phaethornis* hummingbirds at similarly low elevation; (3) hematocrit, 53.7%; (4) red blood cell concentration, 5.48 million per µl; (5) mean erythrocyte volume (MCV), 98.1 fl; and (6) a blood smear that yielded no detectable blood parasites from >10,000 Giemsa-stained red blood cells scanned at 600× magnification.

DISCUSSION

This represents the first reported case of triorchidism for a hummingbird (Trochilidae) and for the entire clade Neoaves, which includes

~95% of avian species diversity. We can find no previous reports of this phenomenon in wild bird species, nor any bird species outside of the Order Galliformes. Triorchidism in birds was first reported by McFarland (1965), who examined >2,000 male Japanese Quail and found one case of triorchidism that was associated with absence of the right kidney. In that case, the right testis was divided into two nearly co-equal sections, both of which were undergoing normal spermatogenesis. Katiyar et al. (1986) first reported a supernumerary right testis in a Domestic Chicken. Shrivastava et al. (1988) subsequently reported a Domestic Chicken with a supernumerary left testis that was severely reduced in size (less than 10% the size of the left and right testes) and ~1 cm posterior to the caudal end of the left testis, ventral to the third lobe of the left kidney. The small round supernumerary testis was softer in consistency, and normal in color and spermatogenic activity by histological examination.

Hocking (1992) systematically examined the testes of 378 male Domestic Chickens and found three cases of triorchidism, each of which was comprised of two left testes. The two left testes in two of the three cases were nearly co-equal in size with normal semen quality and testis histology. The smaller of the two left testes in the third case was much less than half the volume of the larger one and was characterized by strongly reduced spermatogenesis. Hocking (1992) suggests that supernumerary testes probably originate by congenital defect and are fairly common in domestic fowl, as indicated by his finding prominent third testes in 0.8% of males. Supernumerary testes originating by congenital defect should be present throughout life, in contrast to asymmetrical testes, which are known to shift in relative size according to age, season, overall condition, and other, unidentified factors (Møller 1994, Kimball et al. 1997, Graves 2004).

We examined and measured testes in 591 male hummingbirds representing 65 species during fieldwork between 2006 and 2010, including 21 male Great-billed Hermits (MSB and CORBIDI specimen data). We observed only one case of triorchidism (0.17% of male hummingbirds examined), suggesting the phenomenon is significantly less common in wild hummingbirds than in domestic fowl, although the small sample size of triorchid individuals prohibits statistical testing.

The paucity of case reports of supernumerary testes in birds makes it difficult to understand the mechanisms or risk factors that may be associated with this condition. However, ~100 cases of triorchidism in humans have been described (Hassan et al. 2008), and the developmental, pathological, and functional aspects of human triorchidism are useful for understanding avian triorchidism. Approximately two-thirds of human supernumerary testes occurred on the left side as in the triorchid hummingbird, and a slightly smaller proportion (63%) have reproductive potential as evidenced by normal spermatogenesis (Spranger et al. 2002, Bergholz and Wenke 2009). Leung (1988) classified cases of polyorchidism into four types, of which the triorchid hummingbird seems to fit into 'type C', which is characterized by the supernumerary testis having its own epididymis and sharing the ductus deferens with the regular testis in a parallel fashion. This configuration is thought to occur due to incomplete longitudinal division of the genital ridge and the proximal portion of the mesonephric duct during development (Singer et al. 1992).

Two cases of triorchidism have been observed in a rare Malagasy tortoise, *Geochelone yniphora* (Mourgue 1989), suggesting the phenomenon occurs in at least three deep amniote lineages. The present case is the first report for a wild bird and the first for any bird outside of the Order Galliformes. The hummingbird species involved, the Great-billed Hermit, is a promiscuous, lekmating species and is expected to be under selection for sperm volume and quality (Birkhead 1998). It is not known whether this evolutionary pressure may be associated with susceptibility to presumed congenital defects such as the supernumerary testis observed. Investigators performing autopsies on birds should look for and report supernumerary testes to help elucidate the phylogenetic distribution and evolutionary implications of this interesting phenomenon.

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