# Ovarian degeneration resulting in the phenotypic masculinisation of a wild female Mallard *Anas platyrhynchos*

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**Abstract**

Among dichromatic avian species, the loss of sexual organs can induce reversal of sexual features among females and males. In particular, the phenotypic feminisation or masculinisation of males and females, respectively, has been linked to the presence of testosterone or luteinizing hormones. Specifically, females lacking a functional ovary (*e.g.* experience an ovariectomy) or males lacking testes have been found to exhibit male breeding plumage in subsequent moult cycles. We conducted *post mortem* examination on a wild Mallard *Anas platyrhynchos*, determined genetically as a female but displaying male plumage, and found that the ovary was missing despite the remaining sexual organs being intact. We concluded that this individual provided an example of spontaneous ovarian degeneration, and that its male-like plumage was attributable to a resulting lack of oestrogen in its body. Together, these results further establish that plumage expression is not strictly genetically based, but rather dictated by the ability for the timely expression or suppression of these genes via modifiers, begging the question of why both sexes retain the molecular variation required to express the male plumage.

**Key words:** luteinizing hormone, morphology, ovarian degeneration, phenotype, testosterone.

Plumage colouration in birds is a conspicuous component of phenotypic variation, particularly where plumage patterns differ between the sexes (Price 1998; Hudson & Price 2014; Seehausen *et al.* 2014). In sexually dichromatic species, colourful males and "drab" females (Badyaev & Hill 2003) are thought to have diverged through either sexual (*i.e.* female choice) or natural (*i.e.* predation) selection, respectively (Johnson 1999; Figuerola & Green 2000; Dunn *et al.* 2015). Although

infrequent (Chiba & Honma 2011), the spontaneous reversal of sexual features among females and males of a dichromatic species can occur naturally (Forbes 1947). Whilst recent experimental studies have demonstrated that levels of particular cell-autonomous sex identifier genes (*i.e.* DMRT1) present at the embryonic stage can change the predicted sex organs and expression of secondary sexual traits (Lambeth *et al.* 2014; Ioannidis *et al.* 2021), the phenotypic feminisation or masculinisation of adult males and females, respectively, have repeatedly been found to be linked to the presence of testosterone or luteinizing hormones (Mueller 1970; Gibson *et al.* 1975; Kimball & Ligon 1999; Lank *et al.* 1999; Lahaye *et al.* 2013). In particular, female ornamentation within a clade can be highly labile, with elaborate, male-like traits frequently displayed (Eaton & Johnson 2006; Price & Eaton 2014; Gomes *et al.* 2016). In fact, females lacking their ovary (*i.e.* experience an ovariectomy) become phenotypically masculinised across moult cycles (Goodale 1910; Fitzsimons 1912; Goodale 1913; Greij 1973; Gibson *et al.* 1975; Reyss-Brion *et al.* 1982; Chiba & Honma 2011), demonstrating that the repression of male-like trait display in post-embryonic females is controlled by oestrogen levels (Tanabe 1982; Owens & Short 1995; Kimball & Ligon 1999). Conversely, the removal of testes in post-embryonic males results in loss of the eclipse (*i.e.* non-breeding) plumage and continuous expression of breeding-like plumage, indicating that male-type plumage is the default state of plumage development (Walton 1937; Greenwood & Blyth 1938; Owens & Short 1995). Thus, both sexes are clearly capable of expressing male-like traits that proceed via modifiers (*e.g.* modifier alleles, steroids; Lande 1980; Horton *et al.* 2014; Kraaijeveld 2014), with an inability to circulate appropriate levels of particular steroids resulting in the permanent expression of the dichromatic male plumage in both sexes.

Here we describe the results of *post mortem* examination of a wild Mallard *Anas platyrhynchos* harvested by a hunter in Arkansas, USA, which had a combination of male and female characteristics (male breeding plumage; female bill colour), and was immediately frozen whole for further analysis because of its appearance. In particular, we aimed to determine the genetic and morphological sex of the bird, with a view to providing further insight into the factors affecting variation in the plumage exhibited by dimorphic species.

## Methods

### Sample

The Mallard – sample ID BD61 (University of Texas (UTEP) Biological Collections accession ID: UTEP Bird 3349) – was harvested in Arkansas, USA on 23 January 2021. The bird was immediately frozen and sent to the Lavretsky laboratory at the University of Texas at El Paso for genetic testing and *post mortem* examination. Breast tissue was obtained for DNA analysis once the specimen arrived at the laboratory, and genetic assessment (methods described by Davis *et al.* 2022) found the bird to be female. All activities in the study were conducted under a US Fish & Wildlife

 

Service (USFWS) migratory bird salvage permit (No. MB11579C). Information on specimens used in the manuscript are also available on the museum collections management system "Arctos" (https://arctosdb.org/).

### *Post mortem* examination

Prior to the *post mortem* examination, the bird was removed from the freezer to ensure sufficient thaw at room temperature. First, the plumage and moult phase were assessed based on species moult descriptors (Pyle 2008) and compared to a male (UTEP accession ID 3041) and female (UTEP accession ID 2872) Mallard. Next, the examination and preparation of the specimen followed a routine procedure described in Winker (2000). Briefly, in addition to weight, six morphological measurements were obtained and compared to published data for male and female Mallards (Pyle 2008; Drilling *et al.* 2020). Photographs were also taken prior to *post mortem* dissection for comparative purposes. Following this assessment, we made an initial incision in the skin from furculum to belly, then separated the skin from the body using forceps. These incisions into the body cavity allowed the removal of the stomach and intestines for examination of the sex organs, which are located along the dorsal surface of the cavity near the anterior side of the kidneys (Fig. 1). We examined the sex organs with care, to minimise disturbance in the cavity and the possibility of overlooking or destroying relevant structures. We then compared the sex organs to those of reference male and female Mallards.

## Results

### *Post mortem* examination results

The specimen was genetically determined to be both of wild Mallard ancestry and female (Davis *et al.* 2022). Consistent with the genetics, *post mortem* examination of the bronchus (syrinx) further confirmed an intact and morphologically normal female syrinx (Fig. 1). The female Mallard specimen showed slight moulting on the dorsal and ventral sides, as well as on the head and neck, with new feathers resembling the basic male plumage (Fig. 1). Wing characteristics (Pyle 2008), including tertial wear were consistent with the specimen being an adult.

Morphological measurements of the bird were more consistent with general trends found in adult female rather than male Mallards (Pyle 2008); however, the bird's weight was consistent with that of an adult male (Table 1; Drilling *et al.* 2020). The specimen's overall phenotype resembled a male Mallard, including the absence of the extension of the upper white wing-bar into the tertial area which is characteristic of female Mallards (Fig. 1; Pyle 2008). Despite these male-like phenotypic displays, the duck's bill was female-like, being orange-coloured and carrying a black saddle across the bill that is absent in male Mallard (Fig. 1; Pyle 2008).

Finally, *post mortem* examination of sexual organs revealed that the ovary was missing, whilst the oviduct and syrinx were intact and morphologically normal compared to the reference female (Fig. 2). No wounds, including those that could have occurred at the time of harvest, were found that could

 

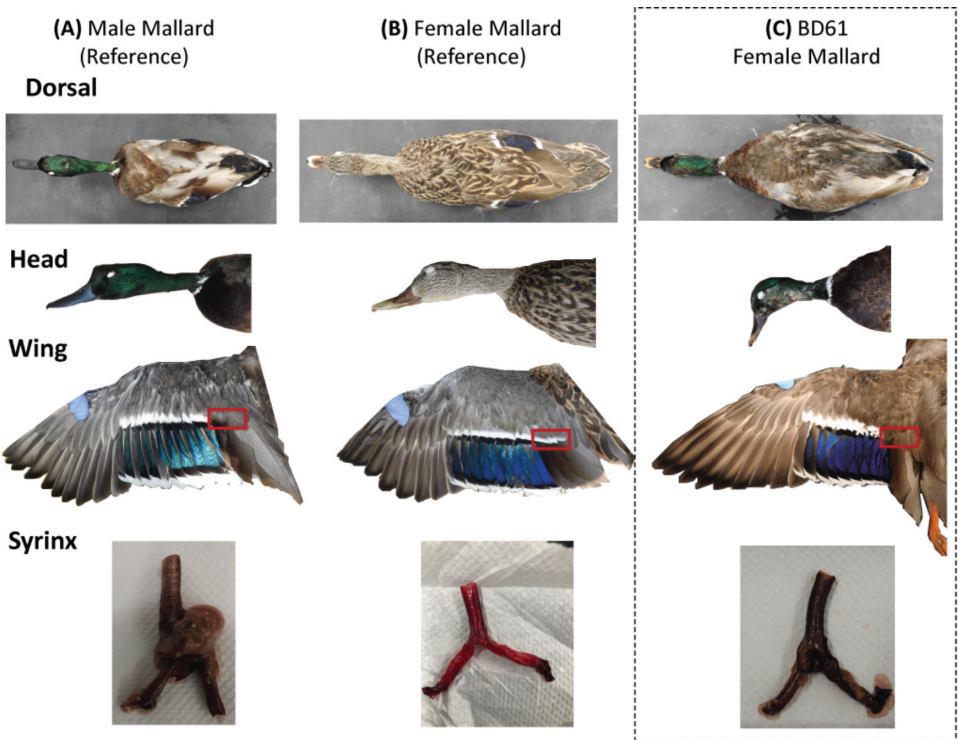


**Figure 1**. Overall dorsal, head, and wing phenotype of (A) reference male Mallard, (B) reference female Mallard, and (C) our sample (BD61). We denote the upper white bar across wings, demonstrating a lack of extension into the tertial covert region in our sample, in comparison with the extension evident for the reference female. Additionally, the bronchus (syrinx) is provided for each of the reference birds and the sample bird, for comparison; note the lack of the bulla syringealis in the syrinx of both the reference female and our sample.

explain the loss of the ovary. Thus, we concluded that this individual is an example of spontaneous ovarian degeneration (Chiba & Honma 2011), and the male-like plumage was due to the resulting lack of oestrogen circulating in the bird.

## Discussion

We present evidence of a female Mallard phenotypically resembling its male counterpart due to substantial ovarian degeneration (Fig. 1). Although ovarian degeneration occurs, it is infrequent among wild populations (Forbes 1947; Chiba & Honma 2011). The reason why this female Mallard experienced ovarian degeneration remains unknown, noting that we did not find any evidence of wounds that could explain the loss of the ovary, and that the individual seemed healthy given its weight (Table 1). It remains possible that ovarian degeneration may have occurred as a result

**Table 1.** Morphological measurements of representative males and females of wild Mallard populations Pyle (2008) and Drilling *et al.* (2020) compared to the bird in our female sample (BD61). Morphological measurements from BD61 fell within the expected female range except for weight, which more closely resembled that of a male Mallard.

| Trait | Pyle (2008) | | Drilling *et al.* (2020) | | |
|---|---|---|---|---|---|
| | Male | Female | Male | Female | BD61 |
| Wing chord (mm) | 271–303 | 255–287 | 292.8 (s.e. ± 0.2) | 275.5 (s.e. ± 0.4) | 264 |
| Tail length (mm) | 84–103 | 80–98 | 88.1 (s.d. ± 5.5) | 82.20 (s.d. ± 4.0) | 85 |
| Exp culmen | 52–59 | 48–55 | N/A | N/A | 49 |
| Bill length (mm) | N/A | N/A | 41.7 (s.d. ± 2.1) | 38.7 ± (s.d. ± 1.6) | 38 |
| Bill depth (mm) | 19.4–23.6 | 18.6–22.6 | N/A | N/A | 16.7 |
| Tarsus length (mm) | 43–50 | 41–47 | 46.1 ± (s.d. ± 1.5) | 43.9 (s.d. ± 1.5) | 41.5 |
| Mass (g) | N/A | N/A | 1,246 g (s.e. ± 3.0) | 1,095 (s.e. ± 5.0) | 1,264 |

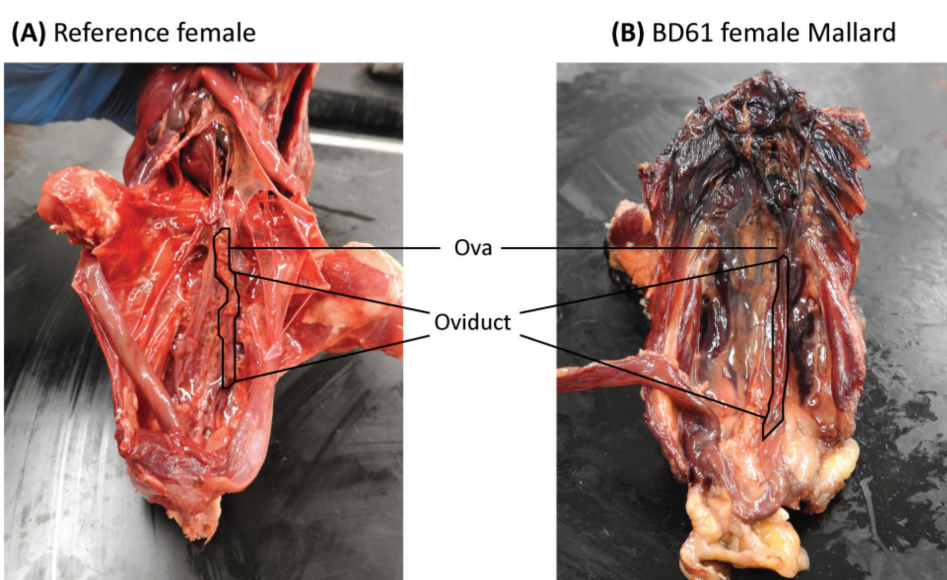


**Figure 2.** The sex organs of (A) wild-type female, and (B) our sample (BD61). Note the missing ovary but intact oviduct in our sample bird, whereas both are present in the reference female.

of age or the individual carrying disease(s), but the histological analysis required to identify disease was outside of the scope of this study. Regardless of cause, ovarian degeneration can lead to phenotypic masculinisation of the female in dimorphic species (Goodale 1910; Fitzsimons 1912; Goodale 1913; Greij 1973; Gibson *et al.* 1975; Reyss-Brion *et al.* 1982; Chiba & Honma 2011), with earlier studies attributing this to a resulting lack of oestrogen (Tanabe 1982; Owens & Short 1995; Kimball & Ligon 1999). Moreover, an inability to produce sufficient steroid levels can result in both males and females being consistently in male breeding plumage during subsequent moult cycles (Walton 1937; Greenwood & Blyth 1938; Owens & Short 1995). Thus, expression of the male Mallard's definitive basic plumage (*i.e.* dichromatic breeding state) in our female Mallard is consistent with her lacking appropriate levels of oestrogen because of the missing ovary. Together, these results demonstrate that sexes of a dichromatic species retain the genetic underpinnings to express the male breeding state, and that the expression or suppression of this state is controlled by appropriate levels of steroids in the respective sex. Given that both sexes evidently retain the molecular variation associated with the expression of male-like plumage, this finding suggests that their retention is selectively important for the species. Full genome analyses will be important to uncover the strength of selection on these regions between sexes, such as whether mutations arise at higher rates, or at all in females compared to males. Whilst we note that expression levels of particular cell-autonomous sex identifier genes (*i.e.* DMRT1) can change sex organs and secondary traits at the embryonic stage (Lambeth *et al.* 2014; Ioannidis *et al.* 2021), how these may interact at the post-embryonic stage requires further research. Additionally, we encourage researchers interested in understanding changes in secondary sexual traits at post-embryonic stages to investigate how variation in luteinizing hormone levels influences the extent to which males and females show the appropriate or alternative plumage characteristics for their sex.

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