Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Female Masculinisation and Reverse Sexual Dimorphism in the North Island Brown Kiwi (*Apteryx mantelli*): A study using wild and captive birds

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

in

Zoology

at Massey University,

Manawatū, New Zealand.

Caitlin McLeod

2024

Abstract

While *sexual dimorphism* is widely known in birds, little is known about when it is reversed. Species with reversed sexual dimorphism and/or sex roles often have females that show *masculinisation* in deviation from traditionally dimorphic pathways. This study investigates the endocrine profile of both male and female North Island brown kiwi across the breeding and non-breeding season as well as captive and wild status. Analysis of these profiles reveals androstenedione as a hormone of interest in female kiwi masculinisation, as well as significant differences between the endocrine profiles of birds in captivity from those in the wild. This study also describes the courtship behaviour of pairs of brown kiwi in the wild, adding nuance to our understanding of sexual dynamics between these birds.

Key words: Reverse sexual dimorphism, reverse sex roles, masculinisation, kiwi courtship, androstenedione

Acknowledgements

I have many people to thank for helping make this thesis happen. First and foremost, I would like to thank my supervisors, Dr. Isabel Castro, Dr. Wei-Hang Chua and Dr. Barbara Durrant. Thank you for your ongoing support throughout this thesis, as it has carried on, and throughout the numerous roadblocks we've hit along the way. There is no way this thesis would be here today without your continued expertise and advice, nor your motivation.

My fieldwork would not have been possible without many people who contributed, including Stephen Marsland, Kayla Purvis, Eliana Ramos Pallares, Jackie Rutherford, Emma Scheltema, Delilah Quedec, Alistair Grant, Monika Nowicki, Yi Luo, Tessa Ure-Bowman and Natasha Bansal. Thank you to Kat Strang for allowing me to use her kiwi videos for this thesis. Thank you to Isabel Castro and Barbara Durrant for permission to use plasma samples for these assays. Thank you to Wei-Hang Chua for his help in the laboratory.

This project would not have been possible without the ongoing support of the Chamberlain family. Thank you for welcoming me so seamlessly to your home, answering my questions and being such a key part of the Ponui experience.

Thank you to Birds NZ for supporting this research with your 2021 research fund - without your support, we would not have been able to embark on such interesting hormonal

investigations. Special thanks to Ingrid Hutzler for being our point of contact and supporting us through the application process. Thank you to Graduate Women Manawatu for awarding me with a post-graduate scholarship that supported me through my study and the ongoing uncertainty of the pandemic. Thank you to the Wildlife and Ecology Group at Massey University, Manawatu, for granting me the Julie Alley Bursary and supporting me as I continued to pursue this research.

I also want to thank Luigi Barbafiera for being an amazing boss and source of motivation during my early thesis. Thank you for covering my shifts without hesitation when I worried about my work and always bringing me coffee and advice on a Saturday morning. Thank you to the team at the Central Energy Trust Wildbase Recovery Centre for supporting me and teaching me so much as we went: Tracie Poole, Martin Steer and Carina Svensson, thank you for your patience with me, trust in me and some absolute laughter along the way. Thank you to Brittany Adams, Anthony Braddock, Geoff Nilsen and Bex Ingram for always making me feel welcome and always bolstering me along the way.

Thank you to Laura for always being one of the best friends anyone could ever ask for. Your love and support have, time and time again, kept me pushing on. Thank you to Bella for being an inspiration in not giving up and always being an ear to vent to. Thank you to Charlotte, Ritika, Emily and Caitlyn for being inspirations in chasing their own study and work and never failing to be there for me when I asked. Thank you to Lainey and Holly for being some of my closest friends and always being the first to share laughter and tears with not a hint of judgment. Thank you to Ava for being an unexpected friend and nothing less than an exceptional future vet. Thank you to Zara, Sapphire and Lee for being my support in Palmerston North, a shoulder to cry on and the people who are always down for any adventure (special thanks to Zara for illustrating our kiwi interactions). Thank you to Olivia for being one of the funniest and brightest humans that I have ever met.

To Callista: who would've thought that one day, the baby I cried under the kitchen table about would become one of my most favourite people in the world. Thank you for being my little sister, an inspiring menace and someone I love more than anything. To Lachlan: Thank you for indulging every single little rabbit hole of information I've ever run down and being the other half of my sense of humour. Thank you for being my little brother. To my mother: Thank you for being one of the most inspiring, endearing, infuriating and strong people I've

ever met - I hope I've inherited even half of these traits from you. I am only able to write this today as a testament to your sacrifices and bravery.

This thesis is dedicated to the memory of Geoff and Ellen Burns, who passed away before they could see it completed. Their unwavering support in me has made this possible. To Ellen, thank you for teaching me to be fierce and unwavering in myself. To Geoff, thank you for being a kindred spirit and encouraging my scientific pursuits from a young age. From my first microscope to fungi dissections in the kitchen, to bone cleaning in one of Grandma's pots, and shooting down seed cones to dissect and display, the spark of interest and passion you ignited in me for the world at a young age will carry on for the rest of my life.

Table of Contents

Table of Figures

Table of Tables

Female Masculinisation and Reverse Sexual Dimorphism: A Review and Introduction to this Thesis

Introduction

Many bird species are known to show distinct sexual dimorphism. Males have typically been characterised as louder, larger, and more colourful, often competing in some way to gain a mate (Promislow, Montgomerie, & Martin, 1992; Dunn et al., 2001; Seddon et al., 2013; Nolazco et al., 2022). However, in some cases, *sex role reversal* occurs, and females perform the roles and behaviours of males (also known as masculinisation) and vice versa (*feminisation*), often accompanied by the reversal of sexual dimorphism (Wheeler & Greenwood, 1983; Eens & Pixten, 2000; Emlen & Wrege, 2004; Barlow, 2005). The North Island brown kiwi (*Apteryx mantelli*) is an example of a species in which this occurs. Females can be distinguished from males by their significantly and disproportionately longer bills and larger body size, while the females of most bird species are smaller than the males. Male kiwi are also the primary egg incubators, a role typically ascribed to females of most avian species (Ziesemann, 2011; Wilson, 2014). Female brown kiwi possess two functional ovaries capable of ovulating, although only the left oviduct is functional (Kinsky, 1971). This may serve as a mechanism for increased hormone production; however, it may not necessarily account for masculinised features driven by androgens such as aggressive behaviour and larger body size. Further investigation into this dynamic and its source could provide novel insight into the mechanisms that control this understudied form of physiological and behavioural dimorphism. This review consists of current knowledge on the masculinisation of females throughout the animal kingdom before focusing on the avian groups. It then reviews current knowledge on how this mechanism is passed on to successive generations, and the possibility that a hormonal basis for this dynamic is inherited in the egg yolk through differential uptake and synthesis of maternal androgens based on the sex of the embryo.

Female Masculinisation and Reversed Sexual Dimorphism in the Animal Kingdom

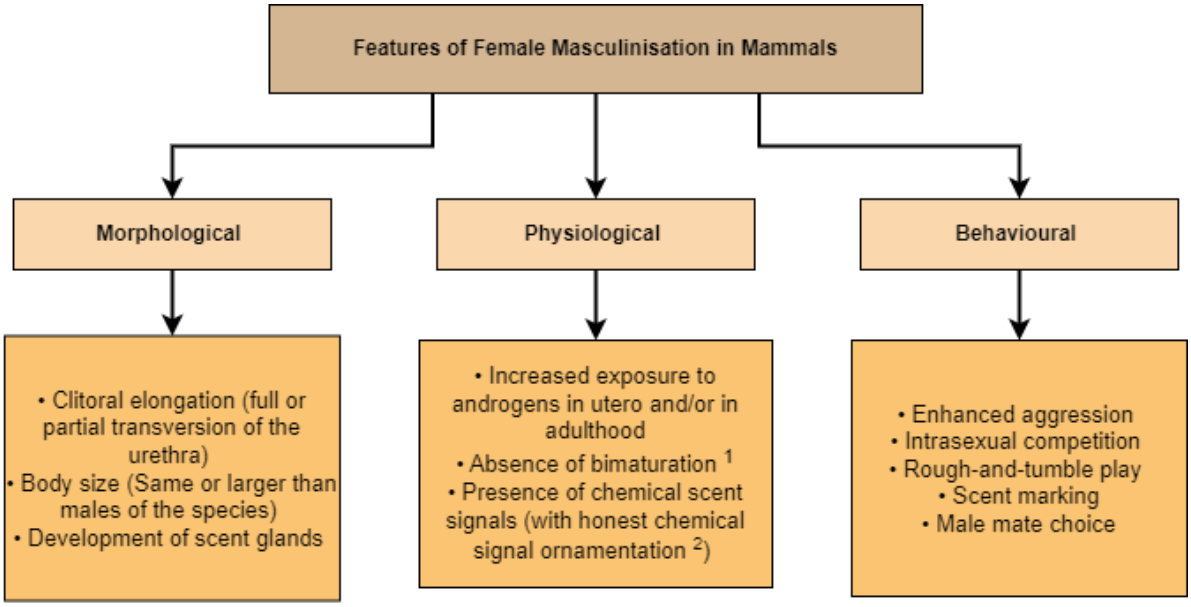
Traditional patterns of sexual dimorphism are associated with features such as bigger body size and greater aggression in males due to circulating androgens acting on hormone-sensitive tissues both pre- and postnatally (Wells, 2007; Drea, 2009; Mori, Mazza & Lovari, 2022). Masculinised females deviate from this pattern and converge with traditional male physiology and behaviour. In many cases, this includes features such as *clitoral elongation*,

large body size, and aggressive dominance over conspecific males (Figure 1; McCarthy & Ball, 2008; Drea, 2009; Auchus & Chang, 2010). While androgens have been implicated in this process at many different levels, for many species, the exact role that sex hormones play in this divergence from standard sexual dimorphism remains uncertain (Drea, 2009).

The *Female Masculinisation Hypothesis* (Glickman et al., 1987; Drea, 2007; French et al., 2013; Drea et al., 2021; Grebe et al., 2022) was developed in terms of, and most often applied to, mammals. Species that fall within the hypothesis are usually *nonseasonal*, with females displaying aggressive social dominance. Aggression and dominance are part of a larger group of traits linked to androgen action, which are associated with increased benefits gained by improved competition between conspecifics for resources such as food and shelter (Glickman et al., 1987). Grebe, Sheikh & Drea (2022) posited that the Female Masculinisation Hypothesis should be applied alongside the *Challenge Hypothesis* developed for seasonally breeding species, stating that androgen concentrations fluctuate to meet differing social demands, augment reproductive competition, and facilitate parenting (Wingfield et al., 1990). Combining these hypotheses offers an integrated framework to examine hormonal action during intersexual social interactions, seasonal relationships, and steroid hormones (Grebe, Sheikh & Drea,2022).

Masculinised traits

Masculinised traits can be separated into multiple categories when describing an animal. These include physiological, hormonal, genetic, evolutionary, behavioural, social, environmental and experimentally induced traits. For this review, they will be summarised into three categories: morphological traits (such as external masculinised genitalia, larger female body size), physiological (such as hormonal variations) and behavioural (such as female aggression, *female social dominance* or female *rough play*) (Figure 1). These traits can vary across different vertebrate groups and different species. Some species may show only behavioural masculinisation; others may show a mix of different traits (Table 1).



[1] Bimaturism = different developmental trajectories between conspecific males and females, leading to sex differences in age at maturity (Teder et al., 2021).

[2] Honest chemical signal ornamentation = ornamentation plays a role in mate choice by broadcasting information about individual genetic quality to potential mates and/or competitors. If this signal is "honest", the quality of the ornament (in this case, chemical, such as scent or pheromone) must be condition dependent, so that only the fittest individuals can bear the cost of producing high-quality signals (Boulet et al., 2010).

Figure 1: Summary of morphological, physiological and behavioural traits consistent with masculinised female mammals according to the Female Masculinisation Hypothesis (adapted from Grebe, Sheikh & Drea, 2022).

Table 1: Examples of mammalian species with masculinised females and characteristic traits that classify them as masculinised (sources in Appendix 1: Sources for masculinised mammals from Table 1 in Chapter 1; Female Masculinisation and Reverse Sexual Dimorphism in the Animal Kingdom; Masculinised Traits).

Species	External masculinised genitalia	Female aggression	Female social dominance	Juvenile female rough play	Female aggressive territorial behaviour	Polyandry	Larger body size (compared to males)
Spotted hyena (*Crocuta crocuta*)	✓	✓	✓	✓	✓	✓	
Ring-tailed lemur (*Lemur catta*)	✓	✓	✓			✓	
Naked mole rat (*Heterocephalus glaber*)		✓	✓		✓		
Syrian hamster (*Mesocricetus auratus*)		✓	✓			✓	
Californian mice (*Peromyscus californicus*)		✓					
Rock hyrax (*Procavia capensis*)		✓	✓				✓
Meerkat (*Suricata suricatta*)		✓	✓				✓

✓ = indicates trait is known to be present in that species

Morphological traits (gonadal, physical)

The female spotted hyena (*Crocuta crocuta*) is known for having the most masculinised genitalia within female mammals. The vagina is fused forming a *pseudoscrotum* while the clitoris is elongated and erectile, known as a *pseudopenis*. The pseudopenis is traversed by a central urogenital canal for urination, copulation, and parturition. While visually the pseudopenis is very similar to the male penis, there are a few differential traits including being shorter, thicker, having an elastic *urogenital meatus* and a rounded glans clitoris (as opposed to the angular glans penis) (Drea et al., 1998; Cunha et al., 2005; Conley et al., 2020).

In ewes (*Ovis aries*), masculinised genitalia are found as an environmental side effect of grazing on oestrogenic pasture. This hormonal exposure leads to the partial fusion of the labia as well as hypertrophy of the clitoris (Adams, 1979; Shackell, Wylie & Kelly, 1993; Pool et al., 2022). Oestrogenic pasture in this case refers to *phytoestrogens*, non-steroidal plant compounds that are structurally and/or functionally similar to mammalian oestrogens. Phytoestrogens are known for exerting oestrogenic effects on the central nervous system and on the reproductive system of male and female animals. They are also known to induce oestrus, stimulate growth of the genital tract and mammary glands in females, and have numerous effects on the ovary, uterus, testis and prostate gland through numerous different mechanisms. It should also be noted that phytoestrogen effects vary with the phytoestrogen that an animal is exposed to, the species, and sex of the animal exposed, the route of exposure, the dose of phytoestrogen and duration of exposure, and the timing of exposure (reproductive development and/or cycles; Mostrom & Evens, 2011).

Physiological traits (hormonal, not immediately visible)

In meerkat (*Suricata suricatta*) populations, there is evidence of increased oestrogen concentrations in males alongside increased androgen concentrations in females. Oestrogen in male meerkats has been linked to behavioural feminisation such as a predisposition towards infant care (Clutton-Brock et al., 2001; Davies et al., 2016). In contrast, androgens in females are linked to aggression. Within females, dominant individuals exhibit higher concentrations of *androstenedione* and *oestradiol* than their subordinate counterparts, and equivalent concentrations of *testosterone*, suggesting that social status is hormonally mediated. These patterns of dominance and hormone profiles become more pronounced during gestation, potentially serving as an evolutionary advantage by enhancing access to

food resources and reducing the risk of infanticide, thereby supporting the health and survival of offspring in masculinised females (Davies et al., 2016).

Behavioural traits (actions, non-actions)

Early in development, human females and males show a preference for toys and methods of interacting socially. Females are more likely to engage in *prosocial* behaviours, maintain eye contact while interacting and show greater social understanding. In contrast, males more often engage in rough play, object play and play in larger groups. In terms of play, females show a preference for social stimuli whereas males show a preference for mechanical stimuli. Human females exposed to higher testosterone levels *in utero* however, spent more time playing with males, showing greater preference for male-associated toys, and overall demonstrating greater male-typical personality features (Paletta et al., 2022; Bono et al., 2018; Bao & Swaab, 2010).

Some species of Malagasy lemurs (*Eulemur* spp.), exhibit female masculinisation. These females all have physically masculinised genitalia and show evidence of behavioural masculinisation such as female social dominance (FSD) and scent marking, which are traditionally male behaviours in comparable species. Additionally, lemur species with female dominance have more complex scent signals than those without female dominance, illustrating a difference between scent use in females compared to masculinised females (Drea, 2007; Petty & Drea, 2015; Kappeler, Fichtel, & Radespiel, 2022; Grebe, Sheikh & Drea, 2022).

Female African Pygmy Mouse (*Mus minutoides*) have three sex chromosomes, with two X variants. These are X, Y and X*. The asterisk represents an unknown x-linked mutation that prevents anatomical masculinisation of X*Y embryos although these females are behaviourally masculinised. They show greater aggression than females of other complements (*XX* and XX*) and have a lower fear response to novelty in the environment. These females also have enhanced reproductive performance, with a greater chance of producing at least one litter, larger litter sizes, a higher breeding probability when paired with a male, and an earlier onset of breeding. Phenotypically, the chromosomal complements cannot be differentiated, and all have similar body mass, *anogenital* structure, and ovarian structure (Saunders et al., 2014; Saunders et al., 2016; Ginot et al., 2017; Heitzmann et al., 2023).

Sexual Development Pathways

Gonadal developmental pathways, and therefore sexual development pathways, can differ between species at different points in development. Regardless, there tends to be a general pattern of development from early embryonic stages with chromosome determination, to gonad differentiation, to a hormone cascade that then leads to the development of primary sex characteristics such as genitalia, secondary sex characteristics such as mammary glands, or sexually dimorphic organs, and sexually driven behaviours (Figure 2).

In mammals, characteristic female embryos have two XX chromosomes while male embryos have an X and a Y chromosome. In birds, this pathway is opposite, with females presenting a Z and a W sex chromosomes and males presenting with two *ZZ* chromosomes (Moore, 1925).

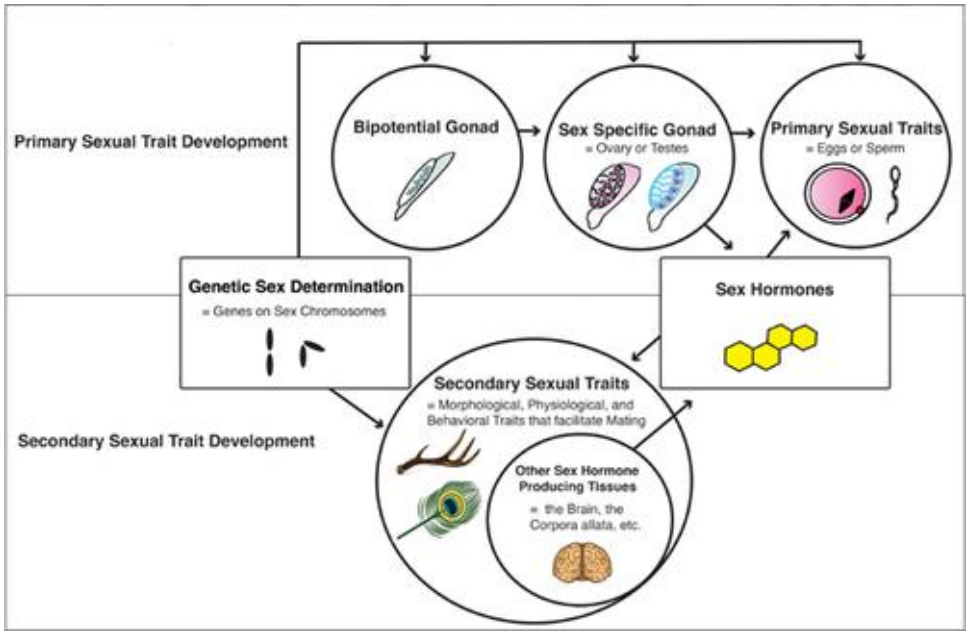


Figure 2: Summary of the interconnectivity of developmental mechanisms that produce primary sexual traits (the gametes) and secondary sexual traits (all other sexually dimorphic traits). From "Both cell-autonomous mechanisms and hormones contribute to sexual development in vertebrates and insects" by Bear and Monteiro, 2013, BioEssays, 35(8), pg. 725-732. Copyright 2013 by John Wiley and Sons. Reprinted with permission (Appendix 2: Copyright log of permissions for reused illustrations, diagrams and photos in this thesis.

Gonadal differentiation into ovaries or testes can vary between vertebrate groups (Table 2). Two general mechanisms are known to exist in vertebrates: *genetic sex determination* (GSD) and *external sex determination (ESD)*, the latter of which in vertebrates is typically confined to *temperature sex determination (TSD)*. Birds and mammals fall under the GSD general pathways. Crocodiles fall under the TSD system exclusively. In comparison, lizards, snakes, turtles, and bony fish species all use one of the aforementioned pathways. Under the GSD mechanisms, there are two typical categories: *heterogametic* males (*XY* in mammals), and

heterogametic females (*ZW* in birds). Both GSD systems are exhibited in amphibian species (Haseltine & Ohno, 1981; Trukhina et al., 2013; Nagahama et al., 2021).

It should be noted that while XX/XY and ZZ/ZW are the most common genetic sex determination systems, there are unusual cases that exist across vertebrates. The XX/*XO* system has been reported in 11 fish species (Arai, 2011). In this system, males are the heterogametic sex, with one less chromosome than females. This system has been found, for example, in *Potamotrygon* sp., a freshwater stingray in the Amazon basin (de Souza Valentim, et al., 2013).

Sex-determining genes are known to vary between taxonomic groups; however, it appears that the genes responsible for gonadal differentiation are conserved across vertebrates. Genes such as *foxl2*, *dmrt1*, *sox9*, *sf1*, *cyp19*, and *dax1* are evolutionarily conserved from fish to mammals (Trukhina et al., 2013; Nagahama et al., 2021). Many of these *conserved genes* that have a role in sex determination across vertebrates are responsible for testis fate (such as *DM-domain genes*, *Sox genes*, and *AMH genes*). In the absence of these genes, ovarian development proceeds instead (Table 2; Herpin and Schartl, 2015; Nagahama et al., 2021; Smaga et al., 2022).

The current belief is that embryonic vertebrates have developing gonads with bipotential genital ridges comprised of cortex and the medulla. Sex determination depends on the development of these areas of the gonad. The cortex is where the ovaries develop, while testes develop from the medulla, and there is antagonism between these two processes (Trukhina et al., 2013; Nagahama et al., 2021) (Figure 3).

There is evidence from female masculinised species (Petty & Drea, 2015; Glickman et al, 2006; Drea, 2009) and clinical studies on individual masculinised females (Conte et al., 1994; Shozu et al., 1991) that suggest that exposure to increased gestational androstenedione (A4) and subsequent placental conversion of this hormone to testosterone may provide an inheritance mechanism for daughters of dominant mothers (Davies et al., 2016).

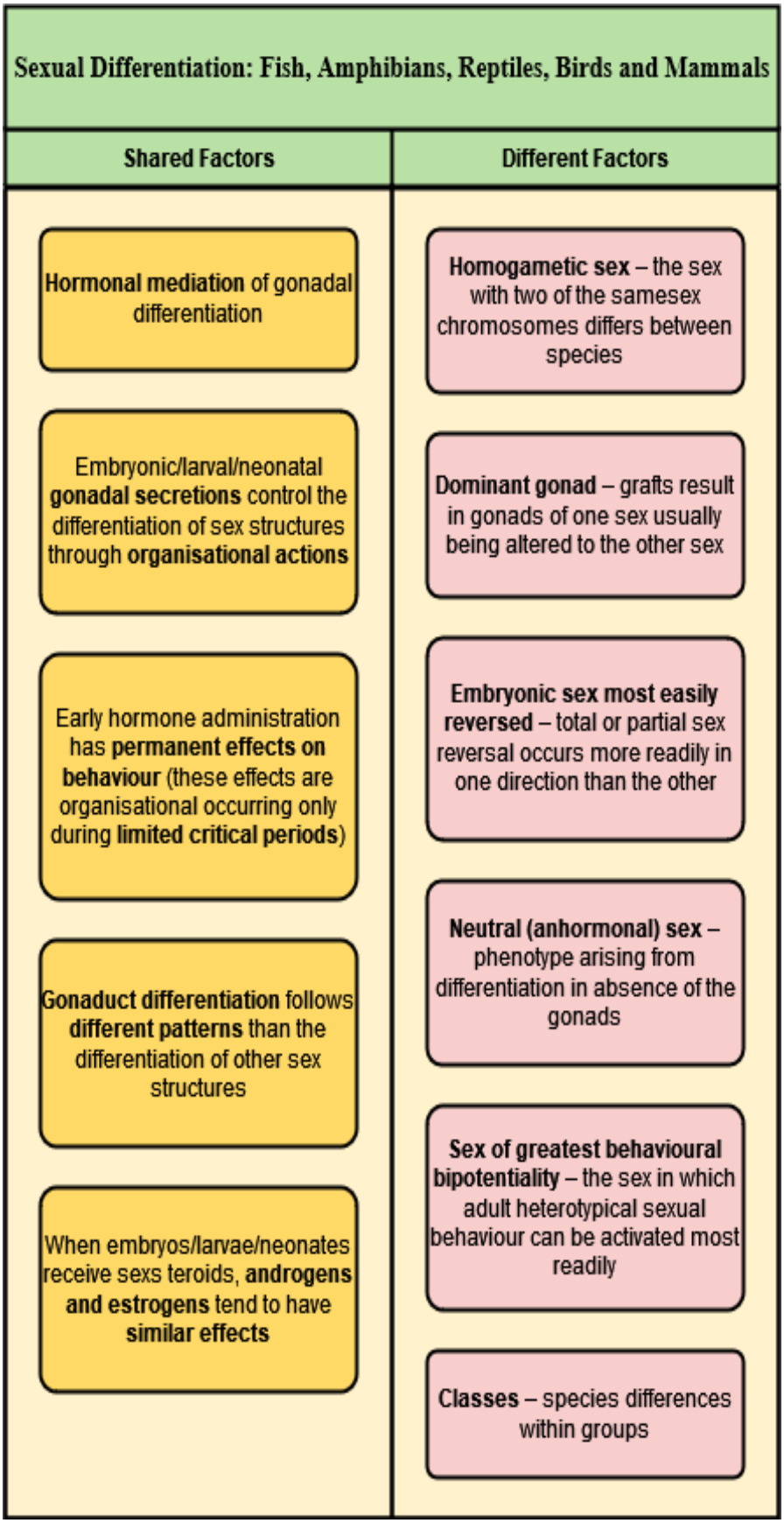


Table 2: Comparisons and contrasts in key features that underlie the sexual differentiation process in vertebrate taxonomic groups (adapted from Adkins-Regan, 1987).

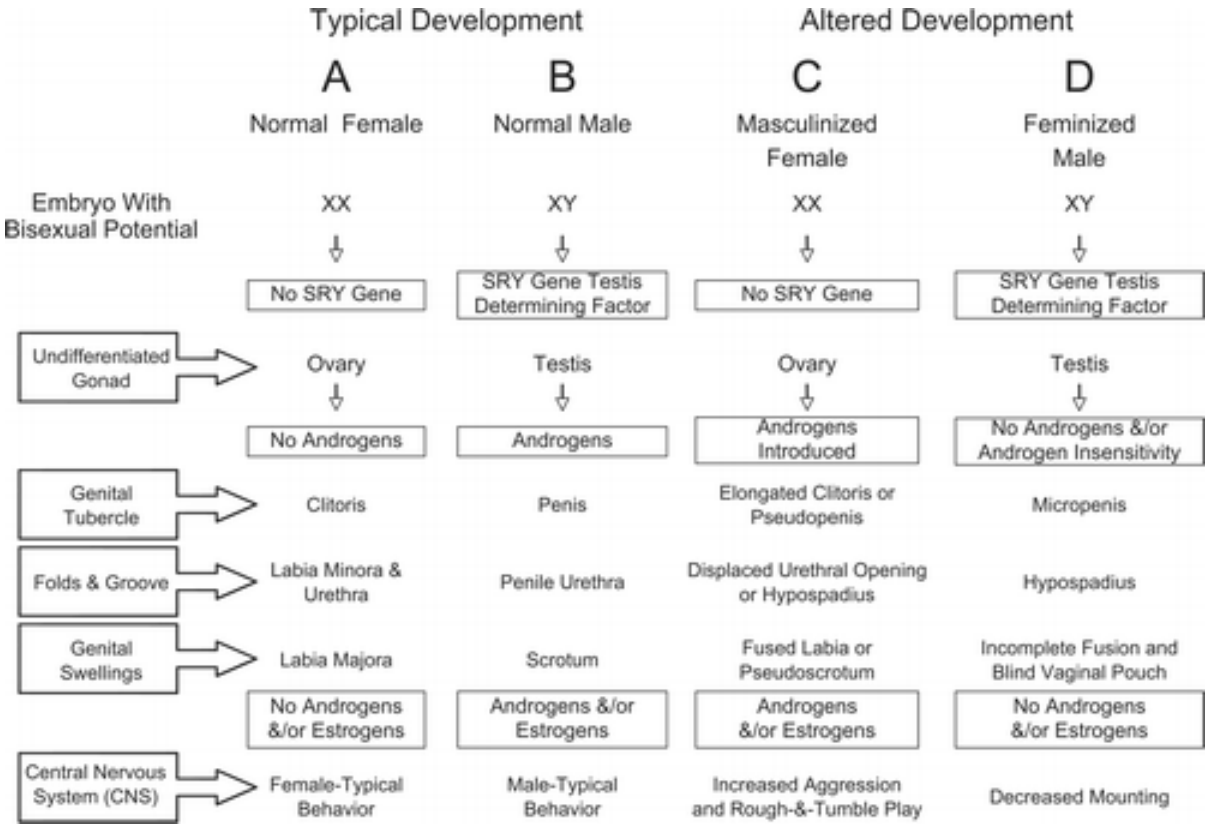


Figure 3: Pathway of sexual development from embryo to full-formed foetus in mammals. From "Endocrine mediators of masculinisation in female mammals" by Drea, 2009, Current Directions in Psychological Science, 18(4), 221-226. Copyright 2009 by SAGE publications.

Fish

Fish are well-known for a wide range of reproductive phenomena. These include *oviparity*, *viviparity*, *unisex species*, all known types of hermaphroditism, *self-fertilisation*, *male parental care*, *female parental care*, *shared parental care*, all extremes of sexual dimorphism and sex ratio influenced by environmental variability (Adkins-Regan, 1987). Flexibility of sexual fate is seen in many fish and is not only induced by *exogenous hormones*. Some fish are sequential hermaphrodites, which begin life as one sex and at some point, in their lifespan, change to another. These sequential hermaphrodites include species where individuals change from female-to-male (protogynous), male-to-female (protandrous), or bidirectional (serial) sex change (Gemmell et al., 2019).

Fish typically possess paired ovaries, except for females in some species, such as the guppy (*Poecilia reticulata*) and medaka (*Oryzias latipes*), which have one. Fish embryonic gonadal tissue distinctly differs from other vertebrates: instead of cortical (future ovarian) and

medullary (future testicular) tissue, only one type of tissue is found in the *gonadal primordium* (Adkins-Regan, 1987).

Some fish, such as guppies, swordtails (*Xiphophorus helleri*), and mosquitofish (*Gambusia affinis*) display gonadal differentiation before birth or hatching. In this case, treatment of fry with sex steroids only results in *partial gonadal sex reversal*. Other fish, such as the medaka, display gonadal differentiation after birth or hatching, and in these species, complete and functional gonadal sex reversal can be induced with sex steroids. Genetic females can be treated with androgens (such as *methyltestosterone* and androstenedione) and mature as males that can produce sperm and mate with mature females. Genetic males can be treated with oestrogens (such as oestradiol, estrone, and *diethylstilbesterol*) and mature as sexually active reproducing females (Adkins-Regan, 1987).

Behaviour linked to sexual hormones is also variable in fish. Some species, such as the shiner perch (*Cymatogaster aggregate*), platyfish (*Xiphophorus variatus*), and female jewel cichlids (*Hemichromis bimaculatus*), among others, cease receptivity and courtship with *gonadectomy*, which can then be restored with homologous sex steroid treatment. In other species, such as male jewel cichlids, frill fin gobies (*Bathygobius soporator*), and male Siamese fighting fish (*Betta splendens*; Adkins-Regan, 1987), gonadectomy does not stop the display or participation in sexual behaviours.

In many fish species, hormonally induced sex reversal can only occur within a limited critical period. However, in this period, the phenotypic and behavioural sex of both fry and adult fish can be completely reversed by sex steroid treatments, highlighting the importance of sex steroid roles in typical sexual development (Adkins-Regan, 1987).

Amphibians

Amphibians display extreme *gonadal bipotentiality*. They also have several sexually dimorphic traits such as body shape, body size, colour, cloacal morphology, courtship, and mating, with male Anurans also having sexually dimorphic nuptial thumb pads, ear sizes, vocal sac sizes, and calls (Adkins-Regan, 1987).

Amphibians have paired internal gonads, and much like other *tetrapod vertebrates*, have a *dual primordium* with medullary (future testicular) and cortical (future ovarian) tissues. However, amphibians also retain large amounts of the *primordial tissue* of the opposite sex, meaning that there is potential for gonadal sex reversal (Adkins-Regan, 1987).

Both male (XX/XY) and female (ZZ/ZW) heterogamety can be found in amphibians. Transitions between the same or different sex chromosome systems are identified even in closely related species, and other combinations are possible, such as systems with multiple sex chromosomes. Most amphibians also possess *homomorphic* sex chromosomes (Roco, Ruiz-García & Bullejos, 2021).

Different experiments utilising grafts and *parabiosis* (a laboratory method that surgically joins two living organisms in such a way that they develop a single, shared physiological system) have demonstrated that hormonal factors can be used to either change or reverse gonadal differentiation. In these experiments, male-produced hormones appear to be dominant. In grafts or *parabionts*, ovaries are masculinised, however, testes are not feminised (Adkins-Regan, 1987). It should be noted that gonadal differentiation in amphibians occurs predominantly or exclusively during the post-hatching larval stage; hormonal experiments are applied to larvae that have pre-determined mechanisms but have not yet differentiated. A testis graft results in the undifferentiated gonad of a female host to develop into a testis, and this method can be used to completely reverse a female into a male. Joining two undifferentiated larvae via parabiosis results in female ovaries becoming masculinised by the male parabiont, with the male's testis remaining unchanged (Adkins-Regan, 1987).

Androgen treatment, such as testosterone and high doses of oestradiol, results in the strong masculinisation of ovaries in larval (pre-differentiation) Ranidae and Hylidae, higher Anurans. Other larval Anurans (primitive) and Urodeles are feminised by oestradiol, but do not show masculinisation when testosterone treatment is used. In these groups, testosterone treatment usually results in the repression of gonadal development (often by the destruction of the medulla tissue) of larvae with undifferentiated gonads. The permanence of sex reversal by hormone treatment is uncertain in many species, as sex is often diagnosed in these cases by necropsy around the time of metamorphosis (Adkins-Regan, 1987; Wallace, Badawy & Wallace, 1999).

Temperature changes during development can also produce similar effects. In many amphibians, egg temperature has a dramatic effect on sexual fate and the sex ratio of hatchlings/juveniles (Adkins-Regen, 1987). Extreme temperatures (both low and high) can cause either masculinisation or feminisation, depending on the specific species undergoing the environmental change. High temperatures trigger female development in some urodelean

species (Dournon, Houillon, & Szmutyk, 1984; Dournon, Houillon, & Pieau, 1990; Sakata, Tamori & Wakahara, 2005). In the same genus, high temperatures trigger the development of males in two species (Dorazi et al., 1995). As a further example, high temperatures (in this case, >30°C) produce males in the Iberian ribbed newt (*Pleurodeles waltl*), but these same temperatures produce females in the Edough ribbed newt (*Pleurodeles poireti*) (Dournon, Houillon, & Pieau, 1990).

Reptiles

In many reptile species, gonadal development is affected by temperature in critical embryonic development stages known as the *thermosensitive period*. Temperature conditions result in two distinct types of sexual differentiation. In temperature-dependent sex determination, the masculinising temperature results in 100% male offspring or a male majority, while the feminising temperature results in 100% female offspring or a female majority. This condition is found in most crocodilians and turtles, as well as many lizards and sphenodons, a lizard-like but distinct reptile lineage (Pieau, Dorizzi & Richard-Mercier, 1999). The male-producing or female-producing temperature is dependent on the specific species. For example, in the European pond turtle (*Emys orbicularis*), it is known that incubating eggs at 25 °C produces males, 28.5 °C is a pivotal point, and 30 °C produces females (Pieau, Dorizzi & Richard-Mercier, 1999). In contrast, the *transition's range of temperature (TRT)* produces both sexes as well as occasional intersexes. Three different patterns arise from the responses to incubation temperatures. In turtle groups, including sea turtles, temperatures below the TRT are masculinising, whereas those above the TRT are feminising. This is the inverse result found in lizards and sphenodons. In some turtles, lizards and crocodilians, intermediate temperatures result in masculinisation, whereas temperatures above and below the TRT result in feminisation (Pieau, Dorizzi & Richard-Mercier, 1999).

It is believed that the transcription of both masculinising and feminising genes is controlled by oestrogens within the reptiles. It is suggested that masculinising genes are downregulated by oestrogen actions, whereas feminising genes are upregulated by oestrogen actions. (Pieau, Dorizzi & Richard-Mercier, 1999).

Treatment of embryonic reptilian gonads in-vivo with sex steroids can modify their development, but the effects are not always permanent. Oestrogen typically feminises embryonic gonads in females, while testosterone has a slight masculinising effect. In both cases, the degree of gonadal reversal is often only partial (Adkins-Regan, 1987).

Treating female lizard embryos with testosterone only slightly masculinises sex structures. Effects are primarily limited to the *Müllerian ducts*, inhibiting their development. This is a notable change as the Mullerian ducts are usually retained in females and degenerate in males. In contrast, estrogenic treatment results in extensive feminisation. Mullerian ducts are stimulated and *Wolffian ducts* are retained (as is normal for females), with feminised cloacae and outer genital regions (Adkins-Regan, 1987).

Male castration of lizards leads to the cessation of sexual behaviours which can be restored with testosterone treatments. Receptivity of females is oestrogen-dependent, however when castrated females are treated with testosterone, they court and pursue other females (Adkins-Regan, 1987).

Birds

Birds are known for marked physical and behavioural sexual dimorphism. Adult females typically have a functional left ovary and a gonadal rudiment on the right. Males typically have a slightly larger left testis. Birds typically have a right gonad that is ambisexual (containing mixed medullary-cortical tissue), and this is often the attributed cause of gonadal intersexuality, in which case a female ceases typical female behaviours, and the right gonad has developed into a testis or *ovotestis*. This condition is often caused by the left ovary becoming inactive due to disease or tumour development (Adkins-Regan, 1987; Alekseevich, 2009; Pick, Hutter & Tschirren, 2017).

Hormone-absent development of gonaducts results in both Mullerian ducts being retained (a condition not seen naturally occurring in either sex, only induced experimentally), with steroidal hormone action necessary for the regression of one or both ducts. Other sex structures, such as the *syrinx* and *genital tubercle*, are feminised by oestrogens, and the neutral state of these organs is male. Behaviour differentiates similarly. Embryonic exposure to oestrogens feminises behaviour, with a male as the neutral state and ovarian oestrogen acting as the organising hormone (Adkins-Regan, 1987).

Secretion of sex hormones from the gonads is known to be critical in the regulation of the development of secondary sexual characteristics. It is believed to be evolutionarily advantageous to regulate secondary sexual development via these hormones with the maturation of the gonads. This hormonal communication means that sexual traits leading to reproduction are only developed when an individual is capable of mating successfully. This is relevant because many sexual traits are costly to produce and/or maintain and are often

conspicuous in birds, which means that they have a negative effect on overall fitness if expressed prematurely (Bear & Monteiro, 2013).

Currently, it is believed that in birds, secondary sexual characteristics are not controlled simply by gonadal hormones but instead show signs of *cell-autonomous sex identity (CASI)*, in which cells determine phenotype via their sex-chromosome content (Clinton et al., 2012; Ioannidis et al., 2021). However, while CASI may direct the phenotypic development of tissues and organs, it can be overridden by other physiological factors. It has been shown that manipulating the pathway from genes to gonadal differentiation, such as altering Doublesex and mab-3 related transcription factor 1 (DMRT1) or blocking *aromatase conversion*, can result in feminised male gonads or masculinised female gonads. Altered gonads (ovaries or testes) will in turn affect the development of reproductive structures (penis, clitoris, etc) and the development of related sexual or secondary sexual characteristics (Elbrecht & Smith, 1992; Burke & Henry, 1999; Bruggeman et al., 2002; Smith et al., 2009; Clinton et al., 2012).

Zebra finches (*Taeniopygia guttata*) have long been a model species for studying sexual dimorphism in avian species because they display dimorphic neural pathways leading to the development of song. Masculinisation of song, and by proxy, masculinisation of the neural pathways, has been induced in female zebra finches by oestradiol, oestrogen and/or testosterone treatment in early life stages (Pohl-Apel, 1985; Simpson & Vicario, 1991).

Masculinisation is known to occur spontaneously in some members of Northern Pintail (*Anas acuta*) populations. Females showed masculinisation of morphological features typically attributed to males, such as male feather colouration, and some mating behaviours directed towards females (Chiba et al., 2004; Chiba & Honma, 2011). Testing of tissues from and dissection of masculinised females also demonstrated low oestradiol levels, and degradation of ovaries to different degrees (Chiba et al., 2004; Chiba & Honma, 2011). The pathway controlling these changes remains unclear in these species, however, masculinisation is observed repeatedly in populations, with an incidence of masculinisation from 0.01% to 0.18% each year (Chiba et al., 2004; Chiba & Honma, 2011).

The black coucal (*Centropus grillii*) is a species that has been investigated heavily for its sex role reversals. Males very rarely vocalise, take one female mate, and care for offspring from incubation to chick feeding. In contrast, females are polyandrous, defend breeding territories, do not contribute to the care of offspring, and are 69% heavier and 39% larger than males on average (Goymann, Wittenzellner & Wingfield, 2004). Male nestlings had higher circulating

testosterone than female nestlings, had rapid reproductive tissue maturation, and began to show interest in mature females as young as three months. While androgen secretion reversal is not currently implicated in the sex role reversal and potential masculinisation of female black coucals, further research is needed to understand if androgens outside of testosterone are having a significant effect. (Goymann, Wittenzellner & Wingfield, 2004; Goymann, Kempenaers & Wingfield, 2005). While studies on masculinisation in birds are uncommon, enough exist to begin to identify and outline what is considered a masculinised behaviour in female birds (Table 3). This includes initiating defence of a territory, attraction of a mate, and lack of incubation and/or parental care. While these behaviours have been observed and recorded, very little work has been done on the mechanisms underlying this dynamic to suggest a driver.

Mammals

The spotted hyena (*Crocuta crocuta*) is one of the most extreme and well-known cases of reversed sexual dimorphism in mammals. Females are characterised by a pseudopenis, enlarged body size compared to males, and aggressive dominance within a matriarchal system (Glickmann et al., 1987). Initially, testosterone was thought to regulate this system, challenging the common pattern of male and female-specific behaviour. However, researchers found that there was minimal difference in circulating testosterone between the two sexes (Glickmann et al., 1992; Glickmann et al., 1993; Glickmann et al., 2006; Antonevich & Naĭdenko, 2007). Further investigation has led to the discovery that female hyenas show considerably higher levels of circulating androstenedione than males, particularly in early infancy (Jaarsveld & Skinner, 1991). This led to the suggestion that androstenedione is a potential vector of the masculinisation of both physiological and behavioural features in female animals (Glickman et al., 1987; Jaarsveld & Skinner, 1991). Additionally, it was noted in experimental testing by Glickman et al. (1987) that *ovariectomy* led to a decrease of both testosterone and androstenedione in females. Ovariectomized females also experienced aggression from intact males, which was a severe deviation from normal social interaction. This implicates the ovaries as a major source of steroid hormones that may be affecting masculinisation (Glickman et al., 1992).

The greater guinea pig (Cavia magna) shows masculinisation of the female genitalia. Unlike many other species with masculinised females, however, the guinea pig shows no behavioural masculinisation; females are not aggressive and do not dominate males (Kraus et al., 2008). In comparison to similar Cavia species, *C. magna* has higher maternal levels of

androstenedione that peak during mid-pregnancy, when physical sexual differentiation occurs in the foetus. It is hypothesised that the masculinised genitalia are therefore a by-product of selection for other advantageous traits that come with high levels of androgens, such as enhanced growth and early development (Kraus et al., 2008). Maternal androstenedione levels show a similar pattern in the spotted hyena; however, staying high into the final trimester, which is believed to account for the behavioural masculinisation as foetal development progresses (Kraus et al., 2008).

In female white-faced marmosets (*Callithrix geoffroyi*), urinary androgens were tested throughout the length of pregnancy to determine if the hormones in circulation were of foetal origin. While androgens rose in the first trimester and peaked in the second trimester, maternal androgen levels were not found to be associated with litter size, number of males in the litter, or proportion of males in the litter. High levels of androgens were subsequently found to be of strictly maternal origin (French, Smith, & Birnie, 2010).

In conclusion, it is believed that androstenedione is a key hormone in the development of female masculinisation in mammals. Androstenedione has been identified as a hormone with elevated levels in masculinised females, whereas other suspected hormones such as testosterone have not provided a mechanism for masculinised traits to arise (Conte et al., 1994; Shozu et al., 1991; Petty & Drea, 2015; Glickmann et al., 2006; Kraus et al., 2008; Drea, 2009). The current belief is that masculinisation serves the purpose of providing females with greater access to resources during pregnancy and that increased gestational androstenedione occurs as females reaffirm social status and resource access. Paired with placental conversion of androstenedione to testosterone, potentially through either enzyme deactivation or differential enzyme saturation, masculinisation then occurs in female offspring prenatally (Davies et al., 2016; Drea et al., 2021).

Table 3: Examples of bird species that have masculinised females (often in comparison to males of the species), and some traits that have been identified as significant to their masculinisation. References in Appendix 3: Sources for masculinised birds in Table 3 in Chapter 1; Sexual

Development Pathways.

Species	Masculinised Trait/s
Northern pintail (*Anas acuta*)	*Some females (0.01-0.18%):* • Masculine plumage • Ovary degeneration • Burping (A male courtship display in this species)
African black coucal (*Centropus grilli*)	*All females:* • Defend large territories • Spend 60% of morning enforcing territory (via territorial perched singing) • Polyandrous • 70% heavier and 40% larger (in overall size) than males *Males:* • Rarely call or sing • Do not initiate courtship • Provide sole incubation • Provide sole parental care
Red Phalarope (*Phalaropus fulicarius*)	*All females:* • Polyandrous • Have plumage that is brighter and more striking than males • Are up to 20% larger (body mass) than males • Fight for mate access *Males:* • Provide sole incubation • Provide sole parental care
Western gull (*Larus occidentalis*)	*Both sexes:* • Provide territory defence • Have identical plumage • Are similar in body size • Have similar sexual and courtship behaviours *Some females:* • Establish their own territory • Mount males and other females
White-necked jacobin (*Florisuga mellivora*)	*Some females (20%):* • Exhibit male-type ornamented plumage
Wattled jacana (*Jacana jacana*)	*All females:* • Are up to 47% heavier than males • Are behaviourally dominant over males • Have greater development of secondary sexual characteristics (fleshy facial ornamentation and spurs) • Are sequentially polyandrous • Compete for mates *Males:* • Provide sole incubation • Provide sole parental care
Spotted sandpiper (*Actitis macularius*)	*All females:* • Fight for and defend territory • Are sequentially polyandrous *Males:* • Predominantly provide parental care

Androstenedione

Androstenedione is an intermediate androgen that leads to the biosynthesis of testosterone. It is produced in the adrenal glands and gonads of animals and is also sometimes referred to as 4-androstene-3-17-dione, 4A, AND, or A4. This hormone is often considered a key intermediate in steroid metabolism in the body. It is synthesised from dehydroepiandrosterone and can be converted into either 1) testosterone by 17β-hydroxysteroid dehydrogenase or 2) *oestrone* via the *aromatase* enzyme complex, depending on the concentrated presence of either of these two enzymes (Mills, 1990). Furthermore, within the adrenal cortex, *dehydroepiandrosterone (DHEA)* is converted into androstenedione, which is then either *aromatised* to produce oestrone or is de-hydrogenated in the liver to produce testosterone (Figure 4; Badaway et al., 2021).

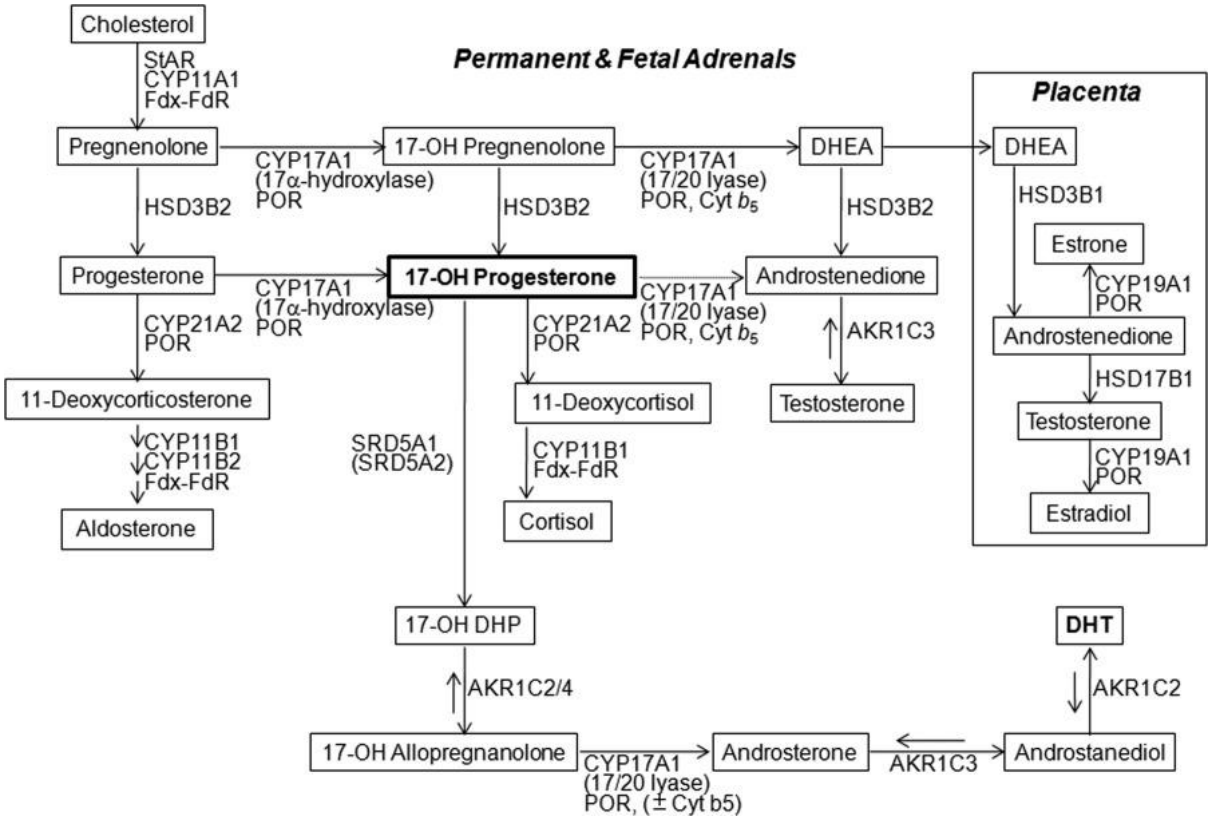


Figure 4: Steroidogenic pathways in the adrenals and the placenta. From "Backdoor pathway for dihydrotestosterone biosynthesis: Implications for normal and abnormal human sex development" by M. Fukami et al., 2012, Development dynamics: An official publication of the American Association of Anatomists, 242(4), pg. 320, 329. Copyright 2012 by John Wiley and Sons. Reprinted with permission (Appendix 2: Copyright log of permissions for reused illustrations, diagrams and photos in this thesis.

Relationship to Masculinisation

Davies et al. (2016) stated that androstenedione has been "revelatory in other female-dominant species" in characterising baseline hormone statuses and sexual characteristics.

Androstenedione became a hormone of interest in the spotted hyena after Frank, Davidson, & Smith (1985) found significant differences in circulating testosterone levels of wild hyenas, offering a possible mechanism for masculinisation after Racey & Skinner (1979) found that testosterone hormone levels were much the same between sexes. Observable masculinisation in the average female hyena, therefore, was much less likely to be attributed to testosterone levels, as males displayed the typical mammalian pattern of higher levels. When levels of androstenedione were tested, it was found that females had much higher levels than males – this is the opposite of the pattern in most mammals (Lindeque & Skinner, 1982; Glickman et al., 1987; van Jaarsveld & Skinner, 1991; Glickman et al., 1993).

Heightened levels of androstenedione in these females appear to originate from the ovaries (Glickman et al., 1993). Further experimentation has shown that within the spotted hyena placenta, there is low aromatase activity and high concentrations of the 17β-hydroxysteroid dehydrogenase enzyme, which indicates that the placenta is extremely efficient at the conversion of androstenedione into testosterone (Yalcinkaya et al., 1993). Placental testosterone is transported to the foetus during development via the umbilical vein. This means that both sexes of the spotted hyena are exposed to high levels of testosterone *in utero*, offering a mechanism for masculinisation (Glickman et al., 1992; Licht et al., 1998).

In bovine research, undifferentiated gonads have been found to metabolise androstenedione, converting it into testosterone in genetically male embryos and oestrogen in genetically female embryos (Juárez-Oropeza et al., 1995).

In the human female, oestradiol is the primary sex steroid originating from the ovary. Levels of oestradiol increase throughout puberty and then vary in women throughout the menstrual cycle. Oestradiol level increases are correlated with developmental changes in oestrogen-sensitive tissue, such as the breast and uterus. Females also have small quantities of androgens present, namely testosterone and androstenedione, which are of ovarian origin. However, circulating testosterone in females is derived primarily from the metabolic conversion of androstenedione (Lerner & Foch., 1987).

During the pubertal stage in humans, androstenedione levels increase twofold in boys and threefold in girls. Serum androstenedione levels are the single strongest hormonal correlate of

measures of pubertal stage in girls (accounting for 34% of the variance in the breast stage, and 52% variance in the pubic hair stage) (Lerner & Foch., 1987).

Androstenedione treatment of 100 mg in 10 human females of good health resulted in abnormal concentrations of plasma testosterone. It is suggested that this treatment is linked to the development of male characteristics such as male pattern baldness, *clitoromegaly*, voice-deepening, hirsutism, abnormal menstrual cycles and bleeding, and metabolic disruption. Additionally, heightened androstenedione levels appear to affect embryo implantation levels negatively (Badaway et al., 2021).

High levels of androstenedione to testosterone ratios in humans are associated with reduced *17β-hydroxysteroid dehydrogenase type 3 (HSD17B3)* function, an enzyme that is critical for testosterone production via conversion of androstenedione to testosterone (Lawrence et al, 2022). 21-hydroxylase deficiency also results in masculinisation of female foetuses. 21-hydroxylase deficiency results in impaired conversions in the adrenal cortex, leading to cortisol deficiency. This then results in increased *adrenocorticotropic hormone* (ACTH) pituitary secretion, and increased production of adrenal androgens – DHEA and androstenedione. These adrenal androgens are then converted into testosterone and *dihydrotestosterone (DHT)*, resulting in masculinisation (Hutson, 2020).

Masculinisation of female mammal foetuses is associated with androstenedione. While direct pathways may yet be uncertain and species dependent, androstenedione is implicated as an androgen that has a direct effect on the process as one of the immediate precursors to testosterone.

Role in Avian Species

Within birds, testosterone is metabolised in the pituitary gland and hypothalamus to *5β-androstane-17β-ol-3-one (5β-DHT)*, 5α-androstan-17β-ol-3-one (5α-DHT), and androstenedione. Testosterone is also aromatised to oestrogen within the brain, primarily within the hypothalamus (Lofts & Holmes, 1981).

Both 5α-DHT and androstenedione are implicated alongside testosterone as active androgens inducing comb growth in chickens as a male secondary sex characteristic, whereas 5β-reduced metabolites are known to be inactive (Massa, 1984). Furthermore, testosterone, androstenedione, and 5α-DHT treatments have been found to result in the development of the cloacal gland in male Japanese quail (*Coturnix japonica*) (Massa, Davies, & Bottoni, 1980). These metabolites (5α-DHT and androstenedione) are implicated in influencing the

androgen-dependent development of accessory sexual organs and secondary sexual characteristics alongside testosterone in both birds and mammals (Massa, 1984).

Within male Japanese quail, it was reported that the conversion of testosterone to androstenedione via the 17-hydroxysteroid-dehydrogenase enzyme was high when plasma testosterone levels were low; this is seen in immature males and castrated birds. In contrast, this conversion is lower in mature quail. Androstenedione was therefore identified as the main metabolite of testosterone in immature birds (Massa, Davies, & Bottoni, 1980).

Minimal research has been done on the role of androstenedione in sexual development pathways in avian species. Androstenedione is known to be related to the development of secondary sexual characteristics, acting as an immediate substrate for testosterone conversion, both of which also hold true in mammalian groups. As androstenedione is implicated as a potential driver of female masculinisation in mammals, this may also hold for birds.

Maternal Androgens and Egg Yolks

Effect of Maternal Hormones on Development

Maternal androgens are known to be deposited into egg yolks (Figure 5; Hegyi et al., 2011). Yolk androstenedione has not been observed to predict hatching success; however, when high androstenedione concentrations are paired with relatively high testosterone concentrations, lower hatching success has been observed in multiple avian species (Hegyi et al., 2011). This increased testosterone is associated with territory intrusions by other unknown individuals of the same species during the laying period of a female and demonstrates the transfer of androgens between mother and egg.

Yolk androstenedione has also been implicated in the prediction of fitness after hatching. Eggs with higher concentrations of androstenedione produced larger chicks, which may be due to increased *embryonic hatch muscle* development and increased *embryonic mass* (Hegyi et al., 2011). It should be noted that these high androstenedione individuals also grew slower and took longer to reach a normal fledging weight than others with comparatively low levels of androstenedione at hatch. This may be for two reasons: if there is nestling competition, maintaining rapid development while physical mass growth is slow means that chicks can fledge earlier than siblings and benefit from the post-fledgling care period. In cases where there is no nestling competition, maintaining rapid development while physical mass growth is slow means that a chick does not have to undergo nutritional stress to keep rapidly growing

an already larger body size, and minimisation of nutritional stressors is linked to greater chances of survival (Metcalfe & Monaghan, 2001). It was also suggested that high androstenedione in chicks with high embryonic mass at hatching and slower growth rate may depend on whole growth, and that heightened androstenedione results in different personality outcomes and mortality outcomes (Hegyi et al., 2011).

Androstenedione by itself is not particularly biologically active but does function as a source for other metabolites and can be converted to oestradiol and testosterone. Species with *altricial* young tend to have high levels of dihydrotestosterone (DHT) in their egg yolks, while birds with *precocial* young tend to have high levels of androstenedione in their eggs (Hegyi & Schawbl, 2010). It is likely that in addition to being credited for development type (precocial vs altricial), maternal androgens are also acknowledged for influencing stages of development and traits in offspring, including synchrony of fledging, *sibling competition*, number of eggs in a clutch, degrees of sexual dimorphism, incubation type, nesting site type and disease risk (Diego, 2003; Gil et al., 2007; Groothuis & Schwabl, 2008; Ruuskanen, 2015; Duckworth, Belloni, & Anderson, 2015). As most studies on maternal yolk androgens have focused solely on testosterone, there are still gaps in identifying how yolk androgens differ in relation to these traits in different species (Groothuis et al., 2005).

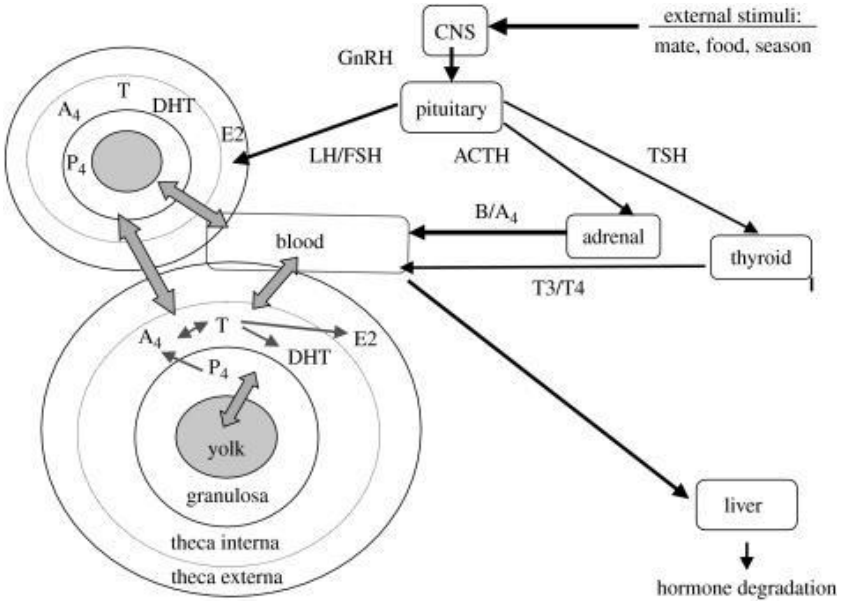


Figure 5: The transfer of maternal hormone pathways into egg, with the smaller concentric circles depicting growing ovarian follicles and larger concentric circles representing the egg (B, corticosterone; P4, progesterone*; A4, androstenedione; T, testosterone; E2, oestradiol;* T3/T4*, thyroid hormones; DHT, dihydrotestosterone;* TSH*, thyroid-stimulating hormone; ACTH, adrenocorticotropic hormone;* LH/FSH*, luteinizing hormone/follicle-stimulating hormone;* GnRH*, gonadotropin-releasing hormone). From "Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them?" by T.G.G Groothuis & H. Schwabl, 2007, Philosophical transactions of the Royal Society B: Biological Sciences, 363(1497), pg. 1647-1661. Copyright 2007 by The Royal Society. Reprinted with permission (Appendix 2: Copyright log of permissions for reused illustrations, diagrams and photos in this thesis.*

In domestic chickens (*Gallus gallus domesticus)*, increased yolk androstenedione concentrations lead to increased growth for female embryos (embryonic body weight and post-hatch wing size, tarsus length and beak size) and reduction of sexually selected traits (comb size) for males (Benowitz-Fredericks & Hodge, 2013). Further research is needed to understand whether this is a result of steroid receptor expression patterns or whether these consequences are driven by sex differences in steroid metabolism pathways (Benowitz-Fredericks & Hodge, 2013).

There is some evidence that female brown kiwi chicks grow faster in body mass, bill length, and tarsus length than male chicks (Wilson, 2014). However, no hormonal studies have been performed alongside an investigation into the growth of male and female brown kiwi chicks, both pre-hatch and post-hatch. Likewise, there have been no studies on the effect of environmental conditions on brown kiwi chick growth. Therefore, it remains unknown whether significant hormone factors result in differential growth of male and female chicks in brown kiwi.

Hormonal Transference: Mammals vs Birds

In both birds and mammals, hormone receptors (for androgens and glucocorticoids) are present before the embryo begins to produce its own hormones (Pedernera et al., 2017; Godsave et al., 2002; Groothuis et al., 2019). Receptors and enzymes for hormone metabolism are also found in the mammalian placenta and the extra-embryonic layers of avian eggs (Albergotti et al., 2009; Griffith et al., 2017; Groothuis et al., 2019).

In both female birds and mammals, it has been noted that elevated plasma cortisol/*corticosterone* concentrations can influence offspring development and have long-term effects on survival, physiology, and behaviour (Kaiser & Sachser, 2005; Weinstock, 2008). Gene expression can be modified by maternal cortisol/corticosterone in multiple ways, with emphasis on maternal genes, offspring genes, and the environment. However, while this topic has been extensively studied in mammals, little is known about these pathways in birds (Henriksen, Rettenbacher, & Groothuis, 2011). Prenatal (pre-hatch) exposure to maternal corticosterone not only reflects maternal condition and environmental conditions but also affects postnatal (post-hatch) development of nestling stress responses and development of a nestling (including the development of the HPA axis). For example, maternal corticosterone has been observed to alter the DNA methylation of offspring via maternal deposition of hormones in eggs, which can alter the ultimate phenotype of the offspring, and therefore

affect survival, fitness, and adaptability (Weaver et al., 2004; Sun et al., 2021; Miltiadous et al., 2024).

Avian embryos can convert maternally deposited androgens found in the yolk into inactive forms, which can no longer influence offspring development (Paitz et al., 2010). The conversion of some maternal hormones into inactive forms is also reversible, suggesting that they can be used to synthesise other hormones or respond to ecological conditions (Kumar et al., 2018). Avian embryos are also capable of regulating the density of hormone receptors (Kumar et al., 2019).

In uterine mammals, it is suggested that embryos are capable of filtering maternal stress hormones out, reducing the sensitivity window for postnatal maternal influences (Del Giudice, 2012). Postnatal maternal influences include the transfer of hormones found in mammalian milk, which can also strongly influence offspring phenotypes (Maestripieri & Mateo, 2009).

Egg Yolks and Hormones: Where A4 Fits In

The primary site of androgen synthesis in female birds is the ovarian follicles. Yolk develops in the ovarian follicle, and therefore it can be assumed that maternal androgen synthesis directly influences elevations or decreases in yolk androgen levels, regardless of detectable circulating hormone levels (Balthazart et al., 1987; Schlinger et al., 1999; Pilz & Smith, 2004).

During a study on hens and chicken embryo development, oestrone (E1), androstenedione and DHT were found to be correlated with offspring sex. These hormones were significantly higher in males than females, and this was particularly obvious in the late hatching stage when the embryos maintained hormone homeostasis. Serum androstenedione increased significantly in the later hatching period of 8-16 days, with a 6.7× increase in females, and an 8.1× increase in males (Yalan et al., 2019).

There was a positive relationship between the body mass of offspring (embryo and hatchling) and yolk androstenedione concentrations (Gil et al., 2007). It has been suggested that this is because offspring need a concentration of androstenedione relative to their size due to other embryonic development functions such as maturing of organs (Starck & Ricklefs, 1998). It has also been suggested that the larger the size of a nestling, the more androgens it requires for development, and therefore, higher levels of androstenedione mean that hatchlings can avoid the detrimental effects of high testosterone (Groothuis & Schwabl, 2002). Further

research into the effects of androstenedione on bird development would be needed to confirm these theories.

Higher androstenedione concentrations in egg yolks have also been linked to an increase in the incubation period and a decrease in the nestling period (Gil et al., 2007). This may be a mechanism of reacting to selection pressures in different stages of development. Nestling period reduction may be driven by increased begging behaviour (Eising & Groothuis, 2003), or the increased incubation period may be driven by the delay of embryonic development (McGivern, Fatayerji & Handa, 1996; Dlugonski & Wilmanska, 1998; Sockmann & Schwabl, 2000) as exact effects of androstenedione on avian development are still not understood, either may be possible (Gil et al., 2007).

North Island Brown Kiwi

Short Overview

North Island brown kiwi are like other kiwi species. All are nocturnal and flightless *ratites*. All lack a keel, have vestigial wings, no external tail, stout legs with three toes and sharp claws, and a long, slightly decurved bill (Reid & Williams, 1975; Colbourne, 2020). Kiwi also have well-adapted senses: small eyes with moderate day/night vision, large ears and good hearing, bill tip nostrils and a well-developed sense of smell, as well as sensory pits in the bill tip to detect underground prey movement (Colbourne et al., 2020). Kiwi are nocturnal ground insectivores that typically forage in soil, leaf litter, rotten logs or on the ground surface (Reid, Ordish & Harrison, 1982). Females tend to be 20-30% heavier than males on average and have a longer bill. They produce one or two eggs in a clutch, which are typically 15-20% of the female body weight and have long incubation periods (70-90 days) (Colbourne et al., 2020).

North Island brown kiwi usually lay from mid-June to late December on mainland Aotearoa, New Zealand. Clutches are usually 1-2 eggs, and there can be 3 to 4 clutches in a season, more commonly two. Males incubate alone for 75-90 days (Sales, 2005; Colbourne et al., 2020). Post-hatching, chicks leave the nest between 5 to 7 days old (initially sustained by large yolk sacs when hatching) but return to the natal nest daily for 2 to 10 weeks post-hatching (Sales, 2005; Colbourne et al., 2020). Males can be identified by drawn-out, ascending whistles that they repeat 15 to 25 times, while females have a hoarse guttural call that they repeat 10 to 20 times (Reid & Williams, 1975; Colbourne et al., 2020).

Birds typically have one functional ovary. Female brown kiwi possess two functional ovaries capable of ovulating, although only the left oviduct is functional (Kinsky, 1971.) If more than one egg is laid by a female during the breeding season, ovulation alternates between the two ovaries. On an individual kiwi ovary, follicles can only grow simultaneously until one is approximately 15 mm. This happens because the size of a follicle at ovulation in brown kiwi is over 80 mm in diameter, and therefore, the available space in the ovary is limited. Once both follicles are 15mm, other follicles stop growing and typically regress. In contrast, if two follicles, one on each ovary, grow simultaneously, this can continue until 40-50 mm in growth. At this point, one can continue to develop while the other lags developmentally until the first egg is laid. This then accounts for the long and inconsistent intervals between eggs a female kiwi can lay in a season (Kinsky, 1971).

Owen (1879) first described the penis in the male kiwi as projecting from below the urethra-sexual cavity into the outer cloacal compartment. It measured approximately 38.1 mm and was described as rapidly diminishing from base to point and spirally retracted. Caithness (1971) described the clitoris of female kiwi as diminutive and measuring around 0.5-1.0 mm and suggested that the birds she measured may not have been entirely mature despite their large size. Caithness (1971) goes on to suggest that clitoral size may reflect sexual maturity in kiwi, as one female with an egg in the oviduct had a larger clitoris measuring 8 mm in length. Much remains to be learnt about kiwi sexual organs, including whether all the species are anatomically similar. Likewise, while the five recognised kiwi species (Great spotted kiwi *Apteryx haasti*; Little spotted kiwi *Apteryx owenii*; Okarito rowi *A. rowi*; southern brown kiwi *A. australis*; and North Island Brown kiwi *A. mantelli*) have larger females, the degree of dimorphism varies between species as does their habitat and social structure (Sales, 2005). Different populations differ in terms of social and mating patterns. Traditionally, brown kiwi have been thought to have exclusively long-term partnerships and high partner fidelity, with any degree of polygamy and territory defence dependent on parental demands (Taborsky & Tarborsky, 1999). Breeding displays of bill-to-bill grunting have been reported in captive North Island brown and Great Spotted kiwi, as well as free-living Little Spotted kiwi (Jolly, 1989). Great Spotted and North Island brown kiwi have also been reported to engage in chasing, leaping, loud screeching and snorting before copulation (Reid & Williams, 1975). Further research has indicated that NI brown kiwi breeding systems are more flexible than predominantly monogamy, with the species having several features characteristic of

monogamous bird species, but also many that are characteristic of species displaying polygamy (Undin & Castro, 2022).

Hormone Cycles and Sexual Dimorphism

Kiwi are seasonal breeders that begin to breed from mid-June (Winter) to late December (Summer) (Robertson, Colbourne & McLennan, 2017). In one of the only published studies, Potter and Cockrem (1992) reported that males in the study population showed low plasma testosterone from February to April, which began to rise in May and peaked for ~4 months, before declining in September and reaching low levels again between November and January (Potter & Cockrem, 1992). This correlated with having low non-breeding season testosterone, peak testosterone in the 2-4 months before laying, and a decline at the start of incubation. Incubating males showed very low levels of plasma testosterone (Potter & Cockrem, 1992).

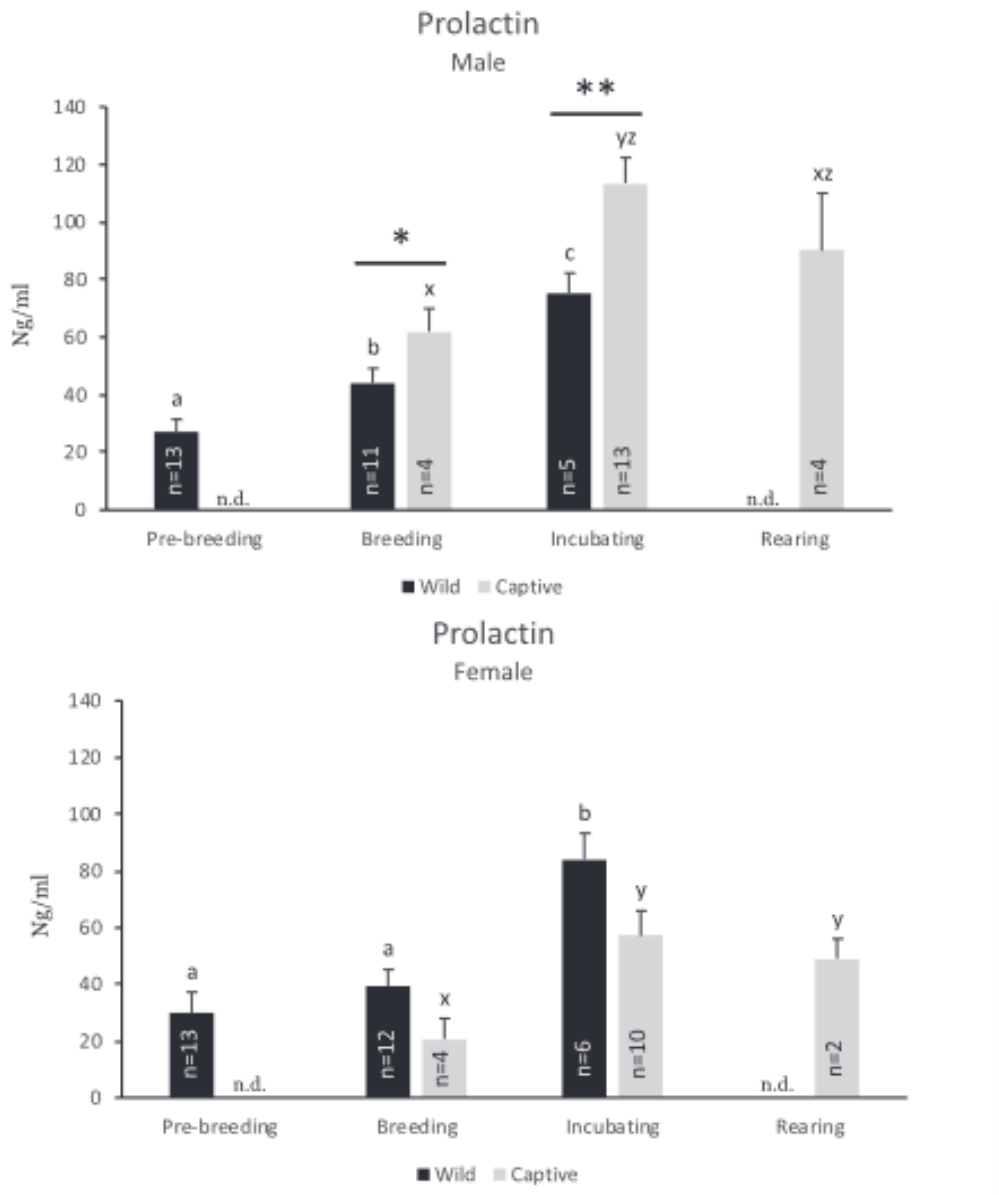
In the same study, oestradiol showed a similar cycle, however, it begins to peak earlier in April. In comparison, female kiwi exhibited low testosterone throughout the year (Potter & Cockrem, 1992). Female plasma oestradiol levels were like those found in males. Oestradiol remained low outside the breeding season before peaking in the 3 months before laying, declining 2 weeks before laying, and then returning to baseline levels. Plasma progesterone levels did not show any significant changes throughout the year for either sex. In all months except March, most males showed higher plasma progesterone levels than females (Potter & Cockrem, 1992).

Both sexes showed an increase in oestradiol in autumn. High oestradiol in males 12 weeks before laying and during incubation is likely to be vital to form the incubation patch found on the male, and to promote incubation behaviours (Potter, 1989). In females, the oestradiol peak that occurs 4 weeks before males may be necessary to facilitate courtship behaviour and egg formation (Potter, 1989).

North Island brown kiwi are known to be an extremely precocial species, with chicks beginning to leave the nest at an average age of 4.3 days (Wilson, 2014) and would be expected to have high levels of DHT in their eggs compared to androstenedione, which is found in higher levels in the yolk in altricial species (Hegyi & Schwabl, 2010).

Prolactin is a protein hormone that also plays a major role in reproductive behaviour (Freeman et al., 2000; Bachelot & Binart, 2007). Prolactin is responsible for maintaining incubation behaviour in birds – a role that is unusually assigned to males in the brown kiwi. In a single study of brown kiwi, males' prolactin increased between the pre-breeding season

and the incubation period (Figure 6) (Jensen et al., 2019). Females' prolactin increased from the point of oviposition. Females may experience an increase in prolactin to encourage nest guarding or to trigger incubation if a mate is lost.



Contrast of levels of serum prolactin between male and female North Island brown kiwi at each stage of their annual breeding cycle (from Jensen et al., 2019).

Figure 6: Serum levels of prolactin (ng/ml) measured across pre-breeding, breeding, incubating and rearing stages of the North Island brown kiwi breeding cycle, in both males (top) and females (bottom). From "Serum prolactin and testosterone levels in captive and wild brown kiwi (Apteryx mantelli) during the prebreeding, breeding and incubation periods" by Jensen et al., 2019, Zoo Biology, 38(3), pg. 316-320. Copyright 2019 by John Wiley and Sons. Reprinted with permission (Appendix 2: Copyright log of permissions for reused illustrations, diagrams and photos in this thesis.

Courtship Behaviour in Kiwi and Other Ratites

Ratites are flightless birds that lack a keel, instead having a flat raft-like breast. The general classification of ratites is the order Struthioniformes, with Struthiones in the African region, Rheae in South America, Casuarii in Australia/New Guinea and Apteryges in New Zealand (Kummrow, 2015). Each family has several species (Box 1).

Box 1: Ratite family and species.

Struthionidae: North African Ostrich (*Struthio camelus camelus*), Somali ostrich (*S. c. molybdophanes*), Maasai Ostrich (*S. c. massaicus*) and the South African ostrich (*S. c. australias*). Many farm populations are *S. c. domesticus*, hybrids of the subspecies.

Rheidae: The Common or Greater Rhea (*Rhea americana*) and Darwin's or Lesser Rhea (*Pterocnemia pennata*).

Casuarii: The emu (*Dromaius novaehollandiae*), the Southern cassowary (*Casuarius casuarius*), the Dwarf cassowary (*C. bennetti*) and the Northern cassowary (*C. upappendiculatus*).

Apteryges: The North Island Brown Kiwi (*Apteryx mantelli*), the Little Spotted Kiwi (*A. owenii*), the Great Spotted Kiwi (*A. haastii*), Tokoeka or Southern kiwi (*A. australis*), and Rowi (*A. rowi*).

In most ratites, males incubate eggs after they are laid, and parental care of chicks is provided by males almost exclusively in all species. The ostrich is an exception, with males and females dividing both incubation and parental care between themselves.

North Island Brown Kiwi and Little Spotted Kiwi (*A. owenii*) have male-only incubation. Brown kiwi has complex social interactions and can breed as pairs or groups, with males sharing incubation. In tokoeka (*Apteryx australis*), males and females incubate and brood the young, and they live in family units composed of several generations. In Rowi (*Apteryx rowi*) both sexes incubate, but the mating system is primarily monogamous (Castro & Morris, 2011). In the emu, cassowary and kiwi, the female is significantly larger than the males (Kummrow, 2015).

Brown Kiwi (*Apteryx mantelli*) behaviour

Interactions between brown kiwi have been historically difficult to study due to the nocturnal nature of the species and the difficulty in tracking through their habitat without disturbing them. However, observations of kiwi species behaviour have been made possible with the

advent of camera traps and infrared video cameras that allow capturing kiwi movements and behaviour (Cunningham & Castro, 2011). Despite this, there is little information about the mating behaviour of wild or captive birds. Cunningham & Castro (2011) videoed the behaviour of kiwi by direct observation using handheld infrared camcorders. Kiwi were estimated to spend most of their time foraging (approximately 75%), while the rest of the time was spent on comfort, walking, and vigilance behaviour. They found that direct social and breeding interactions were rare. They found no differences in foraging behaviour between juveniles, males, or females, with all using the same foraging substrates, microhabitats and foraging techniques (excluding probing depth, due to differences in bill lengths). In captive situations, aggression has been recorded between female chicks, with larger females being the dominant aggressor and displaying behaviours such as pecking and chasing (Wesley & Brader, 2013). As well as pecking, growling and kicking have been described as aggressive behaviours between kiwi (Haeusler, 1923). Males are known to share territory or enclosure if familiar with each other from a young age, but housing adult female kiwi has a high risk of injury or death of an individual. It is also known that adult kiwi in pairs may kill mates even after being together for several years, and this behaviour is often performed by the female (Fraser & Johnson, 2011). The internet has many uploaded videos showing interactions between wild birds, including what could be mating behaviour, but these videos have not been placed into any particular context (Table 4).

Taborsky & Tarborsky (1992) witnessed one aggressive pursuit in three study seasons, between two recently widowed females chasing each other on their 'territorial overlap'. The study also found that territorial behaviour could be artificially induced with playback or imitation calls, with a deliberate approach and/or calling. Fifty-three cases of artificially induced territoriality were recorded, with only three by female mate's calls. It was determined that the main mechanism of territory maintenance appears to be long-distance calling and rare aggressive encounters. Females were also found to be territorial, with a small proportion of responses addressed to other females, and two out of five aggressive combats observed being between females (Taborsky & Taborsky, 1991).

Ostrich (*Struthio camelus*)

In the ostrich, females have a clitoris (sometimes referred to as a female phallus) which has been measured to be anywhere between 20-40 mm in length (Figure 7). The clitoris extends from the genital mound and is visible during both urination and defecation. Females are

known to posture in front of potential mates and begin to develop sexual courtship behaviours earlier than males.

Groups are characterised by having more dominant females at the top of the hierarchy, followed by more submissive females and then males. Dominant females are known to be aggressive to other females and yearlings in the group. This behaviour is alleviated in the group setting by the submissive female assuming a submissive posture with the head down, s-shaped neck, and tail down (Kimwele & Graves, 2003).

While males hold territories that they defend and in which they create nest scrapes, females cover a larger mean range and enter the territories of multiple males. Within a communal nesting system, a dominant female (referred to as 'major') will visit the territory of a male and lay within an established nest. Minor females (subordinate to major females) will also visit the same territory and may lay an egg in an already established nest; minor females in one territory may also be major females in another territory. Both sexes provide incubation, with males predominantly incubating during the night. During the initial stages of incubation, the female will rearrange eggs and actively discard some. These are usually those laid by a minor hen; how these eggs are differentiated by the female is unclear (Kimwele & Graves, 2003).

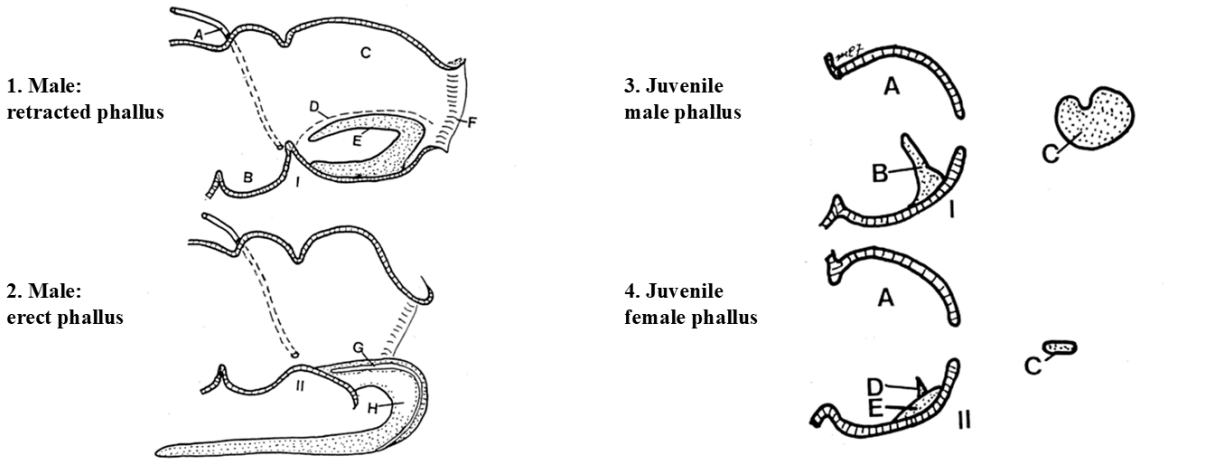
Both males and females provide parental care, and young are usually monitored in creches with other chicks from other nests. The laying season in Africa is broad, reported in different regions between June and October; however, commercial rearing of ostriches in both hemispheres has strongly suggested that ostriches are *opportunistic breeders*. Within farming systems, lower egg production and fertility have been found when more than one female is kept with a male. Similarly, farming systems have also led to the observation of some females displaying to and attempting to mount other females (Kimwele & Graves, 2003).

Australian cassowaries (*Casuarius casuarius*)

Fisher (1968) observed one breeding attempt of captive cassowaries in 1965, where a male began brooding a hollow in which a female laid eggs up until the fifth egg. At this point, the male became aggressive and no longer let the female into the hut where the nest was located. The male incubated these eggs for five weeks, only leaving occasionally to feed, demonstrating parental care. A fight occurred at this point between the male and female – the male sustained significant wounds and highlighted that female cassowaries are capable of physical dominance and aggression over males.

Table 4: Examples of videos uploaded online showing potential courtship interactions between kiwi.

Video Name	Platform	Date	Behaviour Seen	Uploader/s	Link
Kiwi Bird Paso Doble	YouTube	6/11/2020	Female and male kiwi, calling, foraging, preening each other, stepping around and circling each other, clear form of some kind of courtship "dance"	Lindsay Alexander	https://youtu.be/FhNXYFw0Fn8
RARE FOOTAGE - KIWI FIGHT - Stewart Island, NZ	YouTube	28/09/2020	Two birds, shoving and kicking at each other, chasing each other with the pursuing bird placing its beak on the back of the target	South Island Rifle Walkers	https://youtu.be/PE8hYT2nlaI
Fighting (or mating) Kiwi birds on Stewart Island, New Zealand	YouTube	15/03/2009	Two birds chasing each other, grunting, squawking, grabbing at each other	sparvuggla1	https://youtu.be/8x5d_lZtA0U
Two Brown Kiwi fighting - Opito Bay Bay of Islands	YouTube	28/02/2017	Two birds shoving and kicking at each other, running short distances before re-engaging	Dean Wright (Dean Wright Photography)	https://youtu.be/zisdKYL3VVo
Rumble in the Jungle - kiwi birds fighting	YouTube	11/11/2017	Two birds shoving and kicking, chasing each other short distances, potentially an attempted copulation	Kiwi Cam at Russell Nature Walks	https://youtu.be/Gi8fL03Xryg



Left lateral view of the cloaca and phallus of an adult male ostrich

A. Vas deferens
B. Urodeum
C. Proctodeum
D. Uncovered crypt of the floor of the proctodeum
E. Retracted phallus
F. Vent
G. Dorsal sulcus
H. Erect phallus

Left lateral view of the cloaca of chicks and juvenile ostriches (I male ostrich, II female ostrich)

A. Proctodeum
B. Male phallus
C. Cross-sectional view of the phallus
D. Female phallus
E. Genital eminence

Figure 7: Left lateral view of the male ostrich (right) with (1) the retracted phallus and (2) the erect phallus. Left lateral view of the chick/juvenile ostrich and the (3) male phallus and (4) female phallus. From "Comparative clinical anatomy of ratites" by M.E Fowler, 1991, Journal of Zoo and Wildlife Medicine, 22(2), pg. 204-277.

Fisher (1968) observed this same male incubating and aggressively guarding the nest in other breeding attempts. Before mating, the male was also observed scraping the ground and scattering nesting material in preparation for eggs. With a successful clutch, the male was frequently observed breaking down larger food and scattering it for the chicks, plucking and offering them grass, and at one point killing a starling and ripping pieces off to offer to the young birds – this was again, one of the first recorded instances of parental care in cassowaries, an otherwise understudied ratite species.

Greater Rhea (*Rhea americana*)

The Greater Rhea has a mating system in which communal nests are used by several females to lay eggs, and males incubate and provide parental care. Greater Rheas seem to utilise a complicated social mating system that consists of *simultaneous* and *sequential polyandry* and polygyny. *Promiscuity* is also high in this species – males are not the fathers of the majority of offspring from each nest, nor do females act cohesively as a group laying in the same nest even when grouped by the male (Martella et al., 2022).

Rhea males also exhibit other unusual behaviours. This includes stealing eggs from contiguous nests (Fernández & Reboreda, 1995), fighting amongst themselves for care of chicks, and adoption of lost chicks have all been reported previously (Bruning, 1973; Codenotti & Alvarez, 1998; Lábaque, Navarro, & Martella, 1999; Fernández & Reboreda, 2003; Barri et al. 2005). Additionally, subadult males also take on incubation and chick-rearing responsibilities (Codenotti & Álvarez, 1997).

Male Rhea displayed high testosterone levels through their entire breeding period (September to January) even while incubating, indicating that testosterone may not inhibit male parental care and behaviour. Female Rhea displayed high oestradiol levels from October to December, with most eggs laid in October. Juveniles of both sexes displayed high testosterone and oestradiol and did not show seasonal fluctuations (Valdez et al., 2014).

Emu (*Dromaius novaehollandiae*)

Emu mating systems have been a topic of debate. Typically, during breeding, the majority of males and females form monogamous pairs, with over 70% of observed breeding pairs following this mating system. However, while male emus are thought of as predominantly monogamous, female emus are more likely to present cases of polyandry (Gaukrodger, 1925; Fleay, 1936; Long, 1959; Davies, 1976; Handford & Mares, 1985; Coddington & Cockburn, 1995). Additionally, female emu also seems to engage in sequential polyandry, where females are observed mating again while their initial partner is already incubating eggs. This may be due to differences in the overlap of laying and incubation periods; male emu begin to incubate when 5 to 10 eggs have been laid, less than half the number of eggs that many female emu can produce in a season. The female emu can store sperm in their reproductive tract for up to 21 days, meaning they can continue to lay fertilised eggs while their partner has begun incubation and no longer has sexual interest (Blache, Barrett, & Martin, 2000).

Captivity

Selective pressures are relaxed in captivity, which may be why wild and captive animals differ in several traits. Captive birds are typically less genetically diverse, larger, heavier, more colourful, less discriminating during courtship, and less active than their wild counterparts (Sossinka, 1982; Fortstmeier et al., 2007).

If conditions in captivity are suboptimal (such as temperature), animals are forced to allocate resources between functions. Captive zebra finches exposed to a low temperature (7◦C)

maintain body condition, but reproductive performance is greatly impaired when compared to the same birds exposed to 21◦C (Salvante, Walzem & Williams, 2007). Compared to birds from the middle of Australia, birds in cool, temperate zones cannot maintain year-round active reproduction (Perfito et al., 2007). Hormonal effects may also compound these outcomes – experimentally increasing corticosterone levels in captive zebra finches has been found to impair their reproductive performance (Salvante & Williams, 2003). Male zebra finches with high corticosterone levels are unlikely to be selected by females as mates (Roberts et al., 2007), and higher corticosterone levels are associated with greater exploratory and risk-taking behaviour (Martins et al., 2007).

A study on Cape white-eyes (*Zosterops virens*) found that some physiological data collected from birds held in long-term captivity were not directly comparable with freshly wild-caught individuals. The effect of long-term captivity on the whole animal's basal metabolic rate greatly outweighed the effect of season. Body mass was affected more by long-term captivity than by season. Body mass was lower in freshly caught wild birds than captive birds, presumably due to the constant, regular supply of higher quality food available to captive birds compared to the more erratic, lower quality diet in the wild (Thompson, Brown & Downs, 2015).

Captivity and Bird Behaviour

When investigating the ecological significance of personality, researchers often measure behaviour in captivity and then compare the distribution and/or fitness of individuals in the wild as a result (Dingemanse et al., 2004; Bell, 2005). Studying behaviour in captivity has advantages, allowing the control of conditions under which individuals are tested (Campbell et al., 2009). However, there are reasons that applying personality from captive results may be misleading.

Behaviour changes as wild individuals adapt to a captive environment (Butler et al., 2006). Testing in captivity can exaggerate or generate behavioural differences if there are systematic differences in the acclimation times between different personality types. An example of this is risk-averse (or “shy”) individuals taking longer to recover from handling, capture stress, and taking longer to eat in a novel environment than risk-prone (or “bold”) individuals (Wilson, George & Griffin, 1993; Van Oers, Klunder & Drent, 2005). Food is frequently withdrawn before personality trials and returned at a later stage to stimulate behaviour,

residual stress, hunger or condition. This may motivate shy but not bold individuals to a greater extent in captivity than they would be in the wild. This highlights the importance of testing behavioural differences beyond the captive environment (Herbon et al., 2010).

Classifying behaviour in captivity may also be misleading, as behaviour is frequently highly and specifically contextual. Captivity may result in isolation from the appropriate context and suppress or subvert personality traits. In artificially constructed dominance interactions in captive great tits (*Parus major*), there is no linear relationship between rank and exploratory tendency, and an overall negative correlation (Verbeek et al., 1999). Comparatively, in the wild, this trait relationship is only negative between non-territorial juvenile males, and in contests between territorial males on neutral grounds, fast explorers tend to dominate slow explorers (Dingemanse & de Goede, 2004). In their wild territory, males were dominant regardless of personality – the absence of territorial context in captivity may therefore limit the ability to predict the significance of personality traits. Similarly, numerous studies suggest individuals modify risk-taking behaviour in relation to the 1) presence and 2) identity of conspecifics, meaning social isolation in captivity is another important contextual difference (Van Oers, Klunder & Drent, 2005; Boogert et al., 2006; Stöwe et al., 2006; Apfelbeck & Raess, 2008; Pike et al., 2008).

Despite these factors, a study into the personality traits of wild and captive blue tits (*Cyanistes caeruleus*) found that personality traits measured in captivity reflected behavioural differences in wild foraging individuals. Wild variations of exploratory tendencies and neophobia were repeatable in captivity, and analogous traits were repeatable in the wild. Birds that were exploratory in captivity were more likely to find new feeders in the wild, and vice versa. Individual neophobia in captivity correlated with its latency to approach novel feeder colours in the wild. (Herborn et al., 2010).

Captivity and Bird Hormones

Using male and female European starlings (*Sturnus vulgaris*), one study found that when placed in an outdoor aviary, birds bred at the appropriate time of year. Birds placed in an indoor flight aviary experienced reproductive suppression. After ten days, outdoor birds exhibited increases in baseline and stress-induced corticosterone, while also progressing into active breeding stages (pairing, nest building and egg laying). Comparatively, indoor birds

displayed no change in either baseline or stress-induced corticosterone, a few signs of active breeding (Dickens & Bentley, 2014).

Wild-caught birds showed longer corticosterone responses to acute stress than those bred in captivity (Cabezas et al., 2013). These differences in response between wild-caught and captive-bred individuals could be explained by physiological acclimation and/or facilitation (Romero, 2004). Animals reduce their corticosterone response to repeated or chronic exposure to noxious stimuli as a consequence of no longer perceiving the stressor to be dangerous when acclimation occurs (Romero, 2004). Corticosterone elevation interacts with hypothalamus receptors to down-regulate the intensity of hypothalamus-pituitary-adrenal (HPA) activity through negative feedback to avoid chronic corticosterone release negative effects (Dallman et al., 1992). If wild-caught and captive-bred birds are exposed to the same source of stress, birds born in captivity may have a greater degree of physiological acclimation to captivity and human presence than their wild counterparts (Romero, 2004).

Experimental handling of parent-raised parrot chicks (Collete et al., 2000), led to a decrease in corticosterone levels and an increase in tameness. Birds bred in captivity are typically more exposed to frequent human interaction than wild-caught birds, such as close-banding and periodical health checks. Acclimation may then explain why many captive-bred birds do not show a prolonged endocrine response when handled and restrained (Cabezas et al., 2013).

Comparatively, wild-caught birds that were not acclimated to captive stress could show facilitation, whereas captive-bred birds do not. Facilitation refers to animals acclimating to one stressor to improve corticosterone responses to new, unpredictable stimuli (Romero, 2004). Under this scenario, wild-caught birds would show higher corticosterone responses to novel challenges such as experimental restraint protocols (Cabezas et al., 2013).

Prolactin and testosterone have been measured in captive brown kiwi. Jensen et al. (2019) detected significantly higher concentrations of both prolactin and testosterone in captive male kiwi compared to wild male kiwi. The study suggests that elevated testosterone is due to unnaturally high male-male challenge frequencies, supported by the elevated prolactin concentrations, as opposed to captivity-induced stress (via corticosterone). Captive males having prolactin levels higher than their wild counterparts during the breeding season/incubation suggest that their breeding is not impaired by captivity-induced stress.

Conclusions

The identification of androstenedione as a potential catalyst in female masculinisation and reversed sexual dimorphism has been accepted since 1970 (Luttge & Whalen, 1970; Glickman et al., 1987). It is currently suspected that while testosterone may still play a role in this hormonal pathway, it is not directly responsible for masculinisation in many mammalian species currently investigated, and that males and females with reversed sex roles typically do not exhibit any notable deviations from standard sexually dimorphic patterns in plasma testosterone levels (Glickman et al., 1987; Place & Glickman, 2004; Drea, 2009). We also know that the process of masculinisation likely occurs through maternal androgens early in foetal development, during sexual differentiation, and that the ovaries are implicated in maintaining these hormonal differences postpartum in masculinised females (Groothuis et al., 2005; Drea, 2009). However, multiple questions remain in terms of quantifying this direct pathway of development.

In birds, we know that androstenedione has been implicated as an important androgen that induces male secondary sexual characteristics in some species, such as comb growth in chickens (Massa, 1984). We also know that in chickens, increasing yolk androstenedione leads to female embryos experiencing increased body weight, and increased post-hatch wing size, tarsus length and beak size (Benowitz-Fredericks & Hodge, 2013). While minimal study has been done on androstenedione as a masculinising factor in birds, these initial results indicate that it may play a role in the masculinisation process.

In kiwi, hormonal and behavioural studies need to be conducted to provide an understanding of the sex role reversal and sexual dimorphism in the various species. Androstenedione needs to be measured from the plasma of both males and females to quantify any identifiable differences between the sexes, and whether females continuously show higher concentrations than males. Quantification of androstenedione plasma concentrations also allows for a better understanding of existing data on other sex hormone levels (testosterone, progesterone, oestradiol, and prolactin) and their relation to sexual characteristics and behaviour. Behavioural observations during and off the breeding season would provide information on the behavioural differences between males and females, and studies of dead eggs hormones would show potential for identifying sexual differentiation pathways. Quantification of both testosterone and androstenedione levels in brown kiwi may provide answers about the underlying physiological mechanisms that drive reversed sexual dimorphism physically (larger female size and features) and behaviourally (courtship displays, male parental care).

Higher levels of either testosterone or androstenedione in female kiwi (compared to male counterparts) would provide insight into kiwi sexual dimorphism, sexual development, and species-specific behaviours and traits.

In this thesis, I aim to investigate whether female North Island brown kiwi fit some of the parameters of the Female Masculinisation Hypothesis (Glickman et al., 1987; Drea, 2007; French et al., 2013; Drea et al., 2021; Grebe, Sheikh & Drea, 2022) paired with the Challenge Hypothesis as seasonally breeding species where androgen fluctuations serve to meet social demands, reproductive competition and parenting (Grebe, Sheikh & Drea, 2022; Wingfield et al., 1990). Namely, I will:

1. Chapter 2: Investigate the levels of hormones in males and females in captivity and the wild, to observe any differences. I hypothesise that if female brown kiwi are masculinised, they will show higher androstenedione levels than males both inside and outside the breeding season, with higher levels expected within the breeding season.
2. Chapter 3: Investigate social behaviour between male and female North Island brown kiwi around the courtship and breeding season. Describe behaviours performed by individuals and observe for sexual dimorphism in aggression or dominance-driven behaviours. I hypothesise that if female kiwi are masculinised, they will perform behaviours expected from male birds, including being more aggressive, initiating courtship displays and pursuing males.

Chapter 2: Hormone drivers of masculinisation of female North Island brown kiwi physiology, behaviour and sociality

Introduction

Sex steroids and sex determination in birds

At fertilisation, sex is determined in birds by sex chromosomes. Inheritance of two Z (ZZ) chromosomes results in the development of a male embryo, while one Z and one W chromosome results in the development of a female embryo. While gonadal sex is initially determined by sex chromosomes, gonadal differentiation is influenced by multiple genes. Fully developed gonads secrete sex hormones, which influence the masculinisation or feminisation of the entire embryonic body (Kuroiwa, 2017).

Secondary sex characteristic development is regulated by sex hormones from maturing gonads. Sexual trait development can be costly, and in birds can be conspicuous, such that premature expression can have a negative effect on fitness and survival (Bear & Monteiro, 2013).

In birds, testosterone is either produced by *Leydig cells* in the testes in males, theca cells in developing follicles on the ovaries in females or synthesised by the adrenal glands and brain in both sexes. Testosterone from these sources can either bind directly to receptors or undergo further conversion. Testosterone is aromatised primarily within the hypothalamus by aromatase to produce oestradiol, which can then bind to oestrogen receptors. Testosterone metabolism in the hypothalamus and pituitary gland produces 5α-dihydrotestosterone (5α-DHT), and 5β-dihydrotestosterone (5β-DHT), as well as androstenedione, by *5α-reductase* and *5β-reductase* enzymes. Dihydrotestosterone and androstenedione can both bind to testosterone receptors, and receptors are more sensitive to these secondary androgens than directly to testosterone (Goretskaia & Beme, 2021; Lofts & Holmes, 1981) (Figure 8).

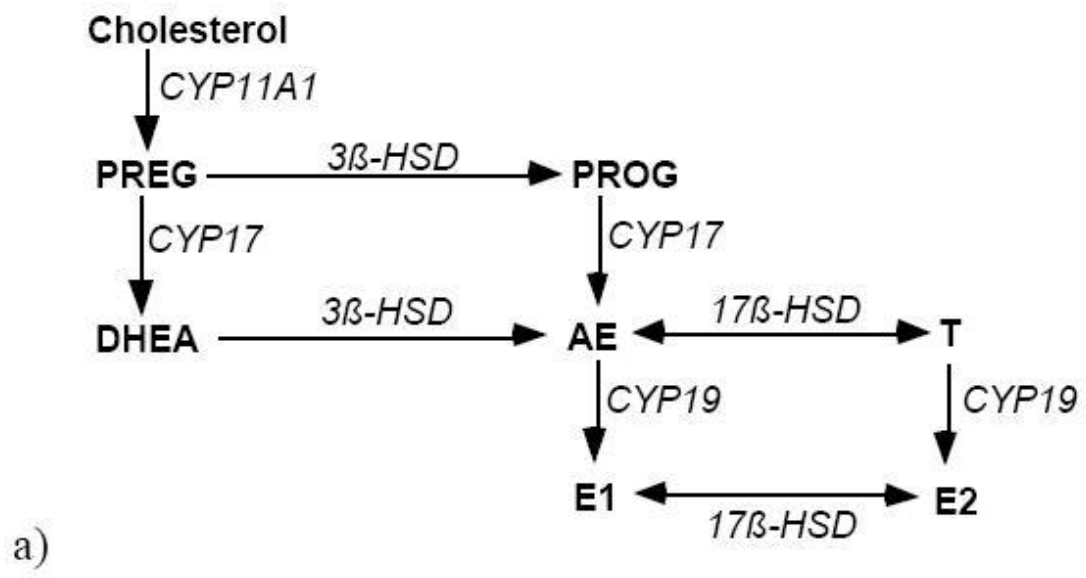


Figure 8: Simplified diagram of sex steroid synthesis that is critical in the control of reproduction. Steroids are in bold; enzymes are in italics. Steroids: PREG = pregnenolone*, PROG = progesterone, DHEA = dehydroepiandrosterone, AE = androstenedione, T = testosterone; E1 = estrone; E2 = oestradiol. Enzymes: CYP11A1 = cytochrome P450 side-chain cleavage;* CYP17 *= cytochrome P450 17α-hydroxylase/C17,20 lyase,* 3β-HSD *= 3β-hydroxysteroid dehydrogenase/isomerase,* 17β-HSD *= 17β-hydroxysteroid dehydrogenase, CYP19 = aromatase. "DHEA effects on brain and behaviour: Insights from comparative studies of aggression" by K.K. Soma, N.M. Rendon, R. Boonstra, H. E. Albers & G.E. Demas, 2008, Neurochemistry International, 52(4-5), pg. 611-620. Copyright 2008 by Elsevier. Reprinted with permission (Appendix 2: Copyright log of permissions for reused illustrations, diagrams and photos in this thesis.*

Maternal effects

Evidence also suggests that maternal corticosterone elevation affects the level of gonadal steroids in the avian egg. These effects were also found to be sex specific, with female bird embryos being less sensitive to elevated maternal corticosterone than males. Female-biased secondary sex ratios in these conditions mean that female birds with higher testosterone produce more daughters (Trivers & Willard, 1973; Henriksen, Rettenbacher, & Groothuis, 2011).

Maternal stress is also associated with greater masculinisation in later development of offspring, including behavioural masculinisation, such as higher frequencies of male-typical courtship and play behaviour performed by masculinised females (Sachser & Kaiser, 1996). Other effects include increased circulating testosterone, elevated *sympathetic adrenomedullary activity* (and greater *adrenal tyrosine hydroxylase* activity) (Kaiser & Sachser, 1998). Male-typical expression patterns of androgen receptors in the *medial preoptic area (MPOA)* and *nucleus arcuatus (ARC)* of the hypothalamus are also noted in these individuals, as well as upregulation of androgen receptors in the *CA1 region of the*

hippocampus, and upregulation of the *oestrogen receptor-alpha in the MPOA, ARC and CA1* (Kaiser et al., 2003; Sachser, Hennessy & Kaiser, 2011).

The relevance of androstenedione

Androstenedione as a potential source of masculinisation has been suggested in mammals, specifically using the model of the spotted hyena (*Crocuta crocuta)*. Androstenedione primarily from the ovaries has been identified as the primary circulating androgen in female hyenas (Lindeque & Skinner, 1982; Glickman et al., 1987; Glickman et al., 1992), who have been compared to androgenised female dogs in terms of their masculinisation (enlarged clitoris resembling a penis, large body size, social dominance and aggression) (Beach, 1983). The androstenedione hypothesis has also been further supported by the discovery of 17b-hydroxysteroid dehydrogenase activity in the hyena placenta, allowing the conversion of androstenedione to testosterone, and the transfer of maternal ovarian and placental androgens to the foetus (Licht et al.,1992; Yalcinkaya et al., 1993).

In birds, maternal androgens are known to be transferred into the egg yolk (Heygi et al., 2011). Androstenedione in yolks has previously been related to post-hatch fitness, with higher concentrations of yolk androstenedione leading to chicks larger than those from eggs with lower concentrations (Heygi et al., 2011).

High concentrations of androstenedione have been found in 9- to 12-week-old prepubertal cockerels (*Gallus gallus domesticus*), likely of testicular origin. Between 9 and 16 weeks, both plasma androstenedione and testicular androstenedione concentrations begin to fall, before the maturation of the testes (able to produce semen) that occurs around 24 weeks. Plasma androstenedione concentrations exceed plasma testosterone concentrations in juvenile cockerels, while androstenedione concentrations in the adult cockerel are roughly half of that of testosterone (Culbert, Sharp, & Wells, 1977).

In castrated male Japanese quail (*Coturnix japonica*), 1 mg/day injections of androstenedione were found sufficient alone to activate copulation in some birds, despite the fact that it had weaker results than testosterone injections. Androstenedione injections also stimulated *proctodeal gland* growth, as well as stimulating sexually motivated strutting behaviour (Adkins, 1977).

These results seem to indicate that in birds, androstenedione produces some masculinised effects, without the full masculinisation found when compared to testosterone. This would support androstenedione as a masculinising factor in female birds, who have masculine traits but are still genetically female and able to reproduce as so.

In this chapter, I look at testosterone, corticosterone and androstenedione measured from North Island brown kiwi plasma samples across males and females, wild and captive, within and outside the breeding season. These results are compared to previously assayed progesterone, oestradiol and prolactin plasma results for the same birds, seasons and conditions, to compare overall sex steroid levels in kiwi.

Objective: To better understand sex roles and sexual dimorphism reversal in kiwi by studying the hormonal profiles of males and females in this species. I hypothesise that female kiwi will have higher androstenedione concentrations than males, supporting the idea that they are masculinised and that they will have higher androstenedione concentrations during the breeding season than outside the breeding season. In contrast, male kiwi will have higher prolactin levels than females in line with the species' parental care mode.

Materials and Methods

Blood Sampling

The samples used in this study were collected as part of a larger study, with the methods of blood collection and storage described by Jensen et al. (2019). In summary, samples were collected from wild kiwi on Ponui Island (36°52′S 175°11′E) and captive birds at Westshore Wildlife Reserve (Napier, New Zealand; 39°28’S 176°52’E) and Kiwi Encounter (Rotorua, New Zealand; 38°06’S 176°13’E). A sterile 25g needle was used to draw up to 3 ml from the jugular or metatarsal vein. Whole blood was centrifuged and separated into plasma and red blood cells. All plasma samples were then stored at -80°C until assay.

Wild kiwi samples comprised 10 females and 17 males, representative of the following mating groupings: one with five birds (3 male, 2 female), two triads (2 male, 1 female) and ten dyads (1 male, 1 female). Samples were collected from March to October 2010, spanning the pre-breeding, breeding and incubation periods.

Samples from captive kiwi represented 5 males and 5 females, including two dyads from Westshore Wildlife Reserve and three dyads from Kiwi Encounter. Samples were collected

during the breeding and incubation periods. Permit regulations did not allow sample collection during the pre-breeding season.

Where possible, repeat samples were collected from individual birds every 2-4 weeks based on the accessibility of each bird.

Hormone Assays

Testosterone, corticosterone and androstenedione

Testosterone, corticosterone and androstenedione were all measured in the plasma samples using commercially available enzyme immunoassay kits (K080, K014, K070; Arbor Assays, Ann Arbor, MI). Assay sensitivity for these assays was 2.97 (testosterone), 20.9 (corticosterone) and 2.30 pg/ml (androstenedione). Kits were validated for use with kiwi plasma. Testosterone and corticosterone were measured in plasma while a liquid solvent extract was prepared from plasma for androstenedione. Briefly, a diethyl ether extract was produced from plasma (as per Arbor Assays steroid liquid sample extraction protocol) and desiccated in a SpeedVac (Savant SC210A SpeedVac Concentrator, Thermo Scientific, Auckland, New Zealand) and reconstituted in assay buffer. Samples for all markers were measured in duplicate and measured by optical density using a colorimetric plate reader (Thermo MultiSkan FC, Thermo Scientific, Auckland, New Zealand) at 450 nm.

Oestradiol and progesterone

Oestradiol and progesterone were measured in kiwi plasma as part of a previous study but were unpublished until now. Both used commercial radioimmunoassay kits (MP Biomedical, Santa Ana, CA) previously validated for kiwi plasma (Potter & Cockrem, 1992). The assay sensitivity was 6 pg/ml for oestradiol and 0.07 ng/ml for progesterone.

Prolactin

Prolactin was measured in males as part of a previous study (Jensen et al., 2019). Prolactin was measured with a radioimmunoassay validated for domestic chickens (Talbot & Sharp, 1994). Assay sensitivity was 0.02 ng/ml.

Statistical Analysis

We used data from adult birds that were sampled more than once. Because of the correlated nature of our data due to the repeated measures, we used Generalised Estimating Equations to

examine the predictive value of two main factors, 1. source (captive or wild), and 2. season (breeding or non-breeding) on each hormone measured (prolactin, corticosterone, progesterone, oestrogen, testosterone and androstenedione for females, and prolactin, testosterone and corticosterone for males). The probability distribution was Gamma with a log link function, and each model used bird identification as a subject effect and sampling time as within within-subject effect. I used Autoregressive (AR1) as the working correlation matrix structure because we expected that samples taken closer in time would be more highly correlated. I used the statistical package IBM SPSS Statistics (Version 29) to run the models.

Results

Captive females had higher prolactin levels compared to wild birds, but this difference was less pronounced during the breeding season (Figure 9, Table 5) and was driven by wild females having significantly lower levels of prolactin during the non-breeding season (Figure 9; left; Table 5; Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.). Captive males had significantly higher prolactin levels compared to wild males, and for both groups, the levels were higher in the breeding season (Figure 9; right; Table 5; Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.). The levels of prolactin in wild males and females followed a similar pattern, with higher prolactin during the breeding season. This was not the case for captive birds. Males had higher prolactin levels than females, and females had constant levels across the seasons.

Captive females had significantly lower corticosterone levels compared to wild birds overall. Wild females had higher corticosterone levels outside the breeding season (Figure 9; B, left; Table 5; Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.). Captive males did not differ from wild males in corticosterone levels overall, but for wild males, corticosterone levels were significantly higher in the breeding season (Figure 9; B, right; Table 5; Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.). Wild females have the highest levels of corticosterone and captive females the lowest. Both captive and wild males showed no

significant difference in corticosterone levels between the breeding and non-breeding season (Table 5).

Captive females had significantly higher androstenedione levels compared to wild birds. The breeding season was associated with significantly higher androstenedione levels in wild females compared to the non-breeding season (Figure 9; C, left; Table 5; Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.). There were no significant differences in androstenedione levels between wild and captive males. Captive males had the highest levels of androstenedione during the breeding season (Figure 9; C, right; Table 5; Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.).

There was no statistically significant difference in testosterone levels in wild females between breeding and non-breeding seasons (Figure 9; D, left; Table 5; Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.). Captive males had significantly higher levels of testosterone compared to wild males (Figure 9; D, right; Table 5; Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.), but for both groups, the levels of testosterone were similar in and outside the breeding season. Female kiwi testosterone levels were comparable to those of wild males.

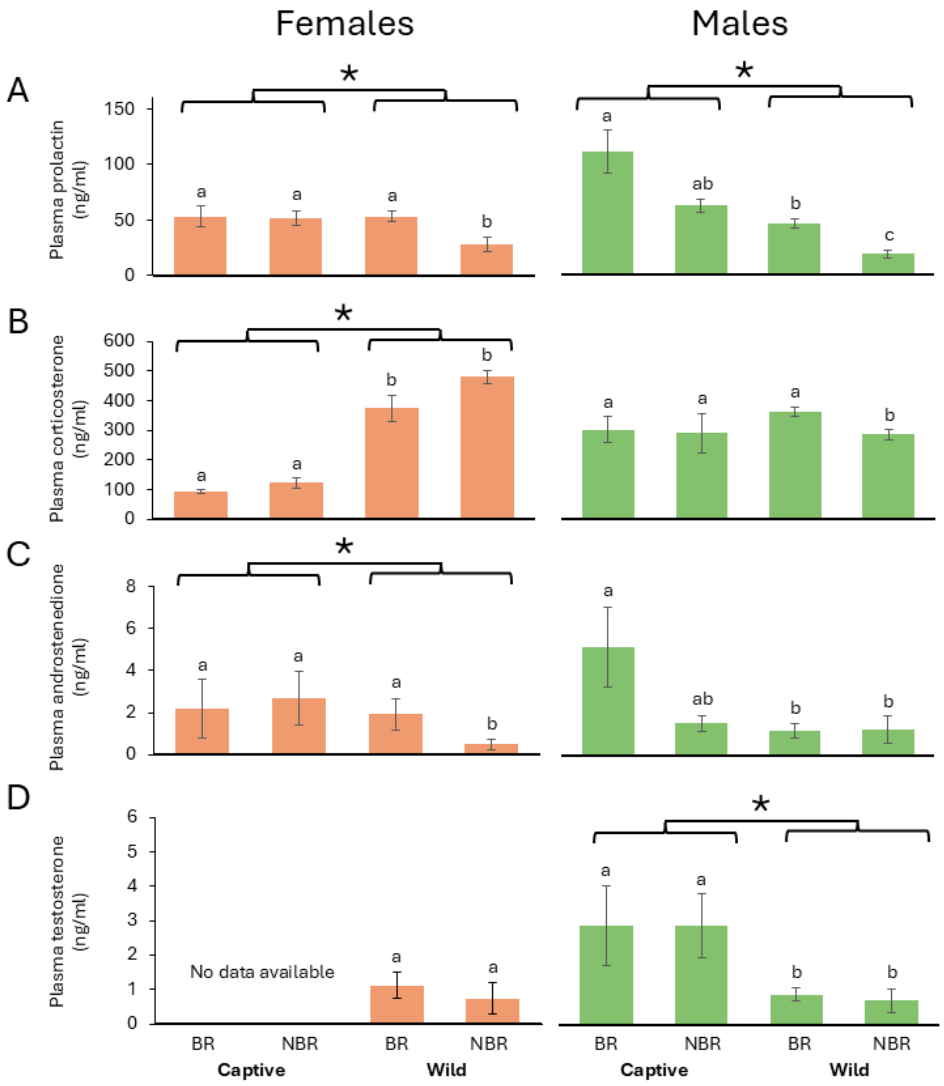


Figure 9: Plasma prolactin (A), corticosterone (B), androstenedione (C) and testosterone (D) for female and male brown kiwi on Ponui Island (Wild) and West Coast Wildlife Centre and Rainbow Springs (Captive) during the breeding (BR) and non-breeding seasons (NBR) in 2010. Mean ± SE. Significant effects between captive and wild kiwi from a generalised estimating equation with hormone concentration as the response variable and captive status and season as independent factors. Significant differences are represented by asterisks, while differences between seasons in each group are given by letters.

Captive females had significantly higher oestradiol levels compared to females in the wild but there were no statistically significant differences between breeding and non-breeding seasons for either group (Figure 10: A; Table 5; Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.).

Captive females had significantly lower progesterone levels compared to wild birds. Wild females had statistically significantly lower progesterone levels in the breeding season when compared to the non-breeding season (Figure 10: B; Table 5; Appendix 4: Generalised Linear

model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.).

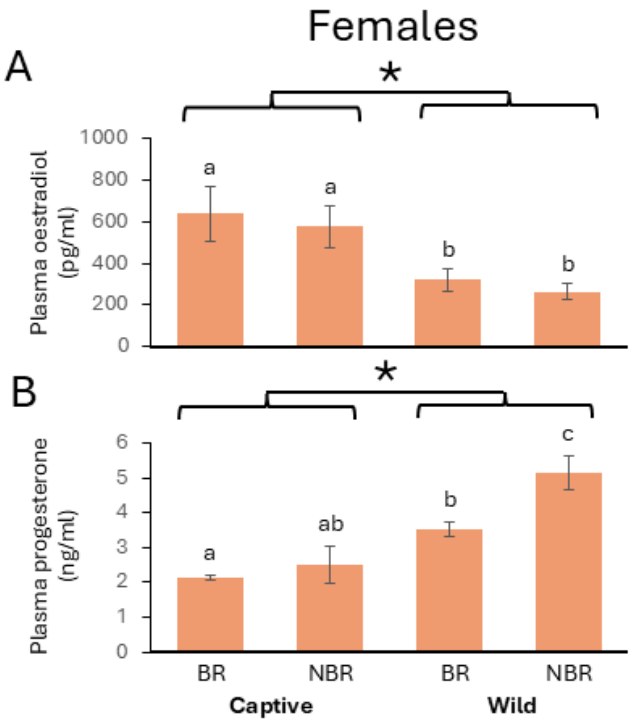


Figure 10: Plasma oestradiol (A) and progesterone (B) for female brown kiwi on Ponui Island (Wild) and West Coast Wildlife Centre and Rainbow Springs (Captive) during the breeding (BR) and non-breeding seasons (NBR) in 2010. Mean ± SE. Significant effects between captive and wild kiwi from a generalised estimating equation with hormone concentration as the response variable and captive status and season as independent factors. Significant differences are represented by asterisks, while differences between seasons in each group are given by letters.

Table 5: Variable information and results of Generalised Estimating Equation models for male and female brown kiwi tested in 2010. Wild (W) birds came from Ponui Island, while captive (C) birds came from Westshore Wildlife Centre and Rainbow Springs. BR = Breeding season; NBR = Non-breeding season. Testosterone was only tested for wild females as there were not enough samples from captive birds. Rest of test output in Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis..

						Hypothesis Testing				Tests of model effects		
	Dependent variable	Factor 1	Factor 2	Mean	Std. Error	Parameter=compared to	Wald Chi-Square	df	Sig.	Wald Chi-Square	df	Sig.
FEMALES												
	Prolactin	Captive	BR	53.32	9.24	Intercept	221.13	1	<.001	1668.78	1	<.001
			NBR	51.96	6.54	[C vs W=Captive]	5.40	1	**0.020**	2.61	1	0.106
		Wild	BR	53.13	4.56	[brSeason=BR]	11.62	1	**<.001**	6.12	1	0.013
			NBR	28.52	6.43	[C vs W=Captive] * [brSeason=BR]	5.18	1	**0.023**	5.18	1	0.023
	Corticosterone	Captive	BR	94.37	7.29	Intercept	16694.85	1	<.001	8202.378	1	<.001
			NBR	123.51	16.73	[C vs W=Captive]	89.38	1	**<.001**	133.421	1	<.001
		Wild	BR	374.96	42.88	[brSeason=BR]	6.90	1	**0.009**	11.518	1	<.001
			NBR	480.25	22.95	[C vs W=Captive] * [brSeason=BR]	0.02	1	0.887	0.02	1	0.887
	Androstenedione	Captive	BR	2.20	1.39	Intercept	1.90	1	0.168	2.31	1	0.128
			NBR	2.71	1.27	[C vs W=Captive]	6.08	1	**0.014**	2.41	1	0.121
		Wild	BR	1.93	0.74	[brSeason=BR]	23.23	1	**<.001**	1.87	1	0.172
			NBR	0.51	0.25	[C vs W=Captive] * [brSeason=BR]	3.52	1	0.061	3.52	1	0.061
	Testosterone	Wild	BR	1.12	0.39	Intercept	0.25	1	0.619	0.04	1	0.842
			NBR	0.74	0.45	[brSeason=BR]	1.62	1	0.203	1.62	1	0.203
	Oestradiol	Captive	BR	636.56	131.19	Intercept	1384.12	1	<.001	3684.07	1	<.001
			NBR	575.19	102.96	[C vs W=Captive]	11.11	1	**<.001**	13.44	1	<.001
		Wild	BR	322.20	53.74	[brSeason=BR]	0.63	1	0.428	0.99	1	0.32
			NBR	264.12	39.59	[C vs W=Captive] * [brSeason=BR]	0.10	1	0.747	0.10	1	0.747
	Progesterone	Captive	BR	2.13	0.05	Intercept	292.38	1	<.001	304.45	1	<.001
			NBR	2.50	0.51	[C vs W=Captive]	10.13	1	**0.001**	21.78	1	<.001
		Wild	BR	3.53	0.22	[brSeason=BR]	31.07	1	**<.001**	6.74	1	0.009
			NBR	5.14	0.49	[C vs W=Captive] * [brSeason=BR]	1.09	1	0.297	1.09	1	0.297
MALES												
	Prolactin	Captive	BR	116.13	20.10	Intercept	194.94	1	<.001	2384.07	1	<.001
			NBR	65.23	6.15	[C vs W=Captive]	26.65	1	**<.001**	40.91	1	<.001
		Wild	BR	48.83	4.10	[brSeason=BR]	20.45	1	**<.001**	28.47	1	<.001
			NBR	19.59	4.17	[C vs W=Captive] * [brSeason=BR]	1.45	1	0.228	1.45	1	0.228
	Corticosterone	Captive	BR	314.81	45.39	Intercept	7460.13	1	<.001	5586.18	1	<0.01
			NBR	302.19	68.23	[C vs W=Captive]	0.00	1	0.949	0.32	1	0.573
		Wild	BR	380.36	16.05	[brSeason=BR]	8.39	1	**0.004**	1.36	1	0.244
			NBR	297.65	19.63	[C vs W=Captive] * [brSeason=BR]	0.69	1	0.405	0.69	1	0.405
	Testosterone	Captive	BR	3.65	1.45	Intercept	0.05	1	0.821	8.91	1	0.003
			NBR	3.62	1.16	[C vs W=Captive]	5.91	1	**0.015**	8.87	1	0.003
		Wild	BR	1.12	0.25	[brSeason=BR]	0.29	1	0.588	0.16	1	0.694
			NBR	0.90	0.43	[C vs W=Captive] * [brSeason=BR]	0.14	1	0.713	0.14	1	0.713
	Androstenedione	Captive	BR	4.74	1.75	Intercept	0.04	1	0.840	7.16	1	0.007
			NBR	1.38	0.36	[C vs W=Captive]	0.12	1	0.725	5.20	1	0.023
		Wild	BR	1.04	0.32	[brSeason=BR]	0.01	1	0.915	2.22	1	0.136
			NBR	1.12	0.60	[C vs W=Captive] * [brSeason=BR]	2.77	1	0.096	2.77	1	0.096

Discussion

Wild females had similar levels of testosterone in both the breeding and non-breeding season to males, and higher levels of androstenedione than males in the breeding season. This supports the idea that 1) female kiwi show some degree of masculinisation and 2) androstenedione plays a role in the masculinisation of female kiwi. Finding females with higher levels of androstenedione than males, paired with comparable testosterone levels, was what initially implicated androstenedione as a hormone of interest in spotted hyena masculinisation research (Frank, Davidson, & Smith, 1985; Lindeque & Skinner, 1982; Glickman et al., 1987). Furthermore, these higher levels of androstenedione are suspected to be of ovarian origin (Glickman et al., 1993). Given that female kiwi possess two functional ovaries, unlike most birds, it seems likely that female brown kiwi could display a degree of hormonal masculinisation.

Wild females and males had similar levels of prolactin, with both groups having higher levels in the breeding season. Comparatively, in captive birds, males had higher prolactin levels than females, and higher levels in the breeding season. Captive females had similar prolactin levels in both seasons. In species where both parents provide for offspring, prolactin levels are typically similar, while in sole parental species, the parent who is providing the parental care typically has higher prolactin levels (Angelier & Chastel, 2009). In brown kiwi, parental care is known to end after egg-laying for females and after hatching for the male (Calder, 1979; Colbourne et al., 2005; Taborsky & Taborsky, 1995). Jensen et al. (2019) suggest that elevated prolactin levels could ensure parental behaviour to protect the large investment that kiwi eggs represent (15-20% of female body weight; Calder et al., 1978). Alternatively, prolactin may be acting to regulate the growth of follicles resulting in small clutch sizes (Sockman, Schwabl & Sharp, 2000; Jensen et al., 2019) as female kiwi have two functional ovaries but limited body-cavity space, prolactin may be a vector of controlling follicular growth until oviposition of the primary follicle in two egg clutches (Figure 11).

Prolactin

In the breeding season, birds showed an increase in prolactin secretion as they transition from the sexual to the parental phase of their breeding cycle (Sharp, Dawson & Lea, 1998; Sockman et al., 2006). Prolactin levels increase at the onset of laying, and stay elevated during the parental phase, before declining back to pre-laying levels (Hall, 1987; Sharp et al., 1988; Vleck, 1998; Lormée et al., 2000; Deviche, Wingfield & Sharp, 2000; Sockman, Sharp & Schwabl, 2006). Prolactin levels are therefore expected to be at their highest during periods of parental care (Buntin, 1996), and this is reflected in circulating prolactin levels in birds based on parental care styles. For example, altricial birds show patterns of elevated prolactin after hatching during which chicks are fed and heavily guarded, while precocial birds show patterns of moderate prolactin levels during brooding, and a decline shortly after hatching. In species of female-only parental care, plasma prolactin is more elevated than in males, while in species with male-only parental care, plasma prolactin is higher in males than in females during the parental phase (Sharp et al., 1988; Vleck, 1998).

Males provide parental care in the brown kiwi species and as expected, male kiwi in this study were found to have 1) higher mean plasma prolactin levels than females, and 2) higher plasma prolactin levels in the breeding season compared to the non-breeding season, which fits the pattern known for species with male-only parental care.

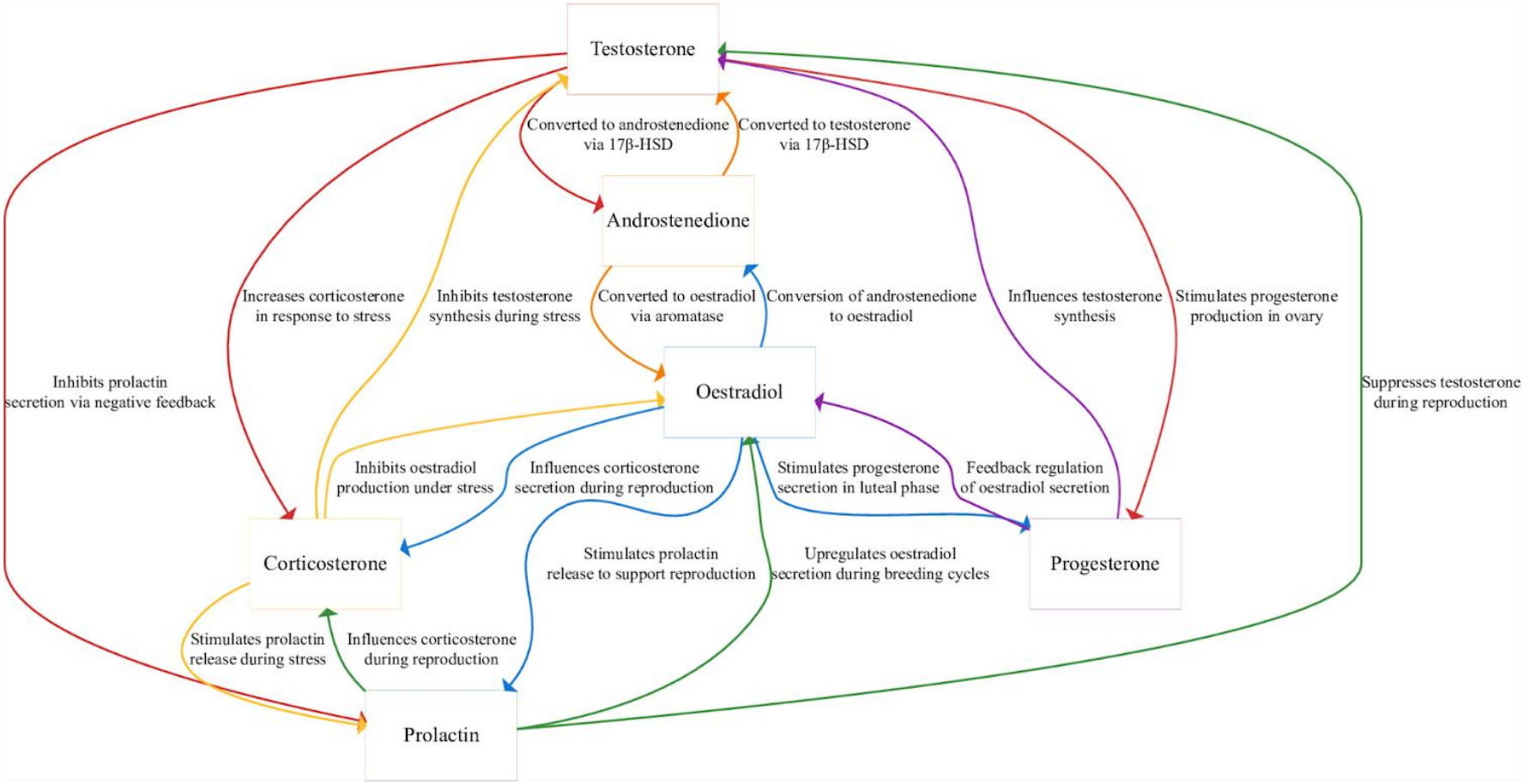


Figure 11:: Interactions of target hormones testosterone, androstenedione, corticosterone, prolactin, oestradiol and progesterone in ratites (created using DiagrammeR in R). Note: "Influences" refers to dynamic interactions that are not a constant increase or decrease, but rather a specific response to physiological conditions.

While prolactin is demonstrated to be essential to the onset of incubation and beginning of the parental phase, elevated prolactin levels do not appear to be solely sufficient to induce incubation in other species under some circumstances (Lehrman, 1963; Silver, 1984; Buntin & Tesch, 1985). Prolactin appears to be unable to stimulate the onset of incubation in turkeys without a preceding increase in both progesterone and oestradiol (El Halawani et al., 1986; El Halawani & Rozenboim, 1993). While prolactin is the main hormone implicated in the initiation of the parental phase, the importance and interaction of other hormones also support the beginning of this phase (El Halawani et al., 1986).

Captive males were found to have significantly higher levels of prolactin than wild males. Captive birds have access to shelter, consistent feeding and water, treatment for parasite loads and lack of predators compared to wild birds. One potential explanation for this difference is that parent birds have been found to have lower prolactin levels associated with poorer body condition (Criscuolo et al., 2006; Jonson et al., 2006; O'Dwyer et al., 2006; Angelier et al., 2007, 2009; Groscolas, Lacroix & Robin, 2008; Spée et al., 2010; Schmid et al., 2013; Riechert, Becker & Chastel, 2014). The lower threshold of body condition appears to have a negative effect on circulating prolactin levels, and long-term fasting or impacted nutritional condition has been confirmed in multiple bird species (Cherel et al., 1994; Criscuolo et al., 2002; Groscolas, Lacroix & Robin, 2008; Spée et al., 2010). Wild males as a result, may have had lower prolactin levels due to more variable body conditions as a result of their environmental challenges, versus captive males who would be expected to have more controlled and stable conditions.

Corticosterone

While males, both captive and wild, did not show a significant difference in corticosterone levels at the time of sampling, captive females had significantly lower corticosterone levels in comparison to wild birds. Seasonal species are associated with the highest glucocorticoid concentrations during their breeding season; however, the regulation of reproductive processes by the hypothalamic-pituitary-adrenal (HPA) axis and increased HPA-axis activity remains poorly understood (Romero, 2002). In a study using male and female European starlings (*Sturnus vulgaris*), two mixed sex groups were housed in either an outdoor aviary (where starlings bred at expected times during the year) or an indoor flight aviary (where reproductive suppression was suspected). Baseline and stress-induced plasma corticosterone concentrations were measured before the separation of the groups and after the experiment.

After 10 days, outdoor birds exhibited an increase in baseline and stress-induced corticosterone levels and progressed into the active breeding stages (including pairing, nest building and egg laying). Indoor birds displayed no change in baseline or stress-induced corticosterone levels and showed few signs of active breeding. It is suggested that the HPA-axis is implicated as a *sex-specific regulatory mechanism* in the transition between early breeding and active breeding phases in wild and seasonal birds, pairing with elements of the hypothalamic-pituitary-gonadal (HPG) axis (Dickens & Bentley, 2014). Given lower corticosterone levels in captive female kiwi, it is possible that they have developed a familiarity with handling, resulting in a stress response that is less pronounced than in wild females.

In breeding kestrels (*Falco sparverius*), mean plasma corticosterone levels are highest before or during the egg laying process (Rehder, Bird & Lague, 1986), a pattern which is also found in other wild (Wingfield & Farner, 1978a, b, 1979; Akesson & Raveling, 1981; Silverin & Wingfield, 1982; Wingfield, 1984) and domestic species (Johnson & van Tienhoven, 1981). Corticosterone is known to initiate the accumulation of egg-laying energy reserves in some species, although it is suspected that high levels of plasma corticosterone may play a role in the initiation of ovulation and/or oviposition or are a result of these processes (Johnson & van Tienhoven, 1981). In the kestrel study, non-breeding females showed no significant change in plasma corticosterone during monitoring (Rehder, Bird & Lague, 1986). Interestingly, wild female kiwi had higher corticosterone levels outside of the breeding season as opposed to within it.

Lower corticosterone levels in bird breeding seasons have been associated with longer breeding seasons (not requiring as much energy reserve mobilisation on a daily basis to sustain increased reproductive intensities), larger body size (lower *mass-specific metabolic rates*; Calder, 1966; Bokony et al., 2009) and tropical species (typically having longer breeding seasons, lower mass specific metabolic rates and more benign temperatures)(Hau et al., 2010). Kiwi on Ponui are in a subtropical climate zone (NIWA, n.d), have larger body sizes for birds (particularly females) and long breeding seasons, which may explain why wild females had higher corticosterone levels outside of the breeding season.

In the related ratite species, the Greater Rhea, males have been found to have higher corticosterone levels within the breeding season, much like what was seen in wild male kiwi. This increase has been associated with an increased likelihood of agonistic encounters

between males when securing mates (Lèche et al., 2014). In Greater Rhea, it has also been suggested that the increased corticosterone levels that occur in both sexes may be a result of increased metabolic activity to meet the increased energetic demands associated with reproductive physiology (Romero, 2002).

Androstenedione

Captive male brown kiwi had the highest levels of androstenedione overall, specifically during the breeding season, but this was not statistically significant. Outside this result, there were no significant differences in androstenedione levels between wild and captive males. In contrast, captive females had higher androstenedione levels compared to wild birds; wild females had higher levels in the breeding season than compared with the non-breeding season.

An example of the role of androstenedione in breeding behaviour is found in ruffs (*Calidris pugnax*). This species has known mutations that have resulted in males having three distinct breeding morphs: Independents (display and defend small territories on *leks*) (van Rhijn, 1991; Widemo, 1998), Satellites (co-display with specific Independents, but do not hold territory) (van Rhijn, 1991; Widemo, 1998), and Faeders (mimic females in order to sneak copulation) (Jukema & Piersma, 2006). Androstenedione concentrations increased alongside testosterone levels in breeding Satellite- and Faeder-morphs, but not in Independent-morphs. During the breeding season, Independents had testosterone concentrations higher than other morphs, while Satellites and Faeders had higher androstenedione concentrations than Independents (Küpper et al., 2016; Loveland et al., 2022, Loveland et al., 2021). Unlike independent morphs, high levels of aggression are not a major feature of Satellite- and Faeder-morph breeding strategies (Giraldo-Deck, Loveland & Goymann, 2024). While there was no statistically significant difference in androstenedione concentrations between the male morphs, one was seen in female ruffs, with a) captive females having higher androstenedione levels and b) wild females having higher levels in the breeding season.

This might also suggest a reproductive benefit to female kiwi that might be associated with elevated androstenedione during breeding. In human studies, androstenedione has been noted to increase during the ovulatory phase of the menstrual cycle (Judd & Yen, 1973). An increase in androstenedione may play a role in preparing female brown kiwi for breeding,

given its importance as a precursor to testosterone (and oestrogens to a lesser degree) in other species.

In domestic chicks (*Gallus gallus domesticus*), yolk androstenedione was detectable in both male and female embryos at day 7 (gonads have just begun to differentiate) and 16 (well-beyond the termination of sexual differentiation) of incubation. Manipulating yolk steroid composition by increasing the androstenedione concentration increased skeletal growth in female, but not male, embryos. Additionally, males showed reduced comb size. Both sexes had reduced *tonic immobility*. This study suggests that sex-dependent enzyme expression and activity may explain the sexually dimorphic response of birds to hormones (Benowitz-Fredericks & Hodge, 2013).

Testosterone

The finding, in this study, that captive-housed male brown kiwi had significantly higher testosterone levels than wild males support the pattern found by Jensen et al. (2019). This effect is possibly linked to the Challenge Hypothesis (Wingfield et al., 1990), which states that males produce higher than necessary (for reproductive purposes) levels of testosterone in response to aggressive male-male interactions. This effect has been specifically linked in species with parental incubation and in species in atypical social and environmental conditions. Further investigation into captive kiwi housing proximity, minimised social structures and lack of mate choice would be required to further test this hypothesis.

While captive males had significantly higher levels of testosterone than wild males, the difference between breeding and non-breeding season levels for each respective group was not statistically significant. In comparison, Potter & Cockrem (1992) found that male kiwi had an annual testosterone cycle, with low levels during non-breeding periods, high levels during the 2-4 months before egg-laying, declining again towards incubation and very low levels in brooding males. Jensen et al. (2019) found a similar pattern, with wild males having elevated testosterone during incubation, and captive males having elevated levels during breeding, incubation and rearing periods.

Evidence suggests that there is coevolution in testosterone levels between adult male and female birds. Peak levels of circulating testosterone in female birds are strongly positively correlated with peak levels of testosterone in males (Møller et al., 2005). Evolutionary analysis from the order of evolutionary events suggests that high levels of male testosterone

precede high levels in females, and that male testosterone levels have influenced the change in female levels over time. Female peak circulating testosterone is relatively lower in species where males have very high levels of testosterone, suggesting that increased testosterone in males is a result of selection by females. Adult female birds from colonially breeding species are known for having particularly high peak testosterone levels compared to those from solitary breeding species (Møller et al., 2005).

This pattern may be due to the consequences on fitness for females as a result of high testosterone, including immunosuppression, and effects of immunosuppression and offspring performance altered by maternal testosterone deposition in eggs. An alternative hypothesis is that higher male testosterone levels are associated with less parental care, and that females can manipulate investment in offspring by boosting development via hormone deposition (Møller et al., 2005). As female kiwi testosterone levels were found to be comparable to wild males, this may be a significant factor.

Oestradiol & Progesterone

While hormones like testosterone are associated with bird aggression and sexual behaviour during the breeding season, bird data suggests that oestradiol may act as a non-genomic steroid regulator of aggression in the non-breeding season. Currently, it is hypothesised that this is because avian non-breeding seasons are usually associated with less abundant food supplies and lower ambient temperatures imposing larger metabolic costs, and shifting to this form of aggression regulation may help reduce these additional metabolic costs (Haller, 1995; Muehlenbein & Watts, 2010) while also maintaining the adaptive value of transient aggression in a species (Heimovics, Trainor & Soma, 2015). Captive female kiwi may show higher levels of oestradiol than wild ones due to the constraints of captivity, meaning that they have more restricted territory options and closer housing with other birds.

If higher levels of oestradiol in captive females are a result of the environment and/or management, further research is recommended to conclusively determine cause and effect. Elevated levels of oestradiol can have unintended effects. For example, a study attempting to increase egg mass in European starlings (*Sturnus vulgaris*) used implants containing oestradiol, as it stimulates the production of yolk precursors by the liver. Treatment of females with oestradiol leads to a decrease in the mass of yolk protein (11%) and lipids (13%) in eggs, suggesting oestradiol may have decreased the uptake of yolk precursors by

stimulating the growth of other follicles. Oestradiol-treated females also displayed accelerated regression of ovaries after a clutch, which may be a result of depressed ovarian yolk precursor uptake. This is also supported by elevated plasma concentrations of yolk precursors in oestradiol-treated females (Christians & Williams, 1999). Further investigation into the effects of elevated oestradiol in brown kiwi would be prudent to ensure that current management is not having an unintended effect on captive breeding efforts.

Progesterone may decrease the incidence of egg fertilisation when elevated. Sperm-storage tubules in laying hens (*Gallus gallus domesticus*) are known to contain progesterone receptors (Yoshimura, Koike & Okamoto, 2000). High progesterone levels are implicated in interfering with sperm release from sperm-storage sites in the infundibulum and/or uterovaginal junction, preventing egg fertilisation (Correa, Adkins-Regan, & Johnson, 2005). This potentially offers an explanation as to why progesterone levels are higher in the non-breeding season for wild female kiwi, and lower during the breeding season.

Summary

- Prolactin levels were higher in wild brown kiwi during the breeding season.
- Captive female brown kiwi had significantly lower corticosterone than wild brown kiwi; wild female brown kiwi had higher corticosterone levels outside the breeding season; wild male brown kiwi had higher levels in the breeding season.
- Captive females have higher androstenedione levels than wild birds; Wild females have higher androstenedione in the breeding season.
- Wild females did not show differences in testosterone levels between the breeding and non-breeding seasons; Female brown kiwi as a whole had testosterone levels compared to wild males.
- Captive males had higher levels of testosterone than wild males; both had similar levels within and outside the breeding season.
- Captive females had higher oestradiol than wild females; there were no differences between the breeding and non-breeding seasons. Captive females had lower progesterone than wild birds; wild females had lower progesterone in the breeding season.

Chapter 3: The dynamics of courting behaviour in brown kiwi: do females run the show?

Introduction

Sexual dimorphism refers to two sexes of the same species differing in phenotype or other features. Males and females can differ in size, colour, body development (appearance of horns, feathers, teeth, fins), scent production, sound production and behaviour. Monomorphism refers to species in which both sexes are identical in appearance; features which are the same between sexes are monomorphic traits. Currently, it is believed that sexual dimorphism in a species results from the difference between the sum of selective pressures (both natural and sexual selection) affecting males and females individually (Ralls & Mesnick, 2009). Since Darwin's 1874 theory that sexual selection drives the differences between sexes, further extensive work has supported this idea. In many species, male size greatly exceeds female size and is highly correlated with intense *male-male competition* for mates (Selander, 2017; Trivers, 1976; Clutton-Brock, Harvey, & Rudder, 1977; Shine, 1989). Reversed sexual dimorphism refers to the opposite of this pattern, where females are driven by *competition* for mates, and as a result are larger than males and/or possess other traits typically associated with males (weaponry, bright colours, scent marking, territoriality, etc). Sexually dimorphic behaviour typically presents in three different forms: courtship, *mating reflexes* (such as copulatory poses and ejaculation) and parental care (Kelley, 1988). The causes of sexually dimorphic behaviour in species are still debated. According to one theory, behavioural sexual dimorphism is a result of *anisogamy*, where the investment in larger gametes by females (compared to males) leads them to invest more in parental care (Schärer et al., 2012). However, it has been argued that instead of focusing on historical investments as a driving source of behaviour dimorphism, the focus may instead be on future payoffs. For example, individual fitness, survival probability and encounter rate of potential mates, rather than anisogamy, result in sexual differences in reproductive decisions such as whether to accept or reject potential mates (Gowaty & Hubbell, 2009; Ah-King & Ahnesjö, 2013).

Sex-role reversal occurs when males or females perform behaviours that are usually carried out by the opposite sex (Vincent et al, 1992). In females, sex-role reversal has been hypothesised to occur when females experience stronger sexual selection than males, and in most species, is associated with polyandry, male-specific mate choice, and male-only paternal

care (Vincent et al., 1992; Kvarnemo & Ahnesio, 1996; Ah-King & Ahnesjö, 2013). Females in sex-role reversed species are also typically masculinised: they have physical traits such as larger body masses than males, larger ornamentation or weapon-like traits (leg or wing spurs), and behavioural traits such as territorial aggression and intense courtship rituals traditionally associated with males (Drea, 2009). It is believed that these reversed traits arise to facilitate *female-female competition* for male mates, and for breeding/nesting territories (Emlin & Oring, 1977; Gwynne, 1991), although it has also been hypothesised that size dimorphism may also be related to fecundity and sex-related selection of traits that affect survival and reproduction of each sex in their specific ecological niches (Blanckenhorn, 2005; Pincheira-Donoso & Hunt, 2017; Lipshutz & Rosvall, 2020). Fecundity (egg size, clutch size and number of clutches) in species where females are larger than males is strongly correlated with female fitness, and there tends to be a trend of positive correlation between female body size and female fecundity (Cassini, 2017).

Sex roles are defined in several different ways throughout behavioural studies: they can be based on reproductive competition, indiscriminate or choice-specific mating behaviour, or parental care. What these definitions all share, though, is that they all combine aspects of behaviour, physiology and morphology into dichotomous categories, resulting in conventional or reversed sex roles. There is also debate about dividing sexually dimorphic behaviour into these roles: if the reversal of sex roles is understood as an exception to a normal pattern, then false impressions of normal may result. It may instead be better to view reversal as the exception to normal within a taxon (Ah-King, 2013).

Sexually dimorphic differences in behaviour are likely driven by both organisational and activational effects of hormones, and the different ratios of sex hormones in females and males of a specific species. Human studies have shown that androgen administration in females leads to an increase in proneness to aggression, sexual arousability and spatial ability performance, while decreasing verbal fluency tasks. The opposite effect was found when males were subjected to androgen deprivation (Goozen et al., 1995). In another example, in African pygmy mice, a species that has both masculinised and typical females (*Mus minutoides*), Saunders et al. (2016) describe female masculinisation as females displaying enhanced aggression, enhanced *anxiogenic responses* to novelty, and enhanced exploratory behaviour that is like that of males.

The testosterone-mediated sexual selection hypothesis posits that sexual selection is based on testosterone-dependent traits. This is because sexually selected male traits are usually associated with testosterone (inducing survival costs of ornamentation/courtship vs increased reproductive success with female preference). However, high testosterone levels are costly in terms of metabolism, immunocompetence, and other energy-dependent processes (Folstad & Karter, 1992; Zuk et al., 1995; Wingfield et al., 2001; Fusani, 2008). This may be applicable in sex-role-reversed species with masculinised females, if female masculinisation is being driven by testosterone (or arguably, other androgens).

Sexual dimorphism and sex role reversal behaviour in Birds

Reverse sexual dimorphism has been observed in several bird species including hawks and vultures (Accipitridae), falcons (Falconidae), sandpipers and snipe (Scolopacidae), phalaropes (Charadriidae), jacanas (Jacanidae), skuas (Sterocoraiidae), boobies (Sulidae), frigate birds (Fregatidae), owls (Strigiformes), cuckoos (Cuclidae), hummingbirds (Trochilidae), manakins (Pipridae) and many ratite species (Swaddle, Karubian & Pruett-Jones, 2000). However, it should be noted that reverse sexual dimorphism does not always mean that the species always has reversed sex roles. Sex-role reversal has been recorded in jacanas (Jacanidae), plovers (Charadriidae), sandpipers (Scolopacidae), coucals (Centropodidae) and buttonquail (Turnicidae) (Eens & Pinxten, 2000) and some species in the ratites (McLennan, 1988). Wattled Jacana (*Jacana jacana*) males provide sole incubation, with females rarely returning to the nest once eggs are laid. Both monogamous and polyandrous mating systems have been observed in different populations and seem to be driven by the local environment. Females solely defend large territories, select the nest site, and perform some initial nest building. However, most nest-building is carried out by the male mate (Osborne & Bourne, 1977). In the Bronze-winged jacana (*Metopidius indicus*), males provided sole incubation and parental care. Monogamy and polyandry were both observed in this species, with polyandrous females having up to 4 males, and heavier females having larger *harems* and producing more clutches per season (Butchart, 2000).

Female African black coucals (*Centropus grillii*) are polyandrous, participating in female-female competition for mates and defending territory from each other. Females defend a larger territory that overlaps with up to four smaller male territories. Males are rarely vocal and provide sole nest-building, incubation and parental care. Females vocalise socially, specifically modifying songs to gauge the threat and performance of intruding females on

their territory and escalating aggression if needed (Geberzahn et al., 2009). Comparatively, in a partially sex-role-reversed species, the Greater Vasa parrot (*Caracopsis vasa*), females incubated and provided parental care. However, they were promiscuous (copulating with at least five males). When females leave the nest, they are fed by multiple males. Females also protect the nest and surrounding territory from other females and produce loud, complex songs from strategic perches near the nest - behaviour which is not present in males (Ekstrom et al., 2007).

Sex-role-reversed females in the class Aves are often associated with female-female competition, female territory defence (and associated female aggression), and female-specific vocal cues (for mate attraction and/or competition). When an avian species is sex-role reversed, the species itself tends to be characterised by some degree of polyandry (although this may not exclusively be the only mating system present), male incubation (often sole) and male parental care (often sole).

One hypothesis on the reversal of sexual dimorphism in birds is that large size is a result of sexual selection on female choice (Mueller, 1990). Greater female social dominance is thought to be the driving cause of reverse sexual size dimorphism. Larger body size, greater dominance, and heightened aggression theoretically allow a female an advantage in establishing a pair bond more quickly than if she had no physical leverage over a male (Mueller, 1990). This would then allow females time to 1) lay more than one egg per clutch with their initial male mate and 2) have the opportunity to lay more than one clutch in a season by moving on to another mate while the initial male incubates.

Sexual dimorphism and sex role reversal behaviour in Ratites

The ostrich displays distinct physical sexual dimorphism in size and plumage, with males being larger and black and white, while females are smaller and greyish brown. Both sexes demonstrate courtship displays and a repertoire of sexual behaviours. Female ostriches incubate during the day (usually a “major” or dominant hen in the group) while male ostriches incubate at night (Handford & Mares, 1985; Blanche et al., 2005). Likewise, male greater rhea are larger than females and compete for access to mates (Clutton-Brock & Vincent, 1991; Codenotti & Alvarez, 2001; Davies, 2002).

In contrast, female emu are larger than males, assume a dominant role in courtship, and fight amongst themselves for access to unpaired males (Davies, 2002). All male ratites possess a

phallus; however, unusually for birds, the female ostrich and emu have a diminutive organ or enlarged clitoris (Fowler, 1991; Machado et al., 2011). Male emu (Buttemer & Dawson, 1989), cassowaries (Fisher, 1968), and Greater rhea (Raikow, 1969; Codenotti & Alvarez, 2001; Fernades & Rebodera, 1998, 2003) incubate and provide sole parental care.

Limited research exists on tinamous, especially pertaining to their mating behaviours. In the Rainforest or Great tinamou (*Tinamous major*), the female performs a display while the male watches. This display includes soft-rolling songs, crouching, neck-stretching, wing-drooping and tail-raising. Females have also been observed feather fluffing during this ritual and making themselves appear much larger to the male. In contrast, the male does not display any of these behaviours, instead standing and watching, while sometimes also flank inspecting, attempting to mount and standing on top of the female. Male tinamou are also known to be the primary care-giving sex (Guo et al., 2024) (Table 6).

Extinct ratites also show evidence of similar patterns. Moa (*Dinornis*) is now known as one of the most notable examples of reversed sexual dimorphism, with males weighing 34-38 kg, and females weighing up to 240 kg (Olsen & Turvey, 2013). Elephant bird (*Aepyornis*) research is still on-going, and reverse sexual size dimorphism has not yet been ruled out (Hansford & Turvey, 2018).

Table 6: : Differences in size, courtship, mating system and parental care in some extant species of ratite.

Common name	Species name	Females larger	Courtship	Mating system	Incubation	Parental Care	References
Great tinamou	*Tinamus major*	Yes	Females neck stretch, sing soft-rolling songs, crouch, wing, droop, tail raise, while males watch and attempt to mount	Polygyandrous	Male only	Male only	Brennan, 2009; Guo et al., 2024
Gray tinamou	*Tinamus tao*	Yes	Chasing, neck-stretching, laying down, exhibiting cloaca, attempted matings	Polygyandrous	Male only	Male only	Solano-Ugalde et al., 2018
Ostrich	*Struthio camelus*	No	Males perform kantling (territorial sitting on the hocks and fanning both swings while swinging the head back and forth), wing swinging and pacing, females cluck	Polygyandrous	Biparental	Biparental	Cooper et al., 2010
Emu	*Dromaius novaehollandiae*	Yes	Males start slow mating dances with snake-like back-and-forth head movements and circling of the female; both sexes strut and circle; females are aggressive and dominant in courtship until incubation begins	Sequential polyandry	Male only	Male only	Patodkar et al., 2009
Southern Cassowary	*Casuarius casuarius johnsonii*	Yes	Males perform intricate rituals, "dancing" around the sitting female, throat swelling, rump scratching and gentle neck preening	Polygyandrous	Male only	Male only	Biggs & Zoo, 2013
Greater Rhea	*Rhea americana*	No	Males approach, "dance" and head bob, females have been observed to form a circle near a male, lower their heads into a "u-shape" and prompt the male to circle and select a female to mount	Polygyandrous	Male only	Male only	Navarro & Martella, 2002; Fernández & Mermoz, 2003

Sexual dimorphism and sex role reversal behaviour in Kiwi

Of the five species of kiwi (*Apteryx*), two have been found to have both sexual dimorphism (larger females) and sex role reversal (male-only incubation): brown kiwi (*A. mantelli*; McLennan 1988) and Little spotted kiwi (*A. owenii*; Colbourne, 2002). In all species, male and female kiwi differ in vocalisation, with males having a repetitive whistle and females a repetitive croak (Heather & Roberston, 2001; Digby, 2013). There are no details of the courtship behaviour of any member of the kiwi group. However, different kiwi species have different mating systems and parental duties (Table 7).

Most of our knowledge of the kiwi group is on the brown kiwi. Research has shown that brown kiwi reproduce as socially monogamous pairs and as groups formed by unrelated males and females (Ziesemann, 2011; Undin & Castro, 2022; Andersson, 1995). Large eggs, long and intensive male-only incubation, and the capacity for females to lay several eggs during the breeding season mean that the number of eggs a male can incubate is less than a female can lay. In addition, brown kiwi are asynchronous breeders, which limits the potential for males to perform mate-guarding while they are incubating and could allow females the opportunity to breed with more than one male.

Female brown kiwi calls exhibit a structural feature consistent with *formants* (Corfield, Gillman, & Parsons, 2008). Formants (characteristic features that resonant in a space, and filter the source of a sound) are currently believed to be an information-sharing element within a call and have been found to accurately communicate body size in Rhesus macaques (*Macaca mulatta*) (Fitch, 1997) and dogs (*Canis familiaris*) (Riede & Fitch, 1999), as well as providing acoustic cues for individual recognition in oilbirds (*Steatornis caripensis*) (Suthers, 1994; Corfield, Gillman, & Parsons, 2008).

Kiwi are nocturnal and inhabit a variety of habitats, but they are now primarily confined to forests. Most of what is known about the species derives from data collected with smart technology radio transmitters that allow clarification of some behaviours. For example, male activity patterns change during incubation as they spend more time sitting in the nest than being active (http://wildtech.co.nz/). This change in activity can be measured and logged into the transmitter. Such information allows scientists to determine the length of incubation and the overall effort a male may put into it.

Table 7: Comparison of kiwi species and mating systems, parental care roles and family groupings.

Common Name	Species	Mating System	Parental Care	Family Groups	Reference
North Island brown kiwi	*Apteryx mantelli*	Monogamous; polyandrous	Male-only	No	Robertson, H.A. 2013 [updated 2022]; Undin & Castro, 2022; Sales, 2005
Great spotted kiwi (Roroa)	*Apteryx haastii*	Monogamous	Biparental	No	Robertson, H.A. 2013 [updated 2022]; Sales, 2005
Little spotted kiwi	*Apteryx owenii*	Monogamous	Male-only	No	Robertson, H.A. 2013 [updated 2022]; Sales, 2005
Rowi	*Apteryx rowi*	Monogamous	Biparental	Yes	Robertson, H.A. 2013 [updated 2022]; Sales, 2005
Tokoeka	*Apteryx australis*	Monogamous	Biparental	Yes	Robertson, H.A. 2013 [updated 2022]; Sales, 2005

Through the use of transmitters and closely following the birds, it is now known that brown kiwi have a variable mating system including monogamy, polyandry, polygyny and *polygynandry* (Undin & Castro, 2022). Direct observations of breeding behaviour have become possible only recently due to the accessibility of camera traps that allow collecting video observations at night with minimal disturbance to the animals (Janisch et al., 2021). Currently, there is a gap in knowledge regarding the role of males and females in courtship and mate guarding, and during nesting. Courtship behaviour includes a set of actions or displays used by animals to attract a mate and facilitate mating. These behaviours include visual, auditory, chemical, or tactile signals, which help individuals assess the suitability of potential partners (Bastock, 2018). Courtship behaviours serve several purposes, such as demonstrating fitness, establishing species identity, reducing aggression between potential mates, and synchronising reproductive readiness. In this study, I utilised videos from camera traps placed at our study site for another species as well camera traps located at kiwi nesting sites to provide information on brown kiwi courtship behaviour for the first time.

Objective: To better understand sex role reversal and sexual dimorphism in kiwi by describing the behaviour of males and females during the breeding season. I hypothesise that

1. Courtship behaviour will be behaviour that is displayed uniquely during the breeding season and will involve both sexes.
2. Males and females will both contribute to courtship.

Prediction 1: It should be possible to video record specific courtship behaviour between kiwi within the breeding season from June to January, with possible peaks during June and October corresponding to the initiation of first and second clutches respectively.

3. I hypothesised that the female kiwi is an example of a masculinised female. If this is correct the following prediction will follow:

***Prediction* 2:** Female kiwi will take the more dominant role in at least some of the courtship behaviour and will be more frequently observed to be the initiator.

Materials and Methods

Study Site and Animals

Ponui Island (-36.86, 175.18) is an 18 km^2 (1,770 ha) island in the Hauraki Gulf located to the east of Auckland and the southeast of Waiheke Island (Figure 12). The study site is approximately 1.2 km^2 (200 ha) at the southern end of the island.

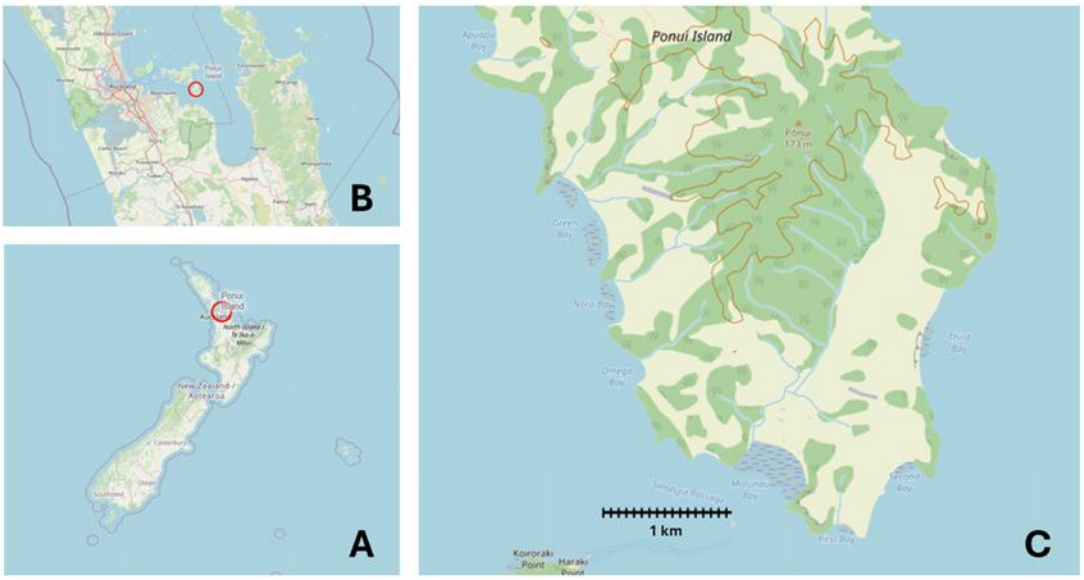


Figure 12: Location of Ponui Island (red circle in A-B) in the Hauraki Gulf off the coast of Aotearoa New Zealand. A = Position of Ponui relative to Aotearoa New Zealand; B = Position of Ponui relative to Auckland and the Hauraki Gulf; C = Southern Ponui where the study site is located.

The study site consists of three forested gullies (Kauri Bush, Red Stoney Hill Gully (RSHG) and Pipe Gully) surrounded by pasture, which all share a similar topography (Figure 13). RSHG and Pipe Gully are located on the eastern side of the study site, facing inland, while Kauri Bush sits on the western side of the main ridgeline and faces out to the Hauraki Gulf. The gullies are covered by broadleaf forests, which meet scrubland at the ridges and include small streams running through each gully that empty into the swampland at their base. The scrublands consist mainly of *Coprosma* spp., kanuka and lancewood, while the broadleaf forest is characterised by taraire (*Beilschmiedia taraire)*, pohutukawa (*Metrosideros excelsa)*, puriri (*Vitex lucens*) and kauri (*Agathis australis*) (Shapiro, 2005).

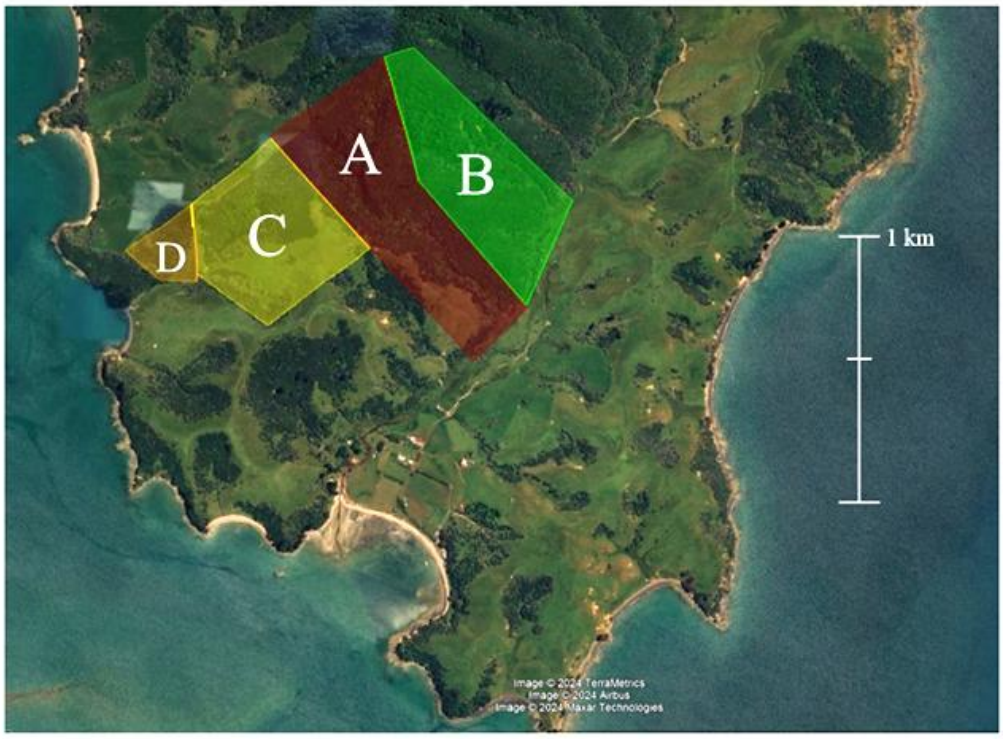


Figure 13: Main study gully sites on Southern Ponui Island. A = Red Stony Hill Gully (RSHG); B = Pipe Gully; C = Kauri; D = Lower Kauri.

The brown kiwi population on this island arose from two translocations in 1964, with the original 14 founding birds coming from Hauturu-o-Toi (Little Barrier Island) in the northern Hauraki Gulf, and the Waipoua Forest in the western Northland region of the North Island of New Zealand (Miles & Castro, 2000; Undin et al., 2021). These founder birds belong to two separate management units currently recognised within *Apteryx mantelli*, representing two genetically separate lineages, Northland and Western. Thus, the Ponui population has a multi-origin background (Undin et al, 2021). The current density of brown kiwi on Ponui Island is approximately 100 birds per km² (Cunningham, Castro, & Alley, 2007).

This population has been part of an ongoing research programme for the last 20 years. From 2004, between 30-50 tagged birds have been followed and their breeding behaviour studied on the island. Birds have a radio transmitter attached to their leg that allows scientists to follow their whereabouts. Each bird is caught twice a year, with data collected on health status, growth, and location and replace transmitters as the battery only lasts around 12 months. Burrows and nests are marked with tape and their GPS location recorded to facilitate collection of data on breeding activity.

Field Work

Videos were collected in two ways:

1. Camera trap grid: Video footage from camera traps placed on a grid over the study site was available for 2014 and 2015 from a study carried out by another student, Kathryn Strang as part of her research on feral cats on the island (Strang, 2018; Figure 14; Appendix 5: Co-ordinates of camera trap locations for kiwi behaviour in 2014 and 2015, provided by Kat Strang, paired with Figure 18, in Chapter 3; Materials and Methods.. These data allowed the observation of kiwi behaviour throughout the year. From January 2014 to August 2015, 6-12 cameras (Bushnell® Trophy Cam) were set up and moved monthly across 27 different locations. From September 2015 to December 2015, 28 trail cameras (12 Bushnell® Trophy Cam and 16 Bushnell® Aggressor) were set in fixed locations in a 500 x 500 m grid, with one camera placed in each 25-ha grid square. GPS locations of the camera sites were taken using a Garmin eTrex® 20x GPS unit (Strang, 2018).

Camera placement was primarily on trails and attached mainly to trees approximately 30 cm above ground. Forest debris was cleared away from the front of the cameras. Each camera was set to record 30 seconds of footage when motion was activated, with an interval (delay between recordings) of one second. Batteries and SD cards were checked and replaced each month (Strang, 2018).

2. Nest Site Cameras: I placed three to four camera traps per site (for a total of 25 Bushnell trophy cameras) at 11 known nest sites of study birds to capture behaviour in the lead up and during the breeding season in 2021 (Table 8; Figure 15). This data allowed me to focus on courtship and sexually specific behaviour occurring at nesting sites.

Each camera was set to record 30 seconds of footage, with an interval (delay between recordings) of one second during nighttime. Camera placement was targeted at the entrance of the nest, or at the nearest flat surface to the entrance, to try to capture behaviour. GPS locations of nest sites were pre-recorded as part of the greater study but were recorded with a Garmin eTrex® 20x GPS unit. Each camera was placed approximately 30 cm off the ground, and surrounding forest debris was cleared for visibility.

Table 8: Locations of camera traps placed at the beginning of the 2021 breeding season by nesting sites known to be used by specific individuals.

Site	Nest co-ordinates	Gully	Birds	Camera	Date placed
Trio	E 179.4344 N 591.5861	Kauri	Louise, Bow & Paul	Cam 31, Cam AL11, Cam B7, Cam SO	1/06/2021
Dario	E 179.4243 N 591.5636	Kauri	Salome & Dario	Cam G1, Cam K6	1/06/2021
Yi Luo	E 179.4772 N 591.5674	RSHG	Yi Luo & Gaia	Cam B1, Cam B6	3/06/2021
Martin	E 179.4489 N 591.5886	RSHG	Martin & Elizabeth	Cam P6, Cam B9	3/06/2021
Nakshatra	E 179.4885 N 591.5701	RSHG	Nakshatra	Cam P3	3/06/2021
Octavia	E 179.4892 N 591.5622	RSHG	Octavia	Cam R5, Cam S1, Cam S2	3/06/2021
Nakshatra & Octavia	E 179 4833 N 591 5662	RSHG	Nakshatra & Octavia	Cam B2, Cam 115	3/06/2021
Ken	E 179 4631 N 591 5997	RSHG	Ken & Betty	Cam B3, Cam R2, Cam S4	3/06/2021
Marc & Jaeden	E 179 4905 N 591 6036	RSHG	Marc & Jaeden	Cam R8, Cam B1, Cam K5	3/06/2021
Anne's Palace	E 179 4668 N 591 5912	RSHG	N/A	Cam K3, Cam P8	3/06/2021
Vincent	E 179 4832.8 N 591 5671	RSHG	Vincent & Laryssa	Cam B5, Cam 116	3/06/2021

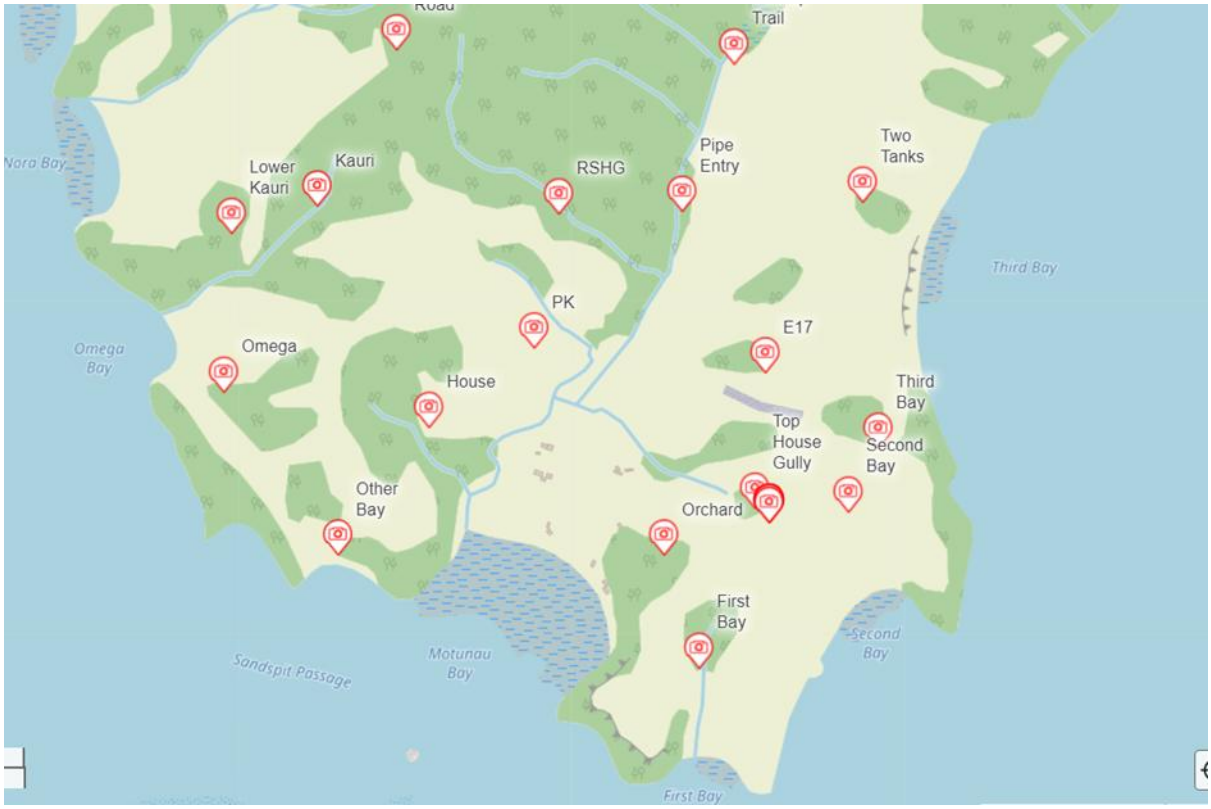


Figure 14: Camera trap grid sites on southern Ponui Island in both 2014 and 2015 (produced with CalTopo, 2024).

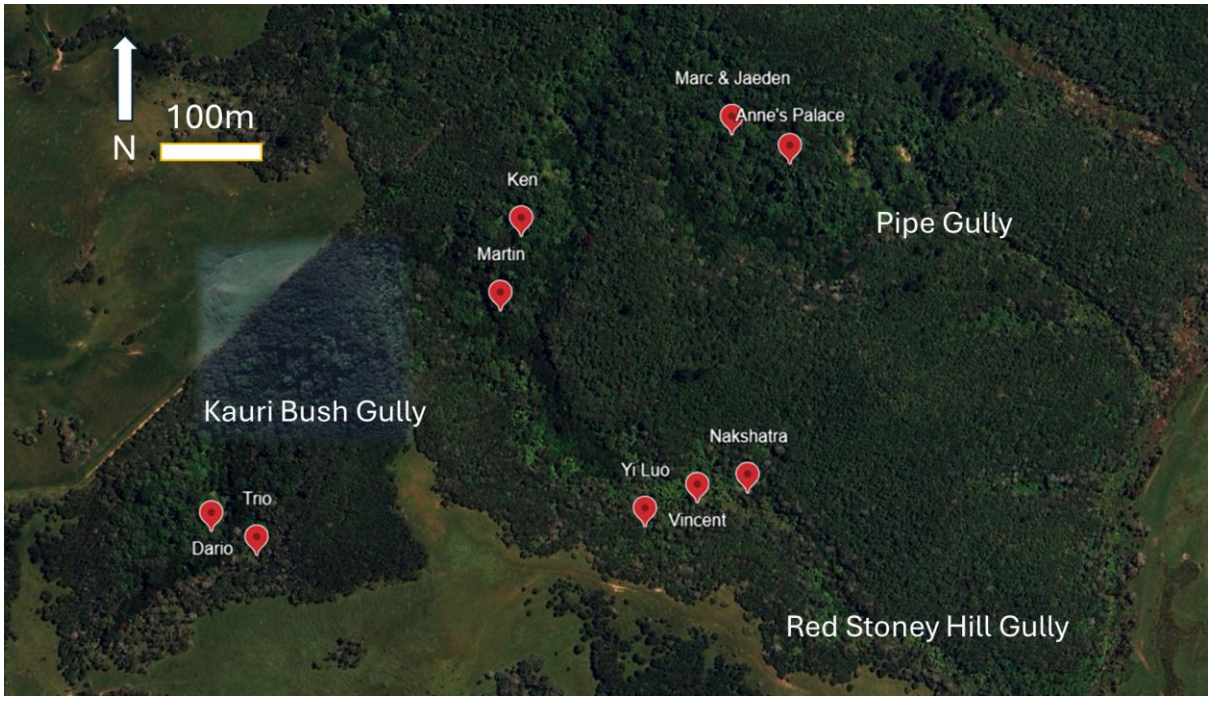


Figure 15: Detail of nest site areas in 2021 (map produced with Google Earth).

Video Storage & Labelling

Camera Trap Grid:

Videos collected as part of Strang's study, from 2014 and 2015, had been previously catalogued as having kiwi or not, and were copied from original files for this study. I named each video in the following format: year/month/day – location taken – time taken – number of birds in the video. If videos were back-to-back, and presumed part of the same interaction across multiple videos, an additional label was added at the end of the name in order (1.0), (2.0), etc.

Nest Site Cameras:

Videos I collected in 2021 were downloaded from SD cards (SanDisk 8GB SDHC Memory Card) onto a Seagate Expansion® 4TB Portable External Drive. I named each video in the same format as the videos from 2014-15.

Video analysis

Each video from 2014, 2015 and 2021 was watched individually. Videos in which brown kiwi were interacting with other individuals on camera, or potentially off camera (including calling or audibly communicating with other birds not visible), were selected for more detailed analyses. Initial summaries of each video included the nest site location (2021), the known individual birds occupying the nest site (2021), the behaviour recorded, and the number of individuals observed in the video (2014-15 and 2021). Each video from camera traps in which kiwi appeared or was heard was transcribed to determine what interactions were occurring. Finally, behaviour was broken down into identifiable sub-behaviours that were seen to be repeated throughout different interactions with different individuals. Behaviours were defined individually. A list was created to include all behaviours seen across all videos, then each video was assessed for the presence or absence of each behaviour. When suspected courtship behaviours were seen, the sequence of each behaviour was noted to determine if there were specific sequences occurring (Appendix 6: Examples of how behaviour was described, categorised and sorted during the video analysis in Chapter 3, Materials and Methods.

For the 2021 videos, behaviour was initially assigned to one basic category: Fighting, chasing, grooming, grunting, calling, parental care or copulation. These basic categories encompassed all the behaviours noted during videos captured at nest sites in 2021. It should

be noted that “fighting” was a category used in earlier analysis for the aggressive interactions between kiwi that later were classified as courtship. Later, this category was renamed because birds do not get injured by the kicking and chasing, they do not disengage despite ample opportunity to do so and rather seem to incite these encounters. Therefore, I redefined the behaviour as courtship, and not fighting, as it would be in terms of territory or resource defence. In total, 33 individual videos were captured across the 2021 breeding season in which birds were interacting with each other. These videos were recorded from June to November, with most interactions occurring in September.

As 2014 to 2015 had a larger number of videos, a different method was used to initially categorise behaviours. Each video was watched and described individually. From these descriptions, individual behaviours were broken down to create a basic ethogram. Initially, a larger basic ethogram was used to break down and assign behaviours. Each video was watched and described individually. From these individual descriptions, behaviours were recorded to create this ethogram. 2014 had 23 different behaviours recorded across the year during interactions. 2015 had 26 different behaviours recorded across the year during interactions. Each of these behaviours was assigned a number in numerical order (i.e. from 1-23, or 1-26). A chart was created with columns for each behaviour. Each video was watched again, and columns were marked “1” if the behaviour was present in the video, or “0” if the behaviour was absent in the video (Appendix 6: Examples of how behaviour was described, categorised and sorted during the video analysis in Chapter 3, Materials and Methods.

Using these behaviour codes also meant that target, longer courtship interactions were noted down individually, and the sequence of behaviours was recorded. This included 6 sequences in 2014 and 17 sequences in 2015. This information was noted down to determine whether the courtship behaviour had a predictable sequence of events every time or was random each time birds interacted (Appendix 6: Examples of how behaviour was described, categorised and sorted during the video analysis in Chapter 3, Materials and Methods.

As part of the greater Ponui study, annual nesting and incubation data collected on birds were provided for this study by Isabel Castro. This information included the number of tracked males that year, the date of the start of incubation, the date of the end of incubation, the duration (days) of incubation and the number of nests. This data was for both the first and second clutch in 2014, 2015 and 2021 (Appendix 7: Examples of incubation data and how it was calculated, in Chapter 3, Materials and Methods.

The percentage of males nesting per month was calculated by dividing the number of males incubating per month by the number of tracked males x 100. This was done for 2014-15 and 2021 for purposes of comparison (Appendix 7: Examples of incubation data and how it was calculated, in Chapter 3, Materials and Methods.

$$\%\ males\ nesting\ per\ month = \frac{no.\ males\ incubating\ per\ month}{no.\ tracked\ males\ that\ year} \times 100$$

Sex Identification

In some cases, sex could be determined in the videos by morphological differences between males and females, including comparative beak length and body size. Transmitter bands also allowed for some individuals to be identified based on the location of the camera and the leg bearing the transmitter (Figure 16).

Figure 16: Examples of visible transmitters on birds at known sites.

The "bill length ratio" (BLR) measurement has been used previously to sex kiwi from video footage. The BLR is the ratio of the distance between the cere and the eye to the bill length. I

took five repeat measurements and averaged them to calculate an individual kiwi's BLR. On Ponui Island, males have previously been found to have a BLR of 3.70 (± 0.22), and females have previously been found to have a BLR of 5.17 (± 0.52) (Cunningham & Castro, 2011). The BLR measurement requires the top of the cere to be visible within a frame of the bird in full lateral view. While females and males do not have overlapping BLR ranges (female BLRs ≥ 4.15), males and juveniles that overlap in bill measurements cannot be distinguished using BLR (Cunningham & Castro, 2011). In this study, stills from videos where birds were in the correct position (Figure 17) were uploaded to Imagej (National Institute of Health, 2024) to allow accurate measurement of the bill tip to cere, and the cere to eye, and to assign sex to birds.

Figure 17: Example of a still from a video in 2015, where a bird was in lateral view and able to be measured. In red are the measurements as determined using the programme Image J.

Sequences of behaviour

In total, 23 courtship sequences were noted in 2014 and 2015 (6 in 2014, 17 in 2015). These 23 sequences consisted of a total of 207 individual behaviours. To see if there was a pattern in these target courtship interactions, I calculated how often one behaviour followed another. For example, how often a kick followed a shove. These sequences were recorded as numbers via the codes given in the basic behaviour ethograms for 2014-15 (Table 9) for ease of

analysis. To calculate the probability of one behaviour following another. I used the following formula:

$$n(x)/207\ x\ 100$$

where n = frequency of behaviour and x = a specific behaviour

There were 22 different patterns of one behaviour following another more than once; if the pattern only occurred once, it was discarded for this analysis. The likelihood of these patterns ranged from 0.97% of occurring (2 occurrences) to 6.76% (14 occurrences). To make a flow chart of the likelihood of one behaviour following another, I only used occurrences 1.45% and above (17 patterns) for visual clarity.

Table 9: Examples of how behaviour sequences were analysed. An example of how one behaviour after another was recorded (count).

	19 (Kicking)
19 to 21	3
19 to 19	14
19 to 14	8
19 to 22	2
19 to 16	6
19 to 9	2

To further analyse patterns of courtship behaviour, the behaviours in the 23 identified sequences were labelled as: S (Start), FG (Feather grab), P (Pivot), RA (Run after), K (Kick), CS (Chest shove), NX (Neck cross) and BB (Beak across back). These codes for representing each differential behaviour observed during the recordings were useful for readability.

A transition matrix was constructed to quantify the likelihood of transitioning from one behaviour to another (Appendix 8: Transition matrix of behaviours recorded in brown kiwi courtship sequences, with the probability of a state transitioning from one behaviour to another,

used for Figure 29 in Chapter 3; Results. The matrix was calculated (using R) based on the frequency of observed transitions between behaviours. Each row of the matrix represented a starting state (the "from" state), and each column represented the ending state (the "to" state). The probabilities were calculated by dividing the number of transitions from a state to another by the total number of transitions from the original state (i.e. P (S -> P) = 0.1739, which meant that the probability of behaviour transitioning from a start to a pivot was 0.1739). This resulted in a probability distribution for each state.

The S (Start) state was treated as a unique case. It occurred once at the beginning of each sequence (when the camera trap began to record) and was observed transitioning to one of four possible states (FG, P, RA or NX) with respective probabilities. These were predefined as follows:

- $P(S \rightarrow FG) = 0.1304$
- $P(S \rightarrow P) = 0.1739$
- $P(S \rightarrow RA) = 0.1739$
- $P(S \rightarrow NX) = 0.1304$

The other states (those that were not S) were allowed to transition to multiple states based on the data, with transition probabilities calculated from the observed frequency of transitions. The state transition diagram was created to visualise the transitions between these states (based on the transition matrix) and was done using the igraph package in R. In the diagram, each node represents a behavioural state, and each edge (the line) between nodes represents the transition from one state to another. Edge weights were calculated to correspond with the previously calculated transition probabilities.

To ensure clarity in the diagram, each node was labelled with the name of the corresponding state (such as P or FG). Each edge was labelled with the probability of transitioning between the two states (the likelihood of state changes). The resulting transition diagram was produced to provide a clear visualisation of behavioural dynamics occurring in courtship sequences over time. The direction and strength of transitions offer insight into the frequency in which specific states follow another, and the possibility of cycles between certain states (which would suggest common behavioural sequences).

I conducted a k-mer analysis to explore the most frequent behaviour transitions in the data (Myers et al., 2022). Behavioural sequences were represented as numerical values where each number corresponds to a specific behaviour. The data consisted of a series of individual behavioural sequences, each containing a varying number of behaviour events. To extract the k-mers, I first defined k = 2, meaning I analysed pairs of consecutive behaviours (i.e., behaviour transitions). For each sequence, k-mers were generated by sliding a window of size 2 over the sequence, creating pairs of adjacent values. For instance, in the sequence [6, 8, 6, 23], the k-mers extracted were "6-8", "8-6", and "6-23". The frequency of each k-mer across all sequences was then computed using the table() function in R. To make the results more interpretable, only the top 10 most frequent k-mers were displayed in the final analysis. The frequency of each k-mer was visualised using a bar plot with the ggplot2 package in R. This analysis allowed us to identify common transitions between specific behaviours and to visualise the relative frequency of these transitions across sequences.

Results

In 2014, 5,157 videos were captured on camera traps, and 20 of these videos captured kiwi behaviour. In 2015, 55,384 videos were captured, and 39 of these videos captured kiwi behaviour. In 2014, 10 out of 20 of these videos captured targeted courtship behaviour; in 2015, 16 out of 39 of these videos captured courtship behaviour.

Describing behaviour

I found several behaviours: some were easily recognisable, such as foraging and calling, but others were initially difficult to categorise as either aggression (fighting/displacement) or courtship (Figure 18; Table 10). Ratite courtship is typically visually aggressive, and kiwi behaviour was reminiscent of interactions seen in similarly related groups. These behaviours were only observed in camera trap grid videos from May to November 2014 and 2015, coinciding with the pre- and breeding season of brown kiwi (Figure 19), which led me to believe they were associated with reproduction vs defence or offence.

Nest Site Cameras 2021

A total of 621 videos were captured at camera trap sites in 2021 across seven known nest sites. Four nests had to be excluded from the analysis; three did not capture any kiwi behaviour, and one was obscured by the environment shortly after placement. Out of these

videos, 64 captured kiwi behaviour at nest sites. Thirty-eight of these videos showed courtship sequences.

Males Nesting

Data on male incubation and nesting (Figure 20) had been collected as part of the greater Ponui study focusing on the population of kiwi in the target gullies. This information was included to give reference to times when birds would be breeding and so would be available to perform courtship behaviours.

Table 10: Ethogram of kiwi behaviours observed in 2014-15.

Behaviour	Sub-behaviour	Description
Environmental interactions	Probing	Touching of objects with beak
	Bird looking around/looking at something specific	Individual bird moving head or body in specific directions to observe something
	General movement/walking	Individual behaviour when a bird is moving with no immediately clear purpose
	Foraging	Visibly pushing beak into soil/leaf litter, visibly eating
Vocalisations	Calling (male)	Recognisable adult male brown kiwi calls
	Calling (female)	Recognisable adult female brown kiwi calls
	Grunting	Grunting/squeaking-like noise made by individual bird (primarily in adult males)
Body care	Preen/scratch/grooming	Individual behaviour when the body and/or feathers are groomed with either the beak or feet
Following (another bird)	Bird following another bird (no touching)	One bird clearly following another bird, with visible space between the birds
	Bird chasing another bird	One bird clearly following another bird at a fast pace
	Bird following another bird (beak on back)	One bird clearly following another bird, bottom of the tip of the beak typically rested on the arch of the back of the bird leading
	One bird possibly following another offscreen	One bird moving in a specific direction, and in a short period of time another bird is moving in that direction
Courtship	Grabbing at feathers	One bird clearly grabbing at the feathers of another bird with the beak, typically the back, thigh or flank feathers
	Pulsing on feet	Individual bird standing on the front of toes, pulsing in an up and down motion, typically seen during intense interaction with other birds
	Pivoting	One bird visible spinning in place, usually 180-degree turn
	Tight circle chases	One bird pursuing another bird in a tight circle in a limited small space
	Necks crossed	Two birds facing chest to chest, necks are crossed as heads are looped over one shoulder of the other bird, and beaks are rested on backs (or often, grabbing at feathers)
	Chest shoving	Two birds face to face using the chest/sternum area to force each other backwards
	Beak movement in front of face	One bird slowly moves its beak in front of the face of the other. Usually from low to high.
	Kicking	One bird striking at another with feet (usually at the chest, thigh or back region)
	Bird falls	A bird falls on its side on the ground, usually from an interaction with another bird
	Shoving bird from side (beak over back)	One bird shoves another from the side, and keeps momentum by placing its head over the other birds back so the beak is looped over the back arch
	Tapping other bird with beak	Brief touching of another bird with a beak

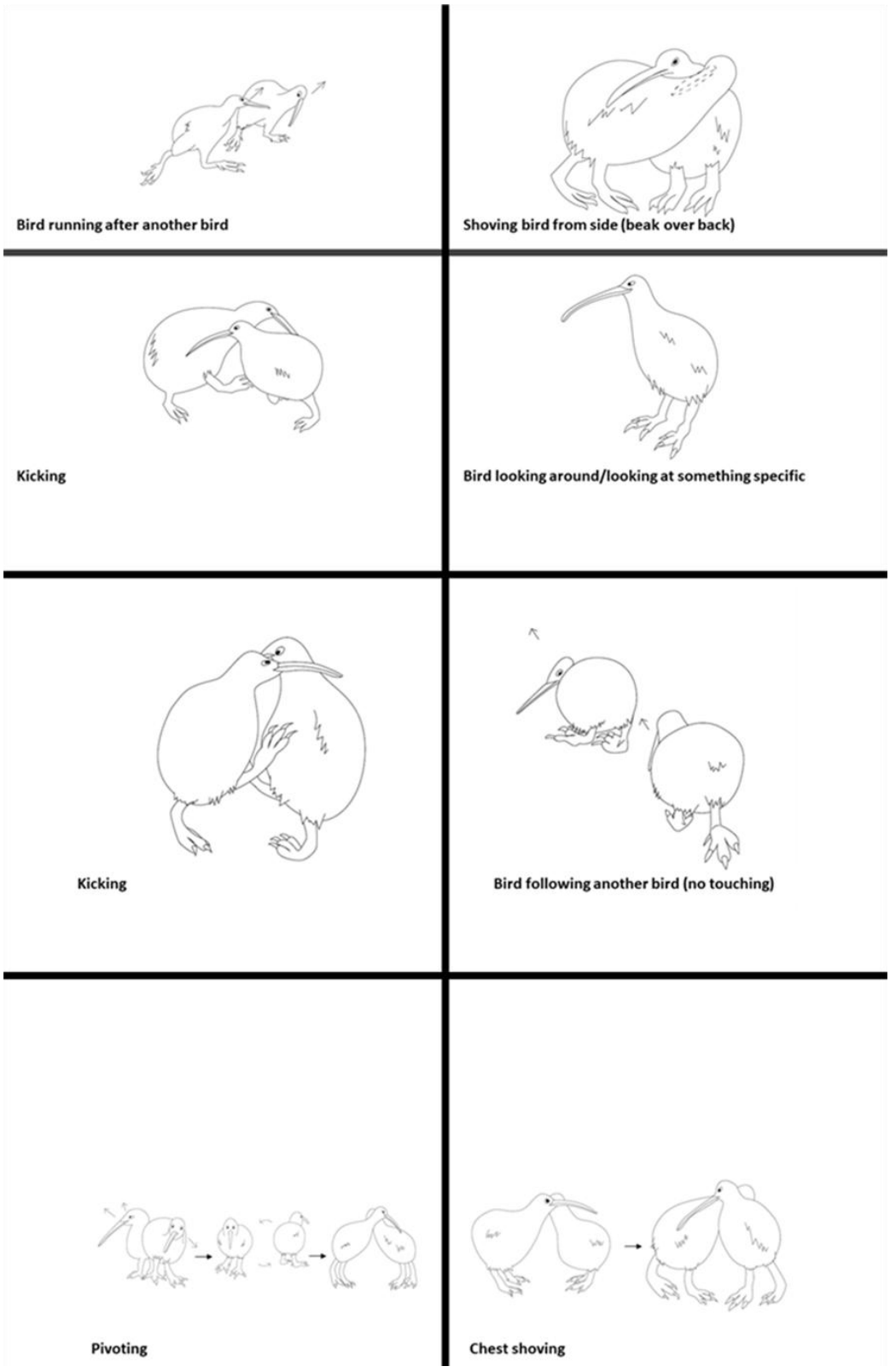


Figure 18: Diagrams of some described interactions between NI brown kiwi. Arrows indicate the direction of movement for each bird (small light arrows) or the direction of a sequence of movements (large bold arrows). Drawings made from video stills of brown kiwi on Ponui Island (Zara Phoenix, 2024).

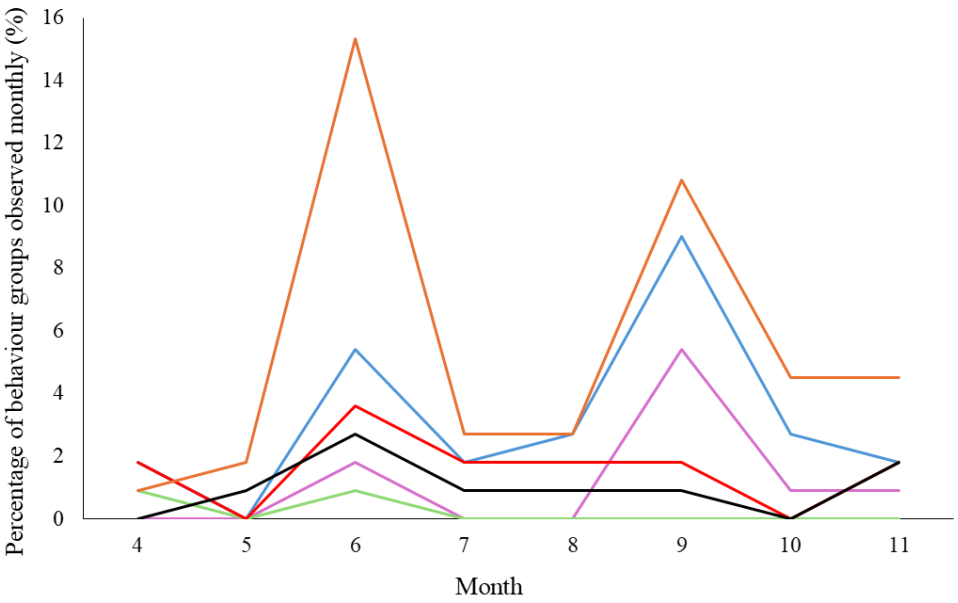


Figure 19:Behaviours observed over the 2014-15 camera trapping period. Orange = social; light blue = vocalizing; red = walking; purple = foraging; black = vigilance; light green = comfort.

Sexing Birds

64 individual birds were noted across the 38 target videos in 2021. Out of these 64, 30 were identified as males, 29 as females, 4 as possibly males, and 1 as possibly a female.

In 2014, 20 individual birds were identified: nine were identified as male and nine were identified as female (45% each of birds observed in that year), while one was identified as a possible male and another one was identified as a possible female (5%). In 2015, 30 individual birds were identified: 10 were identified as male and 10 were identified as female (33%), while five were identified as possible males and another five as possible females (16%). In 2021, 64 individual birds were identified: 30 were identified as male (47%), 29 were identified as female (45%), four were possible females (6%), and one was a possible male (1.5%). In 2014, we observed males instigating chases seven times and contact three times, while in 2015, males instigated chases three times and contact twice, and in 2021, males instigated chases 12 times and contact 13 times. Comparatively, females were not seen instigating chases nor contact in the 2014 videos but were seen instigating chases three times and contact twice in 2015 and 29 chases and two contacts in 2021 (Figure 21).

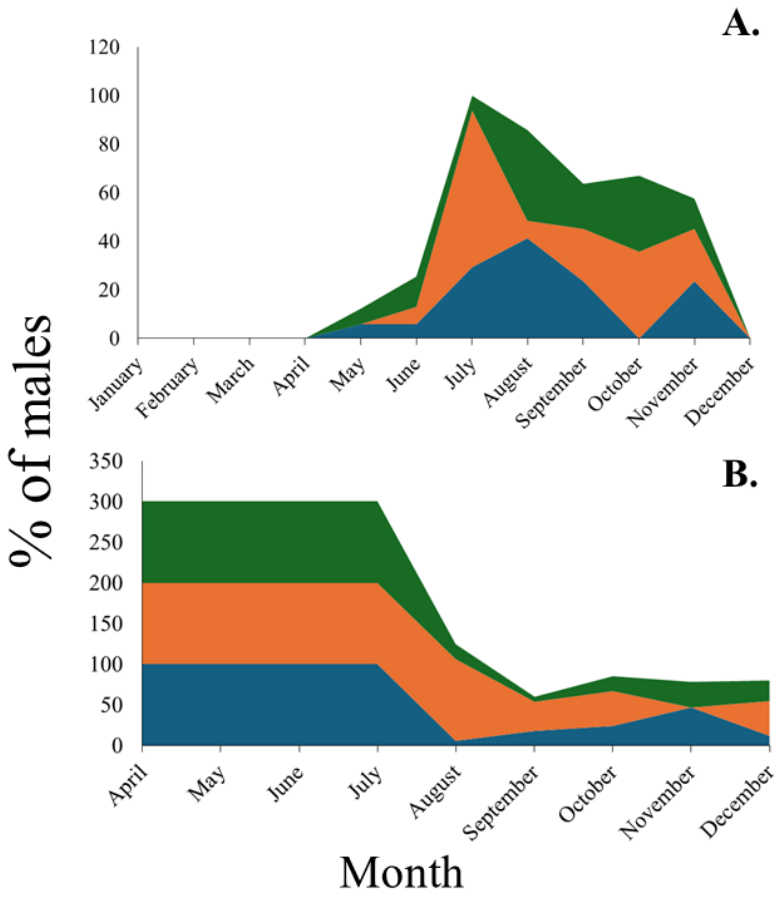


Figure 20: Nesting data on tracked male kiwi on Ponui island during 2014, 2015 and 2021. A = percentage of males starting nests monthly; B = percentage of males available for courtship monthly. Blue = 2014; orange= 2015; green = 2021.

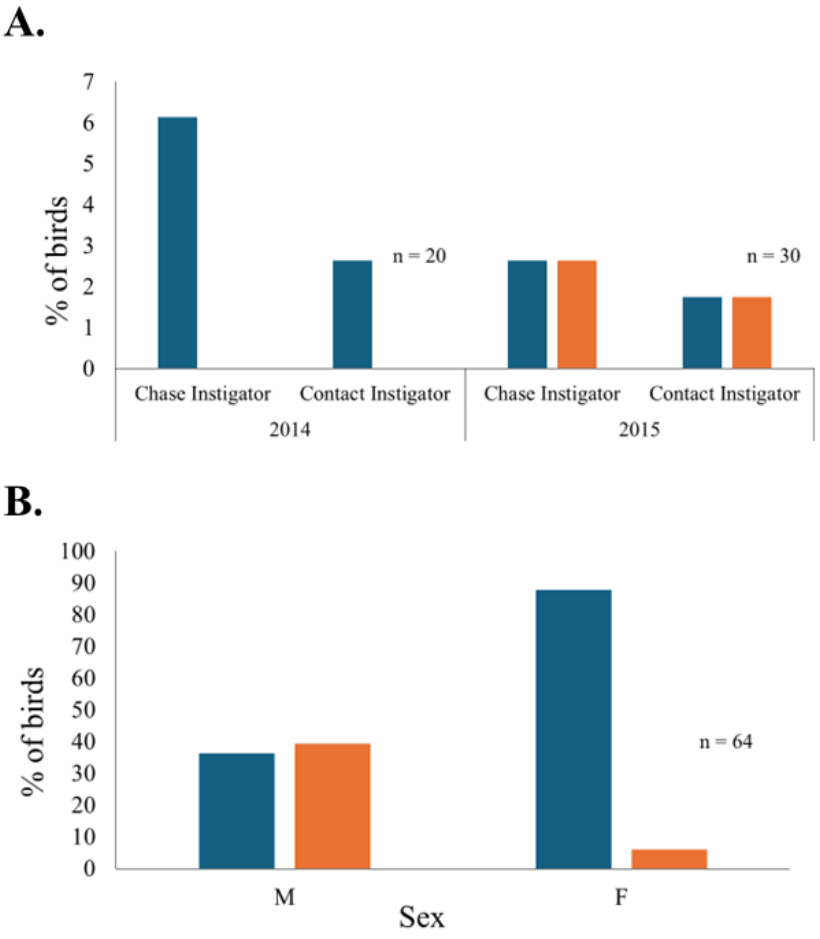


Figure 21: Chase and contact behaviours as identified by sex. A = sex of chase and contact instigators in 2014 and 2015; blue = male; orange = female. B = sex of chase and contact instigators in 2021.

Behaviour sequences

The transition matrix (Appendix 8: Transition matrix of behaviours recorded in brown kiwi courtship sequences, with the probability of a state transitioning from one behaviour to another, used for Figure 29 in Chapter 3; Results. describes the probabilities of moving from one behaviour state to another. Overall, the transition matrix was composed of low probabilities of transitioning for all behaviours. When birds were first seen on camera, they were most often engaging in running after or pivoting behaviour (17.39%). When a bird kicked another, this behaviour was most likely to be followed by another kick (6.76%). The start state (S) only has one transition but begins every sequence to represent the beginning of the recorded behaviour. The (S) state was found to transition into one of four other states in our videos (FG, P, RA or NX). P (Pivot) or RA (Run After) were most likely to follow that start, with a transition probability of 0.1739, followed by RA (Run After) and FG (Feather Grab) with a transition probability of 0.1304.

The most frequent transitions observed were kick to kick (0.0676; 6.76%), followed by pivot to kick (0.0386; 3.86%), neck cross to chest shove (0.0338; 3.38%), and pivot to run after (0.0338; 3.38%) (Figure 22). This suggests that kicking is an important repetitive component in these interactions, as well as pivoting. A less common transition was kick to beak over back (0.0145; 1.45%) and feather grab to pivot (0.0145; 1.45%). Kicking and pivoting appear to be clustered behaviours and important components of these contact interactions between kiwi. This may be due to the fact that kicking dislodges a bird and forces it to pivot to continue the interaction or may be because pivoting allows a bird to avoid being kicked. Some behaviours, such as FG, RA and P, were more interconnected than others, such as more isolated behaviours like S and BB (Figure 22). NX (Neck cross) and BB (Beak over back) showed a tendency to transition to multiple other states, indicating when these behaviours are observed, it is flexible which behaviour will follow them (Figure 22).

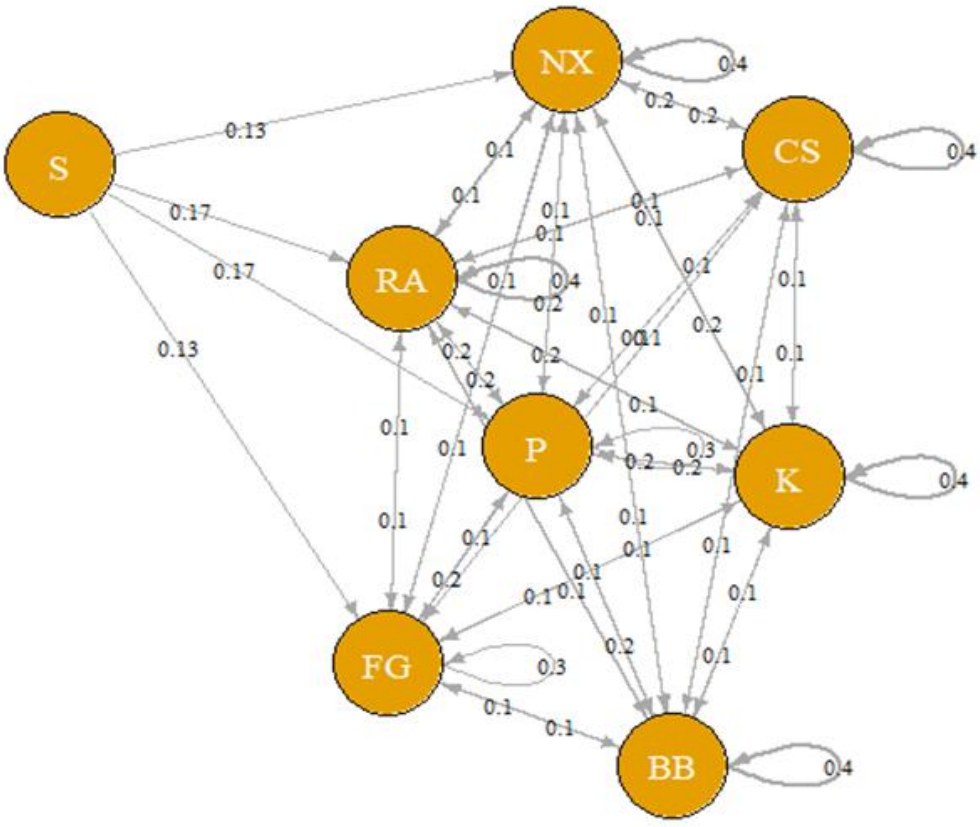


Figure 22: Probabilities of one behaviour transitioning to another. Each node = different behavioural states; edges (lines) represent the likelihood of transitioning between states. Each edge is labelled with the probability of the transition (edge width is proportional to the transition probability). S = start; FG = feather grab; P = pivot; RA = run after; K = kick; CS = chest shove; NX = neck cross; BB = beak over back.

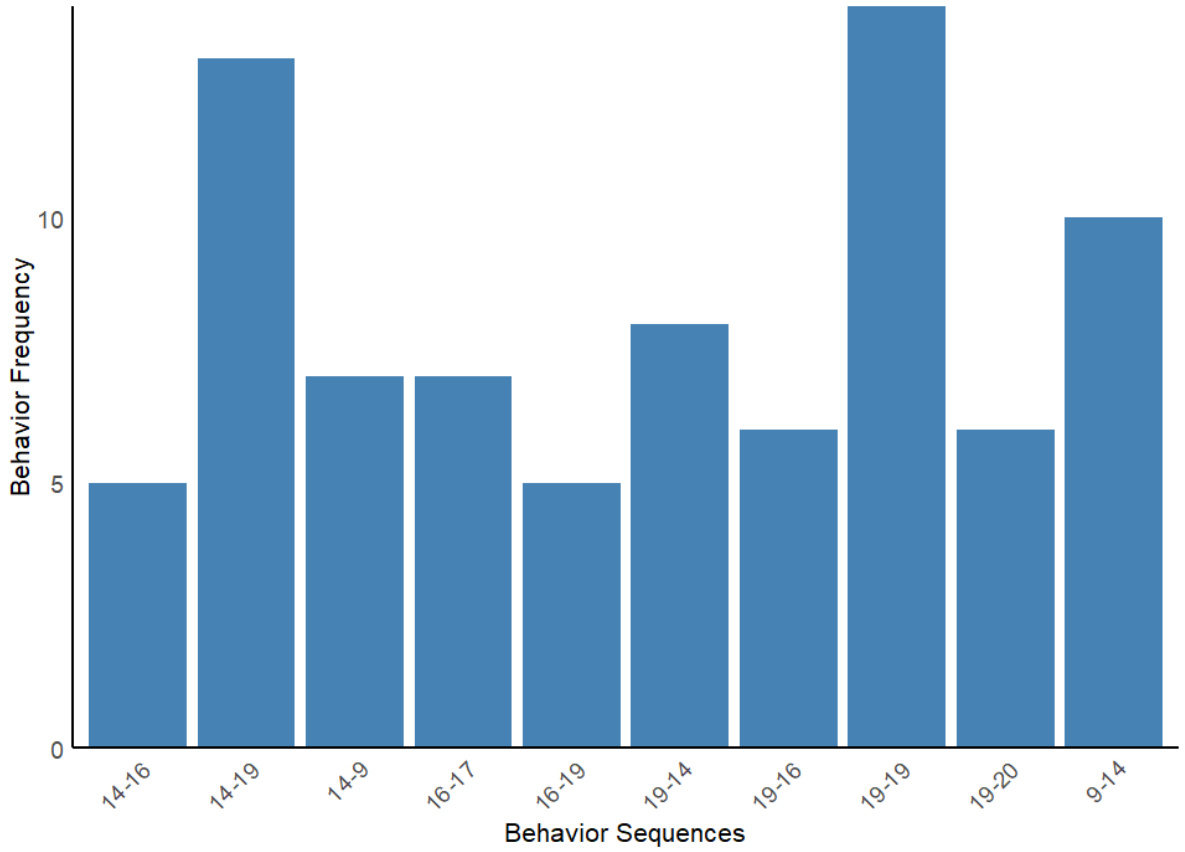


Figure 23: K-mer analysis of the top 10 most frequent behaviour sequences observed. 14-16 = pivot into a neck cross; 14-19 = pivot into a kick; 14-9 = pivot into a run after; 16-17 = neck cross into a chest shove; 16-19 = neck cross into a kick; 19-14 = kick into a pivot; 19-16 = kick into a neck cross; 19-19 = kick into a kick; 19-20 = kick into a fall; 19-14 = kick into a pivot.

The k-mer analysis revealed a range of behaviour transitions across the sequences. The analysis focused on pairs of consecutive behaviours (k=2), and the 10 most frequent k-mers were identified and plotted (Figure 23).

Among the top 10 k-mers, the most frequent transitions involved specific behaviour pairs that occurred consistently across the sequences. For example, the k-mer "14-19" appeared most frequently, indicating that the transition between behaviours "14" and "19" was common. Other frequent k-mers included "16-17" and "9-14", highlighting additional commonly observed transitions.

Discussion

The aim of this videographic study and analysis was to better illustrate the dynamics of sex role reversal and sexual dimorphism found in the brown kiwi. Behaviours that have been previously suspected to be courtship (such as chasing and contact in forms of kicking and shoving) were confirmed to be related to kiwi reproduction as they 1) were found only in the breeding season and 2) were inversely proportional to male nesting rates in the same months. This suggests that this behaviour tapered off when males were in nests and not available for breeding, and spiked when males were available for copulation, strongly supporting my belief that these behaviours are courtship in kiwi.

The Female Masculinisation Hypothesis posits that females in species with reversed sexual dimorphism show behavioural aggression and dominance in social behaviour. Therefore, this courtship behaviour was considered the most important to be identified and described from observations of kiwi interactions. I found that kiwi courtship behaviour was an elaborate and ritualistic display between both male and female, and was visually aggressive, as is found in other species in the ratite group. While the results suggested that males overwhelmingly initiated both chase and contact behaviours during these courtship rituals, females initiated far more chases in 2021, which suggests that both sexes are capable of taking an active role in initiating and/or maintaining these courtship interactions.

To place the behaviours observed into further behavioural context, ratites are known for ritualistic courtship behaviour that may appear initially aggressive and/or territorial (Figure 24). Raikow (1969) described the rhea “Call Display”, in which males assume a call posture, call, and then perform a running display which consists of breaking into a run while alternating flipping wings (https://youtu.be/HWEi7PmomS8). Often during this run, males change directions suddenly, and this behaviour is often compared to the defensive run that the rhea performs. The male will run towards the female from behind, or from the side, at which point the female runs off with the male following. Portielje (1925) has also described the female responding by running at the male. The female response is described as similar to the defence posture, and the courtship ritual initially appears more like an aggressive chase than a mating display. After this interaction, the male does not attempt to copulate and shows no further interest in the female. Similarly, tinamous females have been observed calling to and displaying for males, including making themselves larger by feather fluffing. Males, in contrast, do not engage except to inspect the female closer or try to mount her (Guo et al.,

2024), a possible example of sex-role reversal (https://youtu.be/zwLmItpzrYw; https://youtu.be/KbnkVDBAnwQ)

Greater Rhea have also been reported to exhibit *juvenile play*. Juvenile play is typically characterised as non-serious use of behaviours that are agonistic and species-typical: in Rhea, this looks like pecking each other and wrestling. This reported behaviour is also similar to behaviour reported in courtship in both rhea and kiwi, and provides the basis that these interactions do not necessarily equate aggression or intent to harm. Play behaviour noted in rhea wrestling included feet grappling, beak wrestling and jumping on each other. It has also been noted that play fighting requires a level of social tolerance, self-handicapping behaviours for the sake of the game, taking turns and role-reversal. If juvenile rhea develop these skills, it is not unlikely that they could be applicable in adult life (Zeiträg, Jensen & Osvath, 2023).

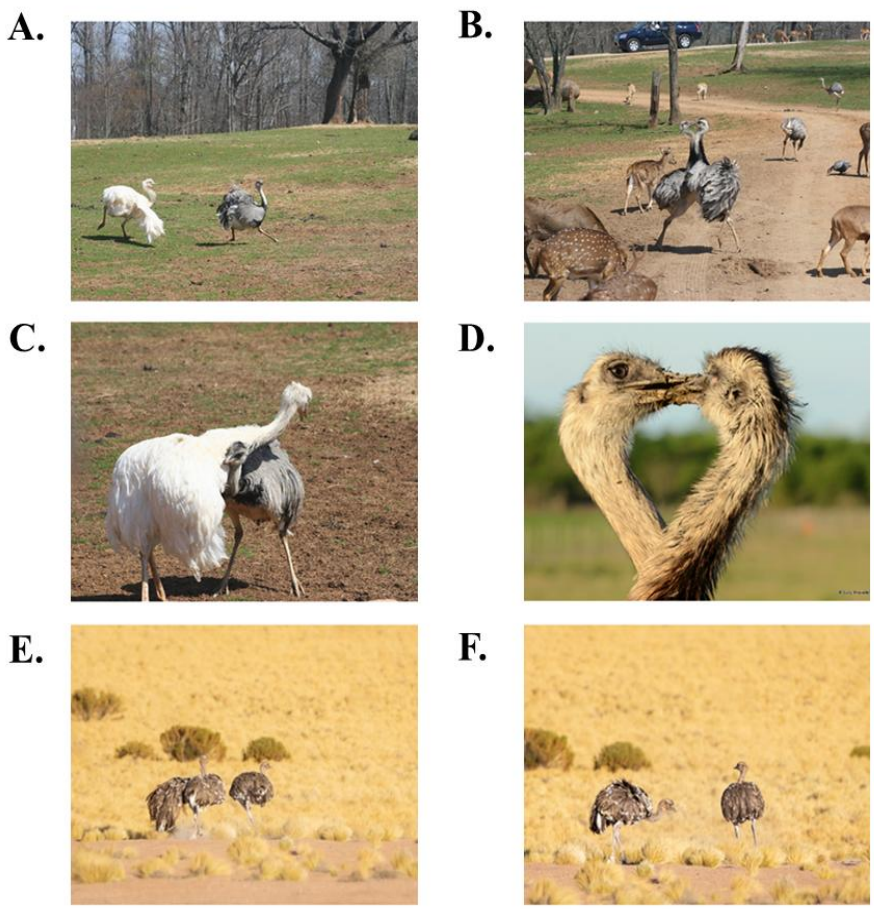


Figure 24: Examples of Rhea behaviour. A = Chase between two Greater Rhea (Higgins, 2009); B = Greater Rhea engaging in chest shoving and kicking (Higgins, 2009); B = Greater Rhea engaging in chest shoving and neck crossing (Higgins, 2009); D = Greater Rhea engaging in neck crossing with grabbing (Prevedel, 2012); E = Puna Rhea (Rhea pennata spp. tarapacensis) chasing each other (Picar, 2022a); F = Puna Rhea pausing during chases to allow each other to catch up (Picar, 2022b).

Only 23 sequences were recorded in 2014 and 2015, which meant there were limited transitions between states. Transition matrices rely on the observed frequencies of transition; when sample sizes are small, the probabilities for each transition are low because there is less data available to support each transition. Even when a behaviour transition occurs multiple times, the probability of that transition is calculated as the frequency of that transition divided by the total possible transitions, which might result in smaller probabilities. As there were 22 original behaviour states recorded from these sequences, there was also a large number of possible transitions, which made the probability of transitioning to any given state from a specific starting state naturally lower because there are many other potential states to transition to. Even though these behaviours were reduced to the eight behaviours that transitioned more frequently, a dilution effect likely occurred, where the transitions are spread across many states, resulting in each transition having a lower probability.

Despite this small sample size, there were clearly frequent behaviours that make up kiwi courtship interactions. As shown by the transition state diagram, kiwi courtship interactions are primarily composed of feather grabbing, pivoting, running after another bird, kicking, chest shoving, neck crossing and locking the beak over the back.

Kiwi displays fit the pattern of being similar to defensive displays and having a chase component that is a significant part of interactions between birds. Kiwi displays often include unusually close contact (chasing or following with beak on the back of the bird in front, necks interlocked, kicking) even among descriptions of ratite interactions. This may be due to the fact that, unlike other ratites, kiwi are nocturnal, have small eyes and visual fields, and have reduced visual centres compared to those processing olfactory and tactile information (Martin et al., 2007). Close contact with another bird allows for the courtship display to continue without losing the other bird from the field of sight, especially in areas with dense bush cover. As well as physical contact, there may be an olfactory component to this behaviour. Both preen oil (from the *uropygial gland*), and feather oils are suspected to play a role in chemical communication in birds (Alves Soares et al., 2024), and repeated, prolonged physical contact would allow birds to read/share these signals. Bill-wiping has been a behaviour implicated in scent communication, as it may allow for the release of waxy preen

Summary

- Kiwi utilise ratite-typical courtship behaviour that falls under chasing and contact.
- This courtship behaviour is found in kiwi during the breeding season and decreases in frequency throughout the mating season when less mates are available as males are incubating on nests.
- Kicking, chest shoving, crossing necks and running after each other are key components that make up courtship behaviour.
- Females are not the sole instigators of courtship behaviour; initiation seems to be shared between sexes.

Link to Google Drive folder where four videos are available as examples of interactions between brown kiwi seen in this study:
https://drive.google.com/drive/folders/1FYOuU83TYTYLWZncQsNp8Cca34eBdesb

Chapter 4: Future Directions

Why Kiwi?

Birds have long been assumed to follow the usual vertebrate paradigm of sexual differentiation and resulting *sexual phenotypes*. The standard model expected across vertebrates is that androgens and oestrogens are synthesised by both embryonic and post-natal gonads, which then masculinise or feminise the brain and other tissues. Embryonic gonads synthesise and secrete gonadal sex hormones according to genetic sex, which then direct the development of sexually dimorphic traits (Major & Smith, 2016).

Yet, in vertebrates, there is also the phenomenon of sex reversal, frequently defined as "a discordance between chromosomal sex and somatic sex" (Major & Smith, 2016). This is widely acknowledged in *teleost fish*, a group susceptible to sex reversals triggered by oestrogens and androgens (Baroiller et al., 2016). Amphibians also show this plasticity, influenced by oestrogens and androgens (Nakamura, 2013). In some reptiles, alteration of egg incubation temperatures can lead to sex reversals and discordance between *gonadal* and sexual genotypes (Georges et al., 2016; Major & Smith, 2016).

There is a wide range of variation in degrees of sexual dimorphism. In some species, sexes are indistinguishable without genetic information, and in others, sexes may be dramatically different in growth, endocrine activity, life expectancy, behaviour and/or physical appearance. A wide range of mechanisms have been proposed to explain these dimorphisms, and what underlies males and females of the same species to appear to be so drastically different. Multiple theories have been proposed, such as sexual selection, including female choice, contest competition and/or sperm competition. Another suggestion is that ecological differences between sexes drive sexual dimorphism, such as the New Zealand huia (*Neomorpha acutirostris*) with sexually dimorphic bill types based on sex-specific foraging (Andersson, 1994). It has also been suggested that sexual dimorphism could be driven by patterns that are important between females and young (such as larger females being better mothers, or urogenital pigment patterns highlighting the mammary glands for young to find) (Ralls, 1976; Ralls & Mesnick, 2009).

Based on studies (primarily in terrestrial mammals), there is a positive correlation generally assumed between the degree of sexual dimorphism in a species and the deviation of the

breeding system from monogamy. In polygynous species, where male competition for females is extreme, males are expected to exhibit traits such as larger sizes, larger teeth or claws, brighter colours, or other traits that allow some males greater access to females than other males (Ralls & Mesnick, 2009). Lack of sexual size dimorphism does not necessarily mean a lack of male-male competition, as different forms of competition between males can include a range of characteristics, including behaviour, vocalisation or agility. Traditionally, the importance of male-male competition has been emphasised to explain the evolution of exaggerated physical and behavioural traits in males. Data suggest that female choice also plays a critical role in birds. Features that appear advantageous in contests between males may also be used by females to select mates or may be utilised to control and/or intimidate females (Ralls & Mesnick, 2009).

Birds are well known for some of the most visually striking sexual dimorphisms in vertebrates. Males are characterised for their bright, and often gaudy, plumage, elaborate courtship dances and rituals, compared to females that are usually more cryptically coloured and behaved (Major & Smith, 2016).

Kiwi are an unusual bird across the five species (and 10 taxa). Their sexual dimorphism is notable: females are 20-30% heavier than males, and their bills are 20-30% longer than males. Males are sole incubators in the Little Spotted Kiwi and North Island brown kiwi (Colbourne et al., 2020), while the other three species share incubation responsibilities. Kiwi are also the most vocal of the ratite species (Davies, 2002). Males produce high-frequency notes that are shrill and piercing. Females produce broadband, low-frequency guttural and hoarse notes (Castro, 2011; Digby, Bell, Teal, 2013; Dent & Molles, 2015).

Hormones

Why androstenedione? Revisiting the Androstenedione Model

Our understanding of mammalian sexual differentiation dates back to work by Jost in the 1940s-50s. Jost (1953; 1972) concluded that male urogenital development arises from the secretion of androgens by foetal testes during critical embryonic development periods. Concomitantly, it was discovered that anti-Mullerian hormone (AMH), a glycoprotein hormone important in fertility regulation and sexual differentiation, is secreted by the foetal testes and is responsible for the regression of the Mullerian duct system, preventing the development of the female reproductive system. This process explains how an

undifferentiated gonad develops into either the male or female reproductive system. The Jost scheme became the accepted model for the development of masculine genitalia in mammals, followed by recognition that the conversion of testosterone to dihydrotestosterone is usually required for male genital development. In contrast, it was understood that the female urogenital system arose through an unidentified process, in the absence of testicular androgens and/or anti-Mullerian hormone (Wilson, George & Griffin, 1981).

Following the publication of the *Jost model*, further experimentation confirmed that exogenous androgen treatment during development often masculinised the external female genitalia in mammals. The spotted hyena (*Crocuta crocuta*) has become one of the primary models for understanding the phenomena of female masculinisation and investigating circulating androgens, as the normal female hyena urogenital system is nearly indistinguishable from an that of an adult male hyena, as well as that of an androgenized female dog (Beach, 1983).

Due to the presence of excess *interstitial tissue* and relatively few follicles, the hyena ovary was initially implicated in high androgen production, leading to masculinised physiology and anatomy (Matthews, 1939). This was later affirmed by demonstration that the foetal ovary did produce testosterone prenatally (Lindeque & Skinner, 1982). Further research however, found that foetal female hyenas had a large genital tubercle with a central urethra extending to the tip of the organ, the beginnings of pseudopenis development, at gestational day 30, before the foetal ovary or foetal adrenal gland had differentiated (at gestational day 45) (Licht et al., 1998). This suggested that the foetal ovary and/or adrenal gland could not be the source of androgen production driving the masculinisation of female hyena urogenital systems. Foetal hyena testes were found to express the enzymes required to synthesise androgens at 30 days of gestation (Browne et al., 2006), further suggesting that androgen production during early development could not explain the urogenital morphology of female hyena.

The androstenedione hypothesis arose as a result of the elimination of the foetal ovary or adrenal gland as a source of masculinisation in the female hyena. This is largely due to the fact that androstenedione (primarily from the ovaries) had been identified as the primary circulating androgen in adult female hyena (Lindeque & Skinner, 1982; Glickman et al., 1987; Glickman et al., 1992). This hypothesis was supported further by the discovery that the spotted hyena placenta has abundant 17β-hydroxysteroid dehydrogenase activity *in vitro*, an enzyme capable of converting androstenedione to testosterone. Further *in vivo* investigations

supported these results, demonstrating that ovarian and placental testosterone can be transferred to the foetus via the umbilical vein, a process which could account for the masculinisation of female hyenas *in utero* (Licht, 1992; Yalcinkaya et al., 1993).

The androstenedione hypothesis has also been supported in humans. Two clinical cases describe genetic women born with masculinised external genitalia, which was correlated with a mutation that activated aromatase production in the human placenta (Shozu et al., 1991; Conte et al., 1994). As a result, *in utero* maternal androgens could not be converted to oestrogens, which would normally pass between the maternal and foetal circulation. Instead, androgens were passed to the developing foetus via umbilical circulation (Siiteri & Seron-Ferre, 1978).

Returning to the hyena, in captivity 60% of first births are stillborn as a result of prolonged retention within the elongated birth canal. In first-time mothers, the meatus has to tear to deliver (Frankand & Glickman, 1994). Foetal retention within the clitoris can result in severe anoxia as the hyena has a short umbilical cord that requires foetal-placental unit detachment early in the process (Flick & Glickman, 1994). Females exposed to anti-androgen treatments *in utero* resulted in easier first births; treated dams had four pregnancies producing seven cubs, with a 0% mortality rate from clitoral delivery. In contrast, control dams had 12 pregnancies producing 20 cubs, with a 60% mortality rate (11 stillbirths and one death within 48 hours) (Drea et al., 2002).

Administration of anti-androgens further tested the hyena androstenedione theory (Drea et al., 1998; Neumann, Elger & Steinbeck, 1970; Neri, 1977). In males, *in utero* anti-androgen exposure completely feminised the external and internal phallus to resemble the pseudopenis (an enlarged, modified clitoris) of the female hyena (Drea et al., 1998; Drea et al., 2002; Cunha et al., 2005). Anti-androgen treatment also feminised the *perineum muscles* of neonatal males and reduced the sex-biased difference in volume and number of motoneurons controlling defecation, urination and ejaculation (Forger et al., 1996).

In females, the exposure to anti-androgens resulted in the development of the clitoris in a way that was described as “feminine”. This resulted in an increased size and elasticity of the urogenital meatus, a decrease in clitoral length, and an increase in glans diameter in adult females (Drea et al., 1998; Drea et al., 2002). Anti-androgen-exposed females also had higher concentrations of plasma oestrogen (Place et al., 2002).

Multiple authors have suggested that androgenisation *in utero* provides the direct benefit of aggressiveness and dominance in females, in this case, leading to first access to a highly competitive resource (food). Adult female hyenas and their sub-adult female offspring completely dominate adult males when feeding on carcasses (Kruuk, 1972; Frank, 1986). There are strong positive correlations between hyena maternal social rank, maternal androgens in late stages of pregnancy, and aggression levels of female juvenile offspring (Hamilton, Tilson & Frank 1986; Glickman et al., 1993; Frank, 1997). Prepubertal ovariectomy reduced the aggressiveness of female hyenas towards males (Glickman et al., 2006). Male-biased sex differences in neural systems underlying aggression (as commonly observed in laboratory rats) did not appear in spotted hyenas, where substantial *vasopressin* (a prohormone produced in the hypothalamus associated with aggression, territoriality, social dominance, bonding and social recognition) innervation is found in the forebrain of the females (Rosen et al., 2006). These findings suggest that aggressive behaviour developed during foetal life is organised further and activated by postnatal hormones, although it has not been ascribed to a specific androgen or oestrogen (Glickman et al., 2006). This poses another key question when investigating the masculinisation of genetic females: what are the associated costs and benefits of deviation from traditionally feminine development?

The Jost theory has also been contested by other research: it seems likely that the formation of the scrotum and penile clitoris of hyenas is androgen-independent, while the morphology of the pseudopenis is controlled by naturally circulating androgens *in utero* (Glickman et al., 2006). Jost's theory does support that sex differences in external genitalia are dependent on androgen secretion by the foetal testes. Yet, androgens cannot be eliminated as a theory for the hyena because alternative mechanisms have not been described (Glickman et al., 2006). Preliminary data suggest oestrogens modulate phallic growth both during pre- and postnatal development, but there is no evidence that the penile growth is oestrogen-dependent (Place & Glickman, 2004; Glickman et al., 1998).

Kiwi represent a novel opportunity to further test the androstenedione masculinisation hypotheses in female animals, especially in this under-investigated avian group. Unlike most birds that possess a single functional ovary, female North Island kiwi possess two, both of which are capable of ovulating (Kinsky, 1971). Therefore, logistically, the two ovaries could be a source of increased hormone production or metabolism, much like paired ovaries are primary producers of androstenedione in female hyenas (Lindeque & Skinner, 1982;

Glickman et al., 1987; Glickman et al., 1992). Unlike many bird species, kiwi possess external genitalia: the penis of the male kiwi is known to project below the urethra-sexual cavity and retract spirally (Owen, 1879). In contrast, the female kiwi has underreported genitalia. However, Caithness (1971) reports identifying the clitoris in kiwi specimens, and our team's observations in the lab and field support Caithness 'observation that this feature is under-investigated rather than non-existent (Castro *et al.* in prep.).

Kiwi also share behavioural and social traits with known masculinised mammals. Female kiwi exhibit polyandry, while leaving males solely in charge of incubation and parental care. Female kiwi are larger (in both body mass and beak size) than their male counterparts and show more aggression. As in masculinised mammals, investigating circulating androgens provides initial insights into the mechanisms driving these traits in kiwi and their potential ecological and evolutionary significance. Based on the hormone information thus far for kiwi (Potter 1989, Jensen et al. 2019), I propose a hormone pathway for kiwi that includes Androstenedione (A4) (Figure 25).

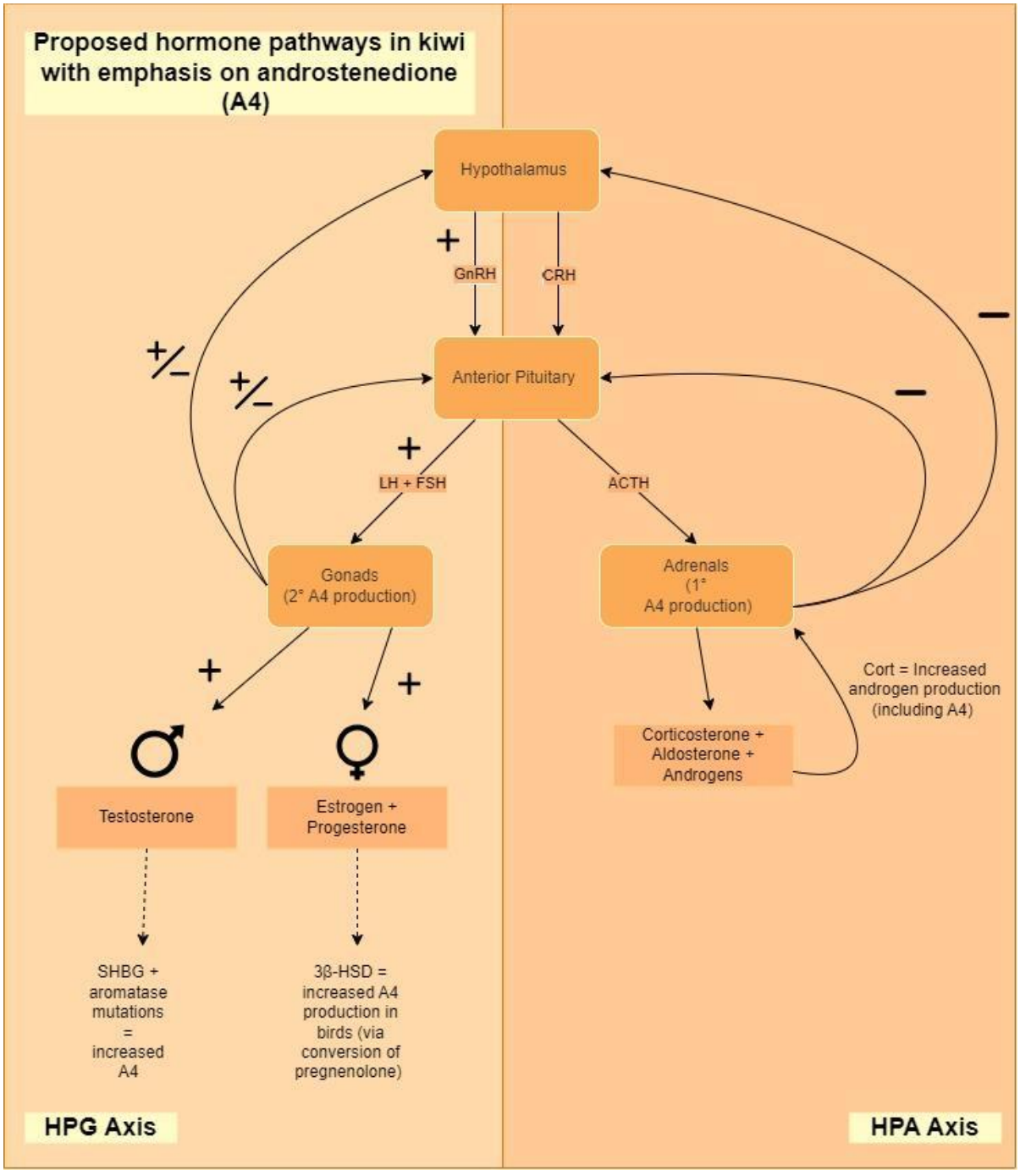


Figure 25:HPA + HPG axis in birds, and how androstenedione (A4) could fit into these pathways in kiwi (Apteryx mantelli).

Potential Alternatives to Androstenedione

Other mechanisms that result in the masculinisation of female animals may be possible. For example, there are direct genetic effects or a special role for nontraditional androgens in the wallaby (*Notamacropus eugenii*). In this species, the formation of the pouch of the female and the scrotum of the male is driven by genetic factors (XX and XY chromosomes,

respectively), without sex steroid intervention (Renfree & Short, 1988). In addition, the formation of the penis is dependent on secretion of *5α-androstane-3α, 17β-diol* from the testes, rather than testosterone (Wilson et al., 2002). Cases such as the wallaby have turned attention to the interaction between direct genetic effects and the effects of gonadal secretions on brain organisation and modulation of behaviour (Arnold et al., 2003; Glickman et al., 2006).

In humans, *46, XX disorders of sexual development (DSD's)* result in the *virilisation*, or masculinisation, of female foetuses (Figure 26). Women with these disorders are genotypically female (XX) but can present phenotypically as male based on the specific disorder. These can include hormone production and levels or physical appearance, including genitalia (Figure 26), reduced or absent mammary gland growth and other secondary sexual characteristics (Auchus & Chang, 2010). While the dominant androgen in human adult males is testosterone, during embryonic development, DHT plays a key role in labioscrotal fusion and phallic growth during week 8-12 of gestation, a critical window of development. Phallic growth occurs throughout gestation, but predominantly in the third trimester. Currently, it is believed that DSDs in genotypic females are caused by excessive exposure to DHT during embryonic development. In addition, the presentation of virilisation in these women is dependent on the amount of DHT in circulation, and the gestational age at the time of exposure (Auchus & Chang, 2010). DHEAs, produced in large quantities by the adrenal glands compared to androstenedione or testosterone, have also been implicated as a nontraditional androgen of relevance in human female masculinisation research.

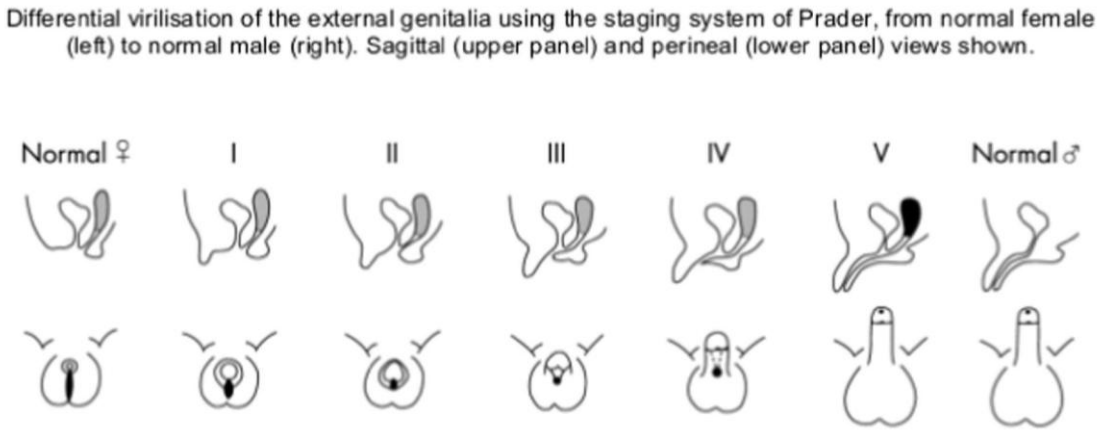


Figure 26: Scale of virilisation of external genitalia in humans from normal female (left) to normal male (right), using the Prader staging system. The top row is the sagittal view; the bottom row is the perineal view. By A.L. Ogilvy-Stuart & C.E Brain, 2004, Early assessment of ambiguous genitalia, Archives of Disease in Childhood, 89(5), pg. 401-407. Copyright 2004 by BMJ Publishing Group Ltd. Reprinted with permission (Appendix 2: Copyright log of permissions for reused illustrations, diagrams and photos in this thesis.

Bird Modelling of Hormonally Mediated Female Masculinisation

The development of the reproductive ducts of birds is similar to other vertebrates. Wolffian and Müllerian ducts also appear in birds during early embryonic stages (Gasc & Stumpf, 1981) and develop into the male or female reproductive tracts under the influence of corresponding hormones. In the chicken, the paired Müllerian ducts regress in male (ZZ) embryos from around day 9 of incubation under the influence of gonadal *anti-Müllerian hormone (AMH)* secreted by developing *Sertoli cells*. The Wolffian ducts develop into male reproductive structures, the vas deferens and epididymis, in response to gonadal androgens. In females (ZW), the right Müllerian duct regresses simultaneously with atrophy of the right gonad, as the female gonads also synthesise AMH, although at lower levels than males (Romanoff, 1960). The left female Müllerian duct develops into a functional oviduct, serving the left ovary. During embryonic life, the left female duct is thought to be protected from AMH by the action of local oestrogens (Tran & Josso, 1977; Hutson, Ikawa, & Donahoe, 1982). In females, due to a lack of gonadal androgens, the Wolffian ducts regress along with the mesonephric kidney (Lambeth & Smith, 2012).

Embryonic gonads synthesise and secrete sex steroid hormones according to genetic sex in birds, as in other vertebrate groups, which then influence the development of sexually dimorphic traits (Tanabe et al., 1979; Clinton & Haines, 2001). Masculinisation of the comb and wattle in males is attributed to testosterone in chickens (Zeller, 1971; Shanbhag & Sharp, 1996; Yoshioka et al., 2010). Differences in body mass between the sexes may be under direct genetic control, as body mass conforms to genetic sex even when sex hormone levels are experimentally manipulated, at least in chickens (Valdez et al., 2010; Lambeth et al., 2016). Sexually dimorphic feathering (or plumage) is generally attributed to sex steroid hormones. In the Galliformes, dull female feathering is induced by oestrogens, and showy male plumage is the default pattern in the absence of significant oestrogens (Owens & Short, 1995).

During embryonic and *perinatal periods*, the avian adrenal gland synthesises significant quantities of corticoids along with sex steroids, such as testosterone and oestradiol. In the embryonic stages, the adrenal gland is a more important source of testosterone than the ovary or testes. The production of these additional steroids by the avian adrenal gland declines rapidly after hatching (de Matos, 2008).

Transport of DHEA, especially between the body and brain, is not well-characterised, especially in birds. In humans and some other vertebrate groups, DHEA travels as DHEA-S (hydrophilic sulphated forms). In birds, DHEA circulates primarily in a non-sulphated form. When reaching the brain, it is uncertain which receptors DHEA binds to, and most are thought to have very low *affinity* for DHEA. It has been suggested that DHEA primarily influences male aggression via local conversion to testosterone, 5α-dihydrotestosterone, and 17β-oestradiol, and subsequent activation of androgen and oestrogen receptors (Wingfield et al, 2018).

Research on male song sparrows (*Melospiza melodia*) and both sexes of spotted antbirds (*Hylophylax naevioides*) suggests DHEA as the source of androgens driving non-breeding aggressive behaviours outside known breeding seasons. In these cases, DHEA is detectable in the circulation and tissues during a period when the gonads are otherwise regressed, while other primary sex steroids such as testosterone and oestrogen are either at their lowest point or undetectable (Soma et al., 2015). High DHEA when gonads are regressed suggests that regulation of aggression outside the specific breeding season may be dissociated from systemic steroid signalling - this reduces the exposure of the peripheral tissues of the bird to the effects of testosterone and oestradiol (Figure 27) (Soma et al., 2015).

To my knowledge at the time of this thesis, DHEA and DHT have not been measured in any *Apteryx* species, nor has androstenedione been measured before this research. Investigation into non-traditional hormones in the brown kiwi may lead to a better understanding of the physiology and behaviour of the species. Understanding hormonal profiles more clearly in kiwi also allows for comparison between wild and captive birds, and improvement in management for captive individuals.

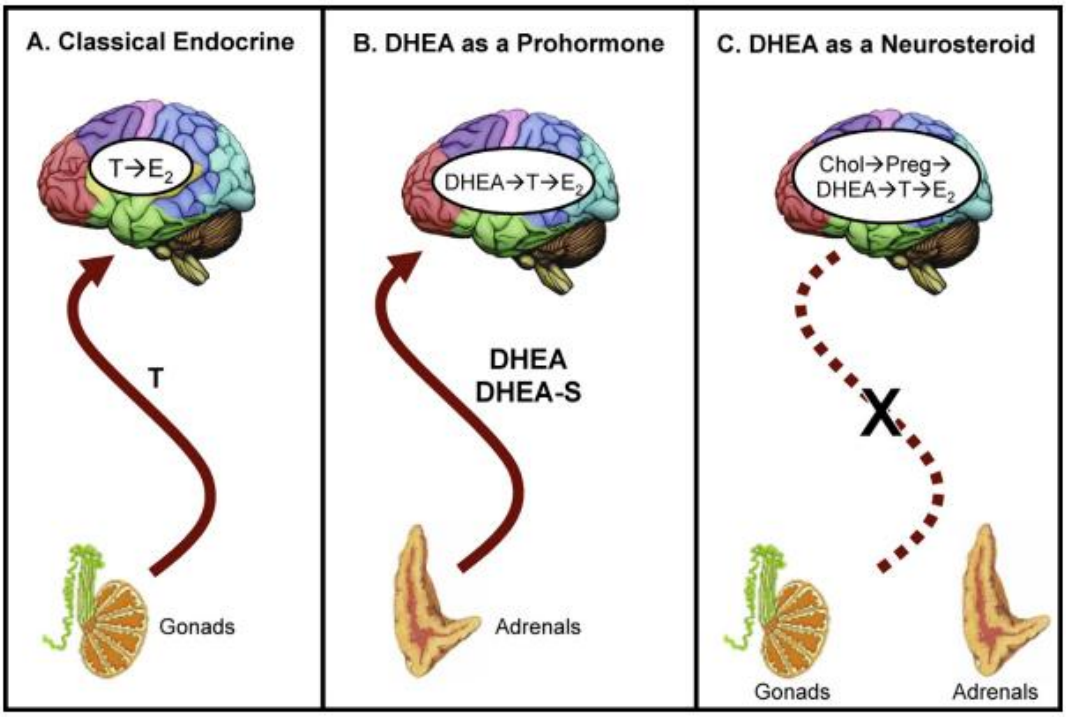


Figure 27: Steroids have the potential to act on the brain and modulate aggression via several pathways.In panel (A), gonadal testosterone (T) either acts directly or via localised conversion to oestradiol (E2). In panel (B), adrenal dehydroepiandrosterone (DHEA) acts via local conversion to T and or E2. In panel (C), DHEA is produced locally in the brain and then is converted into T and/or E2, via cholesterol (Chol) to pregnenolone (Preg) to DHEA to T and/or E2). From "DHEA effects on brain and behaviour: Insights from comparative studies of aggression" by Soma et al., 2015, The Journal of Steroid Biochemistry and Molecular Biology, 145, pg. 261-272. Copyright 2015 by Elsevier. Reprinted with permission.

Recommendations

Further investigation into circulating hormones, both prenatal and postnatal, is required to begin to understand the mechanisms that drive female masculinisation in specific species. While the majority of research has been on mammalian groups, there still remains very little definitive knowledge on this process. While mammalian modelling is useful to gain a basic idea of the potential processes involved, further research would facilitate the construction of a model that could explain this phenomenon in bird species such as the kiwi. An avian model would also elucidate some of the key differences in development between mammals and birds, such as placental foetal growth vs foetal growth in species that lay eggs.

- Investigation into circulating androgen levels other than traditional testosterone monitoring; incorporating the testing of secondary androgens such as androstenedione, DHEA and DHT to identify a possible source of androgenisation supporting masculinised features. Provision of DHT and DHEA concentrations in

kiwi will allow further understanding of the endocrine activity of the species, and the differences in endocrine activity that drive the male and female.

- Investigation into the maternal deposition of hormones in eggs and the effects on chick development when these levels are manipulated. Endocrine, behavioural and anatomical data collected from chicks with different levels of hormone deposition during early critical development will allow further understanding of how male and female kiwi develop.

Recommendations for testing the role of secondary androgens in female kiwi include:

1. Further in-depth literature investigation into levels of DHEA and DHT found in other bird species, how this data was collected, and the hormone levels in bird species that are either closely related to *Apteryx* or share a similar ecological role.

2. Sample collection (sampling of both male and female birds to test the hypothesis that levels of DHEA and/or DHT are higher in female kiwi).

    a. Blood samples taken using established kiwi protocol, considering factors like collecting samples based on timing (such as breeding seasons when hormonal fluctuations are expected) (Robertson, Colbourne & McLennan, 2017).

    b. Faecal samples can be used if permits are unable to be acquired for kiwi blood sampling, or if blood sampling proves to be too difficult/stressful. Faecal metabolites of DHEA and/or DHT can be measured, however, additional validation may be required to ensure correlation with blood hormone levels (Ziegler & Wittwer, 2005).

    c. While less conventional, feather sampling may also allow for hormone sampling. Feather collection is an option for sampling, as hormones like DHEA may be deposited in growing feathers (Freeman & Newman, 2018).

3. Hormone assays (measuring DHEA and/or DHT levels from samples collected from kiwi).

    a. *Radioimmunoassay (RIA)* or *enzyme-linked immunosorbent (ELISA) assays* are standard for monitoring hormone levels, and both these

methods can be used to measure DHEA and/or DHT in blood, faeces or feather samples. Both DHEA and DHT ELISA kits are available for non-avian species but will need to be validated with avian species (Yoon & Kim, 1987; Janse et al., 2011).

b. *Liquid chromatography-mass spectrometry (LC-MS)* is highly sensitive and specific, which may be an option for hormones that can be harder to detect, such as DHEA and DHT. This technique can be used for plasma samples or feather samples but requires specialised equipment and technical expertise.

c. If faecal samples are used, hormone metabolites (such as DHEA metabolites) need to be extracted via organic solvents and then analysed by ELISA or LC-MS.

4. Analytics and comparisons (testing the hypothesis that females have higher levels of DHEA and DHT using collected hormone measurements).

a. Descriptive statistics of DHEA and/or DHT levels in both male and female kiwi, as these results have not been previously published for this species.

b. Analysis of groups using t-tests or ANOVA to compare hormone levels between males and females. More complex statistical models, such as mixed-effects models or ANCOVA, may be needed to account for potential covariates if there are multiple sampling times or groups (age or reproductive status).

5. Consideration of confounding variables

a. Breeding status (seasonal fluctuations of hormonal levels) can be accounted for by i) collecting samples at consistent times relative to the breeding cycle or ii) collecting samples at every point in the breeding cycle for comparison.

b. Age can affect hormonal levels, which can be accounted for by i) stratifying analyses by age or ii) making sure sampled males and females are comparable in terms of age and/or developmental stage.

c. Environmental factors need to be considered (such as temperature, food availability, etc). In captive birds, these can be controlled or accounted for in wild birds.

6. Interpretations of results

    a. Higher levels of DHEA and/or DHT would support the hormonal masculinisation of female brown kiwi.

    b. Further investigation would be needed to correlate these results with behavioural masculinisation (aggression or dominance) and/or morphological traits (such as size).

    c. Further investigation would be needed to correlate these hormones with female-specific behaviours in and out of the breeding season.

    d. Further investigation would be needed to explore the evolutionary significance of hormonal masculinisation in female kiwi, such as dominance in mating systems or reproductive success.

7. Longitudinal monitoring

    a. Longitudinal monitoring would be useful in establishing the relationship between behaviour and hormone levels by tracking hormones over time and measuring corresponding behavioural changes. For example, females with higher DHEA and/or DHEA levels could be correlated with higher frequency of dominance behaviours, or greater reproductive success.

Recommendations for testing the role of maternal hormone deposition in kiwi chicks and resulting sexual development include:

1. Define target hormones of interest (that the amount and/or ratio of a specific hormone deposited into an egg maternally influences sexual differentiation and development of that chick).

    a. Androgens (testosterone, androstenedione, DHEA, DHT) are associated with masculinised traits when found in high levels.

b. Oestrogens (estrone, oestradiol) are associated with feminisation and the development of female sexual traits.

c. Corticosteroids (corticosterone) are associated with stress responses; however, they can alter developmental pathways, particularly growth rates.

2. Sample collection (Eggs and maternal hormones).

a. Eggs can be collected from multiple female kiwi, considering the timing of egg laying and health and body condition of the female (breeding phase, stress levels, parasite load, weight, body condition score, etc).

b. Sampling methods have been detailed previously in recommendations for investigating secondary androgen levels in kiwi. Blood (or faecal samples) from females prior to laying provides information for hormonal sampling to test correlations between maternal hormone levels and egg hormone deposition.

3. Hormone assays of eggs (extracting and measuring hormones deposited in the egg yolk or albumen).

a. The yolk is known to be the primary site for hormone deposition in birds. Yolk extraction protocols can be used to carefully take yolk samples from an egg without destroying the egg and/or foetus. Chemical extraction methods are needed to isolate hormones from the yolk samples (von Engelhardt & Groothuis, 2005; York et al., 2020).

b. Isolated samples after chemical extraction can be quantified by RIA, ELISA or LC-MS as mentioned above.

4. Experimental manipulations (if intending to test causality, manipulation of hormone levels directly in eggs can be used to assess effects on chick sexual development).

a. Injections of hormones of interest can be administered into the egg yolk at different concentrations or different developmental stages.

b. Alternatively, eggs can be immersed in hormone solution to allow the absorption of the target hormone through the shell.

c. Control groups would consist of eggs without added hormones.

d. Post-manipulations, eggs should be incubated under standard brown kiwi protocol. Ideally, incubator use provides environmental control to eliminate the effects of factors such as temperature, humidity, and to eliminate variations in parent incubation.

e. Post-hatch monitoring should include the measurement of chick growth rate, weight, size and morphological developments (such as beak length and tarsus length) to determine if hormone deposition in eggs has influenced these traits.

f. Post-fledging, chicks can be assessed for behavioural changes that are associated with masculinisation or feminisation. This can include aggression, social interactions and courtship.

g. Post-fledging (or at the point of sexual maturity) chicks can be examined for sexual characteristics. This includes changes in morphology (size, colour, beak length, body size and/or mass), reproductive anatomy (gonads, cloacal anatomy) or histological analysis (from gonad tissue samples).

h. Hormone analysis of chicks from blood or feathers at different stages (such as post-hatch, juvenile stages or at sexual maturity) to track effects of egg hormone deposition.

5. Statistical analysis (collected from egg hormone concentrations and/or chick data such as growth, behaviour, hormone levels or degrees of sexual differentiation).

a. ANOVA may be used to compare differences in chick development between control and experimental groups.

b. Regression models may be used to examine the relationship between egg hormone levels and chick data (such as growth rates, behaviour or sexual differentiation).

c. Post-hoc tests can be used to determine if specific hormones are linked to changes in specific traits.

6. Ethical considerations

    a. Research protocols must be investigated and designed to meet ethical guidelines, specifically when dealing with manipulation of eggs via hormone treatment. Harm to embryos or chicks should be minimised.

Anatomy

Genitalia

Very little literature describes the existence of the clitoris in female birds.

In emu, sex assignment has been performed from 16 months of age by eversion of the vent to expose either the phallus or clitoris for visual confirmation of sex (Dash, Malik, & Mohapatra, 2014). Similar sexing methods are also described in greater rhea chicks (Bazzano et al., 2012) and the South African ostrich (Gandini & Keffen, 1985).

One record examines the potential existence of the clitoris in the brown kiwi. Caithness (1971) described the clitoris in brown kiwi as diminutive and measuring around 0.5-1.0 mm in length (Figure 28). However, the author admits that the ages of the birds in the study were unknown, and that the one mature female with an egg in the oviduct post-mortem had a larger clitoris measuring 8 mm in length. Further laboratory investigation because of this thesis indicates that the female brown kiwi has a large clitoris. Histological analysis is necessary to ascertain whether the clitoris in kiwi is a pseudo-penis, and a formal description is pending (Castro *et al.* in prep.).

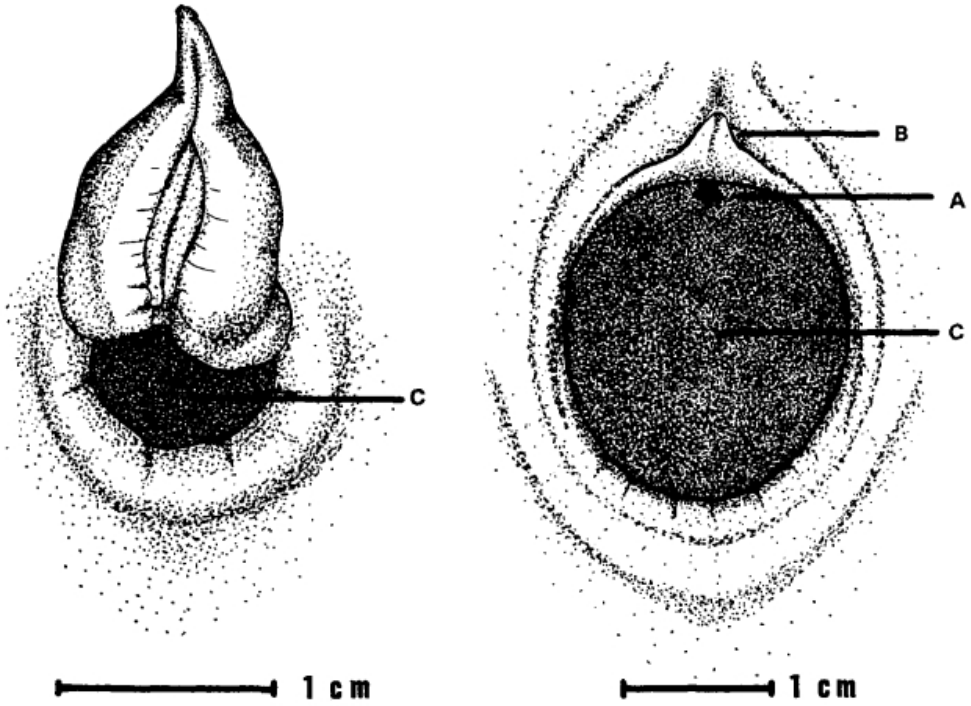


Figure 28: Penis of an adult male brown kiwi (left), and clitoris of an apparently adult female brown kiwi (right). A = anus, B = clitoris, C = cloaca. From "Sexjng kiwis" by T.A Caithness, 1971, International Zoo Yearbook, 11(1), pg. 206-208. Copyright 1959 by John Wiley and Sons. Reprinted with permission (Appendix 2: Copyright log of permissions for reused illustrations, diagrams and photos in this thesis.

Scent Glands

Traditionally, scent has been understudied in avians and not considered important in their behaviour and ecology. However, the uropygial gland produces secretions (preen oil) that are known to differ seasonally in many bird species, as well as between sexes. There is currently no consensus on whether this is a significant trait found across the majority of birds or only in specific groups, nor is there consensus on the function of this sexual dimorphism. The *sex semiochemical hypothesis* suggests that these signals could be used for olfactory communication, particularly during the breeding season (Grieves et al., 2022). Semiochemical refers to a chemical (or pheromone) used to *signal* from one individual to another, with the intent of modifying or influencing the behaviour of the recipient (Grieves et al., 2022).

Current research into avian chemical signals suggests that sexually dimorphic differences are more prominent in females than males. Females are typically characterised by at least one of three patterns: a larger uropygial gland, greater abundances of *volatile* or *semi-volatile preen compounds* or a greater diversity of preen oil compounds or associated microbes. These patterns have been found in 23 of 30 studied bird species (Whittaker & Hagelin, 2021).

Additionally, detection of preen oil changes between seasons is directly related to incubation and nest ecology (Grieves et al., 2022). In ground-nesting species, these changes were more likely in the incubating sex. In contrast, non-ground-nesting species have an equal chance of seasonal changes regardless of incubating sex. Differences in preen oil between sexes have also been found to be more likely during the breeding season than the non-breeding season, and more likely in uniparental than biparental species (Grieves et al., 2022).

Apteryx species are known to possess a uropygial gland at the end of the *coccygeal bone*, with papillae towards the cloaca. These glands are bilobar and possess eight primary sinuses (four per lobe), each possessing an orifice for a total of eight openings in the papillae. Striated muscle is found within the capsule, around the caudoventral part of the gland, suggesting that the birds have control of the secretion (Reynolds et al., 2017).

Large primary sinuses paired with many secondary sinuses offer large areas for secretions to be collected from the gland while preening. It is also suggested that gland volume changes throughout the year in individual birds. Both male and female *Apteryx* were found to have a similar number of cells creating the *follicular epithelium*, however, males had a larger follicle diameter, and females had a larger lumen diameter. Age also predicts follicle cell numbers, with juveniles having fewer than adults, and chicks having fewer than juveniles (Reynolds et al., 2017).

Kiwi also have well-developed olfactory capabilities. Their olfactory structures are large and complex, with a large number of functional olfactory receptor genes (Steiger et al., 2008; Steiger, Fidler, & Kempenaers, 2009). They also display anatomical adaptations to support olfactory capabilities, such as the characteristic long beak with nostrils at the tip. These allow them to navigate and function in their nocturnal, ground-dwelling niche, without heavy reliance on vision (Corfield et al., 2014).

Kiwi have an unusual odour that has been described as “earthy” (Buller, 1888). Kiwi faeces also smell strongly, pungent and incorporate the “earthy” body odour as well as ammonia. Chemical compounds investigated in kiwi faeces are common *hexane-soluble hydrocarbons*, also found in secretions from the kiwi uropygial gland (Jacob, 1982) and are found in the sebaceous and pheromone-secreting glands of other animals (Castro et al., 2010). Kiwi faeces are often found in conspicuous locations: logs, roots, tracks and accumulated inside some burrows. This suggests that faeces could be purposefully deposited in locations where other

birds will encounter them, to communicate information such as breeding condition, burrow occupancy and/or activity (Castro et al., 2010).

Grieves et al. (2022) have suggested further testing to evaluate the hypothesis that females have more pronounced sexual chemical signalling than currently acknowledged, including looking for:

- Evidence for avian olfactory sex discrimination (discrimination between sexes, and between individuals of varying quality). Examples include indicators of genetic quality and compatibility, such as *genome-wide heterozygosity* (Whittaker et al., 2019) and MHC genotype (Grieves et al., 2019).
- Evidence that preen oil becomes more volatile and/or more abundant (for detectability) during breeding seasons compared to non-breeding seasons
- Evidence that preen oil preferences of birds tested in the laboratory translate to mate choice in the wild.
- Evidence that mate choice based on preen oil odour cues can be linked to measures of mate quality and fitness.

Recommendations

There are still many unknowns about kiwi olfaction, olfaction differences between sexes, and specifics of kiwi anatomy (such as preen oil compositions, cloacal differences in secretion, etc). Further work in this area may include:

- Investigation and formal morphological and histological description of the cloacal structure of female North Island brown kiwi with comparison to males at prenatal, juvenile and adult stages of life. This information is needed to fill gaps in current understanding of kiwi anatomy relating to overall reproductive biology.
- Testing the semiochemical hypothesis in kiwi: whether individual kiwi discriminate the sex and "quality of an individual" based on olfactory chemical signals. Analysis of preen oil quantity and content of males and females, both within and outside the breeding season. Analysis of scent production in kiwi, the presence of differences between male and female scents and differences in scents in non-breeding and breeding seasons is critical to understand kiwi social interaction and reproductive behaviour. Further experimental testing would also add to the understanding of kiwi interactions and mate choice.

Recommendations for investigating the presence of a clitoris in female kiwi include:

1. A more in-depth literature review on genital structures and anatomy in avians. Some birds are known to have a phallus (or cloacal protuberance that is classified as a phallus) for copulation, but its structure varies, and some species have been studied much more than others.

    a. Only a few species have had a small, clitoris-like structure observed and/or formally described (Brennan, 2022).

    b. Reviewing case reports of kiwi or similarly related species may provide more evidence (Caithness, 1971).

    c. Reviewing case reports or studies in bird species that are known to have a clitoris-like structure for a baseline comparison (such as ostriches and some species of duck) (Samour, Markham & Nieva, 1984; Gandini & Keffen, 1985; de Oliveira & Mahecha, 2000; Kummrow, 2015).

2. Morphological examination of female birds.

    a. Gross dissection of female kiwi genital tracts, with a focus on the cloaca and oviduct. Examining particularly for anatomical protrusions around or inside the genital opening, or structures that resemble the clitoris.

    b. Examine both the opening of the cloaca (vent) and just inside the cloacal slit. If a clitoris is present, it is likely not to be easily visible without careful dissection and preparation.

    c. External examinations should check for a protuberance (or nub of tissue) near the genital opening that may be a clitoris. In some species where it is documented, it is small yet still discernible.

    d. Internal examinations should examine the cloacal area for erectile tissue or vascular structures that may be a clitoris.

    e. Look for specific sexual dimorphism in genital anatomy compared to males.

3. Histological examination (to determine whether any identified structure of interest resembles the tissue structure of a clitoris, with erectile tissue, nerve endings of vascularisation).

    a. Tissue sampling from areas of interest near the vent/cloaca for histological processing.

    b. Microscopic examination of tissue after staining, examining for vascularisation, nerve endings or erectile tissue.

    c. Comparison with other species that have previously been documented, such as ostriches or quail.

4. Molecular analysis (considering the genetic and molecular basis of clitoral development in birds).

    a. Using techniques like Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) or RNA sequencing, gene expression in the suspected clitoral tissue can be analysed. Genes of interest would be those associated with clitoral development in mammals and those associated with the formation of erectile tissues (Yamada et al., 2003; Gredler, 2016).

    b. Gene expression can be compared in female kiwi to species with well-documented clitorises to document any similarities.

Recommendations for testing the role of chemical communication in kiwi include:

1. Identification of relevant semiochemicals in kiwi (via a more in-depth literature review and/or investigation of chemical markers).

    a. Reviewing known chemical signals such as pheromones, scent marks and glandular secretions used in birds, and those used in closely related species (Rajchard, 2007; Campagna et al., 2012).

    b. Reviewing what signals are used (urine, feather oil, skin secretions) and what information they are known to convey (health, parasite load, genetic quality, dominance status or reproductive status) (Hagelin & Jones, 2007).

c. Identifying specific chemical markers that could change during the breeding season (like composition in skin secretions, urine or feathers) and what they may indicate (such as mating readiness, territory ownership or genetic fitness).

2. Data collection (collecting a baseline of data and information for further analysis).

    a. Pre-breeding and breeding samples for comparison, to test whether birds alter scent production during the breeding season (such as composition of chemical marker changes or scent glands are more productive).

    b. Collection of samples in the non-breeding season as a control in seasonal and/or hormonal investigations.

3. Sampling methods (for data collection as previously mentioned).

    a. Non-invasive methods like swabbing can be used to collect scents, on areas including the feathers, skin or feet (Robertson, Colbourne & McLennan, 2017).

    b. Scent samples can also be isolated from urine or faeces samples (Walker & Valigo, 2021).

    c. Targeted body parts need to be swabbed, including i) areas known to produce scent ii) areas suspected to produce scent, and iii) areas that may come into close contact with other birds, such as the vent or head (Nevitt & Prada, 2015).

    d. Scents can be further isolated using gas chromatography-mass spectrometry (GC-MS) to break down the chemical composition of samples and identify specific chemicals that may play a role in communication (Gabirot et al., 2016).

4. Behavioural experimentation (Conducting behavioural trials can be useful in determining if scent signals influence bird behaviours, especially during the breeding season, and can be used to test species-specific hypotheses).

a. Playback of scent signals experiments can involve exposing an individual or group of kiwi to either a male or female scent sample, and those in different reproductive stages (such as breeding, incubating, and non-breeding). Behavioural observations can be made on responses such as aggression, courtship or territoriality.

b. Exposure to control samples that are unscented or neutral (such as a non-breeding individual or another species) can test if birds are responding specifically to breeding-related scents or if they are responding to other cues.

c. Mate choice can be tested by offering scent-marked objects or substrates (like branches or nest materials) and observing whether the tested bird shows a preference for specific individuals based on sex or reproductive state (Amo et al., 2012a, b).

d. Mate competition can be tested by placing scent cues (like male urine or feather oil) in known territories or near potential mates and observing if these signals influence behaviours including *female mate choice*, male mate choice, male-male competition, female-female competition, or female-male interactions (Amo et al., 2012a, b; Leclaire et al., 2017).

5. Hormonal experimentation (testing the effects of hormone manipulation on scent production, and the behaviour that results in response to scent production).

a. Administration of primary hormones such as testosterone or oestrogen can be used to assess if changes in scent correspond with hormonal fluctuations found during the breeding seasons, and therefore the correlation with behavioural changes.

b. Control groups can consist of individuals that are either artificially hormone-suppressed or kept at normal levels to isolate the effects of breeding-related hormones on scent production and/or communication.

6. Comparative studies (investigating the genetic and evolutionary connections to scent production within kiwi, and whether this is a pattern found more broadly in other avian groups).

    a. Investigation into whether scents are related to genetic quality signals, and if males (or females) with stronger or more attractive scents have more success securing mates.

    b. Investigation into whether scents carry information related to individual bird genetic health or fitness.

    c. Phylogenetic comparisons in closely related species, comparing the presence or absence of scent-based communication and cues. This can be used to investigate if this behaviour is the result of specific ecological pressures (such as mating systems or habitat characteristics).

    d. Cross-species comparison if other species or populations do not use scent communication. Comparison of how behaviours differ in the presence/absence of semiochemical cues can be used to rule out alternative hypotheses about if visual or auditory cues are more important.

7. Analysis of data (to ultimately determine if scent plays a significant role in communication during the breeding season).

    a. Statistical analysis of corresponding behavioural data to determine if scent communication influences mating success, territorial behaviour or social interaction.

    b. Chemical correlations to determine if i) certain chemical compounds are associated with breeding behaviour and ii) if those chemical compounds are being preferentially selected by mates, or if they are influencing aggression, attraction and/or courtship.

Behaviour

Juvenile behaviour, play behaviour and socialisation

Juvenile play is known to be an important developmental step in young animals, and rough-and-tumble play is frequently associated with juvenile males. An indicator that a species may show sex-role reversal when juvenile females partake in this play at the same or greater frequency than similarly aged males. Social play is thought to be a relatively low-cost method for groups to develop dominance relationships, and links are suspected between early play and later dominance between interspecific peers (Blumstein, Chung & Smith, 2013).

Rough and tumble play (RTP) is most ascribed to mammalian species. RTP consists of physically active social behaviour, including chasing and play fighting (Burghardt, 2005). RTP is also characterised by the absence of threat, signalling with play faces or vocalisations that the interaction is not aggressive, and frequent role reversal (Fry, 2005). During RTP, the actions of juveniles are like those performed by adults in serious contexts, such as competition, aggression and social encounters. The similarity to adult behaviour paired with specialised behaviour to communicate benign intent has led to the theory that RTP is used by juveniles to develop social and environmental skills (Marley et al., 2022).

For example, rats (*Rattus norvegicus domestica*) are a common study animal for investigating play-fighting in mammals and other groups. The frequency of play-fighting (or RTP) in this species is traditionally sexually dimorphic. Males engage in RTP more frequently. But if castrated neonatally, RTP is reduced to the female-typical levels of engagement. In contrast, testosterone treatment neonatally in female rats will induce play-fighting at male-typical levels. When this play is further examined, the frequency of attack is found to be sexually dimorphic, unlike the frequency or likelihood of defence. In males, defensive behaviour changes at puberty to become rougher, and only occurs if males undergo normal perinatal androgenisation. If females are ovariectomized at birth or weaning, they also display this male-typical change in defence roughness (Pellis, 2002).

Birds typically do not meet the same requirements for the development of social play as mammals, hence, there are fewer known recorded cases of avian species that engage in social play. These requirements often include ongoing custodial care by parents, cooperative breeding, delayed reproduction, species sociality and larger brain size. However, if provided

with a suitable social environment, young birds appear to be able to develop complex social play like young mammals (Diamond & Bond, 2003).

Allopreening is often closely associated with social play in birds - it both precedes and follows play in many bird species. In an avian-specific context, allopreening may fill the same social role as play behaviours in other vertebrates and seems to be connected to improving social cohesion (Skeate, 1985; Ranford & Plessis, 2006; Wenig, Pacher & Bugnyar, 2022).

In juvenile groups of wild-caught large-billed crows (*Corvus macrorhynchos*), allopreening frequency and duration were studied for patterns of reciprocity to investigate if sex composition and dominance in groups affected the frequency of an individual both preening and being preened. In juvenile males, frequency and duration of allopreening correlated with frequency of aggression. In same sex interactions, allopreening occurred unidirectionally, from dominant individuals to subordinate individuals exclusively. In opposite sex interactions, males reciprocated more frequently and for longer (Miyazawa et al., 2020). Ultimately, findings suggested that allopreening between juveniles served as a dominance signal for same-sex dyads, and as a social bonding function for opposite-sex dyads and may reflect crucial roles for both within-sex competition and opposite-sex attraction (Miyazawa et al., 2020).

Arabian babblers (*Turdoides squamiceps*) are another group in which social play has been described. Commonly described forms of play include wrestling, displacement (via king-of-the-hill type interactions), chases and tug-of-war. Several *play signals* have also been identified, such as crouching, rolling over, elevation of sticks, play bowing and establishing eye contact and freezing during the middle of play. Play also varied depending on the individual: dominant individuals within the group played less than subordinates, however they had a higher frequency of play signalling than subordinates. This is because up to 50% of the time, play invitations were not accepted. It is thought that by play signalling (and therefore initiating play), the initiator handicaps themselves, making it more difficult for them to attack the other party, or making them more vulnerable. It is likely this is how dominant individuals reassure their playmates that no harm is intended and is designed to entice playmates as an honest signal that the intention is play and not harm. Play behaviour was undertaken primarily with individuals close in rank. Social tension within groups resulted in

inhibited play activity. Play bouts were also interspersed with allopreening (Pozis-Francois, Zahavi & Zahavi, 2004).

In ratites, research into juvenile play has shown that greater rhea (*Rhea americana*) engages in contagious locomotor play and often adds social components. Interactive social play is less common but has been observed from 10.5 weeks of age onwards. Social play consisted of pecking each other on the neck and wrestling movements – non-serious use of agonistic behaviour that is species-specific (Zeiträg, Jensen & Osvath, 2023).

While behaviour studies have been conducted on both wild and captive kiwi populations, virtually none have investigated the relationships between juvenile kiwi, and juvenile and adult play behaviour. A videographic study done by the Smithsonian National Zoological Park (2013) observed two juvenile female kiwi. Aggressive behaviour was occasionally noted between the sisters, including mild kicking, stare downs, standing tall, poking the other bird, and chasing it out of the aggressor's "territory" (never more than a few metres). Given that courtship behaviour in kiwi is frequently mistaken for fighting due to its aggressive visuals, it is possible that this sibling behaviour observed was a form of play.

Similarly, during the videographic study of Ponui birds, a singular interaction was noted between a juvenile and a suspected male (Figure 29). This behaviour included prodding and chasing that mimicked courtship in adult birds; however, when charging, physical contact such as kicking was never made. The male appeared to be the aggressor and initiator, however, the juvenile also would wait after running a short distance for the male to re-engage, before starting the behaviour again.

Figure 29: Camera trap footage from the Two Tanks site on Ponui Island on the 7th of November 2015. A male brown kiwi and a juvenile are both seen and appear to be engaging in potential chase-play.

Given these anecdotal observations, there is a possibility that brown kiwi engage in forms of rough-and-tumble play, either between juveniles or between juveniles and adults. This behaviour may have previously been missed due to low population numbers inhibiting this behaviour, or meaning that it was less likely to be observed.

Link to Google Drive folder where this interaction between male and juvenile can be viewed: *https://drive.google.com/drive/folders/18OnAnmNnmm2fbD7ZWq_M7jt7x21PPfdC*

Female Dominance

Social dominance within a species (in this case, sex-specific) is expected to provide advantages in the form of access to resources and greater control of reproduction. Acquiring and maintaining dominance, however, is likely to incur physiological costs such as engaging more frequently in aggressive behaviour, associated injury risk, energy and resources spent to both assert and signal status (Silva et al., 2018).

Social dominance is defined as the relationship between two individuals in which one (subordinate) predictably yields to the other (dominant) during social interactions (Kaufmann, 1983). These social interactions, in turn, are defined as an encounter between two individuals in which the outcome is the loser (subordinate) backing away from the

winner (dominant) (Marolf, McElligott & Müller, 2007). Social dominance is theorised to limit the cost of aggression within social species by limiting aggressive interactions to threats as opposed to physical contact and violence (Fournier & Festa-Bianchet, 1995). The relationship between dominant and subordinate is an adaptive compromise between the costs and benefits of giving in. Familiarity across groups or neighbouring individuals may develop stable dominant-subordinate relationships as a result of individual recognition (Kaufmann, 1983).

Female social dominance (FSD) refers to females consistently winning most agonistic interactions and causing submissive behaviours in males (Pereira et al., 1990). Currently, FSD is considered uncommon and has been primarily studied in mammals, with notable cases being spotted hyenas (*Crocuta crocuta*) and lemurs (*Lemuroidea)* (Frank, 1986; Kappeler, 1993; Dloniak et al., 2006).

Two main hypotheses exist to explain the significance of female social dominance. Hrdy (2009) proposed the "*Male Deference Hypothesis*", that females have a feeding priority over males who do not need higher-quality food resources, and therefore males defer to females (and/or when breeding is seasonal). Jolly (1984) proposed that females should dominate males when ecological variables and metabolic factors challenge female reproductive success.

During my study, recorded brown kiwi were difficult to sex. As feather patterns and colours are monomorphic, being able to differentiate sex often must rely on size or bill length features. In camera trap videos, the larger size of a female is difficult to discern without other birds as comparison. Bill length can be impossible to measure without the correct angle to calculate the full length.

Recorded videos were only able to capture part of the displays of wild brown kiwi. Outside the visual zone, it was impossible in most cases to detect which bird was the initiator of these behaviours. It was also difficult to discern the ultimate outcome of the behaviour, such as whether it ended in 'territorial expulsion' or copulation.

In future research, confirmation of the sexes of all birds engaging in social behaviour, and the full length of behaviours, would allow further understanding of social dominance in brown kiwi.

Recommendations

- Further long-term observations of kiwi with a focus on dynamics between individuals, and whether the species engages in play behaviour.
- Observations of kiwi chick and parent interactions from hatch to dispersal. This may be very difficult to do with a nocturnal species, but with advances in radiotelemetry, there may be an opportunity to track mothers, fathers and offspring in real time over the landscape to infer interactions (Castro pers. comm.).
- Observations of kiwi chick interactions both in the wild and in captivity.
- Further observation of encounters between kiwi in which threat and/or aggression is present, to identify the sex of the instigator, the sex of the dominant and submissive individuals, and determine which sex submits more frequently during encounters.
- Observations to investigate if submission changes by sex during breeding and non-breeding seasons.

Recommendations for investigating play behaviour in kiwi could include:

1. More in-depth literature review (review known kiwi behaviour, review closely related species, determine if play behaviour has ever been documented in kiwi and/or closely related species, identify if there is any potential play behaviour that may have been misidentified).

2. Define hypothesised play behaviours (as play is not as often categorised in birds as other groups such as mammals, define what is and/or could be considered play in the kiwi or closely related species; this could include repetitive non-functional actions like moving things without a direct survival function, solitary play with environmental objects, social play consisting of play-fighting or chasing or developmental play in juvenile birds that may help develop adult behaviours).

3. In-field observation studies (ideally non-invasive and extensive; ideally over multiple months/years and in multiple different seasons to increase the chance of recording this behaviour in a cryptic species).

    a. Motion-activated camera traps and/or hidden observation points allow for recording behaviour without disturbing the birds.

b. Behaviour recordings should target unusual or repetitive behaviours that qualify in the parameters decided for play behaviour in the species (repetitive movements or behaviours with no clear survival purpose, manipulation of objects, social interactions that are non-aggressive and/or non-competitive).

4. Controlled observational experimentation (introducing novel objects and recording interactions with them, such as sticks, leaves, balls, pieces of cloth, or any other object that can be used to stimulate curiosity and/or play).

a. A controlled environment where birds are restricted to a specific open area may be useful for isolating behaviours and providing objects to trigger play behaviour.

b. Identifying and observing specific behavioural markers such as birds engaging with a novel object in ways such as tossing, chasing, or manipulating the object in non-forage-related ways.

c. Social play cues may also be useful to trigger play behaviours. Introduction of conspecifics to a controlled area means that behaviours can be observed that do not result in aggression (chasing, mock fighting, competitive interactions, etc).

5. Analysing behaviour (spontaneous behaviour can be captured by recording and is an ideal option for subtle or infrequent play behaviours being identified without disruption).

a. Coding and categorising behaviour in an ethogram (non-play behaviours such as foraging, mating rituals and defensive behaviours compared to playful behaviour such as object manipulation, play chasing or mock fighting).

b. Statistically analysing whether playful behaviours have seasonal patterns, age-related differences (such as higher frequency in chicks/juveniles) or situational triggers (the presence of conspecifics, certain times of the day, play trigger signals, etc).

6. Comparative studies (Comparing with other closely related species, such as other ratites and/or comparing with other ground-dwelling species that may exhibit play behaviour like quail or grouse for contextualisation).

    a. Phylogenetic analysis may be useful for determining whether play behaviours observed are more common in species with specific ecological or social traits, and whether the behaviour is a specific adaptation or an anomaly.

7. Factors influencing play (such as age and/or hormones)

    a. Play behaviours are more commonly associated with juvenile animals, and so age-related differences are an important investigation point. This could include the frequency with which young birds engage in play, the roles young birds take when engaging in play, the sex of young birds engaging in play (and if there is a difference in frequency) and if adults even engage in play at all.

    b. Hormonal changes occur between seasons and are known to influence behaviour. Investigation into play behaviour between different sexes in different reproductive stages and/or different seasons can be useful for seeing if play behaviour is influenced by specific hormones.

8. Long-term monitoring plan (Repeat observations over multiple seasons would allow for investigation into whether this behaviour is consistent or one-off).

    a. Seasonal variation across multiple years (and if the breeding season, increased conspecific interactions and presence of juveniles increase the frequency of play behaviour).

9. Formal publication (if kiwi exhibit previously unrecorded play behaviour, documentation of findings alongside video and/or photographic evidence would provide greater insight into their behavioural ecology. This also adds to the understudied topic of play behaviour in bird species.)

Recommendations for investigating female social dominance in kiwi could include:

1. Define social dominance in kiwi context (clearly defining what behaviours and/or outcomes will be used to measure dominance). This could include:

    a. Priority access to foraging sites/resources.

    b. Priority access to burrows or nests.

    c. Aggression towards other females in the form of aggressive behaviours (in the case of female social dominance).

    d. Mate selection and/or control (dominant females having the first choice of mate or influencing male behaviours in the case of female social dominance).

2. Observational studies (preliminary observational studies allow further recording of kiwi behaviour for analysis)

    a. Ideally non-invasive and extensive, utilising motion-triggered camera traps and/or observation posts.

    b. Targeting dominance behaviours such as aggressive interactions (females asserting themselves over others with aggressive chasing, pecking or displays), resource monopolisation (if specific females monopolise foraging sites or burrows) and hierarchical behaviours (patterns of certain females always initiating interactions or always having priority access to a resource).

    c. Target sexual and courtship behaviour, such as dominant females being preferred by males for mating, female mate-guarding behaviours, or females having multiple mates.

3. Quantification of dominance (defining how to quantify dominance in kiwi is needed to systematically test if the species shows female social dominance).

    a. Designing an ethogram to categorise specific behaviour that may indicate dominance, such as aggression (fighting or displacement), submission (retreating, lowering body posture or relevant vocalisations) and food access (the order in which a female can access forage or nesting sites).

b. A ranking system can be developed based on the observed frequencies of dominant behaviours (such as the number of aggressive or submissive interactions and the ability to monopolise a resource). This allows for females to be ranked by social status.

4. Controlled experimentation (food competition, nesting site competition, male mate preferences, social interactions).

a. Food competition can be introduced if females are in a controlled and defined environment. Feeding stations with limited resources would allow for observation of female interactions when they all have access to food (whether a particular female drives others away from food, how long each female spends at the food resource, if more dominant females control access, etc).

b. Nesting site competition can be introduced if a situation is created where multiple females are forced to compete for access to preferred sites (such as only a few nesting sites left unblocked). This allows observation of whether certain females can dominate access to the better sites.

c. Male mate preference can be observed by investigating whether males choose specific females more often during mating displays or courtship, such as male engagement, mate guarding or copulation attempts. Experimentation with the choice of mates and manipulation of female traits (to eliminate other traits that may have a greater effect on male choice) also can help investigate male mate preference.

d. Social interactions can be further observed by introducing females of different social status to a neutral area and recording interactions. Under female social dominance, dominant females would be expected to show more aggressive behaviour and chase away fewer dominant females.

5. Hormonal experimentation

a. Further investigation into testosterone levels in females allows for better investigation into aggression. More dominant individuals would be

expected to show elevated levels, and so differences in female hormonal profiles would suggest a hierarchical social set-up.

b. Further investigation into corticosterone levels in females allows for better investigation into stress. More subordinate females would be expected to show higher corticosterone levels due to frequent social stress, again suggesting a hierarchical social set-up.

6. Physical condition (dominant females may be associated with better body condition and/or higher reproductive success).

a. Body condition can be assessed with weight and/or fat reserves (body fat).

b. Reproductive success can be assessed with egg quality, number of clutches per year and number of offspring hatched and fledged.

7. Long-term monitoring plan (Repeat observations over multiple seasons would allow for investigation into whether this behaviour is consistent or one-off).

a. Prolonged observation durations would allow for better capture of seasonal variations in dominance behaviour, especially during breeding behaviours. Prolonged observation also allows for changes in dominance over time and different life stages to be accounted for.

b. GPS tracking can be used to monitor female movement patterns and/or resource use. If there are more dominant females, they would be expected to exhibit more consistent access to better foraging and nesting sites. Radio telemetry may also be used for this purpose.

8. Analysing behaviour (assessing whether there is a significant difference in dominance behaviour frequency between females and whether brown kiwi females can accurately be described as a species with female social dominance).

a. Statistical analysis (such as Chi-square, ANOVA or rank-based analysis) to assess differences between the frequency of specific dominant behaviours (such as aggression or resource access).

b. Correlation testing between dominance behaviours and hormonal levels, reproductive success, body condition and other ecological factors such as mate/food access.

c. Social network analysis can be used to examine the structure of dominance relationships within the population if detailed social interaction data is recorded. This allows for understanding if certain females occupy central and dominant positions socially.

9. Comparative studies (Comparing with other closely related species, such as other ratites and/or comparing with other ground-dwelling species that may exhibit dominance behaviour like quail or grouse for contextualisation).

a. Phylogenetic analysis may be useful for understanding whether the behaviour is species-specific or related to patterns in avian ecology on a broader scale.

Conclusions

This study provides two novel contributions to the study of kiwi physiology and behavioural ecology. First, I found that female brown kiwi hormone levels in the breeding season are consistent with being masculinised; it highlighted the role of androstenedione in kiwi physiology, which has not previously been demonstrated or measured. Secondly, for the first time, it fully described courtship behaviour in the North Island brown kiwi. While the behaviour itself has been noted in studies before, it was not fully described and was incorrectly identified as non-breeding related aggression between individuals. Furthermore, these results are consistent with the idea that brown kiwi are a candidate to be described as a species displaying reverse sexual dimorphism and sex-role reversal, with masculinised females. These results highlight 1) the need to further examine the role of androstenedione in reproduction and courtship, 2) the need to further investigate the sexual dynamics of aggression and submission in kiwi courtship interactions and 3) the need to further brown kiwi for other features frequently associated with species under reverse sexual dimorphism.

References

Adams N. R. (1979). Masculinisation of the external genitalia in ewes with clover disease. *Australian veterinary journal*, *55*(1), 22–24. https://doi.org/10.1111/j.1751-0813.1979.tb09540.x

Adkins, E., K. (1977). Effects of diverse androgens on the sexual behavior and morphology of castrated male quail. *Hormones and Behavior*, *8*(2), 201-207. https://doi.org/10.1016/0018-506X(77)90037-X

Adkins-Regan, E. (1987). Hormones and sexual differentiation. In *Hormones and reproduction in fishes, amphibians, and reptiles* (pp. 1-29). Boston, MA: Springer US. Retrieved from https://link.springer.com/chapter/10.1007/978-1-4613-1869-9_1

Ah-King, M., & Ahnesjö, I. (2013). The "sex role" concept: an overview and evaluation. *Evolutionary biology*, *40*, 461-470. https://doi.org/10.1007/s11692-013-9226-7

Akesson, T. R., and Raveling, D. G. (1981). Endocrine and body weight changes of nesting and non-nesting Canada geese. Biol. Reprod. 25, 792-804. https://doi.org/10.1095/biolreprod25.4.792

Albergotti, L.C., Hamlin, H.J., McCoy, M.W., & Guillette Jr, L.J. (2009). Endocrine activity of extraembryonic membranes extends beyond placental amniotes. *PLOS ONE*, *4*(5), e5452. https://doi-org.ezproxy.massey.ac.nz/10.1371/journal.pone.0005452

Alekseevich, L.A., Lukina, N.A., Nikitin, N.S., Nekrasova, A.A., & Smirnov, A.F. (2009). Problems of sex determination in birds exemplified by *Gallus gallus domesticus*. *Russ J Genet*, *45*, 255–265. https://doi.org/10.1134/S1022795409030016

Amo, L., Avilés, J. M., Parejo, D., Peña, A., Rodríguez, J., & Tomás, G. (2012a). Sex recognition by odour and variation in the uropygial gland secretion in starlings.

Journal of Animal Ecology, *81*(3), 605-613. https://doi.org/10.1111/j.1365-2656.2011.01940.x

Amo, L., López-Rull, I., Pagán, I., & Garcia, C. M. (2012b). Male quality and conspecific scent preferences in the house finch, *Carpodacus mexicanus. Animal Behaviour*, *84*(6), 1483-1489. https://doi.org/10.1016/j.anbehav.2012.09.021

Andersson, M. (1994). "Sexual Selection." Princeton University Press, Princeton.

Angelier, F., & Chastel, O. (2009). Stress, prolactin and parental investment in birds: a review. *General and comparative endocrinology*, *163*(1-2), 142-148. https://doi.org/10.1016/j.ygcen.2009.03.028

Angelier, F., Moe, B., Blanc, S., Chastel, O., 2009. What factors drive prolactin and corticosterone responses to stress in a long-lived bird species: the snow petrel (*Pagodroma nivea*)? *Physiol. Biochem. Zool. 82*, 590-602. https://doi.org/10.1086/603634

Angelier, F., Weimerskirch, H., Dano, S., Chastel, O., 2007. Age, experience and reproductive performance in a long-lived bird: a hormonal perspective. *Behav. Ecol. Sociobiol. 61*, 611–621. https://doi.org/10.1007/s00265-006-0290-1

Antonevich, A. L., & Naĭdenko, S. V. (2007). *Zhurnal obshchei biologii*, *68*(4), 307–317. Retrieved from https://pubmed.ncbi.nlm.nih.gov/17944114/

Apfelbeck, B., & Raess, M. (2008). Behavioural and hormonal effects of social isolation and neophobia in a gregarious bird species, the European starling (*Sturnus vulgaris*). *Hormones and behavior*, *54*(3), 435-441. https://doi.org/10.1016/j.yhbeh.2008.04.003

Arai, R. (2011). *Fish karyotypes: a check list*. Springer Science & Business Media.

Arnold, A. P., Rissman, E. F., & De Vries, G. J. (2003). Two perspectives on the origin of sex differences in the brain. *Annals of the New York Academy of Sciences*, *1007*(1), 176-188. https://doi.org/10.1196/annals.1286.018

Auchus, R. J., & Chang, A. Y. (2010). 46, XX DSD: the masculinised female. *Best Practice & Research Clinical Endocrinology & Metabolism*, *24*(2), 219-242. https://doi.org/10.1016/j.beem.2009.11.001

Bachelot, A., & Binart, N. (2007). Reproductive role of prolactin. *Reproduction*, *133*(2), 361-369. https://doi-org.ezproxy.massey.ac.nz/10.1530/REP-06-0299

Badaway, M.T., Sobeh, M., Xiao, J., & Farag, M.A. (2021). Androstenedione (a natural steroid and a drug supplement): A comprehensive review of its consumption, metabolism, health effects, and toxicity with sex differences. *Molecules, 26*(20), 6210. https://doi-org.ezproxy.massey.ac.nz/10.3390/molecules26206210

Balthazart, J., Delville, Y., Sulon, Y., & Hendrick, J. C. (1987). Plasma levels of luteinizing hormone and of five steroids in photostimulated, castrated and testosterone-treated male and female Japanese quail (*Coturnix coturnix japonica*). *Life Science Advances*, *5*, 31-36.

Bao, A.M., & Swaab, D.F. (2010). Sex differences in the brain, behavior, and neuropsychiatric disorders. *The Neuroscientist*, *16*(5), 550-565. https://doi.org/10.1177/1073858410377005

Barlow, G. W. (2005). How do we decide that a species is sex-role reversed? *The Quarterly review of biology*, *80*(1), 28-35. https://doi.org/10.1086/431022

Baroiller, J. F., & d'Cotta, H. (2016). The reversible sex of gonochoristic fish: insights and consequences. *Sexual Development*, *10*(5-6), 242-266. https://doi.org/10.1159/000452362

Barri, F. R., Navarro, J. L., Maceira, N. O., & Martella, M. B. (2005). Rearing Greater Rhea (Rhea americana) chicks: is adoption more effective than the artificial intensive system? *British Poultry Science*, *46*(1), 22-25. https://doi-org.ezproxy.massey.ac.nz/10.1080/00071660400023888

Bastock, M. (2018). *Courtship: an ethological study*. Routledge.

Bazzano, G., Leche, A., Martella, M. B., & Navarro, J. L. (2012). Efficiency of the cloacal sexing technique in greater rhea chicks (Rhea americana). *British poultry science*, *53*(3), 394-396. https://doi.org/10.1080/00071668.2012.692470

Beach, F.A. et al. (1983). Sexual cycles in female dogs treated with androgen during development. *Behav. Neural Biol. 38*, 1–31. https://doi.org/10.1016/S0163-1047(83)90339-4

Bear, A., & Monteiro, A. (2013). Both cell-autonomous mechanisms and hormones contribute to sexual development in vertebrates and insects. *Bioessays*, *35*(8), 725-732. https://doi-org.ezproxy.massey.ac.nz/10.1002/bies.201300009

Bell, A. M. (2005). Behavioural differences between individuals and populations: Causes and consequences. *Journal of Evolutionary Biology*, *18*(3), 464–473. https://doi.org/10.1111/j.1420-9101.2004.00817.x

Bell, A. M., & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). *Ecology letters*, *10*(9), 828-834. https://doi.org/10.1111/j.1461-0248.2007.01081.x

Benowitz-Fredericks, Z.M., & Hodge, M. *(2013).* Yolk androstenedione in domestic chicks (*Gallus gallus domesticus*): Uptake and sex-dependent alteration of growth and behavior. *General and Comparative Endocrinology*, *193*(1), 48-55. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.ygcen.2013.07.005

Blache, D., Barrett, C. D., & Martin, G. B. (2000). Social mating system and sexual behaviour in captive emus *Dromaius novaehollandiae*. *Emu-Austral Ornithology*, *100*(3), 161-168.Retrieved from Blache_et_al_00_Emu-libre.pdf (d1wqtxts1xzle7.cloudfront.net)

Blanckenhorn, W. U. (2005). Behavioral causes and consequences of sexual size dimorphism. *Ethology*, *111*(11), 977-1016. https://doi.org/10.1111/j.1439-0310.2005.01147.x

Blumstein, D.T., Chung, L.K., & Smith, J.E. (2013). Early play may predict later dominance relationships in yellow-bellied marmots (*Marmota flaviventris*). *Proc. R. Soc. B*, 280. http://doi.org/10.1098/rspb.2013.0485

Bokony, V., Lendvai, A. Z., Liker, A., Angelier, F., Wingfield, J. C., & Chastel, O. (2009). Stress response and the value of reproduction: are birds prudent parents?. *The American Naturalist*, *173*(5), 589-598. https://doi.org/10.1086/597610

Bonadonna, F., Miguel, E., Grosbois, V., Jouventin, P., & Bessiere, J. M. (2007). Individual odor recognition in birds: an endogenous olfactory signature on petrels' feathers?. *Journal of chemical ecology*, *33*, 1819-1829. https://doi.org/10.1007/s10886-007-9345-7

Bono, A.E.J., Whiten, A., Schaik, C., Krützen, M., Eichenberger, F., Scnider, A., & Waal, E. (2018). Payoff- and Sex-Biased social learning interact in a wild primate population. *Current Biology*, *28*(17), 2800-2805. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.cub.2018.06.015

Boogert, N. J., Reader, S. M., & Laland, K. N. (2006). The relation between social rank, neophobia and individual learning in starlings. *Animal Behaviour*, *72*(6), 1229-1239. https://doi.org/10.1016/j.anbehav.2006.02.021

Brennan, P. L. (2022). Bird genitalia. *Current Biology*, *32*(20), R1061-R1062. Retrieved from https://www.cell.com/current-biology/fulltext/S0960-9822(22)01459-2

Browne, P., Place, N. J., Vidal, J. D., Moore, I. T., Cunha, G. R., Glickman, S. E., & Conley, A. J. (2006). Endocrine differentiation of fetal ovaries and testes of the spotted hyena (*Crocuta crocuta*): timing of androgen-independent versus androgen-driven genital development. *Reproduction*, *132*(4), 649-659. https://doi.org/10.1530/rep.1.01120

Bruggeman, V., Van As, P., & Decuypere, E. (2002). Development endocrinology of the reproductive axis in the chicken embryo. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *131*(4), 839-846. https://doi-org.ezproxy.massey.ac.nz/10.1016/S1095-6433(02)00022-3

Bruning, D. F. (1973). Greater Rhea-Chick and egg delivery route. *Natural History*, *82*(3), 68-75.

Buller, W. L. (1888). *A history of the birds of New Zealand* (Vol. 1). London.

Buntin, J.D., 1996. Neural and hormonal control of parental behaviour in birds. In: Rosenblatt, J.S., Snowdon, C.T. (Eds.), Advances in the Study of Behavior, vol. 25. Academic Press, New York, pp. 161–213.

Buntin, J.D., Tesch D., 1985. Effects of intracranial prolactin administration on maintenance of incubation readiness, ingestive behaviour, and gonadal condition in ring doves. Horm. Behave. 19, 188-203. https://doi.org/10.1016/0018-506X(85)90018-2

Burghardt, G. M. (2005). *The genesis of animal play: Testing the limits*. MIT press.

Burke, W.H., & Henry, M.H. (1999). Gonadal development and growth of chickens and turkeys hatched from eggs injected with an aromatase inhibitor. *Poultry Science, 78*(7), 1019-1033. https://doi-org.ezproxy.massey.ac.nz/10.1093/ps/78.7.1019

Butler, S. J., Whittingham, M. J., Quinn, J. L., & Cresswell, W. (2006). Time in captivity, individual differences and foraging behaviour in wild-caught chaffinches. *Behaviour*, 535-548. http://www.jstor.org/stable/4536358

Cabezas, S., Carrete, M., Tella, J. L., Marchant, T. A., & Bortolotti, G. R. (2013). Differences in acute stress responses between wild-caught and captive-bred birds: a physiological mechanism contributing to current avian invasions?. *Biological invasions*, *15*, 521-527. https://doi.org/10.1007/s10530-012-0304-z

Caithness, T.A. (1971). Sexing kiwis. *International Zoo Yearbook, 11*, 206-208. https://doi.org/10.1111/j.1748-1090.1971.tb01907.x

Calder III, W. A. (1979). The kiwi and egg design: evolution as a package deal. *Bioscience, 29*(8), 461-467. https://doi.org/10.2307/1307538

Calder, W. A. (1996). *Size, function, and life history*. Courier Corporation.

Calder, W. I., Parr, C. R., & Karl, D. P. (1978). Energy content of eggs of the Brown Kiwi Apteryx australis; an extreme in avian evolution. cabidigitallibrary.org/doi/full/10.5555/19781474031

Campagna, S., Mardon, J., Celerier, A., & Bonadonna, F. (2012). Potential semiochemical molecules from birds: a practical and comprehensive compilation of the last 20 years studies. *Chemical Senses*, *37*(1), 3-25. https://doi.org/10.1093/chemse/bjr067

Campbell, D. L., Weiner, S. A., Starks, P. T., & Hauber, M. E. (2009, April). Context and control: behavioural ecology experiments in the laboratory. In *Annales Zoologici Fennici* (Vol. 46, No. 2, pp. 112-123). Finnish Zoological and Botanical Publishing Board. https://doi.org/10.5735/086.046.0204

Cassini, M. H. (2017). Role of fecundity selection on the evolution of sexual size dimorphism in mammals. *Anim. Behav*, *128*, 1-4. https://doi.org/10.1016/j.anbehav.2017.03.030

Castro, I. C., & Morris, R. (2011). *Kiwi: a natural history*. New Holland.

Castro, I., Cunningham, S. J., Gsell, A. C., Jaffe, K., Cabrera, A., & Liendo, C. (2010). Olfaction in birds: a closer look at the kiwi (Apterygidae). *Journal of Avian Biology*, *41*(3), 213-218. https://www.jstor.org/stable/25704036

Cherel, Y., Mauget, R., Lacroix, A., Gilles, J., 1994. Seasonal and fasting-related changes in circulational gonadal steroids and prolactin in king penguins, *Aptenodytes patagonicus*. Physiol. Biochem. Zool. 67, 1154–1173. https://doi.org/10.1086/physzool.67.5.30163887

Chiba, A., & Honma, R. (2011). A study on the Northern Pintail (Anas acuta) females with masculinized plumage: their prevalence, morphological and behavioral traits, and reproductive organs. *Journal of Ornithology*, *152*, 733-742. https://doi.org/10.1007/s10336-011-0654-9

Chiba, A., Sakai, H., Sato, M., Honma, R., Murata, K., & Sugimori, F. (2004). Pituitary–gonadal axis and secondary sex characters in the spontaneously masculinized pintail, *Anas acuta* (Anatidae, Aves), with special regard to the gonadotrophs. *General and Comparative Endocrinology*, *137*(1), 50-61. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.ygcen.2004.02.015

Christians, J. K., & Williams, T. D. (1999). Effects of exogenous 17β-estradiol on the reproductive physiology and reproductive performance of European starlings (Sturnus vulgaris). *Journal of Experimental Biology*, *202*(19), 2679-2685. https://doi.org/10.1242/jeb.202.19.2679

Clinton, M., & Haines, L. C. (2001). An overview of factors influencing sex determination and gonadal development in birds. *Genes and Mechanisms in Vertebrate Sex Determination*, 97-115. https://doi.org/10.1007/978-3-0348-7781-7

Clinton, M., Zhao, D., Nandi, S., & McBride, D. (2012). Evidence for avian cell autonomous sex identity (CASI) and implications for the sex-determination process. *Chromosome Research*, *20*(1), 177-190. https://doi-org.ezproxy.massey.ac.nz/10.1007/s10577-011-92

Clutton-Brock, T. H., Harvey, P. H., & Rudder, B. (1977). Sexual dimorphism, socionomic sex ratio and body weight in primates. *Nature*, *269*(5631), 797-800. https://doi.org/10.1038/269797a0

Clutton-Brock, T. H., & Vincent, A. C. J. (1991). Sexual selection and the potential reproductive rates of males and females. *Nature*, 351(6321), 58–60. https://doi.org/10.1038/351058a0

Clutton-Brock, T.H., Brotherton, P.N.M., O'Riain, M.J., Griffin, A.S., Gaynor, D., Kansky, R., Sharpe, L., & McIlrath, G.M. (2001). Contributions to cooperative rearing in meerkats. *Animal Behavior*, *61*(4), 705-710. https://doi.org/10.1006/anbe.2000.1631

Coddington, C.H., & Cockburn, A. (1995). The Mating System of Free-living Emus. *Australian Journal of Zoology*, *43*, 365-372. Retrieved from https://sci-hub.ru/10.1071/zo9950365

Codenotti, T. L., & Álvarez, F. (1997). Cooperative breeding between males in the Greater Rhea *Rhea americana*. *Oecologia*, *46*, 55-62. Retrieved from https://digital.csic.es/bitstream/10261/61815/1/ibis.pdf

Codenotti, T. L., & Alvarez, F. (1998). Adoption of Unrelated Young by Greater Rheas (Adopción de Pollos Extraños por el Ñandü *Rhea americana*). *Journal of Field Ornithology*, 58-65. Retrieved from https://www.jstor.org/stable/4514287

Codenotti, T. L., & Alvarez, F. (2001). Mating behavior of the male Greater Rhea. *The Wilson Bulletin*, *113*(1), 85-89. Retrieved from Codenotti_2001_MatingRhea-libre.pdf (d1wqtxts1xzle7.cloudfront.net)

Colbourne, R. (2002). Incubation behaviour and egg physiology of kiwi (*Apteryx* spp.) in natural habitats. *New Zealand Journal of Ecology*, *26*(2), 129-138. Retrieved from https://www.jstor.org/stable/24055315

Colbourne, R., Bean, E., Coad, N., Fuchs, R., Graham, I., Robertson, H., & Scrimgeour, J. (2020). Kiwi best practice manual. *Wellington, Department of Conservation*.

Colbourne, R., & Digby, A. P. (2016). *Call rate behaviour of brown kiwi (Apteryx mantelli) and great spotted kiwi (A. haastii) in relation to temporal and environmental parameters*. Publishing Team, Department of Conservation. Retrieved from https://www.doc.govt.nz/Documents/science-and-technical/drds348entire.pdf

Colbourne, R., Bassett, S., Billing, T., McCormick, H., McLennan, J., Nelson, A., & Robertson, H. (2005). The development of Operation Nest Egg as a tool in the conservation management of kiwi. *Science for conservation*, *259*, 24. Department of Conservation Science for Conservation.

Conley, A., Place, N. J., Legacki, E. L., Hammond, G. L., Cunha, G. R., Drea, C. M., ... & Glickman, S. E. (2020). Spotted hyenas and the sexual spectrum: reproductive endocrinology and development. *Journal of Endocrinology*, *247*(1), 27-44. https://doi.org/10.1530/joe-20-0252

Conte, F.A., Grumbach, M.M., Ito, Y., Fisher, C.R., & Simpson, E.R. (1994). A syndrome of female pseudohermaphrodism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). *The Journal of clinical endocrinology and metabolism*, *78*(6), 1287–1292. https://doi.org/10.1210/jcem.78.6.8200927

Corfield, J. R., Eisthen, H. L., Iwaniuk, A. N., & Parsons, S. (2014). Anatomical specializations for enhanced olfactory sensitivity in kiwi, Apteryx mantelli. *Brain Behavior and Evolution*, *84*(3), 214-226. https://doi.org/10.1159/000365564

Corfield, J., Gillman, L., & Parsons, S. (2008). Vocalizations of the North Island brown kiwi (Apteryx mantelli). *The Auk*, *125*(2), 326-335. https://doi.org/10.1525/auk.2008.06234

Correa, S. M., Adkins-Regan, E., & Johnson, P. A. (2005). High progesterone during avian meiosis biases sex ratios toward females. *Biology Letters*, *1*(2), 215-218. https://doi.org/10.1098/rsbl.2004.0283

Criscuolo, F., Bertile, F., Durant, J.M., Raclot, T., Gabrielsen, G.W., Massemin, S., Chastel, O., 2006. Body size and clutch size may modulate prolactin and corticosterone levels in eiders. Physiol. Biochem. Zool. 79, 514-521. https://doi.org/10.1086/501065

Criscuolo, F., Chastel, O., Gabrielsen, G.W., Lacroix, A., Le Maho, Y., 2002. Factors affecting plasma concentrations of prolactin in the common eider *Somateria mollissima*. Gen. Comp. Endocrinol. 125, 399–409. https://doi.org/10.1006/gcen.2001.7767

Culbert, J., Sharp, P. J., & Wells, J. W. (1977). Concentrations of androstenedione, testosterone and LH in the blood before and after the onset of spermatogenesis in the cockerel. *Reproduction*, *51*(1), 153-154. https://doi.org/10.1530/jrf.0.0510153

Cunha, G. R., Place, N. J., Baskin, L., Conley, A., Weldele, M., Cunha, T. J., ... & Glickman, S. E. (2005). The ontogeny of the urogenital system of the spotted hyena (*Crocuta crocuta Erxleben*). *Biology of reproduction*, *73*(3), 554-564. https://doi.org/10.1095/biolreprod.105.041129

Cunningham, S. J., & Castro, I. (2011). The secret life of wild brown kiwi: studying behaviour of a cryptic species by direct observation. *New Zealand Journal of Ecology*, 209-219. Retrieved from https://www.jstor.org/stable/24060731

Cunningham, S., Castro, I., & Alley, M. (2007). A new prey-detection mechanism for kiwi (Apteryx spp.) suggests convergent evolution between paleognathous and neognathous birds. *Journal of Anatomy*, *211*(4), 493-502. https://doi.org/10.1111/j.1469-7580.2007.00786.x

Dallman, M. F., Akana, S. F., Scribner, K. A., Bradbury, M. J., Walker, C. D., Strack, A. M., & Cascio, C. S. (1992). Stress, feedback and facilitation in the hypothalamo-pituitary-adrenal axis. *Journal of neuroendocrinology*, *4*(5), 517-526. https://doi.org/10.1111/j.1365-2826.1992.tb00200.x

Dash, S. K., Malik, H. N., & Mohapatra, S. K. (2014). Gender identification in emu (*Dromaius novaehollandiae*)-a review. *Agricultural Reviews*, *35*(4), 271-278. Retrieved from indianjournals.com/ijor.aspx?target=ijor:ar&volume=35&issue=4&article=004

Davies, C.S., Smyth, K.N., Greene, L.K., Walsh, D.A., Mitchell, J., Clutton-Brock, T., & Drea, C.M. (2016). Exceptional endocrine profiles characterise the meerkat: sex, status, and reproductive patterns. *Scientific Reports*, *6*, 35492. https://doi-org.ezproxy.massey.ac.nz/10.1038/srep35492

Davies, S.J. 2002. Ratites and tinamous. Oxford: Oxford University Press.

Davies, S.J.J.F. (1976). *The natural history of the Emu in comparison with that of other ratites*. In: Proceedings of the 16th International Ornithology Congress, 1974. Canberra: Australian Academy of Science. 109-20. Retrieved from: http://hdl.handle.net/102.100.100/304466

de Matos, R. (2008). Adrenal steroid metabolism in birds: anatomy, physiology, and clinical considerations. *Veterinary Clinics of North America: Exotic Animal Practice*, *11*(1), 35-57. https://doi.org/10.1016/j.cvex.2007.09.006

de Oliveira, C. A., & Mahecha, G. A. B. (2000). Morphology of the copulatory apparatus of the spotted tinamou Nothura maculosa (Aves: Tinamiformes). *Annals of Anatomy-Anatomischer Anzeiger*, *182*(2), 161-169. https://doi.org/10.1016/S0940-9602(00)80077-1

de Souza Valentim, F. C., Porto, J. I. R., Bertollo, L. A. C., Gross, M. C., & Feldberg, E. (2013). XX/XO, a rare sex chromosome system in Potamotrygon freshwater stingray from the Amazon Basin, Brazil. *Genetica*, *141*, 381-387. https://doi.org/10.1007/s10709-013-9737-2

Del Giudice, M. (2012). Fetal programming by maternal stress: insights from a conflict perspective. *Psychoneuroendocrinology*, *37*, 1614– 1629. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.psyneuen.2012.05.014

Dent, J., & Molles, L. (2015). Sexually dimorphic vocalisations of the great spotted kiwi (*Apteryx haastii*). https://hdl.handle.net/10182/7375

Deviche, P., Wingfield, J.C., Sharp, P., 2000. Year-class differences in the reproductive system, plasma prolactin and corticosterone concentrations, and onset of prebasic molt in male dark-eyed juncos (*Junco hyemalis*) during the breeding period. *Gen. Comp. Endocrinol. 118*, 425–435. https://doi.org/10.1006/gcen.2000.7478

Diamond, J., & Bond, A. B. (2003). A comparative analysis of social play in birds. *Behaviour*, *140*(8-9), 1091–1115. https://doi.org/10.1163/156853903322589650

Dickens, M. J., & Bentley, G. E. (2014). Stress, captivity, and reproduction in a wild bird species. *Hormones and Behavior*, *66*(4), 685-693. https://doi.org/10.1016/j.yhbeh.2014.09.011

Diego, G. I. L. (2003). Golden eggs: maternal manipulation of offspring phenotype by egg androgen in birds. *Ardeola*, *50*(2), 281-294. https://doi-org.ezproxy.massey.ac.nz/10.1086/519397

Digby, A., Bell, B. D., & Teal, P. D. (2013). Vocal cooperation between the sexes in Little Spotted Kiwi *Apteryx owenii*. *Ibis*, *155*(2), 229-245. https://doi.org/10.1111/ibi.12031

Digby, Andrew. (2013). Whistling in the dark: An acoustic study of little spotted kiwi.

Dingemanse, N. J., & de Goede, P. (2004). The relation between dominance and exploratory behavior is context-dependent in wild great tits. *Behavioral Ecology*, *15*(6), 1023-1030. https://doi.org/10.1093/beheco/arh115

Dingemanse, N. J., Both, C., Drent, P. J., & Tinbergen, J. M. (2004). Fitness consequences of avian personalities in a fluctuating environment. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1541), 847-852. https://doi.org/10.1098/rspb.2004.2680

Dloniak, S. M., French, J. A., & Holekamp, K. E. (2006). Rank-related maternal effects of androgens on behaviour in wild spotted hyaenas. *Nature*, *440*(7088), 1190-1193. https://doi.org/10.1038/nature04540

Dorazi, R., Chesnel, A., & Dournon, C. (1995). Opposite sex determination of gonads in two pleurodeles species may be due to a temperature-dependent inactivation of sex chromosomes. *Journal of Heredity*, *86*(1), 28-31. https://doi.org/10.1093/oxfordjournals.jhered.a111521

Dournon, C., Guillet, F., Boucher, D., & Lacroix, J. C. (1984). Cytogenetic and genetic evidence of male sexual inversion by heat treatment in the newt Pleurodeles poireti. *Chromosoma*, *90*, 261-264. https://doi.org/10.1007/BF00287033

Dournon, C., Houillon, C. H., & Pieau, C. (1990). Temperature sex-reversal in amphibians and reptiles. *The International journal of developmental biology*, *34*(1), 81-92. https://ijdb.ehu.eus/article/2393628

Dournon, C., Houillon, C., & Szmutyk, M. (1984). Genetic demonstration of the functional reversal of the female sexual phenotype under the action of rearing temperature in the Urodele amphibian: *Pleurodeles waltlii* Michah. *Reproduction Nutrition Development*, *24*(4), 361-378. http://pascal-francis.inist.fr/vibad/index.php

Drea, C.M., Weldele, M.L., Forger, N.G., Coscia, E.M., Frank, L.G., Licht, P., & Glickman, S.E. (1998). Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). 2. Effects of prenatal anti-androgens. *Journal of Reproduction and Fertility*, *113*(1), 117-27.

Drea, C. M., Place, N. J., Weldele, M. L., Coscia, E. M., Licht, P., & Glickman, S. E. (2002). Exposure to naturally circulating androgens during foetal life incurs direct reproductive costs in female spotted hyenas, but is prerequisite for male mating. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1504), 1981-1987. https://doi.org/10.1098/rspb.2002.2109

Drea, C.M. (2007). Sex and seasonal differences in aggression and steroid secretion in *Lemur catta*: Are socially dominant females hormonally 'masculinized'? *Hormones and Behavior*, *51*(4), 555-567. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.yhbeh.2007.02.006

Drea, C.M. (2009). Endocrine mediators of masculinization in female mammals. *Current Directions in Psychological Science*, *18*(4), 221-226. https://doi-org.ezproxy.massey.ac.nz/10.1111%2Fj.1467-8721.2009.01640.x

Drea, C.M., Davies, C.S., Greene, L.K., Mitchell, J., Blondel, D.V., Shearer, C.L., Feldblum, J.T., Dimac-Stohl, K.A., Smyth-Kabay, K.N., & Clutton-Brock, T.H. (2021). An intergenerational androgenic mechanism of female intrasexual competition in the cooperatively breeding meerkat. *Nature Communications*, *12*(1), 7332. https://doi.org/10.1038/s41467-021-27496-x

Duckworth, R. A., Belloni, V., & Anderson, S. R. (2015). Cycles of species replacement emerge from locally induced maternal effects on offspring behavior in a passerine bird. *Science*, *347*(6224), 875-877. https://doi-org.ezproxy.massey.ac.nz/10.1126/science.1260154

Dunn, P.O., Whittingham, L.A. & Pitcher, T.E. (2001). Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution*, *55*(1), 161-175. https://doi.org/10.1111/j.0014-3820.2001.tb01281.x

Eens, M., & Pinxten, R. (2000). Sex-role reversal in vertebrates: behavioural and endocrinological accounts. *Behavioural Processes*, *51*(1-3), 135-147. https://doi-org.ezproxy.massey.ac.nz/10.1016/S0376-6357(00)00124-8

Eising, C. M., & Groothuis, T. G. (2003). Yolk androgens and begging behaviour in black-headed gull chicks: an experimental field study. *Animal behaviour*, *66*(6), 1027-1034. https://doi-org.ezproxy.massey.ac.nz/10.1006/anbe.2003.2287

El Halawani, M. E., & Rozenboim, I. (1993). The ontogeny and control of incubation behavior in turkeys. *Poultry Science*, *72*(5), 906-911. https://doi.org/10.3382/ps.0720906

El Halawani, M.E., Silsby, J.L., Behnke, E.J., Fehrer, S.C., 1986. Hormonal induction of incubation behaviour in ovariectomized female turkeys (*Meleagris gallopavo*). *Biol. Repro. 35*, 59-67. https://doi.org/10.1095/biolreprod35.1.59

Elbrecht, A., & Smith, R.G. (1992). Aromatase enzyme activity and sex determination in chickens. *Science*, *255*(5043), 467-470. https://doi-org.ezproxy.massey.ac.nz/10.1126/science.1734525

Emlen, S. T., & Wrege, P. H. (2004). Size dimorphism, intrasexual competition, and sexual selection in Wattled Jacana (*Jacana jacana*), a sex-role-reversed shorebird in Panama. *The Auk*, *121*(2), 391-403. Retrieved from https://academic.oup.com/auk/article-abstract/121/2/391/5562331

Fernandez, G. J., & Reboreda, J. C. (1995). Adjacent nesting and egg stealing between males of the Greater Rhea *Rhea americana*. *Journal of Avian Biology*, 321-324. https://doi-org.ezproxy.massey.ac.nz/10.2307/3677047

Fernández, G. J., & Reboreda, J. C. (2003). Male parental care in greater rheas (*Rhea americana*) in Argentina. *The Auk*, *120*(2), 418-428. https://doi-org.ezproxy.massey.ac.nz/10.1093/auk/120.2.418

Fisher, G.D. (1968). Breeding Australian cassowaries *Casuarius casuarius* at Edinburgh Zoo. *International Zoo Yearbook, 8*(1), 153-156. https://doi.org/10.1111/j.1748-1090.1968.tb00470.x

Fleay, D. (1936) Nesting of the Emu. *Emu - Austral Ornithology*, *35*(3), 202-210. https://doi.org/10.1071/MU935202

Forger, N. G., Frank, L. G., Breedlove, S. M., & Glickman, S. E. (1996). Sexual dimorphism of perineal muscles and motoneurons in spotted hyenas. *Journal of Comparative Neurology*, *375*(2), 333-343. https://doi.org/10.1002/(SICI)1096-9861(19961111)375:2%3C333::AID-CNE11%3E3.0.CO;2-W

Fournier, F., & Festa-Bianchet, M. (1995). Social dominance in adult female mountain goats. *Animal Behaviour*, *49*(6), 1449-1459.https://doi.org/10.1016/0003-3472(95)90066-7

Fowler, M. E. (1991). Comparative clinical anatomy of ratites. *Journal of Zoo and Wildlife Medicine*, 22(2), 204-227. https://www.jstor.org/stable/20095143

Frank, L. G. (1986). Social organization of the spotted hyaena Crocuta crocuta. II. Dominance and reproduction. *Animal Behaviour*, *34*(5), 1510-1527. https://doi.org/10.1016/S0003-3472(86)80221-4

Frank, L. G. (1997). Evolution of genital masculinization: why do female hyaenas have such a large 'penis'?. *Trends in Ecology & Evolution*, *12*(2), 58-62. https://doi.org/10.1016/S0169-5347(96)10063-X

Frank, L.G., Davidson, J.M., & Smith, E.R. (1985). Androgen levels in the Spotted hyaena *Crocuta crocuta:* the influence of social factors. *Journal of Zoology, 206(*4), 525-531. https://doi.org/10.1111/j.1469-7998.1985.tb03556.x

Frankand, L. G., & Glickman, S. E. (1994). Giving birth through a penile clitoris: parturition and dystocia in the spotted hyaena (*Crocuta crocuta*). *Journal of Zoology*, *234*(4), 659-665. https://doi.org/10.1111/j.1469-7998.1994.tb04871.x

Fraser, I., & Johnson, T. (2011). *Brown kiwi (Apteryx mantelli) husbandry manual*. Grey Lynn, Auckland: Kiwi Recovery Group and Zoo and Aquarium Association. From http://www.kiwisforkiwi.org/wp-content/uploads/2012/09/Brown_Kiwi_Husbandry_Manual.pdf

Freeman, M. E., Kanyicska, B., Lerant, A., & Nagy, G. (2000). Prolactin: structure, function, and regulation of secretion. *Physiological reviews*, *80*(4), 1523-1631. https://doi-org.ezproxy.massey.ac.nz/10.1152/physrev.2000.80.4.1523

Freeman, N. E., & Newman, A. E. (2018). Quantifying corticosterone in feathers: validations for an emerging technique. *Conservation Physiology*, *6*(1). https://doi.org/10.1093/conphys/coy051

French, J.A., Mustoe, A.C., Cavanaugh, J., & Birnie, A.K. (2013). The influence of androgenic steroid hormones on female aggression in 'atypical' mammals. *Phil. Trans. R. Soc., 368*, B36820130084. https://doi.org/10.1098/rstb.2013.0084

French, J.A., Smith, A.S., & Birnie, A.K. (2010). Maternal gestational androgen levels in female marmosets (*Callithrix geoffroyi*) vary across trimesters but do not vary with the sex ratio of litters. *General and Comparative Endocrinology*, *165*(2), 309-314. https://doi.org/10.1016/j.ygcen.2009.07.015

Fry, D. P. (2005). Rough-and-tumble social play in humans. *The nature of play: Great apes and humans*, 54-85. Guilford Press.

Fukami, M., Homma, K., Hasegawa, T., & Ogata, T. (2012). Backdoor pathway for dihydrotestosterone biosynthesis: Implications for normal and abnormal human sex development. *Developmental Dynamics*, *242*(4), 320-329. https://doi-org.ezproxy.massey.ac.nz/10.1002/dvdy.23892

Gabirot, M., Mardon, J., Campagna, S., West, N., Bonadonna, F., & Saunders, S. M. (2016). Guidelines for collecting and extracting avian odors in a remote field: case study of a subantarctic seabird. In: *Chemical Signals in Vertebrates, 13*, pp. 435-460. Springer International Publishing.

Gandini, G. C. M., & Keffen, R. H. (1985). Sex determination of the South African ostrich (Struthio camelus). *JS Afr Vet Assoc*, *56*, 209-210.

Garamszegi, L.Z. (2014). Female peak testosterone levels in birds tell an evolutionary story: a comment on Goyman and Wingfield. Behav. *Ecol., 25*, 700–701. https://doi.org/10.1093/beheco/aru048

Gasc, J. M., & Stumpf, W. E. (1981). Sexual differentiation of the urogenital tract in the chicken embryo: autoradiographic localization of sex-steroid target cells during development. *Development*, *63*(1), 207-223. https://doi.org/10.1242/dev.63.1.207

Gaukrodger, D.W. (1925) The Emu at Home. *Emu - Austral Ornithology*, *25*(2), 53-57. https://doi.org/10.1071/MU925053

Gemmell, N. J., Todd, E. V., Goikoetxea, A., Ortega-Recalde, O., & Hore, T. A. (2019). Natural sex change in fish. *Current topics in developmental biology*, *134*, 71-117. https://doi.org/10.1016/bs.ctdb.2018.12.014

Gil, D., Biard, C., Lacroix, A., Spottiswoode, C.N., Saino, N., Puerta, M., & Møller, A.P. (2007). Evolution of yolk androgens in birds: Development, coloniality, and sexual dichromatism. *The American Naturalist*, *169*(6), 802-819. https://doi-org.ezproxy.massey.ac.nz/10.1086/516652

Ginot, S., Claude, J., Perez, J., & Veyrunes, F. (2017). Sex reversal induces size and performance differences among females of the African pygmy mouse, *Mus minutoides*. *Journal of Experimental Biology*, *220*(11), 1947-1951. https://doi.org/10.1242/jeb.157552

Giraldo-Deck, L. M., Loveland, J. L., Goymann, W., Lank, D. B., & Küpper, C. (2024). A supergene affects androgen concentrations during early development in a bird with alternative reproductive morphs. *Hormones and Behavior*, *166*, 105645. https://doi.org/10.1016/j.yhbeh.2024.105645

Glickman, S.E., Frank, L.G., Davidson, J.M., Smith, E.R., & Siiteri, P.K. (1987). Androstenedione may organize or activate sex-reversed traits in female spotted hyenas. *PNAS*, *84*(10), 3444-3447. https://doi-org.ezproxy.massey.ac.nz/10.1073/pnas.84.10.3444

Glickman, S.E., Frank, L.G., Pavgi, S., & Licht, P. (1992). Hormonal correlates of 'masculinization' in female spotted hyaenas (*Crocuta crocuta*). 1. Infancy to sexual maturity. *Journal of Reproduction and Fertility*, *95*(2), 451-462. https://doi-org.ezproxy.massey.ac.nz/10.1530/jrf.0.0950451

Glickman, S.E., Frank, L.G., Holecamp, K.E., Smale, L., & Licht, P. (1993). Costs and benefits of “androgenization” in the female spotted hyena: the natural selection of physiological mechanisms. In. P.P.G Bateson (Eds). (pp. 87-117). Plenum Press.

Glickman, S.E., Cunha, G.R., Drea, C.M., Conley, A.J., & Place, N.J. (2006). Mammalian sexual differentiation: lessons from the spotted hyena. *Trends in Endocrinology and Metabolism, 17*(9), 349-356. https://doi.org/10.1016/j.tem.2006.09.005

Godsave, S.F., Lohmann, R., Vloet, R.P.M., & Gahr, M. (2002). Androgen receptors in the embryonic zebra finch hindbrain suggest a function for maternal androgens in perihatching survival. *Journal of Comparative Neurology, 453*(1), 57-70. https://doi-org.ezproxy.massey.ac.nz/10.1002/cne.10391

Goretskaia, M.Y., & Beme, I.R. (2021). Influence of testosterone on different aspects of bird behaviour and physiology. *Biology Bulletin, 48*, 1323-1331. https://doi.org/10.1134/S1062359021080094

Goymann, W., Kempenaers, B., & Wingfield, J.C. (2005). Breeding biology, sexually dimorphic development, and nestling testosterone concentrations of the classically polyandrous African black coucal, *Centropus grillii*. *Journal of Ornithology, 146*, 314-324. https://doi.org/10.1007/s10336-005-0004-x

Goymann, W., Wittenzellner, A., & Wingfield, J.C. (2004). Competing females and caring males. Polyandry and sex-role reversal in African Black Coucals, *Centropus grillii*. *Ethology, 110*(10), 807-823. https://doi-org.ezproxy.massey.ac.nz/10.1111/j.1439-0310.2004.01015.x

Grebe, N.M., Sheikh, A., & Drea, C.M. (2022). Integrating the female masculinization and challenge hypotheses: Female dominance, male deference, and seasonal hormone fluctuations in adult blue-eyed black lemurs (*Eulemur flavifrons*). *Hormones and Behavior, 139*, 105108. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.yhbeh.2022.105108

Gredler, M. L. (2016). Developmental and evolutionary origins of the Amniote phallus. *Integrative and Comparative Biology*, *56*(4), 694-704. https://doi.org/10.1093/icb/icw102

Grieves, L. A., Gilles, M., Cuthill, I. C., Székely, T., MacDougall-Shackleton, E. A., & Caspers, B. A. (2022). Olfactory camouflage and communication in birds. *Biological Reviews*, *97*(3), 1193-1209. https://doi.org/10.1111/brv.12837

Grieves, L. A., Gloor, G. B., Bernards, M. A., & MacDougall-Shackleton, E. A. (2019). Songbirds show odour-based discrimination of similarity and diversity at the major histocompatibility complex. *Animal behaviour*, *158*, 131-138. https://doi.org/10.1016/j.anbehav.2019.10.005

Griffith, O.W., Brandley, M.C., Whittington, C.M., Belov, K., & Thompson, M.B. (2017). Comparative genomics of hormonal signaling in the chorioallantoic membrane of oviparous and viviparous amniotes. *General and Comparative Endocrinology*, *244*, 19-29. https://doi.org/10.1016/j.ygcen.2016.04.017

Groothuis, T.G & Schwabl, H. (2002). Determinants of within-and among-clutch variation in levels of maternal hormones in black-headed gull eggs. *Functional Ecology*, *16*(3), 281-289. https://doi-org.ezproxy.massey.ac.nz/10.1046/j.1365-2435.2002.00623.x

Groothuis, T.G.G., Müller, W., Engelhardt, N.V., Carere, C., & Eising, C. (2005). Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience and Biobehavioral Reviews*, *29*(2), 329-352. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.neubiorev.2004.12.002

Groothuis, T.G.H., & Schwabl, H. (2008). Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them? *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, *363*(1497), 1647-1661. https://doi.org/10.1098/rstb.2007.0007

Groothuis, T.G.H., Hsu, B.Y., Kumar, N., & Tschirren, B. (2019). Revisiting mechanisms and functions of prenatal hormone-mediated maternal effects using avian species as a model. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*, 1770. https://doi-org.ezproxy.massey.ac.nz/10.1098/rstb.2018.0115

Groscolas, R., Lacroix, A., Robin, J.P., 2008. Spontaneous egg or chick abandonment in energy depleted king penguins: a role for corticosterone and prolactin? *Horm. Behav. 53*, 51–6. https://doi.org/10.1016/j.yhbeh.2007.08.010

Guo, Y., Falk, J. J., Medina-Madrid, J. L., & Wang, S. (2024). The mating ritual of a rainforest tinamou, Tinamus major. *bioRxiv*, 2024-04. https://doi.org/10.1101/2024.04.05.588181

Gwynne, D. T. (1991). Sexual competition among females: what causes courtship-role reversal? *Trends in Ecology & Evolution*, *6*(4), 118-121. https://doi.org/10.1016/0169-5347(91)90089-G

Haeusler, H.R. (1923). Notes on the Habits of the North Island Kiwi (*Apteryx mantelli*). *Emu Austral Ornithology*, *22*(3), 175-179. https://doi-org.ezproxy.massey.ac.nz/10.1071/MU922175

Hagelin, J. C., & Jones, I. L. (2007). Bird odors and other chemical substances: a defense mechanism or overlooked mode of intraspecific communication?. *The Auk*, *124*(3), 741-761. https://doi.org/10.1642/0004-8038(2007)124[741:BOAOCS]2.0.CO;2

Hall, M.R., 1987. External stimuli affecting incubation behavior and prolactin secretion in the duck (*Anas platyrhynchos*). *Horm. Behav. 21*, 269–287. https://doi.org/10.1016/0018-506X(87)90015-8

Haller, J. (1995). Biochemical background for an analysis of cost-benefit interrelations in aggression. *Neuroscience & Biobehavioral Reviews*, *19*(4), 599-604. https://doi.org/10.1016/0149-7634(95)00053-4

Hamilton, W. J. H., Tilson, R. L., & Frank, L. G. (1986). Sexual monomorphism in spotted hyenas, *Crocuta crocuta*. *Ethology*, *71*(1), 63-73. https://doi.org/10.1111/j.1439-0310.1986.tb00570.x

Handford, P. & Mares, M.A. (1985). The mating systems of ratites and tinamous: an evolutionary perspective. *Biological Journal of the Linnean Society*, *25*, 77-104. https://doi.org/10.1111/j.1095-8312.1985.tb00387.x

Hansford, J. P., & Turvey, S. T. (2018). Unexpected diversity within the extinct elephant birds (Aves: Aepyornithidae) and a new identity for the world's largest bird. *Royal Society open science*, *5*(9), 181295. https://doi.org/10.1098/rsos.181295

Haseltine, F. P., & Ohno, S. (1981). Mechanisms of gonadal differentiation. *Science*, *211*(4488), 1272-1278. https://doi.org/10.1126/science.7010601

Hau, M. (2007). Regulation of male traits by testosterone: Implications for the evolution of vertebrate life histories. *BioEssays*, *29*, 133–144. https://doi.org/10.1002/bies.20524

Hau, M., Ricklefs, R. E., Wikelski, M., Lee, K. A., & Brawn, J. D. (2010). Corticosterone, testosterone and life-history strategies of birds. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1697), 3203-3212. https://doi.org/10.1098/rspb.2010.0673

Heather, B., & Robertson, H. (2005). The Field Guide to New Zealand Birds. Penguin, Auckland, New Zealand.

Hegyi, G. and Schwabl, H. (2010). Do different yolk androgens exert similar effects on the morphology or behaviour of Japanese quail hatchlings *Coturnix japonica? Journal of Avian Biology*, 41, 258-265. https://doi.org/10.1111/j.1600-048X.2009.04787.x

Hegyi, G., Herényi, M., Szöllősi, E., Rosivall, B., Török, J & Groothuis, T. (2011). Yolk androstenedione, but not testosterone, predicts offspring fate and reflects parental quality. *Behavioral Ecology*, *22*, 29-38. http://dx.doi.org/10.1093/beheco/arq165

Heimovics, S. A., Trainor, B. C., & Soma, K. K. (2015). Rapid effects of estradiol on aggression in birds and mice: the fast and the furious. *Integrative and comparative biology*, *55*(2), 281-293. https://doi.org/10.1093/icb/icv048

Heitzmann, L. D., Challe, M., Perez, J., Castell, L., Galibert, E., Martin, A. O., Rimbault, M., & Veyrunes, F. (2023). Genotypic sex shapes maternal care in the

African Pygmy mouse, *Mus minutoides*. *Proceedings of the Royal Society B, 290*(2006), 20231224. https://doi.org/10.1098/rspb.2023.1224

Henriksen, R., Rettenbacher, S., & Groothuis, T. G. (2011). Prenatal stress in birds: pathways, effects, function and perspectives. *Neuroscience & Biobehavioral Reviews, 35*(7), 1484-1501. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.neubiorev.2011.04.010

Herborn, K. A., Macleod, R., Miles, W. T., Schofield, A. N., Alexander, L., & Arnold, K. E. (2010). Personality in captivity reflects personality in the wild. *Animal Behaviour, 79*(4), 835-843. https://doi.org/10.1016/j.anbehav.2009.12.026

Herpin, A., & Schartl, M. (2015). Plasticity of gene-regulatory networks controlling sex determination: of masters, slaves, usual suspects, newcomers, and usurpators. *EMBO Rep, 16*(10), 1260–74. https://doi.org/10.15252/embr.201540667

Higgins, Greg. (2009). Emu Fight 027. [Image]. Flickr. https://www.flickr.com/photos/27468976@N02/3381544176/in/album-72157615843368774

Higgins, Greg. (2009). Emu Fight 044. [Image]. Flickr. https://www.flickr.com/photos/27468976@N02/3381590012/in/album-72157615843368774

Higgins, Greg. (2009). Emu Fight 048. [Image]. Flickr. https://www.flickr.com/photos/27468976@N02/3381599244/in/album-72157615843368774

Hrdy, S. B. (2009). *The woman that never evolved: With a new preface and bibliographical updates*. Harvard University Press.

Hutson, J. M. (2020). Hormones Regulating Sex Development. Disorders: Differences of Sex Development: An Integrated Approach to Management, 39-47.

Hutson, J. M., Ikawa, H., & Donahoe, P. K. (1982). Estrogen inhibition of Mullerian inhibiting substance in the chick embryo. *Journal of Pediatric Surgery, 17*(6), 953-959. https://doi.org/10.1016/S0022-3468(82)80474-0

Ioannidis, J., Taylor, G., Zhao, D., & Clinton, M. (2021). Primary sex determination in birds depends on DMRT1 dosage, but gonadal sex does not determine adult secondary sex characteristics. *PNAS, 118*(10). https://doi-org.ezproxy.massey.ac.nz/10.1073/pnas.2020909118

Jaarsveld, A.S.V., & Skinner, J.D. (1991). Plasma androgens in spotted hyaenas (*Crocuta crocuta*): influence of social and reproductive development. *Reproduction*, *93*(1), 195-201. https://doi-org.ezproxy.massey.ac.nz/10.1530/jrf.0.0930195

Jacob, J. (1982). The occurrence of two diester wax types in the uropygial gland secretion of the common kiwi (*Apterix australis*). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *72*(1), 161-164. https://doi.org/10.1016/0305-0491(82)90028-1

Janisch, J., Mitoyen, C., Perinot, E., Spezie, G., Fusani, L., & Quigley, C. (2021). Video recording and analysis of avian movements and behavior: insights from courtship case studies. *Integrative and Comparative Biology*, *61*(4), 1378-1393. https://doi.org/10.1093/icb/icab095

Janse, F., Eijkemans, M. J., Goverde, A. J., Lentjes, E. G., Hoek, A., Lambalk, C. B., ... & Norman, R. J. (2011). Assessment of androgen concentration in women: liquid chromatography–tandem mass spectrometry and extraction RIA show comparable results. *European journal of endocrinology*, *165*(6), 925-933. https://doi.org/10.1530/EJE-11-0482

Jensen, T., Jamieson, S.E., Castro, I., Gartrell, B., Cockrem, J.F., & Durrant, B. (2019). Serum prolactin and testosterone levels in captive and wild brown kiwi (*Apteryx mantelli*) during the prebreeding, breeding, and incubation periods. *Zoobiology*, *38*(3), 316-320. https://doi.org/10.1002/zoo.21484

Johnson, A. V., & Van Tienhoven, A. (1981). Plasma concentrations of corticosterone relative to photoperiod, oviposition, and ovulation in the domestic hen. *General and comparative endocrinology*, *43*(1), 10-16. https://doi.org/10.1016/0016-6480(81)90025-3

Jolly A. 1984. The puzzle of female feeding priority. In: Small MF, editor. *Female primates studies by woman primatologists*. New York: Alan R Liss. p 197-215.

Jolly, J. N. (1989). A field study of the breeding biology of the little spotted kiwi (*Apteryx owenii*) with emphasis on the causes of nest failures. *Journal of the Royal Society of New Zealand*, *19*(4), 433-448. https://doi.org/10.1080/03036758.1989.10421846

Jost, A. (1953) Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Recent Prog. Horm. Res*. 8, 379–418

Jost, A. (1972) A new look at the mechanisms controlling sex differentiation in mammals. *Johns Hopkins Med. J.* 130, 38– 53.

Juárez-Oropeza, M.A., López, V., Álvarez-Fernández, G., Gómez, Y., & Pedernera, E. (1995). Androstenedione metabolism in the indifferent stage of bovine gonad development. *Journal of Experimental Zoology*, *271*(55), 373-378. https://doi-org.ezproxy.massey.ac.nz/10.1002/jez.1402710507

Judd, H. L., & Yen, S. S. (1973). Serum androstenedione and testosterone levels during the menstrual cycle. *The Journal of Clinical Endocrinology & Metabolism*, *36*(3), 475-481. https://doi.org/10.1210/jcem-36-3-475

Jukema, J., & Piersma, T. (2006). Permanent female mimics a lekking shorebird. *Biology Letters*, *2*(2), 161-164. https://doi.org/10.1098/rsbl.2005.0416

Kaiser, S., & Sachser, N. (1998). The social environment during pregnancy and lactation affects the female offsprings' endocrine status and behaviour in guinea pigs. *Physiology & behavior*, *63*, 361-6. http://dx.doi.org/10.1016/S0031-9384(97)00435-6

Kaiser, S., & Sachser, N. (2005). The effects of prenatal social stress on behaviour: mechanisms and function. *Neuroscience & Biobehavioral Reviews*, *29*(2), 283-294. https://doi.org/10.1016/j.neubiorev.2004.09.015

Kaiser, S., Kruijver, F.P.M., Swaab, D.F., & Sachser, N. (2003). Early social stress in female guinea pigs induces a masculinization of adult behavior and

corresponding changes in brain and neuroendocrine function. *Behav. Brain Res.*, *144*, 199–210. https://doi.org/10.1016/S0166-4328(03)00077-9

Kappeler, P. M., Fichtel, C., & Radespiel, U. (2022). The island of female power? Intersexual dominance relationships in the lemurs of Madagascar. *Frontiers in Ecology and Evolution*, *10*, 858859. https://doi.org/10.3389/fevo.2022.858859

Kappeler, PM. 1993. *Female dominance in primates and other mammals.* In Bateson PPG, Klopfer PH, Thompson NS, editors. Perspectives in ethology vol. 10: behavior and evolution. New York: Plenum Press. p 143-158.

Kaufmann, J. H. (1983). On the definitions and functions of dominance and territoriality. *Biological reviews*, *58*(1), 1-20. https://doi.org/10.1111/j.1469-185X.1983.tb00379.x

Kelley, D.B. (1988). Sexually dimorphic behaviors. *Annual Review of Neuroscience*, *11*, 225-251. https://doi.org/10.1146/annurev.ne.11.030188.001301

Kimwele, C. N., & Graves, J. A. (2003). A molecular genetic analysis of the communal nesting of the ostrich (*Struthio camelus*). *Molecular Ecology*, *12*(1), 229-236. https://doi-org.ezproxy.massey.ac.nz/10.1046/j.1365-294X.2003.01727.x

Kinsky, F.C. (1971). The consistent presence of paired ovaries in the Kiwi (*Apteryx*) with some discussion of this condition in other birds. *Journal für Ornithologie*, *112*, 334-357. https://doi.org/10.1007/BF01640692

Kraus, C., Pfannkuche, K., Trillmich, F., & Groothuis, T. G. G. (2008, April). *High maternal androstenedione levels during pregnancy in a small precocial mammal with female genital masculinisation*. (Working Paper No. 2008-017). https://www.demogr.mpg.de/papers/working/wp-2008-017.pdf

Kruuk, H. (1972). *The Spotted Hyaena: A Study of Predation and Social Behavior.* Chicago University Press.

Kumar, N., van Dam, A., Permentier, H., van Faassen, M., Kema, I., Gahr, M. & Groothuis, T. G. (2019). Avian yolk androgens are metabolized rather than taken up by the embryo during the first days of incubation. *Journal of*

Experimental Biology, 222, 193961. https://doi-org.ezproxy.massey.ac.nz/10.1242/jeb.193961

Kumar, N., van Faassen, M., Kema, I., Gahr, M. & Groothuis, T. G. (2018). Early embryonic modification of maternal hormones differs systematically among embryos of different laying order: a study in birds. *General and Comparative Endocrinology, 269*, 53– 59. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.ygcen.2018.08.014

Kummrow, M. S. (2015). Ratites or Struthioniformes: Struthiones, Rheae, Cassuarii, Apteryges (ostriches, rheas, emus, cassowaries, and kiwis), and Tinamiformes (tinamous). *Fowler's Zoo and Wild Animal Medicine, Volume 8*, 75. https://doi.org/10.1016%2FB978-1-4557-7397-8.00009-8

Küpper, C., Stocks, M., Risse, J. E., Dos Remedios, N., Farrell, L. L., McRae, S. B., ... & Burke, T. (2016). A supergene determines highly divergent male reproductive morphs in the ruff. *Nature genetics*, *48*(1), 79-83. https://doi.org/10.1038/ng.3443

Kuroiwa A. (2017). Sex-Determining Mechanism in Avians. *Advances in experimental medicine and biology*, *1001*, 19–31. https://doi.org/10.1007/978-981-10-3975-1_2

Kvarnemo, C., & Ahnesjo, I. (1996). The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution*, *11*(10), 404-408. https://doi.org/10.1016/0169-5347(96)10056-2

Lábaque, M. C., Navarro, J. L., & Martella, M. B. (1999). A note on chick adoption: a complementary strategy for rearing rheas. *Applied Animal Behaviour Science*, *63*(2), 165-170. https://doi-org.ezproxy.massey.ac.nz/10.1016/S0168-1591(99)00006-4

Lambeth, L. S., & Smith, C. A. (2012). Disorders of sexual development in poultry. *Sexual Development*, *6*(1-3), 96-103. https://doi.org/10.1159/000334059

Lambeth, L. S., Morris, K. R., Wise, T. G., Cummins, D. M., O'Neil, T. E., Cao, Y., ... & Smith, C. A. (2016). Transgenic chickens overexpressing aromatase have

high estrogen levels but maintain a predominantly male phenotype. *Endocrinology*, *157*(1), 83-90. https://doi.org/10.1210/en.2015-1697

Lawrence, B. M., O'Donnell, L., Smith, L. B., & Rebourcet, D. (2022). New Insights into Testosterone Biosynthesis: Novel Observations from HSD17B3 Deficient Mice. *International Journal of Molecular Sciences*, *23*(24), 15555. http://dx.doi.org/10.3390/ijms232415555

Lèche, A., Bazzano, G., Hansen, C., Navarro, J. L., Marin, R. H., & Martella, M. B. (2014). Stress in wild Greater Rhea populations Rhea americana: effects of agricultural activities on seasonal excreted glucocorticoid metabolite levels. *Journal of Ornithology*, *155*, 919-926. https://doi.org/10.1007/s10336-014-1074-4

Leclaire, S., Strandh, M., Mardon, J., Westerdahl, H., & Bonadonna, F. (2017). Odour-based discrimination of similarity at the major histocompatibility complex in birds. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1846), 20162466. https://doi.org/10.1098/rspb.2016.2466

Lehrman, D.S., 1963. On the initiation of incubation behavior in doves. Anim. Behav. 11, 433-438. https://psycnet.apa.org/doi/10.1016/0003-3472(63)90257-4

Lerner, R.M & Foch, T.T. (1987). Gonadal and adrenal hormone correlates of adjustment in early adolescence. In Nottelmann, E.D., Susman, E.J., Blue, J.H., Inoff-Germain, G., Dorn, L.D., Loriaux, D.L., Cutler, G.B., & Chrousos (Eds). *Biological-psychosocial interactions in early adolescence* (pp. 303-323). Routledge Press.

Licht, P., Frank, L. G., Pavgi, S., Yalcinkaya, T. M., Siiteri, P. K., & Glickman, S. E. (1992). Hormonal correlates of 'masculinization 'in female spotted hyaenas (*Crocuta crocuta*). 2. Maternal and fetal steroids. *Reproduction*, *95*(2), 463-474. https://doi.org/10.1530/jrf.0.0950463

Licht, P., Hayes, T., Tsai, P., Cunha, G., Kim, H., Golbus, M., Hayward, S., Martin, M.C., Jaffe, R.B., & Glickman, S.E. (1998). Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). 1. Urogenital morphology and

placental androgen production during fetal life. *Journal of Reproduction and Fertility*, *113*(1), 105-116. https://doi.org/10.1530/jrf.0.1130105

Lindeque, M., & Skinner, J.D. (1982). Fetal androgens and sexual mimicry in spotted hyaenas (*Crocuta crocuta*). *Journal of Fertility and Reproduction*, *65*, 405-410. https://doi-org.ezproxy.massey.ac.nz/10.1530/jrf.0.0650405

Lipshutz, S. E., & Rosvall, K. A. (2020). Neuroendocrinology of sex-role reversal. *Integrative and Comparative Biology*, *60*(3), 692-702. https://doi.org/10.1093/icb/icaa046

Lofts, B. & Holmes, W.N. (1981). Current trends in comparative endocrinology. In Massa, R. & Sharp, P.J (Eds). *Steroidal hormones and the control of gonadotropin secretion in male birds* (pp. 199-264). Hong Kong University Press.

Long, J.L. (1959) Some Notes on the Emu in the Northern Wheatbelt of Western Australia. *Emu - Austral Ornithology*, *59*(4), 275-286. https://doi.org/10.1071/MU959275

Lormée, H., Jouventin, P., Lacroix, A., Chastel, O., 2000. Reproductive endocrinology of tropical seabirds: sex-specific patterns in LH, steroids, and prolactin secretion in relation to parental care. *Gen. Comp. Endocrinol. 117*, 413–426. https://doi.org/10.1006/gcen.1999.7434

Loveland, J. L., Giraldo-Deck, L. M., & Kelly, A. M. (2022). How inversion variants can shape neural circuitry: Insights from the three-morph mating tactics of ruffs. *Frontiers in Physiology*, *13*, 1011629. https://doi.org/10.3389/fphys.2022.1011629

Loveland, J. L., Giraldo-Deck, L. M., Lank, D. B., Goymann, W., Gahr, M., & Küpper, C. (2021). Functional differences in the hypothalamic-pituitary-gonadal axis are associated with alternative reproductive tactics based on an inversion polymorphism. *Hormones and Behavior*, *127*, 104877. https://doi.org/10.1016/j.yhbeh.2020.104877

Luttge, W. G., & Whalen, R. E. (1970). Dihydrotestosterone, androstenedione, testosterone: comparative effectiveness in masculinizing and defeminizing reproductive systems in male and female rats. *Hormones and behavior*, *1*(4), 265-281. https://doi.org/10.1016/0018-506X(70)90020-6

Maestripieri, D. & Mateo, J. M. (2009). The role of maternal effects in mammalian evolution and adaptation. In *Maternal Effects in Mammals* (eds D. Maestripieri and J. M. Mateo), pp. 1–10. The University of Chicago Press, Chicago.

Major, A. T., & Smith, C. A. (2016). Sex reversal in birds. *Sexual Development*, *10*(5-6), 288-300. https://doi.org/10.1159/000448365

Marley, C. L., Pollard, T. M., Barton, R. A., & Street, S. E. (2022). A systematic review of sex differences in rough and tumble play across non-human mammals. *Behavioral Ecology and Sociobiology*, *76*(12), 158. https://doi.org/10.1007/s00265-022-03260-z

Marolf, B., McElligott, A. G., & Müller, A. E. (2007). Female social dominance in two Eulemur species with different social organizations. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *26*(3), 201-214. https://doi.org/10.1002/zoo.20135

Martella, M. B., Renny, M., Chiappero, M. B., & Navarro, J. L. (2022). Promiscuity in the Greater Rhea: a genetic approach. *acta ethologica*, *25*(3), 155-164. https://doi.org/10.1007/s10211-022-00398-x

Martin, G. R., Wilson, K. J., Wild, J. M., Parsons, S., Kubke, M. F., & Corfield, J. (2007). Kiwi forego vision in the guidance of their nocturnal activities. *Plos one*, *2*(2), e198. https://doi.org/10.1371/journal.pone.0000198

Martins, T. L., Roberts, M. L., Giblin, I., Huxham, R., and Evans, M. R. (2007). Speed of exploration and risk-taking behavior are linked to corticosterone titres in zebra finches. Horm. Behav. 52, 445–453. https://doi.org/10.1016/j.yhbeh.2007.06.007

Massa, R. (1984). Patterns and biological significance of steroidal hormone metabolism in birds. *Journal of Experimental Zoology*, *232*(3), 531-537. https://doi-org.ezproxy.massey.ac.nz/10.1002/jez.1402320320

Massa, R., Davies, D.T., & Bottoni, L. (1980). Cloacal gland of the Japanese quail: Androgen dependence and metabolism of testosterone. *Journal of Endocrinology, 84*(2), 223-230. https://doi-org.ezproxy.massey.ac.nz/10.1677/joe.0.0840223

Matthews, L.H. (1939). Reproduction of the spotted hyaena (*Crocuta crocuta* Erxleben). *Philos. Trans. R. Soc. London Ser. B,* 230, 1–78. https://doi.org/10.1098/rstb.1939.0004

McCarthy, M. M., & Ball, G. F. (2008). The neuroendocrine control of sex specific behavior in vertebrates: lessons from mammals and birds. *Current topics in developmental biology*, *83*, 213-248. https://doi.org/10.1016/S0070-2153(08)00407-9

McGivern, R. F., Fatayerji, N., & Handa, R. J. (1996). Androstenedione synergizes with stress or prenatal drug exposure to retard fetal growth: role of IGF. *Pharmacology Biochemistry and Behavior*, *55*(4), 549-557. https://doi-org.ezproxy.massey.ac.nz/10.1016/S0091-3057(96)00249-3

Metcalfe, N. B., & Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in ecology & evolution*, *16*(5), 254–260. https://doi.org/10.1016/s0169-5347(01)02124-3

Miles, J. R. G., & Castro, I. (2000). Survey of Northern brown kiwi (Apteryx mantelli) on Ponui Island, Hauraki Gulf–1999. *Unpublished Report. Department of Conservation, New Zealand*.

Mills, M.G.L. (1990). Kalahari Hyaenas: Comparative Behavioural Ecology of Two Species. London: Unwin Hyman.

Miltiadous, A., Callahan, D. L., Dujon, A. M., Buchanan, K. L., & Rollins, L. A. (2024). Maternally derived avian corticosterone affects offspring genome-wide DNA methylation in a passerine species. *Molecular Ecology*, e17283. https://doi.org/10.1111/mec.17283

Miyazawa, E., Seguchi, A., Takahashi, N., Motai, A., & Izawa, E. I. (2020). Different patterns of allopreening in the same-sex and opposite-sex interactions of

juvenile large-billed crows (*Corvus macrorhynchos*). *Ethology*, *126*(2), 195-206. https://doi.org/10.1111/eth.12992

Møller, A. P., Garamszegi, L. Z., Gil, D., Hurtrez-Bousses, S., & Eens, M. (2005). Correlated evolution of male and female testosterone profiles in birds and its consequences. *Behavioral Ecology and Sociobiology*, *58*, 534-544. https://doi.org/10.1007/s00265-005-0962-2

Moore, C. R. (1925). Sex determination and sex differentiation in birds and mammals. *The American Naturalist*, *59*(661), 177-189. Retrieved from https://www.jstor.org/stable/2456357

Mori, E., Mazza, G., & Lovari, S. (2022). Sexual dimorphism. In *Encyclopedia of animal cognition and behavior* (pp. 6389-6395). Cham: Springer International Publishing. Retrieved from https://link.springer.com/referenceworkentry/10.1007/978-3-319-55065-7_433

Mostrom, M., & Evans, T. J. (2011). Phytoestrogens. In *Reproductive and developmental toxicology* (pp. 707-722). Academic Press. https://doi.org/10.1016/B978-0-12-382032-7.10052-9

Muehlenbein, M. P., & Watts, D. P. (2010). The costs of dominance: testosterone, cortisol and intestinal parasites in wild male chimpanzees. *BioPsychoSocial medicine*, *4*, 1-12. https://doi.org/10.1186/1751-0759-4-21

Mueller, H.C. (1990). The evolution of reversed sexual dimorphism in size in monogamous species of birds. *Biological Reviews*, *65*(4), 553-585. https://doi.org/10.1111/j.1469-185X.1990.tb01238.x

Myers, B. M., Rankin, D. T., Burns, K. J., Brelsford, A., & Clark, C. J. (2022). k-mer analysis shows hybrid hummingbirds perform variable, transgressive courtship sequences. *Animal Behaviour*, *186*, 67-84. https://doi.org/10.1016/j.anbehav.2022.01.018

Nagahama, Y., Chakraborty, T., Paul-Prasanth, B., Ohta, K., & Nakamura, M. (2021). Sex determination, gonadal sex differentiation, and plasticity in] vertebrate

species. *Physiological reviews*, *101*(3), 1237-1308. https://doi.org/10.1152/physrev.00044.2019

Nakamura, M. (2013). Is a sex-determining gene(s) necessary for sex-determination in amphibians? Steroid hormones may be the key factor. *Sexual Development*, *7*(1-3), 104-114. https://doi.org/10.1159/000339661

Neri, R.O. (1977). *Studies on the biology and mechanism of action of nonsteroidal anti-androgens.* In Androgens and Antiandrogens (Martini, L. and Motta, L., eds), pp. 179–189. Raven Press.

Neumann, F., Elger, W., & Steinbeck, H. (1970). Antiandrogens and reproductive development. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *259*(828), 179-186. https://doi.org/10.1098/rstb.1970.0057

Nevitt, G. A., & Prada, P. A. (2015). The chemistry of avian odors: an introduction to best practices. *Handbook of olfaction and gustation*, 565-578. https://doi.org/10.1002/9781118971758.ch24

NIWA. (n.d.). *Climate and weather map - North*. National Institute of Water and Atmospheric Research. https://niwa.co.nz/climate-and-weather/map-north

Nolazco, S., Delhey, K., Nakagawa, S., & Peters, A. (2022). Ornaments are equally informative in male and female birds. *Nature communications*, *13*, 5917. https://doi.org/10.1038/s41467-022-33548-7

O'Dwyer, T.W., Buttemer, W.A., Priddel, D.M., Downing, J.A., 2006. Prolactin, body condition and the costs of good parenting: an interyear study in a long-lived seabird, Gould's petrel (*Pterodroma leucoptera*). Funct. Ecol. 20, 806–811. http://www.jstor.org/stable/3806588

Ogilvy-Stuart, A. L., & Brain, C. E. (2004). Early assessment of ambiguous genitalia. *Archives of disease in childhood*, *89*(5), 401-407. https://doi.org/10.1136/adc.2002.011312

Owen, R. (1879). *Memoirs on the extinct wingless birds of New Zealand, with an appendix on those of England, Australia, Newfoundland, Mauritius, and*

Rodriguez. London, Jon van Voorst. Retrieved from http://hdl.handle.net/2152/16251

Owens, I. P., & Short, R. V. (1995). Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends in Ecology & Evolution*, *10*(1), 44-47. https://doi.org/10.1016/S0169-5347(00)88967-3

Paitz, R. T., Bowden, R. M. & Casto, J. M. (2010). Embryonic modulation of maternal steroids in European starlings (*Sturnus vulgaris*). *Proceedings of the Royal Society B: Biological Sciences, 278*, 99– 106. https://doi-org.ezproxy.massey.ac.nz/10.1098/rspb.2010.0813

Paletta, P., Bass, N., Aspesi, D., Choleris, E. (2022). *Sex Differences in Social Cognition*. In: Current Topics in Behavioral Neurosciences. Springer, Berlin, Heidelberg. https://doi-org.ezproxy.massey.ac.nz/10.1007/7854_2022_325

Pedernera, E., Gómora, M.J., Meneses, I., De Ita, M., & Méndez, C. (2017). Androgen receptor is expressed in mouse cardiomyocytes at prenatal and early postnatal developmental stages. *BMC Physiology*, *17*(1), 3294. https://doi.org/10.1186/s12899-017-0033-8

Pellis, S. M. (2002). Sex differences in play fighting revisited: traditional and nontraditional mechanisms of sexual differentiation in rats. *Archives of sexual behavior*, *31*, 17-26. https://doi.org/10.1023/A:1014070916047

Pereira, A. E., Kaufinanº, R., Kappelerº, P. M., & Overdorff, D. J. (1990). Female Dominance Does Not Characterize All of the Lemuridae. *Folia Primato*, *55*, 96-103.

Perfito, N., Zann, R. A., Bentley, G. E., and Hau, M. (2007). Opportunism at work: habitat predictability affects reproductive readiness in free-living zebra finches. *Funct. Ecol. 21*, 291–301. https://doi.org/10.1111/j.1365-2435.2006.01237.x

Petty, J.M.A., & Drea, C.M. (2015). Female rule in lemurs is ancestral and hormonally mediated. *Scientific Reports*, *5*, 9631. https://doi-org.ezproxy.massey.ac.nz/10.1038/srep09631

Picar, C. (2022a). Photo 239423821. [Image]. INaturalist. https://inaturalist.nz/photos/239423821

Picar, C. (2022b). Photo 239423855. [Image]. INaturalist. https://inaturalist.nz/photos/239423855

Pick, J.L., Hutter, P., Tschirren, B. (2017). Divergent artificial selection for female reproductive investment has a sexually concordant effect on male reproductive success. *Evolution Letters, 1*(4), 222–228. https://doi-org.ezproxy.massey.ac.nz/10.1002/evl3.21

Pieau, C., Dorizzi, M., & Richard-Mercier, N. (1999). Temperature-dependent sex determination and gonadal differentiation in reptiles. *Cellular and Molecular Life Sciences, 55*, 887-900. https://doi-org.ezproxy.massey.ac.nz/10.1007/s000180050342

Pike, T. W., Samanta, M., Lindström, J., & Royle, N. J. (2008). Behavioural phenotype affects social interactions in an animal network. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1650), 2515-2520. https://doi.org/10.1098/rspb.2008.0744

Pilz, K.M., & Smith, H.G. (2004). Egg yolk androgen levels increase with breeding density in the European Starling, *Sturnus vulgaris*. *Functional Ecology, 18*(1), 58-66. https://doi.org/10.1111/j.1365-2435.2004.00811.x

Place, N. J., & Glickman, S. E. (2004). Masculinization of female mammals: lessons from nature. *Hypospadias and Genital Development*, 243-253. https://doi.org/10.1007/978-1-4419-8995-6_15

Place, N. J., Holekamp, K. E., Sisk, C. L., Weldele, M. L., Coscia, E. M., Drea, C. M., & Glickman, S. E. (2002). Effects of prenatal treatment with antiandrogens on luteinizing hormone secretion and sex steroid concentrations in adult spotted hyenas, *Crocuta crocuta*. *Biology of reproduction*, *67*(5), 1405-1413. https://doi.org/10.1095/biolreprod.102.004226

Pohl-Apel, G. (1985). The correlation between the degree of brain masculinization and song quality in estradiol treated female zebra finches. *Brain Research, 336*(2), 381-383. https://doi-org.ezproxy.massey.ac.nz/10.1016/0006-8993(85)90673-0

Pool, K. R., Chazal, F., Smith, J. T., & Blache, D. (2022). Estrogenic pastures: A source of endocrine disruption in sheep reproduction. *Frontiers in Endocrinology*, *13*, 880861. https://doi.org/10.3389/fendo.2022.880861

Potter, M.A. (1989). *Ecology and reproductive biology of the North Island Brown kiwi (Apteryx australis mantelli)* [Doctoral dissertation, Massey University]. https://mro.massey.ac.nz/handle/10179/3148

Potter, M.A., & Cockrem, J.F. (1992). Plasma levels of sex steroids in the North Island brown kiwi (*Apteryx australis mantelli*) in relation to time of year and stages of breeding. *General and Comparative Endocrinology*, *87*(3), 416-424. https://doi-org.ezproxy.massey.ac.nz/10.1016/0016-6480(92)90049-P

Pozis-Francois, O., Zahavi, A., & Zahavi, A. (2004). Social play in Arabian babblers. *Behaviour*, *141*(4), 425-450. http://www.jstor.org/stable/4536139

Prevedel, L. (2012). Photo 165169435. [Image]. INaturalist. https://inaturalist.nz/photos/165169435

Promislow, D.E.L., Montgomerie, R., & Martin, T.E. (1992). Mortality costs of sexual dimorphism in birds. *Proceedings of the Royal Society of London B*, *250*(1328), 143-150. https://doi.org/10.1098/rspb.1992.0142

Racey, P.A., & Skinner, J.D. (1979). Endocrine aspects of sexual mimicry in Spotted hyaenas *Crocuta crocuta*. *Journal of Zoology*, *187*(3), 315-326. https://doi.org/10.1111/j.1469-7998.1979.tb03372.x

Raikow, R. J. (1969). Sexual and agonistic behavior of the Common Rhea. *The Wilson Bulletin*, 196-206. http://www.jstor.org/stable/4159838

Rajchard, J. (2007). Intraspecific and interspecific chemosignals in birds: a review. *VETERINARNI MEDICINA-PRAHA-*, *52*(9), 385. https://doi.org/10.17221/2000-VETMED

Ralls, K. (1976). Mammals in which females are larger than males. *The Quarterly Review of Biology*, *51*(2), 245-276. https://doi.org/10.1086/409310

Ralls, K., & Mesnick, S. (2009). Sexual dimorphism. In *Encyclopedia of marine mammals* (pp. 1005-1011). Academic Press. Retrieved from https://repository.si.edu/bitstream/handle/10088/8059/nzp_Sexual_Dimorphism.pdf?sequence=1

Rehder, N. B., Bird, D. M., & Lague, 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), 386-393. https://doi.org/10.1016/0016-6480(86)90048-1

Reid, B., & Williams, G. R. (1975). The kiwi. In *Biogeography and ecology in New Zealand* (pp. 301-330). Dordrecht: Springer Netherlands.

Reid, B., Ordish, R. G., & Harrison, M. (1982). An analysis of the gizzard contents of 50 North Island brown kiwis, *Apteryx australis mantelli*, and notes on feeding observations. *New Zealand Journal of Ecology*, 76-85. https://www.jstor.org/stable/24052678

Renfree, M. B., & Short, R. V. (1988). Sex determination in marsupials: evidence for a marsupial—eutherian dichotomy. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *322*(1208), 41-53. https://doi.org/10.1098/rstb.1988.0112

Reynolds, S. M., Castro, I., Alley, M. R., & Cunningham, S. J. (2017). *Apteryx* spp. (Kiwi) possess an uropygial gland: anatomy and pathology. Eur J Anat, 21(2), 125-139.

Rhijn, J. V. (1991). The ruff. Individuality in a gregarious wading bird.

Riechert, J., Becker, P.H., Chastel, O., 2014. Regulation of breeding behaviour: do energy-demanding periods induce a change in prolactin or corticosterone baseline levels in the common tern (*Sterna hirundo*). Physiol. Biochem Zool. 87, 420-431. https://doi.org/10.1086/675682]

Roberts, M. L., Buchanan, K. L., Bennett, A. T. D., and Evans, M. R. (2007). Mate choice in zebra finches: does corticosterone play a role? Anim. Behav. 74, 921–929. https://doi.org/10.1016/j.anbehav.2006.12.021

Robertson, H. A., Colbourne, R., & McLennan, J. (2017). *Kiwi best practice manual* (p. 116). Wellington, New Zealand: Department of Conservation. Retrieved from https://web.archive.org/web/20180412130143id_/http://www.doc.govt.nz/Documents/science-and-technical/sap262entire.pdf

Roco, Á. S., Ruiz-García, A., & Bullejos, M. (2021). Testis development and differentiation in amphibians. *Genes*, *12*(4), 578. http://dx.doi.org/10.3390/genes12040578

Romanoff AL. 1960. *The Avian Embryo: Structural and Functional Development.* Macmillan, New York.

Romero, L. M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in ecology & evolution*, *19*(5), 249-255. https://doi.org/10.1016/j.tree.2004.03.008

Romero, L., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol. 128*, 1–24. https://doi.org/10.1016/S0016-6480(02)00064-3

Rosen, G. J., De Vries, G. J., Villalba, C., Weldele, M. L., Place, N. J., Coscia, E. M., ... & Forger, N. G. (2006). Distribution of vasopressin in the forebrain of spotted hyenas. *Journal of Comparative Neurology*, *498*(1), 80-92. https://doi.org/10.1002/cne.21032

Ruuskanen, S. (2015). Hormonally mediated maternal effects in birds: lessons from the flycatcher model system. *General and Comparative Endocrinology*, *224*, 283-293. https://doi-org.ezproxy.massey.ac.nz/10.1016/j.ygcen.2015.09.016

Sachser, N., & Kaiser, S. (1996). Prenatal social stress masculinizes the females' behaviour in guinea pigs. *Physiology & behavior*, *60*, 589-94. http://dx.doi.org/10.1016/0031-9384(96)00051-0

Sachser, N., Hennessy, M. B., & Kaiser, S. (2011). Adaptive modulation of behavioural profiles by social stress during early phases of life and adolescence. *Neuroscience & Biobehavioral Reviews*, *35*(7), 1518-1533. https://doi.org/10.1016/j.neubiorev.2010.09.002

Sakata, N., Tamori, Y., & Wakahara, M. (2005). P450 aromatase expression in the temperature-sensitive sexual differentiation of salamander (Hynobius retardatus) gonads. *The International journal of developmental biology*, *49*(4), 417-425. https://doi.org/10.1387/ijdb.041916ns

Sales, J. (2005). The endangered kiwi: A review. *Folia Zoologica*, *54*(1/2), 1–20. https://www.proquest.com/docview/206353860

Salvante, K. G., and Williams, T. D. (2003). Effects of corticosterone on the proportion of breeding females, reproductive output and yolk precursor levels. *Gen. Comp. Endocrinol. 130,* 205–214. https://doi.org/10.1016/S0016-6480(02)00637-8

Salvante, K. G., Walzem, R. L., and Williams, T. D. (2007). What comes first, the zebra finch or the egg: temperature-dependent reproductive, physiological and behavioural plasticity in egg-laying zebra finches. *J. Exp. Biol. 210*, 1325–1334. https://doi.org/10.1242/jeb.02745

Samour, J. H., Markham, J., & Nieva, O. (1984). Sexing ratite birds by cloacal examination. *Veterinary Record (UK)*, *115*(8). https://doi.org/10.1136/vr.115.8.167

Saunders, P. A., Franco, T., Sottas, C., Maurice, T., Ganem, G., & Veyrunes, F. (2016). Masculinised behaviour of XY females in a mammal with naturally occuring sex reversal. *Scientific reports*, *6*, 22881. https://doi.org/10.1038/srep22881

Saunders, P. A., Perez, J., Rahmoun, M., Ronce, O., Crochet, P. A., & Veyrunes, F. (2014). XY females do better than the XX in the African pygmy mouse, *Mus minutoides*. *Evolution*, *68*(7), 2119-2127. https://doi.org/10.1111/evo.12387

Schlinger, B.A., Lane, N.I., Grisham, W., & Thompson, L. (1999). Androgen synthesis in a songbird: A study of Cyp17 (17α-Hydroxylase/C17,20-Lyase) activity in

the Zebra Finch. *General and Comparative Endocrinology*, *113*(1), 46-58. https://doi.org/10.1006/gcen.1998.7179

Schmid, B., Tam-Dafond, L., Jenni-Eiermann, S., Arlettaz, R., Schaud, L., Jenni, L., 2013. Modulation of the adrenocortical response to acute stress with respect to brood value, reproductive success and survival in the Eurasian hoopoe. *Oecologia*, *173*, 33–44. https://doi.org/10.1007/s00442-013-2598-7

Seddon, N., Botero, C.A., Tobias, J.A., Dunn, P.O., MacGregor, H.E.A., Rubenstein, D.R., Uy, J.A.C., Weir, J.T., Whittingham, L.A., & Safran, R.J. (2013). Sexual selection accelerates signal evolution during speciation in birds. *Proceedings of the Royal Society of London B*, *280*(1766). https://doi.org/10.1098/rspb.2013.1065

Selander, R. K. (2017). Sexual selection and dimorphism in birds. In *Sexual selection and the descent of man* (pp. 180-230). Routledge.

Shackell, G. H., Wylie, J. G., & Kelly, R. W. (1993). Effects of prolonged exposure of ewes to oestrogenic pasture 2. Occurrence of abnormalities of the external genitalia and altered mating performance. *New Zealand Journal of Agricultural Research*, *36*(4), 459-464. https://doi.org/10.1080/00288233.1993.10417747

Shanbhag, B. A., & Sharp, P. J. (1996). Immunocytochemical localization of androgen receptor in the comb, uropygial gland, testis, and epididymis in the domestic chicken. *General and Comparative Endocrinology*, *101*(1), 76-82. https://doi.org/10.1006/gcen.1996.0009

Shapiro, L.M. (2005). *Diet overlap and potential competition between North Island brown kiwi chicks (Apteryx mantelli) and ship rats (Rattus rattus) for limited resources on Ponui Island, New Zealand: a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Ecology at Massey University, Palmerston North, New Zealand* [Massey University].

Sharp, P. J., Macnamee, M. C., Sterling, R. J., Lea, R. W., Pedersen, H. C. 1988. Relationships between prolactin, LH and broody behaviour in bantam hens. J. Endocrinol. 118, 279-286. https://doi.org/10.1677/joe.0.1180279

Sharp, P. J., Dawson, A., & Lea, R. W. (1998). Control of luteinizing hormone and prolactin secretion in birds. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *119*(3), 275–282. https://doi.org/10.1016/S0742-8413(98)00016-4

Shine, R. (1989). Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *The Quarterly review of biology*, *64*(4), 419-461. https://doi.org/10.1086/416458

Shozu, M., Akasofu, K., Harada, T., & Kubota, Y. (1991). A new cause of female pseudohermaphroditism: placental aromatase deficiency. *The Journal of clinical endocrinology and metabolism*, *72*(3), 560–566. https://doi.org/10.1210/jcem-72-3-560

Siiteri, P.K. & Seron-Ferre, M. (1978). Secretion and metabolism of adrenal androgens to estrogens. *In Endocrine Functions of the Human Adrenal Cortex (James, V.H.T. et al., eds)*, pp. 251–264. Academic Press.

Silva, L. R., Lardy, S., Ferreira, A. C., Rey, B., Doutrelant, C., & Covas, R. (2018). Females pay the oxidative cost of dominance in a highly social bird. *Animal behaviour*, *144*, 135-146. https://doi.org/10.1016/j.anbehav.2018.08.006

Silver, R., 1984. Prolactin and parenting in the pigeon family. J. Exp. Zool. 232, 617-625. https://doi.org/10.1002/jez.1402320330

Silverin, B., & Wingfield, J. C. (1982). Patterns of breeding behaviour and plasma levels of hormones in a free-living population of pied flycatchers, Ficedula hypoleuca. *Journal of Zoology*, *198*(1), 117-129. https://doi.org/10.1111/j.1469-7998.1982.tb02064.x

Simpson, H.B., & Vicario, D.S. (1991). Early estrogen treatment alone causes female zebra finches to produce learned, male-like vocalizations. *Journal of Neurobiology*, *22*(7), 755-776. https://doi-org.ezproxy.massey.ac.nz/10.1002/neu.480220710

Skeate, S. T. (1985). Social play behaviour in captive white-fronted Amazon parrots Amazona albifrons. *Bird Behavior*, *6*(1), 46-48. https://doi.org/10.3727/015613885792335284

Smaga, C.R., Bock, S.L., Johnson, J.M., & Parrott, B.B. (2022). Sex determination and ovarian development in reptiles and amphibians: From genetic pathways to environmental influences. *Sex Dev*, 1-22. https://doi.org/10.1159/000526009

Smith, C.A., Roeszler, K.N., Ohnesorg, T., Cummins, D.M., Farlie, P.G., Doran, T.J., & Sinclair, A.H. (2009). The avian Z-linked gene *DMRT1* is required for male sex determination in the chicken. *Nature*, *461*, 267-271. https://doi-org.ezproxy.massey.ac.nz/10.1038/nature08298

Sockman, K. W., Schwabl, H., & Sharp, P. J. (2000). The role of prolactin in the regulation of clutch size and onset of incubation behavior in the American kestrel. *Hormones and Behavior*, *38*(3), 168-176. https://doi.org/10.1006/hbeh.2000.1616

Sockman, K.W., Sharp, P.J., Schwabl, H., 2006. Orchestration of avian reproductive effort: an integration of the ultimate and proximate bases for flexibility in clutch size, incubation behaviour, and yolk androgen deposition. *Biol. Rev., 81,* 629–666. https://doi.org/10.1017/S1464793106007147

Soma, K. K., Rendon, N. M., Boonstra, R., Albers, H. E., & Demas, G. E. (2015). DHEA effects on brain and behavior: insights from comparative studies of aggression. *The Journal of steroid biochemistry and molecular biology*, *145*, 261-272. https://doi.org/10.1016/j.jsbmb.2014.05.011

Sossinka, R. (1982). "Domestication in birds," in Avian Biology, eds D. Farner, J. King, and K. Parkes (New York, NY: Academic Press), 373–403.

Spée, M., Beaulieu, M., Dervaux, A., Chastel, O., Le Maho, Y., Raclot, T., 2010. Should I stay or should I go? Hormonal control of nest abandonment in a long-lived bird, the Adelie penguin. *Horm. Behav., 58,* 762–768. https://doi.org/10.1016/j.yhbeh.2010.07.011

Starck, J. M & Ricklefs, R.E. (1998). 3 Structural Variants and Invariants in Avian Embryonic and Postnatal Development J. Matthias Starck 3.1. Introduction Differences in the external appearance of bird hatch. *Avian growth and development: Evolution within the altricial-precocial spectrum*, (8), 59.

Steiger SS, Fidler AE, Kempenaers B. (2009). Evidence for increased olfactory receptor gene repertoire size in two nocturnal bird species with well-developed olfactory ability. *BMC Evol Biol, 9*,117.

Steiger SS, Fidler AE, Valcu M, Kempenaers B. (2008). Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? *Proc Biol Sci 275*, 2309–2317.

Stöwe, M., Bugnyar, T., Loretto, M. C., Schloegl, C., Range, F., & Kotrschal, K. (2006). Novel object exploration in ravens (Corvus corax): effects of social relationships. *Behavioural processes, 73*(1), 68-75. https://doi.org/10.1016/j.beproc.2006.03.015

Sun, D., Layman, T. S., Jeong, H., Chatterjee, P., Grogan, K., Merritt, J. R., Maney, D. L., & Yi, S. V. (2021). Genome-wide variation in DNA methylation linked to developmental stage and chromosomal suppression of recombination in white-throated sparrows. *Molecular Ecology, 30*(14), 3453–3467. https://doi.org/10.1111/mec.15793

Swaddle, J. P., Karubian, J., & Pruett-Jones, S. (2000). A novel evolutionary pattern of reversed sexual dimorphism in fairy wrens: implications for sexual selection. *Behavioral Ecology*, *11*(3), 345-349. https://doi.org/10.1093/beheco/11.3.345

Taborsky, B., & Taborsky, M. (1991). Social organization of North Island Brown Kiwi: Long-term pairs and three types of male spacing behaviour. *Ethology*, *89*(1), 47-62. https://doi.org/10.1111/j.1439-0310.1991.tb00292.x

Taborsky, B., & Taborsky, M. (1992). Spatial organization of the North Island Brown Kiwi Apteryx australis mantelli: sex, pairing status and territoriality. *Ibis, 134*(1), 1-10. https://doi.org/10.1111/j.1474-919X.1992.tb07222.x

Taborsky, B., & Taborsky, M. (1995). Habitat use and selectivity by the brown kiwi (Apteryx australis mantelli) in a patchy environment. *The Auk*, *112*(3), 680-689. https://doi.org/10.1093/auk/112.3.680

Taborsky, B., & Taborsky, M. (1999). The mating system and stability of pairs in kiwi Apteryx spp. *Journal of Avian Biology*, 143-151. https://doi.org/10.2307/3677123

Talbot, R. T., & Sharp, P. J. (1994). A radioimmunoassay for recombinant- derived chicken prolactin suitable for the measurement of prolactin in other avian species. *General and Comparative Endocrinology*, *96*, 361–369. https://doi.org/10.1006/gcen.1994.1191

Tanabe, Y., Nakamura, T., Fujioka, K., & Doi, O. (1979). Production and secretion of sex steroid hormones by the testes, the ovary, and the adrenal glands of embryonic and young chickens (*Gallus domesticus*). *General and Comparative Endocrinology*, *39*(1), 26-33. https://doi.org/10.1016/0016-6480(79)90189-8

Thompson, L. J., Brown, M., & Downs, C. T. (2015). The effects of long-term captivity on the metabolic parameters of a small Afrotropical bird. *Journal of Comparative Physiology B*, *185*, 343-354. https://doi.org/10.1007/s00360-015-0888-6

Tran, D., & Josso, N. (1977). Relationship between avian and mammalian anti-Müllerian hormones. *Biology of reproduction*, *16*(2), 267-273. https://doi.org/10.1095/biolreprod16.2.267

Trivers, R. L. (1976). Sexual selection and resource-accruing abilities in Anolis garmani. *Evolution*, 253-269. https://doi.org/10.2307/2407700

Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science*, *179*(4068), 90-92. https://doi.org/10.1126/science.179.4068.90

Trukhina, A.V., Lukina, N.A., Wackerow-Kouzova, N.D., & Smirnov, A.F. (2013). The variety of vertebrate mechanisms of sex determination. *Biomed Research International*, 587460. https://doi.org/10.1155/2013/587460

Undin, M., & Castro, I. (2022). Predicting breeding systems to guide conservation strategies: A kiwi example. *Ethology*, *128*(7), 538-549. https://doi.org/10.1111/eth.13286

Undin, M., Lockhart, P.J., Hills, S.F.K., Armstrong, D.P., & Castro, C. (2021). Mixed Mating in a Multi-Origin Population Suggests High Potential for Genetic Rescue in North Island Brown Kiwi, *Apteryx mantelli*. *Front. Conserv. Sci.*, 2. https://doi.org/10.3389/fcosc.2021.702128

Valdez Jr., M. B., Mizutani, M., Kinoshita, K., Fujiwara, A., Yazawa, H., Shimada, K., Namikawa, T., & Yamagata, T. (2010). Differential development of sex-related characters of chickens from the GSP and PNP/DO inbred lines after left ovariectomy. *Journal of Reproduction and Development*, 56(1), 154–161. *https://doi.org/10.1262/jrd.09-156S*

Valdez, D. J., Vera Cortez, M., Della Costa, N. S., Leche, A., Hansen, C., Navarro, J. L., & Martella, M. B. (2014). Seasonal changes in plasma levels of sex hormones in the Greater Rhea (*Rhea americana*), a South American Ratite with a complex mating system. *Plos one*, *9*(5), e97334. https://doi-org.ezproxy.massey.ac.nz/10.1371/journal.pone.0097334

van Oers, K., Klunder, M., & Drent, P. J. (2005). Context dependence of personalities: risk-taking behavior in a social and a nonsocial situation. *Behavioral Ecology*, *16*(4), 716-723. https://doi.org/10.1093/beheco/ari045

Verbeek, M. E., De Goede, P., Drent, P., & Wiepkema, P. (1999). Individual behavioural characteristics and dominance in aviary groups of great tits. *Behaviour*, *136*(1), 23-48. https://doi.org/10.1163/156853999500659

Vincent, A., Ahnesjö, I., Berglund, A., & Rosenqvist, G. (1992). Pipefishes and seahorses: are they all sex role reversed? *Trends in ecology & evolution*, *7*(7), 237-241. https://doi.org/10.1016/0169-5347(92)90052-D

Vleck, C.M., 1998. Hormonal control of incubation/brooding behavior: lessons from Wild birds. In: Proceedings of the WSPA European Poultry Conference, Israel, (pp. 163–169).

von Engelhardt, N. K., & Groothuis, T. G. (2005). Measuring steroid hormones in avian eggs. *Annals of the New York Academy of Sciences*, *1046*(1), 181-192. https://doi.org/10.1196/annals.1343.015

Walker, D., & Vaglio, S. (2021). Sampling and analysis of animal scent signals. *JoVE (Journal of Visualized Experiments)*, (168), e60902. https://doi.org/10.3791/60902

Wallace, H., Badawy, G. & Wallace, B. (1999). Amphibian sex determination and sex reversal. *Cell. Mol. Life Sci*, *55*, 901–909. https://doi.org/10.1007/s000180050343

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., Dymov, S., Szyf, M. & Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature neuroscience*, *7*(8), 847-854. https://doi.org/10.1038/nn1276

Weinstock, M. (2008). The long-term behavioural consequences of prenatal stress. *Neuroscience & Biobehavioral Reviews*, *32*(6), 1073-1086. https://doi.org/10.1016/j.neubiorev.2008.03.002

Wells, J. C. (2007). Sexual dimorphism of body composition. *Best practice & research Clinical endocrinology & metabolism*, *21*(3), 415-430. https://doi.org/10.1016/j.beem.2007.04.007

Wenig, K., Pacher, L., & Bugnyar, T. (2022). Testing the contagious nature of allopreening: Bystander ravens are affected by conspecifics' affiliative interactions. *Animal Behaviour*, *184*, 71-80. https://doi.org/10.1016/j.anbehav.2021.12.009

Wesley, K.B., & Brader, K. (2013). Video-graphic study of the behaviour of juvenile Brown kiwi *Apteryx mantelli* females at Smithsonian National Zoological Park, Washington, DC. *International Zoo Yearbook*, *48*(1), 128-138. https://doi-org.ezproxy.massey.ac.nz/10.1111/izy.12034

Wheeler, P., & Greenwood, P.J. (1983). The evolution of reversed sexual dimorphism in birds of prey. *Oikos*, *40*(1), 145-149. https://doi.org/10.2307/3544210

Whittaker, D. J., & Hagelin, J. C. (2021). Female-based patterns and social function in avian chemical communication. *Journal of chemical ecology*, *47*, 43-62. https://doi.org/10.1007/s10886-020-01230-1

Whittaker, D. J., Kuzel, M., Burrell, M. J., Soini, H. A., Novotny, M. V., & DuVal, E. H. (2019). Chemical profiles reflect heterozygosity and seasonality in a tropical lekking passerine bird. *Animal Behaviour*, *151*, 67-75. https://doi.org/10.1016/j.anbehav.2019.03.005

Widemo, F. (1998). Alternative reproductive strategies in the ruff, Philomachus pugnax: a mixed ESS?. *Animal Behaviour*, *56*(2), 329-336. https://doi.org/10.1006/anbe.1998.0792

Wilson, A.L. (2014). *The triumphs, challenges and failures of young North Island brown kiwi (Apteryx mantelli): a study of behaviour, growth, dispersal and mortality* [Master's Thesis, Massey University]. https://mro.massey.ac.nz/handle/10179/5945 '

Wilson, D. S., Coleman, K., Clark, A. B., & Biederman, L. (1993). Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): An ecological study of a psychological trait. *Journal of comparative psychology*, *107*(3), 250. https://psycnet.apa.org/doi/10.1037/0735-7036.107.3.250

Wilson, J. D., Shaw, G., Leihy, M. L., & Renfree, M. B. (2002). The marsupial model for male phenotypic development. *Trends in Endocrinology & Metabolism*, *13*(2), 78-83. https://doi.org/10.1016/S1043-2760(01)00525-2

Wilson, J.D. George, F.W., & Griffin, J.E. (1981). The hormonal control of sexual development. *Science*, 211, 1278–1284. https://doi.org/10.1126/science.7010602

Wingfield, J. C. (1984). Environmental and endocrine control of reproduction in the song sparrow, *Melospiza melodia*. *Gen. Comp. Endocrinol*, *56*, 417-424.

Wingfield, J. C., and Farner, D. S. (1978a). The endocrinology of a natural breeding population of the white-crowned sparrow (*Zonotrichia leucophrys gambelli*). *Physiol. zoo.*, *51*, 188-205. https://doi.org/10.1086/physzool.51.2.30157866

Wingfield, J. C., & Farner, D. S. (1978b). The annual cycle of plasma irLH and steroid hormones in feral populations of the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *Biology of Reproduction*, 19(5), 1046–1056. https://doi.org/10.1095/biolreprod19.5.1046

Wingfield, J. C., & Farner, D. S. (1979). Some endocrine correlates of renesting and loss of clutch or brood in the white-crowned sparrow (*Zonotrichia leucophrys gambelli*). *Gen. Camp. Endocrinol., 38*, 322-331.

Wingfield, J. C., Wacker, D. W., Bentley, G. E., & Tsutsui, K. (2018). Brain-derived steroids, behavior and endocrine conflicts across life history stages in birds: a perspective. *Frontiers in Endocrinology*, *9*, 270. https://doi.org/10.3389/fendo.2018.00270

Wingfield, J.C., Hegner, R.E., Dufty Jr, A.M., & Ball, G.F. (1990). The "challenge hypothesis': theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *American Naturalist*, *136*(6), 829-846. Retrieved from https://www.journals.uchicago.edu/doi/pdf/10.1086/285134

Wingfield, J.C., Ramenofsky, M., Hegner, R.E., & Ball, G.F. (2018). Whither the challenge hypothesis? *Horm. Behav., 123*, 104588. https://doi.org/10.1016/j.yhbeh.2019.104588

Yalan, W., Guofeng, J., Meihu, M., & Xiaole, X. (2019). Sex differences in serum steroid hormone levels during embryonic development in hen eggs. *Poultry Science*, *98*(11), 6053-6062. https://doi.org/10.3382/ps/pez270

Yalcinkaya, T.M., Siiteri, P.K., Vigne, J.L., Licht, P., Pavgi, S., Frank, L.G., & Glickman, S.E. (1993). A mechanism for virilization of female spotted hyenas *in utero*. *Science*, *260*(5116), 1929-1931. https://doi.org/10.1126/science.8391165

Yamada, G., Satoh, Y., Baskin, L. S., & Cunha, G. R. (2003). Cellular and molecular mechanisms of development of the external genitalia. *Differentiation*, *71*(8), 445-460. https://doi.org/10.1046/j.1432-0436.2003.7108001.x

Yoon, Y. D., & Kim, S. R. (1987). Luminescence Immunoassays and Their Applications for Dihydrotestosterone and Testosterone (I); Establishment of LIA. *Clinical and Experimental Reproductive Medicine*, *14*(2), 138-148.

York, J. L., Magnuson II, R. H., & Schug, K. A. (2020). On-line sample preparation for multiclass vitamin, hormone, and mycotoxin determination in chicken egg yolk using LC-MS/MS. *Food chemistry*, *326*, 126939. https://doi.org/10.1016/j.foodchem.2020.126939

Yoshimura, Y., Koike, K., & Okamoto, T. (2000). Immunolocalization of progesterone and estrogen receptors in the sperm storage tubules of laying and diethylstilbestrol-injected immature hens. *Poultry science*, *79*(1), 94-98. https://doi.org/10.1093/ps/79.1.94

Yoshioka, K., Watahiki, Y., Kanie, A., Tsujio, M., Ikadai, H., Kashimoto, T., & Mutoh, K. (2010). Morphology of the cockerel's comb after androgen administration. *British poultry science*, *51*(2), 185-194. https://doi.org/10.1080/00071661003745810

Zeiträg, C., Jensen, T. R., & Osvath, M. (2023). Play in juvenile greater rheas: different modes and their evolutionary and socio-cognitive implications. *International Journal of Play*, *12*(1), 4-19. https://doi.org/10.1080/21594937.2022.2152532

Zeller, F. J. (1971). The effects of testosterone and dihydrotestosterone on the comb, testis, and pituitary gland of the male fowl. *Reproduction*, *25*(1), 125-127. https://doi.org/10.1530/jrf.0.0250125

Ziegler, T. E., & Wittwer, D. J. (2005). Fecal steroid research in the field and laboratory: improved methods for storage, transport, processing, and analysis. *American Journal of Primatology: Official Journal of the American Society of Primatologists*, *67*(1), 159-174. https://doi.org/10.1002/ajp.20175

Ziesemann, B. (2011*). The social organisation and mating system for the brown kiwi (Apteryx mantelli): a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Ecology at Massey University, Albany, New Zealand.* Retrieved from http://hdl.handle.net/10179/2779

Glossary

17β-Hydroxysteroid Dehydrogenase (17β-HSD): An enzyme that plays a critical role in the metabolism and regulation of steroid hormones by catalysing the conversion of 17β-hydroxy steroids (such as testosterone and oestradiol) into their corresponding keto forms (such as androstenedione and oestrone). This enzyme is involved in both the activation and inactivation of various steroid hormones, influencing their biological activity.

17β-Hydroxysteroid Dehydrogenase Type 3 (HSD17B3): An enzyme that plays a critical role in the biosynthesis of testosterone, which is the primary androgen (male sex hormone). HSD17B3 specifically catalyses the conversion of androstenedione to testosterone, a crucial step in the androgen biosynthesis pathway.

3β-HSD (3β-Hydroxysteroid Dehydrogenase): An enzyme involved in the biosynthesis of steroid hormones. It catalyses the conversion of pregnenolone to progesterone and dehydroepiandrosterone (DHEA) to androstenedione, which are critical steps in the production of glucocorticoids, mineralocorticoids, and sex hormones like testosterone and oestrogens. 3β-HSD plays a key role in the synthesis of hormones produced by the adrenal glands, gonads, and placenta, influencing various physiological processes such as stress response, reproductive function, and metabolism. The enzyme is found in tissues involved in steroidogenesis, including the adrenal glands and gonads.

46, XX Disorders of Sexual Development (DSDs): A group of intersex conditions where individuals with 46, XX karyotype (genetically female) are born with ambiguous or atypical genitalia or exhibit signs of male-patterned sexual differentiation. These conditions result from disruptions in the typical processes of hormonal or gonadal development during foetal life. In these individuals, the ovaries may be present, but the external genitalia or internal reproductive organs may not match typical female structures. The causes of 46, XX DSDs can include genetic mutations, hormonal imbalances, or enzyme deficiencies that affect the production or metabolism of sex hormones such as testosterone.

5α-Androstane-3α, 17β-diol: A steroid hormone and a metabolite of testosterone. It is produced through the enzymatic conversion of testosterone by the enzyme 5α-

reductase, which reduces the hormone to its more potent form. This compound is a neurosteroid and has been shown to have various effects on the brain and nervous system, including potential roles in regulating behaviour, mood, and social interactions. It can act as an agonist at androgen receptors and may influence behavioural traits related to aggression, sexual behaviour, and stress responses.

5α-Reductase: An enzyme that converts testosterone into its more potent form, 5α-dihydrotestosterone (5α-DHT). This enzyme plays a critical role in the development of male secondary sexual characteristics, including facial hair, deep voice, and prostate enlargement. 5α-reductase is found in various tissues, including the prostate, skin, and hair follicles.

5β-Androstane-17β-ol-3-one (5β-DHT): A metabolite of dihydrotestosterone (DHT) formed by the action of the 5β-reductase enzyme. It has much less potent androgenic activity compared to 5α-DHT, and it is generally considered an inactive metabolite in the androgenic pathway.

5β-Reductase: An enzyme that catalyses the reduction of testosterone and other androgens into their 5β-reduced metabolites. Unlike 5α-reductase, which converts testosterone to the more potent 5α-dihydrotestosterone (5α-DHT), 5β-reductase produces 5β-dihydrotestosterone and other inactive or less potent metabolites. These products play a role in androgen metabolism and are involved in the clearance of androgens from the body. 5β-reductase is expressed in various tissues, including the liver, skin, and brain, and influences the overall balance of androgenic effects in the body.

Adrenal Tyrosine Hydroxylase: An enzyme found in the adrenal glands, specifically in the adrenal medulla, that catalyses the conversion of tyrosine to L-DOPA, a precursor in the biosynthesis of catecholamines such as dopamine, norepinephrine, and epinephrine. This enzyme plays a crucial role in the production of these important neurotransmitters and hormones that regulate stress responses, blood pressure, heart rate, and metabolism. Tyrosine hydroxylase is the rate-limiting enzyme in catecholamine synthesis, meaning its activity controls the overall production of these hormones. It is regulated by factors such as sympathetic nervous system activity and hormonal signals.

Adrenocorticotropic Hormone (ACTH): A peptide hormone produced and secreted by the pituitary gland, specifically by the anterior lobe (pars distalis). ACTH plays a central role in the body's response to stress and in regulating the function of the adrenal glands, particularly the production of cortisol, the body's primary stress hormone.

Allopreening: Allopreening refers to the behaviour in animals, particularly in birds and primates, where one individual grooms or cleans the body of another individual. This behaviour often serves social functions, such as strengthening bonds, reducing stress, or promoting hygiene. It is typically seen in species that live in social groups and can be reciprocal, meaning individuals take turns grooming each other. Allopreening can also help to remove parasites, dirt, and dead skin from the body of the groomed individual, contributing to the overall well-being of the group.

Altricial: A term used to describe offspring that are born or hatched in an undeveloped, helpless state, requiring extensive parental care and nurturing for survival. Altricial young are typically born or hatched blind, immobile, and unable to feed themselves, relying on their parents for warmth, protection, and nourishment until they reach a more mature developmental stage.

Androstenedione: A steroid hormone that serves as a precursor to both testosterone and oestrogen. It is produced primarily in the adrenal glands, gonads and, to a lesser extent, the placenta. In animals, androstenedione plays a role in the development of secondary sexual characteristics, such as the growth of muscle mass in males and the regulation of reproductive functions. It can also influence behaviour, including aggression and mating behaviours, due to its conversion into more potent sex hormones.

Anisogamy: A form of sexual reproduction in which there is a distinct difference in size and/or form between the male and female gametes (sex cells). In anisogamous species, the female gamete (usually an egg) is typically larger, immobile, and contains more nutrients, while the male gamete (usually a sperm) is smaller, motile, and produced in larger quantities.

Anogenital: Relating to genitals and anus (often in the case of anogenital distance, or distance between genitals and anus).

Anti-Müllerian hormone (AMH): A protein hormone responsible for the regression of Müllerian ducts in male embryos and Wolffian ducts in female embryos. Important for sexual development and regulation of ovarian follicles.

Anti-Müllerian Hormone Genes: Genes that encode the Anti-Müllerian Hormone (AMH), a key protein involved in sexual differentiation. AMH is produced by the Sertoli cells in the testes during male development and plays a crucial role in the regression of the Müllerian ducts, which would otherwise develop into female reproductive organs (such as the uterus and fallopian tubes). The presence of AMH in males contributes to the development of male reproductive structures and the suppression of female reproductive organ development.

Anxiogenic Responses: Physiological and behavioural reactions that are triggered by a stimulus or condition that induces anxiety or fear. These responses are typically characterised by increased stress levels, including changes in heart rate, blood pressure, breathing, and cortisol secretion. Anxiogenic responses can also involve behaviours such as avoidance, hypervigilance, or aggression, and are often observed when an animal perceives a threat or faces an uncertain situation.

Aromatase (CYP19): An enzyme responsible for the biosynthesis (aromatization) of androgens into oestrogens. Found in gonads (granulosa cells), brain, adipose, placenta, blood vessels, skin, and bone tissues.

Aromatase Conversion: Refers to the biochemical process in which the enzyme aromatase converts androgens (male hormones such as testosterone) into oestrogens (female hormones such as oestradiol).

Aromatised: Refers to the process by which a steroid hormone undergoes aromatization, which involves the conversion of an androgen (such as testosterone or androstenedione) into an oestrogen (such as oestradiol or oestrone) through the action of the aromatase enzyme.

CA1 Region of the Hippocampus: A specific area within the hippocampus, a part of the brain involved in memory formation, spatial navigation, and learning. The CA1 (Cornu Ammonis 1) region is one of the subfields of the hippocampus and plays a crucial role in the processing and consolidation of long-term memory. It is

particularly involved in contextual memory and episodic memory, which relate to remembering events and their associated details. The CA1 region receives inputs from the entorhinal cortex and other parts of the hippocampus and sends outputs to various brain regions, including the cortex, helping integrate and coordinate information related to memory and spatial awareness.

Cell Autonomous Sex Identity (CASI): Phenotypes are dependent on the nature of cells making up a specific tissue rather than being imposed by the type of gonad formed/exposure to gonadal products.

Challenge Hypothesis: This hypothesis suggests that testosterone promotes aggression when beneficial in mating contexts, such as mate guarding or prevention of rivals having access to mates. This hypothesis predicts that seasonal testosterone changes are a function of mating system type, paternal care, and male-male aggression interactions in seasonal breeders.

Clitoral Elongation: A biological phenomenon in certain animal species where the clitoris is unusually large or elongated, often resembling a penis.

Clitoromegaly: Abnormal enlargement of the clitoris (also known as macroclitoris).

Coccygeal Bone: The small, triangular bone located at the end of the vertebral column (tailbone) in most vertebrates.

Conserved genes: Genes that have remained relatively unchanged throughout evolution across different species due to their essential biological functions. These genes are highly conserved because they play critical roles in fundamental processes such as cell division, metabolism, and DNA repair. As a result, variations in these genes are often detrimental to the organism's survival, so they are preserved across generations and species

Corticosterone: A steroid hormone produced by the adrenal glands, primarily in amphibians, birds, and reptiles. It plays a key role in the body's stress response by regulating energy metabolism, immune function, and behavioural changes during stressful conditions. Corticosterone helps mobilize energy reserves and modulate immune

activity, but prolonged high levels can have negative effects on health and reproduction.

CYP11A1 (Cytochrome P450 family 11 subfamily A member 1; CYP17): An enzyme encoded by the CYP11A1 gene, which is primarily involved in the first step of steroid hormone synthesis. It catalyses the conversion of cholesterol into pregnenolone, the precursor for all steroid hormones, including cortisol, oestrogens, androgens, and progesterone. This enzyme is located in the mitochondria of cells in the adrenal glands, gonads, and other steroidogenic tissues. CYP11A1 is essential for proper endocrine function and plays a critical role in the production of hormones involved in metabolism, stress response, and reproductive health.

DAX1: A gene that encodes a protein that helps regulate steroidogenesis, as well as functioning as an anti-testis gene. Supports the development of normal hormone-producing tissues such as adrenal glands and pituitary.

Dehydroepiandrosterone (DHEA): A steroid hormone produced primarily by the adrenal glands, with smaller amounts produced by the gonads (ovaries and testes). DHEA serves as a precursor to both androgens (male hormones) and oestrogens (female hormones), playing a key role in the synthesis of sex hormones.

Diethylstilbesterol (DES): A synthetic nonsteroidal oestrogen that was first developed in the 1930s. It was widely prescribed to women during the mid-20th century to prevent miscarriages and preterm births, as well as to treat various hormonal issues. However, later research revealed that DES was associated with a range of adverse health effects, particularly in offspring exposed to the drug in utero.

Dihydrotestosterone (see: 5α-Dihydrotestosterone; 5α-DHT; DHT): A potent androgen derived from testosterone, playing a central role in male sexual differentiation, hair growth, and prostate function. Its binding to androgen receptors in tissues such as the prostate, hair follicles, and skin leads to the development of male secondary sexual characteristics.

DM-Domain genes: A gene family that is responsible for coding transcription factors related to sexual development and regulation.

DMRT1: A gene in the DM domain that regulates male development in vertebrates, that regulates Sertoli cells and germ cells. The majority of this protein is found in Sertoli cells and the testicular cord.

Dual Primordium: The dual primordium exists in the early stages of gonadal development and has the potential to give rise to either testes or ovaries, depending on the genetic and hormonal signals it receives during development. The differentiation process is typically influenced by sex-determining genes (such as the SRY gene in mammals) and the presence or absence of specific hormones.

Embryonic Hatch Muscle: Specialised muscle cells in certain egg-laying species, particularly in birds, reptiles, and fish. These muscles assist the embryo in breaking out of the egg during the hatching process, enabling the young animal to emerge into the outside world. These muscles are temporary and typically degenerate after hatching, as their role is no longer needed once the embryo becomes free-living.

Embryonic Mass: Refers to the total mass of the developing embryo, which includes cells, tissues, and fluids during the early stages of development. It is an important parameter used in embryology to track the growth and health of the embryo, influenced by genetic factors, nutrition, environmental conditions, and species-specific reproductive strategies.

Environmental Sex Determination (see also: External Sex Determination): Sex determination by non-genetic cues, such as light intensity, photoperiod, temperature, nutrient availability, and pheromones by surrounding animals or plants. Under environmental sex determination, once sex is determined, it is fixed and cannot be reversed. Individuals possess genetic coding for both sexes on autosomes, and exposure to environmental cues results in epigenetic changes dictating sex fate.,

Enzyme-Linked Immunosorbent Assay (ELISA): A laboratory technique used to detect and quantify specific substances such as proteins, hormones, or antibodies in a sample. ELISA works by using an enzyme-linked antibody or antigen that binds to the substance of interest. Once the target substance binds to the antibody, a substrate is added, which reacts with the enzyme to produce a colour change, indicating the presence and concentration of the target.

Exogenous Hormones: Hormones that are introduced into an organism from an external source, rather than being naturally produced by the body. These hormones can be administered through various means, such as oral ingestion, injection, or topical application.

Female Masculinisation Hypothesis: This hypothesis suggests that aggressive social dominance in females is part of traits linked to androgens and is associated with gaining benefits such as resource access via enhanced competition. It is often applied to nonseasonal species.

Female Mate Choice: The process by which females select their mating partners based on certain traits or behaviours exhibited by males. This form of sexual selection allows females to influence the genetic quality of their offspring by choosing mates with desirable characteristics, such as physical strength, attractiveness, health, or genetic compatibility. Female mate choice can drive the evolution of secondary sexual characteristics in males, such as bright coloration, elaborate courtship displays, or physical attributes like large size or ornamentation. Female mate choice plays a critical role in shaping the mating systems and reproductive strategies within a species.

Female Parental Care: A reproductive strategy in which females provide significant care, protection, and nurturing of their offspring after fertilization. Is sole if only females are providing this care.

Female Social Dominance (FSD): A social structure in which females hold higher status or greater influence within a group, often influencing access to resources, mates, and decision-making. In species with female social dominance, females may assert authority over males and other females, and this dominance can be linked to factors like size, age, or the ability to control group dynamics.

Female-Female Competition: A form of intrasexual competition where females compete with one another for access to mates, resources, or dominance within a social group. This type of competition is often driven by the need to secure the best reproductive opportunities, such as obtaining the highest-quality mate or a preferred nesting site. Female-female competition can manifest in various ways, including aggressive behaviours, mate guarding, resource monopolization, or social tactics like alliances. It

plays a significant role in shaping reproductive strategies and can lead to the evolution of traits that enhance competitive success among females, such as size, strength, or increased reproductive output.

Feminisation: Development of characteristics that are usually unique to the female of a species, often during the normal developmental stages that contribute to sexual differentiation.

Follicle-Stimulating Hormone (FSH): A hormone produced by the pituitary gland that plays a critical role in the reproductive system. In females, FSH stimulates the growth and maturation of follicles in the ovaries and promotes the production of oestrogen. In males, FSH stimulates spermatogenesis, the production of sperm, by acting on the seminiferous tubules in the testes. FSH works in conjunction with luteinizing hormone (LH) to regulate reproductive functions, with levels fluctuating throughout the menstrual cycle in females to support egg development and ovulation.

Follicular Epithelium: The layer of cells that surrounds and supports the developing oocyte (egg cell) within the follicles of the ovaries. In mammals and other vertebrates, the follicular epithelium consists of granulosa cells (in females) that play a key role in the maturation of the oocyte and the production of hormones such as oestrogens. The follicular epithelium also helps in the formation of the zona pellucida, a protective layer around the oocyte, and is involved in the coordination of hormonal signals that regulate the female reproductive cycle. This epithelium is crucial for the proper development of oocytes and successful reproduction.

Formants: Resonant frequencies in the sound spectrum produced by the vocal tract, which shape the quality and pitch of vocalizations, particularly in humans and some animals. Formants are crucial for speech and communication, as they help distinguish different vowel sounds and other vocal characteristics. In animals, formants in vocalizations can also be used for communication, signalling species identity, emotional states, or mating calls. Formants are created by the resonance of sound waves as they travel through the vocal tract, which includes the throat, mouth, and nasal passages. The specific pattern of formants is influenced by the shape and size of the vocal tract and the way air is pushed through it.

FOXL2: A transcription factor found in the FOX superfamily of genes. Plays an important role in the development of ovaries and their function; postnatally regulates the differentiation of granulosa cells and growth of pre-ovulatory follicles.

Genetic Sex Determination: Different alleles (or genes) specify male and female sexual morphology, through combinations of XY, ZW, XO, ZO chromosomes, or sometimes haplodiploidy. Sexual differentiation is typically triggered by a sex locus (main gene), which introduces a cascade of gene effects.

Genital Tubercle: A small, early developmental structure in embryos that gives rise to the external genitalia during sexual differentiation. The genital tubercle is present in both male and female embryos initially and is located between the developing hind limbs.

Genome-Wide Heterozygosity: A measure of genetic diversity within an individual or population, reflecting the proportion of heterozygous loci (locations in the genome where the two alleles are different) across the entire genome. Higher genome-wide heterozygosity indicates a greater genetic variation, which is often associated with increased fitness, adaptability, and resilience to environmental changes or diseases.

Gonadal Bipotentiality (also: bipotentiality; ambisexual): The initial phase of gonadal development has the bipotential gonad (or genital ridge) which is identical in both sexes. Cell lines that create this gonad can adopt a female or male fate and enter the second phase of development into a testis or ovary based on gene expression.

Gonadal Genotype: The genetic makeup of an individual's gonads (testes or ovaries), which influences their development and function. The gonadal genotype typically refers to the specific combination of sex chromosomes (e.g., XX or XY in mammals) or other genetic factors that determine whether the gonads will develop into male or female reproductive organs.

Gonadal Primordium (also: gonadal rudiment): The early, undifferentiated tissue from which the gonads (ovaries or testes) develop during embryonic or foetal development. The gonadal primordium consists of a cluster of cells that has the potential to differentiate into either male or female reproductive organs, depending on the genetic or environmental signals received.

Gonadectomy: A surgical procedure that involves the removal of the gonads (ovaries or testes) from an organism.

Gonadotropin-Releasing Hormone (GnRH): A hormone produced by the hypothalamus that stimulates the release of gonadotropins—luteinizing hormone (LH) and follicle-stimulating hormone (FSH)—from the pituitary gland. GnRH plays a key role in regulating reproductive function, controlling the menstrual cycle in females and the production of sperm in males. The secretion of GnRH is pulsatile and tightly regulated, influencing the release of LH and FSH, which in turn control the activity of the ovaries and testes.

Harem Polyandry: A group of males is actively defended by a female, and there is female-female competition for control of the group and access to the males.

Heterogametic: Refers to the sex that produces two different types of sex chromosomes in a species with chromosomal sex determination. Examples include XY (male mammals) and ZW (female birds).

Hexane Soluble Hydrocarbons: A group of hydrocarbons (organic compounds made up of hydrogen and carbon atoms) that are soluble in hexane, a non-polar solvent commonly used in chemical extractions. These hydrocarbons are typically lipid-based and can include various alkanes, alkenes, and aromatic compounds. In the context of animals, hexane soluble hydrocarbons can be found in preen oils, sebum, and scent-marking substances, where they may play a role in chemical signalling, territorial marking, or mate attraction.

Homomorphic: Refers to sex chromosomes that are similar in size, shape, and genetic content, meaning there is little to no visible difference between the male and female sex chromosomes. In species with homomorphic sex chromosomes, both males and females may have identical or nearly identical chromosomes that determine their sex.

Honest Chemical Signal Ornamentation: A form of communication in animal reproduction and behaviour where chemical signals, such as pheromones, reliably convey information about an individual's health, genetic quality, or reproductive status. These signals are considered "honest" because they are costly to produce or maintain, ensuring they accurately reflect the sender's condition.

Hormone Binding Affinity: The strength of the interaction between a hormone and its receptor or binding protein. It refers to how tightly a hormone binds to its receptor or target molecule, with a higher binding affinity indicating a stronger, more stable interaction. This property plays a crucial role in the hormone's biological activity and effectiveness in initiating physiological responses. Hormone binding affinity can influence the potency of a hormone and how it regulates various processes such as growth, metabolism, reproduction, and behaviour. It is typically measured through binding assays and is an important factor in understanding hormone signalling pathways and receptor sensitivity.

***In utero*:** A term used to describe events, conditions, or development that occur inside the uterus during pregnancy, before birth. It refers to the period of time from conception to birth, during which an embryo or foetus develops. In utero development is crucial for the formation of organs, tissues, and systems, and influences an individual's growth and health later in life. Hormonal influences, such as exposure to oestrogens, and environmental factors during this period can have significant effects on the animal's development, behaviour, and reproductive health once born.

Interstitial Tissue: The tissue found between the functional cells or structures of an organ or gland, providing structural support and facilitating nutrient exchange. In the context of the gonads, interstitial tissue refers to the space between the seminiferous tubules in the testes or the ovarian follicles in the ovaries. This tissue contains various cells, including Leydig cells in males (which produce testosterone) and theca cells in females (which contribute to hormone production). Interstitial tissue plays a vital role in supporting the function of the reproductive organs, including hormone synthesis, tissue regeneration, and maintaining the proper environment for gamete development.

Intrasexual Competition: A form of competition that occurs between individuals of the same sex within a species, typically for access to mates or reproductive opportunities. This competition can take various forms, such as physical combat, displays of dominance, or the production of elaborate ornaments.

Jost Model: A theoretical model used to explain the sexual differentiation observed in spotted hyenas (*Crocuta crocuta*), where females exhibit masculinised traits in comparison to males. According to the Jost Model, this unusual phenomenon is driven

by the early exposure to high levels of androgens (male hormones) during foetal development. The model suggests that, in hyenas, the foetal environment alters the development of female genitalia, resulting in females having a pseudo-phallus and other characteristics typically associated with males, such as larger body size and more aggressive behaviour. This sexual differentiation is thought to be a consequence of hormonal influences that override typical female development, leading to a unique form of sexual dimorphism in hyenas, where females are dominant in both social and reproductive contexts.

Juvenile Play: Play behaviours exhibited by young animals during their developmental stages, typically involving actions that are not directly related to survival or reproduction but are essential for learning and socialization. Juvenile play often includes activities such as chasing, wrestling, playing with objects, or role-playing behaviours that simulate adult activities. This type of play is believed to help young animals develop essential skills for foraging, predator avoidance, social interactions, and physical coordination. It also plays a role in the development of cognitive and motor functions, and in species with complex social structures, juvenile play can help establish social bonds and hierarchies.

Lekking: Gathering of male animals engaging in competitive displays and courtship rituals, designed to attract surveying females to choose partners and mate. Lekking species have male displays, strong female mate choice, indirect benefits to males and reduced female costs.

Leydig Cells: Specialized cells located in the testes that are responsible for producing testosterone, the primary male sex hormone. Leydig cells are found in the interstitial tissue between the seminiferous tubules, where sperm production occurs. Testosterone produced by Leydig cells is essential for the development of male secondary sexual characteristics (such as facial hair, deep voice, and muscle growth) and for regulating spermatogenesis (sperm production). The activity of Leydig cells is regulated by luteinizing hormone (LH) from the pituitary gland.

Liquid Chromatography-Mass Spectrometry (LC-MS): A powerful analytical technique that combines the physical separation capabilities of liquid chromatography with the molecular identification and quantification abilities of mass spectrometry. LC-MS is

used to analyse complex mixtures of compounds, such as hormones, proteins, drugs, or metabolites, by separating them based on their chemical properties (using liquid chromatography) and then identifying and quantifying them based on their mass-to-charge ratio (using mass spectrometry).

Luteinizing Hormone (LH): A hormone produced by the pituitary gland that plays a key role in the reproductive system. In females, LH triggers ovulation, the release of an egg from the ovary, and stimulates the production of progesterone by the corpus luteum after ovulation. In males, LH stimulates the testes to produce testosterone. LH works in concert with follicle-stimulating hormone (FSH) to regulate the reproductive processes. The levels of LH fluctuate during the menstrual cycle in females, with a surge in LH triggering ovulation.

Male Deference Hypothesis: A theory in behavioural ecology and evolutionary biology that suggests males may show deference or submissive behaviour toward females to increase their chances of mating success. This hypothesis proposes that by deferring to females, particularly in species where females have more control over mating opportunities or are the primary caregivers, males can avoid aggression or rejection and foster positive interactions. In some species, males may offer resources, perform rituals, or display other behaviours that signal respect or submission in hopes of gaining access to reproductive opportunities.

Male Parental Care: A reproductive strategy in which the male contributes significantly to the care, protection, and nurturing of offspring, typically after fertilization. Is sole if only the male provides this care.

Male-Male Competition: A form of intrasexual competition where males compete with each other for access to mates, resources, or reproductive opportunities. This competition can involve physical confrontations, displays of strength or dominance, or other behaviours aimed at establishing social rank or territorial control. Male-male competition is a common driver of the evolution of sexual dimorphism, where males develop traits (such as larger size, weaponry, or elaborate displays) that enhance their chances of winning these competitions.

Masculinisation: Development of characteristics that are usually unique to the male of a species, often during the normal developmental stages that contribute to sexual differentiation.

Mass-Specific Metabolic Rate: A measure of the metabolic rate (the rate at which an organism consumes energy) relative to its body mass. It is often expressed as energy consumption per unit of body mass (e.g., calories per gram per hour). This measurement allows for comparisons of metabolic efficiency between organisms of different sizes. Generally, smaller animals have a higher mass-specific metabolic rate, meaning they use energy at a faster rate relative to their body size, compared to larger animals. This is due to differences in surface area-to-volume ratios, where smaller animals lose heat more quickly and need more energy to maintain their body temperature. Mass-specific metabolic rates are important in understanding energy requirements, thermoregulation, and growth across different species.

Mate Defence Polygyny: Also known as Resource Defence Polygyny. A mating strategy where males support multiple female mates by competing with other males territorially for access to a resource. Larger territory being held leads to greater resource holding power. Female-female competition for mates is also common due to restricted male movement.

Mating Reflexes: Innate, automatic physiological and behavioural responses that are triggered in an animal during the mating process. These reflexes are typically controlled by hormonal signals and neural circuits and are essential for facilitating reproduction. Mating reflexes can include behaviours such as courtship displays, sexual postures, copulatory movements, and other species-specific actions that encourage successful mating.

Medial Preoptic Area (MPA): A region located in the preoptic area of the hypothalamus, involved in the regulation of several important physiological processes, including reproductive behaviours, sexual motivation, and thermoregulation. The MPA is critical for the integration of hormonal and neural signals that govern sexual behaviour, particularly in response to oestrogen and testosterone. It is also involved in parental behaviours, such as nesting and caregiving. The MPA plays a role in the

regulation of circadian rhythms and has connections to brain regions that control autonomic functions like heart rate and blood pressure.

Methyltestosterone: A synthetic anabolic steroid and a derivative of testosterone. Methyltestosterone is often used in medicine to treat conditions related to low testosterone levels, such as delayed puberty in males, hormone replacement therapy, or certain forms of breast cancer in females.

Müllerian Ducts: Paired structures in the embryos of vertebrates that develop into female reproductive organs, such as the uterus, fallopian tubes, and part of the vagina, in mammals. The Müllerian ducts are initially present in both male and female embryos during early development, but their fate is determined by the presence or absence of certain hormones.

Nonseasonal (also: aseasonal): A reproductive strategy in animals where mating and reproduction occur year-round, rather than being confined to specific seasons. This behaviour is often observed in species living in stable environments with consistent resources, such as tropical climates.

Nucleus Arcuatus (ARC): A small cluster of neurons located in the hypothalamus that plays a key role in regulating several physiological processes, including hormonal regulation, appetite, and energy balance. The ARC contains neurons that produce important neurotransmitters and neuropeptides, such as neuropeptide Y (NPY) and pro-opiomelanocortin (POMC), which help control feeding behaviour, metabolism, and body weight. The ARC is also involved in the regulation of the hypothalamic-pituitary-gonadal axis, influencing reproductive function by responding to signals like gonadotropin-releasing hormone (GnRH). Additionally, it has roles in circadian rhythms, stress responses, and growth.

Oestradiol (also: estradiol): The primary oestrogen produced by the ovaries in females and is involved in the regulation of the menstrual cycle, the development of female secondary sexual traits (such as breast development and widening of the hips), and the maintenance of reproductive tissues. In males, oestradiol is present in smaller amounts and plays a role in regulating sperm production and other physiological processes. Oestradiol also influences behaviours related to reproduction, such as mate selection and sexual behaviour, in both males and females.

Oestrogen Receptor-Alpha (ER-α) in the MPA, ARC, and CA1: A type of oestrogen receptor found in various regions of the brain, including the medial preoptic area (MPA), arcuate nucleus (ARC), and CA1 region of the hippocampus (CA1). ER-α is a protein that binds to oestrogen, a key hormone involved in regulating a wide range of physiological processes, including reproductive function, memory, and emotion.

Oestrone (also: estrone): plays a key role in the menstrual cycle, where it helps regulate the development of the endometrial lining and influences other reproductive processes. It is considered a weaker oestrogen compared to oestradiol but still has important biological effects. In addition to its role in reproduction, oestrone has oth6er physiological effects, such as influencing bone health, fat distribution, and cardiovascular function.

Opportunistic Breeder: An organism that breeds in response to favourable environmental conditions, rather than adhering to a specific breeding season. Opportunistic breeders take advantage of temporary conditions, such as the availability of food, water, or favourable weather, to reproduce whenever conditions are optimal. This strategy is common in species that live in unpredictable environments, where the timing of resources can vary.

Ovariectomy: A surgical procedure that involves the removal of one or both ovaries from an organism.

Oviparity: A reproductive strategy in which animals lay eggs that develop and hatch outside of the mother's body. In oviparous species, the fertilized eggs contain all the nutrients the developing embryo needs until it hatches.

Ovotestis: A gonad with both testicular and ovarian segments (often observed as a segment of testicular tissue with seminiferous tubules next to a segment of ovarian tissue with ovarian primordial follicles).

Parabiont: An organism that is part of a symbiotic relationship, specifically one where two organisms are physically connected or attached but not necessarily in a fully integrated or dependent relationship like in some other forms of symbiosis (e.g., mutualism or parasitism).

Parabiosis: The condition in which two entire living organisms are connected and share a single circulatory system. Can result from abnormal development of embryos in monozygotic twins, however, is frequently used in medical research via surgical operation.

Partial Gonadal Sex Reversal: A condition in which the gonads (the ovaries or testes) of an organism show characteristics of both male and female gonads, rather than developing fully into one sex or the other. This can occur as a result of genetic, hormonal, or environmental factors that disrupt the normal process of sexual differentiation during development.

Perinatal Period: The phase in an animal's life that encompasses the time immediately before, during, and after birth, typically covering the period from the late stages of pregnancy (often around the last few weeks of gestation) to a few weeks post-birth, depending on the species. This period is characterised by critical developmental changes, including the final stages of foetal development, birth or hatching, and the newborn's transition to independent life.

Perineum Muscles: The muscles located in the perineal region, which is the area between the genitalia and the anus. In both males and females, the perineum muscles form part of the pelvic floor and are involved in functions such as supporting pelvic organs, controlling urination and defecation, and assisting in sexual activity. These muscles include the bulbospongiosus, ischiocavernosus, and levator ani muscles, among others. In males, these muscles play a role in ejaculation, while in females, they contribute to vaginal tone and may assist in childbirth.

Phytoestrogens: Plant-derived compounds that mimic the action of oestrogen, a primary female sex hormone, in the body. These compounds can bind to oestrogen receptors and influence various biological processes, though they typically have weaker effects than animal-derived oestrogens. Phytoestrogens are found in a variety of plants, especially in soy products, flaxseeds, and certain legumes.

Play Signals: Play signals are specific behaviours or actions used by animals to indicate that their actions are part of a play activity rather than a serious or aggressive interaction. These signals help establish the context of play, allowing participants to understand that the behaviour is non-threatening and meant for enjoyment or social bonding.

Polygynandry: A type of mating system in which both males and females have multiple mating partners during a breeding season. In this system, both sexes engage in multiple pair bonds or sexual relationships with individuals of the opposite sex, and all partners may have an opportunity to reproduce.

Precocial: A term used to describe offspring that are born or hatched in a relatively mature state, capable of independent movement, feeding, and survival shortly after birth or hatching. Precocial species typically produce well-developed young with open eyes, functioning limbs, and the ability to feed themselves, requiring minimal parental care.

Pregnenolone: A steroid hormone produced primarily in the adrenal glands, gonads, and brain. It is a precursor in the biosynthesis of various other steroid hormones, including progesterone, oestrogens, androgens, cortisol, and aldosterone. Pregnenolone plays a key role in the synthesis of hormones that regulate a wide range of physiological processes, including stress response, metabolism, reproductive functions, and immune system activity.

Primordial Tissue: Cells that make up the primordium, tissue, or organs in their earliest recognizable stage of development.

Proctodeal Gland: In some vertebrate species, the proctodeal gland is an exocrine gland located near the anus or rectum, involved in the secretion of substances such as mucus or pheromones. These glands can play a role in various functions, including the lubrication of the digestive tract for easier excretion, as well as in territorial marking or communication via the release of chemical signals.

Progesterone: A steroid hormone produced primarily by the corpus luteum in the ovaries (and by the placenta during pregnancy in mammals). It plays a critical role in regulating the reproductive cycle, fertility, and pregnancy. Progesterone prepares the uterus for implantation of a fertilized egg, supports the maintenance of pregnancy, and helps regulate menstrual cycles. It also influences other physiological processes, such as immune function and nervous system activity.

Promiscuity: A mating system in which individuals' mate with multiple partners without forming long-term pair bonds or exclusive relationships. In promiscuous species, both

males and females may engage in mating with several different individuals during a breeding season.

Prosocial: Refers to behaviours exhibited by animals that benefit others within their social group, enhancing cooperation, group survival, and social bonds. These behaviours can include actions such as grooming, sharing food, cooperative hunting, protection of young, and mutual aid. Prosocial behaviours are often seen in species that live in social groups, where cooperation and reciprocal help can improve the chances of survival and reproduction.

Pseudopenis: A structurally penis-like organ found in some female animals, which resembles a male's penis but does not function in reproduction in the typical sense.

Pseudoscrotum: A structure in some female animals that resembles a scrotum but is not involved in reproductive function.

Radioimmunoassay (RIA): A laboratory technique used to measure the concentration of specific substances, such as hormones, drugs, or proteins, in a sample by using radioactive isotopes and antibodies. The method involves labelling the substance of interest with a radioactive isotope and then measuring the amount of radioactivity in a sample, which correlates to the concentration of the target molecule. The technique relies on the principle of competitive binding, where the labelled and unlabelled substances compete for binding to a specific antibody.

Ratite: A term used to describe a group of large, flightless birds that have a flat, keel-less sternum (breastbone), which distinguishes them from flying birds. The lack of a keel prevents the attachment of large flight muscles, making ratites incapable of flight. Ratites include birds such as ostriches, emus, rheas, kiwis, and cassowaries. These birds are typically characterised by strong legs adapted for running, and they are found mostly in the Southern Hemisphere. The term "ratite" comes from the Latin word *ratis*, meaning "raft," referring to the flat shape of their sternum.

Reverse Sexual Dimorphism (also: Inverse Sexual Dimorphism): Opposite of typical trends of differences between males and females of a species when it comes to traits such as body size, shape, traits, colour and parasitic loads (typically secondary sexual

characteristics). Mostly commonly used to refer to reversed sexual size dimorphism, where females of a species have a larger body size than males.

Rough-and-Tumble Play: A form of social interaction observed in juvenile animals, characterised by physical activities such as chasing, wrestling, pouncing, and play fighting. This behaviour is non-aggressive and serves important developmental purposes, including improving motor skills, establishing social hierarchies, and practicing behaviours needed for survival and reproduction.

Self-fertilisation: A reproductive strategy in which an individual organism fertilizes its own eggs with its own sperm, leading to offspring that are genetically similar to the parent. This form of reproduction is most common in hermaphroditic species, which possess both male and female reproductive organs.

Semi-Volatile Preen Oil: A type of preen oil produced by birds, characterised by its semi-volatile nature, meaning it contains compounds that are less volatile than those in volatile preen oil but can still evaporate at higher temperatures or over time. Semi-volatile components of preen oil typically include fatty acids, waxes, and terpenes. These compounds serve to condition and protect the feathers, maintaining their flexibility and water resistance. In some bird species, semi-volatile preen oil can also contain odoriferous compounds that may function in social signalling or mate selection, though they tend to have a longer-lasting presence compared to the more volatile components. These oils can also help with preening behaviours, assisting birds in the grooming and upkeep of their plumage.

Sequential Polyandry: A female mates and produces offspring with one male and then moves on to another male while the initial mate incubates and/or provides offspring care.

Sertoli Cells: Specialized somatic cells found in the testes that support and nourish developing sperm cells (spermatogenesis). Sertoli cells are located within the seminiferous tubules, where they provide structural support, secrete hormones (such as inhibin), and regulate the process of sperm development. They also form the blood-testis barrier, which helps maintain a controlled environment for sperm production by preventing harmful substances in the blood from reaching the developing sperm.

Sertoli cells play a critical role in male fertility by supporting the growth, maturation, and proper function of sperm cells throughout the process of spermatogenesis.

Sex Role Reversal: A phenomenon where the typical reproductive roles and behaviours of males and females are reversed. Males often invest more in offspring care, becoming the choosier sex in mate selection, while females compete for access to males. This reversal can involve changes in parental care, mate competition, and sexual dimorphism.

Sex Semiochemical Hypothesis: A theory proposing that chemical signals, or semiochemicals, play a critical role in sexual attraction, mate selection, and reproductive behaviours in animals. These chemical cues, which can include pheromones, allomones, and kairomones, are produced by one individual and influence the behaviour or physiology of another individual of the same or different species. According to the hypothesis, these sex-specific semiochemicals convey information about genetic compatibility, mate quality, or reproductive readiness, and are crucial for successful mating and reproduction. The Sex Semiochemical Hypothesis emphasizes the importance of chemical signalling in the sexual and reproductive strategies of many species, particularly in environments where visual or auditory signals may be less effective.

Sex-Specific Regulatory Mechanism: Biological processes or systems that operate differently in males and females, often influencing the expression or activity of certain genes, hormones, or physiological functions. These mechanisms are typically regulated by sex chromosomes, sex hormones (such as oestrogens in females and testosterone in males), and can involve differences in gene expression, metabolism, and behaviour. Sex-specific regulatory mechanisms play a crucial role in the development of secondary sexual characteristics, reproductive organs, and sexual dimorphism. These mechanisms ensure that male and female individuals develop and function in a way that is optimized for their reproductive roles. Examples include the differential expression of sex-linked genes, the regulation of gonadal hormones, and sex-specific neural circuits.

Sexual Dimorphism: Differences between males and females of a species when it comes to traits such as body size, shape, traits, colour and parasitic loads (typically secondary

sexual characteristics). Due to commonality of male-male competition in species, often results in exaggerated male traits.

Sexual Phenotype: The observable physical characteristics and traits related to an individual's sex, which result from the interaction between their genetic makeup (gonadal genotype) and the influence of hormones and environmental factors. The sexual phenotype includes characteristics such as genitalia, secondary sexual characteristics (e.g., body size, coloration, or the presence of certain structures like antlers or manes), and reproductive organs. It reflects the individual's sexual differentiation and is typically categorized as either male or female, although in some species, intersex or other variations may occur. The sexual phenotype is the result of complex genetic and hormonal pathways that determine the expression of sexually dimorphic traits.

SF1: A protein that is responsible for the formation of the bipotential gonad; also appears to support the masculinisation of Leydig and Sertoli cells.

Shared Parental Care (also: Biparental Care): A reproductive strategy in which both parents (male and female) contribute to the care, protection, and nurturing of their offspring. Shared parental care can enhance offspring survival by pooling resources and efforts from both parents, which allows for more efficient care and protection.

Sibling Competition: The competition between siblings for limited resources, such as food, parental care, or attention, which can influence their growth, survival, and development. This competition can occur both in the womb (e.g., in multiple births) and after birth or hatching. Sibling competition often arises in environments where resources are scarce, leading to differences in the survival or success of each sibling. In some species, this competition can result in siblicide (killing of siblings) or other aggressive behaviours as siblings vie for limited resources.

Simultaneous Polyandry: A female controls an area that has multiple males living with in it, and mates with all males simultaneously. Also refers to co-operative simultaneous polyandry, where a female has one nesting site where she mates with multiple males and produces offspring of mixed parentage.

Sox genes: A gene family that is responsible for coding multiple aspects of vertebrate development, including but not limited to, testis development, central nervous system neurogenesis and neural crest cell development.

SOX9: A protein gene that regulates the transcription of the AMH gene; also supports male sexual development, by supporting the production of AMH in Sertoli cells to inhibit female development.

Sympathetic Adrenomedullary Activity: The physiological process in which the sympathetic nervous system stimulates the adrenal medulla (the inner part of the adrenal glands) to release catecholamines, such as epinephrine (adrenaline) and norepinephrine (noradrenaline), into the bloodstream. This activation is a key part of the body's fight-or-flight response to stress, preparing the body for quick action. The release of these hormones increases heart rate, blood pressure, respiratory rate, and blood glucose levels, while also diverting blood flow to muscles and vital organs, enhancing physical performance in stressful situations. Sympathetic adrenomedullary activity plays a critical role in maintaining homeostasis during times of stress or danger.

Syrinx: The specialized vocal organ found in birds and some other animals (e.g., certain reptiles) that allows for the production of complex sounds, including songs and calls. The syrinx is located at the junction of the trachea and the bronchi (the main airways leading to the lungs) and is unique to birds and some non-avian species, such as certain species of frogs and some reptiles.

Teleost Fish: A large and diverse group of bony fish belonging to the subclass Teleostei, which is the largest and most advanced group within the class Actinopterygii. Teleosts include the majority of modern fish species, such as salmon, trout, goldfish, clownfish, and herring. They are characterised by a wide range of adaptations, including a mobile jaw, a swim bladder for buoyancy, and a homocercal tail (a symmetrical tail fin).

Temperature Sex Determination: A type of environmentally dependent sex determination system where ambient temperatures experienced during embryonic or larval development determines the offspring sex. Typically found in reptiles and teleost fish.

Testosterone: A steroid hormone primarily produced in the testes in males (and in smaller amounts in the ovaries and adrenal glands in females). It is the principal androgen (male sex hormone) responsible for the development of male secondary sexual characteristics, such as facial hair, deepening of the voice, muscle mass, and bone density. Testosterone also plays a key role in sperm production, libido, and behavioural traits such as aggression and dominance. In females, testosterone is involved in maintaining muscle strength, bone health, and sexual drive.

Tetrapodal Vertebrates: A group of vertebrates that possess four limbs, which are characteristic of most land-dwelling animals. The term "tetrapod" comes from Greek roots meaning "four feet," and it includes all animals descended from the first four-limbed vertebrates, which evolved from lobe-finned fish. Includes amphibians, reptiles, birds and mammals.

Thermosensitive Period: Used in reference to temperature-dependent sex determination. A period during development where sex is irreversibly determined; typically, during the middle third of incubation. After this point, sex is unresponsive to temperature, and sex reversal is impossible.

Thyroid-Stimulating Hormone (TSH): A hormone produced by the pituitary gland that regulates the production and release of thyroid hormones (T3 and T4) from the thyroid gland. TSH stimulates the thyroid to produce T3 and T4, which are essential for regulating metabolism, growth, and development in the body. The secretion of TSH is regulated by feedback mechanisms involving the levels of T3 and T4 in the bloodstream.

Tonic Immobility: A temporary state of involuntary paralysis or immobility exhibited by certain animals when they are subjected to extreme stress or perceived threats, often as a defence mechanism. During tonic immobility, the animal remains motionless and unresponsive to external stimuli, which may help it avoid detection by predators or reduce the likelihood of being attacked.

Transitional Range of Temperature: Used in reference to temperature-dependent sex determination. The range of temperatures over which sex-ratios shift from 100% male to 100% female.

Triiodothyronine/Thyroxine (T3/T4): Hormones produced by the thyroid gland that regulate the body's metabolism, growth, and development. T3 (Triiodothyronine) is the more active form of the hormone and influences many physiological processes, including heart rate, body temperature, and energy production. T4 (Thyroxine) is the precursor to T3 and is converted into T3 in various tissues of the body. T4 has a longer half-life and is primarily responsible for regulating the metabolic rate.

Unisex Species: Refers to species that consist of only one sex or exhibit a lack of sexual dimorphism, meaning they do not have distinct male and female individuals. This can occur in several forms of reproduction or biological organization. Includes parthenogenesis, hermaphroditism and clonal reproduction.

Urogenital Meatus: The external opening through which both urine and, in males, semen are expelled from the body. It serves as the exit point for both the urinary system and the reproductive system. In males, the urogenital meatus is located at the tip of the penis, while in females, it is found just above the vaginal opening and below the clitoris.

Uropygial Gland (also: preen gland): A specialized sebaceous gland found in most birds, located near the base of the tail. This gland secretes an oily substance that birds use to preen their feathers, keeping them in good condition. The oil produced by the uropygial gland helps to waterproof the feathers, maintaining their flexibility and insulating properties. It also has antibacterial and antifungal properties that protect the bird's feathers from damage and infections. In some species, the oil may contain volatile or semi-volatile compounds that play a role in mate attraction, territorial marking, or species identification through scent.

Vasopressin: A hormone produced by the hypothalamus and secreted by the posterior pituitary gland. It plays a critical role in regulating water balance in the body by promoting water retention in the kidneys. Vasopressin also influences blood pressure by causing vasoconstriction (narrowing of blood vessels), which increases blood pressure. In addition to its physiological roles, vasopressin is involved in social behaviours such as bonding, pair bonding, and territoriality in some animals.

Virilisation: Sometimes also referred to as masculinisation. The development of adult male characteristics in young males or females, typically due to androgens. Prenatally, associated with perineum closure, rugation of the scrotum, penis growth and urethral

groove closure. In postnatal (children), associated with public hair growth, accelerated body growth and bone maturation, increased strength, acne, body odour. In juvenile males, associated with precocious puberty. In genetic females, associated with clitoral enlargement, increased muscle strength, acne, hirsutism, voice deepening, anovulation, and increased libido.

Viviparity: A reproductive strategy in which offspring develop inside the mother's body and are born live, rather than being laid as eggs. In viviparous species, the embryo receives nourishment directly from the mother, typically through a placenta or other specialized structures that provide oxygen, nutrients, and waste removal during development.

Volatile Preen Oil: A type of oil produced and secreted by the preen gland (uropygial gland) found in many bird species. This oil is used by birds to coat and maintain their feathers, providing waterproofing and protection. Some volatile components of preen oil are odoriferous compounds that can serve as olfactory signals in social and mating behaviours. In certain species, the scent of preen oil may play a role in territorial marking, mate attraction, or individual identification. These volatile compounds can also act as chemical signals that influence interactions between birds, especially in species where scent communication is important for reproduction or social organization.

Wolffian Ducts: Paired structures in the early embryo that are precursors to the male reproductive system. These ducts are present in both male and female embryos during early development but differentiate based on the influence of sex hormones.

XO: Refers to a sex determination system where individuals with a single X chromosome and no second sex chromosome (XO) are typically male, and individuals with two X chromosomes (XX) are typically female. In the XO system, the absence of a second sex chromosome (the "O" represents the absence of a second sex chromosome) results in male development, while two X chromosomes lead to female development.

XX: Refers to the chromosomal pattern typically associated with female sex determination in many species, including humans. In organisms with an XX sex determination system, individuals with two X chromosomes (XX) are usually female, while individuals with one X and one Y chromosome (XY) are typically male. The presence of two X

chromosomes is linked to the development of female reproductive organs and secondary sexual characteristics.

XY: Refers to the chromosomal pattern typically associated with male sex determination in many species, including humans. In organisms with an XY sex determination system, individuals with one X chromosome and one Y chromosome (XY) are typically male, while individuals with two X chromosomes (XX) are typically female. The Y chromosome contains the SRY gene, which triggers the development of male reproductive organs and secondary sexual characteristics.

ZW: Refers to the chromosomal pattern typically associated with females in species that use the ZW sex-determination system, such as birds, some reptiles, and certain fish. In this system, individuals with one Z chromosome and one W chromosome (ZW) are typically female, while individuals with two Z chromosomes (ZZ) are typically male.

ZZ: Refers to the chromosomal pattern typically associated with males in species that use the ZW sex-determination system, such as birds, some reptiles, and certain fish. In this system, individuals with two Z chromosomes (ZZ) are typically male, while individuals with one Z chromosome and one W chromosome (ZW) are typically female.

Appendices

Appendix 1: Sources for masculinised mammals from Table 1 in Chapter 1; Female Masculinisation and Reverse Sexual Dimorphism in the Animal Kingdom; Masculinised Traits

Species	Sources
Spotted hyena (*Crocuta crocuta*)	French et al., 2013 Jaarsveld & Skinner, 1991 Drea et al., 1998; Frank et al., 1985 Glickman et al, 1987 Glickman et al., 1993 Boydston, Morelli & Holekamp, 2001 East et al., 2003 Glickman et al., 2006
Ring-tailed lemur (*Lemur catta*)	Parga, 2006 French et al., 2013 Drea, 2007 Petty & Drea, 2015
Naked mole rat (*Heterocephalus glaber*	Clarke & Faulkes, 2001 French et al., 2013
Syrian hamster (*Mesocricetus auratus*)	Gattermann et al., 2002 French et al., 2013
Californian mice (*Peromyscus californi*	Trainor et al., 2010 French et al., 2013
Rock hyrax (*Procavia capensis*)	Koren, Mokady & Geffen, 2006 French et al., 2013
Meerkat (*Suricata suricatta*)	Clutton-Brock et al., 2001 Davies et al., 2016 Drea et al., 2021

Appendix 2: Copyright log of permissions for reused illustrations, diagrams and photos in this thesis.

Details about material used (author, source, URL etc)	**Location in thesis** (e.g. chapter, page number)	**Permission required** (Yes/No)	**Permission granted** (Yes/No)	**Notes** (copyright owner contact information; details of permission request; terms & conditions that apply)
"Both cell-autonomous mechanisms and hormones contribute to sexual development in vertebrates and insects" by A. Bear & A. Monteiro, 2013, BioEssays, 35(8). Figure 4: Summary of the interconnectivity of developmental mechanisms that produce primary sexual traits and secondary sexual traits.	Chapter 1: Sexual development pathways	Yes	Yes	Licensed content publisher: John Wiley and Sons; License number: 5736250636885; License date: Feb 25, 2024; Portion: Figure/table.
Pathway of sexual development from embryo to full-formed foetus in mammals. From "Endocrine mediators of masculinization in female mammals" by C.M Drea, 2009, Current Directions in Psychological Science, 18(4), 221-226. Figure 4: Pathway of sexual development from embryo to full-formed foetus in mammals	Chapter 1; Sexual development pathways	Yes	Yes	*Copyright 2009 by SAGE publications. Specific permissions not required for this use.*
"Backdoor pathway for dihydrotestosterone biosynthesis: Implications for normal and abnormal human sex development" by M. Fukami, K. Homma, T. Hasegawa, et al., 2012, Developmental Dynamics, 242(4). Figure 5: Steroidogenic pathways in the adrenals and the placenta.	Chapter 1; Androstenedione	Yes	Yes	Licensed content publisher: John Wiley and Sons; License number: 5736251092480; License date: Feb 25th, 2024; Portion: Figure/table.
"Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them?" by Ton. G.G Groothuis and H. Schwabl, 2008, Philosophical transactions. Biological sciences, 363(1497). Figure 1: Depiction of hormone accumulation in egg yolk.	Chapter 1; Maternal Androgens and Egg Yolks; Effects of Maternal Hormones on Development	Yes	Yes	Copyright holder: The Royal Society (U.K.); Order License ID 1454089-2; Order Date 26-Feb-2024; Type of Use Republish in a thesis/dissertation; Publisher ROYAL SOCIETY; Portion Image/photo/illustration

"Serum prolactin and testosterone levels in captive and wild brown kiwi (Apteryx mantelli) during the prebreeding, breeding, and incubation periods" by B. Durrant, J.F. Cockrem, B. Gartrell, et al., 2019, Zoo Biology, 38(3). FIGURE 1 The mean ± SEM concentration of serum prolactin in wild (black) and captive (gray) female kiwi + FIGURE 2 The mean ± SEM concentration of serum prolactin in wild (black) and captive (gray) male kiwi.	Chapter 1; North Island Brown Kiwi; Hormone Cycles and Sexual Dimorphism	Yes	Yes	Licensed content publisher: John Wiley and Sons; License number: 5897320883431; License date: Oct 27, 2024; Type of use: Dissertation/thesis; Portion: Figure/table.
From "Comparative clinical anatomy of ratites" by M.E Fowler, 1991, Journal of Zoo and Wildlife Medicine, 22(2), pg. 204-277. Left lateral view of the male ostrich (right) with (1) the retracted phallus and (2) the erect phallus. Left lateral view of the chick/juvenile ostrich and the (3) male phallus and (4) female phallus.	Chapter 1; Courtship Behaviour In Kiwi and Other Ratites; Ostrich (*Struthio camelus*)	Yes	Pending	Permissions to reuse pending.
From "DHEA effects on brain and behavior: Insights from comparative studies of aggression" by K.K. Soma, N.M. Rendon, R. Boonstra, H. E. Albers & G.E. Demas, 2008, Neurochemistry International, 52(4-5), pg. 611-620. Fig. 1. Steroids can act on the brain to modulate aggression via several pathways	Chapter 2; Sex steroids and sex determination in birds	Yes	Yes	License content publisher: Elsevier. License number 5902180733642; License date Nov 04, 2024; Type of use: Reuse in a dissertation/thesis; Portion: Figures/Tables/Illustrations.
Greg Higgins, Mar 2009, Greater Rhea photos via Flickr Emu Fight | Flickr	Chapter 3; Discussion	Yes	Yes	© All rights reserved, permission sought from owner through Flickr messaging and granted December 02, 2024.
Luis Prevedel, Oct 2012, Greater Rhea photo via iNaturalist Photo 165169435. (c) Luis Prevedel, some rights reserved (CC BY-NC), uploaded by Luis Prevedel · iNaturalist NZ	Chapter 3; Discussion	Yes	Yes (additional permission still pending)	CC BY-NC 4.0, permission also sought through iNaturalist from owner, still pending.

César Picar, Feb 2020, Puna Rhea photos via iNaturalist Puna Rhea (Subspecies Rhea pennata tarapacensis) in February 2022 by César Picar · iNaturalist NZ	Chapter 3; Discussion	Yes	Yes	CC BY-NC-ND 4.0, permission also sought through iNaturalist from owner and granted December 04, 2024, 03:54. Bottom of Form
"Early assessment of ambiguous genitalia" by A.L Ogilvy-Stuart & C.E Brain, 2004, Archives of Disease in Childhood, 89(5). Figure 2: Differential virilisation of the external genitalia using the staging system of Prader, from normal female (left) to normal male (right).	Chapter 4; Hormones; Potential Alterantives to Androstenedione	Yes	Yes	Licensed content publisher: BMJ Publishing Group LTD.; License number 5901620231267; License date Nov 03, 2024; Type of use dissertation/thesis; Requestor type individual; Portion figure/table/extract.
"DHEA effects on brain and behavior: Insights from comparative studies of aggression" by K.K. Soma, N.M. Rendon, R. Boonstra, H. E. Albers & G.E. Demas, 2015, The Journal of Steroid Biochemistry and Molecular Biology, 145, 261-272. Fig. 1. Steroids can act on the brain to modulate aggression via several pathways.	Chapter 4; Hormones; Bird Modelling of Hormonally mediated female masculinisation	Yes	Yes	License content publisher: Elsevier; License number: 5902180733642; License date: Nov 04, 2024; Type of use: reuse in a dissertation/thesis; Portion: Figures/tables/illustrations.
"Sexjng kiwis" by T.A Caithness, 1971, International Zoo Yearbook, 11(1), pg. 206-208. Figure 2 & Figure 3: Kiwi genitalia	Chapter 4 Future Directions; Anatomy; Genitalia	Yes	Yes	Copyright owner: John Wiley & Sons; Order License ID 1555264-1; Order Date 12-Dec-2024; Type of Use Republish in a thesis/dissertation Publisher BLACKWELL PUBLISHING; Portion Image/photo/illustration

Appendix 3: Sources for masculinised birds in Table 3 in Chapter 1; Sexual Development Pathways.

Species	Sources
Northern pintail (*Anas acuta*)	Chiba, 2004 Chiba & Honma, 2011
African black coucal (*Centropus grilli*)	Goymann, Wittenzellner & Wingfield, 2004 Goymann, Kempenaers & Wingfield, 2005
Red Phalarope (*Phalaropus fulicarius*)	Schamel, Tracy & Lank, 2004 English, 2014 Giroux et al., 2016
Western gull (*Larus occidentalis*)	Wingfield et al., 1980
White-necked jacobin (*Florisuga mellivora*)	Falk, 2020 Falk, Webster & Rubstein, 2021
Wattled jacana (*Jacana jacana*)	Emlen & Wrege, 2004
Spotted sandpiper (*Actitis macularius*)	Oring & Lank, 1982

Appendix 4: Generalised Linear model tests run for kiwi plasma hormonal data (testosterone, androstenedione, corticosterone, prolactin, progesterone and oestradiol) in Chapter 2; Statistical Analysis.

Generalised Linear Models: Prolactin (PRL)

Notes

Output Created		30-AUG-2024 15:57:47
Comments		
Input	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	80
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN PRLngml BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.08

Model Information

Dependent Variable		PRL (ng/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Categorical Variable Information

			N	Percent
Factor	C vs W	Captive	24	33.3%
		Wild	48	66.7%
		Total	72	100.0%
	brSeason	BR	39	54.2%
		NBR	33	45.8%
		Total	72	100.0%

Case Processing Summary

	N	Percent
Included	72	90.0%
Excluded	8	10.0%
Total	80	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	PRL (ng/ml)	72	1.20	120.80	45.4944	26.86702

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	19
	Within-Subject Effect	sampling	6
Number of Subjects			19
Number of Measurements per Subject	Minimum		2
	Maximum		6
Correlation Matrix Dimension			6

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	44.925
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	45.378

Dependent Variable: PRL (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason
[a]
a. Information criteria are in smaller-is-better form.
b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	1668.776	1	.000
C vs W	2.611	1	.106
brSeason	6.115	1	.013
C vs W * brSeason	5.176	1	.023

Dependent Variable: PRL (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square
(Intercept)	3.351	.2253	2.909	3.792	221.128
[C vs W=Captive]	.600	.2581	.094	1.106	5.397
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	.622	.1825	.264	.980	11.624
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	-.596	.2620	-1.110	-.083	5.176
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	.398				

Parameter Estimates

Parameter	Hypothesis Test df	Sig.
(Intercept)	1	.000
[C vs W=Captive]	1	.020
[C vs W=Wild]	.	.
[brSeason=BR]	1	<.001
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.023
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: PRL (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means 1: C vs W

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	BR	53.3196	9.24022	37.9642	74.8857
	NBR	51.9566	6.54210	40.5940	66.4996
Wild	BR	53.1324	4.55595	44.9129	62.8561
	NBR	28.5236	6.42720	18.3402	44.3613

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
BR	53.2259	5.14567	44.0385	64.3301
NBR	38.4966	4.96844	29.8926	49.5770

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	52.6337	6.25006	41.7048	66.4264
Wild	38.9298	5.60651	29.3559	51.6260

Generalised Linear Models: Estradiol (Oestradiol)

Notes

Output Created		30-AUG-2024 16:03:11
Comments		
Input	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	80
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN Estradiolpgml BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.06

Model Information

Dependent Variable		Estradiol (pg/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Categorical Variable Information

			N	Percent
Factor	C vs W	Captive	24	33.3%
		Wild	48	66.7%
		Total	72	100.0%
	brSeason	BR	39	54.2%
		NBR	33	45.8%
		Total	72	100.0%

Case Processing Summary

	N	Percent
Included	72	90.0%
Excluded	8	10.0%
Total	80	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Estradiol (pg/ml)	72	65.11	1775.75	401.1743	296.23031

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	19
	Within-Subject Effect	sampling	6
Number of Subjects			19
Number of Measurements per Subject	Minimum		2
	Maximum		6
Correlation Matrix Dimension			6

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	37.316
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	31.818

Dependent Variable: Estradiol (pg/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason
a

a. Information criteria are in smaller-is-better form.
b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	3684.065	1	.000
C vs W	13.436	1	<.001
brSeason	.989	1	.320
C vs W * brSeason	.104	1	.747

Dependent Variable: Estradiol (pg/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper	Hypothesis Test Wald Chi-Square
(Intercept)	5.576	.1499	5.283	5.870	1384.119
[C vs W=Captive]	.778	.2335	.321	1.236	11.113
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	.199	.2508	-.293	.690	.628
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	-.097	.3019	-.689	.494	.104
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	.422				

Parameter Estimates

Parameter	Hypothesis Test df	Hypothesis Test Sig.
(Intercept)	1	.000
[C vs W=Captive]	1	<.001
[C vs W=Wild]	.	.
[brSeason=BR]	1	.428
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.747
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: Estradiol (pg/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means 1: C vs W

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
Captive	605.0963	105.15258	430.4316	850.6381
Wild	291.7178	28.31162	241.1863	352.8363

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
BR	452.8813	60.03603	349.2571	587.2508
NBR	389.7652	45.49832	310.0559	489.9661

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
Captive	BR	636.5613	131.18699	425.0291	953.3707
	NBR	575.1866	102.95621	404.9924	816.9032
Wild	BR	322.2022	53.74277	232.3537	446.7942
	NBR	264.1176	39.58805	196.8849	354.3090

Generalised Linear Models: Progesterone

Notes

Output Created		30-AUG-2024 16:06:58
Comments		
Input	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	80
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN Progesteronengml BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.03

Model Information

Dependent Variable		Progesterone (ng/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Case Processing Summary

	N	Percent
Included	72	90.0%
Excluded	8	10.0%
Total	80	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	19
	Within-Subject Effect	sampling	6
Number of Subjects			19
Number of Measurements per Subject	Minimum		2
	Maximum		6
Correlation Matrix Dimension			6

Categorical Variable Information

			N	Percent
Factor	C vs W	Captive	24	33.3%
		Wild	48	66.7%
		Total	72	100.0%
	brSeason	BR	39	54.2%
		NBR	33	45.8%
		Total	72	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean
Dependent Variable	Progesterone (ng/ml)	72	.98	10.48	3.5553

Continuous Variable Information

		Std. Deviation
Dependent Variable	Progesterone (ng/ml)	1.81991

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	21.941
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	18.304

Dependent Variable: Progesterone (ng/ml)

Model: (Intercept), C vs W, brSeason, C vs W * brSeason

[a]

a. Information criteria are in smaller-is-better form.

b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	304.448	1	.000
C vs W	21.780	1	<.001
brSeason	6.738	1	.009
C vs W * brSeason	1.090	1	.297

Dependent Variable: Progesterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square
(Intercept)	1.638	.0958	1.450	1.825	292.380
[C vs W=Captive]	-.720	.2261	-1.163	-.276	10.128
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	-.376	.0674	-.508	-.243	31.066
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	.215	.2064	-.189	.620	1.090
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	.149				

Parameter Estimates

Parameter	Hypothesis Test df	Sig.
(Intercept)	1	.000
[C vs W=Captive]	1	.001
[C vs W=Wild]	.	.
[brSeason=BR]	1	<.001
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.297
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: Progesterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means 1: C vs W

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	2.3116	.25103	1.8685	2.8599
Wild	4.2627	.31325	3.6909	4.9230

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
BR	2.7456	.09252	2.5702	2.9331
NBR	3.5889	.40577	2.8755	4.4792

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	BR	2.1338	.05418	2.0302	2.2427
	NBR	2.5043	.51300	1.6762	3.7416
Wild	BR	3.5329	.22056	3.1261	3.9928
	NBR	5.1431	.49258	4.2629	6.2051

Generalised Linear Models: Corticosterone

Notes

Output Created		30-AUG-2024 16:08:32
Comments		
Input	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	80
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN Corticosteronengml BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.02

Model Information

Dependent Variable		Corticosterone (ng/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Case Processing Summary

	N	Percent
Included	53	66.3%
Excluded	27	33.8%
Total	80	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	19
	Within-Subject Effect	sampling	5
Number of Subjects			19
Number of Measurements per Subject	Minimum		1
	Maximum		4
Correlation Matrix Dimension			5

Categorical Variable Information

			N	Percent
Factor	C vs W	Captive	18	34.0%
		Wild	35	66.0%
		Total	53	100.0%
	brSeason	BR	27	50.9%
		NBR	26	49.1%
		Total	53	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean
Dependent Variable	Corticosterone (ng/ml)	53	29.09	846.70	320.1874

Continuous Variable Information

		Std. Deviation
Dependent Variable	Corticosterone (ng/ml)	209.73703

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	16.229
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	20.203

Dependent Variable: Corticosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason
[a]
a. Information criteria are in smaller-is-better form.
b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	8202.378	1	.000
C vs W	133.421	1	.000
brSeason	11.518	1	<.001
C vs W * brSeason	.020	1	.887

Dependent Variable: Corticosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square
(Intercept)	6.174	.0478	6.081	6.268	16694.849
[C vs W=Captive]	-1.358	.1436	-1.639	-1.076	89.382
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	-.247	.0942	-.432	-.063	6.898
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	-.022	.1522	-.320	.277	.020
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	.226				

Parameter Estimates

Parameter	Hypothesis Test df	Sig.
(Intercept)	1	.000
[C vs W=Captive]	1	.000
[C vs W=Wild]	.	.
[brSeason=BR]	1	.009
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.887
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: Corticosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means 1: C vs W

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	107.9608	10.00147	90.0349	129.4557
Wild	424.3485	31.35722	367.1327	490.4810

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
BR	188.1058	12.97815	164.3140	215.3425
NBR	243.5491	17.49119	211.5705	280.3613

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	BR	94.3676	7.28755	81.1126	109.7885
	NBR	123.5121	16.73021	94.7132	161.0676
Wild	BR	374.9571	42.87793	299.6699	469.1590
	NBR	480.2459	22.94880	437.3092	527.3984

Generalised Linear Models: Androstenedione

Notes

Output Created		30-AUG-2024 16:11:50
Comments		
Input	Active Dataset	DataSet2
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	80
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN Androstenedionengml BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.03

Model Information

Dependent Variable		Androstenedione (ng/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Case Processing Summary

	N	Percent
Included	65	81.3%
Excluded	15	18.8%
Total	80	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	19
	Within-Subject Effect	sampling	6
Number of Subjects			19
Number of Measurements per Subject	Minimum		2
	Maximum		6
Correlation Matrix Dimension			6

Categorical Variable Information

			N	Percent
Factor	C vs W	Captive	18	27.7%
		Wild	47	72.3%
		Total	65	100.0%
	brSeason	BR	36	55.4%
		NBR	29	44.6%
		Total	65	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean
Dependent Variable	Androstenedione (ng/ml)	65	.02	15.73	1.5888

Continuous Variable Information

		Std. Deviation
Dependent Variable	Androstenedione (ng/ml)	3.30954

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	187.713
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	181.914

Dependent Variable: Androstenedione (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason
[a]
a. Information criteria are in smaller-is-better form.
b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	2.314	1	.128
C vs W	2.411	1	.121
brSeason	1.866	1	.172
C vs W * brSeason	3.521	1	.061

Dependent Variable: Androstenedione (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square
(Intercept)	-.676	.4909	-1.639	.286	1.898
[C vs W=Captive]	1.673	.6786	.343	3.003	6.076
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	1.335	.2769	.792	1.877	23.226
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	-1.545	.8232	-3.158	.069	3.521
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	4.113				

Parameter Estimates

Parameter	Hypothesis Test df	Sig.
(Intercept)	1	.168
[C vs W=Captive]	1	.014
[C vs W=Wild]	.	.
[brSeason=BR]	1	<.001
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.061
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: Androstenedione (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means 1: C vs W

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	2.4384	.97786	1.1111	5.3513
Wild	.9909	.41519	.4359	2.2526

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
BR	2.0591	.76423	.9948	4.2618
NBR	1.1735	.39818	.6035	2.2819

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	BR	2.1954	1.39294	.6330	7.6135
	NBR	2.7084	1.26895	1.0812	6.7846
Wild	BR	1.9312	.74409	.9075	4.1095
	NBR	.5085	.24961	.1943	1.3308

Generalised Linear Models: Prolactin (PRL) from Jensen et al. paper

Notes

Output Created		30-AUG-2024 16:25:30
Comments		
Input	Data	C:\Users\iccastro\OneDrive - Massey University\Students\Caitlin McLeod\Data males 2024.sav
	Active Dataset	DataSet3
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	62
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN PRLngml BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.05
	Elapsed Time	00:00:00.05

Model Information

Dependent Variable		PRL (ng/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Case Processing Summary

	N	Percent
Included	62	100.0%
Excluded	0	0.0%
Total	62	100.0%

Categorical Variable Information

		N	Percent
Factor	C vs W	Captive	29	46.8%
		Wild	33	53.2%
		Total	62	100.0%
	brSeason	BR	34	54.8%
		NBR	28	45.2%
		Total	62	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	17
	Within-Subject Effect	sampling	6
Number of Subjects			17
Number of Measurements per Subject	Minimum		2
	Maximum		6
Correlation Matrix Dimension			6

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	PRL (ng/ml)	62	.1	223.2	62.571	47.6816

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	42.001
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	44.601

Dependent Variable: PRL (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason
[a]
a. Information criteria are in smaller-is-better form.
b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	2384.068	1	.000
C vs W	40.912	1	<.001
brSeason	28.470	1	<.001
C vs W * brSeason	1.451	1	.228

Dependent Variable: PRL (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper	Hypothesis Test Wald Chi-Square
(Intercept)	2.975	.2131	2.558	3.393	194.944
[C vs W=Captive]	1.203	.2330	.746	1.659	26.648
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	.913	.2019	.517	1.309	20.452
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	-.336	.2792	-.884	.211	1.451
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	.310				

Parameter Estimates

Parameter	Hypothesis Test df	Hypothesis Test Sig.
(Intercept)	1	.000
[C vs W=Captive]	1	<.001
[C vs W=Wild]	.	.
[brSeason=BR]	1	<.001
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.228
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: PRL (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means 1: C vs W

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	87.037	8.7579	71.459	106.012
Wild	30.929	3.9172	24.130	39.644

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
BR	75.300	7.2439	62.360	90.924
NBR	35.750	4.1651	28.452	44.921

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	BR	116.129	20.1013	82.719	163.034
	NBR	65.233	6.1494	54.229	78.471
Wild	BR	48.825	4.1016	41.413	57.564
	NBR	19.593	4.1749	12.904	29.749

Generalised Linear Models: Testosterone from Jensen et al. paper

Output Created		30-AUG-2024 16:28:18
Comments		
Input	Data	C:\Users\iccastro\OneDrive - Massey University\Students\Caitlin McLeod\Data males 2024.sav
	Active Dataset	DataSet3
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	62
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN Testosteronengml BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.05

Model Information

Dependent Variable		Testosterone (ng/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Case Processing Summary

	N	Percent
Included	62	100.0%
Excluded	0	0.0%
Total	62	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	17
	Within-Subject Effect	sampling	6
Number of Subjects			17
Number of Measurements per Subject	Minimum		2
	Maximum		6
Correlation Matrix Dimension			6

Categorical Variable Information

			N	Percent
Factor	C vs W	Captive	29	46.8%
		Wild	33	53.2%
		Total	62	100.0%
	brSeason	BR	34	54.8%
		NBR	28	45.2%
		Total	62	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean
Dependent Variable	Testosterone (ng/ml)	62	.022	10.141	1.16787

Continuous Variable Information

		Std. Deviation
Dependent Variable	Testosterone (ng/ml)	1.807490

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	79.685
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	75.758

Dependent Variable: Testosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason
[a]
a. Information criteria are in smaller-is-better form.
b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	3.390	1	.066
C vs W	40.535	1	<.001
brSeason	5.520	1	.019
C vs W * brSeason	9.724	1	.002

Dependent Variable: Testosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper	Hypothesis Test Wald Chi-Square
(Intercept)	-2.000	.3005	-2.589	-1.411	44.292
[C vs W=Captive]	2.762	.3694	2.038	3.486	55.900
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	1.659	.3858	.903	2.415	18.488
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	-1.892	.6067	-3.081	-.703	9.724
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	1.404				

Parameter Estimates

Parameter	Hypothesis Test df	Hypothesis Test Sig.
(Intercept)	1	<.001
[C vs W=Captive]	1	<.001
[C vs W=Wild]	.	.
[brSeason=BR]	1	<.001
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.002
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: Testosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means 1: C vs W

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	1.90696	.461064	1.18724	3.06298
Wild	.31015	.046953	.23052	.41728

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
BR	1.09831	.251852	.70071	1.72153
NBR	.53850	.099473	.37493	.77343

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	BR	1.69703	.720777	.73818	3.90139
	NBR	2.14285	.460458	1.40632	3.26512
Wild	BR	.71083	.122984	.50640	.99778
	NBR	.13532	.040669	.07509	.24388

Generalised Linear Models: Corticosterone

Notes

Output Created		30-AUG-2024 16:28:38
Comments		
Input	Data	C:\Users\iccastro\OneDrive - Massey University\Students\Caitlin McLeod\Data males 2024.sav
	Active Dataset	DataSet3
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	62
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN Corticosteronengml BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.06

Model Information

Dependent Variable		Corticosterone (ng/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Case Processing Summary

	N	Percent
Included	44	71.0%
Excluded	18	29.0%
Total	62	100.0%

Correlated Data Summary

Number of Levels	Subject Effect ID Bird	16
	Within-Subject Effect sampling	6
Number of Subjects		16
Number of Measurements per Subject	Minimum	1
	Maximum	5
Correlation Matrix Dimension		6

Categorical Variable Information

			N	Percent
Factor	C vs W	Captive	22	50.0%
		Wild	22	50.0%
		Total	44	100.0%
	brSeason	BR	24	54.5%
		NBR	20	45.5%
		Total	44	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean
Dependent Variable	Corticosterone (ng/ml)	44	49.58	750.90	325.9586

Continuous Variable Information

		Std. Deviation
Dependent Variable	Corticosterone (ng/ml)	143.05863

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	17.615
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	17.132

Dependent Variable: Corticosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason
[a]
a. Information criteria are in smaller-is-better form.
b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	5586.182	1	.000
C vs W	.317	1	.573
brSeason	1.358	1	.244
C vs W * brSeason	.693	1	.405

Dependent Variable: Corticosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	Hypothesis Test df	Sig.
(Intercept)	1	.000
[C vs W=Captive]	1	.949
[C vs W=Wild]	.	.
[brSeason=BR]	1	.004
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.405
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: Corticosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square
(Intercept)	5.696	.0659	5.567	5.825	7460.132
[C vs W=Captive]	.015	.2352	-.446	.476	.004
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	.245	.0847	.079	.411	8.391
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	-.204	.2455	-.685	.277	.693
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	.205				

Estimated Marginal Means 1: C vs W

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	308.4335	46.37598	229.7075	414.1408
Wild	336.4710	12.00418	313.7470	360.8409

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
BR	346.0358	25.99016	298.6680	400.9159
NBR	299.9081	35.27311	238.1638	377.6597

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
Captive	BR	314.8081	45.38627	237.3160	417.6040
	NBR	302.1881	68.23192	194.1253	470.4057
Wild	BR	380.3612	16.04603	350.1766	413.1476
	NBR	297.6454	19.62856	261.5566	338.7136

Generalised Linear Models: Testosterone

Notes

Output Created		30-AUG-2024 16:29:23
Comments		
Input	Data	C:\Users\iccastro\OneDrive - Massey University\Students\Caitlin McLeod\Data males 2024.sav
	Active Dataset	DataSet3
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	62
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN Testosteronengml_A BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.00
	Elapsed Time	00:00:00.05

Model Information

Dependent Variable		Testosterone (ng/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Case Processing Summary

	N	Percent
Included	42	67.7%
Excluded	20	32.3%
Total	62	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	17
	Within-Subject Effect	sampling	6
Number of Subjects			17
Number of Measurements per Subject	Minimum		1
	Maximum		4
Correlation Matrix Dimension			6

Categorical Variable Information

			N	Percent
Factor	C vs W	Captive	18	42.9%
		Wild	24	57.1%
		Total	42	100.0%
	brSeason	BR	21	50.0%
		NBR	21	50.0%
		Total	42	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean
Dependent Variable	Testosterone (ng/ml)	42	.01	9.42	2.1612

Continuous Variable Information

		Std. Deviation
Dependent Variable	Testosterone (ng/ml)	2.54558

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	79.635
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	83.919

Dependent Variable: Testosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason
[a]
a. Information criteria are in smaller-is-better form.
b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	8.913	1	.003
C vs W	8.872	1	.003
brSeason	.155	1	.694
C vs W * brSeason	.136	1	.713

Dependent Variable: Testosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper	Hypothesis Test Wald Chi-Square
(Intercept)	-.108	.4752	-1.039	.824	.051
[C vs W=Captive]	1.395	.5738	.270	2.520	5.912
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	.218	.4027	-.571	1.008	.294
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	-.211	.5735	-1.335	.913	.136
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	1.217				

Parameter Estimates

Parameter	Hypothesis Test df	Hypothesis Test Sig.
(Intercept)	1	.821
[C vs W=Captive]	1	.015
[C vs W=Wild]	.	.
[brSeason=BR]	1	.588
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.713
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: Testosterone (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means 1: C vs W

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
Captive	3.6363	1.08736	2.0236	6.5343
Wild	1.0015	.31353	.5422	1.8498

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
BR	2.0190	.46273	1.2884	3.1639
NBR	1.8038	.51746	1.0280	3.1650

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
Captive	BR	3.6494	1.45453	1.6709	7.9704
	NBR	3.6234	1.16484	1.9296	6.8039
Wild	BR	1.1170	.25286	.7168	1.7408
	NBR	.8980	.42674	.3538	2.2791

Generalised Linear Models: Androstenedione

Notes

Output Created		30-AUG-2024 16:29:48
Comments		
Input	Data	C:\Users\iccastro\OneDrive - Massey University\Students\Caitlin McLeod\Data males 2024.sav
	Active Dataset	DataSet3
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	62
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN Androstenedionengml BY CvsW brSeason (ORDER=ASCENDING) /MODEL CvsW brSeason CvsW*brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=CvsW SCALE=ORIGINAL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /EMMEANS TABLES=CvsW*brSeason SCALE=ORIGINAL /REPEATED SUBJECT=IDBird WITHINSUBJECT=sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.06

Model Information

Dependent Variable		Androstenedione (ng/ml)
Probability Distribution		Gamma
Link Function		Log
Subject Effect	1	ID Bird
Within-Subject Effect	1	sampling
Working Correlation Matrix Structure		AR(1)

Case Processing Summary

	N	Percent
Included	54	87.1%
Excluded	8	12.9%
Total	62	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	17
	Within-Subject Effect	sampling	6
Number of Subjects			17
Number of Measurements per Subject	Minimum		1
	Maximum		6
Correlation Matrix Dimension			6

Categorical Variable Information

			N	Percent
Factor	C vs W	Captive	27	50.0%
		Wild	27	50.0%
		Total	54	100.0%
	brSeason	BR	31	57.4%
		NBR	23	42.6%
		Total	54	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean
Dependent Variable	Androstenedione (ng/ml)	54	.03	31.88	2.2081

Continuous Variable Information

		Std. Deviation
Dependent Variable	Androstenedione (ng/ml)	5.21334

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	122.260
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	120.922

Dependent Variable: Androstenedione (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason
[a]
a. Information criteria are in smaller-is-better form.
b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	7.158	1	.007
C vs W	5.197	1	.023
brSeason	2.221	1	.136
C vs W * brSeason	2.771	1	.096

Dependent Variable: Androstenedione (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper	Hypothesis Test Wald Chi-Square
(Intercept)	.109	.5403	-.950	1.168	.041
[C vs W=Captive]	.211	.6009	-.966	1.389	.124
[C vs W=Wild]	0[a]	.	.	.	.
[brSeason=BR]	-.068	.6369	-1.317	1.180	.011
[brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Captive] * [brSeason=BR]	1.304	.7831	-.231	2.838	2.771
[C vs W=Captive] * [brSeason=NBR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=BR]	0[a]	.	.	.	.
[C vs W=Wild] * [brSeason=NBR]	0[a]	.	.	.	.
(Scale)	2.783				

Parameter Estimates

Parameter	Hypothesis Test df	Hypothesis Test Sig.
(Intercept)	1	.840
[C vs W=Captive]	1	.725
[C vs W=Wild]	.	.
[brSeason=BR]	1	.915
[brSeason=NBR]	.	.
[C vs W=Captive] * [brSeason=BR]	1	.096
[C vs W=Captive] * [brSeason=NBR]	.	.
[C vs W=Wild] * [brSeason=BR]	.	.
[C vs W=Wild] * [brSeason=NBR]	.	.
(Scale)		

Dependent Variable: Androstenedione (ng/ml)
Model: (Intercept), C vs W, brSeason, C vs W * brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means 1: C vs W

Estimates

C vs W	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
Captive	2.5551	.57474	1.6442	3.9708
Wild	1.0778	.32828	.5933	1.9579

Estimated Marginal Means 2: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
BR	2.2217	.53538	1.3854	3.5629
NBR	1.2395	.37242	.6879	2.2336

Estimated Marginal Means 3: C vs W* brSeason

Estimates

C vs W	brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
Captive	BR	4.7388	1.74696	2.3008	9.7603
	NBR	1.3777	.36218	.8230	2.3064
Wild	BR	1.0416	.32336	.5668	1.9141
	NBR	1.1152	.60259	.3867	3.2158

Generalised Linear Models: Testosterone – Wild Females Only (Captive unavailable)

Notes

Output Created		11-OCT-2024 14:29:47
Comments		
Input	Data	C:\Users\iccastro\OneDrive - Massey University\Students\Caitlin McLeod\2024\data for testosterone wild females.sav
	Active Dataset	DataSet4
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	22
Missing Value Handling	Definition of Missing	User-defined missing values for factor, subject and within-subject variables are treated as missing.
	Cases Used	Statistics are based on cases with valid data for all variables in the model.
Weight Handling		not applicable
Syntax		GENLIN Testosteronengml BY brSeason (ORDER=ASCENDING) /MODEL brSeason INTERCEPT=YES DISTRIBUTION=GAMMA LINK=LOG /CRITERIA METHOD=FISHER(1) SCALE=MLE MAXITERATIONS=100 MAXSTEPHALVING=5 PCONVERGE=1E-006(ABSOLUTE) SINGULAR=1E-012 ANALYSISTYPE=3(WALD) CILEVEL=95 LIKELIHOOD=FULL /EMMEANS TABLES=brSeason SCALE=ORIGINAL /REPEATED SUBJECT=ID Bird WITHINSUBJECT=Sampling SORT=YES CORRTYPE=AR(1) ADJUSTCORR=YES COVB=ROBUST MAXITERATIONS=100 PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1 /MISSING CLASSMISSING=EXCLUDE /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.06

Model Information

Dependent Variable	Testosterone (ng/ml)
Probability Distribution	Gamma
Link Function	Log
Subject Effect 1	ID Bird
Within-Subject Effect 1	Sampling
Working Correlation Matrix Structure	AR(1)

Case Processing Summary

	N	Percent
Included	22	100.0%
Excluded	0	0.0%
Total	22	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	ID Bird	11
	Within-Subject Effect	Sampling	4
Number of Subjects			11
Number of Measurements per Subject	Minimum		2
	Maximum		2
Correlation Matrix Dimension			4

Categorical Variable Information

		N	Percent
Factor	brSeason BR	11	50.0%
	NBR	11	50.0%
	Total	22	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Testosterone (ng/ml)	22	.009	5.000	.94271	1.448654

Goodness of Fit[a]

	Value
Quasi Likelihood under Independence Model Criterion (QIC)[b]	83.571
Corrected Quasi Likelihood under Independence Model Criterion (QICC)[b]	85.732

Dependent Variable: Testosterone (ng/ml)

Model: (Intercept), brSeason

a. Information criteria are in smaller-is-better form.

b. Computed using the full log quasi-likelihood function.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
(Intercept)	.040	1	.842
brSeason	1.618	1	.203

Dependent Variable: Testosterone (ng/ml)

Model: (Intercept), brSeason

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval Lower	Upper	Hypothesis Test Wald Chi-Square	df	Sig.
(Intercept)	-.299	.6010	-1.477	.879	.247	1	.619
[brSeason=BR]	.413	.3248	-.223	1.050	1.618	1	.203
[brSeason=NBR]	0[a]	.	.	.	.	.	.
(Scale)	2.846						

Dependent Variable: Testosterone (ng/ml)

Model: (Intercept), brSeason

a. Set to zero because this parameter is redundant.

Estimated Marginal Means: brSeason

Estimates

brSeason	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
BR	1.12100	.385474	.57135	2.19940
NBR	.74165	.445745	-.22835	2.40872

Appendix 5: Co-ordinates of camera trap locations for kiwi behaviour in 2014 and 2015, provided by Kat Strang, paired with Figure 18, in Chapter 3; Materials and Methods.

Location Name	Latitude	Longitude
Trail to trig path	-36.87147	175.19629
Trig	-36.87185	175.18805
King fern	-36.87356	175.19253
Base of KF	-36.87493	175.19458
Bush opposite PF	-36.87699	175.20405
Main swamp	-36.87726	175.19577
Waterfall	-36.87771	175.19329
Road2	-36.87691	175.18686
Hayshed	-36.87973	175.20177
Trail near hayshed	-36.88053	175.19958
Swamp trail	-36.88221	175.19334
End of pipe	-36.88217	175.18648
Road	-36.88183	175.18219
Two tanks	-36.88583	175.19758
Pipe entry	-36.88606	175.19162
RSHG	-36.88613	175.18757
Kauri	-36.88594	175.17957
Lower Kauri	-36.88667	175.17676
Omega	-36.89084	175.17648
House	-36.89176	175.18326
PK	-36.88968	175.18674
Other Bay	-36.89515	175.18025
E17	-36.89033	175.1944
Third Bay	-36.89232	175.19811
Second Bay	-36.89399	175.19713
Top house gully	-36.8939	175.19405
Orchard	-36.89515	175.19104
First Bay	-36.89812	175.1922

Appendix 6: Examples of how behaviour was described, categorised and sorted during the video analysis in Chapter 3, Materials and Methods.

A.

- End of Pipe, 04/06/2015, 05:35, "kiwi fight 2" → "2015/06/04 - End of Pipe - 05:35 - Two Birds (1.0)"

A transmitter bird (Bird 1) walks from the middle of the shot to the back right hand corner. At 11 seconds, Bird 1 is engaged by another bird (Bird 2). Foliage interferes with viewing the interaction, but Bird 2 appears to push Bird 1 from the right to the left of the frame by the side of the body, with Bird 2 resting their head over Bird 1s back. Bird 1 separates this motion with a sideways kick at Bird 2, before pivoting to re-engage head on. Necks are interlocked.

B.

Behaviour	Description	Behavioural Code
Probing	Touching of objects with beak	1
Foraging	Visibly pushing beak into soil/leaf litter, visibly eating	2
Calling (male)	Recognisable adult male brown kiwi calls	3
Calling (female)	Recognisable adult female brown kiwi calls	4
Preen/scratch/grooming	Individual behaviour where the body and/or feathers are groomed with either the beak or feet	5

C.

Original File Name	New File Name (In Drive)	Location	Single or Series	Month	Individuals	Males	Females	Sex Unknown	Probing	Foraging	Calling (male)	Calling (female)
"male kiwi calling"	2014/04/23 - Road Scrub - 21:52 - One Bird	Road Scrub	Single	4	1	1	0	0	1	0	1	0
"male kiwi calling"	2014/04/29 - Swamp Trail - 19:04 - One Bird	Swamp Trail	Single	4	1	1	0	0	0	0	1	0

D.

Original File Name	New File Name	Month	Sequence of Behaviour
"kiwi chase"	2014/06/23 - Swamp Paddock - 04:32 - Two Birds (1.0)	6	6 8 6 23
"kiwi chase 2"	2014/06/23 - Swamp Paddock - 04:33 - Two Birds (2.0)	6	23 6 8 6
"kiwi fight"	2014/06/28 - RSHG-BT - 02:33 - Two Birds	6	12 14 15 13 15 16 12 17
"kiwi fight 2"	2014/08/27 - Road - 23:34 - Two Birds	8	12 20 12 16 17 14 12 10
"kiwi fight"	2014/10/30 - RSHG Fork - 20:27 - Two Birds (1.0)	10	16 17 19 14 19 14 19 14 17 20 3 2
"kiwi fight 2"	2014/10/30 - RSHG Fork - 20:28 - Two Birds (2.0)	10	12 14 21 19 19 16 14 12 14 14 19 9 19 14 16 17 19 16 19 17

: Examples of how behaviour was sorted and categorized during video analysis. A = An example of an individual description given to a single video; B = An example of how behaviours were broken down in 2014-15 and how a number was assigned to categorise it further; C = An example of how each video in 2014-2015 was noted as "1" for present or "0" for absent for each behaviour seen overall during the year; D = An example of how behaviour codes in 2014-2015 were used to note sequences.

Appendix 7: Examples of incubation data and how it was calculated, in Chapter 3, Materials and Methods.

A.

Clutch 1					
Year	Number Tracked males	Start incubation	End incubation	Duration (days)	No. nests
2014	17	28/05/2014	5/12/2014	191	15
2015	14	19/06/2015	7/01/2016	202	13
2021	16	29/05/2021	19/01/2022	235	13

B.

Month	Year		
	2014	2015	2021
January	0	0	0
February	0	0	0
March	0	0	0
April	0	0	0
May	6	0	6
June	6	7	13
July	29	64	6
August	41	7	38
September	24	21	19
October	0	36	31
November	24	21	13
December	0	0	0

Examples of how incubation data was collected and calculated. A = A example of data collected for the first clutches of each year; B = The calculation of the percentage of each male incubating per month in each year.

Appendix 8: Transition matrix of behaviours recorded in brown kiwi courtship sequences, with the probability of a state transitioning from one behaviour to another, used for Figure 29 in Chapter 3; Results.

From \ To	S	FG	P	RA	NX	K	CS	BB
S	0	0.1304	0.1739	0.1739	0.1304	0	0	0
FG	0	0	0.0145	0	0	0	0.0242	0
P	0	0	0.0193	0.0338	0	0.0386	0.0242	0
RA	0	0	0	0	0	0	0	0
NX	0	0	0	0	0	0.0242	0.0338	0
K	0	0	0.0193	0	0	0.0676	0	0.0145
CS	0	0	0	0	0	0	0.0242	0
BB	0	0	0	0	0	0	0	0.0193