ASPECTS OF GENETIC AND MORPHOLOGICAL VARIATION

IN SELECTED NEW WORLD LAND BIRDS

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ASPECTS OF GENETIC AND MORPHOLOGICAL VARIATION IN SELECTED NEW WORLD LAND BIRDS

A

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Abstract

The objective of this thesis is to examine variation in certain New World land birds, focusing on morphological differences at the intraspecific level and genetic differences at the intra- and interspecific levels. First, I investigate sexual dimorphism in the Wilson's Warbler (*Wilsonia pusilla*), a Nearctic-Neotropic migrant parulid. Using museum specimens, I quantify the degree of dimorphism and devise a method to distinguish the sexes using morphological measurements. Second, I outline a new method of approximating Weir and Cockerham's θ (1984, 1993), an unbiased estimator of genetic population structure. The method uses commonly published parameters and obviates the need to recode existing allozyme data sets to calculate θ. The estimation algorithm is shown to be useful for both model populations and real-world avian populations.

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Chapter 1: Introduction

To understand evolution in birds (or in any class of organisms, for that matter), one must first understand its cornerstone, variation. Without variation within natural populations, there is no basis for evolutionary change, whether through natural selection, genetic drift, or any other mechanism. Biological variation is a coin with two sides: genetic variation and morphological variation. In this thesis, I approach variation in birds through both aspects, using morphological differences at the intraspecific level and genetic differences at the intra- and interspecific levels.

First, I examine sexual dimorphism in the Wilson's Warbler (*Wilsonia pusilla*), a Nearctic-Neotropic migrant parulid. The evolution of sexual dimorphism in birds has been attributed to the influence of several selective factors, including sexual selection, survival selection, and fertility selection (Owens and Hartley 1998). While others have suggested reasons for sexual dimorphism in the Wilson's Warbler (Rappole 1986), I use museum specimens to quantify the degree of dimorphism in this bird and devise a method to distinguish the sexes using morphological measurements. Ultimately the purpose is to expose problems in the current methods of sexing this species and help field workers make their data collection more productive and accurate.

This chapter adds to a growing body of work on the extent of avian sexual

dimorphism. In particular, it adds to a series of studies on dimorphism in birds from Veracruz, Mexico initiated by Kevin Winker and others (Winker et al. 1994, Winker et al. 1996). As co-author on this manuscript, Winker suggested the extension of the series with the species Wilson's Warbler, solicited the loan of specimens from the Bell Museum of Natural History, and provided guidance in the selection of appropriate analyses.

The third chapter of this thesis provides a new method of quantifying genetic variation in birds. Over the past thirty years, many allozyme studies of genetic variation within avian species have been done that have suffered the bias of small population sample sizes. A more modern method for the analysis of population structure was devised by Weir and Cockerham (1984), which eliminates this bias. Unfortunately, the difficulties of reconstructing complete data sets preclude the reanalysis of most of the older allozyme studies. In this chapter, I explore a method whereby Weir and Cockerham's unbiased estimator of population structure may be approximated from the minimal information that is usually available in the literature, without the need to reconstruct the original data matrices. This estimation algorithm is examined for its utility, using empirical data from Neotropical land birds. By removing the confounding effects of sample size bias, a wealth of data in the literature can be revived for different uses, such as the comparison of levels of population structure across species and studies.

Kevin Winker, the co-author on this manuscript, suggested the potential of this approach and helped secure original data sets with which to test the estimation equation. As with the other chapter, the writing and analyses are my own.

Chapter 2: Sexual Dimorphism in Wilson's Warblers[1]

Abstract.— Using museum specimens from Mexico, Canada, and the western United States, I examined sexual dimorphism in Wilson's Warbler (Wilsonia pusilla), a Nearctic-Neotropic migrant (Passeriformes: Parulidae). On average, males had longer tails, wing chords, and eighth and ninth primaries than females. Three methods for quantifying cap plumage showed that differences in cap size and pattern alone could not definitively separate the sexes. Discriminant functions are presented for sexing individuals using cap category, cap length, wing chord, tail length, and ninth primary length. More specific functions are provided for samples from Alaska and eastern Mexico. For each group, equations are included for assigning individual probabilities of belonging to either sex.

[1]Prepared for submission to Journal of Field Ornithology, by Jacqueline J. Weicker and Kevin Winker

INTRODUCTION

Wilson's Warbler (Parulidae: Wilsonia pusilla) is a Nearctic-Neotropic migrant passerine with a broad breeding range in northern North America and a wintering range from southern Texas and Baja California south to Costa Rica and western Panama (AOU 1998). This small warbler is recognizable by its olive-green upperparts and yellow underparts and forehead. Males generally have a distinctive black cap, but females can also have dark crowns, although their caps, when present, tend to be shorter or more patchy than those of males. Ridgway (1902) concluded that females of the three subspecies may have duller plumage, a more restricted black crown-patch, and smaller average tail and wing measurements than males, but the sexes are often not distinguishable. Dwight (1900) also found little definitive difference between female and male plumages.

The current protocol for determining the sex of captured Wilson's Warblers prepared by the U.S. Fish and Wildlife Service and the Canadian Wildlife Service (1980) advocates separation by size differences in cap length and wing chord, following Stewart (1972). Pyle (1997) offered a combination of cap lengths and patterns to discern males from females. However, the examination of plumage differences cannot reliably sex all breeding season adults (Chase et al. 1997).

This study was undertaken to explore sexual size dimorphism in Wilson's Warblers, to critically examine plumage dimorphism (dichromatism), and to create discriminant functions to help distinguish the sexes in the field. This approach has been

used successfully in other species where plumage or external soft parts have been inadequate for distinguishing males from females (e.g., Anderson 1975, Winker et al. 1994, 1996).

METHODS

My analyses are based on 255 W. pusilla museum specimens collected during breeding, wintering, and migration. Specimens were collected from Mexico, the United States, and Canada from 1952 to 1998. Over half (180) were collected in Veracruz, Mexico in the mid 1970's (for site description see Rappole and Warner 1980). Two were collected in Morelos, Mexico, and four in British Columbia, Canada. Of the 69 specimens from the United States, 47 are from Alaska, 11 from Texas, 8 from Minnesota, two from Wisconsin, and one from Arizona.

I only used specimens for which sex had been determined by gonadal inspection (n=255). Specimens of questionable or unknown sex were excluded, regardless of external morphological characteristics. Of the specimens I examined, 9 from the University of Alaska Museum and 80 from the Bell Museum of Natural History (25.9% of the specimens in those two collections) did not have gonadal information on their labels and were not included in any analyses. Degree of skull ossification was determined when skulls were available or from the specimen labels when recorded. W. pusilla generally reach complete ossification between September 15 and November of their first year (Pyle 1997). However, this study included specimens with incomplete ossification

that had been collected through November and as late as 23 December.

Measurements taken were the lengths of the wing chord (unflattened wing), tail, tarsometatarsus (tarsus), and bill (from tip to anterior edge of nostril), bill width and height (at the anterior edge of nostrils), and the length of the eighth and ninth outermost primaries (Baldwin et al. 1931; Jenni and Winkler 1989). To avoid bias, all measurements were completed before noting and recording the sex of each individual. Specimens with heavy feather wear or damage that would preclude accurate measurement were excluded. Measurements were made to the nearest 0.1 mm with vernier calipers, except for primaries P8 and P9, which were measured to the nearest 0.5 mm with a highly flexible insert (Jenni and Winkler 1989). To assure consistency, all measurements were performed by a single observer (JJW). Body mass was determined from specimen labels when possible.

Caps were evaluated in three ways. First, the extent of black feathers from the front to the back of the head was measured to the nearest 0.1 mm with vernier calipers. This measurement, called "cap length," is the distance from the front of the anterior-most dark feather to the back of the posterior-most dark feather, regardless of the dark feathers' positions on the crown and the amount of olive feathers interspersed. Second, caps were assigned a "cap category." This value separated caps into one of four classes, ranging from solid olive-green to solid black (Figure 1). Third, caps were evaluated for their level of demarcation or distinctiveness at the trailing, or posterior, edge. They were divided into three classes: those showing no black, those with a gradual transition from black to

green, and those with a strong, even demarcation between the posterior end of the black feathers and the beginning of the green feathers. Ranking and measurements of caps were done independently of other measurements and blindly with regard to the sex of the bird.

Means of male and female measurements were compared using t-tests (SPSS for Windows 1995). Specimens of different subspecies were pooled for all analyses because it can be difficult or even impossible to distinguish these accurately based on subtle plumage characteristics, especially during migration. Age classes were also pooled to accommodate the uncertainty in assessing a bird's age in the field, which can be particularly difficult after ossification is completed. Equality of variance was not assumed for the t-tests unless Levene's Test was satisfied for that condition.

Discriminant analyses were performed on untransformed data using a stepwise selection for "good" predictor variables through the minimization of Wilks' lambda. Specimens with missing values were excluded from analyses requiring those variables. The variable with the most missing values was mass, which had not been recorded for 27 (10.6%) of the specimens. Other variables with missing values, with the number of specimens in parentheses, included bill height (12), bill width (8), bill length (6), tarsus (5), and tail length (1).

In calculating the discriminant scores of individuals, the sex ratios of the samples were used rather than an assumption of a 1:1 ratio. It is fair to assume that the sex ratios of the samples reflect sex ratios encountered in similar field work. While it is unwise to confound relative sample sizes with prior probabilities in nature (Williams 1983), it is

important not to add incorrect information, such as a sex ratio of unity where none exists.

Multivariate normality (indirectly) and equality of group covariance matrices were examined using Box's M Test (Norušis 1988). Discriminant equations were derived from unstandardized canonical discriminant function coefficients (Norušis 1988:B7). The ability of these equations to identify males and females accurately is reported here as the percent of individuals correctly classified from the sample that generated the discriminant function. In addition, I used the jackknife approach to estimate how well the final discriminant functions performed (Sokal and Rohlf 1981:796).

RESULTS

Male Wilson's Warblers, on average, have significantly longer tails, wing chords, eighth and ninth primaries, and caps (Table 1). I found no difference between the sexes in mass, tarsus, or bill measurements.

The distribution of cap categories (Fig. 2) differed between males and females (Mann Whitney U = 1845.5, Z = ⁻11.237, two-tailed significance = 0.000). 60.3% of the 131 males examined (79) had Category 4 caps, 38.9% (51) had Category 3 caps, none had a Category 2 cap, and 0.8% (1) had a Category 1 cap. Of the 124 females examined, 4.8% (6) had Category 4 caps, 37.9 (47%) had Category 3 caps, 25% (31) had Category 2 caps, and 32.3% (40) had Category 1 caps.

The proportion of individuals whose cap had a distinctive trailing edge also varied between males and females. Of 131 males, 48.9% (64) had a strong posterior line of

demarcation on the cap, 50.4% (66) did not, and 0.8% (1) had no dark cap at all. Of 124 females, 62.1% (77) had no distinct trailing edge to their caps, 5.6% (7) did have strong delineation, and 32.3% (40) had no dark caps to be evaluated.

Discriminant analysis of the entire sample correctly assigned the sex to 91.3% of the specimens (117 of 125 males and 103 of 116 females). However, this function required nine variables, which would be onerous to measure in the field. Removing four weak predictor variables (eighth primary length, bill length, bill height, and bill width) yielded the following discriminant function, which correctly sexed 89.8% of the specimens (a 1.5% loss in discriminating ability):

$$\underline{D} = 0.5977\ CAPC + 0.1172\ CAPL + 0.0646\ P9 + 0.0454\ TL + 0.1805\ WCH - 17.0779 \quad (1)$$

where $\underline{D}$ is the discriminant score, CAPC is the cap category, CAPL is cap length, P9 is ninth primary length, TL is tail length, and WCH is wing chord. When applied to the sample that generated it, this equation correctly classified 93.9% of the males (123 of 131) and 85.4% females (105 of 123). The sample size here is larger because with fewer characters, there were fewer missing values. Jackknifing the data yielded a success rate of 88.6%, correctly classifying 92.4% of the males and 84.6% of the females. The assumptions of multivariate normality and equality of covariance matrices were violated in this analysis (Box's $\underline{M} = 137.20$, approximate $\underline{F} = 8.95$, df = 15, 253496, P = 0.0000).

Focusing on birds from Mexico (mostly from Veracruz), I was able to classify 87.9% of the 182 specimens using the following discriminant function:

$$\underline{D} = 0.6423\ CAPC + 0.0938\ CAPL + 0.0467\ TL + 0.2632\ WCH - 18.8946 \quad (2)$$

This equation correctly sexed 88.9% males (80 of 90) and 85.9% females (78 of 92). Jackknifing these data gave an 86.8% success rate, correctly classifying 90.0% of the males and 83.7% of the females. The assumptions of multivariate normality and equality of covariance matrices were also violated in this analysis (Box's $\underline{M} = 72.23$, approximate $\underline{F} = 7.05$, df = 10, 154728, P = 0.0000).

When specimens collected in Alaska and British Columbia were pooled, they yielded a discriminant function with a much higher success rate of 96.0%:

$$\underline{D} = 0.9189\ CAPC + 0.1800\ CAPL + 0.0977\ TL + 0.0938\ WCH - 13.9426 \quad (3)$$

This function correctly classified 100% of the males in the sample (29) and 90.5% females (19 of 21). The jackknife procedure yielded the same success rates. Again, the standard assumptions were violated (Box's $\underline{M} = 46.82$, approximate $\underline{F} = 4.24$, df = 10, 8720, P = 0.0000).

Cap length, cap category, and demarcation level were all significantly different for males and females, but none of these variables could effectively differentiate the sexes alone or in combination. At best, cap length alone correctly classified 80.0% of the entire sample, while the other cap-related variables were less successful. Using just one cap variable had the added disadvantage of disparate success rates for the two sexes. Cap length, when applied alone, misidentified 37.9% of the females compared to misidentifying only 3.1% of the males. In contrast, using level of demarcation alone misidentified 51.9% of the males but only 5.6% of the females.

DISCUSSION

Using dimorphism to sex Wilson's warblers is not as straightforward as is generally assumed. For instance, U.S. Fish and Wildlife Service and Canadian Wildlife Service (1980) guidelines classify birds with a black cap greater than 11 mm and wing chord 53 mm or greater as male, and those with a cap length less than 8 mm as female. This method would incorrectly classify 1 of the males and 6 of the females in my sample (n=255), a failure rate of 2.7%. It would leave more than one third unknown, with an overall success rate of just 62%. Although Pyle (1997) stated that all males after August of their hatching year (i.e., in first basic plumage) have caps of 12 mm or greater, 26% of the male specimens in this study had caps under 12 mm, often retaining short caps into their second year. Black-capped females exist; one in this study had a solid cap of 14.2 mm (Table 1). The idea that males can be differentiated by a stronger demarcation between black and green feathers in the crown is also unreliable, misidentifying nearly a third of the specimens when used as the sole criterion.

Although only gonadally sexed specimens were used, the possibility of missexed specimens remains (Clench 1976). The examination of the ten longest-capped females and shortest-capped males revealed a steady gradation of cap lengths in both sexes, suggesting a natural and plausible progression to the extremes in this species' cap coloration. However, one specimen (Bell MNH 33458) labeled as male with a cap length of 0.0 mm represented a marked break from the next shortest-capped male at 8.8 mm. The preceding discriminant functions classified it as female with a likelihood of over

99%, suggesting an error in sexing. Removing this anomalous specimen from the study would lower the success rate by 0.5% in the Mexican sample yet would improve it by 0.5% in the overall sample. Because excluding it does not alter the functions or their discriminating ability significantly, this specimen was included in all analyses.

There were two other specimens (that appear to be correctly sexed) that warrant attention for their unusual colorations. One immature October male from Veracruz, Mexico (Bell MNH 33567) lacks the deep black pigment usually seen in dark crowns. Instead of having deep Black feathers, its crown is Citrine, with some feathers edged and shafted in Olivaceous Black (color standards of Ridgway 1912). Adjusting for the paler variation of dark crown feathers, its cap measurements were not unusual for a male. Another specimen, an immature August female from Alaska (UAM 6372), displays a striking lack of yellow pigment, or a hypoxanthic condition. While its wings and tail are much like those of other Wilson's Warblers, its belly and undertail coverts are Olive-Buff, washed with Baryta Yellow on the throat and sides. Its back is a Dark Olive-Gray, changing to Olive Citrine at the crown; the superciliary line and eye ring are Barium Yellow. Its identification as a Wilsonia pusilla lacking yellow pigment was confirmed by K. C. Parkes in 1993, and its measurements are within expectation for a female of the species.

The differences in the discriminant functions (1-3) and in the success rates of the sexing algorithms reflect morphological differences between the sampled populations. Some 17% of the total sample was identified as subspecies pusilla, 60% as pileolata, and

1% as chryseola; 22% of the sample was not identified to subspecies. Determination of subspecies was based on label annotations, with the exception of all Alaska and British Columbia birds, which were designated pileolata (AOU 1957). The Mexico sample is more heterogeneous, including different subspecies, and is representative of the migrant stream encountered in eastern Middle America. The eigenvalue for that group's discriminant function is therefore lower than that for the Alaska-British Columbia group (Table 2). The functions appear robust despite the lack of multivariate normality. The violations of multivariate normality stem from the categorical nature of some characters, the sexually bimodal nature of the data, and the within-sex lack of normality in all of the individual morphological characters except wing chord and tail length even when males and females were considered separately (not shown).

The discriminant functions presented can be used to determine an individual's probability of being male (p_m) with the following equation:

$$p_m = (1 + e^{q})^{-1}, \tag{4}$$

where the sex ratio (r) of the sample generating the discriminant equation is incorporated through the calculation of

$$q = -D \times [1.9 + |\ln(r^4)| - (r \times \ln(r))]. \tag{5}$$

The probability that an individual is female is $p_f = 1 - p_m$. These equations allow the close approximation of the posterior probabilities generated with the more complex Bayes' theorem (see discussion in Winker et al. 1994). It is optimized for Equation 1. To estimate probabilities for the Mexican sample, substitute the following value of

$$\underline{q} = -\underline{D} \times [1.75 + |\ln(\underline{r}^4)| - (3\underline{r} \times \ln(\underline{r}))]. \quad (6)$$

To estimate probabilities for the Alaska-British Columbia group, replace the constant 1.75 in Equation 6 with 1.0. Sex ratios are calculated from Table 2. Readers can review an example of how to use discriminant functions to calculate probabilities in Winker et al. (1994).

The value $\underline{r}$ can be modified in Equations 5 and 6 to reflect different sex ratios if such are found to occur in one's sample (through examination of gonads, or cloacal protuberances and incubation patches during the breeding season). Although the ratios of the samples used here are unbiased and approximate what mist netters might encounter, habitat and season may call for an adjustment in the sex ratio if evidence supports it. For example, Chase et al. (1997) reported a male bias of 2:1 in summer breeding ground captures. On the wintering grounds the sex ratio is closer to unity (Ramos 1986), but there are deviations depending on habitat. Rappole (1986) found males to be less common in Veracruz seral stages and females to be rare in primary rain forest. Timing is also a factor; for example, males migrate north significantly earlier in the spring than females, which might result in a stronger male bias earlier during spring in northern regions (Ramos 1986, Otahal 1995).

Modifications in the equations may also be made to account for shrinkage in museum specimens (Winker 1993, Winker et al. 1994). A subsample of 27 Wilson's Warblers were measured before preparation as museum specimens and then again four or more weeks later after they had dried. The measurements were performed by a single

observer (JJW) and included wing chord, tail length, tarsus length, and bill height, width, and length. Although limited by small sample size, paired t-tests showed statistically significant shrinkage in two measurements. The mean tail length shrank by 3.34%, while the mean bill width shrank by 2.54%. Investigators applying these sexing algorithms to living or recently dead birds may wish to multiply values for tail length by the correction factor of 0.967. Slight shrinkage in the other bill measurements and slight increase in wing chord and tarsus were not significant. Shrinkage in the measured characters may have been affected by freeze-drying, however, because not all specimens were fresh. Mean ninth primary length and mean cap length were not examined for shrinkage. Primary feathers have been shown not to shrink (Jenni and Winkler 1989), and shrinkage in cap length may be difficult to determine precisely, depending on whether specimens were prepared with skull in or out of the skin.

Sexual size dimorphism observed in the lengths of wing chord, tail, and eighth and ninth primaries may be influenced by a number of factors, including sexual selection, fertility selection, and survival selection. Sexual differences in microhabitat preference on the wintering grounds may also explain the evolution of size dimorphism in this species (Rappole 1986). Because W. pusilla demonstrates size differences in morphological characters associated with flight (e.g., wing chord, primaries, tail) rather than in mass or bill size, it is possible that these characters reflect differences in flight styles demanded by spatial differences in microhabitats. However, this study cannot differentiate among the possible reasons for the observed dimorphism or determine any functional explanation.

Instead of being considered sexually dimorphic, W. pusilla may be more properly termed polymorphic, as suggested by Rappole (1986). Males have larger black caps on average, but there is an intergradation between the sexes. This "andromimesis" may provide females with a competitive edge in agonistic displays on the wintering grounds (Rappole 1986). There is some evidence that dark head plumage in females of the congener Wilsonia citrina is not related to age (Morton 1989), but the development and adaptive value of dark caps in some female Wilson's Warblers remain poorly understood. Regardless, there is substantial overlap in male and female plumage characteristics, and size dimorphism also shows enough sexual overlap to defy complete separation.

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TABLE 1. Comparative measurements (mm) and body mass (g) of male and female Wilsonia pusilla.

	Males			Females			Dimor-	t-test	
	$\bar{x}$ (n)	SD	Min.—Max.	$\bar{x}$ (n)	SD	Min.—Max.	phism[1]	t	P
Cap length	12.8 (131)	1.87	0.0—16.6	6.3 (124)	4.88	0.0—14.2	2.03	13.98	0.000
Wing chord	54.8 (131)	2.04	47.9—59.2	52.6 (124)	1.85	47.6—57.4	1.04	9.10	0.000
Primary 9	38.7 (131)	2.15	34.5—45.0	36.8 (124)	1.99	31.5—46.5	1.05	7.18	0.000
Primary 8	41.3 (131)	2.00	36.5—46.5	39.4 (124)	1.85	36.0—48.0	1.05	7.83	0.000
Tail length	46.4 (131)	1.63	41.8—50.1	45.0 (123)	2.05	39.3—50.7	1.03	5.75	0.000
Mass[2]	6.9 (111)	0.65	5.4—9.0	6.8 (117)	0.89	5.3—10.4	1.01	1.52	0.131
Tarsus	16.9 (129)	0.69	14.8—19.3	16.8 (121)	0.69	15.3—19.2	1.01	0.29	0.774
Bill length	6.0 (128)	0.28	5.4—6.9	6.1 (121)	0.31	5.3—7.0	0.98	⁻0.12	0.904
Bill height	2.8 (126)	0.24	2.0—3.5	2.8 (117)	0.21	2.3—3.5	1.00	⁻0.14	0.886
Bill width	3.0 (128)	0.25	2.4—3.7	3.0 (119)	0.24	2.2—3.5	1.00	⁻0.03	0.976

[1] Index of dimorphism calculated by dividing mean male value by mean female value.

[2] Includes individuals in migration with substantial fat deposits.

TABLE 2. Statistics for the individual discriminant scores (D) generated by Equations 1-3.

Region	Sex	Equation	n	Mean	SE	Min.—Max.	95 % CI[1]	Eigenvalue[2]
North America	Males	1	131	1.06	0.07	−2.28—2.87	−1.32—4.01	1.2186
	Females	1	123	−1.13	0.11	−3.31—2.06	−4.11—1.84	1.2186
Mexico	Males	2	90	0.96	0.08	−2.20—2.29	−1.80—3.73	0.9219
	Females	2	92	−0.94	0.12	−3.14—2.22	−3.71—1.82	0.9219
Alaska and	Males	3	29	1.75	0.12	0.61—2.78	−2.85—6.35	4.4033
British Columbia	Females	3	21	−2.42	0.29	−3.98—0.75	−7.02—2.18	4.4033

[1] 95 % confidence interval; mean ± 2 SD, where SD is that of the population.

[2] This eigenvalue is the ratio of between-groups to within-groups sums of squares. Generally, the larger the value, the better the discriminant function.

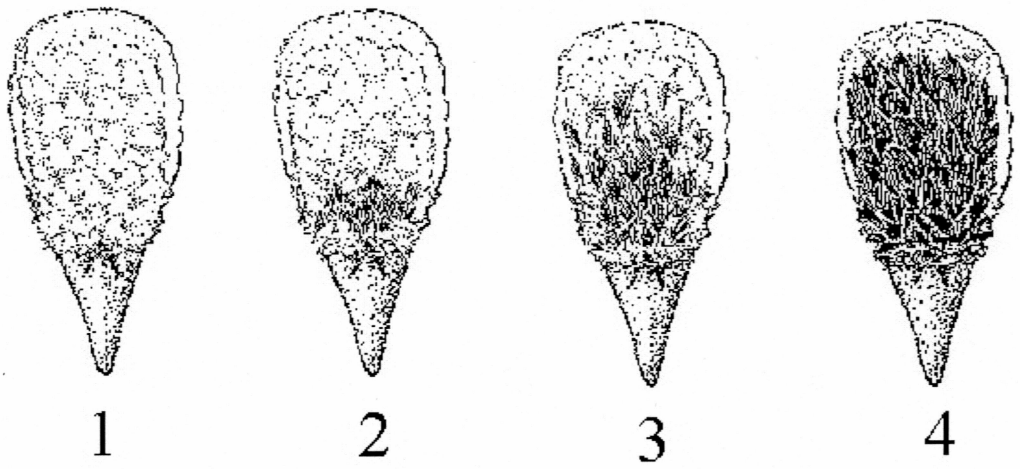

Figure 1. Depiction of the four cap categories for Wilson's Warbler. The categories are 1) all olive with no black feathers; 2) black feathers in anterior half of crown only; 3) black feathers in posterior half of crown, regardless of presence of black feathers in anterior half; and 4) solid black cap with no olive feathers. Caps in categories 2 and 3 may have olive feathers interspersed with the black ones.

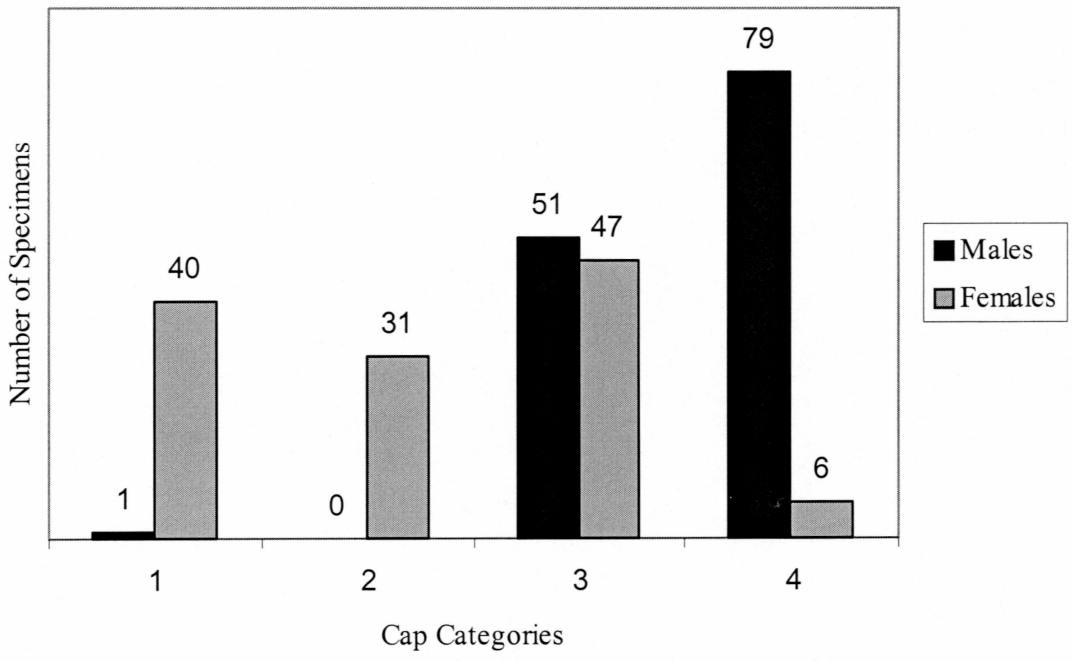

Figure 2. The distributions of males and females across the four cap categories. The total number of males and females examined are 131 and 124, respectively.

Chapter 3: Can a Biased Estimator Be Made Unbiased?[2]

Key words.—Wright's F_{ST}, Weir and Cockerham's θ, population structure

It has been a long-standing interest of biologists to understand and quantify how natural populations are genetically structured. Understanding how genetic differences within populations compare to those among populations can provide insight into what separates populations, what levels of gene flow are necessary to consolidate populations, and how the early stages of speciation proceed. The prevailing method of describing this hierarchical structure has been the use of *F*-statistics, introduced by Wright (1943, 1951, 1965). F_{ST} measures the amount of genetic variation in the total sample that is due to differences among subpopulations in that sample; this proportion can range between 0 and 1. F_{ST} is also functionally equivalent to Nei's (1973) G_{ST}, which was derived using expected panmictic heterozygosity rather than the variance in allele frequencies among subpopulations. Both of these measures have been used extensively in the past thirty years to describe genetic diversity among populations, particularly in allozyme studies (e.g., Nevo 1978, Evans 1987).

[2]Prepared for submission to Evolution, by Jacqueline J. Weicker and Kevin Winker

Although elegant in its simplicity, the estimation of F_{ST} does not account for sampling error. Weir and Cockerham (1984) developed the estimator θ to correct for the error associated with differences in allele frequency distributions between the population samples and the total set of populations. Simulations confirmed that θ is independent of the number of groups sampled and the number of individuals sampled in each group (Weir and Cockerham 1984, Cockerham and Weir 1993). While these authors concurred that the use of F_{ST} or G_{ST} is valid when examining diversity among populations for which there has been a complete census, they advocated the use of an unbiased estimator such as θ for most empirical studies, where sample sizes tend to be small.

An ultimate goal in quantifying population structure is to understand how different species are partitioned genetically and to learn whether there are patterns in different groups of organisms and life zones. There are several reasons why making these comparisons among different allozyme studies can be problematic, such as differences in loci used and in abilities to distinguish "hidden" alleles (for caveats, see Barrowclough 1983 and Evans 1987). These limitations are mitigated because many of the same gene loci are used in vertebrate studies (Nevo 1978). However, one serious reason not to compare allozyme studies are the differences in sampling designs, a problem that is exacerbated because the vast majority of studies report population structure in terms of F_{ST}. It would seem that among-study comparisons using the unbiased θ would be more robust.

However, calculating θ from published allele frequency tables is tedious or

impossible, with hindrances such as missing data and typographic errors. In many cases, original data are simply unavailable; one study in which data were requested from authors of thirty publications with incomplete data sets resulted in only one researcher providing the full data set (Leberg and Neigel, 1999). While this study involved mitochondrial data, retrieving allozyme data for reanalysis would be even more difficult considering that most allozyme research is older than mtDNA research.

In this paper I consider a method of approximating θ using values readily gleaned from published reports: F_{ST} (or G_{ST}), the number of populations sampled, and the average number of individuals sampled per population. This approximation is derived from the relationship between G_{ST} and the intraclass correlation β put forth by Cockerham and Weir (1993: 858). I then show how well this approximation corresponds with the actual calculated θ and investigate the utility of the transformation in eliminating sampling bias from estimates of genetic population structure in empirical data.

METHODS

Cockerham and Weir (1993) presented the following formulation of G_{ST} (their Equation 4), which they noted was the same formulation used by Slatkin and Barton (1989):

$$G_{ST} = \frac{(r-1)\beta + \frac{r-1}{2M-1}}{r-\beta + \frac{r-1}{2M-1}} \qquad (7)$$

where r is the number of populations, and M is the number of individuals per population. β is a ratio of functions involving the two measures F_0 and F_1, and has the same statistical calculations as θ. For this approximation, they may be regarded as equivalent. So, solving for θ (or β), the equation becomes

$$\theta = \frac{G_{ST}(r + \frac{r-1}{2M-1}) - \frac{r-1}{2M-1}}{G_{ST} + r - 1} \qquad (8)$$

This approximation of θ can be calculated if the values of G_{ST}, r, and M are known.

To demonstrate the efficacy of this approximation, four different model data sets were constructed (see Appendix). Each data set contained eighteen individuals, divided into three (r) populations of equal size (M = six individuals). For each model, individuals were assigned one of three alleles at each of thirty-two loci. These numbers of populations, sample sizes, loci, and alleles all approach the average of several representative allozyme studies on birds (Capparella 1987, 1988; Peterson 1990, 1992; Bates 1993; Brumfield and Capparella 1996; Winker et al. 2000). One model consisted of populations that shared all alleles at identical frequencies, as in a case of panmixia. A

second model consisted of populations with only private alleles, suggesting fixation of those alleles. The other two models had varied frequencies of shared alleles.

For each model data set, Wright's F_{ST} was calculated using BIOSYS-1 (Swofford and Selander 1981). This program was selected because it has been the predominant tool for analyzing allozyme data and was used in the empirical studies cited. In addition, Weir and Cockerham's θ (1984) was calculated for each data set using the program GDA 1.0 (Lewis and Zaykin 1999). This value shall be referred to as "actual θ". Bootstrap estimates of upper and lower limits for actual θ were obtained after 5000 repetitions.

Finally, the conversion algorithm above (Equation 8) was used to calculate the approximation of θ, or estimated θ, based on F_{ST} (from BIOSYS-1), r, and M values for each model data set. Actual θ and estimated θ values for each model data set were compared using linear regression.

Empirical data were gathered from allozyme studies of New World land birds (Capparella 1987, 1988; Peterson 1990, 1992; Bates 1993; Brumfield and Capparella 1996; Winker et al. 2000). From these studies, 39 species were selected for which the allozyme frequency data were available or could be derived. In some cases where species/subspecies distinctions are unclear, data from "different" species were combined to form single species groups, following the lead of the author. For the purpose of this study, as long as populations are closely related enough to make allozyme comparisons meaningful, it does not matter whether the populations are considered different subspecies or species.

As with the model data, F_{ST} values were calculated using BIOSYS-1 (Swofford and Selander 1981). To eliminate any discrepancies due to typographical or data entry errors, these values were checked against those reported in the literature. When the F_{ST} value was not published but Nei's genetic distance was, this measure was calculated to provide confirmation. GDA 1.0 (Lewis and Zaykin 1999) was used to calculate actual θ for each set of populations, and Equation 8 was used to estimate θ, based on directly calculated values for F_{ST}, r, and M. Actual θ and estimated θ values for each empirical data set were compared using linear regression.

RESULTS

Under the parameters of model populations, Equation 8 produced estimations of θ that correlated nearly perfectly with actual θ (Fig. 3). When the estimations were not the same as the actual values of θ, they were well within the 95% confidence intervals determined by bootstrapping (Table 3).

Using the empirical data sets, Equation 8 performed almost as well in estimating θ (Fig. 4), with an adjusted r^2 of 0.96. Only one of 39 taxa had an estimated θ that fell outside of the 95% confidence intervals around actual θ; the estimated θ of *Amazona farinosa* was much higher than its actual θ (Table 4).

One might assume that those data sets that produced the largest confidence intervals around θ when bootstrapped would have the largest residuals in Fig. 4; that is, they would have the least accurate estimations of θ. This is not the case, as there is no

correlation between the magnitude of the residuals (whether positive or negative) around estimated θ and the magnitude of the bootstrapped 95% confidence intervals associated with actual θ (Fig. 5). The bootstrapping is performed across loci and reflects inconsistencies that may arise from the effects of a particular locus. The problematic effects of specific loci are not revealed in the process of estimating θ because the differences in the effects of loci influence Equation 8 only indirectly through the value of F_{ST}.

DISCUSSION

The estimation algorithm for θ (Equation 8) appears to be a sound method for approximating θ when full data sets are not available for the actual calculation of θ. In cases where only the number of populations, the number of individuals, and the value of Wright's F_{ST} are known, Equation 8 is a feasible way of estimating θ. However, although the correlation between estimated and actual θ was significant for both model and real-world populations, it did not perform quite as well with the empirical data (Figures 3 and 4).

What explains the difference in how well Equation 8 approximates θ for natural data sets? One reason is that Equation 7 is simplified by the use of M, an average of the number of individuals per population sample. Data sets composed of equal-sized population samples are not compromised by the use of an "average" population size in the estimation of θ, whereas data sets that include population samples of different sizes

may be affected by the use of an arithmetic mean.

Data sets with equal population samples had, on average, a smaller difference between actual θ and estimated θ than those with unequal population samples (comparing a mean difference of 0.00026 to 0.045; paired independent *t*-test not assuming equal variances, df = 31.007, 2-tailed significance = 0.00). The six taxa that showed the greatest differences between actual and estimated θ (*Amazona farinosa*, *Pionus menstruus*, *Myiobius* sp., *Dendrocolaptes certhia*, *Cyphorhinus* sp., and *Chlorospingus ophthalmicus*) all had unequal population sample sizes. Of the six taxa that showed the least differences between actual and estimated θ, five had equal population sample sizes (*Leucopternis* sp., *Tersina viridis*, *Glyphorhynchus spirurus*, *Micrastur* sp., and *Nyctiphrynus* sp.). The one with unequal population sample sizes, *Xenops minutus*, is not far from equality, with two population samples of three individuals and one population sample of two individuals.

Ultimately, the titular question regards the utility of Equation 8. While removing the sampling bias inherent in Wright's F_{ST} is important for making cross-study comparisons, the bias itself may be negligible compared to the error involved in the approximation of θ. It is clear that the correlation between Wright's F_{ST} and Weir and Cockerham's θ (shown using the same 39 empirical data sets) is strong (Fig. 6). The difference between the two values (which would include the adjustment for bias) is not much greater than the difference between estimated and actual θ. It would seem, then, that the danger of sampling bias in real-world data sets may be overstated. Although actual θ

may be the best way to measure population structure in small populations, estimated θ does not seem as useful when its associated error is considered.

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APPENDIX

The four models are documented below. The numbers refer to absolute numbers of individuals in each population sample, with each sample having a total of six individuals. The letters refer to different alleles at each locus.

MODEL 1

	Population				Population		
Locus	1	2	3	Locus	1	2	3
LOC1				LOC5			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC2				LOC6			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC3				LOC7			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC4				LOC8			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2

MODEL 1. Continued.

Locus	Population 1	2	3	Locus	Population 1	2	3
LOC9				LOC14			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC10				LOC15			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC11				LOC16			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC12				LOC17			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC13				LOC18			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2

MODEL 1. Continued.

Locus	Population 1	2	3	Locus	Population 1	2	3
LOC19				LOC24			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC20				LOC25			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC21				LOC26			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC22				LOC27			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC23				LOC28			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2

MODEL 1. Continued.

Locus	Population 1	2	3	Locus	Population 1	2	3
LOC29				LOC31			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2
LOC30				LOC32			
A	2	2	2	A	2	2	2
B	2	2	2	B	2	2	2
C	2	2	2	C	2	2	2

MODEL 2

Locus	Population 1	2	3	Locus	Population 1	2	3
LOC1				LOC3			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC2				LOC4			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6

MODEL 2. Continued.

Locus	Population 1	2	3	Locus	Population 1	2	3
LOC5				LOC10			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC6				LOC11			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC7				LOC12			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC8				LOC13			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC9				LOC14			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6

MODEL 2. Continued.

	Population				Population		
Locus	1	2	3	Locus	1	2	3
LOC15				LOC20			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC16				LOC21			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC17				LOC22			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC18				LOC23			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC19				LOC24			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6

MODEL 2. Continued.

	Population				Population		
Locus	1	2	3	Locus	1	2	3
LOC25				LOC29			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC26				LOC30			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC27				LOC31			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC28				LOC32			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6

MODEL 3

Locus	Population 1	2	3	Locus	Population 1	2	3
LOC1				LOC6			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC2				LOC7			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC3				LOC8			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC4				LOC9			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC5				LOC10			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6

MODEL 3. Continued.

	Population				Population		
Locus	1	2	3	Locus	1	2	3
LOC11				LOC16			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC12				LOC17			
A	6	0	0	A	3	2	2
B	0	6	0	B	2	2	2
C	0	0	6	C	1	2	2
LOC13				LOC18			
A	6	0	0	A	3	2	2
B	0	6	0	B	2	2	2
C	0	0	6	C	1	2	2
LOC14				LOC19			
A	6	0	0	A	3	2	2
B	0	6	0	B	2	2	2
C	0	0	6	C	1	2	2
LOC15				LOC20			
A	6	0	0	A	3	2	2
B	0	6	0	B	2	2	2
C	0	0	6	C	1	2	2

MODEL 3. Continued.

	Population				Population		
Locus	1	2	3	Locus	1	2	3
LOC21				LOC26			
A	3	2	2	A	2	3	2
B	2	2	2	B	2	2	1
C	1	2	2	C	2	1	3
LOC22				LOC27			
A	3	2	2	A	2	3	2
B	2	2	2	B	2	2	1
C	1	2	2	C	2	1	3
LOC23				LOC28			
A	3	2	2	A	2	3	2
B	2	2	2	B	2	2	1
C	1	2	2	C	2	1	3
LOC24				LOC29			
A	3	2	2	A	2	3	2
B	2	2	2	B	2	2	1
C	1	2	2	C	2	1	3
LOC25				LOC30			
A	2	3	2	A	2	3	2
B	2	2	1	B	2	2	1
C	2	1	3	C	2	1	3

MODEL 3. Continued.

Locus	Population 1	2	3	Locus	Population 1	2	3
LOC31				LOC32			
A	2	3	2	A	2	3	2
B	2	2	1	B	2	2	1
C	2	1	3	C	2	1	3

MODEL 4

Locus	Population 1	2	3	Locus	Population 1	2	3
LOC1				LOC4			
A	4	0	0	A	4	0	0
B	2	4	2	B	2	4	2
C	0	2	4	C	0	2	4
LOC2				LOC5			
A	4	0	0	A	3	1	1
B	2	4	2	B	2	3	3
C	0	2	4	C	1	2	2
LOC3				LOC6			
A	4	0	0	A	3	1	1
B	2	4	2	B	2	3	3
C	0	2	4	C	1	2	2

MODEL 4. Continued.

Locus	Population 1	2	3	Locus	Population 1	2	3
LOC7				LOC12			
A	3	1	3	A	6	0	0
B	2	3	2	B	0	6	0
C	1	2	1	C	0	0	6
LOC8				LOC13			
A	3	1	3	A	6	0	0
B	2	3	2	B	0	6	0
C	1	2	1	C	0	0	6
LOC9				LOC14			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC10				LOC15			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6
LOC11				LOC16			
A	6	0	0	A	6	0	0
B	0	6	0	B	0	6	0
C	0	0	6	C	0	0	6

MODEL 4. Continued.

	Population				Population		
Locus	1	2	3	Locus	1	2	3
LOC17				LOC22			
A	3	2	2	A	3	2	2
B	2	2	2	B	2	2	2
C	1	2	2	C	1	2	2
LOC18				LOC23			
A	3	2	2	A	3	2	2
B	2	2	2	B	2	2	2
C	1	2	2	C	1	2	2
LOC19				LOC24			
A	3	2	2	A	3	2	2
B	2	2	2	B	2	2	2
C	1	2	2	C	1	2	2
LOC20				LOC25			
A	3	2	2	A	2	3	2
B	2	2	2	B	2	2	1
C	1	2	2	C	2	1	3
LOC21				LOC26			
A	3	2	2	A	2	3	2
B	2	2	2	B	2	2	1
C	1	2	2	C	2	1	3

MODEL 4. Continued.

	Population				Population		
Locus	1	2	3	Locus	1	2	3
LOC27				LOC30			
A	2	3	2	A	2	3	2
B	2	2	1	B	2	2	1
C	2	1	3	C	2	1	3
LOC28				LOC31			
A	2	3	2	A	2	3	2
B	2	2	1	B	2	2	1
C	2	1	3	C	2	1	3
LOC29				LOC32			
A	2	3	2	A	2	3	2
B	2	2	1	B	2	2	1
C	2	1	3	C	2	1	3

TABLE 3. Actual and estimated θ calculated for each model set of populations. Actual values for θ (Weir and Cockerham 1984) and their associated 95% confidence intervals were determined using GDA (Lewis and Zaykin 1999). Estimated θ was determined using the following variables: Wright's F_{ST} (from BIOSYS-1: Swofford and Selander 1981), *r* (the number of populations sampled), and *M* (the average number of individuals sampled per population).

Model	Actual θ	Confidence Interval[1]	Estimated θ	F_{ST}	*r*	*M*
1	⁻0.09091	⁻0.09091— ⁻0.09091	⁻0.09091	0.000	3	6
2	1.00000	1.00000— 1.00000	1.00000	1.000	3	6
3	0.58298	0.41329— 0.73269	0.58338	0.519	3	6
4	0.35341	0.18270— 0.52788	0.35319	0.314	3	6

[1] Estimated using the bootstrap, 5000 repetitions.

TABLE 4. Weir and Cockerham's (1984) θ (actual and estimated) calculated for empirical data sets. See Methods for details.

Taxon	Actual θ	Confidence Interval	Estimated θ	F_{ST}	*r*	*M*
Crypturellus berlepschi/C. cinereus[a]	0.87346	0.65812— 1.00000	0.90341	0.879	2	1.50000
Leucopternis plumbea/L. schistacea[a]	1.00000	1.00000— 1.00000	1.00000	1.000	2	1.00000
Micrastur plumbeus/M. gilvicollis[a]	0.89630	0.64103— 1.00000	0.89655	0.856	2	2.00000
Pyrrhura melanura[b]	0.57143	0.00000— 1.00000	0.59875	0.514	2	2.50000
Pionus menstruus[b]	0.20805	−0.02280— 0.46253	0.40119	0.413	3	2.66667
Amazona farinosa[b]	0.04280	−0.09091— 0.14286	0.24019	0.274	2	2.00000
Nyctiphrynus ocellatus/N. rosenbergi[a]	0.72157	0.52903— 0.85135	0.72126	0.623	2	3.00000
Threnetes ruckeri/T. leucurus[b]	0.58932	0.33908— 0.78690	0.57216	0.527	3	4.00000
Eutoxeres aquila[b]	0.37965	0.00848— 0.60036	0.43506	0.440	3	2.66667
Trogon rufus[b]	0.28958	−0.08571— 0.55066	0.36225	0.421	3	2.00000
Baryphthengus martii[b]	0.49099	0.02697— 0.76088	0.48251	0.437	3	4.66667
Glyphorhynchus spirurus[b]	0.31855	0.05759— 0.61069	0.31862	0.296	3	5.00000
Glyphorhynchus spirurus[c]	0.16231	0.08319— 0.23825	0.20790	0.230	8	8.50000
Dendrocolaptes certhia[b]	0.36719	0.04801— 0.57968	0.47674	0.463	3	3.00000
Automolus rubiginosus[b]	0.10300	−0.04234— 0.23261	0.13592	0.149	2	3.50000
Sclerurus mexicanus[b]	0.85739	0.56331— 1.00000	0.83368	0.816	3	2.33333
Xenops minutus[b]	0.64648	0.44286— 0.81887	0.64665	0.615	3	3.00000

TABLE 4. Continued.

Taxon	Actual θ	Confidence Interval	Estimated θ	F_{ST}	*r*	*M*
Myrmotherula axillaris[b]	0.53512	0.06245— 0.86469	0.52153	0.476	3	4.33333
Microrhopias quixensis[b]	0.09160	−0.03704— 0.16712	0.10184	0.198	3	2.66667
Myrmoborus myotherinus[d]	0.23305	0.05054— 0.46120	0.27159	0.261	4	7.50000
Pithys albifrons[d]	0.03283	−0.01976— 0.09538	0.03757	0.057	4	13.00000
Tityra semifasciata[b]	0.29905	0.07407— 0.48123	0.29966	0.376	3	2.00000
Pipra coronata[d]	0.26419	0.03800— 0.48397	0.26326	0.233	5	30.80000
Chiroxiphia pareola[d]	0.40955	0.27711— 0.61941	0.40192	0.262	2	22.50000
Myiobius barbatus/M. sulphureipygius[b]	0.44267	0.21013— 0.64049	0.55951	0.571	4	2.75000
Mionectes olivaceus[b]	0.39584	0.05843— 0.65630	0.40575	0.371	3	4.66667
Henicorhina leucosticta[e]	0.56808	0.28136— 0.76209	0.54006	0.563	4	2.50000
Microcerculus marginatus/M. luscinia[b]	0.58548	0.14914— 0.78779	0.56246	0.518	3	4.00000
Cyphorhinus aradus/C. phaeocephalus[b]	0.44289	−0.07243— 0.66093	0.54290	0.512	3	3.33333
Turdus albicollis/T. assimilis[b]	0.38304	0.09070— 0.57927	0.40285	0.385	3	3.66667
Microbates cinereiventris[b]	0.57037	0.20833— 0.81373	0.57083	0.513	2	2.00000
Atlapetes brunneinucha[f]	0.26645	0.11529— 0.39383	0.31672	0.287	4	10.50000
Pitylus grossus[b]	0.00842	−0.03490— 0.05872	0.03003	0.152	3	2.66667
Chlorospingus ophthalmicus[f]	0.25702	0.06311— 0.47566	0.33748	0.297	4	14.50000

TABLE 4. Continued.

Taxon	Actual θ	Confidence Interval	Estimated θ	F_{ST}	*r*	*M*
Chlorophanes spiza[b]	0.47259	0.12566— 0.71169	0.54618	0.533	3	2.66667
Tersina viridis[b]	0.20000	0.00000— 0.38462	0.20000	0.250	2	2.00000
Limnothlypis swainsonii[g]	0.02938	0.00167— 0.07585	0.03095	0.043	5	21.80000
Aphelocoma coerulescens[h]	0.12456	0.04986— 0.15973	0.12875	0.126	5	18.20000
Aphelocoma unicolor[h]	0.75771	0.29411— 1.00000	0.74615	0.686	3	6.33333

[a] Brumfield and Capparella (in prep), [b] Brumfield and Capparella (1996), [c] Bates (1993), [d] Capparella (1987), [e] Brumfield (pers.comm.), [f] Peterson (1992), [g] Winker et al. (2000), [h] Peterson (1990).

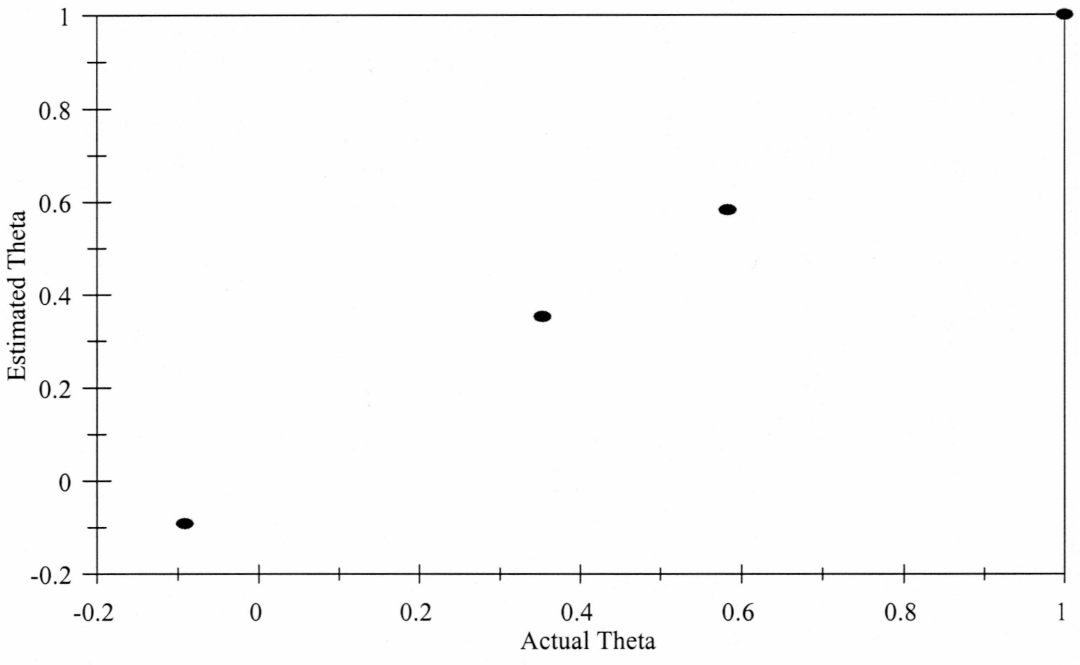

Figure 3. Actual θ vs. estimated θ, using model populations. This shows the relationship between the full calculation of Weir and Cockerham's θ (1984), called actual θ, and θ as estimated through Equation 8. Adjusted $r^2 = 1.00$, $p < 0.01$, $n = 4$.

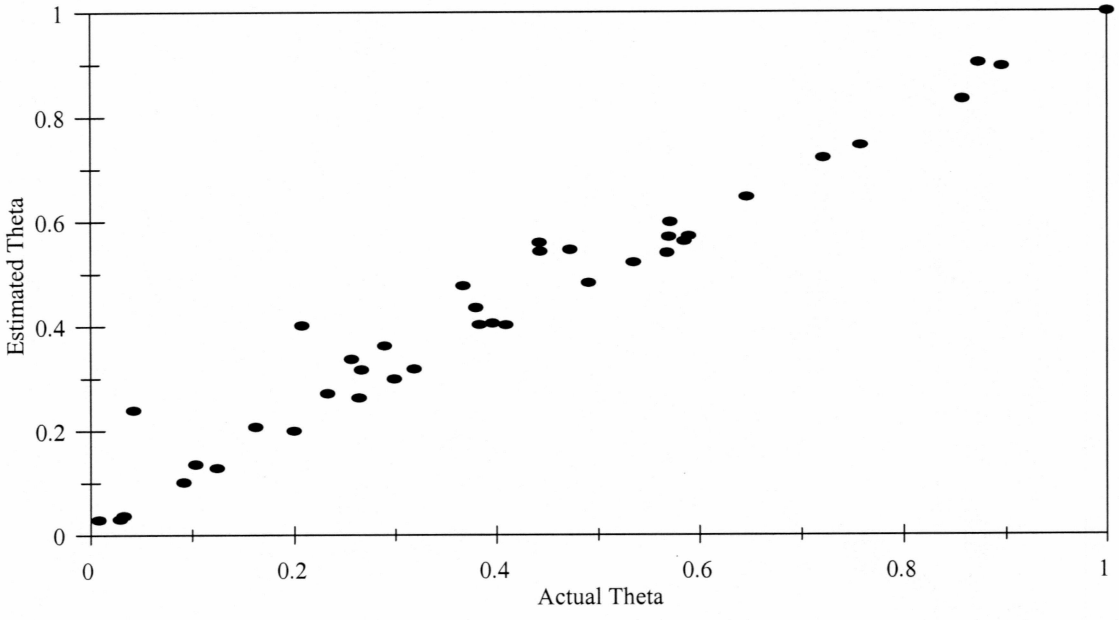

Figure 4. Actual θ vs. estimated θ, using real populations. This shows the relationship between the full calculation of Weir and Cockerham's θ (1984), called actual θ, and θ as estimated through Equation 8. Adjusted $r^2 = 0.96$, $p < 0.01$, $n = 39$.

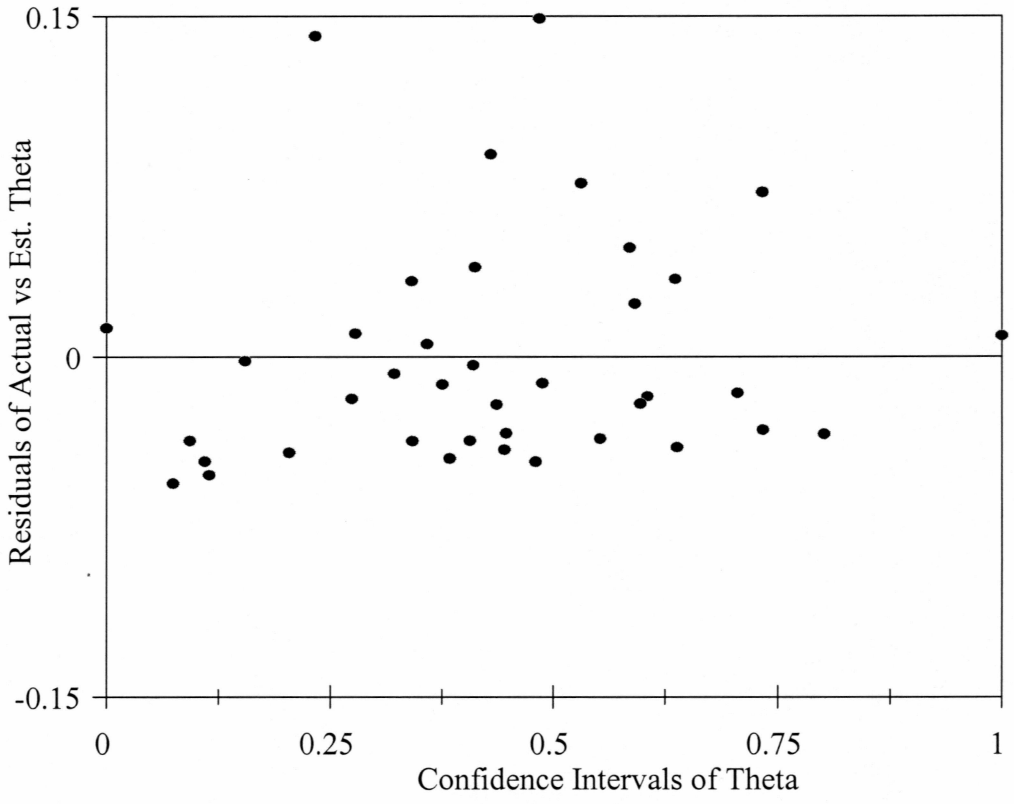

Figure 5. The magnitude of residuals from Figure 4 do not correspond with the 95% bootstrap confidence intervals around actual θ.

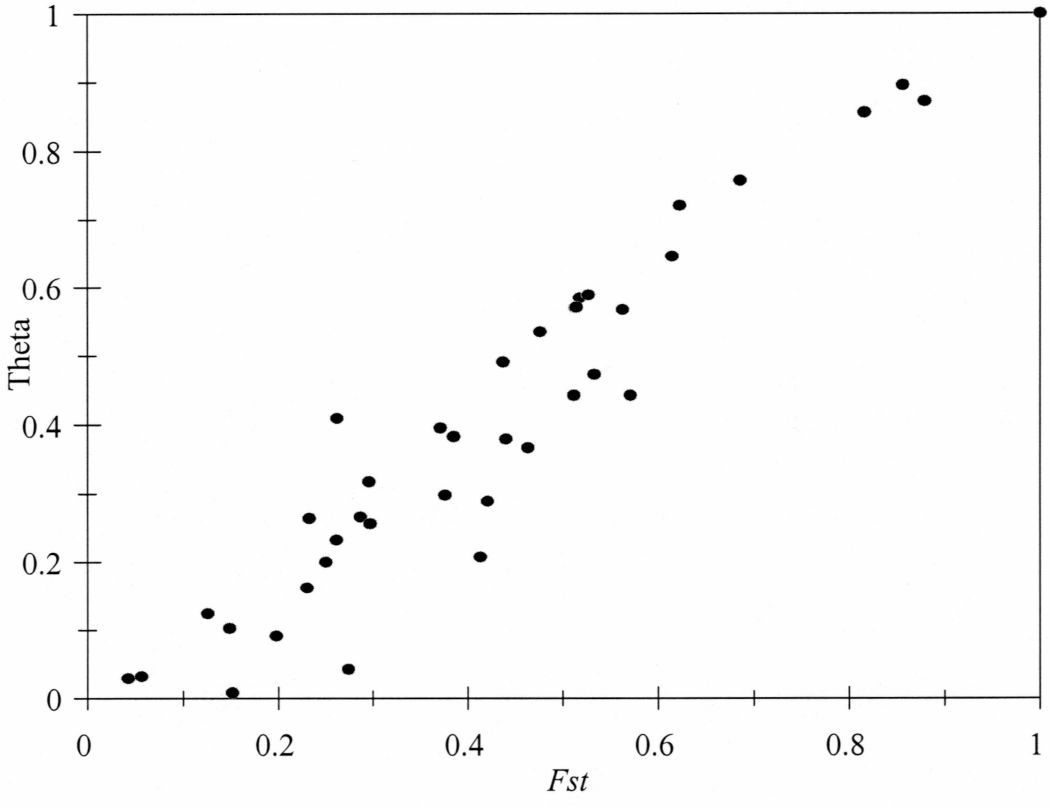

Figure 6. Wright's (1943) F_{ST} or Nei's (1993) G_{ST} (as reported in each study and recalculated here) vs. Weir and Cockerham's (1984) θ. Adjusted $r^2 = 0.91$, $p < 0.01$, n = 39.

Chapter 4: Conclusions

Through the preceding two chapters, I have examined not only the nature of variation in birds, but have shown how these differences in genetics and morphology can be used. There is indeed size dimorphism and plumage polymorphism in Wilson's Warblers, which can be used in discriminant equations to distinguish the sexes. Also, the new method of approximating Weir and Cockerham's θ (1984, 1993) obviates the need to recode existing avian allozyme data sets to estimate θ, an unbiased estimator of genetic population structure. Although the estimation algorithm is successful for both model and real-world bird populations, the advantage of this method is unclear when examined in light of the small effect of θ's bias-removing power on empirical data sets. More research needs to be done to confirm the theoretical efficacy of this estimator of population structure in empirical studies.

While the object of this thesis was to examine variation in birds, I also have shown the great promise in two under-utilized sources of biological information: museum collections and genetic information in the scientific literature. Museum specimens in natural history collections, which originally contributed to and documented an individual collector's research, continue to provide data for other researchers for decades, even centuries after their "original purpose" was met. With modern techniques such as stable

isotope analysis and the amplification of DNA through the polymerase chain reaction, these specimens are being given new life in ways their collectors could not have foreseen. My Wilson's Warbler study would not have been feasible without the great series of specimens available in museum collections.

The other resource from the past that should see greater use is published genetic data. Whether the data are pieced together from printed journal articles or found on an internet database such as Genbank, they can be reviewed, reanalyzed, and used in ways that may not have been possible when the data were collected. My revival of allozyme data with the application of a modern method of analysis in an example of this. These two reservoirs of past scientific endeavors have growing value for new lines of hypothesis testing in the study of evolutionary principles and population genetics.

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