THE EFFECTS OF DOMESTICATION AND ARTIFICIAL SELECTION ON BRAIN ANATOMY OF THE DOMESTIC CHICKEN (*GALLUS GALLUS DOMESTICUS*) AND DOMESTIC PIGEON (*COLUMBA LIVIA*)

KELSEY JOLYNN RACICOT
Bachelor of Science, University of Lethbridge, 2019

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

MASTER OF SCIENCE

in

NEUROSCIENCE

Department of Neuroscience
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

THE EFFECTS OF DOMESTICATION AND ARTIFICIAL SELECTION ON BRAIN ANATOMY OF THE DOMESTIC CHICKEN (*GALLUS GALLUS DOMESTICUS*) AND DOMESTIC PIGEON (*COLUMBA LIVIA*)

KELSEY JOLYNN RACICOT

Dr. A. Iwaniuk Supervisor	Associate Professor	Ph.D.
Dr. S. M. Pellis Thesis Examination Committee Member	Professor	Ph.D.
Dr. G. Pyle Thesis Examination Committee Member	Professor	Ph.D.
Dr. E. Hecht External Examiner Harvard University Boston, Massachusett	Assistant Professor	Ph.D.
Dr. I. Whishaw Chair, Thesis Examination Committee	Professor	Ph.D.

ABSTRACT

Domestication occurs due to captivity and artificially selecting animals for human benefit. One effect of domestication is a reduction in brain and brain region sizes of domesticated animals compared with their wild ancestors. However, little is known about the neuroanatomical effects of domestication on the world's most common birds: chickens (*Gallus gallus*) pigeons (*Columba livia*). This study revealed that chickens and junglefowl share similar telencephalon composition, but they both differ significantly from wild galliform species. In addition, I show that homing pigeons have larger olfactory bulbs than show breeds, but not sporting or feral pigeons, suggesting that all free-flying pigeons might use olfactory based navigation. Taken together, these results revealed that captive breeding can exert complex effects telencephalon anatomy and how artificial selection can alter the size of sensory regions in the brain.

ACKNOWLEDGEMENTS

I would first like to thank my committee members (Serge Pellis and Greg Pyle) for their contributions to this thesis and their feedback throughout the process of data collection and results interpretations. Without their expertise and advice, this thesis would not have been possible.

Second, I want to thank my lab mates who helped me throughout this entire (sometimes grueling) process that is a graduate degree. I want to thank Ben Brinkman, who has worked with me for my entire time in Dr. Iwaniuk's lab and has been the one to show me nearly everything I know from histology, perfusions, statistics, and most importantly, how to not take working "too" seriously. This thesis would not have been possible without Ben because the perfusions of over 80 birds would not have been done if it wasn't for him driving 9 hours (one way) to pick up our birds for us, crushing a 5-hour energy, and then perfusing all of them while Audrey and I tried not to cry.

I also want to thank Audrey Guyonnet, who was by my side for my entire thesis as we processed over 80 pigeon brains together and counted cells almost to the point of failure. Without her ability to make everything more enjoyable and our dark humor that was our only reliable coping mechanism, I don't think this thesis would have been possible. I also want to thank the undergraduate students (Sydney Irvine, Grace Lake, and Jocelyn Kaloa) who helped with so much of the tissue processing and with the workload of sectioning 8 brains per week (at the end, sorry Andy, it is called cramming…).

Finally, I want to thank my supervisor, Andy. I want to thank him for supervising me not only for this thesis, but for my entire time in his lab since January of 2018. If it wasn't for Andy's reassurance that a master's degree isn't the "worst" thing ever, I would not have started

this program in the first place. Andy has spent countless hours meeting with me when I was worried about statistical analyses, thesis progress, and everything in-between. He has spent so much time (probably too much) editing my thesis, helping me with statistical analyses that I had never done before, giving me feedback on every aspect of my thesis without questioning why he took me on as a student… At least to my knowledge. Without Andy, I would not be the scientist and researcher I have become, and I definitely would not be as far into my academic career as I am without his relentless support and encouragement throughout the last four and a half years. So, for that, I am deeply grateful for my opportunity to work with such an amazing scientist, supervisor, and mentor for over half of my post-secondary career, and I will always be thankful for the effort that Andy has put into making me a better person, student, and scientist.

TABLE OF CONTENTS

LIST OF TABLES

number of mitral cells, and size of mitral cells. Telencephalon size was used as a covariate for OB size, and OB size was used as a covariate for mitral cell number and mitral cell size. Significant p-values ($p < 0.05$) are denoted with (*). Post-hoc analyses were carried out to determine which breeds were different if the p-value was significant. F-values, degrees of freedom (df) and p-values are reported. Data were log-transformed prior to statistical analysis.

Table 3.4 Results from Tukey's Honest Significant Difference (HSD) post-hoc analyses showing the significant differences between relative OB volume between homing pigeons and 7 other pigeon breeds (feral, Capuchine, show homer, show roller, cropper, roller, highflyer). degrees of freedom (df) and p-values are reported. Significant p-values ($p < 0.05$) are denoted with (*).

LIST OF FIGURES

LIST OF ABBREVIATIONS

A – arcopallium
ANCOVA – analysis of covariance
ANOVA – analysis of variance
APH – area parahippocampalis
E – entopallium
GRPR – greater prairie chicken
H – hyperpallium
HF – hippocampal formation
Homer – homing pigeon
LEPC – lesser prairie chicken
M – mesopallium
MTC – mitral cells
N – nidopallium
OB – olfactory bulb
ORN – olfactory receptor neuron
PCA – principal component analysis
RJF – red junglefowl
RUGR – ruffed grouse
St – striatum
STGR – sharp-tailed grouse
Tele – telencephalon
TSHR – thyroid stimulating hormone receptor
WL – white leghorn chicken

CHAPTER ONE: GENERAL INTRODUCTION

Domestication is the process by which wild animals are modified for human use (Kruska, 1988b; Price, 1999b). Humans began domesticating animals at least 10 000 years ago (Zeder, 2008), although some estimate that humans began domesticating dogs even earlier (Perri et al., 2021). In all cases, a domestic stock is created by intense selection by humans of a small, isolated population of a species in a captive environment (Kruska, 1988b). Humans impose a high degree of control over this isolated population, controlling many aspects of their life, including survival and reproduction (Kruska, 1988b). Humans also exert intense selection for certain traits of these animals, which effectively alters their phenotypes including physical appearance, as well as behaviour, over many generations of selection (Kruska, 1988b). This selection ultimately results in a divergence in anatomy and behaviour (i.e., phenotype) of domesticates from their wild counterparts, a process that was crucial the development of domesticated species (Darwin, 1859; Darwin, 1868). Although domestication of animals has been occurring for tens of thousands of years, it is only the past few decades that scientific studies have focused on the wealth of anatomical and behavioural effects of domestication, and most of these studies focus on mammals.

A prime example of how domestication affects anatomy and behaviour was the Farm Fox experiment carried out in Russia by Dmitri Belyaev and Lyudmila Trut (Belyaev, 1979; Trut et al., 2009). In this experiment, Belyaev bred silver foxes (*Vulpes vulpes*) over 40 generations while selecting for the least aggressive ones. The outcome was foxes that resembled domestic dogs (*Canis familiaris*) not only in their appearance, but also in their behaviour. The foxes developed floppy ears, curly tails, lighter coats, and were more tolerant of human proximity (Belyaev, 1979). This longitudinal experiment showed that the domesticated phenotype can be

achieved through selection for a single behavioural trait: tameness (Belyaev, 1979; Trut et al., 2009). Whether it is selection for one trait (such as tameness), or multiple traits, domestication is typically associated with a host of unselected changes in phenotype, including coat or plumage color, decreased body and organ size, progenesis, and neoteny (Agnvall, 2016; Francis, 2015; Wilkins et al., 2014). These traits are collectively referred to as "domestication syndrome" (Wilkins et al., 2014) and are thought to arise from the over-expression of specific genes as a direct result of selection. For example, *TSHR*, a gene involved in photoperiod regulation in birds and mammals (Albert et al., 2012; Rubin et al., 2010; Saravanan et al., 2020), is often overexpressed in domesticated strains and likely linked to changes in reproductive physiology (Karlsson et al., 2016).

In addition to the changes in external morphology that occur under domestication, there are also behavioural changes. Domestic animals tend to be more docile, less aggressive towards conspecifics, less athletic (Francis, 2015), less fearful of novel situations (Blanchard et al., 1986), and have reduced fear responses overall when compared to their wild counterparts (Campler et al., 2009). There is also a marked difference in stimulus response thresholds in domesticates (Price, 1999b). For example, the wild cavy (*Cavia aperea*) only vocalizes in high arousal situations, but the domesticated guinea pig (*Cavia porcellus*) vocalizes in response to many stimuli (Rood, 1972). All of these behavioural changes are thought to arise from changes in neurochemistry (Marliave et al., 1993) and brain anatomy. In fact, a common trait of domestication syndrome is a reduction in the size of the brain and its constituent regions (Ebinger, 1995; Kruska, 2005; Rehkamper et al., 2008). Although many different brain regions undergo reductions in volume, reductions in the size of telencephalic regions are both common and typically of large magnitude in domesticated mammals (Kruska, 2005). The telencephalon is

a diverse brain region responsible for a host of sensory processing functions as well as executive functions, learning and memory, and motor control (Karten, 1969; Reiner et al., 2005; Shimizu et al., 2010). The telencephalon tends to be the most malleable in response to evolutionary selection pressures in both birds and mammals (Kruska, 2005; Rehkämper et al., 1988) and some of the biggest volumetric changes in the brain that result from domestication are telencephalic, such as the sensory cortices, hippocampus and amygdala (Brusini et al., 2018; Ebinger, 1984; Kruska, 1988b; Kruska, 2005). That said, changes in volumes arising from domestication are not universal across telencephalic regions, some regions change in size and others do not. Even among those telencephalic regions that become smaller in domesticated strains, the amount of shrinkage varies across anatomical regions and species (Kruska, 1988b). That said, details on whether these changes arise from fewer neurons, smaller neurons, or both, is lacking.

Differences in brain anatomy between wild and domesticated mammals have been studied extensively, but far less is known about the effects of domestication in birds despite their equally long history of domestication (Sossinka, 2013). We do know that birds adhere to the general mammalian trend of brains becoming smaller during domestication (Mehlhorn & Rehkämper, 2013) and that some brain regions become smaller, but which brain regions and to what degree vary across species. For example, the arcopallium, a premotor region in the telencephalon, is greatly reduced in volume in both domestic ducks (*Anas platyrhynchos*) and geese (*Anser anser*), but not in domestic pigeons (Ebinger, 1984) Conversely, hyperpallial regions, multisensory regions of the telencephalon, are reduced in domestic pigeons and geese, but not in ducks (Ebinger, 1984). Chickens (*Gallus gallus*) are even more complicated in that the telencephalon and the cerebellum are larger, rather than smaller than in the wild-type, the red junglefowl (Henriksen et al., 2016). It therefore appears that domestication affects the brain

differently dependent across bird species and that these effects differ from that observed in mammals (Kruska 2005).

In this thesis, we will address two major knowledge gaps in our understanding of the effects of domestication on birds, focusing on the two most widespread and common species: the chicken and the pigeon. Both species have been domesticated for thousands of years (Al-Nasser et al., 2007; Darwin, 1868) and have diversified into hundreds of breeds (Mehlhorn & Petow, 2020; Shapiro & Domyan, 2013), making them useful species in the study of how artificial selection affects both the brain and behaviour. Chickens and pigeons are also interesting because breeds of both species have increased the size of specific brain regions compared with their wild ancestors (Henriksen et al., 2016; Rehkamper et al., 2008; Rehkämper et al., 1988), the opposite of what is typically observed in mammals (Kruska, 1988a, 1988b). We will address two primary questions on these species, both of which focus on the telencephalon, the brain region most affected by domestication in mammals (Ebinger, 1974; Kruska, 1988b). First, does the telencephalon of the chicken differ from junglefowl and other members of the Order Galliformes? Second: do homing pigeons have enlarged olfactory bulbs, a highly specialized telencephalic structure, with more neurons?

Project 1: How has domestication shaped the chicken telencephalon?

The majority of birds on the planet are domestic chickens, all of which are derived from the red junglefowl (Al-Nasser et al., 2007). Like other domesticated birds, chickens undergo a decrease in relative brain size compared to their wild counterpart, the red junglefowl (Henriksen et al., 2016). In contrast to mammals and most other birds, however, Henriksen et al. (2016) found that chickens have proportionately enlarged the cerebellum and telencephalon compared to

junglefowl. This initial study only used brain mass to compare brain regions sizes between chickens and junglefowl, so it remains unclear to what extent chickens and junglefowl differ in the sizes of individual regions of the telencephalon, as found in pigeons, ducks, geese and turkeys (Ebinger, 1995; Ebinger & Löhmer, 1987; Ebinger, 1984). Because some telencephalic regions may be larger and others smaller in domesticated strains, changes in telencephalon composition would go undetected in a gross comparison of telencephalon size. Thus, a detailed volumetric comparison of the major telencephalic regions comparing chickens and junglefowl could reveal more specific and behaviorally relevant changes. By conducting a more detailed quantification of eight subregions that make up the telencephalon, including several pallial regions, hippocampal formation, septum, and striatum, I will be able to draw conclusions about the importance of various sensory modalities in an artificial environment, as well as gain insight into the relative importance of spatial memory and limbic structures in these species. By examining regions that are sensory, limbic, and multi-functional, we can gain a better understanding of how domestication has affected not only the brain, but also behaviour.

In Chapter 2, I therefore compare the volumes of eight telencephalic subregions between red junglefowl and white leghorn chickens. In addition, I compare the relative size of these telencephalic subregions with that of several grouse species, all of which are grouped in the same order and family as the chicken and junglefowl. Comparisons with other wild, closely related species is important because the junglefowl have been captive bred for several generations. By comparing domestic chickens and wild captive-bred junglefowl with completely wild grouse species, I can determine if the chicken brain is more similar to its ancestral form, the red junglefowl, or if chicken brains are more similar in composition to other related grouse species.

Project 2: Domestication effects on olfactory bulb of homing, non-homing, and feral pigeons

Of all birds, pigeons are accepted to have the most diverse array of phenotypes across breeds (Gilbert & Shapiro, 2014; Price, 2002b), so much so that they have almost been accidentally classified as different species due to their wide range of phenotypic diversity (Darwin, 1868). The massive breed diversity makes studying domestic pigeon breeds very useful, especially for studying differences in neuroanatomy. Pigeons have been selected for aerial maneuvers, plumage complexity, feather color, body shape and size, homing behaviour, and many more traits. Although there is a plethora of phenotypes that pigeon breeds possess, this thesis specifically focused on one: homing behaviour. Homing has been intensely selected over many generations to create the super navigator, the homing pigeon. The homing pigeons' mechanisms for navigation has been highly contested, with olfaction recently gaining light in terms of sensory modalities that aid in navigation. Given the very strong selection for one behaviour (homing), and that homing pigeons are known to use olfactory cues as a major indicator of position relative to their home loft (Gagliardo, 2013; Papi, 1982; Papi et al., 1971) homing pigeons are an ideal model to study how navigational ability can have correlated changes in OB anatomy, and how other domesticated breeds of pigeon differ from homing pigeons in their olfactory systems.

As stated above, pigeons have been selected for a wide range of behaviours, the most well-known of which is homing ability (Meskenaite et al., 2016). Homing is the ability to navigate back to a home loft after being displaced at distances of thousands of kilometers of unfamiliar terrain (Mehlhorn & Rehkämper, 2009). Homing is one of the most remarkable examples of animal navigation that we know of (Bingman et al., 2005) and a critical part of homing behaviour is a detailed and accurate map. Through training, homing pigeons create a

spatial map within the hippocampus and rely on spatial memory and visual cues to navigate home (Bingman et al., 2005). A map, however, is only one part of navigation. Effectively navigating over long distances also requires a compass that is dependent on available and relevant sensory cues. Magnetoreception is believed to play at least a partial role in homing pigeon navigation (Mora et al., 2004). It is hypothesized that homing pigeons detect the earth's magnetic field via specialized receptors to orient themselves in the direction of their home loft and find their way back, although the precise mechanism underlying magnetoreception in pigeons remains highly controversial (Gagliardo et al., 2006; Mora et al., 2004; Nimpf et al., 2019).

Olfactory cues are also of critical importance for homing pigeons to orient themselves in the direction of their home loft and maintain the proper course of direction for the flight home (Ioalè et al., 1990; Papi, 1991; Papi et al., 1971, 1972). To support olfactory-based navigation, homing pigeons are reported to have enlarged olfactory bulbs compared to other domesticated, non-homing breeds (Rehkamper et al., 2008; Rehkämper et al., 1988). However, these comparisons were based on relatively few breeds and data on neuron numbers are lacking. Obtaining quantitative data on neuron numbers and sizes would provide much needed insight into whether homing pigeons have greater olfactory sensitivity and/or processing power than other breeds.

In Chapter 3, we quantify the size of the olfactory bulbs and the number and size of mitral cells across homing pigeons, feral pigeons, non-homing sporting breeds, and show breeds. By comparing homing pigeons and feral pigeons to a whole host of other sporting and show breeds including highflyers, rollers, show homers, show rollers, Capuchines, and Norwich

croppers, we can conduct a more rigorous test of whether the olfactory bulbs of homing pigeons differ from other domestic pigeon breeds and, more importantly, how they differ.

CHAPTER 2: THE EFFECTS OF DOMESTICATION ON TELENCEPHALON COMPOSITION OF THE CHICKEN: A COMPARISON WITHIN AND ACROSS SPECIES

INTRODUCTION

Domestication of animals began approximately 15 000 years ago with wolves (*Canis lupus*), soon to be followed by many other plants and animals (DeMello, 2021; Vigne, 2011). The process of domesticating animals is almost identical across species: a subset of a wild population is isolated and then selected for specific traits within a captive environment across several generations. Some of the desirable traits that are actively selected for include tameness towards humans, larger body sizes (in meat producing animals), certain coat or plumage colors, and specific behavioural traits (Price, 1984; Trut, 1999). In addition to artificial selection, animals must also adapt to a human-made environment and, in most instances, different food sources than what they would normally eat in the wild (Price, 1984). Over time, artificial selection and captive breeding ultimately changes their phenotype such that they differ significantly in phenotype from the wild type (DeMello, 2021).

Domestication not only alters those traits under direct selection by humans, but there are also indirect effects. One of the more common indirect effects of domestication is a reduction in brain size of domesticates (i.e., domesticated strains of a species) compared with their wild ancestors. Domesticated mammals have decreased overall brain size compared to their wild counterparts, ranging from 8% to up to 30% (Kruska, 1988b). Similar patterns are observed in many domesticated bird species. For example, in ducks (*Anas platyrhyncos*) and pigeons (*Columba livia*), overall brain size is decreased by 14% and 7%, respectively (Ebinger, 1995; Ebinger & Löhmer, 1984). These reductions in overall brain size are largely driven by the

shrinkage of telencephalic regions. Indeed, the most drastic volumetric changes in the brain arising from domestication are decreases in the sizes of the sensory cortices, hippocampus, and amygdala (Brusini et al., 2018; Ebinger, 1984; Kruska, 1988b). Although the telencephalon is most affected by domestication, changes in the size of individual regions are not consistent across species and some regions do not change in size at all. For example, the arcopallium is greatly reduced in volume in both domestic ducks and geese (*Anser anser*), but not in domestic pigeons (Ebinger, 1995). Similarly, hyperpallial regions are reduced in domestic pigeons and geese, but not in ducks (Ebinger & Löhmer, 1984). There are even examples of the expansion of telencephalic regions in domesticates, despite a reduction in brain size, such as the enlarged hippocampal formation of homing pigeons relative to wild rock doves (Rehkamper et al., 2008).

Although many domesticated species have been the focus of neuroanatomical studies, data is lacking for the world's most common bird species: the chicken (*Gallus gallus domesticus*). All domestic chicken breeds are derived from wild red junglefowl (*Gallus gallus gallus*), a species found in the Indus Valley of south-east Asia. Red junglefowl were first domesticated in 5000 B.C. and their original purpose was cockfighting, a form of entertainment in earlier human civilizations (Al-Nasser et al., 2007; Perry-Gal et al., 2015). Three-thousand years after their original domestication, they had spread to Middle East and Europe (Wang et al., 2020), at which point chickens had become a food source selected for both larger body size and egg production. Today, there are hundreds of chicken breeds that differ in size, behaviour, and morphology from the red junglefowl (Al-Nasser et al., 2007; Perry-Gal et al., 2015). Brain composition varies among many of these breeds, and some of that variation may reflect behaviour (Rehkämper et al. 2003). For example, the White Crested Polish chicken has the most divergent brain of the breeds examined thus far, with an enlarged telencephalon, optic

tract, and diencephalon compared with other breeds (Frahm & Rehkamper, 1998). Despite these studies of neuroanatomical variation across chicken breeds, relatively little is known about differences between chickens and their predecessor, the red junglefowl. Compared to the wild red junglefowl, domestic chickens are tamer towards humans and generally have larger bodies (Campler et al., 2009; Henriksen et al., 2016), but relatively smaller brains (Henriksen et al., 2016). Despite having relative smaller brains, white leghorn chickens have relatively larger cerebella than junglefowl (Henriksen et al. (2016)), which is at least partially due an enlarged granule cell layer (Racicot et al., 2021). There is also a difference in telencephalon size between junglefowl and white leghorn chickens (Henriksen et al., 2016), but a more detailed size comparison of regions within the telencephalon is lacking. Such an analysis could reveal changes similar to those reported in other domesticate-wild comparisons in birds (Ebinger, 1995; Ebinger, 1984; Rehkämper et al., 1988), including region specific expansions/reductions correlated with behavioural differences.

Here, we address this knowledge gap by quantifying the volumes of eight telencephalic regions of junglefowl and white leghorn chickens from the same populations used by Henriksen et al. (2016). The junglefowl were captive bred and captive breeding can cause volumetric decreases in brain size (Guay, 2008) and individual brain regions (Day et al., 2008; LaDage et al., 2009). To account for this, we compare these data with that of four grouse species collected in the wild: ruffed grouse (*Bonasa umbellus*), sharp-tailed grouse (*Tympanuchus phasianellus*), lesser prairie chicken (*Tympanuchus pallidicinctus*), and greater prairie chicken (*Tympanuchus cupido*). There are two main reasons for comparing chickens and junglefowl with wild grouse. First, all of the grouse were collected in the wild, so there are no effects of captivity or crossbreeding with domesticated strains/species. Second, these four grouse species are similar

in body size to chickens and junglefowl, so that intraspecific allometry can be readily compared across species and strains.

We hypothesize that there will be differences among chickens, junglefowl, and wild grouse species in the relative size of telencephalic brain regions that reflect behavioural and sensory processing abilities among them. Based on previous work (Ebinger, 1995; Ebinger & Löhmer, 1987; Ebinger, 1984; Ebinger & Röhrs, 1995; Kruska, 1988a, 1988b; Kruska & Schott, 1977; Rehkamper et al., 2008; Rehkämper et al., 1988; Rehkämper et al., 2003) and the functions of the telencephalic regions measured (Shanahan et al., 2013), we can make a few predictions. First, limbic regions will be smaller in domestic chickens because they are selected for reduced fear of humans (Agnvall et al., 2017), a function modulated by the limbic system (Reiner et al., 2005). Second, chickens have poorer visual acuity than junglefowl (Roth & Lind, 2013), so we expect the entopallium, the telencephalic target of the tectofugal visual pathway (Shimizu et al., 2010), to be reduced in domestic chickens compared to junglefowl. Third, we predict that all four grouse species will have larger sensory and limbic telencephalic regions than both chickens and junglefowl to support their navigational, sensory, and predator evasion needs in the wild because chickens and junglefowl do not need to detect or avoid predators in captivity, and foraging behaviour differs markedly between wild and captive housed birds.

METHODS

Specimens

To assess the effects of domestication on telencephalon composition, we measured the volumes of eight telencephalic regions across 6 male white leghorn chickens (*Gallus gallus domesticus*), 6 male red junglefowl (*Gallus gallus*) and 4 species of grouse (greater prairie chicken, *Tympanuchus cupido*, n = 3*;* lesser prairie chicken, *Tympanuchus pallidicinctus*, n = 3*;*

ruffed grouse, *Bonasa umbellus*, n = 6; sharp-tailed grouse, *Tympanuchus phasianellus*, n = 3). The chickens and junglefowl were provided from breeding colonies maintained at Linköping University (Linköping, Sweden). The red junglefowl were derived from a Swedish zoo population and have been kept at Linköping University since 1998. The domesticated chickens originated from a selection line, SLU13, bred at the Swedish University of Agricultural Sciences. The sharp-tailed grouse specimens were obtained from hunters in Canada, the ruffed grouse were trapped in the field (Corfield et al., 2013) and both prairie chicken species were donated by Dr. J. Augustine (Audubon Kansas). Prior to quantification, all brains were sectioned on a freezing stage microtome in the coronal plane at 40 µm. Every second section was mounted onto gelatinized slides and were subsequently stained with thionin acetate (Nissl) stain.

Quantification of Telencephalon Subregions

The volumes of eight telencephalic regions were measured including: sensory regions (hyperpallium, entopallium), motor (arcopallium), limbic (septum, hippocampus, striatum) and associative (mesopallium, nidopallium) as well as total telencephalon volume. The volumes were quantified using unbiased stereology with the Cavalieri estimator in StereoInvestigator (Microbrightfield, Williston, VT) using a Zeiss Axio Imager M2 microscope (Carl Zeiss, MicroImaging GmBH, Germany), with borders of regions delineated by the chick (Puelles et al., 2018) and pigeon brain atlases (Karten & Hodos, 1967). For hyperpallium volume, we took the combined volumes of both the dorsal and ventral hyperpallium as the two regions could not be consistently differentiated within and across specimens. Similarly, nucleus basalis could not always be discerned due to variation in staining quality, so we included nucleus basalis as part of our nidopallium measurements. We were, however, able to distinguish the arcopallium from

the rest of the nidopallium, so this region was measured separately. The entire hippocampal formation was measured and included hippocampus proper (Hp), area parahippocampalis (APH), as well as the dorsal lateral corticoid area (CDL). To estimate the volumes of each brain region, a 1x objective was used and a grid size of either 400 µm x 400 µm or 450 µm x 450 µm depending on the size of the brain. Every 16^{th} section throughout the rostro-caudal extent of the telencephalon was measured for every specimen.

Statistical Analyses

All data were log-transformed prior to statistical analysis. One-way analyses of covariance (ANCOVAs) were used to test for significant differences in relative telencephalon region size among chickens, junglefowl, and ruffed grouse. We used only ruffed grouse as our third group to contrast against the chickens and junglefowl as they have similar brain size to chickens and junglefowl and we could obtain the same sample sizes across all three. For the ANCOVA, species was used as a fixed factor, the telencephalon subregion of interest was used as the dependent variable (y-axis), and the telencephalon minus the region of interest was used as the covariate (x-axis). For all significant p-values ($p < 0.05$), Tukey's post hoc tests were used to identify which groups were significantly different from each other (i.e., pairwise differences).

Because the telencephalon is heterogenous and different brain regions could be expanding/reducing in different species, we used multivariate analyses to determine how telencephalon composition varies among all specimens. Specifically, we used both principal component analysis (PCA) and cluster analysis in a similar fashion to previous studies of brain composition (Iwaniuk and Hurd 2005; Rehkamper et al. 2003). For both sets of analyses, we

ran data for each individual specimen and expressed telencephalic region volume as a proportion of total telencephalon volume (i.e., region volume divided by total telencephalon volume). The PCA allowed us to reduce the total number of variables such that a smaller number of principal components (PCs) could be plotted. The cluster analysis was used to identify groups within the data set and organize them based on specific properties, thus revealing "clusters" of species that have similar telencephalon composition (Yim & Ramdeen, 2015). For the cluster analysis, we used Euclidian distance measures with the ward D.2 clustering method. The Euclidian distance measure (or the squared Euclidian distance measure) is recommended when using continuous variables (Yim & Ramdeen, 2015). The ward D.2 method is recommended for most datasets as it creates clusters with the smallest variance between members of the same cluster, allowing groups to form based on similarity to one another (Eszergár-Kiss & Caesar, 2017).

RESULTS

Comparisons of chickens, junglefowl, and ruffed grouse

When compared with ruffed grouse, both junglefowl and chickens have significantly larger telencephalon volumes than ruffed grouse relative to the rest of the brain ($F = 45.93$, $df = 2, 14$, $p < 0.001$) (Figure 2.2, Table 2.1). No significant interaction effects were detected (all p-values > 0.2), so we removed the interaction effects and tested for differences among groups, using telencephalon remainder as a covariate. There were significant effects of group (i.e., species) on the relative size of the hyperpallium ($F = 9.26$, $df = 2, 14$, $p < 0.005$), nidopallium ($F = 12.37$, $df = 2, 14$, $p < 0.001$), and striatum ($F = 5.71$, $df = 2,14$, $p < 0.05$). (Figure 2.2, Table 2.1). More specifically, red junglefowl have significantly smaller hyperpallial volumes

for their telencephalon size than ruffed grouse (Figure 2.2B) and both red junglefowl and white leghorns have significantly larger nidopallial volumes than ruffed grouse (Figure 2.2D). Finally, red junglefowl and white leghorn have larger striatal volumes than ruffed grouse, but only junglefowl were significantly larger (Figure 2.2E).

Principal Component Analysis (PCA)

Of the eight principal components, the first three make up nearly 76% of the variation in the scores (Table 2.2). Based on the loadings, the first principal component (PC1) is positively correlated with the hyperpallium, mesopallium, hippocampal formation and septum, and negatively correlated with nidopallium, entopallium, arcopallium and striatum (Figure 2.3D). The second principal component (PC2) is mainly driven by entopallium, septum, and mesopallium: it is positively correlated with the mesopallium and entopallium, and negatively correlated with the septum (Figure 2.3E). PC3 is driven most strongly by a negative correlation with the hippocampal formation (Figure 2.3F). In the plot of PC2 against PC1 (Figure 2.3A), individuals are primarily separated along the PC1 axis (x-axis) with chickens and junglefowl on the left, indicating proportionally larger hyperpallium, mesopallium, hippocampal formation and septum than in other species. There was not, however, any apparent pattern or clustering along the PC2 axis. The second plot (Figure 2.3B) of PC3 against PC1 separates the sharp-tailed grouse from the other species along PC3, potentially reflecting relatively larger nidopallium and HF volumes in them. The third plot (Figure 2.3C) with PC3 against PC2 is less clear than the other two plots as all of the species cluster together in the top left of the figure, but the sharp-tailed grouse tend to be lower on the PC3 axis.

Cluster Analysis

The cluster analysis produced a dendrogram with two main groupings we labelled ‘A’ and ‘B’ (Figure 2.4). Group ‘A’ is composed of the ruffed grouse, lesser prairie chickens, and greater prairie chickens and is characterized by larger hyperpallial, mesopallial, hippocampal, and nidopallial volumes, but smaller striatal, entopallial, and arcopallial volumes (Table 2.3). Group ‘B’ is composed of the red junglefowl, white leghorns, sharp-tailed grouse, and one greater prairie chicken. This cluster is characterized by smaller hyperpallial, mesopallial, and nidopallial volumes, but larger striatal, entopallial, and arcopallial volumes (Table 2.3). Thus, the chickens and junglefowl tend to be more similar to one another than they are to the other species and the sharp-tailed grouse tends to differ from the other grouse species examined.

DISCUSSION

Overall, when compared with ruffed grouse, chickens and junglefowl differed in relative size of several telencephalic regions. This potentially indicates an effect of captive breeding on junglefowl brains or an effect of *Gallus* species being more like each other despite the effects of domestication on the chicken brain. Further, our multivariate analyses corroborate that chickens and junglefowl are more similar to one another than wild grouse species. Before discussing the potential implications of these results, it is important to note three potential limitations of this study. First, we only examined males. Although this was advantageous for this study because our analyses were not confounded by varying numbers of males and females within each species or strain, there is potential for our results to differ when analyzing only females or including both sexes in a study, particularly given how common sex differences in brain region sizes are across bird species (MacDougall-Shackleton & Ball, 1999; Nottebohm et al., 1976).

Second, we used grouse as our "wild-type" group. Although grouse and chickens are both within the same family (Phasianidae), they are not each other's closest relatives. Ideally, the wild species used in such a comparison would be the Chinese bamboo partridge (*Bambusicola thoracicus*) or francolins (Kimball et al., 2021), but obtaining these species in the wild is difficult logistically and captive-bred specimens would not allow us to disentangle the effects of captive breeding. The grouse represent wild specimens that are similar in body and brain size, even though they are not as closely related to the genus *Gallus* as other species (Kimball et al., 2021).

Third, we used captive-bred junglefowl whereas other species comparisons used wild-caught individuals. Captivity can have effects on brain anatomy, even after as few as eight generations (Guay, 2008) and the junglefowl have been maintained in captivity far longer (Henriksen et al., 2021; Henriksen et al., 2016; Johnsson et al., 2018). However, it should be noted that true wild junglefowl are nearly impossible to source; almost all wild junglefowl populations have been hybridized with domesticated chickens (Peterson & Brisbin, 1998; Wu et al., 2020). Considering the difficulty in obtaining truly wild junglefowl, captive bred junglefowl are the closest comparison with chickens that can be made, but this means that we cannot discount the possibility of captive breeding effects.

Chickens vs. Junglefowl

Based on our analyses, chickens have larger telencephalon volumes for the rest of their brain size compared to red junglefowl. This is consistent with the findings from (Henriksen et al., 2016) where domestic chickens had larger telencephala than junglefowl. In other domesticated birds, however, the telencephalon undergoes the greatest reduction of all brain regions (ducks -15%, geese -16%, turkeys -24-29%) (Ebinger, 1995; Ebinger & Löhmer, 1984;

Löhmer & Ebinger, 1983), with the exception of pigeons, where the telencephalon only decreases by about 7% (Ebinger & Löhmer, 1984). Chickens had larger pallial regions than junglefowl, but these were absolutely larger in chickens and there were no relative differences in any of the telencephalon regions measured. In other words, none of the telencephalon regions were relatively larger in the chickens than in junglefowl and therefore the chicken telencephalon could be considered a 'scaled-up' version of that of the red junglefowl.

In comparative analyses of telencephalic evolution in birds, these same regions scaled in a concerted way with body size across species such that they contribute more to not only absolutely larger brains, but also relatively larger brains (Sayol et al., 2016), and the same principle appears to apply to chickens and junglefowl. This was somewhat unexpected given the marked differences in behaviour between the two strains (Agnvall et al., 2012; Schütz et al., 2001; Schütz, 2001). However, we only measured the volume of telencephalic regions and there may be other differences between chickens and junglefowl that are associated with their behavioural differences. For example, changes in neuron number or neuronal density are often associated with cognition (Sol et al., 2022) but would not have been detected in this study.

There may also be changes in neurochemistry without any associated changes in volume, neuron numbers or neuron sizes. Neuropeptides (e.g., oxytocin, mesotocin, vasotocin) in vertebrates can have profound effects on behaviour, sociality, and aggression (Bales et al., 2004; Carter et al., 2008; Goodson & Bass, 2001; Goodson et al., 2004). For example, infusions of mesotocin in zebra finches (*Taeniopygia guttata*) caused them to be more social and spend more time in social groups (Goodson et al., 2009). Given that chickens are much more tolerant of threats than junglefowl (Agnvall et al., 2014; Agnvall et al., 2012), it is possible, and even likely, that changes in neuropeptides are responsible for the behavioural differences between the two strains. It is also possible that there are differences in central nervous system

neurotransmitters, particularly GABA and serotonin, as these neurotransmitters seem to play a role in tameness (Albert et al., 2008). More detailed neurochemical studies are therefore warranted to address chicken-junglefowl behavioural differences in relation to the brain.

Chickens and Junglefowl vs. Ruffed grouse

Compared with ruffed grouse, both chickens and junglefowl had significantly larger telencephala for their brain size. This is an unusual finding as one would expect the telencephala of chickens and junglefowl to be relatively smaller due to captive breeding (Ebinger, 1995; Ebinger, 1984; Guay, 2008). Ruffed grouse appear to have smaller telencephala, which could either be explained by a larger "rest of brain", meaning everything but the telencephalon could be enlarged (i.e., brainstem, cerebellum, tectum), or that the chickens and junglefowl have smaller "rest of brain" sizes, making their telencephalon relatively larger than the rest of their brain. The directionality of these changes cannot be determined from a comparison of two species (Garland Jr & Adolph, 1994), so we instead focus our interpretation on the three telencephalic regions were disproportionately different in size in chickens and junglefowl compared to ruffed grouse.

First, junglefowl and chickens have a relatively smaller hyperpallium than ruffed grouse. This is consistent with previous work in pigeons where domestic homing pigeons had smaller hyperpallia volumes than wild rock doves (Rehkamper et al., 2008). The hyperpallium comprises around 12% of the telencephalon in chickens and processes primarily somatosensory and visual information (Atoji et al., 2018). The functional relevance of a relatively larger or smaller hyperpallium is challenging to interpret given its multisensory nature (Reiner et al., 2005; Shanahan et al., 2013). However, we speculate that the captive environment of chickens and junglefowl may have resulted in a decreased reliance on visual and somatosensory

processing because the need for predator detection/evasion as well as complex foraging behaviour are largely eliminated.

Second, both junglefowl and chickens have larger nidopallia than ruffed grouse. The nidopallium is the largest subregion within the telencephalon and has many functions and numerous subdivisions (Shanahan et al., 2013). In general, the nidopallium, is responsible for higher-level processing, sensory integration, and cognition (Shanahan et al., 2013), potentially suggesting differences in cognition and/or sensory processing. We expected that the nidopallium would be larger in ruffed grouse than chickens and junglefowl as a captive environment would require, in theory, less cognitive processing, so why the reverse occurs is uncertain. The use of immunohistochemistry or *in situ* hybridization could potentially enable the delineation of individual nidopallial regions that are not discernible in Nissl stained tissue (Güntürkün, 2005; von Eugen et al., 2020), but this would still not address why nidopallial regions are larger in chickens and junglefowl. One potential avenue to explore would be the use of a battery of behavioural tests of chicken, junglefowl, and wild Galliformes to assess if there are cognitive or sensory perception differences across species/strains (Cauchoix et al., 2017; Shaw, 2017).

Third, junglefowl and chickens have larger striatal volumes than ruffed grouse. Domestic mammals have relatively smaller striatal and limbic brain regions compared to their respective wild counterparts (Kruska, 1988a) and domestic birds show the same trend; the striatum is smaller in domestic ducks, turkeys, and to a lesser extent, pigeons (Ebinger, 1995; Ebinger & Röhrs, 1995; Löhmer, 1984). Our findings in chickens seem to contradict the trend once again compared to other domesticated species. The striatum is part of the basal ganglia circuitry, and does not have one specialized function, but does play a significant role in movement control and coordination (Reiner et al., 1998; Reiner et al., 2005). This could reflect

a difference in motor control or coordination between chickens and junglefowl and ruffed grouse, although once again this requires behavioural testing.

Although each of these three regions, hyperpallium, nidopallium, and striatum, differ in size between ruffed grouse and chickens/junglefowl in our analyses, it is important to note that a change can occur in either direction. That is, a region can appear larger due to other regions getting smaller, or it could mean that the region of interest is truly larger. Determining directionality of these differences is not possible with such a small number of species/strains. We therefore urge caution is extrapolating functional consequences of our results until such time that our results can be corroborated by behavioural or physiological testing.

Multivariate Analyses of Telencephalon Composition

The principal component analysis (PCA) revealed that chickens and junglefowl have a different telencephalon composition than other grouse species. To elaborate, junglefowl and chickens group together with sharp-tailed grouse, largely due to differences in hyperpallium, mesopallium, and nidopallium sizes compared to other grouse. To some extent, this corroborates our comparisons with ruffed grouse (see above) and indicates that chickens and junglefowl differ in telencephalon composition from grouse species.

Our cluster analysis further revealed that chickens and junglefowl are more like each other than prairie chickens or ruffed grouse (Figure 2.6). Given that cluster analysis groups individuals solely based on similarities within the data, we can infer that chickens and junglefowl have different telencephalon composition compared to wild grouse, with the exception of sharp-tailed grouse in our limited dataset. With such a small number of species sampled, it is not possible to determine why the sharp-tailed grouse groups with the chickens and junglefowl and not with other members of its own genus (i.e., the prairie chickens).

Despite the lack of difference between chickens and junglefowl, the fact that both differ from wild Galliformes reveals that changes may have occurred through the captive breeding process, or that *Gallus* species are different from grouse species. First, given that captive environments presumably require less reliance on sensory processing and cognition, this might explain why we see junglefowl and chickens with smaller sensory processing (hyperpallium) regions than wild ruffed grouse. Although not intuitive, the larger nidopallium and striatum in chickens and junglefowl may be due to the hyperpallium decreasing in size. The other possibility is that these regions are indeed larger, so there may be some element of behavioural change in captivity that has not yet been studied. Second, given that junglefowl are the ancestor to domestic chickens, it is possible that their similarity in telencephalon composition is simply due to genetic relatedness. Thus, future research in this field should not only compare neuroanatomy across and within species, but also behaviour, neurochemistry, and phylogeny to better disentangle the interactions and consequences of captive breeding, domestication, and genetics.

Table 2.1. Results of one-way analysis of covariance (ANCOVAs) of species, telencephalon volume minus subregion volume (covariate), and their interaction on eight measurements of telencephalon subregion volumes: hyperpallium, mesopallium, nidopallium, striatum, entopallium, hippocampal formation, septum, and arcopallium. The three groups examined were Red Junglefowl (RJF), White Leghorn (WL), and Ruffed Grouse (RUGR). ANCOVA results are reported as F-ratios (F), degrees of freedom (df) and p-values (p), where (*) denotes a significant effect. For all significant p-values of group (p-value < 0.05) effects were further tested with Tukey Honest Significant Difference (HSD) post-hoc analyses. In the post-hoc column, differences between strains are indicated using greater than (>) or less than (<) signs. All data were log transformed before analysis to improve normality.

Brain Region	Group				Telencephalon Size		
	F	df	p	Tukey	F	df	p
Hyperpallium	9.26	2, 14	**0.003***	RJF>RUGR	12.41	1, 14	**0.003***
Mesopallium	4.49	2, 14	**0.031***		11.47	1, 14	**0.004***
Nidopallium	12.37	2, 14	**<0.001***	RJF, WL>RUGR	3.57	1, 14	0.080
Striatum	5.71	2, 14	**0.015***		5.59	1, 14	**0.033***
Entopallium	0.08	2, 14	**0.031***	RJF>RUGR	0.0255	1, 14	0.875
Hippocampal formation	2.16	2, 14	0.152		1.28	1, 14	0.277
Septum	3.05	2, 14	0.080		13.73	1, 14	**0.002***
Arcopallium	1.72	2, 14	0.215		2.41	1, 14	**0.143**
Total Tele	45.93	2, 14	**<0.001**	RJF, WL>RUGR	6.62	1, 14	0.022

Table 2.2. Loadings, cumulative variation, and eigenvalues arising from the principal component analysis of the proportional sizes of 8 telencephalic regions across chickens, junglefowl, and all four grouse species. H = hyperpallium, M = mesopallium, N = nidopallium, St = striatum, E = entopallium, HF = hippocampal formation, S = septum, A = arcopallium.

Tele. Region	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
H	0.766	-0.181	0.388	-0.215	0.031	-0.409	-0.101	-0.078
M	0.762	0.463	0.230	-0.046	0.183	0.316	0.066	-0.116
N	-0.841	-0.241	-0.404	-0.098	0.035	0.053	-0.192	-0.146
St	-0.830	-0.044	0.253	-0.328	-0.134	-0.067	0.335	-0.055
E	-0.475	0.637	0.349	0.243	-0.406	-0.049	-0.143	-0.024
HF	0.639	-0.003	-0.548	0.424	-0.235	-0.140	0.179	-0.067
S	0.306	-0.760	0.383	0.148	-0.308	0.254	-0.026	-0.017
A	-0.561	-0.151	0.362	0.633	0.345	-0.089	0.055	-0.028
Cumulative % variation	45.06%	61.46%	75.59%	85.92%	92.01%	96.62%	99.37%	100.00%
Eigenvalues	1.312	1.131	0.8264	0.8264	0.4875	0.3689	0.2199	0.05012

Table 2.3. The mean proportions of each of the eight telencephalic subregions in each cluster as created by a hierarchical cluster analysis (see text for details). The brain regions are as follows: H (total hyperpallium), M (mesopallium), N (nidopallium), Str (striatum), E (entopallium), HF (hippocampal formation), Sept (septum), A (arcopallium). The proportions were calculated by dividing the volume of each individual structure by that of the total telencephalon volume minus the subregion volume. The proportions for each cluster are the total averages from every individual making up that cluster. As shown in Figure 2.5, Cluster A is composed of ruffed grouse, lesser prairie chicken, and greater prairie chicken whereas Cluster B is composed of red junglefowl, white leghorn, sharp-tailed grouse, and one greater prairie chicken. SD = standard deviation.

Species		H	M	N	Str	E	HF	Sept	A
Cluster A	Mean	0.1274	0.1901	0.4008	0.1116	0.0185	0.0628	0.0102	0.0508
	SD	± 0.020	± 0.028	± 0.032	± 0.007	± 0.006	± 0.009	± 0.003	± 0.004
Cluster B	Mean	0.1174	0.1634	0.4391	0.1239	0.0204	0.0525	0.0110	0.0536
	SD	± 0.020	± 0.017	± 0.032	± 0.013	± 0.006	± 0.012	± 0.001	± 0.008

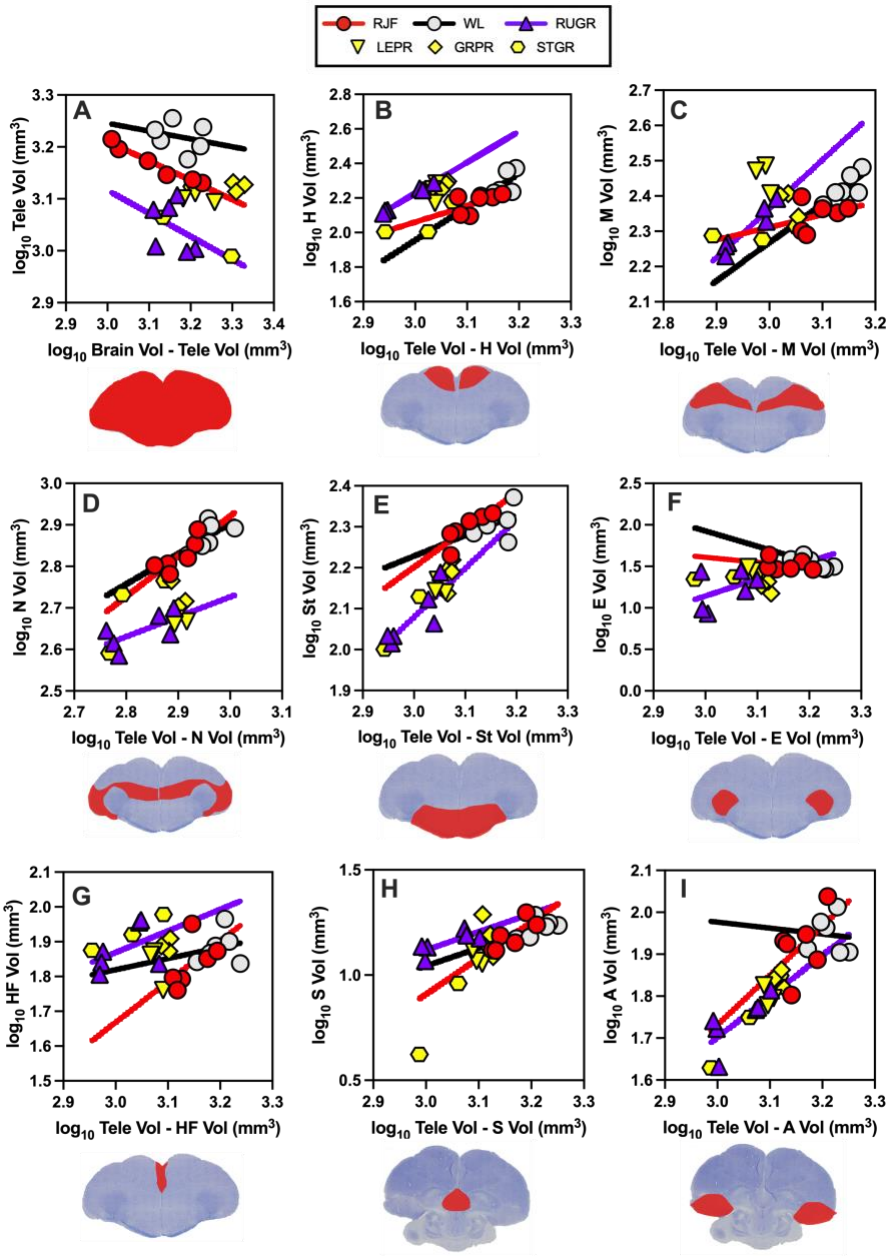

Figure 2.1. Double log-transformed scatter plots showing the interspecific relationship between (A) telencephalon volume (y-axis) and total brain volume minus telencephalon volume (x-axis). Volume, (B) telencephalon volume to hyperpallium volume, (C) telencephalon volume to mesopallium volume, (D) telencephalon volume to nidopallium volume, (E) telencephalon volume to striatum volume, (F) telencephalon volume to entopallium volume, (G) telencephalon volume to hippocampal formation volume, (H) telencephalon volume to septum volume, and (I) telencephalon volume to arcopallium volume. RJF = red junglefowl, WL = white leghorn chicken, RUGR = ruffed grouse, GRPC = greater prairie chicken, LEPC = lesser prairie chicken, STGR = sharp-tailed grouse. Representative sections with region of interest highlighted are shown below each respective scatter plot. Red, black, and purple regression lines represent junglefowl, chicken, and ruffed grouse, respectively.

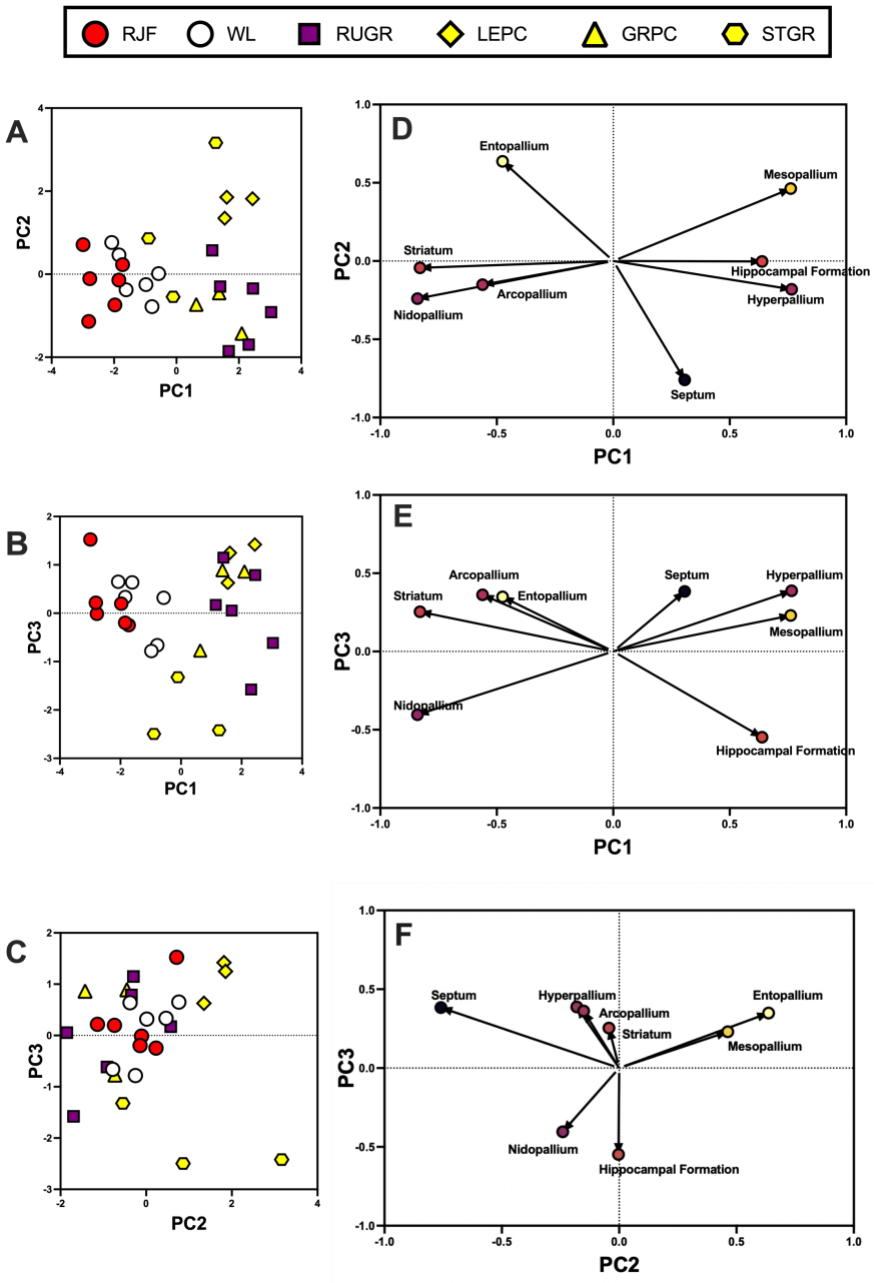

Figure 2.2. Scatter plots of PC scores for all groups (junglefowl, chickens, grouse). (A) Scatter plot showing the clustering of groups along PC1 and PC2. (B) Scatter plot showing the clustering of groups along PC1 and PC3. (C) Scatter plot showing the clustering of groups along PC2 and PC3. (D) Loadings plot showing the positive and negative correelations of each telencephalic region for PC1 (x-axis) and PC2 (y-axis). (E) Loadings plot showing the positive and negative correelations of each telencephalic region for PC1 (x-axis) and PC3 (y-axis). (F) Loadings plot showing the positive and negative correelations of each telencephalic region for PC2 (x-axis) and PC3 (y-axis). RJF = red junglefowl, WL = white leghorn chicken, RUGR = ruffed grouse, GRPC = greater prairie chicken, LEPC = lesser prairie chicken, STGR = sharp-tailed grouse.

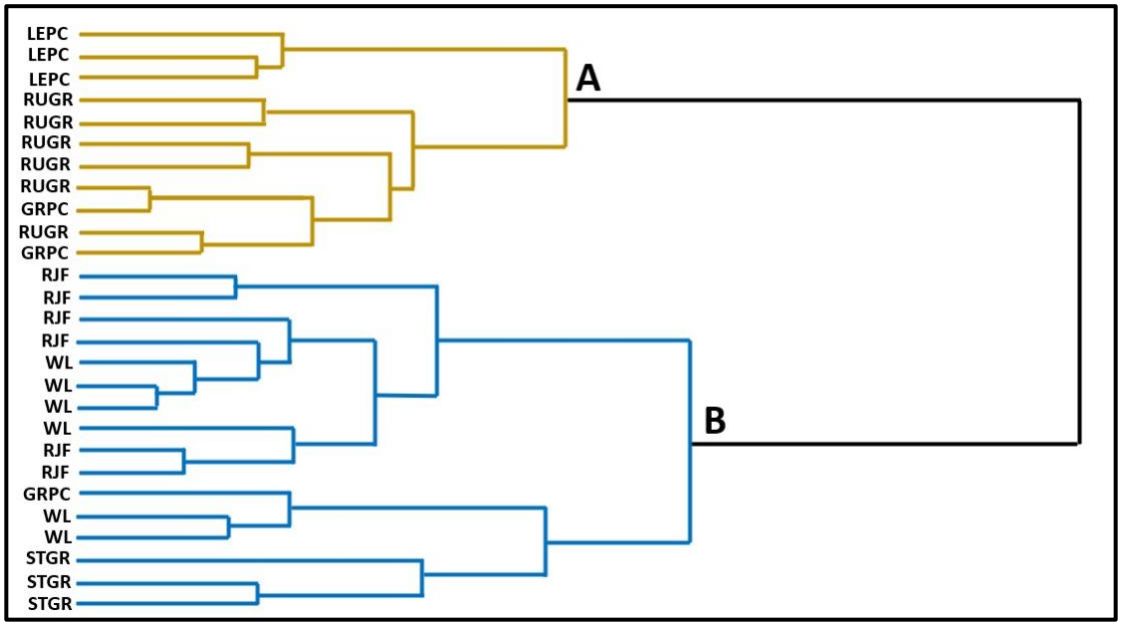

Figure 2.3. A dendrogram resulting from a UPGMA hierarchical cluster analysis. The two distinct clusters are indicated by the letter's "A" (yellow, top) and "B "(blue, bottom). RJF = red junglefowl, WL = white leghorn chicken, RUGR = ruffed grouse, GRPC = greater prairie chicken, LEPC = lesser prairie chicken, STGR = sharp-tailed grouse. Cluster A is primarily composed of ruffed grouse and prairie chickens. Cluster B is primarily composed of chickens, junglefowl and sharp-tailed grouse.

CHAPTER 3: HAS SELECTION FOR HOMING CAUSED CHANGES IN THE OLFACTORY SYSTEM OF THE HOMING PIGEON (*COLUMBA LIVIA*)?

INTRODUCTION

The process of natural selection is described as "a process by which organisms that are better-adapted to their environments produce more offspring to transmit their genetic characteristics", or in other words, improve an organism's fitness in the wild. This concept was first described and published in 1858 by Charles Darwin and Alfred Wallace (Darwin & Wallace, 1858). Although natural selection was one of the first types of selection identified, artificial selection was also described by Charles Darwin in his detailed study of the phenotypic variation of domestic pigeons (*Columba livia*) (Darwin, 1868). Artificial selection is one of the most important pressures placed on plants and animals in the initial steps of domestication (Price, 1999a), and is responsible for the variation across breeds of domesticated species.

Typically, we recognize breeds by their external characteristics, but behavioural selection can be equally intense. One of the most prominent examples of artificial selection for behaviour is the selection for homing ability in homing pigeons (Meskenaite et al., 2016). Homing is the term used to describe an animal's ability navigate to a home region after being displaced (Mehlhorn & Rehkämper, 2009). In homing pigeons, the definition is more specific in that homing is the pigeon's ability to navigate to a home loft over thousands of kilometers of unfamiliar terrain (Mehlhorn & Rehkämper, 2009). Based on decades of research, homing pigeons rely on a combination of spatial memory and visual cues (Bingman et al., 2005), magnetoreception (Gagliardo et al., 2006), sun-compass (Mehlhorn & Rehkamper, 2009), and olfactory cues (Gagliardo et al., 2011; Gagliardo et al., 2007; Patzke et al., 2010) to

successfully navigate. The relative importance of the sun-compass and magnetoreception remain hotly contested (Biro et al., 2007; Gagliardo et al., 2006). The hippocampus is important for the initial orientation during homing as the bird relies on visual landmarks to determine the direction home, but once on course, the hippocampus appears less important (Bingman et al., 2005; Bingman & Mench, 1990; Mehlhorn & Rehkämper, 2009). In contrast, olfactory cues seem to be the one cue that allows homing pigeons to maintain course over long-distance homing and, importantly, from unfamiliar locations (Benvenuti et al., 1973; Ioalè et al., 1990; Papi, 1976; Papi et al., 1971, 1972). Previous studies on the use of olfactory mediated navigation in pigeons have shown that olfactory nerve sectioning impairs homing (Papi et al., 1971, 1972) and pigeons that are given artificial olfactory cues (i.e. direction of olfactory information changed) oriented the wrong way home (Papi, 1991). From these and other studies (see review by Gagliardo (2013)), we can conclude that pigeons use olfaction for successful homing.

Because homing is reliant on olfactory cues and homing pigeons have been subject to intense selection for fast and efficient homing, the olfactory system of homing pigeons might differ from that of other pigeons. Comparisons of brain composition across domestic pigeon breeds partially support this prediction; homing pigeons have larger olfactory bulbs than some show breeds (Rehkämper et al., 1988), but not larger than wild rock doves (Ebinger, 1984). However, previous work only compared olfactory bulb (OB) volume of homing pigeons (Rehkamper et al., 2008) and did not include a sufficient diversity of breeds that spanned show and active breeds or the inclusion of feral pigeons. Active pigeon breeds have been selected for various ariel maneuvers and endurance in flight (Levi, 1965). For example, rollers were bred to do somersaults in the air and highflyers were bred to fly at extreme altitudes circling the home loft for hours (Kabir, 2015). These active breeds are regularly flown freely and therefore must

be able find their way home, albeit over much shorter distances than homing pigeons, and that navigation may be at least partially dependent on olfaction. Show breeds, in contrast to active breeds, rarely leave the loft and are primarily selected for their appearance, such as plumage, color, crop size, and body shape/size. Because the show breeds do not leave their home, there is no selection for improved olfactory navigation. Feral pigeons are derived from domesticated breeds and likely have ongoing introgression from lost or released domestic pigeons, including homing pigeons, other active breeds, and even some show breeds (Giunchi et al., 2020; Shapiro & Domyan, 2013). Like the active breeds, feral pigeons would be dependent on olfaction for some aspects of navigation, but without the intense selection that occurs in homing pigeons. Feral pigeons are, however, subject to natural selection and may be more reliant on olfaction for mate selection, foraging, and even predator detection, most of which would not apply to domestic pigeons of any breed.

In addition to ensuring that a sufficient diversity of pigeon breeds are examined, an anatomical comparison of the olfactory system requires more than volumetric data. There are multiple mechanisms of increasing brain region volumes: a region can develop more neurons, larger neurons, or both (Herculano-Houzel et al., 2014). Without data on neuron numbers and sizes, it will be unclear if any changes in homing pigeon olfactory bulb volumes are "scaled up" from other domestic breeds, or if they have altered neuronal density or neuron sizes. In the olfactory bulb, mitral cells are particularly important to quantify because they receive input from olfactory receptor neurons and project to the piriform cortex for processing of olfactory information (Patzke et al., 2011). The mitral cells therefore represent an information bottleneck for olfactory information to be sent from the olfactory bulbs to other parts of the brain. Having relatively more or larger mitral cells could therefore be indicative of faster processing and/or improvements to olfactory acuity that would improve the accuracy and precision of homing.

Larger or more mitral cells could also indicate higher processing power for olfactory information, or a generalized increase in olfactory input to telencephalic regions, which would inform navigational decisions (Ioalè et al., 1990).

Here, we provide a comprehensive test of whether the OBs of homing pigeons differ quantitatively from that of other pigeon breeds. We compared homing pigeons against feral, active, and show pigeons and quantified the number of mitral cells, the size of mitral cells, and the size of the OBs. Based on behavioural and housing variation across breeds, we predicted that homing pigeons will have the largest OBs and the most mitral cells compared to all other domestic breeds and feral pigeons. We also presume that feral pigeons and active breeds would have larger OB's than inactive breeds, as their reliance on olfaction would likely be more demanding. In addition, we predicted that homing pigeons will have larger mitral cells to accommodate faster olfactory processing compared to all domestic breeds and feral pigeons.

METHODS

Animals

Feral pigeons (n = 6) were trapped in downtown Lethbridge, AB, and Hanna, AB with baited walk-in traps. Homing pigeons (n = 6) were donated from a regional pigeon racing club and the other breeds purchased from breeders in Alberta and Saskatchewan (Table 3.1, Figure 3.1). All pigeons were euthanized with an overdose of sodium pentobarbital (Euthansol) and subsequently perfused with 0.9% phosphate-buffered saline (PBS) solution followed by 4% paraformaldehyde (PFA) solution (pH 7.4). The birds were then decapitated, and the heads were placed in fresh 4% PFA overnight and then the brains were extracted from the skull and immediately weighed. All procedures adhere to the Canada Council for Animal Care and were approved by the University of Lethbridge Animal Care Committee (protocol #2011).

Histology

The cerebellum was first removed from each brain by severing the connective fibers and cerebellar peduncles. The brains were then placed in fresh 4% PFA (pH = 7.4) 2-3 days post fixation and then in 30% sucrose and 0.1M phosphate buffered saline (PBS) solution as a cryoprotectant. Once the brains had sunk, we embedded each brain in gelatin and sectioned them on a freezing stage microtome at a thickness of 40µm in the coronal plane. Each section was placed in a tissue well with 0.1M PBS. For the entire brain through the rostro-caudal extent, every fourth section was mounted onto gelatinized slides and set to air dry for several days. For the olfactory bulbs, every second section was mounted to ensure that we had sufficient sections for accurate neuron counts and volume estimates. Once dried, the slides were stained with thionin acetate (AcrosOrganics, LOT: A0393256) for Nissl substance and then coverslipped with Permount (Fisher Scientific, LOT: 216064). Representative sections through the olfactory bulbs of all 8 breeds are shown in Figure 3.1.

Stereological estimates

We used a 1x objective on a Zeiss AxioImager M2 microscope (Carl Zeiss, MicroImaging GmBH, Germany) to measure the volumes of the whole brain, telencephalon, and olfactory bulb. The volumes for the entire brain and telencephalon were measured using the Cavalieri estimator (Mouton, 2013; West, 2012) in StereoInvestigator (MBF Bioscience, Williston, VT USA). We sampled between 25 and 30 sections throughout the entire extent of the brain (rostro-caudal extent) (i.e., every 4^{th} mounted section) and a grid size of 400 µm x 400 µm. This was the same for all specimens. The volumes for the OB were also measured using a

350 x 350 µm grid size through 10 to 20 sections through the entire extent of the olfactory bulb by measuring every mounted section (i.e., every 2nd section). We took the OB volume as the total volume from both the left and right sides. The Coefficients of Error (Gunderson m=1) were all below 0.02.

The number of mitral cells were quantified using the same system, but with a 40x immersion oil lens (NA = 1.4) and the optical fractionator (Mouton, 2013; West, 2012) in StereoInvestigator. Mitral cells were identified by three criteria: 1) their large size compared to the granule cells and cells in the external plexiform layer; 2) their signature "angular" shape; and 3) were found within the mitral cell layer of the OB (Figure 3.2). Only cells with their entire soma within the boundary of the counting frame or touching the inclusion lines were counted. The counting frame was 40 µm x 40 µm and the grid size was 300 µm x 300 µm, with an optical dissector height of 15 µm and top and bottom guard zones of 1 µm. Coefficients of error (Gunderson m = 1) ranged from 0.05 to 0.09.

To measure mitral cell size, we used the same parameters as for counting cells (frame = 40 µm x 40 µm, grid size = 350 µm x 350 µm). To quantify the soma size, we used the nucleator probe in StereoInvestigator with six rays and placed the marker in the center of the mitral cell and the points where the ray intersected the edge of the soma were identified as the edge of the cell. From the nucleator probe, we extracted soma volume (μm^3) of the mitral cells. For each specimen, we measured the size of ~50 mitral cells.

Statistical Analyses

We first used a one-way analysis of variance (ANOVA) to test for absolute differences in OB volume, number of mitral cells, and size of mitral cells among pigeon breeds. Tukey's HSD post-hoc tests were used to determine which breeds were significantly different from one

another. One-way analyses of covariance (ANCOVAs) were then used to test for relative differences across breeds on the same variables: OB size, number of mitral cells, and size of mitral cells. For the ANCOVA for OB size, we used breed as a fixed factor, telencephalon volume minus OB volume as the covariate, and OB volume as the dependent variable. For the ANCOVA for mitral cell number and mitral cell size, we used breed as a fixed factor, OB volume as the covariate, and mitral cell number/size as the dependent variables. For all significant p-values ($p < 0.05$), Tukey's post-hoc tests were run to determine which breeds were significantly different. All statistical analyses were run in Jamovi (Jamovi, 2021).

RESULTS

OB Volumes

Across the breeds that we sampled, homing pigeons had the largest OB's overall and show rollers had the smallest (Table 3.1, Figures 3.3, 3.4A). Despite the homing pigeons having large OBs (Figure 3.4A), there were no significant differences in absolute OB volumes among breeds ($F = 2.65$, $df = 7, 32$ $p = 0.063$). Figure 3.4B represents the ratio of OB to telencephalon (OB size divided by telencephalon size), and Figure 3.4C shows the scaling relationship between OB size (y-axis) and telencephalon size (x-axis). Both figures are useful to visualize the difference in relative OB size across pigeon breeds as the boxplot (Figure 3.4B) shows the magnitude of difference (i.e., effect size), and the scatter plot (Figure 3.4C) shows whether breeds are above or below the allometric expectation of OB size for brain size. Relative to telencephalon size (covariate), OB volume did differ significantly among breeds ($F = 3.18$, $df = 7, 32$, $p = 0.012$). Post-hoc tests revealed that homing pigeons had relatively larger OBs than three of the four show breeds, with the one exception being show homers (Figure 3.4B, Table

3.4). No significant differences were present between homing pigeons and the active breeds or feral pigeons (or among any of the other breeds) (Figures 3.4B, 3.4C, Table 3.4).

Mitral Cells

Overall, homing pigeons had the most mitral cells overall, and croppers had the fewest (Table 3.1), but no significant differences were detected across any of the breeds ($F = 2.68$, $df = 7, 32$ $p = 0.063$, Figure 3.5A). As shown in Figure 3.6, croppers had the largest mitral cells with most cells measured in the 1500-2000 μm^3 range, with show rollers having the smallest with most cells measuring in the 1000 μm^3 range. However, the differences in cell size among breeds was small and did not vary significantly among them ($F = 1.80$, $df = 7, 32$, $p = 0.17$, Figure 3.5B). Active breeds had mitral cells that were 7% bigger compared with homing pigeons (Table 3.1). Similarly, show breeds had larger mitral cells than homing pigeons (6%) with croppers having the largest cells (Table 3.1, Figure 3.5B).

We also tested whether there were differences in breeds for mitral cell number and size relative to the size of the OBs. Relative mitral cell number (mitral cell density) was calculated by dividing the number of mitral cells by the OB volume. There were no relative differences in mitral cell number among breeds (Table 3.3., $F = 0.91$, $df = 7, 32$, $p = 0.51$). Relative mitral cell size was calculated by dividing the mitral cell size by the OB volume. There were no significant differences among any breeds on relative mitral cell size (Table 3.3, $F = 1.68$, $df = 7, 32$, $p = 0.151$). The boxplots for relative mitral cell size (Figure 3.5D) show no significant difference in means between breeds. However, both show breeds and active breeds, on average, had mitral cells that were 32% larger than homing pigeons relative to the size of their OB (Figure 3.5D). The scatter plot (Figure 3.5F) shows a cloud of data points with active and show breeds, with

no discernable trend in the data, suggesting a larger OB is not associated with a significant change in average mitral cell size.

DISCUSSION

Larger brain structures are typically associated with higher processing capacity (Jerison, 1973). This appears to be especially true of sensory systems in which the acuity and sensitivity of a sensory modality are higher in species with larger sensory brain regions (Corfield et al., 2015; Iwaniuk et al., 2004; Iwaniuk & Hurd, 2005; Krebs, 1990; Wylie et al., 2015). Given that homing is heavily reliant on olfaction (Papi, 1976; Papi, 1991; Papi et al., 1971, 1972), we predicted that homing pigeons would have enlarged olfactory bulbs compared to other breeds. Overall, our results confirm previous work showing that homing pigeons have relatively larger OBs than show breeds (Rehkämper et al., 1988), but homing pigeon OBs did not differ significantly from active breeds or feral pigeons. In fact, there is considerable overlap in the relative size of the OBs homing pigeons with most breeds (Figure 3.4) We propose several possible reasons for this overlap and why the relative size of the homing pigeon OBs only differs from some breeds.

Homing pigeons did have larger OBs than three of the show breeds but did not differ from American show homers. Show homers were derived from homing pigeons, but were selected only for body size and appearance, with no selection for homing ability (Woodfield, 1892). In fact, early accounts of the breed describe how "there are some (show homers) descended from strains well known for their achievements in long-distance flying… however …. the flying properties of the Show Homer are degenerating at a rapid rate…" (page 6, Woodfield, 1892). Given that all show homers are descended from racing homers, it is possible that they have retained some genes associated with homing behavior (Shao et al., 2020), even though they are not flown freely or engage in homing. OB size could then develop in show homers and be maintained despite the lack of homing experience. In support of this hypothesis, previous work has shown that homing experience increases hippocampal volume, but neither

relative nor absolute OB size is affected (Cnotka et al., 2008). Whether show homers have similar olfactory abilities to that of racing homers or show homers can be trained to home has not been tested but would shed light on the similarity in OB anatomy between the two breeds.

The lack of difference between homers with active breeds and feral pigeons could also be associated with introgression of genes associated with homing, but it may also indicate that olfaction is used for homing over any distance, not just the long distances (100 – 1000 km) that homing pigeons are selected for and trained to do. All free flying pigeons, to some extent, need to find their way home and of the captive pigeons examined, only the rollers and highflyers were free flown. Feral pigeons tend to stay very close to their feeding site/home loft and seldom travel further than 5km away (Rose et al., 2006). The same is likely true of highflyers and rollers as their flight practice typically occurs immediately over their loft. Although pigeons can use other navigational cues, such as visual landmarks over short distances (<10km) (Benvenuti et al., 1973; Ioalè et al., 1978; Papi, 1982), olfaction may still be important in conjunction with these other cues. For example, pigeons may rely more heavily on other navigational mechanisms like visual landmarks when they are closer to the loft (Papi, 1982; Wallraff & Wallraff, 2005), yet still use olfactory cues for initial orientation (Nacci et al., 1994). If correct, then feral, roller, and highflyer pigeons could have larger OBs to support short-distance homing so they can return to their loft after free flight. However, tests of homing behavior and/or olfactory based navigation have not been tested directly in other non-homing pigeon breeds.

It is also important to note that although the OB is important for navigation (Gagliardo, 2013; Papi, 1976, 1982; Papi et al., 1972; Patzke et al., 2010), pigeons presumably use olfactory information for other behaviours. As demonstrated in other species, some of these could include foraging (Wenzel, 1968), mate choice/conspecific identification (Balthazart & Schoffeniels, 1979; Mihailova et al., 2014; Zelano & Edwards, 2002), and scent marking

(Castro et al., 2010). However, nothing has been reported about how pigeons use olfaction for behaviours other than navigation. If pigeons are using olfaction for non-homing related behaviours, this could explain why homing pigeons only differed from the majority of show breeds and not active or feral pigeons. Show breeds likely do not need to use olfaction to search for food or choose mates as show breeds are often housed in small cages with human-chosen mates.

Despite homing pigeons not differing in relative OB size than all other breeds, they were relatively larger than most show breeds and tended to have the largest OBs for their telencephalon size of any of the breeds sampled (Figure 3.4). Our results therefore partially support the hypothesis that selection for homing has driven an increase in relative OB size in homing pigeons. OB enlargement did not lead to changes in mitral cell size or density (Figures 3.5 and 3.6), contrary to our prediction, but this result is consistent with previous work showing that mitral cell density does not vary across species (Grigg et al., 2017). The moderate enlargement of the homing pigeon OBs can therefore be considered a scaled-up versions of the smaller OBs found in show breeds (or *vice versa*), but note, that we did not quantify other cell types or glomeruli. Identifying glomeruli from coronal sections could not be done consistently across our samples; the borders of the glomeruli were often indistinct due to minor tissue damage and staining intensity. Similarly, the borders of other layers of the OBs are not always distinct within and across specimens, which made it difficult to reliably quantify other cell types. There is therefore the potential for homing pigeons to differ from other breeds in the number and/or density of glomeruli or granule cells, either of which could contribute to breed specific differences in olfactory acuity and/or sensitivity (Egger & Kuner, 2021; Liu, 2020; Wang et al., 2022).

Unfortunately, we still know little about olfactory acuity and sensitivity in pigeons. Early experiments probed the acuity and sensitivity of pigeon olfaction with *n*-amylacetate and a limited range of other chemicals using both behavioural testing and physiological responses (Shumake at al., 1969; Stattleman et al., 1975; Tonosaki and Shibuya 1985). Pigeons are certainly sensitive to some chemicals more than others, but what odors they are attending to during homing remains unknown. We do know that the number and concentration of odors can change based on wind speed, direction, humidity, and temperature (Jinn et al., 2020), so the homing pigeon olfactory system must be sensitive enough to detect an odor "cocktail" at varying concentrations and discern their home cocktail from that of other odor cocktails that can change rapidly and frequently. As mentioned above, the remaining question then is whether homing pigeons have higher sensitivity and/or acuity than other breeds. One potential means of addressing this would be to quantify the type and number of olfactory receptor neurons (ORNs) in the olfactory epithelium across breeds (Meisami, 1989; Steiger et al., 2008; Wang et al., 2022). ORN's respond to specific chemicals and as the sensory receptors of the olfactory system, homing pigeons might have a higher density of ORN's or a greater diversity of them. The former would increase sensitivity, and the latter would increase acuity, both of which would increase the olfactory processing ability of homing pigeons. Thus, homing pigeons would be expected to have a greater diversity of ORNs (Steiger et al., 2009) and/or more ORNs than other pigeon breeds to support homing over great distances.

Table 3.1. Sample size (n), means, and standard deviations (SD) for each of the three OB measurements: OB volume, mitral cell number, mitral cell size (μm^3) as well as telencephalon volume.

Measurement	Breed	n	Mean	SD
Telencephalon Volume (mm^3)	Homer	4	955.77	98.99
	Feral	6	1003.86	166.78
	Capuchine	4	960.91	114.82
	Show Homer	5	928.31	75.29
	Show Roller	4	904.03	86.60
	Cropper	4	1140.94	46.35
	Roller	6	907.18	71.94
	Highflyer	7	931.37	58.64
OB Volume (mm^3)	Homer	4	8.97	1.82
	Feral	6	6.57	1.31
	Capuchine	4	6.02	1.26
	Show Homer	5	7.31	1.26
	Show Roller	4	5.65	0.36
	Cropper	4	6.38	0.80
	Roller	6	6.76	1.49
	Highflyer	7	6.32	1.13
Mitral Cell Number	Homer	4	24811.59	4009.04
	Feral	6	20001.68	6553.04
	Capuchine	4	19002.45	1421.10
	Show Homer	5	22394.97	5234.86
	Show Roller	4	18434.18	473.64
	Cropper	4	16842.94	2269.15
	Roller	6	20699.60	4140.41
	Highflyer	7	22359.48	3941.79
Mitral Cell Size (μm^3)	Homer	4	1239.13	98.16
	Feral	6	1287.74	166.58
	Capuchine	4	1393.51	227.47
	Show Homer	5	1362.81	108.84
	Show Roller	4	1211.28	157.27
	Cropper	4	1487.94	125.52
	Roller	6	1319.24	165.49
	Highflyer	7	1461.99	176.05

Table 3.2. Results from one-way analyses of variance (ANOVAs) comparing means among all 8 pigeon breeds (homer, feral, Capuchine, show homer, show roller, cropper, roller, highflyer) on our three measures: OB volume, number of mitral cells, and size of mitral cells. Significant p-values ($p < 0.05$) are denoted with (*). Post-hoc analyses were carried out to determine which breeds were different if the p-value was significant. F-values, degrees of freedom (df) and p-values are reported. Data were log-transformed prior to statistical analysis.

Variable	F-ratio	df	p
OB Volume	2.65	7, 32	0.063
Mitral Cell Number	2.68	7, 32	0.063
Mitral Cell Size (μm^3)	1.80	7, 32	0.173

Table 3.3. Results from analyses of covariance (ANCOVAs) comparing means between 8 pigeon breeds (homer, feral, Capuchine, show homer, show roller, cropper, roller, highflyer) on our three measures: OB volume, number of mitral cells, and size of mitral cells. Telencephalon size was used as a covariate for OB size, and OB size was used as a covariate for mitral cell number and mitral cell size. Significant p-values ($p < 0.05$) are denoted with (*). Post-hoc analyses were carried out to determine which breeds were different if the p-value was significant. F-values, degrees of freedom (df) and p-values are reported. Data were log-transformed prior to statistical analysis.

	Breed			Telencephalon Size (Covariate)			OB Size (Covariate)		
Variable	**F-ratio**	**df**	**p**	**F-ratio**	**df**	**p**	**F-ratio**	**df**	**p**
OB Volume	3.18	7, 32	**0.012***	5.80	7, 32	**0.022***	-	-	-
Mitral Cell Number	0.91	7, 32	0.510	-	-	-	6.04	7,32	**0.020***
Mitral Cell Size (μm^3)	1.68	7, 32	0.151	-	-	-	0.31	7,32	0.585

Table 3.4. Results from Tukey's Honest Significant Difference (HSD) post-hoc analyses showing the significant differences between relative OB volume between homing pigeons and 7 other pigeon breeds (feral, Capuchine, show homer, show roller, cropper, roller, highflyer). degrees of freedom (df) and p-values are reported. Significant p-values ($p < 0.05$) are denoted with (*).

Homers vs:	df	p-value
Feral	7, 32	0.063
Capuchine	7, 32	**0.034***
Show Homer	7, 32	0.727
Show Roller	7, 32	**0.029***
Cropper	7, 32	**0.025***
Roller	7, 32	0.309
Highflyer	7, 32	0.057

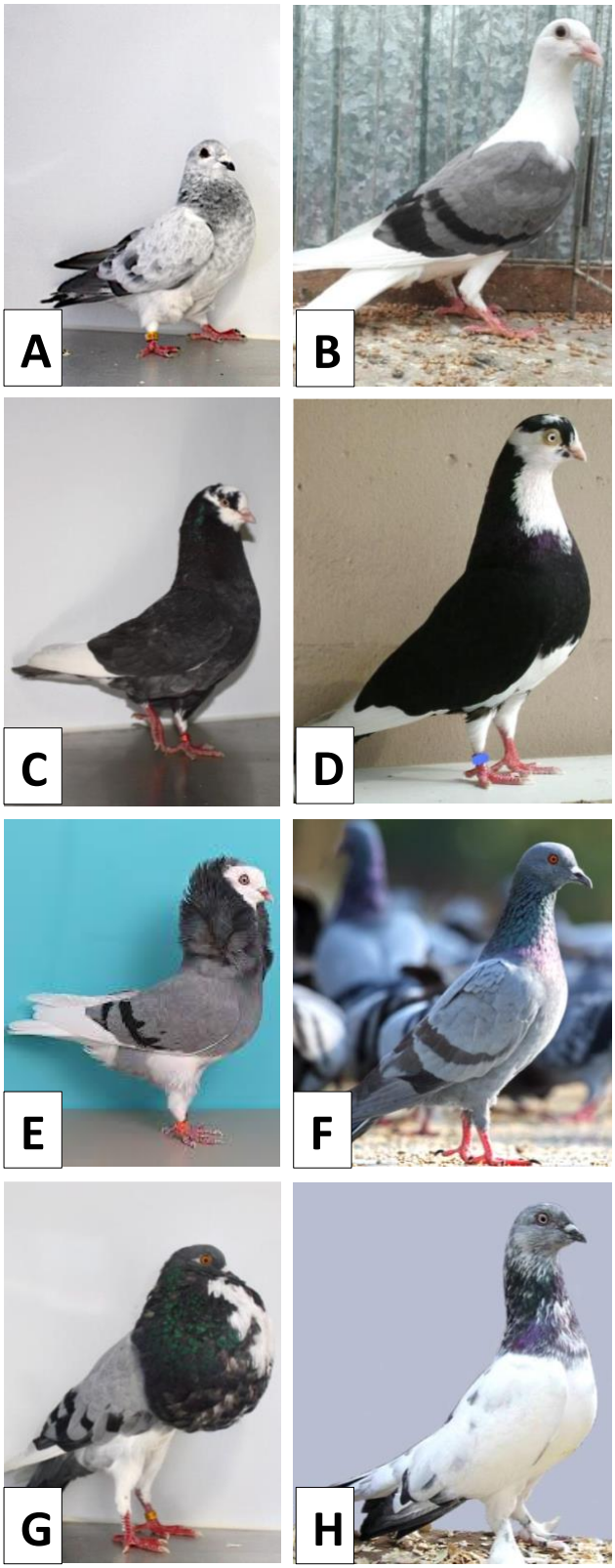

Figure 3.1. Images showcasing the diversity of pigeon breeds compared in this study. (A) show homer, (B) homing pigeon, (C) show roller, (D) roller, (E) Capuchine, (F) Feral, (G) Norwich cropper, (H) Highflyer. Note: images are not to scale.

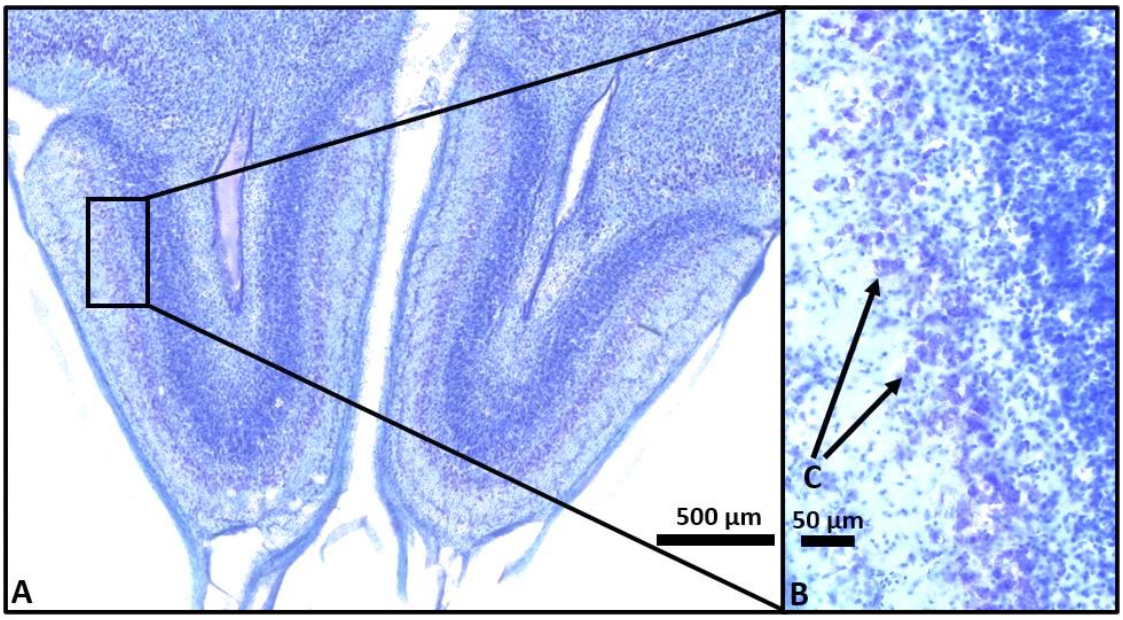

Figure 3.2. Photomicrograph of (A) Homing pigeon OB taken with a 10x objective on an Olympus VS120 Slide Scanning Microscope. (B) Mitral cell layer of homing pigeon OB. (C) Arrows indicating mitral cells of homing pigeon.

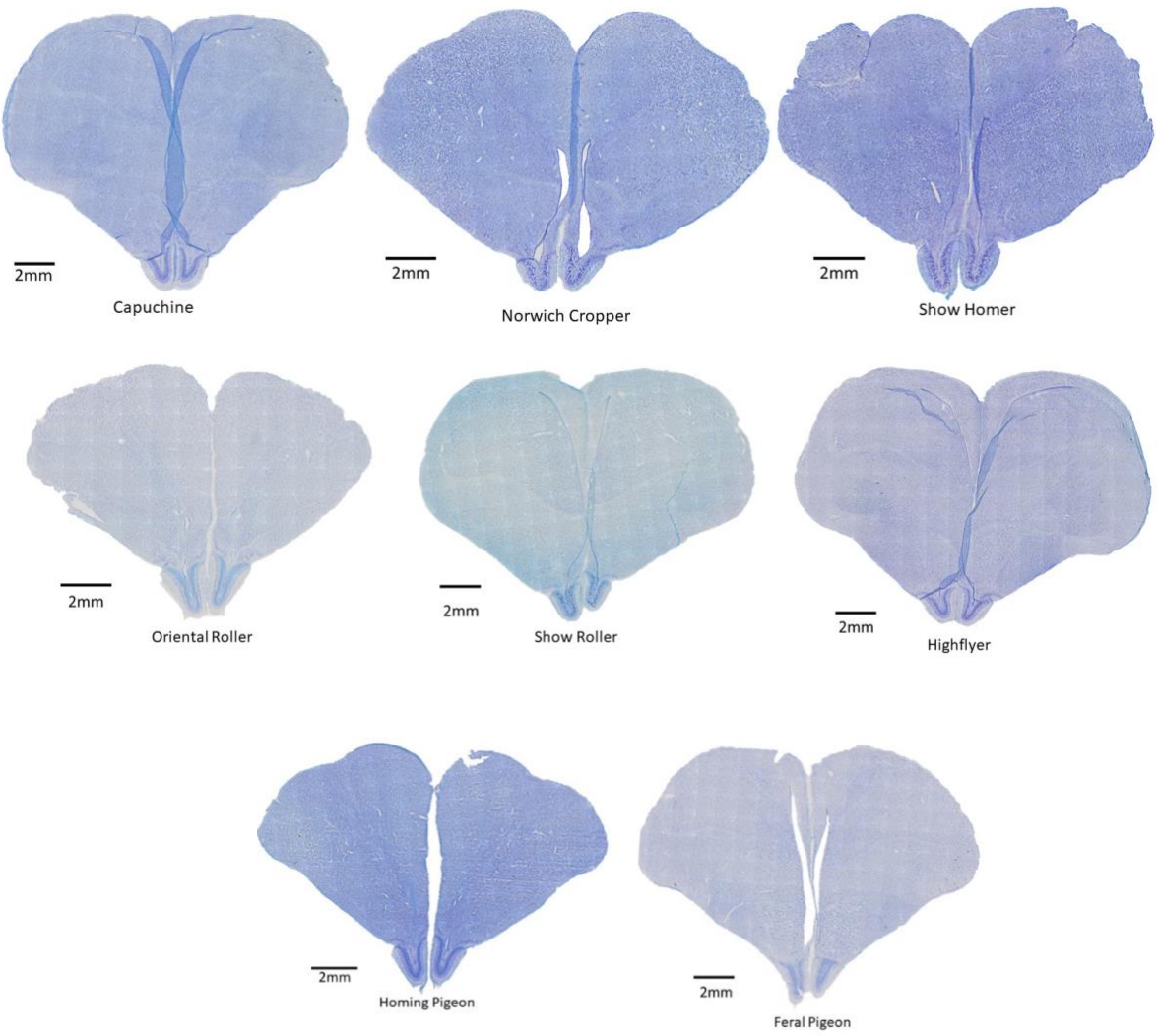

Figure 3.3. Photos of Nissl-stained coronal sections through the rostro-caudal midpoint of the olfactory bulbs of all of the pigeon breeds sampled.

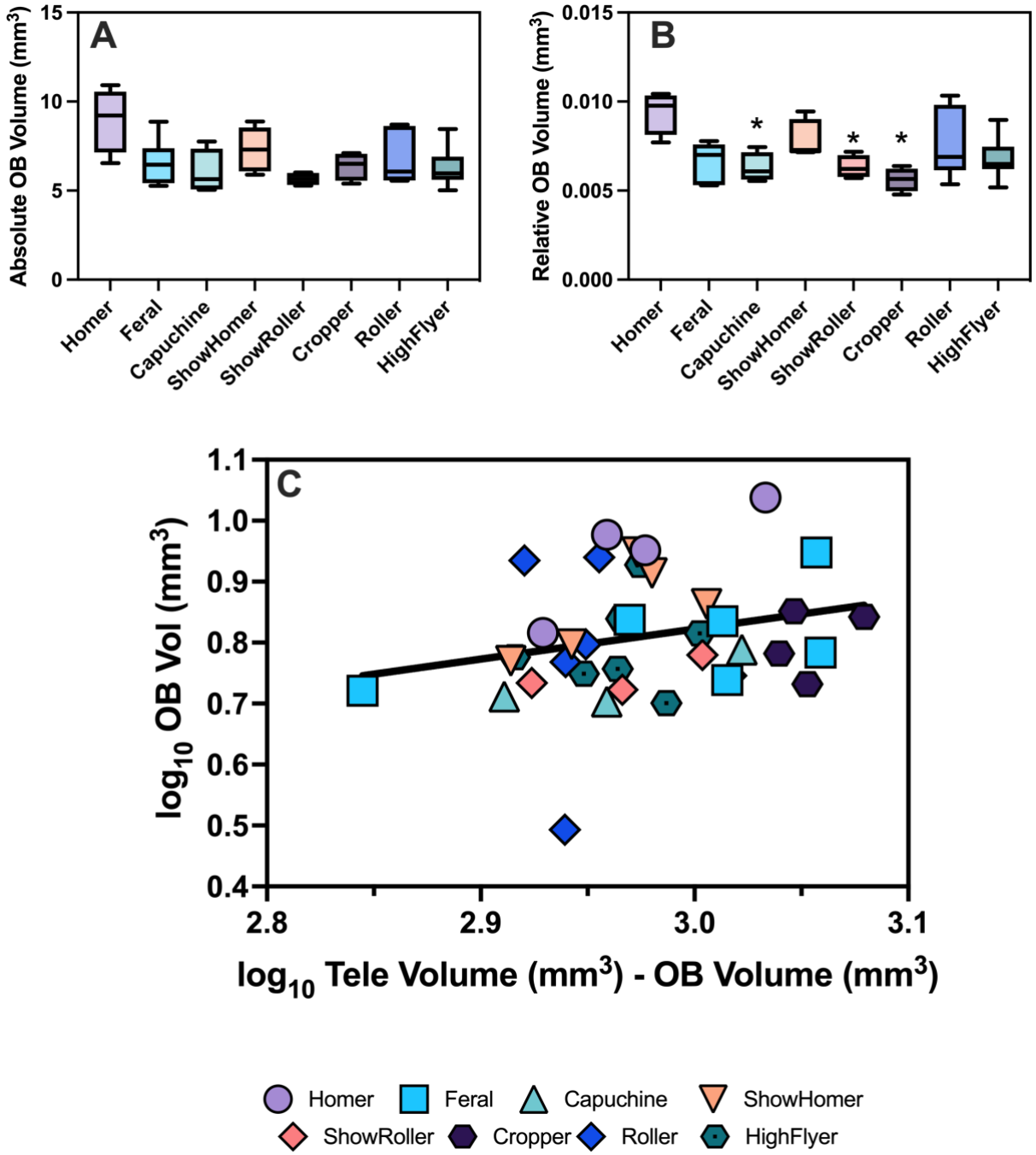

Figure 3.4. Boxplots showing (A) absolute differences in OB size across pigeon breeds, (B) ratio of OB size to telencephalon size (OB volume divided by Tele volume), (C) double log-transformed scatter plot showing the relationship between OB volume (y-axis) and telencephalon volume (x-axis) across pigeon breeds. Significant differences are denoted with (*) and are between homing pigeons and all other breeds. Significance in absolute and relative differences were determined with ANOVAs and ANCOVAs, respectively. Significance was between homing pigeons and the other breeds; no significant differences were determined within other domestic breeds.

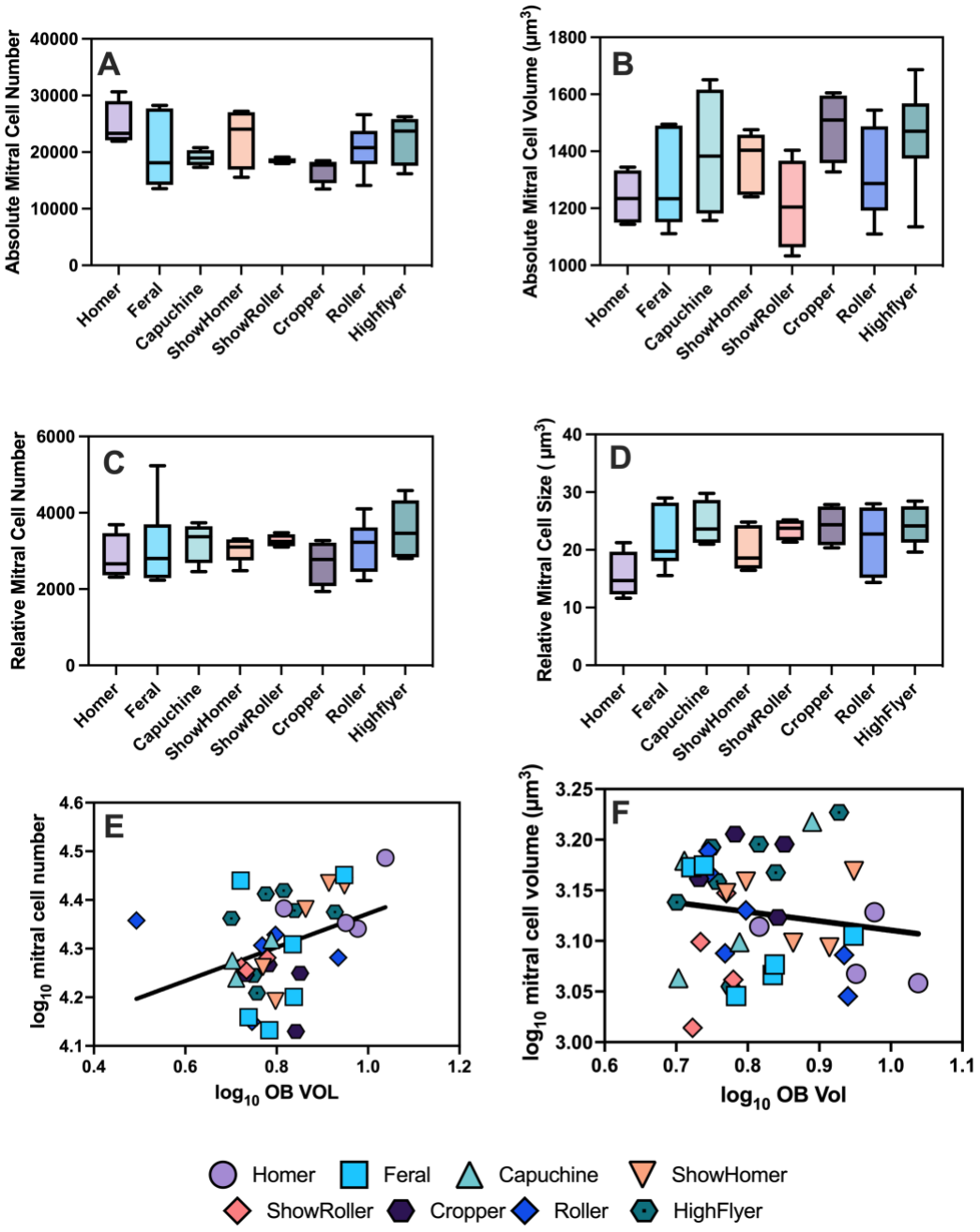

Figure 3.5 (A) box plot showing absolute mitral cell number, (B) boxplot absolute mitral cell size, (C) box plot showing relative mitral cell number, (D) box plot showing relative mitral cell size, (E) double-log transformed scatter plot showing the relationship between mitral cell number and olfactory bulb (OB) size, and (F) double log-transformed scatter plot showing the relationship between mitral cell size and OB size. Relative mitral cell number (mitral cell density) was determined by dividing the number of mitral cells by the OB volume (mm^3). Relative mitral cell size was determined by dividing the size of mitral cells by the OB volume (mm^3).

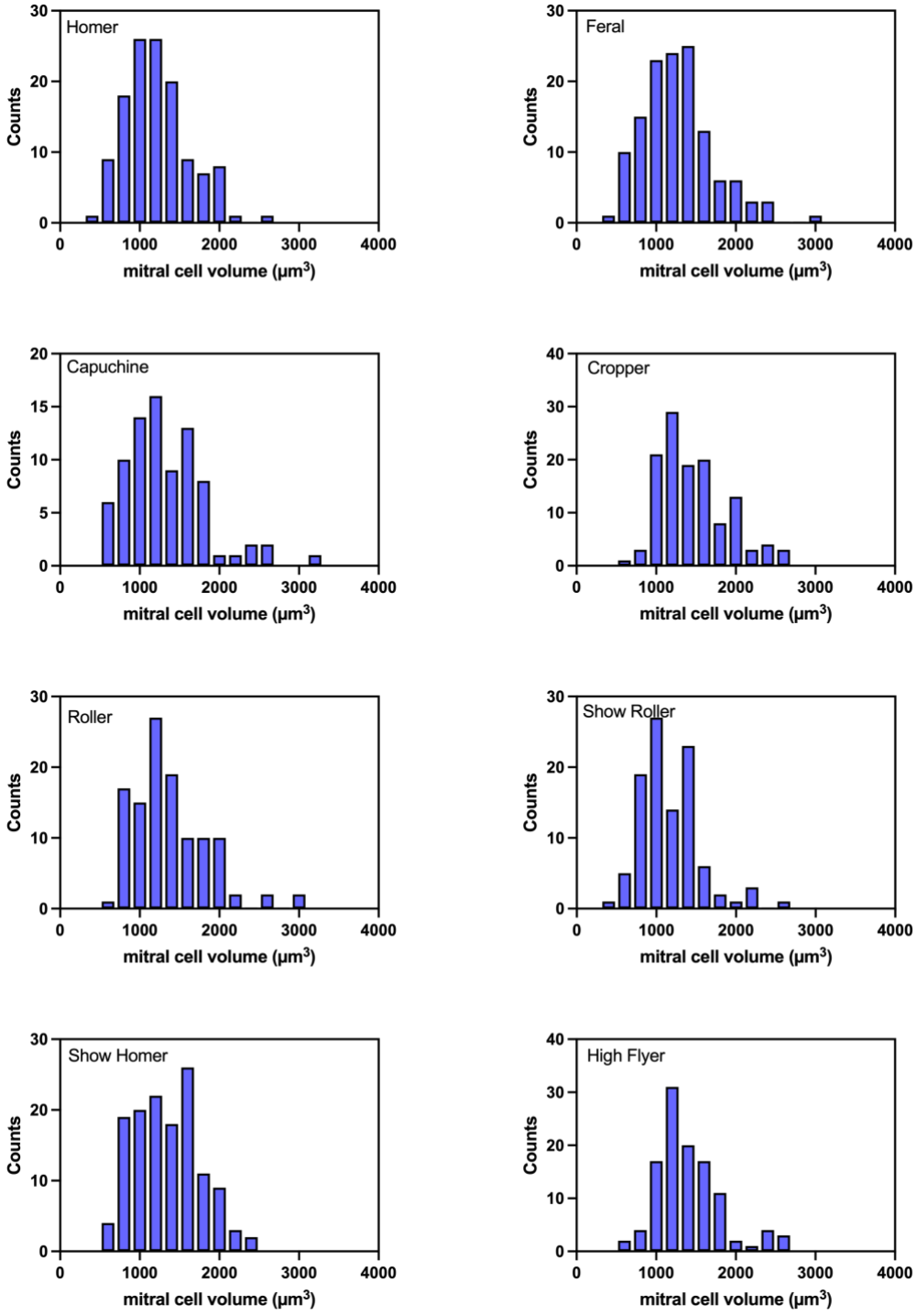

Figure 3.6. Frequency distributions of mitral cell soma size (mm^3) in homers, ferals, capuchines, croppers, rollers, show rollers, show homers, and highflyer pigeons. Graphs are labelled in the upper left corner indicating which breed each histogram represents. X-axis represents mitral cell volume (μm^3) and ranges from 0 to 4000 μm^3.

CHAPTER 4: GENERAL DISCUSSION

Domestication is one of the most widespread "accidental" experiments in evolutionary science that humans have conducted all over the world. Numerous species of birds and mammals are domesticated, and scientists have been utilizing domestication as a model for studying evolution of behavior, anatomy, and neuroscience for decades (Kruska, 2005; Price, 2002a; Price, 1984, 1999a). Charles Darwin, the "father of evolution", was fascinated with domestication and how quickly it could change phenotypes and give rise to diverse breeds over a relatively short time (Darwin, 1868). Domestication is a particularly useful tool as domestic animals have been in existence for a very short time (in evolutionary terms), and thus are a prime example of rapid evolution and diversity. Compared to their wild counterparts, it is widely known that domestics differ in many phenotypic traits including external morphology, behavior, and neuroanatomical changes (Kruska, 1988b; Kruska, 2005; Price, 2002a; Price, 1999b). In this thesis, the goal was to use domestication and artificial selection to examine how the brain changes under selection pressures, with a primary focus on the forebrain in general (i.e., telencephalon), and the specialized olfactory bulb (OB). Two of the most important first steps in domestication of animals are captive breeding and artificial selection, which coincide with the two concepts that this thesis addresses. We used chickens (*Gallus gallus*) and pigeons (*Columba livia*) as model organisms to study these effects on overall and specialized (i.e., OB) telencephalic brain regions.

Chickens and Junglefowl

In Chapter 2, the goal was to determine if the chicken and junglefowl telencephalon differ from one another, and if so, which regions are different? And if not, then do chickens and

junglefowl differ from wild, but closely related, gallinaceous species? Our results revealed that chickens and junglefowl have similar telencephalon anatomy to each other, with the chicken telencephalon being a larger version of the junglefowl telencephalon. Compared to ruffed grouse, we found that chickens and junglefowl have differential enlargement of the telencephalon compared to ruffed grouse. The main regions within the telencephalon that differed between chickens and junglefowl with ruffed grouse were the hyperpallium (-), nidopallium (+), and striatum (+).

One possible explanation for the similarity between chickens and junglefowl is that there are differences in neurochemistry (i.e., neurotransmitters or their receptors) that explain the difference in behaviour between the two, without changing neuroanatomy. It has been documented that chickens and junglefowl have marked differences in behaviour, particularly in their fear responses, with junglefowl being more fearful of open spaces, novel stimuli, predators, and humans (Campler et al., 2009; Schütz et al., 2001). Differences in neurotransmitters that play a role in fear responses (i.e., serotonin, GABA) or neuropeptides (i.e. oxytocin and vasopressin) and their receptors can have a profound effect on behaviour (Bales et al., 2004; Carter et al., 2008) that would go undetected in a study comparing only the size of brain regions in Nissl stained tissue. It is widely accepted that changes in neuropeptide and neurohormone receptors and levels can alter sociality in birds and mammals (Donaldson & Young, 2008; Goodson, 1998; Goodson & Bass, 2001; Goodson et al., 2009). Including information on differences in neurochemistry between chickens and junglefowl, and further between both *Gallus* breeds and ruffed grouse, would create a more "full picture" analysis, thus explaining behavioural between these species that are undetected in a comparison of telencephalon anatomy alone.

Second, there are neuroanatomical differences between *Gallus* species and ruffed grouse in more generalized (rather than specialized) telencephalic regions that are involved in sensory processing (hyperpallium), cognition (nidopallium), and motor coordination (striatum) between chickens and junglefowl with ruffed grouse. This suggests that there are likely different sensory demands and behaviours (visual processing, decision making, and movement, respectively) between captive and domestic birds than there are in wild birds, which is something we predicted. Whether ruffed grouse have vastly different behaviours to chickens and junglefowl that would explain the differences in telencephalic anatomy remains untested. The telencephalon in vertebrates is primarily responsible for voluntary behaviour and decision making (Reiner et al., 2005; Shimizu et al., 2010). Thus, a succession of behavioural response tests comparing chickens, junglefowl, and ruffed grouse regarding behaviors controlled primarily by the telencephalon would help explain the forebrain changes reported here.

An example of one such behavioural test is response of chickens, junglefowl, and ruffed grouse to the presence of predators, in a similar fashion to Palleroni (2005) or Campler (2009). In Palleroni et al. (2005), there were differential responses between chicken breeds based on the body size of the predator threat (various hawk species). Campler et al. (2009) and Schütz et al. (2001) also found different responses to predators between chickens and junglefowl as well, indicating that differences across species in fear responses are likely to occur. For example, ruffed grouse may respond to predator threats from a much further distance than chickens and junglefowl because they may need extra time to evade an attack in the wild, where this would not be necessary in a captive setting. Predator evasion is known to be heavily reliant on visual information (Butler & Fernández-Juricic, 2018; Devereux et al., 2006; Fernández-Juricic et al., 2008), and the hyperpallium in birds has major roles in visual processing (Shimizu et al., 2010),

so it is possible that differential visual processing abilities explain the differences in telencephalon anatomy, and would also be reflected in their behavior.

Third, another important factor to consider with these data is the influence that phylogeny has on brain anatomy. It is well known that numerous factors influence phenotype (including brain anatomy), some of which include developmental mode, ecology, feeding habits, habitat, experience, phylogeny, and other factors (Corfield et al., 2015; Iwaniuk & Hurd, 2005; Iwaniuk et al., 2000; Wylie et al., 2015). However, phylogeny also plays an important role in how brains develop and evolve (Iwaniuk & Hurd, 2005; Iwaniuk et al., 2006; Iwaniuk et al., 2007). Ruffed grouse are not the closest relative to chickens and junglefowl, so adding in genetic analyses to these results could improve our understanding of how genetics influence our data. In terms of brain anatomy, phylogeny could explain some of the differences we have reported between *Gallus* species and ruffed grouse based on genetic disposition.

Pigeons

Chapter 3 confirmed our prediction and previous work (Rehkämper et al., 1988) that homing pigeons have a relatively larger OB compared to show breeds; however, they were not larger than active breeds or feral pigeons. We also found that there were no significant differences between mitral cell number or size among breeds, despite our predictions that homing behaviour would require more and/or larger mitral cells. Given the lack of significant difference between homing pigeons and other active breeds and ferals, we can presume several things.

First, we assume that activity (i.e. leaving the loft in any degree) may require some degree of preliminary navigation that requires olfactory input, but reliance on other information such as visual landmarks may be playing a bigger role in short distance navigation (<10km)

(Papi, 1982; Papi et al., 1972; Wallraff & Wallraff, 2005) rather than long distance (>100 km), thus decreasing the reliance on olfactory cues from short distances. Although this is a possibility, we also presume that exercise could be causing an increase in the size (volume) of certain brain structures in active and homing breeds by increasing neurogenesis. Many regions of the telencephalon in birds undergo adult neurogenesis (Barnea & Pravosudov, 2011), including the olfactory bulbs (OBs) and hippocampus (Hp). However, previous work comparing the effects of exercise on hippocampal and OB size revealed that exercise enlarges the hippocampus in homing pigeons that are given the opportunity to fly compared to homing pigeons simply kept in a loft (Cnotka et al., 2008). Despite an increase in hippocampus size with increased activity, there were no differences in OB size between experienced homers and inexperienced homers. It is therefore possible than that genes influence OB volume more than exercise, and these genes preserve the size of the OB regardless of experience and exercise. This presumption would also explain why we saw a general lack of difference between homing pigeons and show homers; the OB size has been relatively maintained in show homers given that they are descendant from homing pigeons despite that the selection for homing behaviour has been mostly eliminated in them (Woodfield, 1892). To summarize, further genetic analyses comparing differences in the genomes, or gene regulation (i.e., genes up or downregulated) between breeds could reveal how genes and gene expression differences are influencing the OB size across pigeon breeds.

Second, the olfactory bulb is a relatively simple network, with ORN's receiving olfactory stimuli directly from the environment via the olfactory epithelium and synapsing directly on mitral cells (Greer et al., 2008; Kosaka & Kosaka, 2016; Macrides, 1982). Mitral cells then project directly to associative brain regions including the piriform cortex where the olfactory information is processed, and decisions can be made regarding this information (Atoji

& Wild, 2014). Interestingly, ORN's are the only example of a neuron exposed to the external environment (Getchell, 1986), so a more comprehensive analysis of these ORN's (i.e., number or diversity) between homing pigeons and other breeds could provide insight to the enhanced olfactory abilities of homing pigeons.

Because ORN's are responsible for the initial detection of olfactory stimuli, the number and variety of ORN's would be very useful knowledge in determining if homing pigeons have a larger diversity of receptors, and if these receptors are more numerous. If homing pigeons have increased ORN diversity, we can assume that the olfactory discrimination abilities of homing pigeons are enhanced. Unfortunately, to our knowledge, there are no studies testing the odor discrimination (i.e., acuity) abilities of homing pigeons, so it is unknown whether homing pigeons have enhanced olfactory acuity compared to other breeds. Additionally, having more ORN's would increase odor detection in homing pigeons, thus allowing them to detect odors at lower levels than "normal". Some studies have examined the olfactory sensitivity in pigeons (Tonosaki & Shibuya, 1985), but these studies used other pigeon breeds (King and Carneau) that have not been intensely selected for homing behaviour, and the odors used were very potent and artificial. Therefore, how homing pigeons actually detect odors in a natural environment during flight has not been examined, and how they compare to other breeds (show, active, and feral) has not been reported. Therefore, examining ORN number and diversity would allow us to predict the acuity and sensitivity of the homing pigeon olfactory system, which would help explain how homing pigeons are able to navigate and potentially explain why there are little differences in OB size and mitral cell number between homing pigeons and active/feral pigeon breeds.

Overall, our hope is that the present study provides insight to the effects of captive breeding on brain anatomy so future work on domestic-wild comparisons will take this into

account as a potential confounder to their results. Further, we hope that comparisons between species and between breeds will consider the effects of neurochemical differences, and how it can alter behaviour without any correlated changes in neuroanatomy. Lastly, we hope that future research examining how homing pigeons navigate effectively consider how the diversity and number of ORN's could be playing a major role in the elite olfactory abilities of homing pigeons, and not only OB anatomy alone. In conclusion, these results should be taken as further evidence for domestication as a strong selection pressure that can and should be used in comparative neuroanatomical, neurochemical, and behavioural studies for decades to come.

REFERENCES

Agnvall, B. (2016). Early domestication? Phenotypic alterations of Red Junglefowl selected for divergent fear of humans. *PhD diss., Linköping University Electronic Press*.

Agnvall, B., Ali, A., Olby, S., & Jensen, P. (2014). Red Junglefowl (Gallus gallus) selected for low fear of humans are larger, more dominant and produce larger offspring. *Animal, 8*(9), 1498-1505.

Agnvall, B., Belteky, J., & Jensen, P. (2017). Brain size is reduced by selection for tameness in Red Junglefowl- correlated effects in vital organs. *Scientific Reports, 7*(1), 3306.

Agnvall, B., Jongren, M., Strandberg, E., & Jensen, P. (2012). Heritability and genetic correlations of fear-related behaviour in Red Junglefowl--possible implications for early domestication. *PLoS One, 7*(4), e35162.

Al-Nasser, A., Al-Khalaifa, H., Al-Saffar, A., Khalil, F., Albahouh, M., Ragheb, G., Al-Haddad, A., & Mashaly, M. (2007). Overview of chicken taxonomy and domestication. *World's Poultry Science Journal, 63*(2), 285-300.

Albert, F. W., Shchepina, O., Winter, C., Römpler, H., Teupser, D., Palme, R., Ceglarek, U., Kratzsch, J., Sohr, R., & Trut, L. N. (2008). Phenotypic differences in behavior, physiology and neurochemistry between rats selected for tameness and for defensive aggression towards humans. *Hormones and behavior, 53*(3), 413-421.

Albert, F. W., Somel, M., Carneiro, M., Aximu-Petri, A., Halbwax, M., Thalmann, O., Blanco-Aguiar, J. A., Plyusnina, I. Z., Trut, L., Villafuerte, R., Ferrand, N., Kaiser, S., Jensen, P., & Paabo, S. (2012). A comparison of brain gene expression levels in domesticated and wild animals. *PLoS Genetics, 8*(9), e1002962.

Atoji, Y., Sarkar, S., & Wild, J. M. (2018). Differential projections of the densocellular and intermediate parts of the hyperpallium in the pigeon (Columba livia). *Journal of Comparative Neurology, 526*(1), 146-165.

Atoji, Y., & Wild, J. M. (2014). Efferent and afferent connections of the olfactory bulb and prepiriform cortex in the pigeon (Columba livia). *Journal of Comparative Neurology, 522*(8), 1728-1752.

Bales, K. L., Kim, A. J., Lewis-Reese, A. D., & Carter, C. S. (2004). Both oxytocin and vasopressin may influence alloparental behavior in male prairie voles. *Hormones and behavior*, *45*(5), 354-361.

Balthazart, J., & Schoffeniels, E. (1979). Pheromones are involved in the control of sexual behaviour in birds. *Naturwissenschaften*, *66*(1), 55-56.

Barnea, A., & Pravosudov, V. (2011). Birds as a model to study adult neurogenesis: bridging evolutionary, comparative and neuroethological approaches. *European Journal of Neuroscience*, *34*(6), 884-907.

Belyaev, D. K. (1979). Destabilizing selection as a factor in domestication. *Journal of Heredity*, *70*(5), 301-308.

Benvenuti, S., Fiaschi, V., Fiore, L., & Papi, F. (1973). Homing performances of inexperienced and directionally trained pigeons subjected to olfactory nerve section. *Journal of comparative physiology*, *83*(1), 81-92.

Bingman, V. P., Gagliardo, A., Hough, G. E., Ioalé, P., Kahn, M. C., & Siegel, J. J. (2005). The avian hippocampus, homing in pigeons and the memory representation of large-scale space. *Integrative and Comparative Biology*, *45*(3), 555-564.

Bingman, V. P., & Mench, J. A. (1990). Homing behavior of hippocampus and parahippocampus lesioned pigeons following short-distance releases. *Behavioural Brain Research*, *40*(3), 227-238.

Biro, D., Freeman, R., Meade, J., Roberts, S., & Guilford, T. (2007). Pigeons combine compass and landmark guidance in familiar route navigation. *Proceedings of the National Academy of Sciences*, *104*(18), 7471-7476.

Blanchard, R. J., Flannelly, K. J., & Blanchard, D. C. (1986). Defensive behaviors of laboratory and wild Rattus norvegicus. *Journal of Comparative Psychology*, *100*(2), 101.

Brusini, I., Carneiro, M., Wang, C., Rubin, C.-J., Ring, H., Afonso, S., Blanco-Aguiar, J. A., Ferrand, N., Rafati, N., & Villafuerte, R. (2018). Changes in brain architecture are consistent with altered fear processing in domestic rabbits. *Proceedings of the National Academy of Sciences*, *115*(28), 7380-7385.

Butler, S. R., & Fernández-Juricic, E. (2018). European starlings use their acute vision to check on feline predators but not on conspecifics. *PLoS One*, *13*(1), e0188857.

Campler, M., Jongren, M., & Jensen, P. (2009). Fearfulness in red junglefowl and domesticated White Leghorn chickens. *Behavioural Processes*, *81*(1), 39-43.

Carter, C. S., Grippo, A. J., Pournajafi-Nazarloo, H., Ruscio, M. G., & Porges, S. W. (2008). Oxytocin, vasopressin and sociality. *Progress in brain research*, *170*, 331-336.

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.

Cauchoix, M., Hermer, E., Chaine, A., & Morand-Ferron, J. (2017). Cognition in the field: comparison of reversal learning performance in captive and wild passerines. *Scientific Reports*, *7*(1), 1-10.

Cnotka, J., Möhle, M., & Rehkämper, G. (2008). Navigational experience affects hippocampus size in homing pigeons. *Brain, behavior and evolution*, *72*(3), 233-238.

Corfield, J. R., Krilow, J. M., Ligt, M. N. V., & Iwaniuk, A. N. (2013). A quantitative morphological analysis of the inner ear of galliform birds. *Hearing Research*, *304*, 111-127.

Corfield, J. R., Price, K., Iwaniuk, A. N., Gutiérrez-Ibáñez, C., Birkhead, T., & Wylie, D. R. (2015). Diversity in olfactory bulb size in birds reflects allometry, ecology, and phylogeny. *Frontiers in Neuroanatomy*, *9*, 102.

Darwin, C. (1859). On the Origin of Species. *London: John Murray*.

Darwin, C., & Wallace, A. (1858). On the variation of organic beings in a state of nature. *Journal of the Proceedings of the Linnean Society of London (Zoology)*, *3*, 45-52.

Darwin, C. R. (1868). Variation of plants and animals under domestication.

Day, L. B., Guerra, M., Schlinger, B. A., & Rothstein, S. I. (2008). Sex differences in the effects of captivity on hippocampus size in brown-headed cowbirds (Molothrus ater obscurus). *Behavioural Neuroscience*, *122*(3), 527-534.

DeMello, M. (2021). The Domestication of Animals. In *Animals and Society* (pp. 102-116). Columbia University Press.

Devereux, C. L., Whittingham, M. J., Fernández-Juricic, E., Vickery, J. A., & Krebs, J. R. (2006). Predator detection and avoidance by starlings under differing scenarios of predation risk. *Behavioral Ecology*, *17*(2), 303-309.

Donaldson, Z. R., & Young, L. J. (2008). Oxytocin, vasopressin, and the neurogenetics of sociality. *Science*, *322*(5903), 900-904.

Ebinger, P. (1974). A cytoarchitectonic volumetric comparison of brains in wild and domestic sheep. *Zeitschrift fuer Anatomie und Entwicklungsgeschichte*, *144*(3), 267-302.

Ebinger, P. (1995). Domestication and plasticity of brain organization in mallards (Anas platyrhynchos). *Brain, behavior and evolution*, *45*(5), 286-300.

Ebinger, P., & Löhmer, R. (1984). Comparative quantitative investigations on brains of rock doves, domestic and urban pigeons (Columba 1. livia) 1. *Journal of Zoological Systematics and Evolutionary Research*, *22*(2), 136-145.

Ebinger, P., & Löhmer, R. (1987). A volumetric comparison of brains between greylag geese (Anser anser L.) and domestic geese. *Journal fur Hirnforschung*, *28*(3), 291-299.

Ebinger, P., Löhmer, R. . (1984). Comparative quantitative investigations on brains of rock doves, domestic and urban pigeons (Columba 1. livia). *Journal of Zoological Systematics and Evolutionary Research*, *2*, 136-145.

Ebinger, P., & Röhrs, M. (1995). Volumetric analysis of brain structures, especially of the visual system in wild and domestic turkeys (Meleagris gallopavo). *Journal fur Hirnforschung*, *36*(2), 219-228.

Egger, V., & Kuner, T. (2021). Olfactory bulb granule cells: specialized to link coactive glomerular columns for percept generation and discrimination of odors. *Cell and tissue research*, *383*(1), 495-506.

Eszergár-Kiss, D., & Caesar, B. (2017). Definition of user groups applying Ward's method. *Transportation Research Procedia*, *22*, 25-34.

Fernández-Juricic, E., Gall, M. D., Dolan, T., Tisdale, V., & Martin, G. R. (2008). The visual fields of two ground-foraging birds, House Finches and House Sparrows, allow for simultaneous foraging and anti-predator vigilance. *Ibis*, *150*(4), 779-787.

Frahm, H. D., & Rehkamper, G. (1998). Allometric comparison of the brain and brain structures in the white crested polish chicken with uncrested domestic chicken breeds. *Brain, behaviour and evolution*, *52*(6), 292-307.

Francis, R. C. (2015). *Domesticated: evolution in a man-made world*. WW Norton & Company.

Gagliardo, A. (2013). Forty years of olfactory navigation in birds. *Journal of Experimental Biology*, *216*(12), 2165-2171.

Gagliardo, A., Filannino, C., Ioalè, P., Pecchia, T., Wikelski, M., & Vallortigara, G. (2011). Olfactory lateralization in homing pigeons: a GPS study on birds released with unilateral olfactory inputs. *Journal of Experimental Biology*, *214*(4), 593-598.

Gagliardo, A., Ioale, P., Savini, M., & Wild, J. M. (2006). Having the nerve to home: trigeminal magnetoreceptor versus olfactory mediation of homing in pigeons. *Journal of Experimental Biology*, *209*(Pt 15), 2888-2892.

Gagliardo, A., Pecchia, T., Savini, M., Odetti, F., Ioalè, P., & Vallortigara, G. (2007). Olfactory lateralization in homing pigeons: initial orientation of birds receiving a unilateral olfactory input. *European Journal of Neuroscience*, *25*(5), 1511-1516.

Garland Jr, T., & Adolph, S. C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiological Zoology*, *67*(4), 797-828.

Getchell, T. V. (1986). Functional properties of vertebrate olfactory receptor neurons. *Physiological reviews*, *66*(3), 772-818.

Gilbert, M. T. P., & Shapiro, M. D. (2014). Pigeons: domestication. *Encyclopedia of global archaeology*, 1382-1384.

Giunchi, D., Mucci, N., Bigi, D., Mengoni, C., & Baldaccini, N. E. (2020). Feral pigeon populations: their gene pool and links with local domestic breeds. *bioRxiv*.

Goodson, J. L. (1998). Territorial aggression and dawn song are modulated by septal vasotocin and vasoactive intestinal polypeptide in male field sparrows (Spizella pusilla). *Hormones and behavior*, *34*(1), 67-77.

Goodson, J. L., & Bass, A. H. (2001). Social behavior functions and related anatomical characteristics of vasotocin/vasopressin systems in vertebrates. *Brain research reviews*, *35*(3), 246-265.

Goodson, J. L., Lindberg, L., & Johnson, P. (2004). Effects of central vasotocin and mesotocin manipulations on social behavior in male and female zebra finches. *Hormones and behavior*, *45*(2), 136-143.

Goodson, J. L., Schrock, S. E., Klatt, J. D., Kabelik, D., & Kingsbury, M. A. (2009). Mesotocin and nonapeptide receptors promote estrildid flocking behavior. *Science*, *325*(5942), 862-866.

Greer, C., Whitman, M., Rela, L., Imamura, F., & Gil, D. R. (2008). Architecture of the olfactory bulb. In *Olfaction* (pp. 623-640). Elsevier Inc.

Grigg, N. P., Krilow, J. M., Gutierrez-Ibanez, C., Wylie, D. R., Graves, G. R., & Iwaniuk, A. N. (2017). Anatomical evidence for scent guided foraging in the turkey vulture. *Scientific Reports*, *7*(1), 17408.

Guay, P. J., Iwaniuk, A. N. . (2008). Captive breeding reduces brain volume in waterfowl (Anseriformes). *The Condor*, *110*(2), 276-284.

Güntürkün, O. (2005). The avian 'prefrontal cortex'and cognition. *Current opinion in neurobiology*, *15*(6), 686-693.

Henriksen, R., Holm, A.-C. S., & Jensen, P. (2021). Effect of contact incubation on stress, behavior and body composition in the precocial Red jungle fowl. *Hormones and behavior*, *128*, 104892.

Henriksen, R., Johnsson, M., Andersson, L., Jensen, P., & Wright, D. (2016). The domesticated brain: genetics of brain mass and brain structure in an avian species. *Scientific Reports*, *6*, 34031.

Herculano-Houzel, S., Manger, P. R., & Kaas, J. H. (2014). Brain scaling in mammalian evolution as a consequence of concerted and mosaic changes in numbers of neurons and average neuronal cell size. *Frontiers in Neuroanatomy*, *8*, 77.

Ioalè, P., Nozzolini, M., & Papi, F. (1990). Homing pigeons do extract directional information from olfactory stimuli. *Behavioral Ecology and Sociobiology*, *26*(5), 301-305.

Ioalè, P., Papi, F., Fiaschi, V., & Baldaccini, N. (1978). Pigeon navigation: effects upon homing behaviour by reversing wind direction at the loft. *Journal of comparative physiology*, *128*(4), 285-295.

Iwaniuk, A. N., Dean, K. M., & Nelson, J. E. (2004). A mosaic pattern characterizes the evolution of the avian brain. *Proceedings of the Royal Society B, 271 Suppl 4*, S148-151.

Iwaniuk, A. N., & Hurd, P. L. (2005). The evolution of cerebrotypes in birds. *Brain, behavior and evolution*, *65*(4), 215-230.

Iwaniuk, A. N., Hurd, P. L., & Wylie, D. R. (2006). Comparative morphology of the avian cerebellum: I. Degree of foliation. *Brain, behaviour, and evolution*, *68*(1), 45-62.

Iwaniuk, A. N., Hurd, P. L., & Wylie, D. R. (2007). Comparative morphology of the avian cerebellum: II. Size of folia. *Brain, behavior and evolution*, *69*(3), 196-219.

Iwaniuk, A. N., Pellis, S. M., & Whishaw, I. Q. (2000). The relative importance of body size, phylogeny, locomotion, and diet in the evolution of forelimb dexterity in fissiped carnivores (Carnivora). *Canadian Journal of Zoology*, *78*(7), 1110-1125.

Jamovi. (2021). The jamovi project. *version 1.6.23.0*.

Jerison, H. (1973). Evolution of the brain and intelligence Academic Press.

Jinn, J., Connor, E. G., & Jacobs, L. F. (2020). How ambient environment influences olfactory orientation in search and rescue dogs. *Chemical senses*, *45*(8), 625-634.

Johnsson, M., Henriksen, R., Fogelholm, J., Höglund, A., Jensen, P., & Wright, D. (2018). Genetics and genomics of social behavior in a chicken model. *Genetics*, *209*(1), 209-221.

Kabir, M. A. (2015). Worlds highflyer, tumbler and roller pigeons. *Platinum Global Journal of Agriculture and Food Science, 1*(1), 1-4.

Karlsson, A.-C., Fallahshahroudi, A., Johnsen, H., Hagenblad, J., Wright, D., Andersson, L., & Jensen, P. (2016). A domestication related mutation in the thyroid stimulating hormone receptor gene (TSHR) modulates photoperiodic response and reproduction in chickens. *General and Comparative Endocrinology*, *228*, 69-78.

Karten, H. J. (1969). The organization of the avian telencephalon and some speculations on the phylogeny of the amniote telencephalon. *Annals of the New York Academy of Sciences*, *167*(1), 164-179.

Karten, H. J., & Hodos, W. (1967). Stereotaxic atlas of the brain of the pigeon (Columba livia).

Kimball, R. T., Hosner, P. A., & Braun, E. L. (2021). A phylogenomic supermatrix of Galliformes (Landfowl) reveals biased branch lengths. *Molecular Phylogenetics and Evolution*, *158*, 107091.

Kosaka, T., & Kosaka, K. (2016). Neuronal organization of the main olfactory bulb revisited. *Anatomical Science International*, *91*(2), 115-127.

Krebs, J. R. (1990). Food-storing birds: adaptive specialization in brain and behaviour? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *329*(1253), 153-160.

Kruska. (1988a). Effects of Domestication on Brain Structure and Behaviour in Mammals. *Human Evolution*.

Kruska. (1988b). Mammalian domestication and its effect on brain structure and behavior. In *Intelligence and evolutionary biology* (pp. 211-250). Springer.

Kruska, D., & Schott, U. (1977). Comparative-quantitative investigations of brains of wild and laboratory rats. *Journal fur Hirnforschung*, *18*(1), 59-67.

Kruska, D. C. (2005). On the evolutionary significance of encephalization in some eutherian mammals: effects of adaptive radiation, domestication, and feralization. *Brain, behaviour and evolution*, *65*(2), 73-108.

LaDage, L. D., Roth II, T. C., Fox, R. A., & Pravosudov, V. V. (2009). Effects of captivity and memory-based experiences on the hippocampus in mountain chickadees. *Behavioral neuroscience*, *123*(2), 284.

Levi, W. M. (1965). *Encyclopedia of pigeon breeds*.

Liu, S. (2020). Dopaminergic modulation of glomerular circuits in the mouse olfactory bulb. *Frontiers in Cellular Neuroscience*, *14*, 172.

Löhmer, E. a. (1984). Comparative quantitative investigations on brains of rock doves, domestic and urban pigeons (Columba l. livia). *Journal of Zoological Systematics*, *22*(2), 136-145.

Löhmer, R., & Ebinger, P. (1983). Ergänzende Untersuchungen zur Hirn-Körpergewichtsbeziehung bei Graugänsen (Anser anser) vom Dümmer (Niedersachsen). *Journal für Ornithologie*, *124*(2), 195-196.

MacDougall-Shackleton, S. A., & Ball, G. F. (1999). Comparative studies of sex differences in the song-control system of songbirds. *Trends in neurosciences*, *22*(10), 432-436.

Macrides, F., & Schneider, S. P. (1982). Laminar organization of mitral and tufted cells in the main olfactory bulb of the adult hamster. *Journal of Comparative Neurology*, *208*(4), 419-430.

Marliave, J. B., Gergits, W. F., & Aota, S. (1993). F10 pandalid shrimp: sex determination; DNA and dopamine as indicators of domestication; and outcrossing for wild pigment pattern. *Zoo biology*, *12*(5), 435-451.

Mehlhorn, J., & Petow, S. (2020). Smaller brains in laying hens: New insights into the influence of pure breeding and housing conditions on brain size and brain composition. *Poultry Science*, *99*(7), 3319-3327.

Mehlhorn, J., & Rehkamper, G. (2009). Neurobiology of the homing pigeon--a review. *Naturwissenschaften*, *96*(9), 1011-1025.

Mehlhorn, J., & Rehkämper, G. (2009). Neurobiology of the homing pigeon—a review. *Naturwissenschaften*, *96*(9), 1011-1025.

Mehlhorn, J., & Rehkämper, G. (2013). Some remarks on bird's brain and behavior under the constraints of domestication. *International Scholarly Research Notices*, *2013*.

Meisami, E. (1989). A proposed relationship between increases in the number of olfactory receptor neurons, convergence ratio and sensitivity in the developing rat. *Developmental Brain Research*, *46*(1), 9-19.

Meskenaite, V., Krackow, S., & Lipp, H.-P. (2016). Age-dependent neurogenesis and neuron numbers within the olfactory bulb and hippocampus of homing pigeons. *Frontiers in behavioral neuroscience*, *10*, 126.

Mihailova, M., Berg, M. L., Buchanan, K. L., & Bennett, A. T. (2014). Odour-based discrimination of subspecies, species and sexes in an avian species complex, the crimson rosella. *Animal Behaviour*, *95*, 155-164.

Mora, C. V., Davison, M., Wild, J. M., & Walker, M. M. (2004). Magnetoreception and its trigeminal mediation in the homing pigeon. *Nature*, *432*(7016), 508-511.

Mouton, P. R. (2013). *Neurostereology: unbiased stereology of neural systems*. John Wiley & Sons.

Nacci, L., Ioalè, P., & Benvenuti, S. (1994). A new experiment to verify the spatial range of pigeons' olfactory map. *Behaviour*, *131*(3-4), 277-292.

Nimpf, S., Nordmann, G. C., Kagerbauer, D., Malkemper, E. P., Landler, L., Papadaki-Anastasopoulou, A., Ushakova, L., Wenninger-Weinzierl, A., Novatchkova, M., & Vincent, P. (2019). A putative mechanism for magnetoreception by electromagnetic induction in the pigeon inner ear. *Current Biology*, *29*(23), 4052-4059. e4054.

Nottebohm, F., Stokes, T. M., & Leonard, C. M. (1976). Central control of song in the canary, Serinus canarius. *Journal of Comparative Neurology*, *165*(4), 457-486.

Papi, F. (1976). The olfactory navigation system of the homing pigeon.

Papi, F. (1982). Olfaction and homing in pigeons: ten years of experiments. In *Avian navigation* (pp. 149-159). Springer.

Papi, F. (1991). Olfactory navigation. *Orientation in birds*, 52-85.

Papi, F., Fiore, L., Fiaschi, V., & Benvenuti, S. (1971). The influence of olfactory nerve section on the homing capacity of carrier pigeons. *Monitore Zoologico Italiano-Italian Journal of Zoology*, *5*(4), 265-267.

Papi, F., Fiore, L., Fiaschi, V., & Benvenuti, S. (1972). Olfaction and homing in pigeons. *Monitore Zoologico Italiano-Italian Journal of Zoology*, *6*(1), 85-95.

Patzke, N., Manns, M., & Güntürkün, O. (2011). Telencephalic organization of the olfactory system in homing pigeons (Columba livia). *Neuroscience*, *194*, 53-61.

Patzke, N., Manns, M., Güntürkün, O., Ioale, P., & Gagliardo, A. (2010). Navigation-induced ZENK expression in the olfactory system of pigeons (Columba livia). *European Journal of Neuroscience*, *31*(11), 2062-2072.

Perri, A. R., Feuerborn, T. R., Frantz, L. A., Larson, G., Malhi, R. S., Meltzer, D. J., & Witt, K. E. (2021). Dog domestication and the dual dispersal of people and dogs into the Americas. *Proceedings of the National Academy of Sciences*, *118*(6).

Perry-Gal, L., Erlich, A., Gilboa, A., & Bar-Oz, G. (2015). Earliest economic exploitation of chicken outside East Asia: Evidence from the Hellenistic Southern Levant. *Proceedings of the National Academy of Sciences*, *112*(32), 9849-9854.

Peterson, A. T., & Brisbin, I. L. (1998). Genetic endangerment of wild Red Junglefowl Gallus gallus? *Bird Conservation International*, *8*(4), 387-394.

Price. (2002a). *Animal domestication and behavior*. Cabi.

Price. (2002b). Domesticated birds as a model for the genetics of speciation by sexual selection. *Genetics of Mate Choice: From Sexual Selection to Sexual Isolation*, 311-327.

Price, E. O. (1984). Behavioral aspects of animal domestication. *The quarterly review of biology*, *59*(1), 1-32.

Price, E. O. (1999a). Behavioral development in animals undergoing domestication. *Applied Animal Behaviour Science*, *65*(3), 245-271.

Price, E. O. (1999b). Behavioural development in animals undergoing domestication. *Applied Animal Behaviour Science*, *65*, 245-271.

Puelles, L., Martinez-de-la-Torre, M., Martinez, S., Watson, C., & Paxinos, G. (2018). *The Chick Brain in Stereotaxic Coordinates and Alternate Stains: Featuring Neuromeric Divisions and Mammalian Homologies*. Academic Press.

Racicot, K. J., Popic, C., Cunha, F., Wright, D., Henriksen, R., & Iwaniuk, A. N. (2021). The cerebellar anatomy of red junglefowl and white leghorn chickens: insights into the effects of domestication on the cerebellum. *Royal Society Open Science*, *8*(10), 211002.

Rehkamper, G., Frahm, H. D., & Cnotka, J. (2008). Mosaic evolution and adaptive brain component alteration under domestication seen on the background of evolutionary theory. *Brain, behaviour and evolutionl*, *71*(2), 115-126.

Rehkämper, G., Haase, E., & Frahm, H. D. (1988). Allometric comparison of brain weight and brain structure volumes in different breeds of the domestic pigeon, Columba livia fd (fantails, homing pigeons, strassers). *Brain, behavior and evolution*, *31*(3), 141-149.

Rehkämper, G., Kart, E., Frahm, H. D., & Werner, C. W. (2003). Discontinuous variability of brain composition among domestic chicken breeds. *Brain, behavior and evolution*, *61*(2), 59-69.

Reiner, A., Medina, L., & Veenman, C. L. (1998). Structural and functional evolution of the basal ganglia in vertebrates. *Brain research reviews*, *28*(3), 235-285.

Reiner, A., Yamamoto, K., & Karten, H. J. (2005). Organization and evolution of the avian forebrain. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology: An Official Publication of the American Association of Anatomists*, *287*(1), 1080-1102.

Rood, J. P. (1972). Ecological and behavioural comparisons of three genera of Argentine cavies. *Animal Behaviour Monographs*.

Rose, E., Nagel, P., & Haag-Wackernagel, D. (2006). Spatio-temporal use of the urban habitat by feral pigeons (Columba livia). *Behavioral Ecology and Sociobiology*, *60*(2), 242-254.

Roth, L. S., & Lind, O. (2013). The impact of domestication on the chicken optical apparatus. *PLoS One*, *8*(6), e65509.

Rubin, C.-J., Zody, M. C., Eriksson, J., Meadows, J. R., Sherwood, E., Webster, M. T., Jiang, L., Ingman, M., Sharpe, T., & Ka, S. (2010). Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature*, *464*(7288), 587-591.

Saravanan, K., Panigrahi, M., Kumar, H., Bhushan, B., Dutt, T., & Mishra, B. (2020). Genome-wide analysis of genetic diversity and selection signatures in three Indian sheep breeds. *Livestock Science*, 104367.

Sayol, F., Lefebvre, L., & Sol, D. (2016). Relative brain size and its relation with the associative pallium in birds. *Brain, behavior and evolution*, *87*(2), 69-77.

Schütz, K. E., Forkman, B., & Jensen, P. (2001). Domestication effects on foraging strategy, social behaviour and different fear responses: a comparison between the red junglefowl (Gallus gallus) and a modern layer strain. *Applied Animal Behaviour Science*, *74*(1), 1-14.

Schütz, K. E., Jensen, P. . (2001). Effects of Resource Allocation on Behavioural Strategies: A Comparison of Red Junglefowl (Gallus gallus) and Two Domesticated Breeds of Poultry.

Shanahan, M., Bingman, V. P., Shimizu, T., Wild, M., & Güntürkün, O. (2013). Large-scale network organization in the avian forebrain: a connectivity matrix and theoretical analysis. *Frontiers in computational neuroscience*, *7*, 89.

Shao, Y., Tian, H.-Y., Zhang, J.-J., Kharrati-Koopaee, H., Guo, X., Zhuang, X.-L., Li, M.-L., Nanaie, H. A., Dehghani Tafti, E., & Shojaei, B. (2020). Genomic and phenotypic analyses reveal mechanisms underlying homing ability in pigeon. *Molecular Biology and Evolution*, *37*(1), 134-148.

Shapiro, M. D., & Domyan, E. T. (2013). Domestic pigeons. *Current Biology*, *23*(8), R302-303.

Shaw, R. C. (2017). Testing cognition in the wild: factors affecting performance and individual consistency in two measures of avian cognition. *Behavioural Processes*, *134*, 31-36.

Shimizu, T., Patton, T. B., & Husband, S. A. (2010). Avian Visual Behavior and the Organization of the Telencephalon [Article]. *Brain, behavior and evolution*, *75*(3), 204-217.

Sol, D., Olkowicz, S., Sayol, F., Kocourek, M., Zhang, Y., Marhounová, L., Osadnik, C., Corssmit, E., Garcia-Porta, J., & Martin, T. E. (2022). Neuron numbers link innovativeness with both absolute and relative brain size in birds. *Nature Ecology & Evolution*, 1-9.

Sossinka, R. (2013). Domestication in birds. *Avian biology*, *6*, 373-403.

Steiger, S. S., Fidler, A. E., & Kempenaers, B. (2009). Evidence for increased olfactory receptor gene repertoire size in two nocturnal bird species with well-developed olfactory ability. *BMC evolutionary biology*, *9*(1), 1-11.

Steiger, S. S., Fidler, A. E., Valcu, M., & Kempenaers, B. (2008). Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? *Proceedings of the Royal Society B: Biological Sciences*, *275*(1649), 2309-2317.

Tonosaki, K., & Shibuya, T. (1985). Olfactory receptor cell responses of pigeon to some odors. *Comparative Biochemistry and physiology. A, Comparative Physiology*, *81*(2), 329-333.

Trut, L., Oskina, I., & Kharlamova, A. (2009). Animal evolution during domestication: the domesticated fox as a model. *Bioessays*, *31*(3), 349-360.

Trut, L. N. (1999). Early Canid Domestication: The Farm-Fox Experiment: Foxes bred for tamability in a 40-year experiment exhibit remarkable transformations that suggest an interplay between behavioral genetics and development. *American Scientist*, *87*(2), 160-169.

Vigne, J.-D. (2011). The origins of animal domestication and husbandry: a major change in the history of humanity and the biosphere. *Comptes rendus biologies*, *334*(3), 171-181.

von Eugen, K., Tabrik, S., Güntürkün, O., & Ströckens, F. (2020). A comparative analysis of the dopaminergic innervation of the executive caudal nidopallium in pigeon, chicken, zebra finch, and carrion crow. *Journal of Comparative Neurology*, *528*(17), 2929-2955.

Wallraff, H. G., & Wallraff, H. G. (2005). *Avian navigation: pigeon homing as a paradigm*. Springer Science & Business Media.

Wang, I.-H., Murray, E., Andrews, G., Jiang, H.-C., Park, S. J., Donnard, E., Durán-Laforet, V., Bear, D. M., Faust, T. E., & Garber, M. (2022). Spatial transcriptomic reconstruction of

the mouse olfactory glomerular map suggests principles of odor processing. *Nature Neuroscience*, *25*(4), 484-492.

Wang, M. S., Thakur, M., Peng, M. S., Jiang, Y., Frantz, L. A. F., Li, M., Zhang, J. J., Wang, S., Peters, J., Otecko, N. O., Suwannapoom, C., Guo, X., Zheng, Z. Q., Esmailizadeh, A., Hirimuthugoda, N. Y., Ashari, H., Suladari, S., Zein, M. S. A., Kusza, S., . . . Zhang, Y. P. (2020). 863 genomes reveal the origin and domestication of chicken. *Cell Research*.

Wenzel, B. M. (1968). Olfactory prowess of the kiwi. *Nature*, *220*(5172), 1133-1134.

West, M. J. (2012). Introduction to stereology. *Cold Spring Harbor Protocols*, *2012*(8), pdb. top070623.

Wilkins, A. S., Wrangham, R. W., & Fitch, W. T. (2014). The "domestication syndrome" in mammals: a unified explanation based on neural crest cell behavior and genetics. *Genetics*, *197*(3), 795-808.

Woodfield, V., Chambers, J. . (1892). *The Show Homer Pigeon: Pigeon Breeds Book 7.* [Book].

Wu, M. Y., Low, G. W., Forcina, G., van Grouw, H., Lee, B. P. Y. H., Oh, R. R. Y., & Rheindt, F. E. (2020). Historic and modern genomes unveil a domestic introgression gradient in a wild red junglefowl population. *Evolutionary applications*, *13*(9), 2300-2315.

Wylie, D. R., Gutierrez-Ibanez, C., & Iwaniuk, A. N. (2015). Integrating brain, behavior, and phylogeny to understand the evolution of sensory systems in birds. *Frontiers in Neuroscience*, *9*, 281.

Yim, O., & Ramdeen, K. T. (2015). Hierarchical cluster analysis: comparison of three linkage measures and application to psychological data. *The quantitative methods for psychology*, *11*(1), 8-21.

Zeder, M. A. (2008). Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. *Proceedings of the National Academy of Sciences*, *105*(33), 11597-11604.

Zelano, B., & Edwards, S. V. (2002). An MHC component to kin recognition and mate choice in birds: predictions, progress, and prospects. *The American Naturalist*, *160*(S6), S225-S237.