Contrary to phylogenetic predictions, crown coloration of the Eared Dove is not regulated by sex hormones

Leila M. López[1§], Agustín I. Quaglia[2§¶], Santiago M. Benitez-Vieyra[3], Verónica I. Cantarelli[4], Marina F. Ponzio[4], Diego J. Valdez[1,5*]

1 Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Centro de Zoología Aplicada, Córdoba, Argentina.

2 Laboratorio de Arbovirus, Instituto de Virología "Dr. J. M. Vanella," Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

3 Instituto Multidisciplinario de Biología Vegetal (IMBIV), Universidad Nacional de Córdoba–Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina.

4 Instituto de Investigaciones en Ciencias de la Salud (INICSA-CONICET), Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

5 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Diversidad y Ecología Animal (IDEA), Córdoba, Av. Vélez Sarsfield 299, X5000JJC, Argentina.

*Corresponding Author: Diego J. Valdez, Centro de Zoología Aplicada, Rondeau 798, X5000AVP Córdoba, Argentina Tel-Fax: (+54 9 0351) 433-2055 and 433-2054.

E-mail: dvaldez@unc.edu.ar

ORCID ID LML: 0009-0004-6945-0904

ORCID ID AIQ: 0009-0006-7275-2545

ORCID ID SMB-V: 0000-0003-4116-7969

ORCID ID VIC: 0000-0002-2974-886X

ORCID ID MFP: 0000-0002-3549-2063

ORCID ID DJV: 0000-0002-4467-6761

Running title: Non hormonal control of the Eared Dove's coloration.

§ Equal contributions

¶ Present addresses: Laboratorio de Biología Integrativa, IMIBIO-SL-CONICET, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina.

*Corresponding author

Abstract

The mechanisms regulating plumage coloration appear to be phylogenetically conserved in avian species: the plesiomorphic character state is estrogen-dependent regulation while testosterone, luteinizing hormone and non-hormonal control are derived states. Limited data exist on the underlying regulatory processes of sexual dichromatism in the Eared Dove (*Zenaida auriculata* des Murs, 1847). Since the Columbiformes order is close to basal branches, we hypothesised that estrogen and testosterone play a central role in the regulation of crown plumage coloration in the Eared Dove. To test this, we subjected adult males to a forced molt accompanied by an exogenous increase of estradiol and testosterone to determine whether the presence of these hormones during molting modified the spectrophotometric characteristics of the plumage. No significant differences were found between treatments and controls in the colorimetric variables hue, ultraviolet saturation and brightness. Similarly, the avian visual model showed no perceptible changes in chromatic and achromatic signals in the individual male adults. We also analysed the effects of estrogen and testosterone on the growth speed of the new feathers. The hormone-treated groups increased in the speed of molting compared to the control group. These results suggest that the Eared Dove is an exception to basal branch models: rather than regulating coloration, sex hormones speed up the growth of the new feathers.

Keywords: *Zenaida auriculata*, Eared Dove, plumage, crown, molt, coloration, avian visual model.

Introduction

Feathers are one of the primary features that distinguish birds. Besides being crucial for flight, they serve various functions such as thermal insulation and protection against solar radiation and parasites (Terril and Shultz 2022). Additionally, they play a prominent role in both intra- and interspecific communication, being involved in attracting potential mates (through display or sound production), maintaining or acquiring social status, defending territory, and camouflage (Hill and McGraw 2006; Terril and Shultz 2022).

Over time, the plumage and its coloration are degraded by both biotic (bacterial activity and ectoparasites) and abiotic factors (mechanical abrasion, accumulation of fat and dust, solar radiation, among others) (Delhey et al. 2007; Tökölyi et al. 2008; Shawkey et al. 2009; Griggio et al. 2011; Surmacki et al. 2011; Valdez and Benitez-Vieyra 2023). When a feather deteriorates, its function can be compromised, making its replacement fundamental for restoring full functionality (Jenni and Winkler 2020). In this regard, molting is a key physiological process in the life history of birds, as it allows old feathers to be replaced with new ones (Lindström et al. 1993). The molting period enables birds to change their overall color. Feather coloration is determined by two main factors: the deposition of pigments, predominantly carotenoids and melanins and the ultrastructure of the feather, which influences how light is absorbed and reflected within the keratin layers, giving rise to structural colours (Hill and McGraw 2006).

Hormones are proposed as the primary regulatory element of plumage coloration, as they can vary sexually, seasonally, and ontogenetically (Ralph, 1969; Kimball, 2006; Valdez et al. 2014; Maldonado et al. 2020). In numerous bird species, the influence of the sex hormones estradiol and testosterone on feather coloration and growth rate of new feathers has been observed (Haase and Schmedemann 1992; Hahn et al. 1992; Hall et al. 1993; Haase et al. 1995; Evans et al. 2000; Stoehr and Hill 2001; Fargallo et al. 2007; Kurvers et al. 2008). These steroid hormones affect feather development in two ways: directly, through their interaction with steroid receptors located in the feather papilla, and indirectly, via their pro-oxidant properties. Consequently, these hormones can influence the growth speed, colour, and structure of new feathers (Peczely 1992; Peters et al. 2006; Alonso-Alvarez et al. 2007; Roberts et al. 2009). Increased concentration of sexual hormones such as estrogen and testosterone have been found to delay the onset of molt across the avian groups. Conversely, when hormone levels return to species-specific basal concentrations, the molt proceeds normally (Hahn et al. 1992; Hall et al. 1993; Nolan et

al. 1992; Stoehr and Hill 2001). These observations suggest that elevated levels of sexual hormones have an inhibitory effect on the molt process across the avian phylogeny. Added to this, in numerous bird species exhibiting carotenoid-based coloration, testosterone often plays a role in enhancing the quality of plumage or bare parts (Romero-Diaz et al., 2022). In contrast, estrogen generally reduces investment in color expression, resulting in a duller appearance (Casagrande et al. 2011; Lindsay 2016; Romero Diaz et al. 2022). In melanic coloration, testosterone appears to have a significant influence on melanocyte function and melanization, at least in bird species that have androgen-dependent dichromatism (McGraw 2006; Bókony et al. 2008). The role of estrogen in melanic coloration remains understudied, however existing evidence suggests that it affects the different forms of melanin (eumelanin-pheomelanin), which impacts pigment deposition (McGraw 2006).

The estrogen-dependent mechanism appears as the plesiomorphic state in avian phylogeny, present in groups such as paleognaths, galliformes, and anseriformes (see Fig. B10.1 Hormonal control of coloration, Kimball 2006). In addition to this hormonal regulation, other control mechanisms are acquired in more derived avian groups (Neoaves - passeriformes), involving, for example, regulation by testosterone, luteinizing hormone, or even the loss of hormonal control (see Fig. B10.1 Hormonal control of coloration. Kimball, 2006). The columbiformes order emerges as an avian group of particular interest due to its basal positioning within the Neoaves (Prum et al. 2015), suggesting the potential retention of plesiomorphic characteristics in coloration regulation. Specifically, it is postulated that species within this order may uphold the ancestral condition of estrogenic regulation of their coloration resulting in a duller plumage with slower growth speed. However, despite its basal position within Neoaves, there exists an extensive evolutionary timespan between Galloanserae and the Neoaves. This evolutionary gap raises the possibility that coloration in the columbiformes order may have evolved towards a derived trait, possibly under the control of testosterone, resulting in birds with brighter plumage colors but slower growth rates.

In the *Zenaida* genus, species are typically sexually monochromatic; however, The Eared Dove (*Zenaida auriculata* des Murs, 1847) is sexually dichromatic (Valdez and Benitez Vieyra 2016). The Eared Dove, a species of dove native to South America, exhibits a melanistic coloration, with a slight pinkish hue and greyish tones (Narosky et al. 2010). In males, the crown is a particularly conspicuous region which is exposed during

courtship and appears bluish-grey melanic colour, while in juveniles and females, it tends to be brownish-grey (Fig 1A). The differences in crown coloration between males and females make this species interesting to study, particularly due to the potential influence of sex hormones such as estradiol and testosterone in regulating their melanin-based coloration. Given its phylogenetic position, two possibilities exist: either estrogen drives the coloration, resulting in a dull plumage similar to observed in females or testosterone drives it, resulting in a bright plumage coloration similar to observed in males.

The molting period of crown feathers in the Eared Dove occurs between the months of January and June (Valdez and Benitez-Vieyra 2023). Additionally, during this same period, Eared Dove males exhibit plasma testosterone levels ranging from 250 pg/ml in February to less than 100 pg/ml for the remainder of the period (Maldonado et al. 2020). What is noteworthy is that even at low plasma testosterone levels, the males remain sexually active (Maldonado et al. 2020).

Given the aforementdioned phylogenetic context, we hypothesise the existence of sex hormone-mediated (either estradiol or testosterone) control of coloration in the crown feathers of the Eared Dove. Moreover, we also hypothesise that increased levels of sexual hormones will delay the onset of the molt process in this species. We therefore assessed the effect of estradiol and testosterone on the coloration and growth speed of new crown feathers in adult male Eared Dove during the molting period.

Materials and Methods

The study meets all the requirements of the Argentine legal system and was carried out in strict accordance with the Guidelines for Ethical Research on Laboratory and Farm Animals and Wildlife Species; the study also had the prior approval of the ethics committee of CONICET (Resolution No. 1047 ANNEX II, 2005) and CICUAL (Institutional Committee for the Care and Use of Experimental Animals, Act. N°. 33/2023, dated 10/04/23). The necessary permits to capture specimens of Eared Dove were provided by the Ministry for Water, the Environment and Public Services of the Province of Córdoba, Argentina, through the Secretariat for the Environment and Climate Change.

153 **Animals**

154 The sampling of Eared Dove (*Z. auriculata*) was carried out in the vicinity of the
155 Biodiversity Park of Córdoba, Argentina (Ex-Córdoba Zoo; 31°25'31.79" S, 64°10'29.92"
156 W) between April and May 2023, during the crown feather molting period (Valdez and
157 Benitez-Vieyra, 2023). For the capture of birds, passive traps baited with commercial bird
158 food, as stipulated by Navarro (1986), were employed. Only those individuals displaying
159 a strong bluish-grey plumage on their crown, a characteristic of males of this species,
160 were selected (Valdez and Benitez-Vieyra 2016; 2023).

161 A total of 23 Eared Dove adult males were captured and randomly distributed among the
162 three experimental groups. The control group (*C*, with hormone-free implants) consisted
163 of 7 animals, and the groups treated with estradiol (*E*) and testosterone (*T*) each had 8
164 birds.

165 The specimens underwent an acclimation period of at least 7 days in a partially closed
166 room, suitable for small to medium-sized wild birds. Within this partially closed room,
167 individual cages measuring 50x40x40cm were provided, exposed to semi-natural
168 conditions of temperature (approximately 22°C) and light (approximately 600 lux of cool
169 white light). Individuals had access to food and water ad-libitum. To prevent any potential
170 dietary influence on the coloration of the new feathers, we only used canary seed
171 ("alpiste") instead of commercial bird food.

172

173 **Hormone Treatments**

174 The captured males were separated into three experimental groups: control (implant
175 without hormones) and implanted with estradiol or testosterone. Implants were made
176 using beeswax (volume = 63 mm^3) as specified by Quispe et al. (2015), at a concentration
177 of 5 mg of hormones each. The estradiol and testosterone standards used to develop the
178 implants were obtained from Sigma Aldrich, Inc. USA.

179 Each bird was immobilised using an elastic fabric tube to expose a patch of skin on the
180 midsection of their back. To remove the feathers in this area and expose the skin, a topical
181 anaesthetic was administered. After allowing the anaesthetic to take effect (approximately
182 5 minutes) and removing the feathers, an incision using a surgical scalpel was made in

183 the skin, creating a pocket where the subcutaneous implant was inserted. The incision
184 was closed with surgical adhesive (90% 2-ethyl cyanoacrylate) (Fig. 1B).

185 While the individuals were still immobilised, a section of ½ cm² of crown feathers was
186 removed to induce a forced molt (Fig. 1C). A post-operative behavioral observation was
187 conducted over the following 24 hours.

188

189 **Initiation and completion of the growth of the new feathers**

190 Following the forced molt procedure and the implant insertion, the growth of new feathers
191 in the crown patch of the three experimental groups was monitored, determining the exact
192 date on which the tips of the first feathers began to appear (initiation) and the point at
193 which the patch was completely covered by new feathers (completion).

194

195 **Spectrophotometric Analysis of Feathers**

196 The new crown feathers resulting from the forced molt were analysed by
197 spectrophotometry following the protocol established by Valdez and Benitez-Vieyra
198 (2016; 2023). Reflectance was measured within the avian visual spectrum range, covering
199 wavelengths from 300 to 700 nm. An OceanOptics USB4000 spectrophotometer
200 equipped with both a halogen and deuterium light source (839 Douglas Ave., Dunedin,
201 FL, USA 34698), both connected to the sensor via a bifurcated optical fibre, was used for
202 this purpose.

203 The spectrophotometric data (hue [H3], brightness [B2], saturation [S1U] and an avian
204 vision model) were processed using the PAVO package (Maia et al. 2013) within the R
205 software (R Core Team 2023).

206 The variables in the avian visual model were adjusted based on the approach used in
207 Valdez and Benitez-Vieyra (2016; 2023), where the quantum catch (Q) for each cone and
208 the standardised daylight (D65) representative of ambient light at midday in an open space
209 were established. The generalised spectral sensitivity of violet-type avian cones (VS),
210 characteristic of Columbiformes (Odeen and Håstad 2013), was used for this purpose.

211 The cone excitation values were used to calculate the plumage coordinates of the
212 experimental groups in the tetrachromatic energy space. Finally, to estimate chromatic

and achromatic distances, the avian visual model was applied, which states that receptor noise limits discrimination in each cone. Distances were characterised in units of JNDs (Just Noticeable Differences), where one JND represents the discrimination threshold for color perception.

Statistical Analysis

The reflectance spectra obtained from different experimental groups (control, estradiol and testosterone implants) were examined and compared to determine the presence of spectral overlaps.

The classic colorimetric variables, including hue, UV saturation, and brightness, were analysed using a one-way ANOVA to test for significant differences. Differences between coordinates in the tetrachromatic space as provided by the avian visual model were examined using a PERMANOVA analysis (Valdez and Benitez-Vieyra 2016).

The onset of molting and the growth speed data were analysed using a Student's t-test to determine significant differences between the treatments.

For all the statistical analyses conducted, the significance level (α) used was 0.05.

Blood Sampling and Hormone Quantification

To assess the proper functioning of the implants and the stress experienced by Eared Doves during the experiment, blood samples were extracted from the jugular vein of each bird using heparinized 1 ml syringes (Tuberculin syringe, TERUMO, Terumo Corporation) between 10:00 and 13:00 h to avoid circadian variations (Turek and Gwinner, 1982). The extractions were carried out after 4 days of acclimation in the partially closed room, before implantation, 2 days after implant placement, and then once a week for the following two weeks.

All blood samples were centrifuged at 5000 G for 25 minutes in a refrigerated centrifuge (Presvac EPF-12R). The resulting plasma was stored at -20°C until the corresponding hormonal assays were conducted. As the quantity of plasma obtained was insufficient to analyse all parameters, the sample sizes are not equal for all determinations. Therefore, samples from 16 individuals were randomly selected (6 from the control group and 5 from

each hormonal treatment) for the measurement of the sex hormones estradiol and testosterone. For the quantification of corticosterone, samples from 7 birds were randomly chosen (1 from the control group and 3 from each hormone treatment).

Estradiol and Testosterone

The concentration of estradiol and testosterone in plasma was determined by immunoassay, following the procedure described in the commercial electrochemiluminescence immunoassay kits Elecsys Testosterone II and Elecsys Estradiol II by ROCHE (specifications available in Valdez et al. 2014). Both hormones were analysed on a Cobas 6000 system with an immunoassay module e 601 (HITACHI High Technology Corporation-ROCHE Diagnostic GmbH).

Corticosterone

One clear sign of stress in birds is an increase in corticosterone levels (Sapolsky *et al.*, 2000; Romero and Romero, 2002; Bilková, 2020). This can be detected by comparing with basal corticosterone values, which can be safely measured if the blood sample is taken within the first 3 minutes after capture, a protocol applied in this study (Schoech et al. 1999; Romero and Romero 2002).

Corticosterone concentration was measured with an in-house enzyme immunoassay (EIA) using polyclonal antibody, corticosterone standard and the corresponding horseradish peroxidase conjugate (anti-Corticosterone CJM006, CJ Munro, UC Davis, CA, USA), as described in Rojas et al. (2019). Absorbance was measured at 405 nm using a Bio-Tek Synergy LX microplate reader (Bio-Tek Instruments, Winooski, VT, USA). Cross-reactivities reported for CC antibody are: corticosterone 100%, desoxycorticosterone 14.25%, progesterone 2.65%, tetrahydrocorticosterone 0.90%, testosterone 0.64%, cortisol 0.23%, prednisolone 0.07%, 11-deoxycortisol 0.03%, prednisone <0.01%, cortisone <0.01% and estradiol 17β <0.01%.

The sensitivity of the assay was 0.078 ng/ml. The intra-assay coefficient of variation was <12%.

Animal Sacrifice

Once the crown patch in all experimental groups was fully covered with new feathers, the individuals were kept in their cages for an additional week, reaching day 50. After this period, the animals were euthanized by decapitation using a guillotine, as the same experiment was designed to answer two different questions. The first question refers to the current study, while the second linked to our recent work (Marchese, N. A., Ríos, M. N., Guido, M. E., & Valdez, D. J. (2024). Three different seasonally expressed opsins are present in the brain of the Eared Dove, an opportunist breeder. Zoology, 162, 126147). For this reason, after collecting the necessary data for the current study, the birds were sacrificed at the end of the experiment to obtain and preserve their brains, eyes, and gonads.

Plasma was collected from each individual in 1,5 ml Eppendorf tubes, previously heparinized. These samples were treated according to the specifications outlined in the Hormone Quantification section, following the established selection criteria. Immediately afterwards, gonadal inspection was performed to confirm the sex of the animals.

Coloration and Phylogeny

Given the limited information regarding hormonal control of coloration across avian orders, Figure 5 was constructed based on the literature cited in the chapter “Hormonal control of coloration” by Kimball (2006) in Bird Coloration I, as well on the data available to date, which are cited within this work.

RESULTS

Spectrophotometric Analysis

Reflectance Spectra

Reflectance spectra for the crown plumage (Fig. 2A) show a reflectance peak near 340 nm in all three treatments, which corresponds to the ultraviolet (UV) region of the electromagnetic spectrum. However, it is important to note the greater variability in the ultraviolet region (from 300 to 400 nm), whereas in the rest of the spectrum, variability was limited and behaved similarly in all the experimental groups.

303 ***Classic Spectrophotometric Variables***

304 Table 1 displays the classic colorimetric variables studied: hue, ultraviolet saturation (S
305 UV), and brightness. No significant differences were observed for any of the variables or
306 between treatments (in all cases $F_{(2,20)} < 2.294$; $p > 0.127$).

307 ***Avian Visual Model***

308 Figure 2B-C shows the mean chromatic and achromatic distances, respectively, measured
309 in JND (just noticeable differences) between pairs of different treatments. For birds to be
310 able to perceive differences between groups, the values of each comparison should exceed
311 the discrimination threshold. We observed that chromatic distances showed significant
312 differences (PERMANOVA, $F_{(2, 19)}$=5.034; p=0.013) between all pairs of treatments (*C-T*, *C-E* and *E-T*), but all the values obtained were below the discrimination threshold. As
313
314 for achromatic distances, they did not display significant differences between any pairing
315 of treatments (PERMANOVA, $F_{(2, 19)}$=1.192; p=0.324).

316

317 **Initiation and completion of new feather growth**

318 After the forced molt (day 0), new feathers began to grow in the control group (*C*) at 3.21
319 ± 0.39 days (mean ± s.e.m), while the estradiol-treated group (*E*) started at 3.25 ± 0.38
320 days, and the testosterone-treated group (*T*), initiated growth at 3.13 ± 0.35 days. The
321 statistical analysis revealed no significant differences between any of the groups *(**C-E**:*
322 *t=-0.18; 95% CI [0.47; 0.39]; p = 0.905; **C-T**: t=0.46; 95% CI [0.51; 0.77]; p = 0.651;*
323 ***E-T** (t=0.68; 95% CI [0.52; 0.86]; p = 0.505)*.

324 The day of completion of new feather growth was considered when the previously created
325 patch was 100% covered with new feathers (Fig. 3A). Animals in the group *C* took 33 ±
326 5.86 (mean ± s.e.m) days to achieve 100% patch coverage, the group *E* took 25.87 ± 6.49
327 days, and the group *T:* 22.37 ± 3.33 days (Fig. 3B). Differences between means revealed
328 significant differences between the control group and the estradiol *(t=2.22; 95% CI [0.18;*
329 *14.07]; p = 0.045)* and testosterone *(t= 4.39; 95% CI [5.40;11.13] p=0.0007)* groups.
330 There were no significant differences between the groups *E* and *T (t=1.360; 95% CI [-*
331 *2.030; 9.030]; p = 0.196).*

332

Assessment of the proper functioning of implants

Hormone Quantification

In the group *C* (with hormone-free implants), basal concentrations of both hormones corresponding to the acclimation period (day -3) are observed in Figure 4A (estradiol: 13.455 ± 2.056 pg/ml; testosterone: 0.082 ± 0.058 ng/ml). After the implant placement (day 0), a slight increase in estradiol concentration was noticeable, reaching 27.510 ± 13.448 pg/ml and then rapidly decreasing to 11.570 ± 2.214 pg/ml, followed by 9.756 ± 1.846 pg/ml. The last measurement event for estradiol showed a slight increase, indicating a concentration of 16.858 ± 8.486 pg/ml. Testosterone, on the other hand, remained at 0.023 ± 0.002 ng/ml in the two weeks following day 0, except for the last measurement, which recorded a concentration of 0.144 ± 0.111 ng/ml. Thus, concentrations of both, estradiol and testosterone, remained at minimum levels, with no noticeable changes throughout the experiment in the control group.

Initially, the group *E* (Fig. 4B) showed estradiol concentrations similar to those observed for the acclimation period (15.804 ± 4.462 pg/ml). On day 2, an increase in the estradiol concentration can be observed, with a peak at 220.400 ± 34.054 pg/ml, followed by a slowly decreased over time. Estradiol reached values of 44.420 ± 10.776; 26.544 ± 6.354 and 22.218 ± 7.481 pg/ml in the subsequent weeks. In contrast, the testosterone concentration remained constant at all recorded time points (from day -3 to the last measurement on day 50), similar to the basal levels indicated for the control group, even after the surgical intervention.

A similar pattern is observed in the group treated with testosterone (Fig. 4C). Before the implant (day -3), testosterone showed a concentration of 0.022 ± 0.002, similar to that observed in the control group. Two days after implantation, testosterone levels reached 2.253 ± 1.398 ng/ml and then decreased rapidly. On day 9, levels were at 0.131 ± 0.057 ng/ml and on day 16, 0.089 ± 0.044 ng/ml. In the last measurement, the testosterone concentration increased slightly to 0.213 ± 0.165 ng/ml. Despite the different kinetics of this hormone in the blood, the values remained above those reported for the control group. The estradiol concentration remained within baseline concentration values throughout the experiment.

The monitoring of plasma corticosterone (Fig. 4D) shows a wide variability among individuals during the acclimation period (day -3). After the surgical intervention and the

forced molt, there was a general reduction in hormone concentration, showing a clear trend of decreasing corticosterone (and therefore, stress) in the blood throughout the experiment.

Behavioral appreciation

Despite the absence of a formal ethological analysis, notable behavioral changes were observed among the experimental groups. While control and estradiol-treated birds displayed no significant behavioral changes before and after implant insertion; testosterone-treated birds exhibited increased activity, object interaction and restlessness following implant insertion. This heightened reactivity was also evident during blood sampling and upon returning to their cages.

DISCUSSION

Here we report on the first study addressing the role of the sex hormones estradiol and testosterone in the mechanisms regulating the melanic coloration of the crown in the Eared Dove, the most abundant member of the *Zenaida* genus in South America (del Hoyo et al. 1992). Within the columbiform order, specifically in this species, the crown region is particularly important in courtship as males exhibit a characteristic courtship behavior: they pursue the female with puffed neck feathers, displaying their crown and vocalising (Goodwin 1966).

Our findings reveal that sex hormone treatment does not affect the spectrophotometric characteristics of the new crown plumage in terms of classic colorimetric variables (Table 1). However, analysis of the data using a columbiform visual model reveals two significant findings. Firstly, groups subjected to hormone treatment show differences among themselves and with the control group in terms of their chromatic distances (Fig. 2B). However, none of the obtained values exceeded the discrimination threshold (JND = 1), indicating that, at the biological level, birds may not perceive any differences among them. Secondly, in terms of achromatic distances it was observed that despite the statistical analysis not showing any significant differences, two of the groups exceeded the discrimination threshold (Fig. 2C), though not the 1 JND threshold. Birds appear to be unable to distinguish between signals with such minimal variations. Treatment with

sex hormones (estradiol and testosterone) therefore did not produce any appreciable changes in the coloration of the crown feathers of the Eared Dove. Furthermore, treatment with sex hormones significantly impacted the molting speed. Both estradiol and testosterone accelerated the molting of the crown feathers in the Eared Dove compared to the control group, with testosterone exerting a pronounced effect on this process (Fig. 3 A-B).

Additionally, these results were found to be unaffected by stress (corticosterone levels) resulting from experimental procedures or implant insertion (Fig. 4D).

Effect on Coloration

Estradiol

Estrogen-modulated changes in plumage coloration is the plesiomorphic state in the avian phylogeny (Kimball 2006). The effects are clear and consistently observed across three avian orders, encompassing different types of coloration, including melanic coloration in struthioniformes, and estructural coloration in galliformes and anseriformes (Kimball 2006). Considering the results obtained in our experiment, and in contrast with our previous expectations, we can observe that plumage coloration regulation in the Eared Dove is not estrogen-dependent. This conclusion is based on the fact that estrogen did not alter classical colorimetric variables and did not induce noticeable changes in either chromatic or achromatic signals.

In taxonomic groups where coloration is estrogen-dependent, the presence or absence of this hormone determines plumage color and pattern in dichromatic species (Kimball 2006). In both females and males of Common Quail (*Coturnix coturnix* Linnaeus, 1758), Domestic Chicken (*Gallus gallus domesticus* Linnaeus, 1758), Ring-necked Pheasant (*Phasianus colchicus* Linnaeus, 1758) and Mallards (*Anas platyrhynchos* Linnaeus, 1758), regardless of whether gonadectomy was performed beforehand, the plumage became paler and more female-like if oestrogen was present during the molt (see table 10.1 in Kimball 2006). Conversely, in its absence, individuals acquired bright, masculine-like plumage. Of particular interest is that treatment with androgens such as testosterone in no way affects plumage coloration in estrogen-dependent regulation (Kimball 2006). It is important to note that the Eared Dove does not conform to either of the scenarios

outlined: plumage was not affected neither by testosterone treatment nor by an exogenous increase in estradiol.

The plumage coloration in the Eared Dove appears to be more closely aligned with groups found in branches of the phylogeny, such as passerines. This order exhibits controls by androgens, luteinizing hormones, and hereditary (genetic) mechanisms, similar to what was observed in the Barn Swallow (*Hirundo rustica rustica* Linnaeus, 1758) where some coloration components appear to be highly heritable (Kimball 2006; Lindsay et al. 2011; Saino et al. 2013; Price-Waldman and Stoddard 2021). Unfortunately, the study of hormonal control of coloration has been conducted only in a few avian groups, with the majority remaining largely unknown. This complicates arriving at a precise phylogenetic conclusion, thus necessitating further studies to elucidate the effects of estradiol on plumage coloration.

Testosterone

What is known about the interaction between testosterone and secondary sexual traits, such as plumage coloration, indicates a complex interaction that varies with phylogeny and different types of coloration (Owens and Short 1995; Kimball 2006). In the charadriiformes group, the presence of testosterone is necessary to obtain melanistic colorful plumage, and in its absence, a dull and more female-like molt is obtained (Wingfield et al. 1980; Groothuis and Meeuwissen 1992; Bókony et al. 2008).

In the columbiform order, specifically in the Eared Dove, males exhibit a courtship behavior where they pursue the female with puffed neck feathers, displaying their crown and vocalizing. This body region shows marked sexual dichromatism (Valdez and Benitez-Vieyra 2016; 2023). As reported by Maldonado et al. (2020), Eared Dove males experience an increase in testosterone levels that reach around 200 pg/ml during February, which is when the first half molting period occurs (January, February and March). Thus, testosterone levels may regulate the coloration of male plumage, acting as a reliable signal of quality for females. However, the increase in testosterone did not lead to a significant or perceptible change in crown coloration during a forced molting.

Research on the correlation between coloration and testosterone levels during molting in passeriformes remains ongoing, and thus far, no consistent general trend has emerged. While some studies have observed a relationship between testosterone levels and

458 coloration changes, others have found no significant connection, highlighting the
459 complexity of this topic (Evans et al. 2000; Peters et al., 2006; Roberts et al., 2009;
460 Sieferrman et al., 2013).

461 The crown plumage of the Blue Tit (*Cyanistes caeruleus* Linnaeus, 1758), which is
462 subject to sexual selection pressure, increased its UV saturation after exogenous
463 testosterone treatment (Roberts et al. 2009). However, this difference was observed in
464 juveniles and in the breeding season following implant placement, not immediately after
465 molting. Treated birds also showed an increase in preening and in surrounding
466 testosterone levels in the months after implant placement. In our case, all Eared Dove
467 males were adults, and the samples were taken immediately after molting, so there is no
468 record of what might occur in a subsequent season. In the Eastern Bluebird (*Sialia sialis*
469 Linnaeus, 1758) an increase in testosterone did not affect the structural plumage
470 coloration of the crown but did affect the melanin-based plumage of the chest, making
471 the males browner and duller (Siefferman et al. 2013). In House Finches (*Haemorhous*
472 *mexicanus* Müller, 1776) an increase in testosterone directly affects the tone of carotenoid
473 coloration, making them less red (Stoehr and Hill 2001).

474 Against this background, we think that the seasonal increase in testosterone observed by
475 Maldonado et al. (2020) at the time of molting in the crown plumage of the Eared Dove
476 is a coincidence and not due to it being a regulatory element in coloration. Further studies
477 could address whether the increase in testosterone changes the coloration in other body
478 regions such as the beak and legs, as observed in the House Sparrow (*Passer domesticus*
479 Linnaeus, 1758) and the Australian Red-backed Fairy-wren (*Malurus melanocephalus*
480 Latham, 1801). In both these species, the darkening of the beak functions as a reliable
481 signal of their quality as a mate (Laucht et al. 2010; Karubian et al. 2011). More studies
482 will be necessary to evaluate the sex hormones effect on the coloration of beaks and legs
483 in the Eared Dove.

484 The information obtained in our experiment suggests that the mechanisms regulating the
485 coloration of the Eared Dove's crown would be similar to those in more derived
486 phylogenetic groups (genetics control). Here arises the question of whether the
487 columbiformes group constitutes an exception among the basal phylogenetic branches of
488 Neoaves, or if the absence of hormonal regulation of coloration is to be expected, given
489 the evolutionary timespan between Galloanserae and Neoaves. Unfortunately, there is

insufficient information available from other avian groups to reach a robust phylogenetic conclusion. (Fig. 5). It would be of interest for future studies to investigate the role of sex hormones and other hormones (such as luteinizing hormone), as well as the genetic mechanisms of coloration in additional avian orders (Kimball 2006; Price-Waldman & Stoddard 2021).

Corticosterone

Plasma corticosterone is an indicator of stress levels in birds (Sapolsky et al. 2000; Romero and Romero 2002; Bilková 2020). Corticosterone affects coloration differently, depending on the type of coloration (carotenic or melanic). In species with carotenoid-based plumage, such as the House Finch, individuals with elevated corticosterone concentrations during molting displayed redder feathers than individuals with lower hormone levels (McGraw et al. 2011; Lendvai et al. 2013). In contrast, in melanin-based plumage, such as that of Barn Owls (*Tyto alba* Scopoli, 1769), this glucocorticoid has a negative impact on plumage, resulting in a duller and more monotonous coloration (Roulin et al. 2007). To date, there are no studies analysing the effects of corticosterone on structural coloration. In our study, a decrease in corticosterone levels was observed throughout the experiment, which would indicate that this hormone does not affect the coloration of the new plumage similar to what was observed in the American Kestrel (*Falco sparverius* Linnaeus, 1758) (Butler et al. 2009). This phenomenon may be due to the Eared Dove having an attenuated stress response owing to its opportunistic behavior, in which they take advantage of their interactions with humans to obtain food and shelter (Murton et al. 1974; Bucher and Ranvaud 2006; Bucher 2016).

Effect on Growth speed

Estradiol

Estradiol appear to exert a general inhibitory effect on molting processes across various avian species belonging to different taxonomic groups. In the Gifujidori hens (Gallus gallus domesticus Linnaeus, 1758), a native Japanese chicken, molting only occurred when plasma estradiol levels were undetectable (Kono et al. 1986). Added to this, in both sexes of Japanese quail (*Coturnix japonica* Temminck & Schlegel, 1849) and in female Song Sparrows (*Melospiza melodia* Wilson, 1810), molting was delayed in response to an

exogenous increase in estradiol. However, once the implants were removed, they reached molting scores similar to the control group (Runfeldt and Wingfield 1985; Hahn et al. 1992; Hall et al. 1993). Similarly, during the transition from reproduction to molting in Humboldt Penguins (*Spheniscus humboldti* Meyen, 1834), low estradiol levels were necessary for a correct molt (Otsuka et al. 2004). Female American Kestrels (*F. sparverius*) began molting once estradiol levels decreased to species-specific basal levels (close to 10 pg/ml), indicating an inhibitory effect of estradiol on this physiological process (Rehder et al. 1986).

Contrary to our predictions, our results indicate a stimulatory effect of estradiol on the molting speed in the Eared Dove. In terms of the duration of molting, the elevation of estradiol levels resulted in accelerated growth of plumage (Fig. 3B) and in the group treated with this hormone, the patch was covered approximately a week earlier than in the control group. We lack understanding as to whether the increase in molting speed observed in the Eared Dove is a direct result of estradiol acting on its specific receptors, or if it is the outcome of interaction between estradiol and other hormones, such as thyroid hormones (Otsuka et al. 2004).

Testosterone

In the same way as the effect produced by estradiol, testosterone seems to delay molting in different bird species, such as females and males Shelducks (*Tadorna tadorna* Linnaeus, 1758), the European Starling (*Sturnus vulgaris* Linnaeus, 1758), the song sparrow, the House Finch (*H. mexicanus*), the Blue Tit and the Golden-collared Manakins (*Manacus vitellinus* Gould, 1843) (Runfeldt and Wingfield 1985; Schleussner et al. 1985; Hahn et al. 1992; Diittmann et al. 1999; Stoehr and Hill 2001; Day et al. 2006; Kurvers et al. 2008; Dawson 2015); and even prevents it from occurring, as is the case with the Dark-eyed Junco (*Junco hyemalis* Linnaeus, 1758) (Nolan et al. 1992).

In contrast, in the Superb Fairy-wren (*Malurus cyaneus* Ellis, 1782), subcutaneous testosterone implants induced prenuptial molt within weeks post-implantation, even outside the natural molt period. Additionally, removal of the implants before completion of the nuptial plumage halted the molt process (Peters et al. 2000). Similarly, in Black Redstart males (*Phoenicurus ochruros* Gmelin, 1774), analysis of blood samples collected during molting revealed significantly higher testosterone levels in males that molted into

552 adult plumage compared to those that molted into subadult plumage (Schwarzová et al.
553 2010).

554 The impact of testosterone on feather growth speed in Eared Doves is evident: the
555 hormone-treated group completed coverage of 100% of the molted patch nearly ten days
556 earlier than the control group (Fig. 3B). The observed variability in the effects of
557 testosterone on feather growth during molting indicates that these effects may be species-
558 specific, rather than representing a conserved trait across taxonomic groups. This further
559 emphasizes the importance of studying hormone-feather relationships throughout the
560 avian phylogeny to achieve a comprehensive understanding of this complex phenomenon.

561 ***Corticosterone***

562 Corticosterone can also significantly affect the normal growth of new feathers.
563 Administered corticosterone affected feather structure and reduced growth rate in feral
564 Pigeons (*Columba livia domestica* Gmelin, 1789) (Jenni-Eiermann et al. 2015) and
565 elevated levels of corticosterone in blood caused a delay in feather growth in the European
566 Starling and the White-crowned Sparrow (*Zonotrichia leucophrys* Forster, 1772) after a
567 forced molt (Romero et al. 2005). In contrast, our results show that the hormone-treated
568 groups completed the molt within a shorter period than the control group, indicating that
569 corticosterone-mediated stress levels during the course of the experiment would not be
570 involved in the speed of feather growth.

571

572 **Conclusion**

573 This is the first study to assess the influence of sex steroids on the coloration of the
574 newly developed plumage in the Eared Dove. Our findings suggest that the sex
575 hormones estradiol and testosterone do not contribute to the regulation of crown feather
576 coloration. However, these hormones do play a crucial role in controlling the growth
577 speed of emerging feathers. Furthermore, this species serves as a compelling example,
578 highlighting that hormonal control mechanisms governing bird coloration are not
579 consistently maintained in accordance with their phylogenetic position.

580

581 **Acknowledgments**

We extend our special thanks to Drs. Alicia Sércic and Andrea Cocucci of the Multidisciplinary Institute of Plant Biology (IMBIV-CONICET-UNC) for their generosity in lending us the spectrophotometer. Our gratitude also goes to the members of the Endocrinology Laboratory of the Santísima Trinidad Children's Hospital, especially Dra. Gabriela Sobrero, for their assistance conducting the sexual hormone determinations. We are equally grateful to Manuel Sosa, technical illustrator of our institute, for his help in constructing Figures 1 and 3. Additionally, we wish to express our appreciation to Guillermo Sferco, a biologist from our institute, for kindly providing the photos of the female and male Eared Doves featured in Figure 1.

Author contributions

Conceptualization: L.M.L., D.J.V; Methodology: L.M.L., A.I.Q., S.M.B-V, M.F.P., D.J.V; Validation: L.M.L., A.I.Q., D.J.V; Formal analysis: L.M.L., S.M.B-V, D.J.V; Investigation: L.M.L., D.J.V.; Resources: L.M.L., D.J.V.; Writing - original draft: L.M.L., D.J.V.; Writing - review & editing: L.M.L., A.I.Q., S.M.B-V, M.F.P., D.J.V.

Funding

The authors declare no specific funding for this work

Data availability

All relevant data can be found within the article and its supplementary information.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to have influenced the work reported in this paper.

References

Alonso-Alvarez, C., Bertrand, S., Faivre B., Chastel O. and G. Sorci. 2007. Testosterone and oxidative stress: the oxidation handicap hypothesis. *Proceedings*

of the Royal Society B: Biological Sciences **274**(1611): 819–25. http://dx.doi.org/10.1098/rspb.2006.3764.

Bilková Z. 2020. Analyses of steroids in the feather and plasma of birds. *pHD thesis*. Masaryk University, Brno, Czech Republic.

Bókony, V., Garamszegi, L. Z., Hirschenhauser, K. and Liker, A. 2008. Testosterone and melanin-based black plumage coloration: a comparative study. *Behavioural Ecology and Sociobiology* **62**(8): 1229–38. http://dx.doi.org/10.1007/s00265-008-0551-2.

Braun, E. L. and Kimball, R. T. 2002. Examining Basal Avian Divergences with Mitochondrial Sequences: Model Complexity, Taxon Sampling, and Sequence Length. *Systematic Biology* **51**(4): 614–25. http://dx.doi.org/10.1080/10635150290102294.

Bucher, E. 2016. La paloma torcaza. *Ciencia Hoy Argentina* 16-20.

Bucher, E. and Orueta, A. 1977. Ecología de la reproducción de la paloma *Zenaida auriculata*. II. Época de cría, suceso y productividad en las colonias de nidificación de Córdoba. *Ecosur.* **4**(8): 57–185

Bucher, E. and Ranvaud, R. 2006. Eared dove outbreaks in South America: pattern and characteristics. *Dong wu xue bao.* **52**: 564-567.

Butler, M. W., Leppert, L. L. and Dufty Jr., A. M. 2009. Effects of Small Increases in Corticosterone Levels on Morphology, Immune Function, and Feather Development. Physiological and Biochemical Zoology **83**(1): 78–86. http://dx.doi.org/10.1086/648483.

Casagrande, S., Dijkstra, C., Tagliavini, J., Goerlich, V. C. and Groothuis, T. G. G. 2010. Differential effects of testosterone, dihydrotestosterone and estradiol on carotenoid deposition in an avian sexually selected signal. *Journal of Comparative Physiology A* **197**(1): 1–13. http://dx.doi.org/10.1007/s00359-010-0579-4.

Dawson, A. 2015. Chapter 38: Avian molting in *Sturkie's avian physiology* (sixth edition). 907–917

Day, L. B., McBroom, J. T. and Schlinger, B. A. 2006. Testosterone increases display behaviors but does not stimulate growth of adult plumage in male golden-collared manakins (*Manacus vitellinus*). Hormones and Behavior **49**(2):223–232. http://dx.doi.org/10.1016/j.yhbeh.2005.07.006.

644 **Diittmann, H., Dieleman, S. and Groothuis, T. G. G.** 1999. Timing of moult in male
645 and female Shelducks *Tadorna tadorna*: effects of androgens and mates. Ardea
646 **87**: 33-39.
647 **Delhey, K., A. Peters and Kempenaers, B.** 2007. Cosmetic Coloration in Birds:
648 Occurrence, Function, and Evolution. *The American Naturalist* **169**(S1): 145–58.
649 http://dx.doi.org/10.1086/510095.
650 **del Hoyo, J., Elliott, A., Christie, D.** 1992. *Handbook of the birds of the world*, vol. 4.
651 Barcelona: Lynx edicions.
652 **Evans, M. R., Goldsmith, A. R. and Norris, S. R. A.** 2000. The effects of testosterone
653 on antibody production and plumage coloration in male house sparrows (*Passer*
654 *domesticus*). *Behavioural Ecology and Sociobiology* **47**(3): 156–63.
655 https://doi.org/10.1007/s002650050006
656 **Fargallo, J. A., Martínez-Padilla, J., Toledano-Díaz, A., Santiago-Moreno, J. and**
657 **Dávila, J. A.** 2007. Sex and testosterone effects on growth, immunity and melanin
658 coloration of nestling Eurasian kestrels. *Journal of Animal Ecology* **76**(1): 201–9.
659 DOI: 10.1111/j.1365-2656.2006.01193.x
660 **Goodwin, D.** 1966. The Bowing Display of Pigeons in Reference to Phylogeny. *The*
661 *Auk* **83**(1): 117–23.
662 **Griggio, M., Serra, L. and Pilastro, A.** 2011. The possible effect of dirtiness on
663 structurally based ultraviolet plumage. *Italian Journal of Zoology* **78**(1): 90–95.
664 DOI:10.1080/11250003.2010.504238
665 **Groothuis, T. and Meeuwissen, G.** 1992. The influence of testosterone on the
666 development and fixation of the form of displays in two age classes of young
667 black-headed gulls. *Animal Behaviour* **43**(2): 189–208.
668 **Haase, E. and Schmedemann**, **R**. 1992. Dose-dependent effect of testosterone on the
669 induction of eclipse coloration in castrated wild mallard drakes (Anas
670 platyrhynchos L.). *Canadian Journal of Zoology* **70**(3): 428–31.
671 **Haase, E., Ito, S. and Wakamatsu, K.** 1995. Influences of sex, castration, and
672 androgens on the eumelanin and pheomelanin contents of different feathers in
673 wild mallards. *Pigment Cell Research* **8**(3): 164–70.
674 **Hahn, T. P., Swingle, J., Wingfield, J. C and Ramenofsky, M.** 1992. Adjustments of
675 the Prebasic Molt Schedule in Birds. *Ornis Scandinavica* **23**(3): 314.
676 DOI:10.2307/3676655

677 **Hall, M. R., Laouar, L. and Ivings, W**. 1993. Effects of reproductive state on molt in
678 the Japanese quail. *Journal of Experimental Zoology* **265**(3): 240–51.
679 **Hill, G. E. and McGraw, K. J.** (Eds.). 2006. *Bird coloration, volume 1: mechanisms*
680 *and measurements.* Harvard University Press, Cambridge.
681 **Jenni-Eiermann, S., Helfenstein, F., Vallat, A., Glauser, G. and Jenni, L.** 2015.
682 Corticosterone: effects on feather quality and deposition into feathers. (Ed. D.
683 Fisher). Methods in Ecology and Evolution **6**(2):237–246.
684 http://dx.doi.org/10.1111/2041-210x.12314.
685 **Jenni, L. and Winkler, R.** 2020. The Biology of Moult in Birds. Bloomsbury
686 Publishing, London. https://doi.org/10.22621/cfn.v135i1.2845
687 **Karubian, J., Lindsay, W. R., Schwabl, H. and Webster, M. S**. 2011. Bill coloration,
688 a flexible signal in a tropical passerine bird, is regulated by social environment
689 and androgens. *Animal Behaviour* **81**(4): 795–800.
690 https://doi.org/10.1016/j.anbehav.2011.01.012
691 **Kimball, R. T.** 2006. Hormonal control of coloration. In: *Bird coloration: mechanisms*
692 *and measurements* (Ed. G. E Hill. and K. L. McGraw). Harvard University Press,
693 Cambridge. 431–468.
694 **Kono, T., Kuwayama, T., Ichinoe, K. and Itoh, H.** 1986. Changes in plasma
695 concentrations of progesterone, testosterone and estradiol during molting period in
696 the Gifujidori hens, native Japanese chicken. Japanese poultry science **23**(2): 104–
697 107. http://dx.doi.org/10.2141/jpsa.23.104.
698 **Kurvers, R. H. J. M., Roberts, M. L., McWilliams, S. R. and Peters, A.** 2008.
699 Experimental manipulation of testosterone and condition during molt affects
700 activity and vocalizations of male blue tits. *Hormones and Behaviour* **54**(2): 263–
701 69. DOI:10.1016/j.yhbeh.2008.03.011
702 **Laucht, S., Kempenaers, B. and Dale, J.** 2010. Bill color, not badge size, indicates
703 testosterone-related information in house sparrows. *Behaviooral Ecology and*
704 *Sociobiology* **64**(9), 1461–71. 10.1007/s00265-010-0961-9
705 **Lendvai, Á. Z., Giraudeau, M., Németh, J., Bakó, V. and McGraw, K. J.** 2013.
706 Carotenoid based plumage coloration reflects feather corticosterone levels in male
707 house finches (*Haemorhous mexicanus*). *Behavioural Ecology and Sociobiology*
708 **67**(11): 1817–24. DOI:10.1007/s00265-013-1591-9
709 **Lindsay, W. R., Webster, M. S. and Schwabl, H.** 2011. Sexually Selected Male
710 Plumage Color Is Testosterone Dependent in a Tropical Passerine Bird, the Red-

711 Backed Fairy-Wren (*Malurus melanocephalus*). Iwaniuk, A. ed. PLoS ONE
712 **6**(10). http://dx.doi.org/10.1371/journal.pone.0026067.
713 **Lindsay, W. R., Barron, D. G., Webster, M. S. and Schwabl, H.** 2016. Testosterone
714 activates sexual dimorphism including male-typical carotenoid but not melanin
715 plumage pigmentation in a female bird. *Journal of Experimental Biology* **219**(19):
716 3091–3099. http://dx.doi.org/10.1242/jeb.135384.
717 **Lindström, Å., Visser, G. H. and Daan, S.** 1993. The energetic cost of feather
718 synthesis is proportional to basal metabolic rate. *Physiological Zoology* **66**(4):
719 490–510. DOI:10.2307/30163805
720 **Maia, R., Eliason, C. M., Bitton, P. P., Doucet, S. M. and Shawkey, M. D.** 2013.
721 pavo: an R package for the analysis, visualization and organization of spectral
722 data. Ed. Andrew Tatem. *Methods in Ecology and Evolution* **4**(10):906–13.
723 DOI:10.1111/2041-210X.12069
724 **Maldonado, L., Tempesti, T. C., Somoza, G. M., Peluc, S. I. and Valdez, D.J.** 2020.
725 Reproduction in the Eared Dove: An exception to the classic model of seasonal
726 reproduction in birds? *Zoology* **140**: 125769. DOI: 10.1016/j.zool.2020.125769
727 **Marchese, N. A., Ríos, M. N., Guido, M. E., and Valdez, D. J.** 2024. Three different
728 seasonally expressed opsins are present in the brain of the Eared Dove, an
729 opportunist breeder. *Zoology* **162**: 126147.
730 **McGraw, K. J.** 2006. Mechanics of carotenoid-based coloration. In: *Bird coloration:*
731 *mechanisms and measurements* (Ed. G. E Hill. and K. L. McGraw). Harvard
732 University Press, Cambridge. 177–243.
733 **McGraw, K. J., Lee, M. D and Lewin, A.** 2011. The effect of capture-and-handling
734 stress on carotenoid-based beak coloration in zebra finches. *Journal of*
735 *Comparative Physiology A* **197**(6): 683–91. DOI: 10.1007/s00359-011-0631-z
736 **Murton, R. K., Bucher, E. H., Nores, M. and Reartes, J.** 1974. The Ecology of the
737 Eared Dove (Zenaida auriculata) in Argentina. *The Condor* **76**(1): 80–88.
738 **Narosky, T., Yzurieta, D. and Matarasso, H..** 2010. *Aves de Argentina y Uruguay.*
739 *Guía de identificación/Birds of Argentina and Uruguay. A field guide.* Editora
740 Vazquez Mazzini, Buenos Aires.
741 **Navarro, J. L., L. Martin and E. H. Bucher.** 1986. Una nueva trampa portátil para
742 capturar palomas torcazas. *Vida Silvestre Neotropical* **1**: 79-80.
743 **Nolan, Val, Ketterson, E. D., Ziegenfus, C., Cullen, D. P. and Chandler, C. R.** 1992.
744 Testosterone and Avian Life Histories: Effects of Experimentally Elevated

Testosterone on Prebasic Molt and Survival in Male Dark-Eyed Juncos. *The Condor* **94**(2): 364–70. DOI:10.2307/1369209

Ödeen, A. and Håstad, O. 2013. The phylogenetic distribution of ultraviolet sensitivity in birds. *BMC evolutionary biology* **13**(1):36. doi: 10.1186/1471-2148-13-36

Otsuka, R., Machida, T. and Wada, M. 2004. Hormonal correlations at transition from reproduction to molting in an annual life cycle of Humboldt penguins (*Spheniscus humboldti*). General and Comparative Endocrinology **135**(2); 175–185. http://dx.doi.org/10.1016/j.ygcen.2003.09.007.

Owens, I. P. F. and Short, R. V. 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends in Ecology and Evolution* **10**(1): 44–47. DOI: 10.1016/s0169-5347(00)88967-3

Peczely, P. 1992. Hormonal Regulation of Feather Development and Moult on the Level of Feather Follicles. *Ornis Scandinavica* **23**(3): 346–54.

Peters, A., Astheimer, L. B., Boland, C. R. J. and Cockburn, A. 2000. Testosterone is involved in acquisition and maintenance of sexually selected male plumage in superb fairy-wrens, *Malurus cyaneus*. Behavioral Ecology and Sociobiology **47**(6): 438–445. http://dx.doi.org/10.1007/s002650050688.

Peters, A., Delhey, K., Goymann, W. and Kempenaers, B. 2006. Age-dependent association between testosterone and crown UV coloration in male blue tits (*Parus caeruleus*). *Behavioural Ecology and Sociobiology* **59**(5): 666–73. DOI https://doi.org/10.1007/s00265-005-0095-7

Price-Waldman, R. and Stoddard, M. C. 2021. Avian Coloration Genetics: Recent Advances and Emerging Questions. Ed. Bridgett vonHoldt. *Journal of Heredity* **112**(5): 395– 416. DOI: 10.1093/jhered/esab015

Prum, R. O., Berv, J. S., Dornburg, A., Field, D. J., Townsend, J. P., Lemmon, E. M. and Lemmon, A. R. 2015. A comprehensive phylogeny of birds (Aves) using targeted nextgeneration DNA sequencing. *Nature* **526**(7574): 569–73. DOI: 10.1038/nature15697

Quispe, R., Trappschuh, M., Gahr, M. andGoymann, W. 2015. Towards more physiological manipulations of hormones in field studies: Comparing the release dynamics of three kinds of testosterone implants, silastic tubing, time-release

pellets and beeswax. *General and Comparative Endocrinology* **212**: 100–105. OI: 10.1016/j.ygcen.2015.01.007

Ralph, C. L. 1969. The Control of Color in Birds. *American Zoologist* **9**(2): 521–30.

Rehder, N. B., Bird, D. M. and Laguë, P. C. 1986. Variations in plasma corticosterone, estrone, estradiol-17β, and progesterone concentrations with forced renesting, molt, and body weight of captive female American kestrels. *General and Comparative Endocrinology* **62**(3): 386–93.

Roberts, M. L., Ras, E. and Peters, A. 2009. Testosterone increases UV reflectance of sexually selected crown plumage in male blue tits. *Behavioural Ecology* **20**(3): 535– 41. https://doi.org/10.1093/beheco/arp028

Rojas, T. N., Vergara-Tabares, D. L., Valdez, D. J., Ponzio, M. F. and Peluc, S. I. 2019. Food supplementation by an invasive fleshy-fruited shrub sustains body condition of a native frugivorous bird during winter. *Integrative Zoology* **14**(3): 259–69. DOI: 10.1111/1749-4877.12353

Romero-Diaz C., Silva, P. A., Soares, M. C, Cardoso, G. C. and Trigo, S. 2022. Oestradiol reduces female bill colour in a mutually ornamented bird. *Proceedings of the Royal Society B: Biological Sciences* **289**(1984). http://dx.doi.org/10.1098/rspb.2022.1677

Romero, L. M. and Romero, R. C. 2002. Corticosterone Responses in Wild Birds: The Importance of Rapid Initial Sampling. *The Condor* **104**(1): 129–35. https://doi.org/10.1093/condor/104.1.129

Romero, L. M., Strochlic, D. and Wingfield, J. C. 2005. Corticosterone inhibits feather growth: Potential mechanism explaining seasonal down regulation of corticosterone during molt. *Comparative Biochemistry and Physiology Part A: Molecular y Integrative Physiology* **142**(1): 65–73. https://doi.org/10.1016/j.cbpa.2005.07.014

Roulin, A., Almasi, B., Rossi-Pedruzzi, A., Ducrest, A. L., Wakamatsu, K., Miksik, I., Blount, J. D., Jenni-Eiermann, S. and Jenni, L. 2008. Corticosterone mediates the conditiondependent component of melanin-based coloration. *Animal Behaviour* **75**(4): 1351–58. DOI:10.1016/j.anbehav.2007.09.007

Runfeldt, S. and Wingfield J. C. 1985. Experimentally prolonged sexual activity in female sparrows delays termination of reproductive activity in their untreated mates. *Animal Behaviour* **33**(2): 403–10.

Saino, N. et al. 2013. Sexual Dimorphism in Melanin Pigmentation, Feather Coloration and Its Heritability in the Barn Swallow (*Hirundo rustica*). Roulin, A. ed. PLoS ONE **8**(2). http://dx.doi.org/10.1371/journal.pone.0058024.

Sapolsky, R. M., Romero, L. M. and Munck, A. U. 2000. How Do Glucocorticoids Influence Stress Responses? integrating permissive, suppressive, stimulatory, and adaptive actions. *Endocrine Reviews* **21**(1): 55–89. https://doi.org/10.1210/edrv.21.1.0389

Schleussner, G., Dittami, J. P. and Gwinner, E. 1985. Testosterone Implants Affect Molt in Male European Starlings, Sturnus vulgaris. *Physiological Zoology* **58**(5): 597–604.

Schoech, S. J., Ketterson, E. D. and Nolan, V. 1999. Exogenous Testosterone and the Adrenocortical Response in Dark-Eyed Juncos. *The Auk* **116**(1): 64–72. https://doi.org/10.2307/4089454

Shawkey, M. D., Pillai, S. R. and Hill, G. E. 2008. Do feather-degrading bacteria affect sexually selected plumage color? *Naturwissenschaften* **96**(1): 123–28. DOI: 10.1007/s00114-008-0462-0

Schwarzová, L., Fuchs, R. and Frynta, D. 2010. Delayed Plumage Maturation Correlates with Testosterone Levels in Black Redstart *Phoenicurus ochruros* Males. Acta Ornithologica **45**(1): 91–97. http://dx.doi.org/10.3161/000164510x516146.

Siefferman, L., Liu, M., Navara, K. J., Mendonça, M. T. and Hill, G. E. 2013. Effect of Prenatal and Natal Administration of Testosterone on Production of Structurally Based Plumage Coloration. *Physiological and Biochemical Zoology* **86**(3): 323– 32. DOI: 10.1086/670383

Stoehr, A. M. and Hill, G. E. 2001. The effects of elevated testosterone on plumage hue in male House Finches. *Journal of Avian Biology* **32**(2): 153–58. DOI:10.1034/j.1600-048X.2001.320208.x

Surmacki, A., Siefferman, L. and Yuan, H. W. 2011. Effects of Sunlight Exposure on Carotenoid-Based and Structural Coloration of the Blue-tailed Bee-eater. *The Condor* **113**(3): 590–96. https://doi.org/10.1525/cond.2011.100167

Terrill, R. S. and Shultz, A. J. 2022. Feather function and the evolution of birds. *Biological Reviews* **98**(2): 540–66. https://doi.org/10.1111/brv.12918

842 **Tökölyi, J., Bókony, V. and Barta, Z.** 2008. Seasonal colour change by moult or by
843 the abrasion of feather tips: a comparative study. *Biological Journal of the*
844 *Linnean Society* **94**(4): 711–21. DOI:10.1111/j.1095-8312.2008.01027.x
845 **Turek, F. and Gwinner, E.** 1982. Role of hormones in the circadian organization of
846 vertebrates. Vertebrate Circadian Systems. Springer, Berlin, Heidelberg. 173–182.
847 **Valdez, D. J., Cortez, M. V., Della Costa, G. E., Lèche, A., Hansen, C., Navarro, A.**
848 **and Martella, M. B.** 2014. Seasonal Changes in Plasma Levels of Sex Hormones
849 in the Greater Rhea (Rhea americana), a South American Ratite with a Complex
850 Mating System. *PLoS ONE* **9**(5), e97334.
851 https://doi.org/10.1371/journal.pone.0097334
852 **Valdez, D. J. and Benitez-Vieyra, S. M.** 2016. A Spectrophotometric Study of
853 Plumage Color in the Eared Dove (*Zenaida auriculata*), the Most Abundant South
854 American Columbiforme. *PLoS ONE* **11**(5): e0155501.
855 https://doi.org/10.1371/journal.pone.0155501
856 **Valdez, D. J. and Benitez-Vieyra, S. M.** 2023. Annual molt period and seasonal color
857 variation in the Eared Dove´s crown. *PLoS ONE* **18**(2): e0280819.
858 http://dx.doi.org/10.1371/journal.pone.0280819.
859 **Wingfield, J. C., Newman, A., Hunt, G. L. and Farner, D. S.** 1980. Androgen in high
860 concentrations in the blood of female western gulls, *Larus occidentalis*.
861 *Naturwissenschaften* **67**(10): 514–15

862

863 **Figures legends**

864 **Figure 1. Implantation and forced molting procedure**. A) Eared Dove (*Z. auriculata*
865 des Murs, 1847) female and male in the wild, showing the more bluish-grey plumage in
866 the crown compared with the brownish-grey crown of females. B) Immobilised
867 individual after the operation, with the subcutaneous implant visible. C) Visualization of
868 the area where the crown feathers were removed (forced molting); the asterisk indicates
869 where the spectrophotometric analysis was conducted once the growth of the new
870 feathers was complete.

871

Figure 2. Spectrophotometric analysis of the crown plumage of the Eared Dove (*Z. auriculata* des Murs, 1847). A) Reflectance spectra for the three experimental groups (qualitative analysis) in the avian visual range (300 to 700 nm). B) Chromatic distances expressed in JNDs. C) Achromatic distances expressed in JNDs. Results are plotted as mean ± s.e.m. Significant differences are indicated at α=0.05* $p<0.05$; *** $p<0.001$. * $p<0.05$. Abbreviations: C: Control Group; E: Estradiol-treated Group; T: Testosterone-treated Group.

Figure 3. Characterization of the molting process of the crown plumage of the Eared Dove (*Z. auriculata* des Murs, 1847). A) The individual feather growth process and delineation of the measurement area for forced molting. B) Time of completion of 100% molting in the experimental groups. Results are plotted as mean ± s.e.m. Significant differences are indicated at α=0.05* $p<0.05$; *** $p<0.001$. * $p<0.05$.

Figure 4. Hormonal determination in plasma for each experimental group. A) Plasma levels of estradiol and testosterone in the control group (hormone-free implant). B) Plasma levels of estradiol and testosterone in the estradiol-treated group. C) Plasma levels of estradiol and testosterone in the testosterone-treated group. D) Plasma levels of corticosterone throughout the experiment for the three experimental groups. Note that the days on which the surgical intervention for implant placement and the forced molting took place are counted as day 0. Results are presented as mean ± s.e.m.

Figure 5. Phylogeny of birds and their relationship with mechanisms of hormonal regulation of coloration known to date, with the inclusion of the Eared Dove (*Z. auriculata* des Murs, 1847). Transitions between various hormonal mechanisms are denoted (from estradiol to testosterone, testosterone to LH, etc.) in the few groups of birds studied to date. The dashed line represents the phylogenetic position and signifies the absence of hormonal regulation of coloration in a South American member of the columbiform order, the Eared Dove within the group of Neoaves.

	Control	Estradiol	Testosterone	F ; *p*
Hue	400.714±7.566	394.875±12.733	445.875±87.521	$F_{(2, 20)}$=2.230 ; *p=0.134*
UV S	0.349±0.022	0.331±0.011	0.330±0.023	$F_{(2, 20)}$=2.294 ; *p=0.127*
Brightness	21.692 ± 2.932	19.772±3.881	23.341±5.761	$F_{(2, 20)}$=1.312 ; *p=0.292*

Table 1. **Classical colorimetric variables (hue, UV saturation and brightness) of the new crown feathers of the Eared Dove (*Z. auriculata*) under different hormonal treatments.** No significant differences were observed in the classic colorimetric variables between the treated groups. Group differences were tested using one-way ANOVAs. Data is presented as mean ± s.e.m.

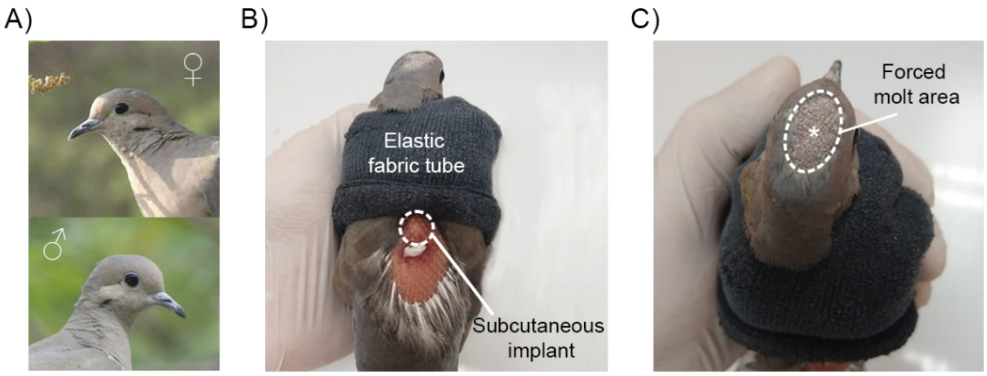

Figure 1. Implantation and forced molting procedure. A) Eared Dove (Z. auriculata) female and male in the wild, showing the more bluish-grey plumage in the crown compared with the brownish-grey crown of females. B) Immobilised individual after the operation, with the subcutaneous implant visible. C) Visualization of the area where the crown feathers were removed (forced molting); the asterisk indicates where the spectrophotometric analysis was conducted once the growth of the new feathers was complete.

102x39mm (300 x 300 DPI)

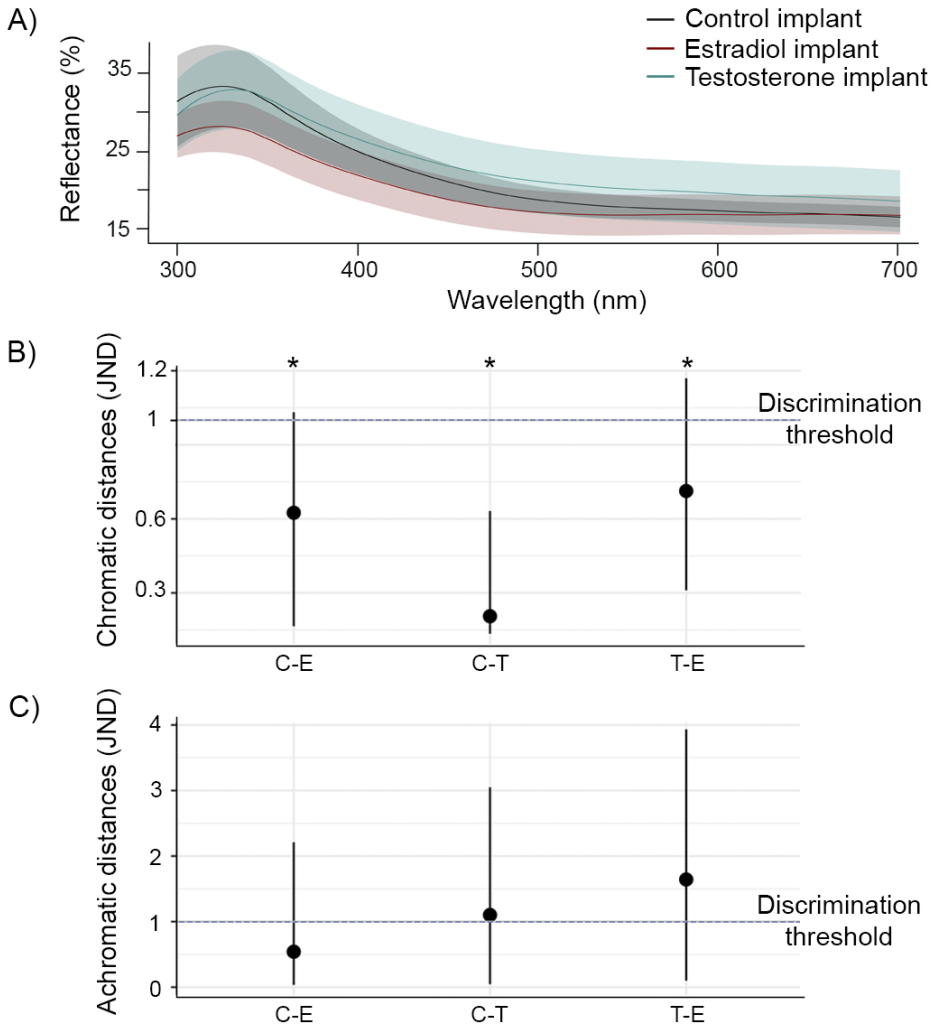

Figure 2. Spectrophotometric analysis of the crown plumage of the Eared Dove (Z. auriculata). A) Reflectance spectra for the three experimental groups (qualitative analysis) in the avian visual range (300 to 700 nm). B) Chromatic distances expressed in JNDs. C) Achromatic distances expressed in JNDs. Results are plotted as mean ± s.e.m. Significant differences are indicated at α=0.05* p<0.05; *** p<0.001. * p<0.05. Abbreviations: C: Control Group; E: Estradiol-treated Group; T: Testosterone-treated Group.

93x101mm (300 x 300 DPI)

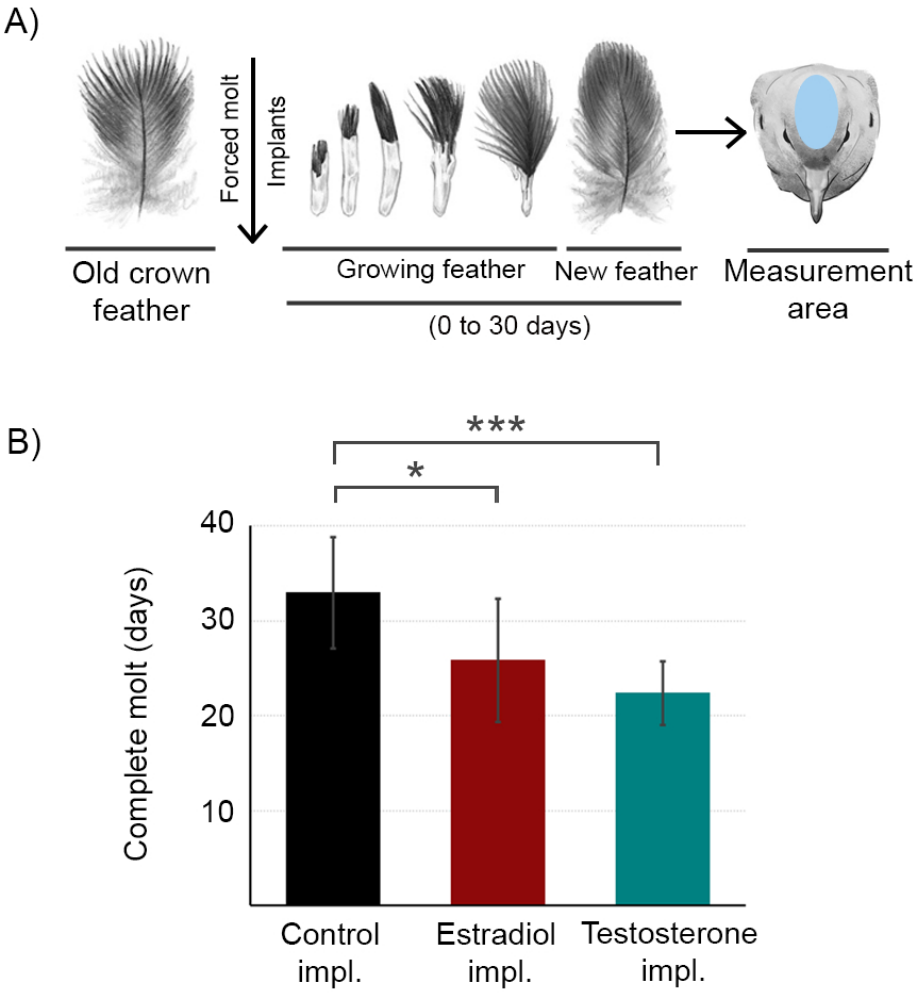

Figure 3. Characterization of the molting process of the crown plumage of the Eared Dove (Z. auriculata). A) The individual feather growth process and delineation of the measurement area for forced molting. B) Time of completion of 100% molting in the experimental groups. Results are plotted as mean ± s.e.m. Significant differences are indicated at α=0.05* p<0.05; *** p<0.001. * p<0.05.

79x79mm (300 x 300 DPI)

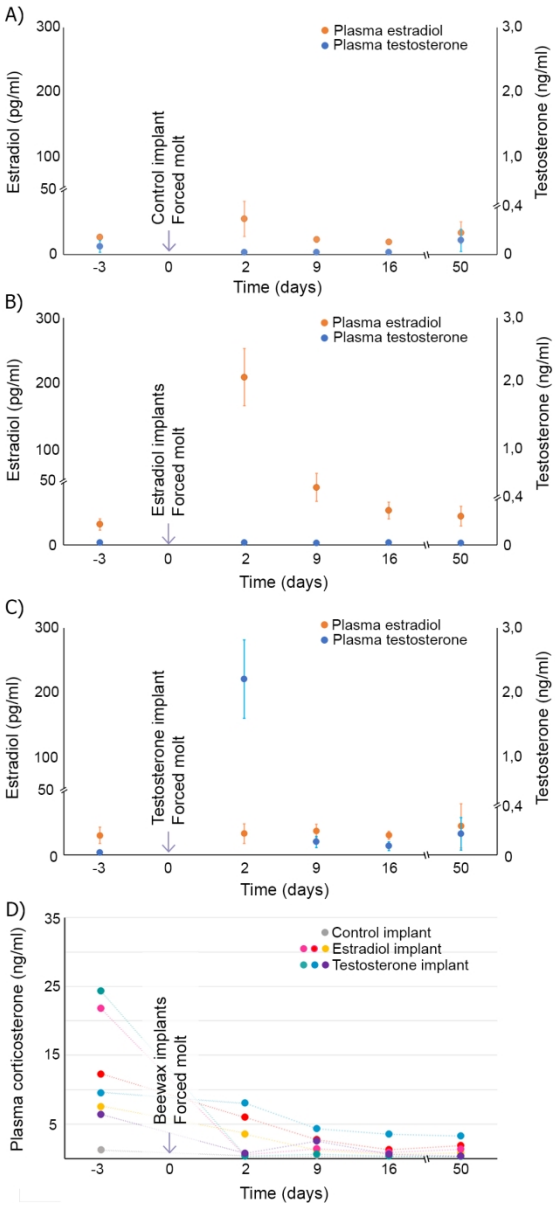

Figure 4. Hormonal determination in plasma for each experimental group. A) Plasma levels of estradiol and testosterone in the control group (hormone-free implant). B) Plasma levels of estradiol and testosterone in the estradiol-treated group. C) Plasma levels of estradiol and testosterone in the testosterone-treated group. D) Plasma levels of corticosterone throughout the experiment for the three experimental groups. Note that the days on which the surgical intervention for implant placement and the forced molting took place are counted as day 0. Results are presented as mean ± s.e.m.

94x199mm (300 x 300 DPI)

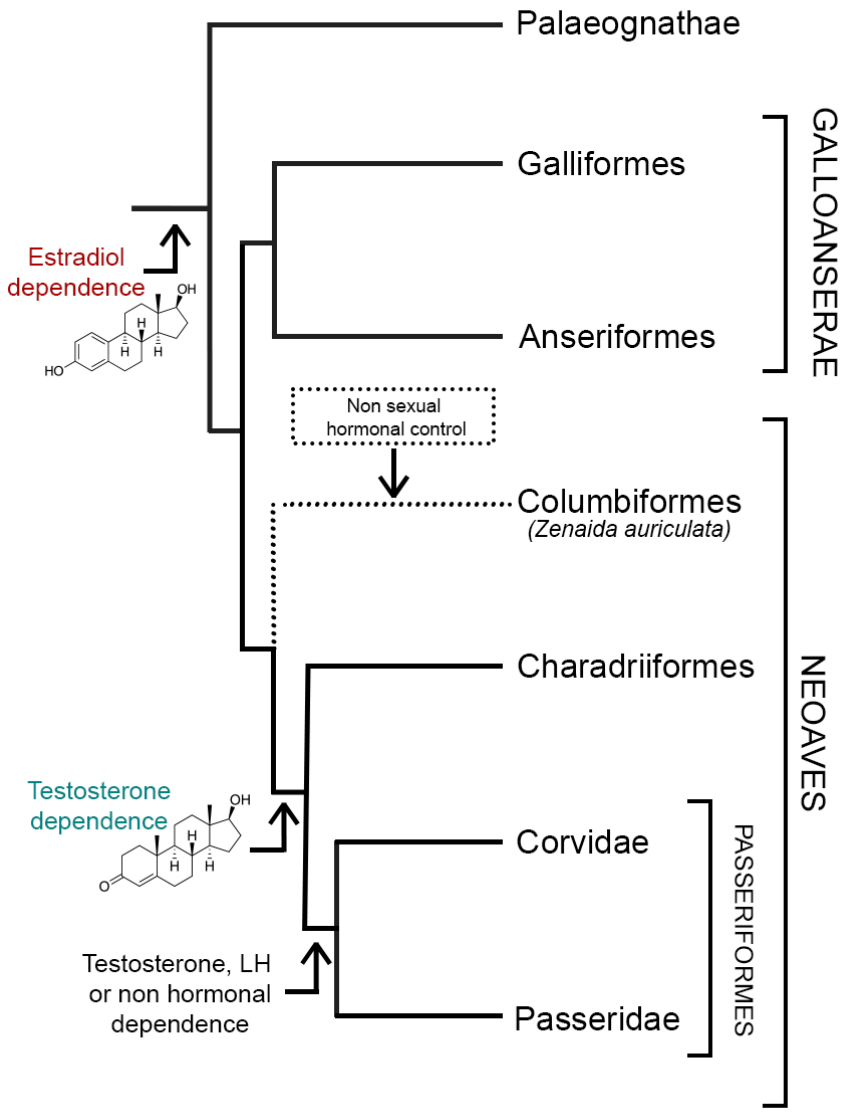

Figure 5. Phylogeny of birds and their relationship with mechanisms of hormonal regulation of coloration known to date, with the inclusion of the Eared Dove (Z. auriculata). Transitions between various hormonal mechanisms are denoted (from estradiol to testosterone, testosterone to LH, etc.) in the few groups of birds studied to date. The dashed line represents the phylogenetic position and signifies the absence of hormonal regulation of coloration in a South American member of the columbiform order, the Eared Dove within the group of Neoaves.

76x101mm (300 x 300 DPI)