Spring 2012

Morphological Detection of Genetic Introgression in Red Junglefowl (Gallus Gallus)

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MORPHOLOGICAL DETECTION OF GENETIC INTROGRESSION IN RED JUNGLEFOWL (*GALLUS GALLUS*)

by

TOMAS CONDON

(Under the Direction of C. Ray Chandler)

ABSTRACT

The Red Junglefowl (*Gallus gallus*) is generally considered to be the primary wild ancestor of the domestic chicken (*Gallus gallus domesticus*). Because they are common in much of the forested habitat of South and Southeast Asia, they have never been granted any formal conservation status. However, evidence suggests that genetic introgression from free-ranging, escaped, and feral domestic chickens might be so widespread and pervasive that Red Junglefowl may be endangered or even extinct in the wild. Because genetic markers have yet to be identified, detection of introgression has been limited to qualitative morphological traits. Thus, there is a need to identify simple quantitative traits that might be used to detect such introgression. Between October 2010 and May 2011, I measured external morphological characters on 94 putatively unintrogressed Indian Red Junglefowl (*G. g. murghi*) – 44 museum specimens and 50 captive birds. The latter were descended from Red Junglefowl collected from remote areas of north-central India between 1960 and 1961, and this population is considered to be one of the only captive flocks of this species with little or no introgression from domestic chickens. I also measured 44 junglefowl-chicken hybrids with known levels of introgression, and 14 domestic chickens from the population that was used to create these hybrids. Female comb size and male spur width both increased in size with increasing

levels of introgression, as did bill length and mass in both sexes. Using a discriminant analysis, I found that bill length, comb height and comb length are effective characters for identifying introgression in females, and bill length and spur width are most effective for males. Based on these results I propose a more complete and quantitative suite of traits that could be used to characterize the level of introgression in populations of Indian Red Junglefowl.

INDEX WORDS: Hybridization, Introgression, Gene flow, Endangered species, Red Junglefowl, *Gallus gallus*, Domestication, Chicken

MORPHOLOGICAL DETECTION OF GENETIC INTROGRESSION IN RED JUNGLEFOWL (*GALLUS GALLUS*)

by

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Natural Resources and the Environment, University of Connecticut, 2009

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial Fulfillment

of the Requirements for the Degree

MASTER OF BIOLOGY

STATESBORO, GEORGIA

2012

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IN RED JUNGLEFOWL (*GALLUS GALLUS*)

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Electronic Version Approved:
May 2012

ACKNOWLEDGMENTS

I would first like to thank my graduate advisor Dr. C. Ray Chandler for all of his assistance with this project, including advice about statistics and many edits to the proposal and thesis manuscripts. I would also like to thank the rest of my graduate committee, including Dr. Stephen Vives, Dr. Lance McBrayer, and Dr. I. Lehr Brisbin, Jr. for all of their input and advice about the project. A special thanks to Mr. and Mrs. Leggette Johnson for their kindness and generosity. Much of the project would not have been possible without their assistance, and for that I am very grateful. I am also appreciative for the hospitality of Mr. Charles Hill who allowed me to work with his captive colony of Red Junglefowl. I would like to thank all of museum curators who allowed me access to their collections – Dr. David Willard (Field Museum of Natural History), Dr. Kristof Zykowski (Yale/Peabody Museum of Natural History), Dr. Paul Sweet (American Museum of Natural History), and Dr. Tom Webber (Florida Museum of Natural History) as well as Dr. A. Townsend Peterson and Dr. Mark Robbins (University of Kansas Natural History Museum). Finally, I would be remiss if I did not thank my friends and family who have supported me all the way through. Funding for this study was provided by the Georgia Southern University College of Graduate Studies.

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CHAPTER 1

INTRODUCTION

There are four extant species of junglefowl (genus *Gallus*), the Grey Junglefowl (*G. sonnerati*), Ceylon Junglefowl (*G. lafayettei*), Green Junglefowl (*G. varius*), and the Red Junglefowl (*G. gallus*). The Red Junglefowl is usually split into five subspecies (Johnsgard 1999) – the Indian (*G. g. murghi*), Burmese (*G. g. spadiceus*), Cochin-Chinese (*G. g. gallus*), Tonkinese (*G. g. jabouillei*), and the Javan (*G. g. bankiva*). These populations are distributed across much of South and Southeast Asia, from portions of eastern Pakistan, east to parts of southeastern China, and south to Bali, Indonesia. They are also found on many Pacific Islands, including the Philippines and Hawaii, but populations east of Wallace's Line are generally attributed to introductions by humans (Beebe 1926a; Ball 1933; Delacour 1977; Johnsgard 1999). Red Junglefowl occur in a wide variety of habitats from sea level up to about 2000 m (Baker 1928, Bump and Bohl 1961, Delacour 1977, Johnsgard 1999).

The Red Junglefowl is a small to medium-sized galliform, similar in appearance to a few breeds of domestic chicken. The sexes are highly dimorphic. Males average 850 g and are spectacularly colored, with yellow-golden to orange neck hackles, red saddles, a black breast and belly, and an iridescent greenish-black tail. They also have a single comb and a pair of gular wattles, both of which are bright crimson red, and sharp, curved spurs on each leg. Females average 550 g and are much drabber, their plumage being various shades of yellow, brown, and black. Unlike the males, female Red Junglefowl have neither spurs nor wattles, and have combs that are greatly reduced. Red Junglefowl

are gregarious and are typically found in pairs or small groups, although groups as large as several dozen birds may not be uncommon, particularly outside of the breeding season (Bump and Bohl 1961, Collias and Collias 1967, Delacour 1977, Johnsgard 1999).

The Red Junglefowl is generally considered to be the primary wild ancestor of the modern domestic chicken (*Gallus gallus domesticus*) and is classified as the same species. The details of domestication, however, including the time and location, and the potential contributions from the other members of the genus, remain the subjects of continued debate (Hutt 1949, Morejohn 1974, West and Zhou 1988, Fumihito et al. 1996, Liu et al. 2006, Eriksson et al. 2008). However, there is general agreement, that the Red Junglefowl is the primary wild ancestor, and that domestication occurred no less than 8000 years ago (West and Zhou 1988).

Because they are common across most of their native range, the Red Junglefowl has never been of conservation concern. Recently, however, concern has been expressed for the genetic integrity of the species. It has been suggested that hybridization between wild Red Junglefowl and free-ranging, escaped, and feral chickens has been so common, that introgression from domestics might be swamping out wild gene pools. The fear is that this has already been so common that Red Junglefowl might be endangered or possibly even extinct in the wild (Brisbin 1996, Peterson and Brisbin 1999, Brisbin et al. 2002). There is the risk then, not only of losing a genetically distinct species and its ecological role, but also the wild ancestor of the domestic chicken.

There are many captive flocks of Red Junglefowl in both zoos and private avicultural collections around the world. However, most have undocumented or poorly documented origins, and morphological and behavioral characteristics that suggest past

introgression from domestic chickens (Brisbin et al. 2002). One captive population, however, has remarkably well documented origins, and its members show no signs of introgression. The founders of this flock, which is now known as the JFW population, were collected between 1960 and 1961 from remote areas of north-central India by the United States Fish and Wildlife Service under the Foreign Game Investigation Program (Bump 1959, 1960, 1961a, 1961b, 1962; Bump and Bohl 1961). Today this population is maintained and propagated in a small number of private avicultural collections, mostly in the southeastern United States. The JFW population is an important source of this species for biological studies, and might even be used for reintroduction programs if it is determined that the Red Junglefowl is in fact endangered in the wild (Brisbin 1996, Peterson and Brisbin 1999, Hawkins 2001, Brisbin et al. 2002).

This thesis uses the JFW population to address two important issues. First, Chapter 2 provides a detailed history of Red Junglefowl introductions in the southern United States and traces the history of the JFW population that stemmed from the program. Essentially, Chapter 2 establishes the provenance of this population as genetically unintrogressed representatives of wild Red Junglefowl. Second, Chapter 3 describes an experiment, using JFW birds, to determine if simple external characters can be used to effectively discriminate populations of unintrogressed Red Junglefowl from those carrying domestic genes.

CHAPTER 2

RED JUNGLEFOWL INTRODUCTIONS IN THE SOUTHERN UNITED STATES: HISTORY AND MODERN RESEARCH LEGACY

The Foreign Game Investigation Program

In the mid 1940s, there was increasing interest in recreational hunting in the United States, as well as the idea of pursuing new, exotic game species (Bump 1968). Because of concern over the potentially disastrous consequences of unregulated introductions of exotics carried out by the public, the International Association of Game Fish and Conservation Commissioners (IAGFCC) requested that the United States Fish and Wildlife Service (USFWS) set up a program to investigate foreign game species that might be used to meet this increasing public demand (Bump 1968). In 1948 the USFWS established the Foreign Game Investigation Program (FGIP). The purpose of the program was to study, collect, import, propagate, and release foreign game species into areas of the United States that were deemed deficient in huntable populations of native game (Bump 1968). Dr. Gardiner Bump was hired as a Wildlife Research Biologist by the Branch of Wildlife Research and appointed leader of the FGIP (Department of the Interior News Release 1949).

Although the FGIP began in 1948, most of the releases were made between 1960 and 1970. During this time, two dozen species, subspecies, and hybrids were released in at least 27 states and the U.S. territory of Guam (Bump 1968, Banks 1981). In all, more than 340,000 birds were released (Bump 1962; Bump and Bohl 1964; Chambers 1965, 1966; Bohl and Bump 1970; Banks 1981). Poor results, combined with a shift in mindset

regarding the ecological appropriateness of such introductions (e.g. Gullion 1965), resulted in the discontinuation of the FGIP in 1970 (Banks 1981). However, a number of state agencies continued to make releases for several years thereafter (Banks 1981). Despite these efforts, only seven of the species eventually became established. Five species became established on Hawaii – the Black Francolin (*Francolinus francolinus*), Grey Francolin (*Francolinus pondicerianus*), Erckel's Francolin (*Francolinus erckelii*), Chestnut-Bellied Sandgrouse (*Pterocles exustus*), and the Kalij Pheasant (*Lophura leucomelana*) (Lever 2005). In the continental U.S. only two species were successful. The Himalayan Snowcock (*Tetraogallus himalayensis*) persists in parts of the Ruby Mountains in Nevada, and the Green Pheasant (*Phasianus versicolor*) still occurs in parts of Virginia and Tennessee (Lever 2005).

The Red Junglefowl of the FGIP

One of the species selected for introduction to the southern United States was the Red Junglefowl (*Gallus gallus*). It was praised as a challenging bird to hunt, with good-tasting meat, and the ability to withstand relatively heavy hunting pressure (Bump and Bohl 1961). It was also thought that they would do little damage to agricultural crops and compete minimally with native species (Bump and Bohl 1961). It was concluded then that the Red Junglefowl would make an ideal game bird in parts of the south where the climate and habitats are similar to their native South Asia (Bump and Bohl 1961, Bump 1968).

Dr. Bump and fellow USFWS biologist Mr. Wayne Bohl began their investigation of the Red Junglefowl in India in the late 1950s. In July of 1959 the team traveled to

Bihar where they conducted a week-long study of the Red Junglefowl. By the fall of 1959 all of the necessary permits were approved, access to the foothills was granted, and trapping of junglefowl began (Bump 1960).

The exact locations in India where Red Junglefowl were collected remains unclear. It does appear, however, that at least one of the areas was 24 to 32 km southeast of Dehradun, in Uttarakhand Pradesh (based on I.L. Brisbin, Jr.'s notes from interviews with G. Bump, 20 Jan 1969; I.L. Brisbin, Jr., pers. comm.). It is also known that the original stock was collected from nests found between 305 and 610 m in elevation (I.L. Brisbin, Jr., pers. comm.). Given this information, it is very likely that at least one of the collection sites was in, or near, present-day Rajaji National Park (30°N, 78°E). Regardless of the exact locations, Bump assured Brisbin that all of the Red Junglefowl were collected after taking great care to ensure that no free-ranging, village chickens were in the vicinity (I.L. Brisbin, Jr., pers. comm.). To ensure this was the case, eggs and chicks were collected from areas at least 5 km from any village, and most were collected 16 to 24 km away or farther (I.L. Brisbin, Jr., pers. comm.). Bump did note the difficulties in locating Red Junglefowl that had not interbred with domestic chickens (Bump and Bohl 1961, F. Parrish and I.L. Brisbin, Jr., pers. comm.), and these precautions were undoubtedly due to this fact. A chronological account of all Red Junglefowl collection, importation, quarantine, and distribution activities between 1959 and 1961 follows. A summary of these activities can be seen in Tables 2.1 and 2.2.

The first trapping season began on 1 October 1959 and ended in the following January, during which time only two junglefowl were collected. Although the exact date cannot be found, it is known that that these two birds were shipped from India to the

United States sometime between 22 March and 19 May 1960 (Bump 1960). After thirty days in quarantine, at the U.S. Animal Quarantine Station in Clifton, New Jersey, the birds were received by one of the cooperating states to be used as breeder stock (Bump 1960). Which state received these two birds is also not clear from the existing records.

Collection of Red Junglefowl resumed in the spring of 1960, this time on a larger scale. Local game wardens and trappers were hired to search for nests and collect eggs and young chicks (Bump 1960, I.L. Brisbin, Jr., pers. comm.). The eggs were set under domestic hens, and twice a week the chicks were transported to FGIP headquarters in New Delhi (I.L. Brisbin, Jr., pers. comm.). Here, the birds were raised in homemade brooders by Bump, his wife, and other FGIP personnel (Bump 1960). At 3-4 weeks of age, the birds were transferred to outdoor pens, and at 12-14 weeks they were loaded into crates and shipped to the U.S. (Bump and Bohl 1961). By May 1960, 107 junglefowl had hatched and been brought to New Delhi. Of these, five birds died during rearing, 32 (mostly extra males) were distributed to several Indian zoos, and the remaining 70 were sent to the U.S. (Bump 1960). After quarantine the birds were distributed to state-operated game farms in Alabama, Georgia, Oklahoma, and Virginia (Bump 1961b).

Collection of Red Junglefowl continued in the spring of 1961. Bump and his wife left India in May, and operations in New Delhi were left under the management of Mr. Wayne Bohl (Bump 1961b). By 31 October 1961, Bohl had shipped 47 more junglefowl to the states. Two of the birds died during the quarantine period, and the 45 that remained were distributed to state game farms in Florida, Kentucky, Tennessee, and Virginia (Bump 1962).

In all, nine states received Red Junglefowl under the FGIP. Although most received birds that had been collected directly from the wild, Louisiana and South Carolina both aquired their stock later, from states that had already sucessfully propogated the species. Between 1960 and 1971 approximately 10,000 Red Junglefowl were raised in Alabama, Florida, Georgia, Kentucky, Louisiana, Oklahoma, South Carolina, Tennessee, and Virginia (Bump 1961a; Bump and Bohl 1964; Chambers 1965, 1966; Bohl and Bump 1970, Banks 1981). Typical of these breeding operations was the Bowen's Mill hatchery in Fitzgerald, Georgia where more than 2,200 junglefowl were raised between 1961 and 1970 (Figure 2.1).

Between 1961 and 1971 an estimated 9,912 Red Junglefowl were released in at least 52 areas in eight states (Table 2.3). Virginia was the only state that did not make trial releases. Despite these sustained efforts, wild junglefowl populations established in only two areas. One population was established on Avery Island, in Iberia County, Louisiana and another in Fitzgerald, in Ben Hill County, Georgia (Hopkins 1981, F. Parrish and R. Rogers, pers. comm.). The population on Avery Island persisted until the early 1990s before disappearing for unknown reasons (R. Rogers, pers. comm.). In Fitzgerald it appears that the birds existed in a relatively wild state through the late 1970s or early 1980s before breeding with domestic chickens and taking on a more or less feral existence (Hopkins 1981, F. Parrish, pers. comm.).

It has also been suggested that a few people living in Fitzgerald persuaded some of the employees to let them have a few junglefowl eggs from the hatchery. It is thought that that the eggs were hatched under domestic chickens, the junglefowl hybridized with the domestic birds in captivity, and the hybrid progeny either escaped or were released.

Today, a large population of feral chickens can be found living in Fitzgerald's suburban neighborhoods, some or all of which are likely carrying genes from the Red Junglefowl raised under the FGIP. Based on surveys taken in March and April 2010, I conservatively estimated the population to be >900 birds in the 4 km^2 downtown area alone. This population could be used for a wide variety of ecological, behavioral, and evolutionary studies. Because they are likely descended from wild Red Junglefowl, they might also be used to study the morphological and genetic changes that occur due to gene flow between wild junglefowl and domestic chickens.

Mrs. and Dr. Gardiner Bump both retired in the early 1970s during the last days of the FGIP, and have since passed away. Before their retirement Leslie Glasgow, former Assistant Secretary of the Department of Interior said, "The Bumps will be remembered and will be the source of vital information for many years to come because of the reliability of their studies…it will take years for biologists to use fully the amount of scientific data they have acquired" (Department of the Interior News Release 1970). This thesis is a confirmation of this statement made over four decades ago.

Start of the JFW population

During the mid to late 1960s Dr. I. Lehr Brisbin, Jr. was a doctoral graduate student at the University of Georgia in Athens. Upon the completion of his degree, Brisbin took a position at the Savannah River Ecology Laboratory (SREL) in Aiken, South Carolina. There he studied, among other things, the fate and effects of radioactive contaminants in game birds at the Department of Energy's Savannah River Site (SRS). He began by using domestic chickens as an analog for wild galliforms that could leave

the SRS and be harvested and consumed by hunters. He quickly switched from using domestic chickens to Red Junglefowl when they became available at several of the nearby state propagation facilities under the FGIP. Brisbin acquired his first group of Red Junglefowl on 24 May 1966, when he picked up 48 day-old chicks from the Georgia Fish and Game Commission's Bowen's Mill facility (Figure 2.1). Although this line eventually died out, and none of these birds contributed to the present day JFW population, this was the start of Brisbin's Red Junglefowl studies at the SREL.

The JFW population began a few years later when Bump helped Brisbin obtain five Red Junglefowl from the South Carolina Department of Wildlife Resources on 21 January 1969 (Figure 2.2). These birds came from the state's propagation facility at the Belmont Game Management Area in Belmont, South Carolina. Although only one of these original birds remained by March of 1970, Brisbin was able to hatch three young birds in 1969 and raise them to maturity by the start of the 1970 breeding season. He added to his flock in April of 1970 when he picked up 101 eggs from the Bowen's Mill facility upon the discontinuation of Red Junglefowl program in Georgia (Figure 2.2); 26 of these eggs hatched. By February of 1971 Brisbin's flock had been reduced to eight birds, four males and four females. However, on 11 May 1971 his flock increased in size again when he received 69 day-old junglefowl from Dr. Dave Anderson and Dr. Stanley Vezey at the University of Georgia (UGA). They had picked up the eggs from the state game farm in Prattville, Alabama (Figure 2.2) and hatched them on the UGA campus in Athens. Brisbin continued to keep the flock at the SREL until June of 1972. Although he was able to maintain the population, Brisbin's flock experienced high mortality rates over

these three years (1969-1972), which he attributed primarily to outbreaks of avian leucosis, also known as Marek's Disease (I.L. Brisbin, Jr., pers. comm.).

Red Junglefowl Obtained by Mr. Isaac Richardson

In the spring of 1972, Brisbin accepted a temporary position in Washington, D.C., and he began searching for individuals who would be able to give the Red Junglefowl the high level of care that they required. He located two experienced aviculturalists, Mr. Oscar Wallace in Dora, Alabama, and Dr. Michael Dam in Haines City, Florida. Wallace was friends with another bird enthusiast named Mr. Isaac Richardson in Tuscaloosa, Alabama. Wallace told Richardson that he could have the birds if he drove to the SREL and picked them up. On 24 June 1972, Richardson drove to the SREL and Brisbin transferred most of his remaining junglefowl to him (Figure 2.2); twelve birds, including eight adult males, one adult female, and three female chicks. Two months later, in August, Brisbin dropped off a few additional birds, which Richardson added to his flock.

Richardson was very successful with the junglefowl, and by March of 1975 he had raised over 75 young birds. He began to farm out the offspring to other aviculturalists in the southeast, but it appears that all of these lines eventually died out (I. Richardson, pers. comm.). Richardson, in contrast, remained very successful raising the junglefowl, and he maintained a breeding colony of 5-20 birds at his home in Alabama for over thirty years (I.L. Brisbin, Jr. and I. Richardson, pers. comm.).

Distribution of JFW birds to Groups of Private Aviculturalists

In July 1998 Richardson donated 65 Red Junglefowl to the Georgia Game Bird Breeders Association (GGBBA) for distribution amongst its members (Figure 2.2) (Hawkins 2001, S. Colomb and A. Cuming, pers. comm.). The birds, of various ages, from quail-sized juveniles to adults, were picked up by several members of the club and taken to the home of Mr. Alfred Cuming in Watkinsville, Georgia (A. Cuming, S. Colomb, and B. Shamblin, pers. comm.). Most of these birds were then distributed to GGBBA members in Florida, Alabama, Georgia, South Carolina, and Virginia, as well as to a few members of the Carolina Virginia Pheasant and Waterfowl Society (CVPWS) (Hawkins 2001, A. Cuming and W. Hawkins, pers. comm.). Unfortunately, most of the junglefowl died from stress and disease shortly after their distribution (A. Cuming and S. Colomb, pers. comm.). In 1999 Mr. Wayne Hawkins of the CVPWS established a studbook for the population, and a few people also listed their holdings with the International Species Information System. Unfortunately, the studbook quickly became outdated and inaccessible to holders of the birds following Hawkins's passing in 2004.

The current population of JFW birds originated from holdings of a few successful aviculturalists, most notably Mr. Elton Housley, Mr. Johnny Wise, Mr. Keith Burnam, Mr. Leggette Johnson, Mr. Al Cuming, Mr. Wayne Hawkins and the continued efforts of Richardson himself.

Current Status and Modern Research Legacy

Fourteen years have passed since Richardson donated the Red Junglefowl to the GGBBA. As of January 2012, there are four main flocks that are direct descendents of

this important population. The largest flock today is being maintained by Mr. Don Shadow, a private aviculturalist in Winchester, Tennessee (Figure 2.2). Three smaller flocks are being maintained by Mr. Leggette Johnson in Cobbtown, Georgia, Mr. Charles Hill in Commerce, Georgia, and by Dr. Brisbin at the SREL (Figure 2.2). There are a number of even smaller flocks scatted throughout the country, but these lines are difficult to track. The total population of JFW Red Junglefowl today is probably 100-200 birds.

Because of their well-documented origins and history the JFW flock is considered one of the purest captive populations of this species (Brisbin 1996, Peterson and Brisbin 1998, Brisbin et al. 2002). They were recently used to document the morphological changes resulting from introgression from domestic chickens (Brisbin and Peterson 2007) and will undoubtedly be used in future studies of this species. There is also the possibility that the JFW population might be used for reintroduction programs, if it is determined that pure Red Junglefowl are in fact endangered in the wild.

Table 2.1. Red Junglefowl collected, reared, and shipped from India to the United States under the Foreign Game Investigation Program, 1959-1961.

Group No.	Season/Year Collected	No. Collected	No. Lost in Rearing	No. Shipped From India	Season/Year Shipped	No. Lost In Shipping
1	Winter, 1959-1960	2	0	2	Spring, 1960	0
2	Spring, 1960	107	5	70*	Summer-Fall, 1960	0
3	Spring, 1961	47	0	47	Summer-Fall, 1961	0
Summary/Totals	**1959-1961**	**156**	**5**	**119**	**1960-1961**	**0**

* The remaining 32 birds (mostly extra males) were distributed to several Indian zoos

Table 2.2. Red Junglefowl quarantined and distributed to state agencies under the Foreign Game Investigation Program.

Group No.	No. Quarantined	No. Lost in Quarantine	No. Shipped to States	States	Use
1	2	0	2	Unknown	Breeders
2	70	0	70	AL, GA, OK, VA	Breeders
3	47	2	45	FL, KY, TN, VA	Breeders
Summary/ Totals	**119**	**2**	**117**	**AL, FL, GA, KY, OK, TN, VA**	**Breeders**

Table 2.3. Red Junglefowl released in the United States under the Foreign Game Investigation Program, 1961-1971.

State	Release Years	No. Birds Released	No. of Counties, No. Release Sites
Georgia	1961-1970	2248 (est.)	≥ 9 counties, ≥ 9 sites
Alabama	1962-1971	1813 (est.)	10 counties, ≥ 10 sites
South Carolina	1965-1971	1380 (est.)	11 counties, ≥ 11 sites
Oklahoma	1961-1967	1283	1 county, 4 sites
Louisiana	1963-1967	1151	5 counties, 6 sites
Florida	1963-1968	1002	7 counties, ≥ 7 sites
Tennessee	1964-1966	566	3 counties, ≥ 3 sites
Kentucky	1964-1967	469	2 counties, ≥ 2 sites
Summary/ Totals	**1961-1971**	**9912 (est.)**	**≥ 48 counties, ≥ 52 sites**

Figure 2.1. Red Junglefowl in a breeding pen at the Georgia Fish and Game Commission's Bowen's Mill hatchery in Fitzgerald, Georgia (above). After hatching, the young birds were raised in the brooder house (below). The brooder building is still standing in its original location, but the breeder pens have long since been dismantled. Photographs were taken by I. Lehr Brisbin, Jr. on 17 January 1967 and 15 February 1967 respectively.

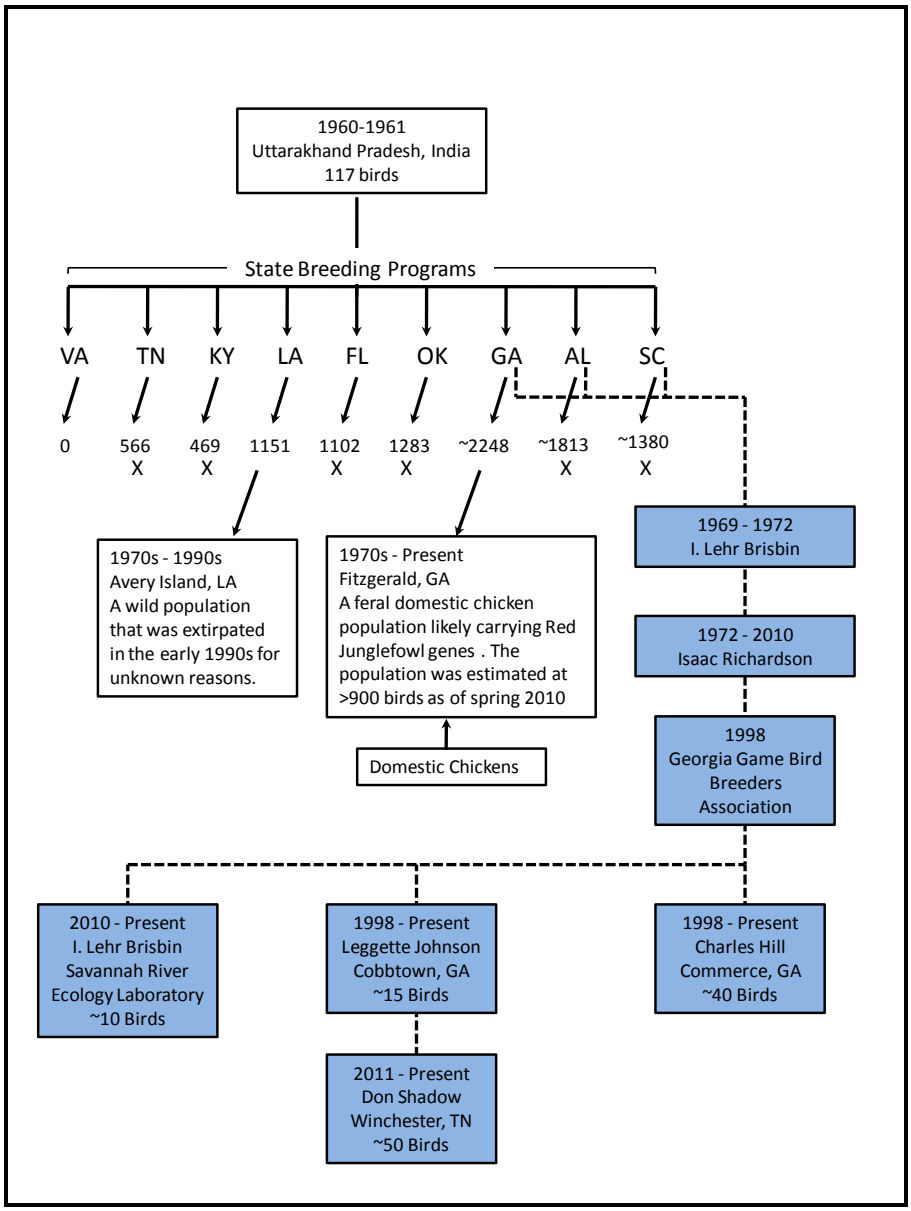

Figure 2.2. Introductions of Red Junglefowl under the Foreign Game Investigation Program and the provenance of the captive JFW population. The number of junglefowl released is shown below each state. 'X's indicate that no populations established from these releases. Blue boxes represent the JFW population that stemmed from the program. The four major flocks that make up the present day population are shown at the bottom with the estimated number of birds in each.

CHAPTER 3

MORPHOLOGICAL DETECTION OF GENETIC INTROGRESSION IN RED JUNGLEFOWL

Introduction

The loss of genetically distinct populations due to introgression is an increasingly important threat to biodiversity. Introgression is the process by which hybridization and repeated backcrossing leads to gene flow between two or more species, subspecies, or populations (Anderson 1949, Rhymer and Simberloff 1996, Allendorf et al. 2001). This gene flow can occur naturally and is an important source of genetic variation in populations. However, conservation concerns arise when introgression is caused by human activities, such as introducing organisms into new areas or removing natural reproductive barriers by modifying habitats (Allendorf et al. 2001). In some cases introgression can be so extensive that endangerment or extinction of one or both of the parental groups can result (Rhymer and Simberloff 1996, Allendorf et al. 2001). Rhymer and Simberloff (1996) reviewed many examples of endangerment and extinction that have occurred as a result of introgression.

There is even greater concern when the species faced with such introgression is the wild ancestor of one of our modern domestic crops or animals (Brisbin 1995, Ellstrand et al. 1999, Randi 2008). According to Ellstrand et al. (1999), 12 out of 13 of our most important food crops are known to hybridize with their wild relatives. Two of these, wild rice (*Oryza* spp.) and wild cottonseed (*Gossypium* spp.), are thought to be endangered because of introgression from their domestic cultivars (Ellstrand et al. 1999).

Introgression from domestic corn (maize) into their wild relatives (teosintes) has also been documented, which has raised concerns for the continued existence of the latter in their pure, wild form (Ellstrand et al. 2007). In such cases, there is not only the risk of losing a distinct species and its ecological role, but also a reservoir of potentially invaluable genetic variation – resources that could be used to improve domestic varieties by increasing vigor, hardiness, performance, productivity, or resistance to emerging diseases (Andersson 1994, Brisbin 1995).

The problem, however, is not limited to crops. It has long been known that the Red Junglefowl (*Gallus gallus*) readily hybridizes with their descendent, the domestic chicken (*Gallus gallus domesticus*), both in captivity and in the wild. In fact, Beebe (1926b) gave the first warning nearly a century ago that wild Red Junglefowl might become increasingly rare because of hybridization with free-ranging domestics. Only recently, however, has it been suggested that introgression of domestic genes into wild populations might be so common that Red Junglefowl could be endangered or possibly even extinct in the wild (Brisbin 1996; Peterson and Brisbin 1999, 2005; Brisbin et al. 2002; Brisbin and Peterson 2007). Thus, methods are needed to distinguish unintrogressed populations of Red Junglefowl from those carrying domestic genes.

Of course, the most direct way to assess introgression in Red Junglefowl would be to use genetic analyses. Unfortunately, at this time there are no diagnostic loci that can be used to accurately distinguish unintrogressed birds from hybrids. Rubin et al. (2010) recently identified a single-nucleotide polymorphism in the Thyroid Stimulating Hormone Receptor gene that might be useful for this purpose, and whole genome sequencing on a captive population of Red Junglefowl (JFW population) is underway

which may lead to the discovery of additional markers (L. Andersson et al., in prep). However, in the meantime, simple morphological characters could provide a quick and economical way to detect introgression. Such characters would be especially useful for field biologists in remote parts of the Red Junglefowls' native range. Putatively unintrogressed populations, as identified by morphological traits, could then be targeted for more intensive analyses.

Several morphological traits have been proposed as a means of distinguishing unintrogressed Red Junglefowl from intergrades. One of the most well-known and frequently used of these traits is presence of an annual "eclipse plumage" in males. The eclipse is a basic plumage (sensu Humphrey and Parkes 1959) that follows a prebasic molt at the end of the breeding season. During this time (usually June-September) the males molt their bright yellow, lanceolate-shaped neck hackles, and quickly replace them with short, black, spatulate-shaped feathers (Baker 1928, Delacour 1947, Kimball 1958, Morejohn 1968, Delacour 1977). The two long sickle-shaped tail streamers are also molted, but are not immediately replaced (Delacour 1977). Although it appears that the absence of this plumage from a population may indicate introgression from domestics, the presence of this plumage in intergrades has also been reported (Brisbin and Peterson 2007).

Another trait that has been used to assess introgression in Red Junglefowl is the size of the female comb and wattles. On most varieties of domestic fowl the female comb and wattles are well-developed. In Red Junglefowl, however, females have no wattles, and the comb is usually so small it can only be seen with the bird in hand and the crown feathers parted. Because of this, there have been many conflicting descriptions of this

character. Baker (1928: 297), for example, described female comb and wattles as "sometimes absent." Delacour (1977: 125-126) said that wild Red Junglefowl females have a "comb reduced to a small fold, and lappets invisible," and then later on in the same description said "pure hens have neither combs nor lappets." Brisbin and Peterson (2007: 431) examined the study skin of a single female Red Junglefowl from the JFW population (KUNHM 110221). They described the comb as a "small thickened ridgeline… [with] 3 or 4 minute nubs, the largest of which might be 0.5 x 0.5 x 1 mm." Although better than previous accounts, they reported the estimated measurements of only one specimen. Thus, to date, evaluation of Red Junglefowl using female comb and wattle size have been limited to qualitative, rather than quantitative, characterization (e.g., Cornwallis 2002, Kaul et al. 2004, Peterson and Brisbin 2005, Platt et al. 2009, Fernandes et al. 2009, Vijh et al. 2009).

A third trait that has been used to distinguish unintrogressed Red Junglefowl from hybrids is tarsus color. Red Junglefowl have dark slate to dark gray tarsi, although they have also been described as "greenish-grey to deep slaty-brown" (Baker 1928: 296) and "plumbeous brown to bluish grey" (Delacour 1977: 125). Bump (1961) suggested that any yellow in the legs was the result of hybridization with domestics. Thus, deviation from dark slate or black tarsi in a population could be indicative of introgression from domestics.

There are a number of other traits that have been described as a means of distinguishing unintrogressed Red Junglefowl from hybrids or intergrades. These include a low tail carriage in both sexes (Beebe 1926b, Bump and Bohl 1961, Delacour 1977), as well as a high pitched and truncated crow (Beebe 1926b, Delacour 1947, Delacour 1977)

and a tuft of white down feathers at the base of the tail of the males (Bump and Bohl 1961). A number of behavioral differences compared to domestic fowl have also been described (Beebe 1926b, Brisbin 1969, Delacour 1977). However, these traits and behaviors are more difficult to assess, and therefore less frequently used in these characterizations.

Several authors have attempted to use morphological traits to evaluate both wild and captive populations of Red Junglefowl, as well as museum specimens. Peterson and Brisbin (1999), for example, analyzed eclipse plumage, tarsus color, and female comb size on 745 Red Junglefowl in 19 museum collections. They concluded that mixing of domestic and wild populations had occurred before intensive scientific collecting began (about 1860). They also suggested that the loss of original gene pools began in the easternmost portion of the species' range prior to 1860, and spread westward reaching India and Nepal by the mid to late 1960s (Peterson and Brisbin 1999). More recently, Peterson and Brisbin (2005) analyzed 87 Red Junglefowl collected from Hawaii and a number of other South Pacific islands between 1891 and 1933. They found clear signs of introgression in 78 out of the 87 birds (89.66%) that they analyzed. The other nine birds, which were collected from the Caroline Islands in 1930-1931, showed no obvious signs of introgression from domestics.

Cornwallis (2002) analyzed wild populations of Red Junglefowl on Kauai (Hawaii), Rarotonga (Cook Islands), and Palau Ubin (Singapore). Kauai and Rarotonga are both outside the natural range of the Red Junglefowl and the birds that occur on these islands were most likely introduced by the Polynesians about 3000 years ago (Ball 1933). Palau Ubin is on the edge of the species' distribution and is likely a naturally occurring

population. Cornwallis used five traits to estimate the amount of introgression that had occurred in these populations. He concluded that extensive mixing with domestics had occurred on both Kauai and Rarotonga, but found minimal signs of introgression in the Palau Ubin population (Cornwallis 2002).

More recently, Platt et al. (2009) analyzed the photographs of 14 Red Junglefowl trapped from the forests of northern Sulawesi in 1996 and in 2002. Like Kauai and Rarotonga, Sulawesi is outside the natural range of the Red Junglefowl, and this population was probably introduced by the Austronesian-speaking people who first colonized the island (Bellwood 1980, Whitten et al. 1987). Of the 14 birds examined, 13 showed clear signs of domestic influence. It was concluded that introgression from domestics is ubiquitous, at least on the northern part of the island, and it was suggested that since birds there were probably introduced, Sulawesi likely never had pure populations of Red Junglefowl.

Several authors have attempted to characterize and evaluate both captive and wild populations of Red Junglefowl in India. Kaul et al. (2004) used female comb size, eclipse plumage, tarsus color, and tail carriage to assess 63 Red Junglefowl in six Indian zoos and pheasantries. They found no clear signs of introgression in any of these captive populations. Vijh et al. (2009) also characterized captive flocks in India. They described hackle color, female comb size, eclipse plumage, and spur size on Red Junglefowl in three pheasantries in Himachal Pradesh. The authors also measured and reported the ranges for mass, tail length, and wing length, but ultimately did not make any conclusions regarding the status of these populations. In an attempt to characterize wild populations, Fernandes et al. (2009) used hackle color and tarsus color, along with tail carriage,

female comb size, and eclipse plumage to evaluate 563 Red Junglefowl across India. They reported no clear signs of introgression in any of these populations.

Although there have been many attempts to characterize and evaluate populations of Red Junglefowl of uncertain genetic status, Brisbin and Peterson (2007) were the first to document the external morphology of putatively unintrogressed birds and hybrids with known levels of introgression. They concluded that phenotype alone, specifically external morphology, could not be used to distinguish unintrogressed Red Junglefowl from hybrids past the first or second generation of backcrossing. Unfortunately, at the time they completed their study very few Red Junglefowl were available for assessment. Therefore their samples for the putatively unintrogressed groups were small (n = 4 males, n = 1 female), and their simple univariate statistics likely had little power to detect possible differences. Thus, the extent to which external morphological characters can be used to estimate the level of introgression in Red Junglefowl remains an open question. My objective, using a large sample size for the putatively unintrogressed groups, was to determine whether multiple characters can be used, in concert, to discriminate unintrogressed Red Junglefowl from hybrids carrying domestic genes.

Methods

Descriptions of the Populations

Captive RJF – The captive Red Junglefowl used in this study were members of the junglefowl wild (JFW) population. The JFW population is a captive flock of Indian Red Junglefowl (*Gallus gallus murghi*) whose founders were collected from north-central India between 1960 and 1961 under the Foreign Game Investigation Program (Bump

1959, 1960, 1961a, 1961b, 1962; Bump and Bohl 1961). Because of their well-documented origins (see Chapter 2) and their unique morphological and behavioral characteristics, the JFW population is generally considered one of the purest strains of Red Junglefowl in captivity (Brisbin 1996, Peterson and Brisbin 1999, Brisbin et al. 2002, Brisbin and Peterson 2007). Preliminary genetic studies have provided additional evidence that this flock is different from other captive populations of this species (Brisbin et al. 2002, Andersson et al., in prep.). The JFW population has also been called the "Bump birds" or "Richardson strain," but here they will be referred to as the "Captive RJF."

Carolina Bantams – The Carolina Bantam is a strain of domestic chicken developed by Dr. I. Lehr Brisbin, Jr., beginning in the mid to late 1960s. The population was started from a flock of pure-bred bantam chickens that included Black Cochins, Golden Seabrights, Mille Fleurs, Old English Silver Duckwings, Rhode Island Reds, Silver Seabrights, and White Crested Black Polish (for photos and complete descriptions of these breeds see *The American Standard of Perfection 2010*). Brisbin released the chickens in a barnyard, along with a small number of domestic-hybrid Red Junglefowl that he had obtained from Dr. Peter Klopfer at Duke University (Brisbin 1993). After a few years of free-choice breeding, the survivors of this population (n = 15-20 birds) were moved to Milledgeville, Baldwin County, Georgia, and released on the edge of a bottomland hardwood swamp (Brisbin 1993). The flock was still present after two years of free-choice breeding at this new location, at which time three males and six females were trapped and brought back to the Savannah River Ecology Laboratory (SREL). The

birds have since been used in a number of behavioral, evolutionary, and ecological studies (Brisbin 1993).

The Carolina Bantam is a medium-sized bantam chicken with females averaging 867 g (Table 3.1) and males 1167 g (Table 3.2). The females are typically either black, or white with black spangling, and the males are black-breasted red or black and silver. Both sexes have a single comb and tarsi that range from pearl to dark gray, without feathering. The Carolina Bantams used in this study are specimens at the University of Kansas Natural History Museum and Biodiversity Research Center (KUMNH 90607, 90609 and 110209-110220).

Hybrids – The experimental hybrids used in this study were created by crossing a Captive RJF male with a small number of Carolina Bantam females as described by Brisbin and Peterson (2007). The females produced from initial crossing (F1 generation) were then backcrossed to the same male to produce the first generation backcrosses (F2 generation). At this point the male died and was replaced with another male from the JFW population. This new male was used to create the second (F3) and third (F4) generations of backcrosses. Between 6 and 15 birds per generation were raised to maturity, sacrificed, and made into study skins. They are currently stored at the University of Kansas Natural History Museum and Biodiversity Research Center (KUMNH 110166-110208). This series of specimens represents a gradient of introgression of domestic genes into a putatively unintrogressed line of Red Junglefowl. From here forward the experimental hybrids will be referred to as "Hybrids." The hybrid generation (F1–F4) will be specified when necessary.

Ancestral RJF – Indian Red Junglefowl in four museum collections were also used in this study. These collections include (1) the Florida Museum of Natural History (FLMNH), (2) the Yale/Peabody Museum of Natural History (YALE), (3) the American Museum of Natural History (AMNH) and (4) the Field Museum of Natural History (FMNH). The 44 birds used in the analyses were collected from 23 locations across India and Nepal. They were collected between 1900 and 1967, and presumably pre-date significant introgression from domestic chickens (Peterson and Brisbin 1999). Museum specimens will be referred to as "Ancestral RJF" from here forward.

Measurements

I measured 50 putatively unintrogressed Captive RJF in two private avicultural collections in Georgia and at the Savannah River Ecology Laboratory in Aiken, South Carolina. All of these birds were healthy adults (≥1.5 years of age). I also measured 44 Ancestral RJF in four museum collections. I determined the age of each specimen (juvenile or adult), so that only adults were included in the analyses. I also noted the tarsus color of each bird, as well as any abnormalities in the plumage, such as brown flecking in the breast or abdomen of the males. Birds with green, yellow, or pearl tarsi, and birds having any plumage defects were considered possible hybrids and were excluded from the analyses. I excluded one additional museum bird (FMNH 745) because it was collected from the "Changchang River, Naga Hills" an area outside of the range delineated as *G. g. murghi* (Johnsgard 1999). Finally, I measured 14 Carolina Bantams and 44 Hybrids at the University of Kansas Natural History Museum and Biodiversity Research Center. Although these birds were measured previously by Brisbin

and Peterson (2007), I re-measured them to eliminate any error introduced by having multiple researchers taking the measurements.

For females I measured bill length, comb height, comb length, tail length, tarsus length, wing length, and mass. For the males I measured bill length, spur length, spur height, spur width, tail length, tarsus length, wing length, and mass. Bill length was measured on both sexes as the straight line (chord) distance from the anterior edge of the nostril to the tip of the maxilla (Baldwin et al. 1931, Brisbin and Peterson 2007). I measured comb height as the distance from the top of the head to the tip of the tallest comb point (Baldwin et al. 1931). Because the female comb grades almost seamlessly into the cere (the fleshy covering at base of the maxilla), I measured this character as the distance from the anterior edge of the cere (a v-shape notch) to the posterior edge of the comb. I measured the tarsometatarsus (hereafter "tarsus") as the distance from the proximal end of the tarsus to lowest undivided scute before the foot (Baldwin et al. 1931, Brisbin and Peterson 2007). Spur length was measured in straight line (chord) from the base of where the spur meets the tarsus to the tip, on the outside of the spur (Baldwin et al. 1931, Brisbin and Peterson 2007) and spur height and spur width were both measured at the base of the spur. I took all of the above measurements with a dial caliper to the nearest 0.1 mm (Winker 1998).

I measured the tail length of each bird from the base of the tail to the end of the longest rectrix, not including the two long sickle feathers in males. I measured the wing length as the straight line (chord) distance on a closed, unflattened wing, from the wrist joint to the tip of the longest primary (Baldwin et al. 1931, Brisbin and Peterson 2007). A flat ruler with a vertical stop at zero and 0.5-mm markings was used to measure wing

length, and a flat ruler with a vertical stop at zero and 1-mm markings was used to measure tail length. I used a 2500-g Pesola spring scale and mesh holding bag to measure the mass of each bird in the Captive RJF to the nearest 10 g.

Unfortunately, many of the characters were either not available (e.g., mass for Ancestral RJF) or were not directly comparable between the groups because the Captive RJF were measured alive and the Ancestral RJF were measured as dried study skins. For comparison between live and dead birds, I only compared the characters that shrink minimally following study skin preparation, which included the bill, tarsus, and spur.

Blood/Tissue Collection and DNA Extractions

Once morphological measurements were taken, I collected 1 ml of blood from the large, brachial vein of each Captive RJF (n = 50). The blood was dispensed into 4-ml collection tubes containing EDTA (BD Vacutainer®) and inverted so that the blood and EDTA were thoroughly mixed together. I placed the samples in a small cooler and carried them back to the lab where they were stored (≤ -75 °C) until processing. I also sub-sampled tissues from the four generations of Hybrids, which were shipped on dry ice from the University of Kansas Natural History Museum and Biodiversity Research Center to Georgia Southern University in May 2011. Genomic DNA was extracted from the blood and tissue samples using a Classic™ Genomic DNA Isolation Kit (Lamda Biotech).

Data Analyses

I began by calculating basic descriptive statistics (mean, standard deviation, minimum, maximum, interquartile range, and coefficient of variation) for each character for each of the seven groups of interest (Tables 3.1, 3.2). Next I used an ANOVA (pooled variances), a Welch's ANOVA (un-pooled variances), and a t-test (pooled variances) to determine if characters differed between Captive RJF, Ancestral RJF, and Carolina Bantams. Next, I used linear regression to determine the relationships between character sizes and level of introgression, or percentage of domestic genes. I tested linearity (e.g. "lack of fit" test on JMP® 9.0) as described by Sokal and Rohlf (1995: 477-478) and re-plotted the data with a quadratic fit when necessary. Next, I used a linear discriminant function analyses (DFA) to determine if multiple external morphological characters can be used together to better distinguish putatively unintrogressed Red Junglefowl (Ancestral RJF) from domestic chickens (Carolina Bantams) and experimental hybrids (Hybrids). Finally, I tested the assumption that the Ancestral RJF used in this study are unintrogressed. I did this by plotting character size by year of collection. All analyses were performed on JMP® 9.0.

Results

I first compared members of the Captive RJF population to the Ancestral RJF specimens. Because I classified both the groups as putatively unintrogressed, I was surprised to find several significant differences between them. Ancestral RJF had longer tarsi (both sexes) and the males had taller and narrower spurs than the Captive RJF

(Tables 3.3, 3.4, 3.5; Figures 3.1, 3.2). I found no differences in bill length or spur length between the two groups (Tables 3.3, 3.4).

Next I asked whether the Ancestral RJF differed from the domestic Carolina Bantams. In this case both groups were measured as study skins and were therefore more directly comparable. Carolina Bantam males had longer bills and wider spurs than Ancestral RJF (Table 3.3, Figure 3.1), but tarsus length, tail length, spur height, and spur length did not differ between the two groups (Table 3.3). Sample sizes for Carolina Bantam females (all characters) and male wing length were too small to perform parallel analyses and for these I report only summary statistics (Table 3.5). However, even with small samples, female Carolina Bantams clearly had larger combs than Ancestral RJF, and longer bills than both Ancestral and Captive RJF.

Having compared the groups directly, next I quantified the relationship between the size of each character and the level of introgression, or percentage of domestic genes. I found significant relationships in 7 of the 15 characters that I measured, 6 of which increased as the level of introgression increased (Table 3.6). For females, bill length, comb height, comb length, and mass all increased in size as the percentage of domestic genes increased (Figure 3.3). For males, bill length, spur width, and mass increased with level of introgression (Figure 3.4). Male wing length was the only character that decreased in size as the level of introgression increased. Interestingly, further analyses revealed that some of the relationships between character size and level of introgression were nonlinear. I found significant deviations from linearity for female comb height ($F = 4.20$, $df = 4, 37$, $P = 0.0067$) and female bill length ($F = 5.65$, $df = 4, 36$, $P = 0.0012$), as

well as mass for both sexes (females; F= 5.63, df = 4, 42, P = 0.001 and males; F = 2.77, df = 4, 38, P = 0.041). I re-plotted these characters with a quadratic fit (Figures 3.3, 3.4).

Next, I used a discriminant function analyses to determine if the measurements of multiple characters, taken together, could be used to effectively discriminate the putatively unintrogressed Ancestral RJF from the Carolina Bantam and Hybrid groups. Because many of the birds were missing at least one measurement, and to keep the sample sizes from becoming too small, I conducted these analyses using the three characters per sex that had the strongest relationship with level of introgression during previous regression analyses.

For females, virtually all of the variation between the groups (97.84%) was explained by the first canonical axis, which was defined mainly by comb height and bill length (Table 3.7; Figure 3.5). The second canonical axis explained most of the small amount of between-group variation that remained (1.48%) and was explained primarily by bill length (Table 3.7; Figure 3.5). Individual birds were then classified into one of the six groups (Carolina Bantam, F1, F2, F3, F4 or Ancestral RJF) according to its shortest Mahalanobis distance. Five out of the 31 females (16.13%) were classified incorrectly (Table 3.9). However, these misclassifications were primarily within hybrid groups. In fact, none of the Carolina Bantam or Hybrid females were classified as Ancestral RJF and only one of the 14 Ancestral RJF (7.14%) was classified incorrectly as a Hybrid (F4).

In males, most of the variation between the groups was also explained by the first canonical axis (90.20%). This axis was defined mainly by spur width and bill length (Table 3.8; Figure 3.6). The second canonical axis explained 7.70% of the between-group variation and was defined primarily by wing length (Table 3.8; Figure 3.6). Twelve out of

the 33 males (36.36%) were misclassified, over twice the misclassification rate of females (Table 3.10). Again, none of the misclassifications placed Carolina Bantams or Hybrids into the Ancestral RJF group. This time, however, 6 of the 18 of the Ancestral RJF (33.33%) were classified as Hybrids, three as F4s, one as an F3, and two as F2s (Table 3.10).

One of the major assumptions underlying all of my previous analyses was that the Ancestral RJF that I measured were in fact unintrogressed. One of the only ways to explore this assumption with the data available was to determine whether or not characters were changing in the wild during this time. I tested this by plotting character size by year for each of the characters of interest. As expected no significant trends were observed for either sex (Table 3.11).

The blood samples that I collected from the Captive RJF and the DNA extracted from these samples and from the Hybrid tissues were sent to Dr. A. Town Peterson at the UKNHM. These samples will be sent to Uppsala University where they will be typed for the TSHR gene by Dr. Leif Andersson and colleagues.

Discussion

Recent concern for the genetic integrity of the Red Junglefowl has resulted in a number of attempts to characterize both wild and captive populations, as well as ancestral museum specimens (e.g., Peterson and Brisbin 1999, 2005; Cornwallis 2002; Kaul et al. 2004; Platt et al. 2009; Fernandes et al. 2009; Vijh et al. 2009). All of these studies, however, have used qualitative morphological traits to describe populations with uncertain origins and unknown levels of introgression. Brisbin and Peterson (2007) were

the first to quantify the external morphology of a putatively unintrogressed population of Red Junglefowl (JFW population) and hybrids with known levels of introgression. They concluded that external characters could not be used to discriminate unintrogressed birds from hybrids past the first generation of backcrossing to the unintrogressed line (i.e., the F2 generation).

My results show that there are, in fact, several external characters that can be used to detect introgression in Red Junglefowl. I found that comb height and length (females), as well as bill length (both sexes) and mass (both sexes), all increase in size with increasing levels of introgression (Table 3.6; Figures 3.3, 3.4). In other words, as populations of Red Junglefowl accumulate more and more domestic genes, both sexes tend to become heavier and have longer bills, and the females tend to have longer and taller combs. Furthermore, these results are based on analysis of a captive population of Red Junglefowl with well-established provenance as unintrogressed, hybrids with known levels of introgression, and museum specimens that presumably predate significant introgression from domestics.

Of course, the rate and direction of these changes are, presumably, related to the breed or type of domestic chicken from which introgression occurs. The trends observed in my study were found despite the fact that bantam chickens (i.e., a small breed) were used to create the Hybrids. In South and Southeast Asia, most people are probably keeping standard-sized domestic chickens (see breeds listed by Tantia et al. 2006, Pirany et al. 2007), since bantam varieties are mainly ornamental and have less productive value. Introgression from standard breeds, which are larger than the bantams used to create the hybrids used in this study, would presumably exaggerate the patterns that I observed.

Thus, because my results are probably conservative, introgression should result in populations of Red Junglefowl with an average increase in the size of these characters.

A few breeds of domestic chicken (i.e., some bantam breeds) are smaller than Red Junglefowl. However, most varieties have a greater mass (both sexes) and, from what I observed, longer bills (both sexes) and larger combs (females) than Red Junglefowl. Increased body mass in domestics is undoubtedly due to selection by humans because of the advantages size provides in sport (i.e., cockfighting) and in food production (i.e., eggs and meat). Larger combs might also have been deliberately selected for by humans. However, it has also been suggested that comb size has been subject to sexual selection in *Gallus gallus* (Zuk et al. 1990, Ligon and Zwartjes 1995), and presumably this character had the opportunity to change in captivity in the absence of opposing natural pressures. Still another possible explanation is that, at least for early breeds, larger combs were simply a consequence of an overall larger body size and that proportionally this character was no larger than those of wild Red Junglefowl. This seems to be the most likely explanation for the change observed in bill length, although it is certainly possible that lengthening of the bill was due in part to the dietary changes associated with captivity.

Regardless of the reasons for these differences, the relationships between size and level of introgression were clear for several of the characters that I measured. My data do support the conclusion that any one of these characters could not be used by itself to accurately detect introgression in Red Junglefowl (Brisbin and Peterson 2007). However, using a few of these characters together I was able to achieve significant discrimination between the putatively unintrogressed Ancestral RJF and the Carolina Bantam and Hybrid groups. In my discriminant analyses, none of the 6 Carolina Bantams or the 29

Hybrids was misclassified as Ancestral RJF, and only 7 of the 29 Ancestral RJF (24.24%) were classified incorrectly as Hybrids. Overall, females were discriminated much more effectively than males, and only one of the Ancestral RJF females (7.14%) was classified incorrectly, as a Hybrid (F4 generation). Even though this appears fairly robust, it must be kept in mind that the birds used in these classifications were in fact the same birds that were used to construct the functions used to classify them. Therefore, these classification rates must be considered a "best-case-scenario." The real misclassification rate is probably slightly higher and would be more accurately determined by measuring and classifying new specimens (i.e., additional museum specimens). However, my discriminant analyses provide promise that field workers in South and Southeast Asia could accurately classify introgressed junglefowl as such.

Another important consideration is that the results and applicability of this study hinge on the assumption that the Captive RJF used to make the Hybrids are in fact free of domestic genes, and that their external morphology has not changed appreciably from their founders collected from India. Although the origin and history of this flock is well documented (see Chapter 2), it is relatively small population that has been in captivity for more than five decades and undergone several tight bottlenecks. Therefore, the potential effects of both inbreeding and genetic drift on the morphology of these birds cannot be ignored. In fact, several differences were found between the Captive RJF and the Ancestral RJF (Tables 3.3, 3.4). However, bill length, one of the characters deemed important for detecting introgression by this study, did not differ between the two groups. Unfortunately mass and female comb size, the two other relevant characters, could not be compared.

Although female comb size has been used by a number of authors to characterize and evaluate populations of Red Junglefowl, this character has only been assessed qualitatively (e.g., Cornwallis 2002, Kaul et al. 2004, Peterson and Brisbin 2005, Platt et al. 2009, Fernandes et al. 2009, Vijh et al. 2009). Using the data provided here, researchers and wildlife managers can now compare combs of Red Junglefowl with unknown levels of introgression to putatively unintrogressed populations. Unfortunately, in order for assessment of this character to be completed for live birds, they would have to be sacrificed, made into study skins, and given sufficient time to dry. The next step then should be to document how drying changes comb size so that quick and accurate comparisons between live birds and museum skins can be made.

In addition to female comb size, I also suggest that bill length and mass be used to characterize populations of Red Junglefowl. Both of these measurements are easily taken, and can be measured quickly and accurately in the field. Further, because the bill shrinks minimally following specimen preparation, the bill length of live birds could be compared directly to museum specimens (e.g. the Ancestral RJF group). Mass could also be compared, in this case to the mass of the putatively unintrogressed Captive RJF, without the need for any corrections.

Screening populations of Red Junglefowl using external characters should be the first step in determining the extent to which introgression from domestic chickens is threatening this species. To effectively use the characters I have suggested here, researchers would need to trap a sample of birds from a population of *Gallus gallus murghi* (perhaps ≥20 of each sex). The population could be described as "potentially unintrogressed" if a large percentage of the males go through an annual eclipse plumage,

all the birds have dark slate to black tarsi, and average comb height (females), comb length (females), bill length (both sexes) and mass (both sexes) fall within the 95% confidence intervals of the putatively unintrogressed groups (Table 3.12). If a large portion of the males do not eclipse, there are birds with pearl, yellow, or green tarsi, or the mean of one or more of these characters falls outside of the 95% CI, this could indicate that introgression from domestics has occurred, and the population should be described as "likely introgressed." Linear combinations of these measurements (i.e. Canonical1 score, Table 3.12) could also be used to more accurately characterize these populations.

Ultimately, the most accurate way to detect introgression in Red Junglefowl would be via genetic analyses. At this time, however, there are no markers that can be used to accurately discriminate unintrogressed birds or populations from those carrying domestic genes. A single-nucleotide polymorphism has recently been identified that might be useful for this purpose (Rubin et al. 2010), and continuation of this work is underway. Also in progress is whole genome sequencing of the JFW population, which will hopefully lead to the discovery of additional markers that can be used to detect introgression (L. Andersson et al. in prep). In the meantime, however, simple morphological characters could provide a quick and economical way for wildlife researchers and managers to screen populations of Red Junglefowl, and determine which to protect and subject to genetic analyses when they become applicable.

Table 3.1. Descriptive statistics of females for the populations used in the study. “Bantams” refer to the domestic Carolina Bantam chickens, “F1 (50%)” through “F4 (6.25%)” refer to the Hybrid generations, “Ancestral” refers to the Red Junglefowl measured in museum collections, and “Captive” refers the Captive RJF (JFW population). The statistics reported are; mean, standard deviation (SD), minimum value (Min), median (Med), maximum value (Max), interquartile range (IR), coefficient of variation (CV), and sample size (N) respectively.

Character	**Group**	**Mean**	**SD**	**Min**	**Med**	**Max**	**IR**	**CV**	**N**
Bill Length (mm)	Bantam	14.88	0.98	13.90	14.75	16.10	1.88	6.60	4
	F1 (50%)	15.03	0.50	14.30	15.00	15.60	0.88	3.35	8
	F2 (25%)	14.20	0.95	13.20	14.30	15.10	1.90	6.72	3
	F3 (12.5%)	13.98	0.67	13.10	14.25	14.60	1.35	4.77	6
	F4 (6.25%)	13.10	0.35	12.70	13.30	13.30	0.60	2.64	3
	Captive	12.49	0.57	11.80	12.40	13.90	0.80	4.55	30
	Ancestral	12.73	0.70	11.40	12.95	13.90	1.05	5.47	18
Comb Height (mm)	Bantam	12.83	1.42	10.80	13.20	14.10	2.53	11.05	4
	F1 (50%)	8.99	1.78	6.20	9.20	10.90	3.10	19.82	7
	F2 (25%)	4.90	0.31	4.40	4.50	6.00	1.20	14.29	5
	F3 (12.5%)	5.78	1.73	3.90	5.50	8.30	3.43	29.88	6
	F4 (6.25%)	5.37	0.65	4.70	5.40	6.00	1.30	12.12	3
	Captive	4.54	1.01	1.70	4.40	6.90	1.23	22.32	30
	Ancestral	2.99	0.83	1.80	2.90	4.20	1.58	27.69	18
Comb Length (mm)	Bantam	29.27	3.45	26.20	28.60	33.00	6.80	11.78	3
	F1 (50%)	23.84	1.07	21.90	23.90	25.20	1.40	4.48	7
	F2 (25%)	21.83	2.32	19.20	22.70	23.60	4.40	10.65	3
	F3 (12.5%)	20.37	2.20	19.00	19.20	22.90	3.90	10.78	3
	F4 (6.25%)	17.63	0.74	16.80	17.90	18.20	1.40	4.18	3
	Captive	20.46	2.68	16.30	20.80	27.70	4.90	13.12	29
	Ancestral	16.38	2.89	12.30	16.20	21.90	4.43	17.65	14

Table 3.1 Continued…

Character	Group	Mean	SD	Min	Med	Max	IR	CV	N
Mass (g)	Bantam	866.75	121.07	724.0	872.0	999.0	233.75	13.97	4
	F1 (50%)	880.71	99.48	782.0	875.0	1018.5	217.50	11.30	7
	F2 (25%)	814.00	216.37	661.0	814.0	967.0	306.00	26.58	2
	F3 (12.5%)	632.56	70.96	550.0	602.0	711.0	133.50	11.22	5
	F4 (6.25%)	575.50	-	575.5	575.5	575.5	-	-	1
	Captive	564.48	70.39	440.0	540.0	780.0	85.00	12.47	29
Tail Length (mm)	Bantam	123.25	6.18	115.00	125.00	128.00	11.25	5.02	4
	F1 (50%)	131.63	3.89	125.00	132.00	138.00	5.00	2.95	8
	F2 (25%)	133.80	6.98	124.00	135.00	143.00	12.00	5.22	5
	F3 (12.5%)	140.50	6.22	130.00	142.50	146.00	10.00	4.43	6
	F4 (6.25%)	136.00	1.73	135.00	135.00	138.00	3.00	1.27	3
	Captive	131.67	5.38	122.00	132.00	141.00	7.50	4.08	29
	Ancestral	128.94	10.94	111.00	125.50	147.00	18.25	8.48	18
Wing Length (mm)	Bantam	192.75	1.06	192.00	192.75	193.50	1.50	0.55	2
	F1 (50%)	197.83	4.16	194.50	196.50	202.50	8.00	2.10	3
	F2 (25%)	191.50	4.08	185.50	193.00	194.50	7.00	2.13	4
	F3 (12.5%)	197.33	4.22	189.50	198.75	201.00	5.87	2.14	6
	F4 (6.25%)	190.83	3.55	187.00	191.50	194.00	7.00	1.86	3
	Captive	187.80	4.53	176.50	188.00	193.00	7.25	2.41	29
	Ancestral	195.58	6.28	183.50	196.00	205.50	7.50	3.21	17

Table 3.2. Descriptive statistics of males for the populations used in the study. “Bantams” refer to the domestic Carolina Bantam chickens, “F1 (50%)” through “F4 (6.25%)” refer to the Hybrid generations, “Ancestral” refers to the Red Junglefowl measured in museum collections, and “Captive” refers the Captive RJF (JFW population). The statistics reported are; mean, standard deviation (SD), minimum value (Min), median (Med), maximum value (Max), interquartile range (IR), coefficient of variation (CV), and sample size (N) respectively.

Character	Group	Mean	SD	Min	Med	Max	IR	CV	N
Bill Length (mm)	Bantam	17.53	1.38	15.20	17.65	20.00	2.00	7.89	10
	F1 (50%)	16.39	0.50	15.60	16.50	16.90	0.90	3.06	7
	F2 (25%)	14.85	0.60	14.20	14.80	15.60	1.15	4.02	4
	F3 (12.5%)	15.38	0.79	14.40	15.45	16.20	1.53	5.16	4
	F4 (6.25%)	14.63	0.68	14.10	14.40	15.40	1.30	4.65	3
	Captive	14.02	0.57	13.30	14.00	15.00	0.78	4.07	20
	Ancestral	14.39	0.62	13.30	14.35	15.80	0.88	4.31	20
Mass (g)	Bantam	1167	246.49	730	1180	1505	392	21.15	9
	F1 (50%)	1203	90.72	1190	1188	1375	143	7.54	8
	F2 (25%)	849	171.40	615	891	1000	318	20.19	4
	F3 (12.5%)	950	79.45	860	979	1011	151	8.36	3
	F4 (6.25%)	850	-	850	850	840	-	-	1
	Captive	850	136.42	550	890	1040	210	16.05	19
Spur Height (mm)	Bantam	6.96	0.48	6.40	6.85	7.90	0.78	6.85	10
	F1 (50%)	6.78	0.23	6.50	6.75	7.10	0.38	3.32	8
	F2 (25%)	6.23	0.52	5.70	6.05	7.10	0.88	8.35	6
	F3 (12.5%)	6.68	0.17	6.50	6.65	6.90	0.33	2.56	4
	F4 (6.25%)	5.77	0.15	5.60	5.80	5.90	0.30	2.65	3
	Captive	6.30	0.72	5.30	6.20	8.30	0.85	11.48	20
	Ancestral	6.77	0.50	5.80	6.80	7.60	0.65	7.44	24

Table 3.2 Continued…

Character	Group	Mean	SD	Min	Med	Max	IR	CV	N
Spur Length (mm)	Bantam	25.66	11.99	13.00	24.15	41.20	25.70	46.74	10
	F1 (50%)	24.23	8.23	11.50	24.75	35.40	13.85	33.97	8
	F2 (25%)	20.20	14.39	5.30	16.65	46.00	21.58	71.25	6
	F3 (12.5%)	37.50	3.53	33.40	37.65	41.30	6.80	9.42	4
	F4 (6.25%)	23.10	1.01	22.20	22.90	24.20	2.00	4.39	3
	Captive	22.43	5.28	10.20	23.20	31.40	4.90	23.53	20
	Ancestral	25.35	5.51	13.20	25.55	36.70	6.88	21.75	24
Spur Width (mm)	Bantam	6.11	0.43	5.70	5.95	6.90	0.65	7.05	10
	F1 (50%)	5.74	0.34	5.30	5.80	6.20	0.70	5.85	7
	F2 (25%)	5.20	0.54	4.30	5.40	5.70	0.95	10.46	6
	F3 (12.5%)	5.13	0.39	4.60	5.20	5.50	0.73	7.54	4
	F4 (6.25%)	4.57	0.06	4.50	4.60	4.60	0.10	1.26	3
	Captive	5.43	0.56	4.80	5.20	6.60	0.78	10.30	20
	Ancestral	4.60	0.49	3.40	4.55	5.40	0.60	10.76	22
Tail Length (mm)	Bantam	167.20	12.75	149.00	163.50	185.00	25.00	7.63	10
	F1 (50%)	177.29	14.73	149.00	180.00	192.00	20.00	8.31	7
	F2 (25%)	162.60	17.30	148.00	159.00	191.00	29.00	10.64	5
	F3 (12.5%)	165.40	32.06	110.00	172.00	188.00	47.50	19.38	5
	F4 (6.25%)	165.33	2.08	163.00	166.00	167.00	4.00	1.26	3
	Captive	173.90	11.60	156.00	172.50	197.00	19.25	6.67	20
	Ancestral	166.48	8.00	155.00	165.00	184.00	10.00	4.80	21

Table 3.2 Continued…

Character	Group	Mean	SD	Min	Med	Max	IR	CV	N
Tarsus Length (mm)	Bantam	75.42	4.23	70.20	74.45	82.30	7.95	5.61	10
	F1 (50%)	81.21	2.44	76.50	81.60	84.30	3.13	3.00	8
	F2 (25%)	75.20	2.51	72.20	74.95	78.60	4.98	3.33	6
	F3 (12.5%)	73.08	1.58	71.10	73.10	75.00	3.05	2.17	5
	F4 (6.25%)	73.57	0.35	73.20	73.60	73.90	0.70	0.48	3
	Captive	71.92	3.26	66.30	72.90	77.50	4.90	4.53	19
	Ancestral	77.01	3.81	71.00	77.35	84.40	5.80	4.94	24

Table 3.3. Comparison of morphology of male Carolina Bantams (skins), Captive RJF (live), and Ancestral RJF (skins) for characters that do not shrink or shrink minimally following study skin preparation. Bill length, spur width, tarsus length, and spur height were compared using an ANOVA (equal variances) and spur length was compared with a Welch's ANOVA (unequal variances). Pair-wise comparisons were done with a Tukey-Kramer HSD (Figure 3.1).

Character	Carolina Bantam	N	Captive RJF	n	Ancestral RJF	n	F	df	P
Bill length	17.53 ± 1.38	10	14.02 ± 0.57	20	14.39 ± 0.62	20	68.97	2, 47	<0.0001
Spur width	6.11 ± 0.43	10	5.43 ± 0.56	20	4.60 ± 0.49	22	33.08	2, 49	<0.0001
Tarsus length	75.42 ± 4.23	10	71.92 ± 3.26	19	77.01 ± 3.81	24	10.16	2, 50	0.0002
Spur height	6.96 ± 0.48	10	6.30 ± 0.72	20	6.77 ± 0.50	24	5.42	2, 51	0.0074
Spur length	25.66 ± 11.99	10	22.43 ± 5.28	20	25.35 ± 5.51	24	1.65	2, 20.84	0.2165

Table 3.4. Comparison of morphology of female Captive RJF (live) and Ancestral RJF (skins) for characters that do not shrink or shrink minimally following study skin preparation. Ancestral RJF females had longer tarsi compared to captive birds. Bill length did not differ between the groups.

Character	Captive RJF	n	Ancestral RJF	n	T	df	P
Tarsus length	61.07 ± 1.47	29	64.48 ± 3.07	17	4.30	20.37	0.0003*
Bill length	12.49 ± 0.57	30	12.73 ± 0.70	18	1.27	30.41	0.2138

Table 3.5. A comparison of female Carolina Bantams (skins), Ancestral RJF (skins), and Captive RJF (live). The sample sizes for the bantam group were too small for effective analysis, so I report only mean ± standard deviation.

Character	Carolina Bantam	n	Ancestral RJF	n	Captive RJF	n
Bill length	14.88 ± 0.98	4	12.73 ± 0.70	18	12.49 ± 0.57	30
Tarsus length	61.20 ± 2.05	4	64.48 ± 3.07	17	61.07 ± 1.47	29
Tail length	123.25 ± 6.18	4	128.94 ± 10.94	18	-	
Comb height	12.83 ± 1.42	4	2.99 ± 0.83	18	-	
Comb length	29.27 ± 3.45	3	16.38 ± 2.89	14	-	
Wing length	192.75 ± 1.06	2	195.58 ± 6.28	17	-	

Table 3.6. Bill length and mass increased in both sexes as the percentage of domestic genes increased. For females comb height and comb length increased in size with increasing levels of introgression, and for males spur width increased and wing length decreased with introgression. Female bill length, comb height, and mass deviated significantly from linearity and were re-plotted with a quadratic fit, as was male mass.

	Male			Female		
Character	**P**	**R^2**	**b**	**P**	**R^2**	**b**
Bill length (L)	<0.0001*	0.71	0.03			
Bill length (Q)				<0.0001*	0.68	
Mass (Q)	<0.0001*	0.46		<0.0001*	0.72	
Comb height (Q)				<0.0001*	0.84	
Comb length (L)				<0.0001*	0.75	0.13
Spur width (L)	<0.0001*	0.63	0.02			
Wing length (L)	0.0449*	0.09	-0.09	0.7194	0.004	
Spur height (L)	0.1131	0.05				
Tail length (L)	0.5656	<0.01		0.2323	0.03	
Spur length (L)	0.8446	<0.01				
Tarsus length (L)	0.8693	<0.01		0.9528	<0.01	

L = linear regression, Q = quadratic regression

Table 3.7. The percentage of between-group variation described by each canonical axis and the standardized scoring coefficient for each variable (females).

	Canonical Axis		
Character	**I**	**II**	**III**
Comb length	0.21	-0.40	0.96
Comb height	0.87	-0.22	-0.56
Bill length	0.57	0.91	-0.09
Percent	**97.84**	**1.48**	**0.68**

Sample sizes: Carolina Bantams = 3, F1 Hybrids = 6, F2 Hybrids = 2, F3 Hybrids = 3, F4 Hybrids = 3, Ancestral RJF = 14

Table 3.8. The percentage of between-group variation described by each canonical axis and the standardized scoring coefficient of each variable (males).

	Canonical Axis		
Character	**I**	**II**	**III**
Bill length	0.72	0.37	-0.64
Spur width	0.52	-.22	0.87
Wing length	-0.22	0.95	0.25
Percent	**90.20**	**7.70**	**2.11**

Sample sizes: Carolina Bantams = 3, F1 Hybrids = 4, F2 Hybrids = 4, F3 Hybrids = 4, F4 Hybrids = 3, Ancestral RJF = 15

Table 3.9. Classification accuracy of females based on a discriminant function using bill length, comb height, and comb length. None of the Carolina Bantams or Hybrids was misclassified as Ancestral RJF and only one Ancestral RJF was misclassified, as a F4 Hybrid. (N = 31 birds). Asterisks indicate misclassifications.

	Predicted					
Actual	Carolina Bantams	F1 Hybrids	F2 Hybrids	F3 Hybrids	F4 Hybrids	Ancestral RJF
Carolina Bantams	3	0	0	0	0	0
F1 Hybrids	0	6	0	0	0	0
F2 Hybrids	0	0	0	1*	1*	0
F3 Hybrids	0	0	1*	1	1*	0
F4 Hybrids	0	0	0	0	3	0
Ancestral RJF	0	0	0	0	1*	13

Table 3.10. Classification accuracy of males based on a discriminant function using bill length, spur width, and wing length. None of the Carolina Bantams or Hybrids was misclassified as Ancestral RJF, however six Ancestral RJF were misclassified, three as F4 Hybrids, one as an F3 Hybrid, and two as F2 Hybrids. (N = 33 birds). Asterisks indicate misclassifications.

	Predicted					
Actual	Carolina Bantams	F1 Hybrids	F2 Hybrids	F3 Hybrids	F4 Hybrids	Ancestral RJF
Carolina Bantams	2	1*	0	0	0	0
F1 Hybrids	0	3	0	1*	0	0
F2 Hybrids	0	1*	1	1*	1*	0
F3 Hybrids	0	0	1*	3	0	0
F4 Hybrids	0	0	0	0	3	0
Ancestral RJF	0	0	2*	1*	3*	9

Table 3.11. Morphological characters did not vary by year for Ancestral RJF.

Sex	Character	Equation	R^2	P
Female	Tarsus length	y = 178.570 – 0.059x	0.089	0.263
	Comb length	y = 115.934 – 0.051x	0.042	0.485
	Comb height	y = -3.151 + 0.003x	0.003	0.823
	Wing length	y = 229.781 – 0.018x	0.002	0.877
	Tail length	y = 186.707 – 0.030x	0.002	0.878
	Bill length	y = 13.745 – 0.001x	<0.001	0.967
Male	Tarsus length	y = -128.928 + 0.106x	0.087	0.162
	Bill length	y = 40.207 – 0.013x	0.061	0.295
	Tail length	y = 473.682 – 0.158x	0.049	0.337
	Wing length	y = 506.245 – 0.143x	0.033	0.422
	Spur width	y = 14.631 – 0.005x	0.014	0.606
	Spur height	y = -0.022 + 0.003x	0.005	0.733
	Spur length	y = 64.676 – 0.020x	0.002	0.852

Table 3.12. A proposed suite of characters for evaluating populations of Indian Red Junglefowl (*Gallus gallus murghi*). For quantitative characters, the 95% confidence interval and maximum value for putatively unintrogressed populations are provided.

Character	**Male**	**Female**
Eclipse plumage [live]	Present, July-September	Not detectable
Tarsus color [live]	Slate blue to black	Slate blue to black
Comb length [skins]	-	14.71-18.05 mm (21.9 mm)
Comb length [live]	-	19.44-21.48 mm (27.7 mm)
Comb height [skins]	-	2.58-3.41 mm (4.2 mm)
Comb height [live]	-	4.16-4.91 mm (6.9 mm)
Bill length [skins]	14.09-14.68 mm (15.8 mm)	12.39-13.08 mm (13.9 mm)
Bill length [live]	13.75-14.28 mm (15.0 mm)	12.27-12.70 mm (13.9 mm)
Bill length [total]	14.00-14.40 mm (15.8 mm)	12.4-12.76 mm (13.9 mm)
Mass [live]	784.25-915.75 g (1040 g)	537.71-591.26 g (780 g)
Canonical1 score [skins]*	11.96-13.05 (14.32)	12.79-13.81 (14.51)

*Canonical1 (males) = 0.9897(bill length) + 1.1139(spur width) – 0.0298(wing length)
*Canonical1 (females) = 0.6989(comb height) + 0.7863(bill length) + 0.0827(comb length)

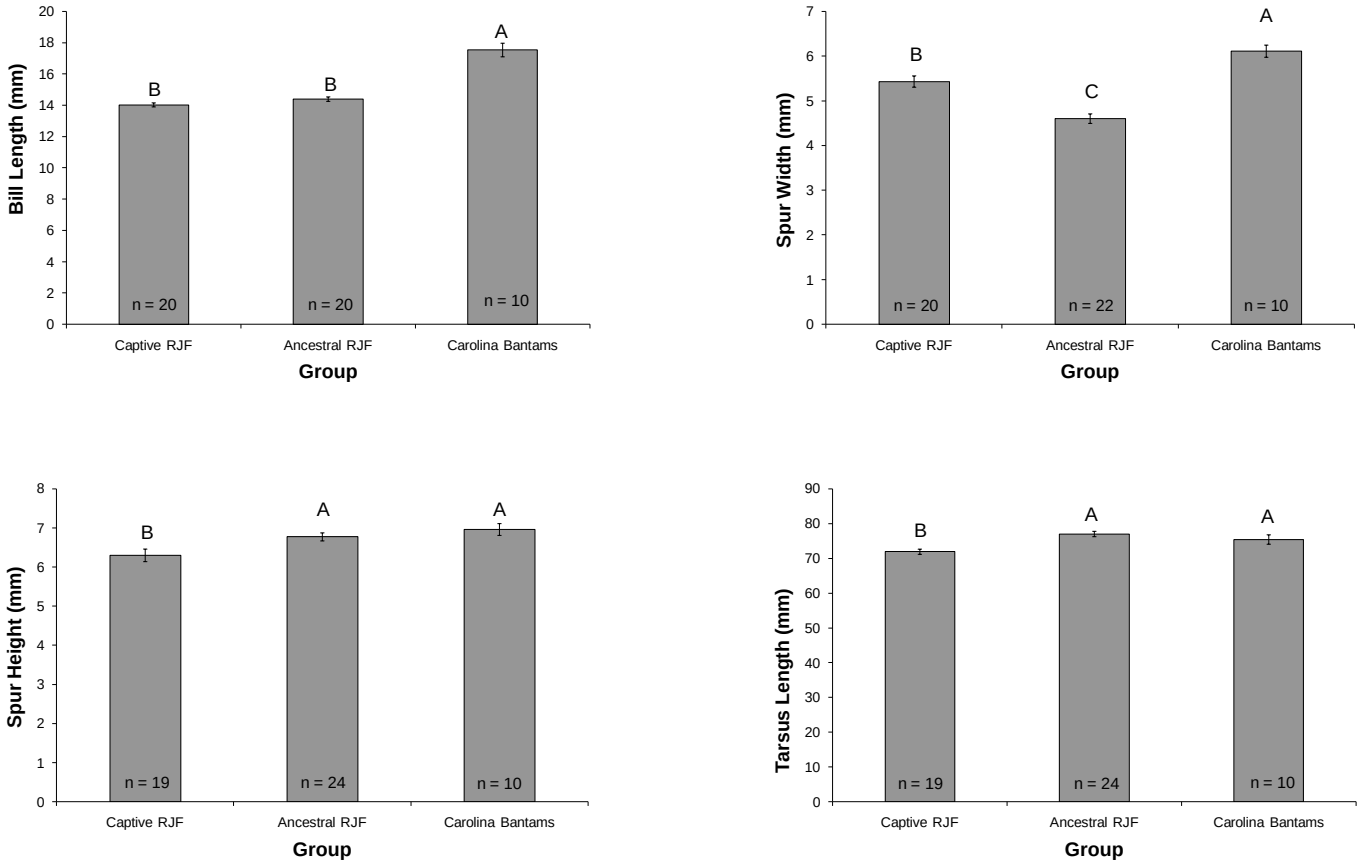

Figure 3.1. The four characters for which significant differences were found among male Captive RJF (live), Ancestral RJF (skins) and Carolina Bantams (skins). The means ± 1 SE are shown. Letters indicate significant differences ($P<0.05$) between the groups (Tukey-Kramer HSD).

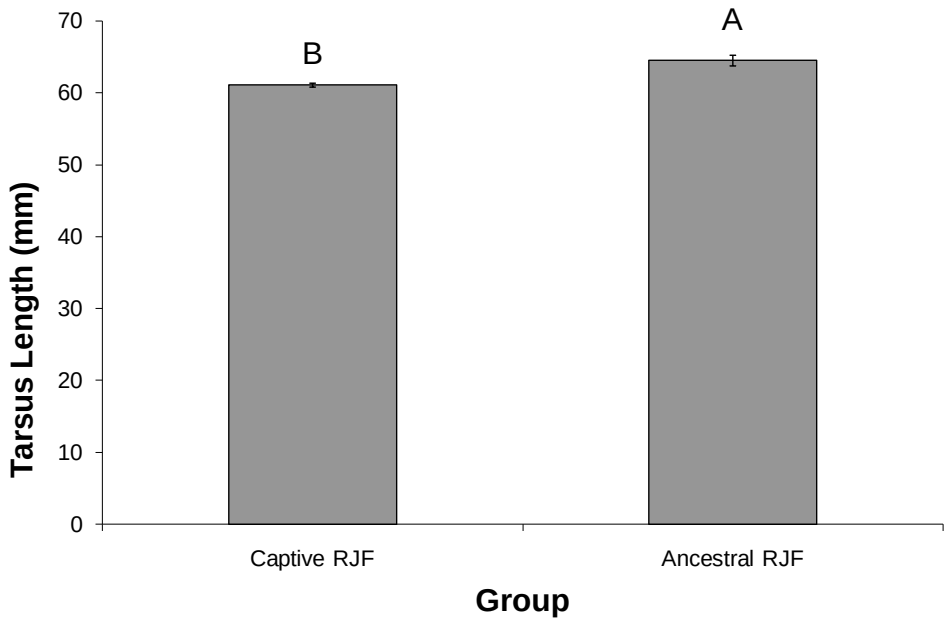

Figure 3.2. Ancestral RJF females (skins) had longer tarsi than Captive RJF females (skins) (unequal variance, t-tests, $P < 0.05$). The means ± 1 SE are shown. Only tarsus length and bill length were directly comparable between the groups and the latter was not significantly different. Due to their small sample size (n = 4) the female Carolina Bantams (skins) could not be compared in this analysis.

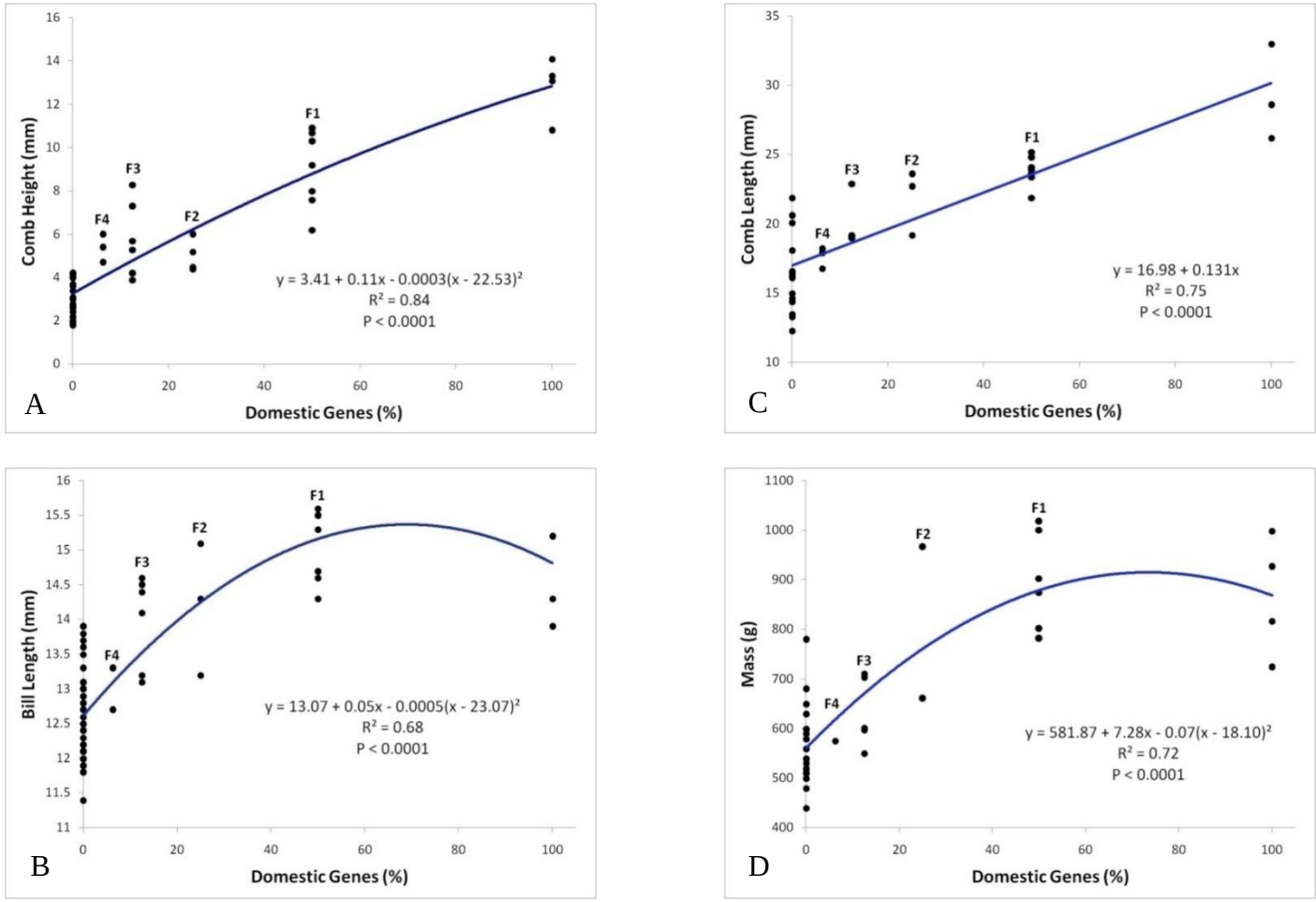

Figure 3.3. Female comb height, comb length, bill length, and mass all increased in size as the percentage of domestic genes increased. In graphs A and C the Ancestral RJF (skins) were used for the putatively unintrogressed group, whereas in graph B the Ancestral RJF and Captive RJF (live) groups were pooled, and in graph D Captive RJF was used as the unintrogressed group.

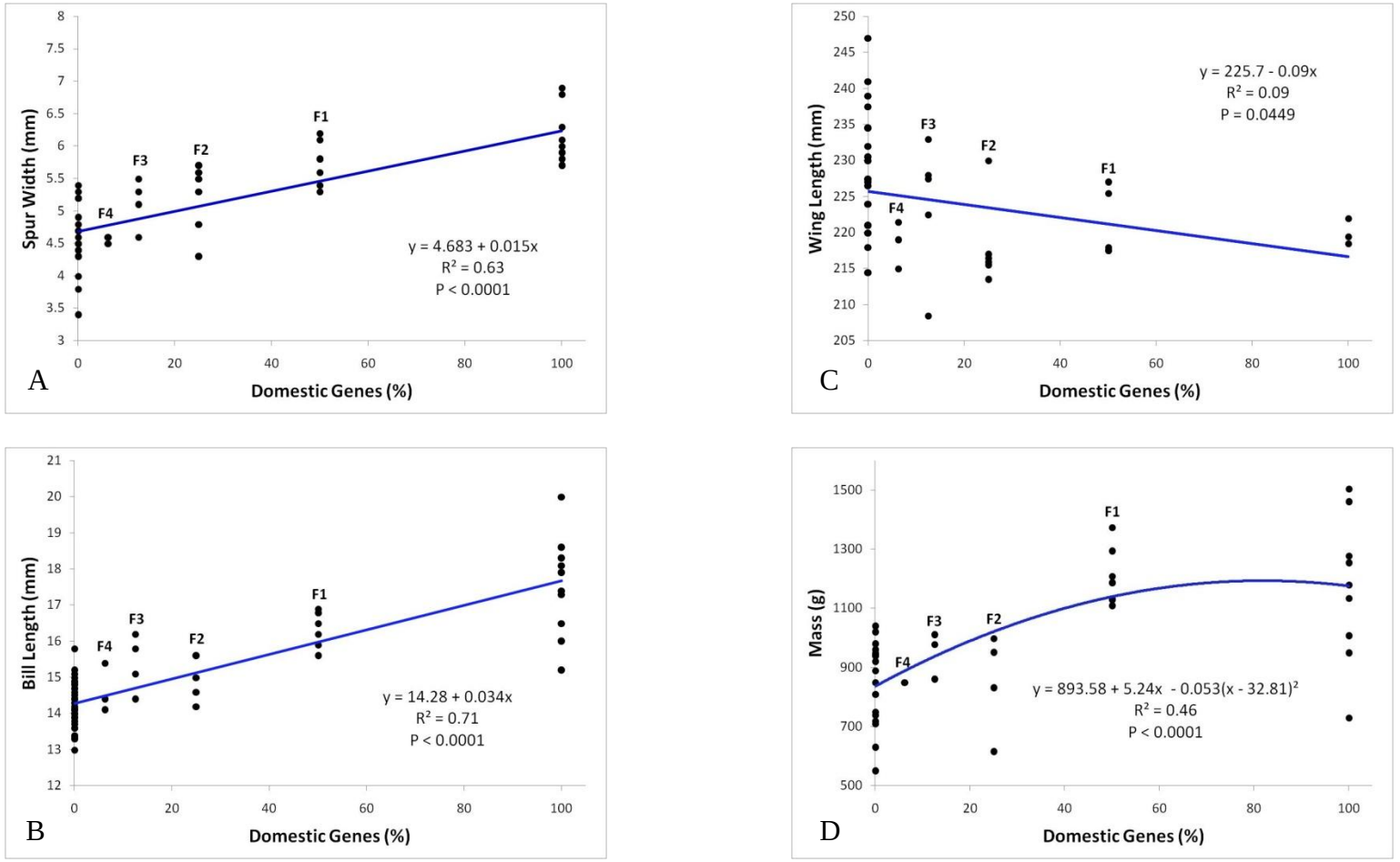

Figure 3.4. Male spur width, bill length, and mass all increased in size as the percentage of domestic genes increased. Only wing length increased with level of introgression. In graphs A and C Ancestral RJF (skins) were used for the putatively unintrogressed group, whereas in graph B the Ancestral RJF and Captive RJF (live) were pooled and in graph D the Captive RJF was used as the unintrogressed group.

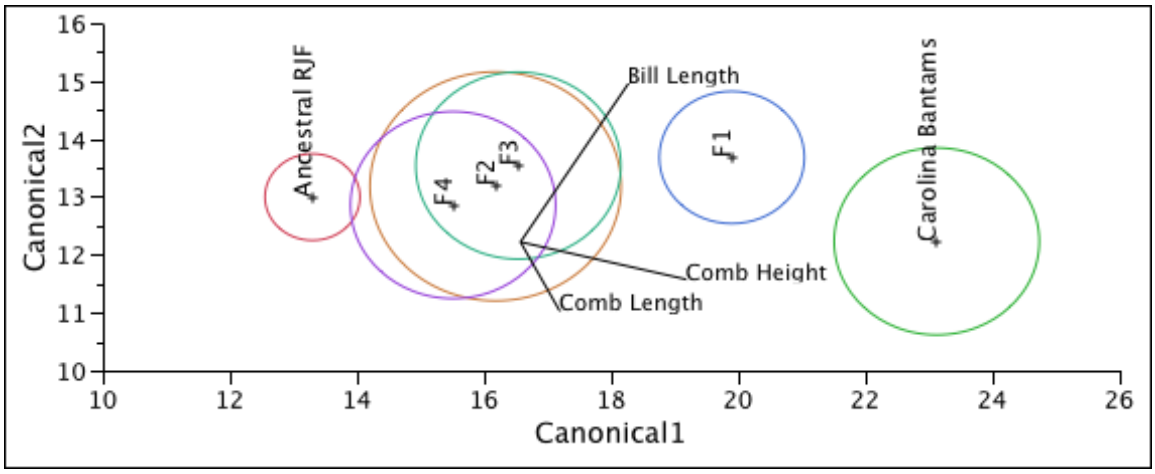

Figure 3.5. A canonical plot for a discriminant function analysis using the measurements of three female characters; bill length, comb height, and comb length. The centroid and 95% confidence ellipse for each of the six groups are indicated. There was significant discrimination between the groups (Wilks' Lambda; $F_{aprox.}$= 8.26, df = 15, P < 0.0001*), and the first canonical axis described virtually all of the between-group variation (97.84%).

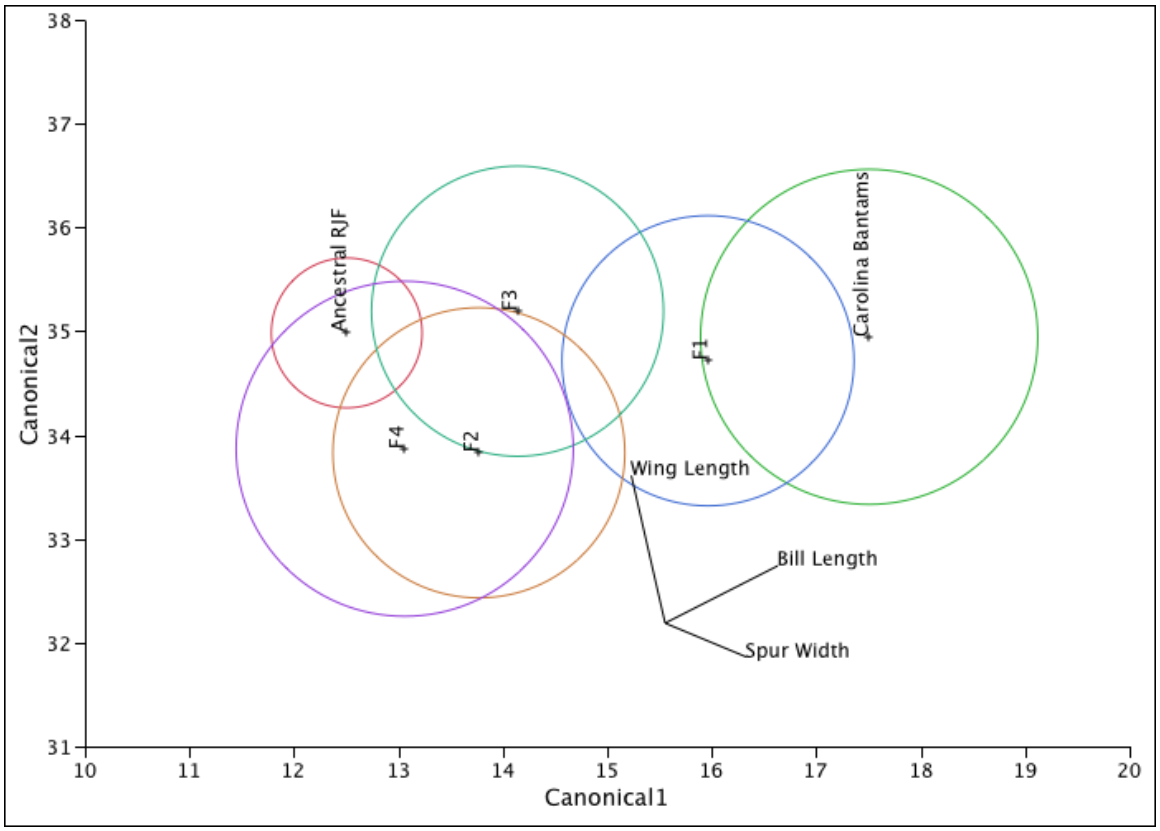

Figure 3.6. A canonical plot for a discriminant function analysis using the measurements of three male characters; bill length, wing length, and spur width. The centroid and 95% confidence ellipse for each of the six groups are indicated. There was significant discrimination between the groups (Wilks' Lambda; $F_{aprox.}$= 4.12, df = 15, P < 0.0001*). The majority of the between-group variation (90.20%) was described by the first canonical axis, and most of the remaining variation (7.70%) was described by the second axis.

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