

Biology of a critically endangered species, the Toki (Japanese Crested Ibis) *Nipponia nippon*

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Populations of the Toki, or Japanese Crested Ibis *Nipponia nippon* are now reduced to a single female in Japan, and captive and free-living populations of about 80 individuals in Yang Xian, Shaanxi Province and the Beijing Zoo, People's Republic of China. Very little has been published about the biology of this species except for Japanese journals and popular articles. Here we outline some unique aspects of the breeding biology of this species as well as introduce endocrine methods as a way of determining the source of reproductive problems in captive breeding programmes and, tentatively, how hormone manipulations based on these observations may help to enhance reproductive output. The Toki has a unique nuptial plumage that is acquired by the cosmetic application of a black tar-like substance secreted by a well-defined patch of skin in the throat and neck region. Apparent specialized down feathers develop in this region during a localized moult in November. The tar-like substance is secreted in January and February and is applied to the head, neck and upper torso by a combination of bathing and daubing behaviour. Development of nuptial colour is thus a combination of morphological development, secretion and a unique behaviour. This combination is found only in adults. Single plasma samples collected from captive Toki at known times in the breeding cycle suggest normal hormone patterns in some individuals, and abnormal levels in those that fail to breed. These conclusions are strengthened by endocrine investigations of a non-endangered ibis species, *Threskiornis melanocephalus*, that reveal normal patterns of circulating concentrations of gonadotrophins and sex steroids as expected for a seasonally breeding bird. Measurement of faecal hormone levels may be a less invasive way of monitoring reproductive function in critically endangered species. Recent work on Japanese Quail *Coturnix japonica* shows that hormone therapy using purified avian gonadotrophins in non-reproductive individuals results in gonadal maturation, egg-laying and production of reproductively active offspring. Although these techniques are still in their formative stages, results to date strongly suggest that carefully applied endocrine techniques can indicate potential problems in captive breeding programmes and may be useful for hormone therapy in critically endangered species.

There can be few more critically endangered species than the Toki or Japanese Crested Ibis *Nipponia nippon* (order Ciconiiformes, family Threskiornithidae). In the 18th century, this ibis was widely distributed through

Honshu and neighbouring small islands, part of Hokkaido, Korea, adjacent parts of China and eastern Siberia. In the 19th century, they may even have expanded their range to the islands of Kyushu and Shikoku, Japan (Yamashina & Nakanishi 1983). The Toki was formerly a common sight on ponds and paddyfields where it foraged for much of the year.

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Most populations underwent a short migration to breeding colonies in forested foothills (Yamashina & Nakanishi 1983). Probably as a result of hunting and habitat loss, populations of the Toki began to decline at the end of the 19th century and by 1920 they were thought to be extinct in Japan. Many wetland habitats were lost and most paddyfields became surrounded by dense human habitations. It also appears that the Toki was rather tame and thus easily approached and captured (Austin 1948). As a result it was hunted and harassed to extinction over most of its range (Austin & Kuroda 1953). In 1929 and 1930, two small populations were rediscovered on the Noto Peninsula and Sado Island, Niigata Prefecture. Apparently these populations remained in forested areas and did not migrate to open wetlands outside the breeding season. Additionally, they nested as single pairs and not in highly vulnerable colonies. It is assumed that for these reasons remnant populations survived in Japan (Yamashina & Nakanishi 1983). Although the Japanese Government gave the Toki protected status in 1934, the Second World War prevented any conservation measures from being applied (Anderson 1984). By 1950 there were 29, and in 1980 only five were alive (Yamashina & Nakanishi 1983, Ishii *et al.* 1994). In 1981 the Sado Japanese Ibis Preservation Centre was established, and all remaining Toki in Japan were captured and housed here. Despite concerted efforts to breed them in captivity, the population continued to decline. Today, only one remains in Japan, an old female – 'Kin' (Ishii *et al.* 1994).

At the time the Japanese birds were being captured and transferred to the Preservation Centre, two nesting pairs of Toki were unexpectedly discovered in Yang Xian, Shaanxi, China (Ishii *et al.* 1994). As of 1997, there appear to be about 44 in the wild, eight immatures and six adults in a breeding colony in Beijing Zoo, and 25 in captivity at a field station near the wild population (see Table 1). Clearly the future of the Toki lies with the Chinese population.

Despite the critically endangered status of the Toki, surprisingly little has been published on this species in the western literature. One major reason is that most facts about the natural history of the Toki are published in the Japanese literature. For example, of 639 articles cited by Yasuda (1984a, 1985) as referring to the Toki, 72 were in English, 14 in French, 12 in German, two in Russian, one in Spanish and one in Latin. These comprise only 16% of the extant literature. Papers that focus entirely on the biology of the Toki, and present most of what is known of this species, are all in Japanese. Here we wish to review this

information in English as a means of drawing attention to this spectacular and biologically intriguing species. We also review new information, including multidisciplinary approaches that combine morphology, ecology, behaviour and endocrinology of non-threatened species, as a way of identifying and addressing problems of breeding in critically endangered species. Whether the Toki will survive the current population bottleneck remains to be seen, but we hope that these data will emphasize the potential power of multidisciplinary research in conservation biology and that this will help other endangered species recover before their populations become so critically low.

Reproductive biology of the Toki

In historic times, the Toki was known to frequent paddyfields (dry and wet) and ponds and swamps, where they foraged for fish, amphibians and large invertebrates. Nearby forested hills were preferred as roost sites. They often formed breeding colonies, particularly in pines *Pinus* spp. and chestnut trees *Castanea* spp., where they built platform nests of twigs. Many populations underwent short migrations from low swampy areas to forested breeding grounds (Austin & Kuroda 1953, Yamashina & Nakanishi 1983). Typically, four speckled eggs comprised a clutch that was laid in April (Yamashina & Nakanishi 1983).

In the last century, it was thought that the Toki had two colour morphs: one white and one grey. Later it became clear that the grey morph was actually the nuptial plumage of both sexes (Yasuda 1984b). Typical plumages of non-breeding and breeding Toki are

Table 1. Captive and wild populations of the Toki *Nipponia nippon* in China in the spring of 1996.

| Province | Status | Age | No. of individuals |
|-------------|---------|--------|--------------------|
| Yang Xian | Wild | Adults | 30 |
| | | Young | 14 |
| | Captive | Adults | 15 |
| | | Young | 10 |
| Beijing | Captive | Adults | 8 ^a |
| | | Young | 6 ^a |
| Subtotal | Wild | | 44 |
| | Captive | | 39 ^a |
| Grand Total | | | 83 ^a |

In 1997, both the Yang Xian and Beijing populations are reported to have increased.

^aNot definite.

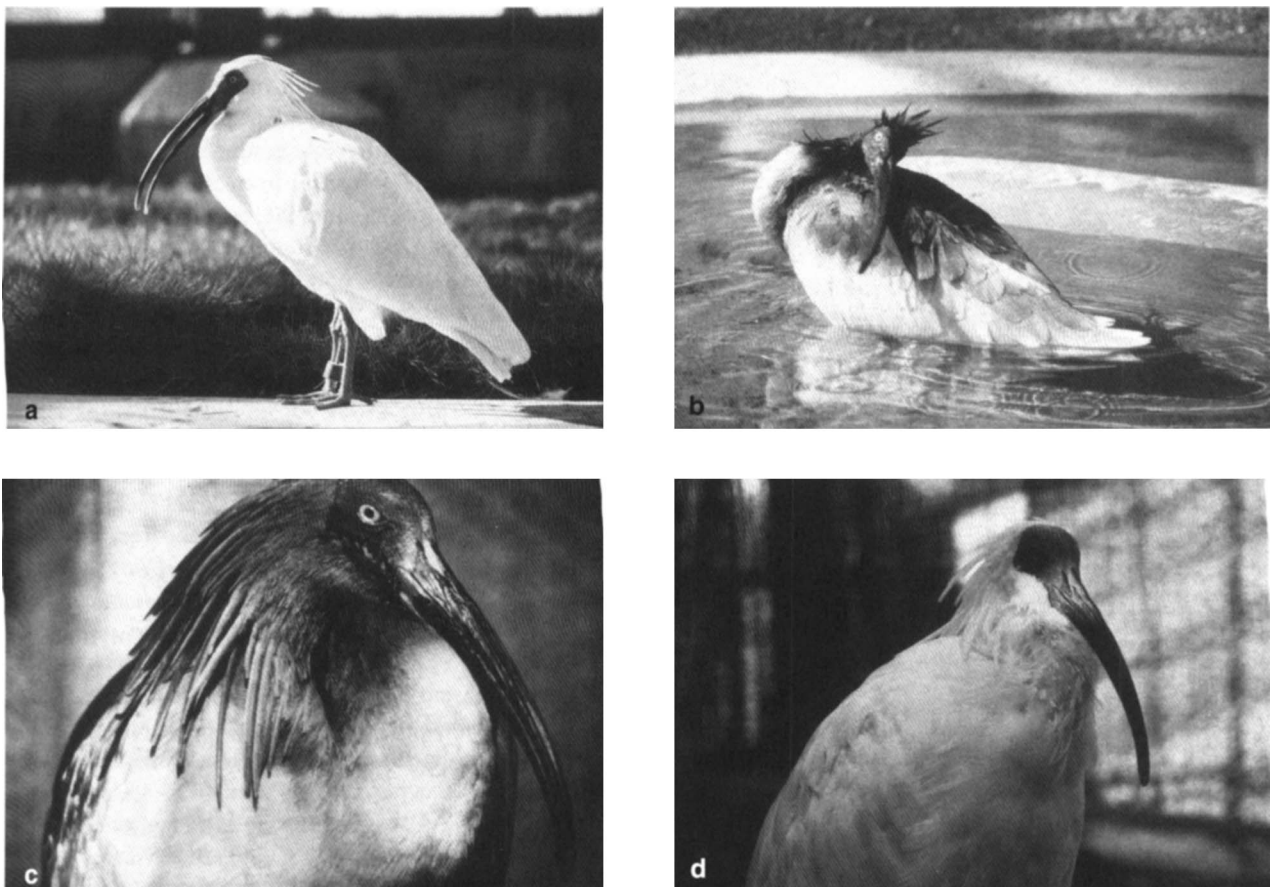


Figure 1. Typical sequence of non-breeding and breeding plumages in the Toki *Nipponia nippon* during cosmetic acquisition of colour. (a) Typical non-breeding plumage; (b) dabbing behaviour (spreading of black tar-like substance); (c) nuptial plumage; and (d) localized moult of contour feathers in the neck and gular region.

presented in Figure 1. They are white in the non-breeding season and grey on the head and upper body when breeding. The salmon-coloured flush (Toki colour in Japanese) to the primaries and secondaries is present all year, as is the red colour of the face and legs. Development of this nuptial plumage may be a unique phenomenon in birds. The grey colour is not acquired through a moult, but by cosmetic application of a tar-like secretion from a specialized patch of skin on the neck and head (Uchida 1970, Yamashina & Nakanishi 1983, Yasuda 1984b). The white plumage characteristic of the non-breeding season is acquired through a normal postnuptial (prebasic) moult in late summer. The sequence of plumages in the Toki is represented in Figure 1 (after Uchida 1970 and pers. obs.) Note also that the plumage colour and structure, as well as the skin colour of the legs, head (anterior to the eyes) and eye-rings appear to be identical in males and females. As far as we are aware, development of

nuptial colour in the Toki has not been described previously in western journals.

Nuptial plumage

Development of nuptial plumage in the Toki is by cosmetic application of colour rather than through a prenuptial (prealternate) moult. Skin from a specialized region of the head and neck (Fig. 2, after Uchida 1970, Yasuda 1984b) secretes a black tar-like substance or 'cosmetic' that is then applied to feathers of the head, neck, back and upper wing coverts resulting in a plumage that can vary from light grey to a dark 'gun-metal' grey. Examination of specimens (see Table 2 for a list of specimens examined for this paper) and photographs of living Toki indicate substantial variation in extent of the grey tint. In some individuals, grey pigment may also extend to the primaries, rectrices and contour feathers of the upper breast, whereas in others it may be limited to the head, neck,

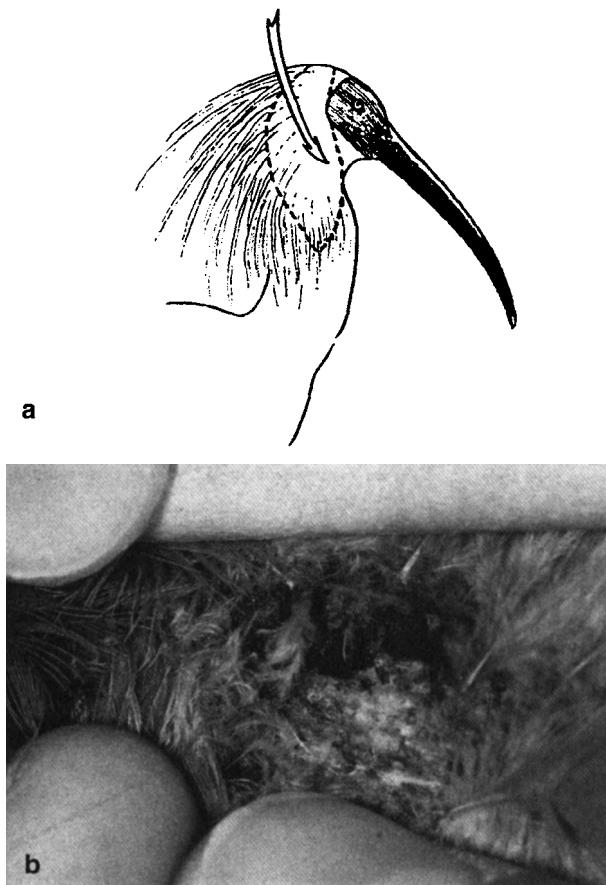


Figure 2. (a) Extent of the pigmented area of skin in the throat region of the Toki *Nipponia nippon*. From Uchida (1970) with permission of the author and the Yamashina Institute of Ornithology. (b) Edge of the pigmented area of skin in the throat region.

upper back, scapulars and some upper wing coverts (Fig. 1). Whether this variation is a function of the amount of cosmetic secreted, age and possible experience in application, remains unknown. In the non-breeding season, the plumage is always white.

Extent of the pigmented skin area

Measurements of specimens that are in good enough condition to handle indicated that the extent of specialized, pigmented skin (source of the black tar-like substance) is also variable (Fig. 2a). The edge of the skin patch is very well defined (Fig. 2b) and its extent can easily be measured. In one specimen, a limited throat patch is present, while another has a small patch on the nape only. Seven other specimens examined have large patches of black skin that extend from the crown down to the nape but posterior to the

eye, then around the sides of the neck to the throat and below the eye (Fig. 2a). In some individuals there may be bilateral skin patches, but most appear to have one patch with two connected lobes. On average, the black skin extends along the side of the head 76 mm (right side) and 81 mm (left side) from the base of the beak (starting from just behind the eye, range 50–100 mm on the right side, 56 to 120 mm on the left side, coefficient of variation = 23.7% and 27.5% respectively). With one exception, the extent of pigmented skin is greater on the left side. On the crown, the pigmented skin begins at 62 mm from the base of the culmen (range 53–67 mm, cv 9.9%). On the throat, black skin extends 47.2 mm from the base of the lower bill to its posterior edge (range 21–96 mm, cv 58%). Others who have examined the skin of fresh specimens (i.e. after death and skinning) confirm the restricted and well-defined shape of this patch (Yamashina & Nakanishi 1983, Yasuda 1984b). It is not known if variation in the extent and shape of the pigmented skin patch is related to age. One immature specimen examined has no pigmented skin on the head and neck. It was collected in February (Table 2), and was thus in its first winter – a time when adults apply the cosmetic to their plumage.

Secretions of the pigmented skin area

Beginning in January, the pigmented skin patch begins to secrete the cosmetic. Microscopic analysis revealed that 'chunks' of this black substance are secreted along feather pores in the specialized skin region and accumulate around the rachis and on the barbules of down feathers. Uchida (1970) describes a black ring near the root of the rachis. The cosmetic appears to ooze out around the rachis and soaks into the rest of the feather. However, the possibility that some epidermal cells containing the black tar may slough off and burst cannot be ruled out. Although the chemical composition of the cosmetic is unknown, it appears to consist of a black pigment (melanin?) in a non water-miscible base (oil, wax?).

Possible morphological adaptation for application of nuptial colour

Close examination of the specimens listed in Table 2 reveals a potentially specialized down feather that may aid the application of nuptial colour. All adults with nuptial colour, or just prior to its development, have long down feathers with short lateral barbs in the region of pigmented skin. Down feathers from other regions of the neck have much longer lateral barbs (Fig. 3). It is possible that these specialized down

Table 2. Specimens of the Toki *Nipponia nippon* examined: location and origins.

| Specimen number | Location | Date | Sex and age |
|--|------------------------|--------------------------|--------------|
| <i>Yamashina Institute for Ornithology</i> | | | |
| 32 | Korea | Jan 1919 | U adult |
| 213 | Korea | 12 Jan 1912 | U adult |
| 2497 | Korea | 24 Mar 1929 | M adult |
| 2683 | Korea | 30 Mar 1929 | M adult |
| 2684 | Korea | 30 Mar 1929 | F adult |
| 3710 | Korea | 4 Feb 1930 | M immature |
| 25805 | Echigo, Niigata, Japan | Feb 1893 | U adult |
| 25806 | Echigo, Niigata, Japan | Feb 1893 | U adult |
| 25808 | Kaishu, Korea | 19 Jan 1912 | F adult |
| 25809 | Korea | Feb 4 1902 | U adult |
| <i>National Museum Research Institute</i> | | | |
| No number | Korea/Manchuria | Pre W.W. II ^a | U adult |
| 1 | Sado Island, Japan | 21 Jan 1981 | F adult |
| 7 | Sado Island, Japan | 13 Apr 1983 | F adult |
| 12175 | Korea | Mar 1919 | F immature ? |

M, Male; F, female; U, unknown sex.

^aSpecimen was collected at an unknown date before the Second World War.

feathers reduce the surface area to which the cosmetic can adhere, thus assisting application to other regions of the plumage. The long lateral barbs of typical down feathers may soak up most tar secreted and become clogged with chunks thus reducing the efficiency of application to other areas. Uchida (1970) reports that the cosmetic may drop off the throat and head region onto the ground during the period of heaviest secretion. This is consistent with the presence of specialized down feathers with short barbs that provide much less surface area to soak up the cosmetic secretion allowing more copious application

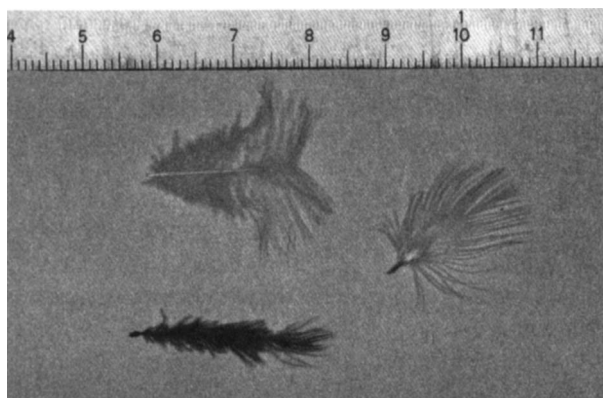


Figure 3. Specialized down feathers in the throat region of the Toki *Nipponia nippon*. Right, normal down feather; top left, down feather from the throat of an immature; bottom left, pigmented down feather from an adult with nuptial colour.

elsewhere. By analogy, given equal volumes of paint on a brush, the one with short bristles drips more easily than one with long bristles.

Museum specimens examined in post-breeding plumage have normal down feathers with long barbs in the pigmented (but not actively secreting) skin area (Fig. 3). The one immature bird examined also does not have the specialized down feathers on the throat and head regions where pigmented, secretory skin would eventually have developed. These observations support the possibility that specialized down feathers occur in the pigmented skin area, but also suggest that they are not always present, and must develop prior to the cosmetic application of pigment. Recently, it has been observed that adult Toki undergo a highly restricted moult of feathers in the throat and head regions in November (Fig. 1d). We suggest that it is at this time that many normal down feathers are shed, and the specialized 'short-barbed' down grows – but only in the pigmented skin area. Thus it appears that there is a morphological change associated with acquisition of nuptial plumage in the Toki, which involves development of a 'tool' for cosmetic application.

Application of nuptial pigment

In addition to a morphological change related to development of nuptial colour, and physiological changes associated with secretion of the cosmetic, there are also distinct behaviour patterns ('daubing') associated with cosmetic application. These behaviours usually occur

in association with bathing, and the grey tint becomes darker with each application. Five or six bathing–daubing sessions are usually needed to complete development of nuptial colour (Uchida 1970). Observations of Toki during daubing sessions reveal two major components to the behaviour patterns (Fig. 1). First the throat and sides of head are rubbed on the upper back and wings thus depositing cosmetic on the outer feathers (Fig. 1). Secondly, the head is turned so that the crown and back of the head (as well as the beak) are rubbed over the back and wings to spread the pigment (Fig. 1). The neck also becomes coated with pigment picked up from the back and wings. The daubing behaviour clearly serves to apply pigment to contour feathers and then to spread it uniformly.

Why is daubing behaviour associated with bathing?

Because of its composition, the cosmetic cannot be diluted by water. Uchida (1970) showed that the tar becomes lodged as small fragments on the proximal barbules of the feathers (i.e. those projecting back from the lateral barbs) rather than the distal barbules (i.e. those projecting forward from the lateral barbs and interlocking with the proximal barbules). It is possible that bathing in water may help emulsify the cosmetic as it is daubed onto wet feathers. Very small fragments of pigment may result, that would then adhere to the surface of barbules to give a more uniform finish to the colour. This hypothesis could be tested by applying cosmetic to dry versus wet feathers. Note, that the feathers are not 'stained' in the sense that the keratin structures become pigmented themselves, but small fragments of pigment adhere to their surface.

After breeding, the Toki undergoes a complete post-nuptial (prebasic) moult. At this time, the pigmented skin area on the head and throat is not actively secreting pigment, and birds show no daubing behaviour. Thus all contour feathers develop as pure white or salmon-coloured structures (Uchida 1970). Bathing continues to occur all year, but secretion of the cosmetic, and daubing behaviour following bathing, are restricted to a discrete period in January and February just prior to the first courtship behaviours and onset of breeding.

Development of nuptial colour in the Toki appears to be unique among birds. Cosmetic application of colour may occur in a few other species (see van Tyne & Berger 1959), but the combination of a morphological change (restricted autumn moult and development of a specialized down feather), physiological change (secretion of the cosmetic) and behavioural changes

(the bathing–daubing patterns) is truly unique.

Given the unusual breeding biology of this critically endangered species, we consider below the possible factors leading to the failure of the current captive breeding programme to re-establish the species in Japan. Specifically we address how endocrine investigations of non-threatened species might be used as a tool to facilitate breeding programmes of endangered species by identifying problems, as well as by increasing the likelihood of successful reproduction.

Reproductive endocrinology of an endangered species

Following capture in 1981 of all six remaining Toki in Japan, two females died within a year. Another female was crippled with arthritis and eventually died in 1986 without breeding. A remaining female paired with a male displayed breeding behaviour and copulation but suddenly died and was found to be egg-bound (i.e. an egg had lodged in the oviduct, eventually causing death). After 1983, only the male and an old female remained, and although the female remained sexually active (as judged by behaviour and development of nuptial colour) no breeding occurred (Ishii *et al.* 1994). Even the introduction of a male from the population in China failed to result in breeding. Now the old female is the only Toki left in Japan and currently she fails to develop nuptial colour, suggesting that she is now too old to breed.

Given the critically low numbers of Toki it is clearly important to determine as accurately as possible the reasons for reproductive failure. Anderson (1984) reports that the old female and the only remaining male were not compatible and they fought. However, it is difficult to determine exactly what such observations mean. Were these birds reproductively mature (i.e. were the testes and ovaries developed to a state where breeding could commence) or had one individual failed to mature and thus failed to respond to courtship from the other, resulting in conflict? Assessment of circulating reproductive hormone levels would be one way to gain further critical information on reproductive status. Individuals that apparently have failed to mature and show deficits in endocrine secretions or patterns might then be brought into breeding condition by hormone treatment. To do this requires considerable background information on changes in plasma levels of appropriate reproductive hormones during gonadal development, onset of laying and termination of breeding. Obviously, this is not practical for critically endangered species where only

a handful of individuals remain. An alternative is to collect samples from a closely related, but not endangered, species as a model. Then single spot samples from the endangered species can be compared to the template developed for the related species to identify possible deficits of endocrine function that could in turn point to revisions of the captive breeding programme, or even suggest therapy with reproductive hormones.

Comparisons of circulating hormone levels.

As a template for the reproductive cycle of the Toki, Ishii (1983, 1984) and Ishii *et al.* (1994) collected a series of blood samples from captive populations of White Ibises *Threskiornis melanocephalus* breeding in the Tama Zoo, Tokyo. They then measured plasma levels of gonadotrophic hormones as an indicator of normal reproductive development in ibises. Gonadotrophins are glycoprotein hormones secreted by the anterior pituitary gland and control gonadal function. These are: (a) luteinizing hormone (LH) which acts primarily on the endocrine gonad to regulate synthesis and release of sex steroid hormones and also induces ovulation; and (b) follicle-stimulating hormone (FSH) which acts primarily on the gametogenic gonad but can influence sex steroid hormone release as well. It was found that both male and female White Ibises show a dramatic rise in plasma levels of LH and FSH during late winter and early spring coincident with gonadal development and eventual onset of nesting (Fig. 4a, 4b). Levels of these hormones are minimal in late summer and early autumn when the breeding season is over (Ishii 1983, 1984, Ishii *et al.* 1994). Furthermore, changes in sex steroid (androgens) levels parallel the patterns of LH (Fig. 4c).

These patterns are typical of many species of birds that have been studied both in the field and in captive populations (see Wingfield & Farner 1993 for review). Furthermore, comparisons of LH, FSH and sex steroid patterns in individuals that are breeding normally, versus those that are not, show marked differences (Wingfield & Farner, 1993), suggesting that monitoring hormone levels can provide information on hormone deficits in individuals that fail to breed. Adjustments can then be made in the breeding programme to correct the deficit, or hormone treatment may be applied to induce breeding.

Changes in plasma levels of LH and FSH in the Toki (Table 3, Fig. 5) are consistent with the data obtained for White Ibises. The individual code-named Blue (Ao) showed higher levels of FSH and LH between December and March – the period of presumed gonadal development prior to nesting in April. However, this bird failed to breed and this is reflected in the generally low and erratic levels of the sex steroid hormone oestradiol-17 β (Table 3) compared with many other avian species breeding naturally (Wingfield & Farner 1993). Ao was the Toki crippled with arthritis. She never developed full nuptial plumage and never laid eggs, although some gonadal development probably occurred. Three other birds (Table 3) had variable levels of reproductive hormones in October. The bird code-named Gold (Kin) is the old female that did not breed and she had lower levels of hormones, particularly LH (Table 3, Fig. 5). White (Shiro) had the highest levels of LH and also elevated FSH. This bird initiated egg-laying but died because she became egg-bound.

These data, although sparse because of the very few data points available, are nonetheless encouraging and

Table 3. Plasma gonadotrophin and sex steroid concentrations in the plasma of Toki *Nipponia nippon*.

| Individual identification | Date | Hormones (ng/ml plasma) | | | |
|---------------------------|-------------|-------------------------|------|------|------|
| | | FSH | LH | E2 | T |
| Blue (Ao) | 2 Feb 1982 | – | 0.67 | – | – |
| | Oct 1982 | 1.99 | 0.61 | 0.23 | – |
| | 21 Dec 1982 | 3.86 | 1.89 | 0.15 | – |
| | 25 Mar 1983 | 3.34 | 2.34 | 0.11 | – |
| | 19 Oct 1983 | 0.39 | 0.99 | 0.12 | – |
| | 29 Feb 1984 | 2.90 | 2.02 | 0.08 | – |
| | 22 May 1984 | 2.31 | 1.31 | 0.02 | – |
| White (Shiro) | Oct 1982 | 1.51 | 2.52 | 0.20 | – |
| Gold (Kin) | Oct 1982 | 1.22 | 0.06 | 0.12 | – |
| Green (Midori) | Oct 1982 | 1.94 | 1.45 | – | 0.26 |

FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, oestradiol-17 β ; T, testosterone. All concentrations of hormones are expressed as ng/ml of plasma. From Ishii *et al.* (1994) and unpubl. data.

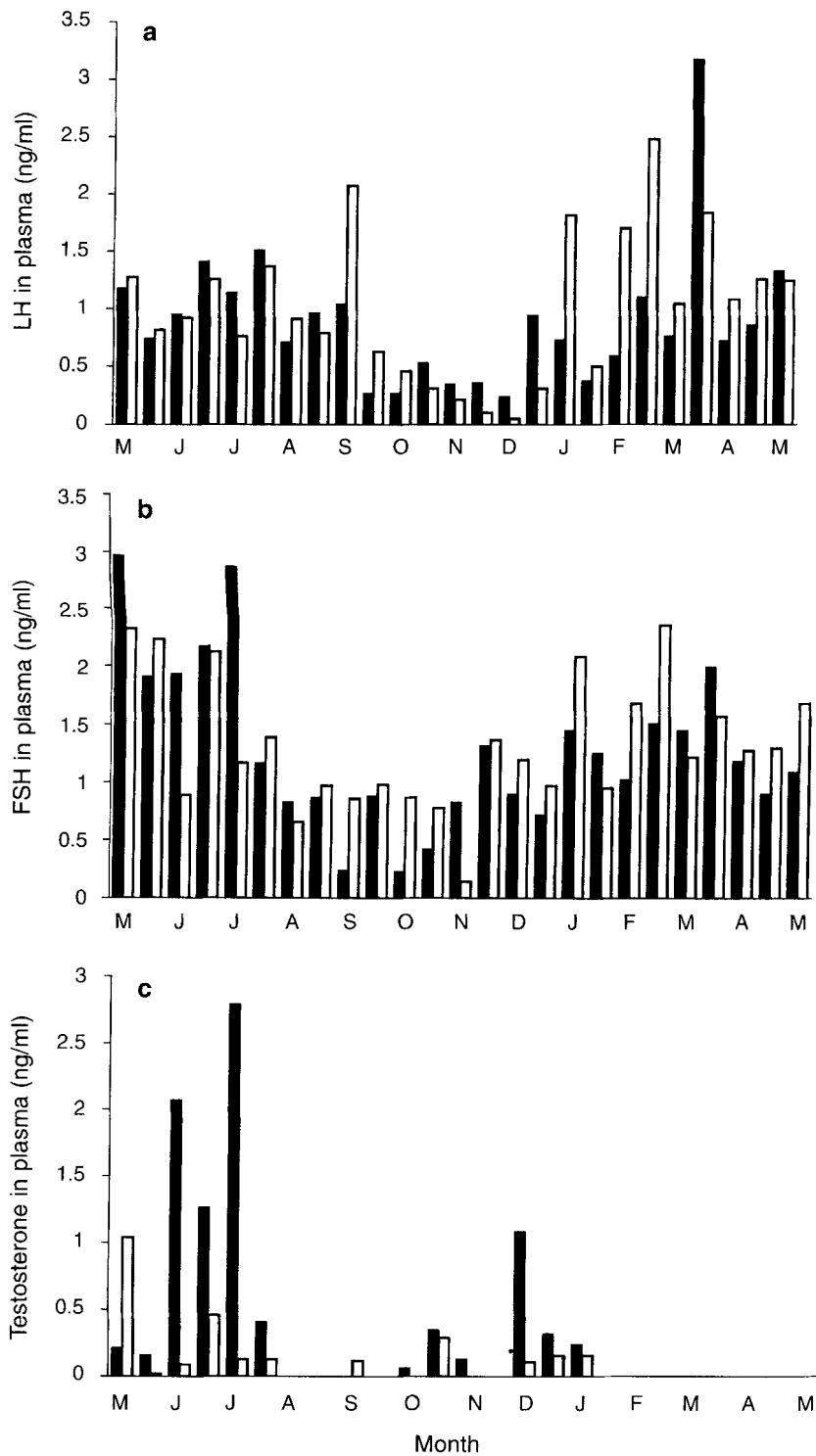


Figure 4. Hormone levels in two individual White Ibis *Threskiornis melanocephalus* males (□ and ■). (a) Luteinizing hormone (LH); (b) follicle-stimulating hormone (FSH); (c) androgen levels. From Ishii *et al.* (1983, 1984).

suggest that hormone levels in single spot samples can help to indicate reproductive status when compared

with a template derived from multiple samples of closely related, non-threatened species.

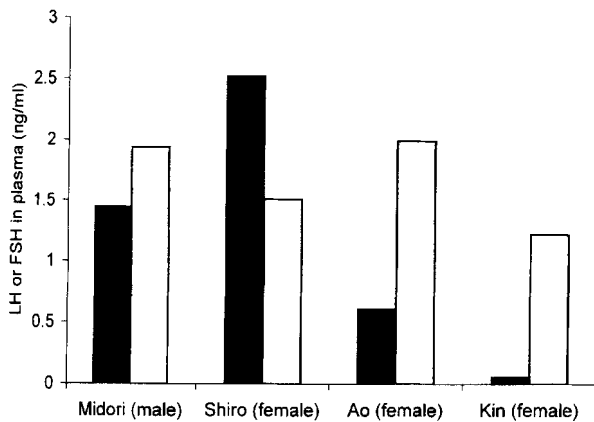


Figure 5. Plasma levels of luteinizing hormone (■) and follicle-stimulating hormone (□) in blood of individual captive Toki, *Nipponia nippon*. From Ishii *et al.* (1994).

Measurements of faecal steroids as indicators of reproductive status

Frequent blood sampling of critically endangered species is clearly out of the question, particularly if these animals are in the field. Recent work has shown that changes in faecal (i.e. excreted) steroid hormone levels may be an accurate measure of gross changes in circulating levels. Obviously, faecal samples can be collected without handling the individual, and they represent a non-invasive way of obtaining critical endocrine information. It may also be possible to measure gonadotrophins in faecal samples, although methods have yet to be validated.

Measurement of steroid levels in faeces is not a new technique, but particular attention has recently been paid to validating the methods by comparing changes in plasma levels of steroid hormones with faecal levels collected from the same animal. This has proved useful for determining if and when female mammals ovulate or become pregnant (Wasser 1995). It is important to validate the faecal steroid procedure in this way so that precise conclusions can be drawn from the data. In female Japanese Quail *Coturnix japonica*, faecal levels of oestradiol parallel ovarian development and plasma oestradiol levels. Similarly in male Japanese Quail, plasma and faecal levels of testosterone increase in parallel during reproductive development and are extremely low in castrated individuals (Ishii 1985, Ishii *et al.* 1994). Further studies show that if female Japanese Quail are given subcutaneous implants of oestradiol or progesterone in capsules that release the hormones at constant rates, then both plasma and faecal levels of oestradiol and progesterone increase in parallel (Kubodera *et al.* 1988, Ishii *et al.* 1994).

These data suggest that changes in faecal levels of steroid hormones can be used as indicators of reproductive development. These studies may then be used as templates to compare changes in faecal levels of steroids in endangered species such as the Toki (Fig. 6). The male code-named Green (Midori) had elevated LH and FSH levels (Table 3, Fig. 5) and showed a normal pattern of faecal androgen levels (Fig. 6) compared with the pattern of circulating androgens in White Ibises (Fig. 4c). Faecal testosterone levels increase in December and January coincident with beginning gonadal maturation and when nuptial colour is developed. Midori, the only male in captivity, developed full nuptial colour. We do not know whether the secretion of the cosmetic and its application are testosterone-dependent, but in other species in which males

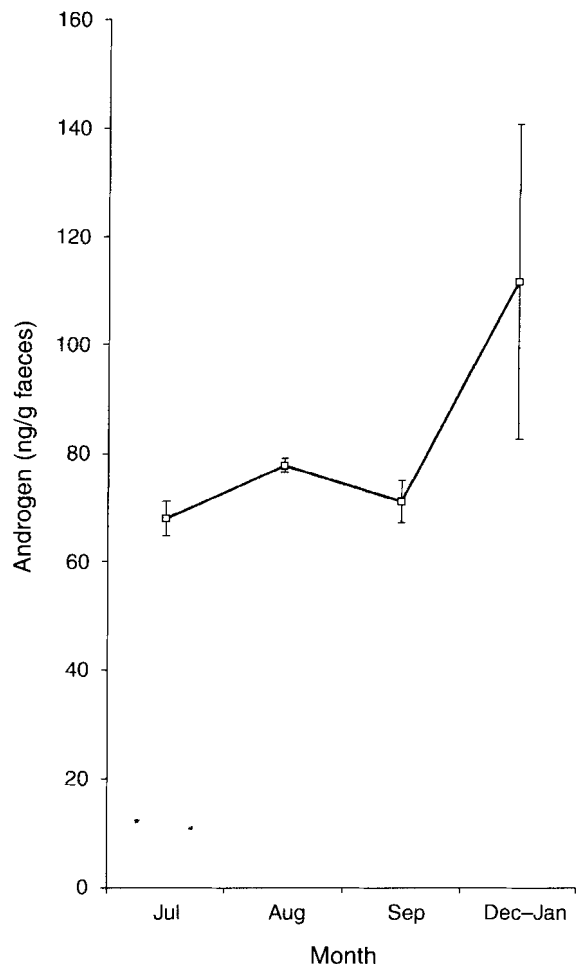


Figure 6. Faecal levels of androgen in 'Midori', a male Toki *Nipponia nippon*. Each point represents the mean level from faeces collected on four consecutive days in each month. Vertical bars are the standard errors. From Kikuchi and Ishii (1997).

and females develop a nuptial plumage, testosterone regulates the process in both sexes (e.g. many species of the genus *Larus*, Witschii 1961, Terkel *et al.* 1976, Groothuis & Meeuwissen 1992). We shall not be able to determine by experiment whether development of nuptial plumage in the Toki is testosterone-dependent, but deductions made from other species as well as from changes in faecal steroid levels allow us to make predictions. For example, if individual Toki fail to develop nuptial colour, or do so only partially, then treatment with testosterone may help to complete the process and enhance the possibility of successful breeding. This could easily be tested by feeding low doses of testosterone in food (steroids can be absorbed from the gut).

Faecal steroid analysis may have further uses. Yamaguchi and Ishii (1991) have shown that faecal levels of progesterone can be used to determine whether female Japanese Quail have ovulated. Females that laid eggs showed a large surge of progesterone in faeces during the late evening. In non-laying females, no such surge in faecal progesterone levels is observed. Further analyses of this type will undoubtedly shed light on reproductive function that can be used to monitor breeding status in endangered species.

Using reproductive hormones to enhance reproductive output

Plasma and faecal levels of steroids coupled with behaviour and morphological observations can be used to pin-point deficits in reproductive function. The first step is to adjust the captive breeding programme to correct those deficits without invasive treatment, so that the birds breed normally. However, if this fails then the next step is to provide hormone therapy as a means of enhancing reproductive output. As mentioned above, brief hormone treatment may be successful in completing development of nuptial colour or triggering courtship behaviour. More extensive treatment may be necessary if individuals still do not breed.

Hormone therapy of this type is fraught with difficulties because endocrine manipulations may affect other processes as well. For example, hormone treatment may disrupt parental behaviour so that young die, or may influence the phenotype, even sex, that develops. It is possible that many offspring generated following hormone therapy of adults may not be viable for reintroduction into the wild but they may, nonetheless, be useful as captive breeding stock to induce natural breeding without further hormone treatment. In cases where populations are reduced to

critical levels there may be few options other than to try hormone therapy.

It has been more than 20 years since avian gonadotrophins (from chickens) were first isolated and purified but, even now, preparations of these hormones are not generally available and certainly not in sufficient amounts to support a major hormone therapy programme. Molecular techniques will provide the answer as genes for these hormones are cloned, sequenced and recombinant techniques used to generate large quantities of gonadotrophins for therapeutic purposes. Recent research suggests that treatment with avian gonadotrophins can indeed induce reproductive maturation and even ovulation. Much work remains to be done, but results are encouraging.

Multiple injections of hormones over a period of many days or even weeks are likely to be counterproductive because of the stress of handling. However, Wakabayashi *et al.* (1987, 1989, 1992) used ALZET osmotic pumps filled with a mixture of avian LH and FSH to treat female quail kept on short, winter-like, days that normally do not support reproductive development. These pumps can be implanted intraperitoneally through minor surgery and deliver hormone preparations at a constant rate for up to two weeks. Measurement of plasma levels of LH and FSH in these females revealed that the pumps successfully elevated circulating gonadotrophin levels over control females that received pumps filled with saline only. Then some of the treated females were given additional injections of avian gonadotrophins and three out of seven laid a total of eight eggs. These females were housed with males and, when the eggs were incubated, two out of eight hatched. Both chicks were males, grew normally and at least one proved to be fertile (Wakabayashi *et al.* 1987). The yield may not be impressive, but for an initial experiment the results are encouraging. Since some of the female Toki in captivity failed to lay at all, the possibility certainly exists that in the future it may be possible to enhance reproductive output with gonadotrophin treatment and produce viable offspring.

Wakabayashi *et al.* (1987) also applied the osmotic pump techniques to administer chicken gonadotrophins to a passeriform, the Chinese Grosbeak *Eophona migratoria*. Treatment resulted in marked development of the ovary and oviduct suggesting that chicken gonadotrophins may be used even in unrelated avian species. Ishii *et al.* (1994) obtained preliminary evidence that chicken gonadotrophin preparations may also stimulate ovarian development

in the Sacred Ibis and Scarlet Ibis *Eudocimus ruber*. However, we should be cautious because over longer periods (weeks to months) it is likely that the recipient of chicken gonadotrophins may eventually form antibodies (as a response to alien chicken protein) that could neutralize biological effects.

The Toki is a critically endangered species with a fascinating and novel breeding biology that enriches our knowledge of reproductive biology in general. It is a typical example of how biodiversity includes not only a web of many species intertwined in a habitat, but also diversity of morphology, physiology and behaviour. All of these aspects are important to consider when attempting to bring back such critically endangered species from the brink of extinction. It is possible that, by incorporating endocrine techniques, we may be able to identify reproductive malfunction and then use hormone therapy in a limited way to enhance reproductive output. Studies of closely related species to provide a template and investigations of hormone therapy procedures in other species may be new ways in which to pursue conservation-endocrine techniques.

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