

AN EVALUATION OF FLUCTUATING ASYMMETRY AS A TOOL
IN IDENTIFYING IMPERILED BIRD POPULATIONS

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DEDICATION

I dedicated this thesis to my wife Kerry, my mother Nancy, my father Jim, and my sister Wendy. Without your love, strength, and encouragement I would not have made it this far.

TABLE OF CONTENTS

	PAGE
LIST OF TABLES	vii
LIST OF FIGURES	viii
INTRODUCTION	1
Developmental Stability and Fluctuating Asymmetry.....	2
LITERATURE REVIEW	4
Environmental Stressors	4
Nutritional Stress	4
Temperature Stress.....	5
Population Density - Induced Stress	5
Chemical Stress.....	6
Genetic Stressors.....	7
Habitat Fragmentation and Fluctuating Asymmetry.....	8
Objective.....	10
METHODS	13
Study Sites and Species	13
Measurement of Fluctuating Asymmetry	16
Fluctuating Asymmetry Analyses.....	17
Contemporary vs. Historic Asymmetry	19
Species of Like Conservation Status.....	22
FA and Conservation Status.....	22
RESULTS	24
Contemporary vs. Historic Asymmetry	28
Species of Like Conservation Status.....	40
FA and Conservation Status.....	41
DISCUSSION	42
Contemporary vs. Historic Asymmetry	42
FA and Conservation Status.....	46
Recommendations Concerning FA Analyses	46

DISCUSSION (continued)	
Conclusions and Implications for Conservation.....	47
ACKNOWLEDGEMENTS.....	50
REFERENCES	51
APPENDICES	
A. TRAITS EVALUATED FOR INCLUSION IN STUDY AND REASON(S) FOR THEIR EXCLUSION.....	56
B. POWER ANALYSIS AND SAMPLE SIZE DETERMINATION.....	58
C. DETERMINATION OF THE NUMBER OF REPEATED MEASUREMENTS PER SIDE IN RELATION TO MEASUREMENT PRECISION	60
D. FLUCTUATING ASYMMETRY VS. MEASUREMENT ERROR ANOVA TABLES	63
E. COMPARISON OF F-STATISTICS AND P-VALUES GENERATED BY THE FA10 AND FPA ANALYSES	75
ABSTRACT	77

LIST OF TABLES

TABLE	PAGE
1. Sample sizes of live birds and museum skins measured by species and trait.....	24
2. Statistically significant results of Grubb’s test on individual repeated measurement differences	25
3. Fluctuating asymmetry outliers identified by Grubb’s test.....	27
4. Sample sizes by species, trait, and collection type	28
5. Summary results from two-way mixed model ANOVAs to test whether fluctuating asymmetry could be separated from measurement error within the 0.05 dataset	29
6. Summary results from two-way mixed model ANOVAs to test whether fluctuating asymmetry could be separated from measurement error within the 0.01 dataset	30
7. Summary results from two-way mixed model ANOVAs to test whether fluctuating asymmetry could be separated from measurement error within the Uncorrected dataset.....	31
8. Results from the FA10 analyses testing for differences in bill and tarsus length fluctuating asymmetry between contemporary and historic populations	36
9. Results from the FPA analyses testing for differences in bill and tarsus length fluctuating asymmetry between contemporary and historic populations	37
10. Two-sample t-test power analysis results	59
11. Pearson correlation statistics for the mean difference of two, three, four and five repeated measurements for tarsus and bill	61

LIST OF FIGURES

FIGURE	PAGE
1. Locations of collection sites for live birds and museum skins within southern California and northern Mexico.....	15
2. Average length of left side vs. right side of bill and tarsus for all species	26
3. Bill and tarsus asymmetry as a function of mean trait size.....	32
4. Contemporary and historic levels of unsigned tarsus length asymmetry in five species of southern Californian riparian birds	33
5. Contemporary and historic levels of unsigned bill length asymmetry in five species of southern Californian riparian birds	34
6. Distribution of differences between repeated measurements of bill and tarsus length taken on live specimens and museum skins.....	35
7. FA10 results comparing changes in tarsus and bill length fluctuating asymmetry between historic and contemporary populations.....	38
8. Contrast of P-values generated from the Uncorrected and 0.01 datasets	39
9. Mean unsigned tarsus and bill length fluctuating asymmetry scores of contemporary populations of five species of southern California riparian birds.....	40
10. FA10 results comparing contemporary bill and tarsus length fluctuating asymmetry levels of the five focal bird species	41
11. Comparison of F-statistics and P-values generated by the FA10 and FPA analyses for the tarsus trait of five avian species.....	76

INTRODUCTION

Presently, wildlife populations are exposed to numerous anthropogenic sources of stress not previously encountered during their evolutionary history. The expansion of human populations has led to an increase in habitat loss and degradation through activities such as land conversion for agriculture, urban growth, and deforestation, potentially impacting species viability. Wildlife populations can be stressed further through exposure to heavy metals, pollutants and pesticides. All of these factors can have negative impacts on wildlife that can lead to a reduction in population size, and a loss of genetic diversity through inbreeding and genetic drift. These impacts can eventually lead to regional extirpation or the extinction of entire species (Meffe et al. 1997).

These environmental and genetic stressors have caused a large-scale human-induced decline in species, and a biodiversity crisis comparable to the five known natural mass extinctions of the past 3.5 billion years (Meffe et al. 1997). The World Conservation Union's (IUCN) year 2003 Red List identifies over 12,250 plants and animals as threatened, with 2,187 of these species designated as critically endangered (IUCN 2003). In the United States, the U.S. Fish and Wildlife Service currently lists 1,265 species of plants and animals as either threatened or endangered, with California ranking second only to Hawaii in the number of threatened or endangered wildlife, at 299 species (USF&WS 2004). This number has increased dramatically from 20 years ago when the USF&WS listed only 80 species as threatened or endangered within California (Beatley 1994). Once listed, the road to recovery is long. Since the passage of the U.S. Endangered Species Act (ESA) in 1973, only fourteen species have been recovered once their populations declined to a point where they warranted listing (USF&WS 2004).

With many more species being proposed for listing than are recovered, the need for an "early warning system" that can identify stressed populations prior to their experiencing precipitous population declines becomes obvious. If imperiled populations could be identified early and management actions taken to prevent declines, the benefits to the conservation of biodiversity (in the form of preservation of genetic diversity and preventing

extinction), and to society (by avoiding the regulations associated with species listing), become apparent. As the U.S. Fish and Wildlife Service (1995; page 1) has stated:

...an ounce of prevention is worth a pound of cure.” We fervently believe that a well-designed program that addresses resource-management at an early stage, thereby preventing species from having to be listed as Threatened or Endangered, will be more cost-effective than a full blown recovery effort required once a species is Federally listed.

The above USF&WS quote is compelling, but to date no cost-effective method has been developed to identify imperiled populations of wildlife, which is the first step in formulating “a well-designed program that addresses resource-management at an early stage.” Traditionally, population viability and status have been measured using labor-intensive monitoring programs that directly track life history parameters such as survival, fecundity, reproductive success, community structure, species diversity, and relative abundance or density. One major problem with these approaches is that indications of population problems typically do not manifest themselves until after declines have occurred (Clarke 1995). The assessment of species’ developmental stability, via the measurement of fluctuating asymmetry, has been put forth as a possible tool to provide the “early warning system” that conservation desperately needs (Leary and Allendorf 1989, Parsons 1992, Clarke 1995, Lens et al. 1999).

Developmental Stability and Fluctuating Asymmetry

Developmental stability (DS) is the capacity of an individual to buffer its development against random errors (Palmer and Strobeck 1986, Moller and Swaddle 1997). This has the effect of reducing the phenotypic variation of characters that could be expressed within a population (Clarke 1995). In an ideal, stress-free environment, bilaterally symmetric characters (e.g. right vs. left arms in humans) would be produced that are morphometrically identical. In reality, no such system exists, as there will always be some element of randomness in an organism’s development, resulting in asymmetry (Leamy 1984, Moller and Swaddle 1997). Developmental stability therefore relates to the capacity of an organism’s developmental pathways to resist accidents and perturbations during the growth process (Moller and Swaddle 1997). Normally, small perturbations during development are corrected by stability mechanisms, resulting in the expression of a genetically predetermined

phenotype. When stressed, developmental mechanisms that buffer against the expression of asymmetric characters may break down, leading to the production of deviant phenotypes (Leamy 1984, Clarke 1995). As organisms are placed under greater stress, less energy is available to buffer their development compared to unstressed individuals, and increasing levels of asymmetry can be expected (Parsons 1992). These deviations are expressed as minor discrepancies between right and left sides of bilaterally symmetric characters and are termed fluctuating asymmetries (Van Valen 1962).

Fluctuating asymmetry (FA) is the most commonly used index of developmental stability (Moller and Swaddle 1997, Whitlock 1996). The underlying assumption of FA is that the same genetic and developmental programs control the left and right sides of bilaterally symmetric traits (Whitlock 1996) and that any resulting asymmetry is a product of random, local disturbances that disrupt developmental pathways (Van Valen 1962). Therefore, individual asymmetry scores within a population can be used as a measure of an organism's ability to buffer its development against both genetic and environmental perturbations and can be considered an indirect measurement of individual fitness (Moller and Swaddle 1997, Clarke 1995). The spread of the distribution of the individual asymmetry scores for a population can be used as a robust measure of the stability of the population as a whole (Moller and Swaddle 1997). Since the metrics for conducting fluctuating asymmetry analysis are derived from taking the difference between right and left traits of bilaterally symmetric organisms, they are often simple and straightforward to measure, and can be statistically very powerful at detecting differences among populations (Moller and Swaddle 1997).

LITERATURE REVIEW

During development, stressors that can disrupt the developmental process and lead to the expression of FA are either genetic or environmental in origin (Clarke 1995). Genetic stressors are a consequence of such things as inbreeding depression (Clarke 1992, Clarke 1995, Moller and Swaddle 1997), extreme homozygosity (Leary and Allendorf 1989, Moller and Swaddle 1997), outbreeding depression (Clarke 1995), and the introduction of novel gene mutations (Leary and Allendorf 1989, Clarke 1995, Moller and Swaddle 1997). The consequences of these factors are a breakdown of possible co-adapted gene complexes and reduction of an organism's ability to buffer its development against random errors (Moller and Swaddle 1997). Environmental stressors can include, but are not restricted to, poor nutrition (Swaddle and Witter 1994), unusual temperatures (Parsons 1992, Moller and Swaddle 1997), starvation (Parsons 1992, Moller and Swaddle 1997), chemical pollutants (Leary and Allendorf 1989, Clarke 1995, Moller and Swaddle 1997), noise pollution (Moller and Swaddle 1997), and habitat fragmentation/degradation (Lens et al. 1999).

Environmental Stressors

Nutritional Stress

A number of studies illustrate how nutritional stress can lead to increased levels of fluctuating asymmetry. Swaddle and Witter (1994) investigated how sequential food deprivation affected primary feather and chest plumage symmetry of molting European starlings (*Sturnus vulgaris*). Starlings were randomly allocated into four feeding regimes, the control group (which had food available continuously throughout the experiment), the morning food deprived treatment (which had food removed for four hours beginning at dawn), the afternoon food deprived treatment (where food was removed for four hours, four hours after dawn) and the variable-time food deprived treatment (where food was randomly removed for four hours corresponding with either the dawn or afternoon deprivation treatments). Swaddle and Witter (1994) found that asymmetries were greatest in birds allocated to the variable-time treatment groups, followed by the fixed-time deprivation treatments. The control groups were determined to be the most symmetric. Therefore, results

indicated that food deprivation and the consistency with which food was provisioned could influence fluctuating asymmetry levels. In a similar fashion the investigators also found that primary feather length asymmetry was inversely related to the amount of subcutaneous fat stored during molt for all birds when treatment group and dominance were controlled for. Presumably birds that entered the experiment with fat reserves were better able to buffer their development against the energetic stress of the food deprivations. Increased nutritional stress has been shown to have comparable affects on fluctuating asymmetry levels in *Drosophila* (Parsons 1964), mice (Erway et al. 1970) and rats (Sciulli et al. 1979).

Temperature Stress

A variety of species express increased FA when raised outside of their optimal rearing temperature; these species include *Drosophila melanogaster* (Beardmore 1960, Parsons 1962, Wakefield et al. 1993), rainbow trout (*Salmo gairdneri*) (Leary et al. 1992), garter snakes (*Thamnophis elegans*) (Fox et al. 1961), and rats (Riesenfeld 1973). For example, McKenzie and Yen (1995) demonstrated that rearing Australian sheep blowflies (*Lucilia cuprina*) in temperatures above and below the norm results in elevated levels of fluctuating asymmetry. Additionally, fluctuating asymmetry levels were found to be greatest in individuals that were under multiple stressors. For example, Parsons (1962) found that fluctuating asymmetry levels varied with maternal age in *Drosophila* offspring, but were always greatest when reared at 30° C compared to 25° C, while Thoday (1955) found a positive correlation between FA, the degree of inbreeding, and temperature. Similarly, Beardmore (1960) and Bradley (1980) both demonstrated that fluctuating the temperature above and below the norm during development could cause a decrease in developmental stability in *Drosophila*.

Population Density - Induced Stress

It is clear from a variety of studies that increasing population density leads to elevated levels of fluctuating asymmetry. Moller et al. (1995) found FA levels in the length of the tarsometatarsus and radius were positively correlated with population density of chickens (*Gallus gallus*) reared in identical enclosures at three treatment densities. Asymmetries of chickens raised in the densest enclosures were 30 percent greater than in chickens reared at the lowest population density. Similarly, Clarke and McKenzie (1992) demonstrated that

larval Australian sheep blowflies were negatively affected by overcrowding, presumably due to increased competition for resources, producing adults with elevated asymmetry levels. Within natural systems, Zakharov (et. al 1991) found similar trends in a cyclic population of common shrews (*Sorex araneus*). The researchers found a high correlation between population density and developmental stability, with fluctuating asymmetry levels increasing positively with density. Fluctuating asymmetry has also been shown to be an indicator of population stress in plants (Rettig et al. 1997). Rettig et al. (1997) used a factorial design to investigate population density, and stress induced from weed competition, on *Populus euramericana* leaf asymmetry. Results indicated a positive relationship between fluctuating asymmetry levels and both population density of trees and competition from weeds.

Chemical Stress

Wildlife exposure to chemicals has increased dramatically since the Industrial Revolution and the introduction of pesticide and herbicides into agriculture. Exposure to chemical pollutants has negatively impacted a variety of wildlife species, and has even led to the decline of some species such as the bald eagle (*Haliaeetus leucocephalus*) and peregrine falcon (*Falco peregrinus*) (Gill 1995). It is apparent from the literature that organismal developmental stability can be impaired through exposure to chemical pollutants and these stressors can result in an increase in fluctuating asymmetry. For example, Ames et al. (1979), Zakharov (1981), and Jagoe and Haines (1985) all found increased levels of fluctuating asymmetry in fish species inhabiting ponds with high concentrations of mercury and/or low pH. Similarly, Clarke (1993a) found elevated FA levels in invertebrates surrounding an area polluted by a fertilizer manufacturing facility, while elevated heavy metal concentrations have been shown to decrease developmental stability in the common shrew (*Sorex araneus*) (Pankakoski et al. 1992). In a similar fashion, p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) exposure under controlled conditions has been shown to increase FA levels in grunion (*Leuresthes tenuis*) fry (Valentine and Soule 1973), while PCB's (polychlorinated biphenyls) have been implicated in causing bill abnormalities in double-crested cormorants (*Phalacrocorax auritus*) in the Great lakes region (Fox et al. 1991).

Genetic Stressors

While examples of how environmental stress can increase fluctuating asymmetry within a population tend to be straightforward, the ways by which genetic stressors potentially influence asymmetry are more complex. One of the major genomic stressors that can result in an increase in asymmetric character expression is the loss of genetic variability, resulting in the expression of recessive deleterious alleles through processes such as inbreeding (Moller and Swaddle 1997). For example, Clarke (1992), Clarke et al. (1986) and Beardmore (1960) found increased levels of fluctuating asymmetry in inbred populations of the marine copepod (*Tisbe holothuriae*) and in two inbred strains of *D. melangaster* compared to outbred control populations. In *T. holothuriae*, statistically significant increases in asymmetry were detected for four out of five characters measured after only a single generation of inbreeding, while the fifth character showed significant increases after the second generation of inbreeding (Clarke 1992). However, a reduction in heterozygosity does not always result in increases in fluctuating asymmetry. For example, Kieser and Groeneveld (1991) found no difference in dental dimensions taken from skulls of the South African cheetah (*Acinonyx jubatus jubatus*) when compared to two other African felids, *Felis lybica* and *F. caracal*, despite the fact that cheetah populations are suspected to have gone through a severe evolutionary bottleneck in recent history as indicated by their low genetic variability (Kieser and Groeneveld 1991). To explain this discrepancy, it has been theorized that cheetahs may have displayed elevated levels of asymmetry around the time of the initial bottleneck, when deleterious alleles were first expressed, but enough time has passed for those alleles to be removed from the population through selection, restoring the cheetah's genetic balance and reducing asymmetry levels (Moller and Swaddle 1997).

Greater genetic variability does not always lead to greater character symmetry. For example, the opposite effect has been observed when closely related species hybridize, or historically isolated populations interbreed (Bader 1965, Graham and Felley 1985, Leary et al. 1985, Ferguson 1986, Ross and Robertson 1990). The resulting decrease in fitness has been theorized to be a consequence of the breakup of co-adapted gene complexes, resulting from what is referred to as outbreeding depression (Clarke 1993b, Meffe et al. 1997). The formation of co-adapted gene complexes results from the selection of groups of genes (and their gene products) that harmoniously and efficiently function together (Clarke 1992, Moller

and Swaddle 1997, Meffe et al. 1997). These complexes are established over evolutionary time and maintained through stabilizing selection. Through reproductive isolation, population genomes can evolve independently, establishing gene complexes adapted to local or regional environments. The reuniting or interbreeding of such populations can have the effect of destroying these complexes and disrupting developmental processes (Clarke 1992, Moller and Swaddle 1997). Once disrupted, the newly formed allelic combinations, which have not been subjected to natural selection, may not function as efficiently as before, resulting in greater fluctuating asymmetry (Moller and Swaddle 1997). This has been seen in numerous studies, where fluctuating asymmetry levels were elevated after “hybridization”, even in cases where genetic diversity was increased in the offspring (Bader 1965, Graham and Felley 1985, Leary et al. 1985, Ferguson 1986, Ross and Robertson 1990). In summary, although the response may be unpredictable, or difficult to interpret in uncontrolled conditions, it is clear that changes to a population’s genome can influence its developmental stability (Moller and Swaddle 1997).

As previously stated, under normal conditions, buffering mechanisms within an organism are able to correct developmental perturbations that occur during ontogeny. However, as stressors are introduced into a system, exposed organisms must expend more metabolic energy to perform the same functions as unstressed individuals (Parsons 1992). As the effects of genetic and environmental stressors are not independent, the strain they impose becomes cumulative. Therefore, in a developmental framework, all stressing agents can be considered cumulative (Clarke 1995). This can result in potentially even minor perturbations having a significant impact on FA levels when an organism is already stressed (Clarke 1995).

Habitat Fragmentation and Fluctuating Asymmetry

Habitat fragmentation and degradation can apply a variety of environmental and genomic stresses to populations. Fragmentation occurs when large tracts of habitat are broken into small, isolated patches that may no longer be able to support species into the indefinite future (Meffe et al. 1997). Fragmentation can have a negative effect on population genomes by reducing gene flow and promoting the loss of genetic variation by isolating once-connected populations and creating barriers to dispersal, in effect leading to inbreeding and genetic drift. In a similar manner, physical changes brought about by fragmentation can

have adverse and often unpredictable consequences that can cascade through the remaining habitat. For example, research in Peru investigating the effect of fragmentation on rainforest ecosystems found that after fragmentation, wind and sunlight penetration into the remaining patches increased dramatically. This had the effect of drying out areas 50 to 100 meters into the patches, resulting in a total change in community dynamics, causing birds, some insects, and primate species to decline while causing small mammal, amphibian and butterfly populations to increase. Also, interspecific interactions changed as decomposition rates declined, and key pollinators dropped out of the system, resulting in a subsequent decline of some plants due to a reduction in seed set (Lovejoy et al. 1984).

Another example of the complex web of changes that can be brought about through habitat fragmentation can be seen in the Kellerberrin Project in Western Australia (Saunders 1989, Saunders 1993). Historically, the 1680 km² area under study consisted of vast tracts of Eucalyptus (*Eucalyptus* spp.) and scrub forest. Due to deforestation, a mere six percent of the forest remains in 459 patches varying in size from less than 1 hectare to 1030 hectares. Annual peak runoff has increased, facilitating soil loss and flooding, and the water table has risen, water-logging the soil and increasing its salinity. Furthermore, 34 species of birds have declined while the abundance of nine species has increased. Additionally, the new matrix of habitat has influenced kangaroo population dynamics. Kangaroos prefer to move between habitat patches through corridors of native vegetation. The new environment of disturbed/native habitat impedes kangaroo movements, fragmenting large, once contiguous populations and making the resulting isolated groups more susceptible to the effects of inbreeding and genetic drift

Other consequences of habitat fragmentation not mentioned in the above examples include: (1) the introduction of invasive, exotic species that can compete with native species for limited resources, and (2) an effect referred to as “crowding of the ark” where remnant patches of habitat experience an initial population increase as displaced species retreat into the remaining vegetation (Meffe et al. 1997).

As can be seen from the above examples, habitat fragmentation/degradation can have a variety of unexpected effects on wildlife populations and impose numerous stresses on the remaining populations. Since these stressors can be genetic and/or environmental in origin, they should be reflected in a population’s fluctuating asymmetry level, and populations under

more stress would be expected to have elevated FA levels in comparison to those under less or no stress, assuming species' abilities to buffer against stress are equal. This prediction is similar to what Lens et al. (1999) observed when they investigated the developmental stability of seven forest bird species in a fragmented system. Their study consisted of capturing and measuring birds from three remnant fragments of cloud forest in southeastern Kenya. The largest fragment (220 ha) was judged to be the highest quality, while the smallest fragment (50 ha) was considered to contain the most degraded habitat. Individuals from the most degraded fragment showed four- to seven-fold higher asymmetry levels than those from the least degraded fragment, with birds from the intermediate sized fragment displaying moderate levels of asymmetry. Lens et al. (1999) also compared current FA levels to historic measurements derived from museum skins collected 50 years ago, and found significantly higher fluctuating asymmetry levels in contemporary populations of species from the most degraded fragments, but no increase from the least degraded one. From this they conclude that "these strikingly parallel spatial and temporal patterns across species confirm that...FA can provide a sensitive early warning system for monitoring stress effects in highly threatened ecosystems".

Objective

Results from previous research (Ames et al. 1979, Zakharov 1981, Jagoe and Haines 1985, Zakharov et al. 1991, Clarke 1992, Pankakoski et al. 1992, Clarke 1993a, Clarke 1993c, Lens et al. 1999, Lens et al. 2002) indicate that FA techniques may be a sensitive and cost effective method of assessing the current "health" of populations. However, a greater understanding of how FA is expressed in wildlife populations through time, and across species, is desirable prior to implementing FA techniques as a possible management tool in identifying species/populations in peril. The majority of the studies in which we might desire to draw conclusions about the applicability of FA to applied management have: 1) focused on a single species in a system (Beardmore 1960, Clarke and Mckenzie 1987, Leamy 1992, Leary et al. 1992, Moller et al. 1995, Fair and Ricklefs 2002, Frechette and Daigle 2002), 2) quantified contemporary levels of fluctuating asymmetry with no reference to FA scores for that species at other times in its history (Beardmore 1960, Leamy 1984, Leamy 1992, Leary et al. 1992, Moller et al. 1995, Fair and Ricklefs 2002, Frechette and Daigle 2002), or 3)

derived FA scores under controlled laboratory conditions with no reference to the wild (Beardmore 1960, Leamy 1984, Clarke and Mckenzie 1987, Leamy 1992, Moller et al. 1995, Fair and Ricklefs 2002).

The objective of this study was to evaluate the use of fluctuating asymmetry as a tool for identifying imperiled avian populations and to investigate whether the temporal and spatial patterns identified by Lens et al. (1999) hold for avian species of differing conservation status in a southern Californian riparian ecosystem. Results from Lens et al. (1999) suggest that species of like conservation status (i.e. endangered/threatened, sensitive, healthy/abundant) should possess similar fluctuating asymmetry levels in response to environmental and/or genetic stressors. Also, current theory within the field, and results from other investigators, suggest that healthy species/populations should show lower FA scores when compared to imperiled populations. If valid, these tenets make it possible to formulate statistically testable hypotheses about patterns of FA scores between species, and within a species over time.

By deriving fluctuating asymmetry scores for contemporary and historic populations of five avian, riparian-obligate species of differing conservation status within the same region, I sought to (1) determine if the trends observed by Lens et al. (1999) in a cloud forest ecosystem in Kenya hold for a lowland riparian ecosystem in southern California, (2) evaluate the assumption that FA levels within contemporary populations are similar for species of like conservation status, and (3) evaluate the assumption that FA scores and conservation status are correlated. If this last assumption is correct, then FA levels should be greatest in populations of threatened/endangered species, followed by species of special concern, with abundant species possessing the lowest asymmetry.

Lowland riparian habitat is one of the most highly threatened ecosystems in California. Since the beginning of the last century, fragmentation and modification have led to large-scale losses of habitat attributable to numerous causes (many of which are still occurring), including urban, recreational, and agricultural development, livestock grazing, water diversion and impoundment, river channelization, and the introduction of nonnative plant and animal species (RHJV 2004). Current figures estimate that only 2 to 15 percent of California's native riparian community remains unimpacted (RHJV 2004). The result has been the decline and State and/or Federal listing of numerous vertebrate species that depend

on riparian communities for at least part of their life cycle. At present, approximately 40 percent of all vertebrates listed as state or federally endangered in California are riparian obligates (CDFG 2001).

Because the reasons for decline of southern California's riparian species are diverse, riparian systems make an excellent natural laboratory in which to investigate the use of fluctuating asymmetry as a tool for the identification of imperiled species. If fluctuating asymmetry provides a true measure of the "health" of a species, then imperiled species should exhibit higher asymmetry levels than abundant (presumably "healthier") species. Furthermore, contemporary asymmetry scores should be elevated when compared to those of historic populations (i.e. populations existing prior to habitat degradation) of the imperiled species, compared to the healthy taxa.

Riparian obligate birds make excellent focal species for investigating these questions due to their diversity, distribution, contemporary variability in species health, and the ability to sample them in large numbers using an existing framework of bird banding stations. Furthermore, riparian bird skins collected in the early twentieth century tend to be well represented in museum collections, facilitating the comparison of fluctuating asymmetry levels within and between species through time.

METHODS

Study Sites and Species

Eight riparian bird species were initially evaluated as possible focal species to assess the effectiveness of using fluctuating asymmetry to identify imperiled avifauna. Focal species were chosen in an attempt to cover the widest range of “health” of extant species. In the context of this study, the terms health and conservation status are used synonymously to refer to the level of concern associated with populations of a species. Species labeled as “threatened or endangered” are currently considered vulnerable to extinction within California due to declining populations, limited ranges and/or continuing threats; “Sensitive” species have recently shown population declines within California, but are not in danger of extinction in the short term; while “healthy” species are those that have not shown marked declines from historic population sizes and are therefore not considered vulnerable to extinction. A species’ final inclusion in the study was determined based on its (1) current State and/or Federal endangered species status, (2) California Species of Special Concern status, (3) abundance within museum collections, and (4) the ease with which contemporary individuals of the species could be sampled. Five of the eight species were selected for inclusion and grouped into three categories: (1) Threatened/Endangered, (2) Sensitive, (3) Healthy. The Federal and State listed endangered Least Bell’s Vireo (*Vireo bellii pusillus*) and Southwestern Willow Flycatcher (*Empidonax traillii extimus*) were selected as contemporary Threatened/Endangered species. The Yellow-breasted Chat (*Icteria virens*), a Federal and State listed Species of Concern, was selected as a “Sensitive” species, and the Common Yellowthroat (*Geothlypis trichas scirpicola*) and Song Sparrow (*Melospiza melodia cooperi*), which have no State or Federal conservation designation, were selected as “Healthy” species.

Within the context of this study, southern California is designated to include the following counties: Los Angeles, Orange, Riverside, San Diego, and the southwest portions of San Bernardino and Ventura Counties. Collection locations of museum skins were identified within these counties, and contemporary populations of the five focal species were

sampled within the same area. However, large numbers of the museum skins were originally collected from areas close to urban centers. As a result of urban expansion, and restrictions on accessing private lands, much of the historic habitat where these birds were collected no longer exists or is inaccessible. Therefore, contemporary field sampling sites do not exactly match the locations of historically collected birds, but were located on accessible lands containing lowland riparian habitat.

Field sampling for live birds was conducted in 2002 at 21 sites on 15 drainages within southern California, and in 2003 at two sites on two drainages (Figure 1). Because of the large effort required to live-capture passerines, six established banding stations were used to aid in data collection, while 15 other sites were selected to increase the sampling distribution of contemporary populations. Passive mist-netting was used to catch contemporary samples of all five focal species. In addition, target-netting using taped vocalizations to attract birds to nets were employed to increase the sample sizes of the Yellow-breast Chat, Least Bell's Vireo, and Southwestern Willow Flycatcher. All birds captured were processed following standardized protocols (DeSante and Burton 1997). Sampling sites ranged from the Santa Clara River in Ventura County south to the Tijuana River in San Diego County, approximately 2.4 kilometers north of the United States/Mexico border. All sites sampled were within 64 kilometers of the Pacific Ocean.

Museum skins from 92 collection locations were selected for measurement. Historic collection locations were generally located within the same region described for contemporary sampling, but also included sites further inland. To increase the sample size of historically collected Southwestern Willow Flycatcher museum skins, it was necessary to include birds collected as far north as Kern (one specimen) and Inyo (two specimens) Counties, as far south as northern Baja Mexico (four specimens), and as far east as Imperial County (nine specimens), up to 260 kilometers from the Pacific Ocean. Skins included in this study were part of the ornithological collections of the following museums/universities: Natural History Museum of Los Angeles County, San Diego Natural History Museum, San Diego State University, University of California Berkeley Museum of Vertebrate Zoology, and the University of California Los Angeles Dickey Bird and Mammal Collections. Museum specimens included in this study were originally collected by shotgun between 1887 and 1938.

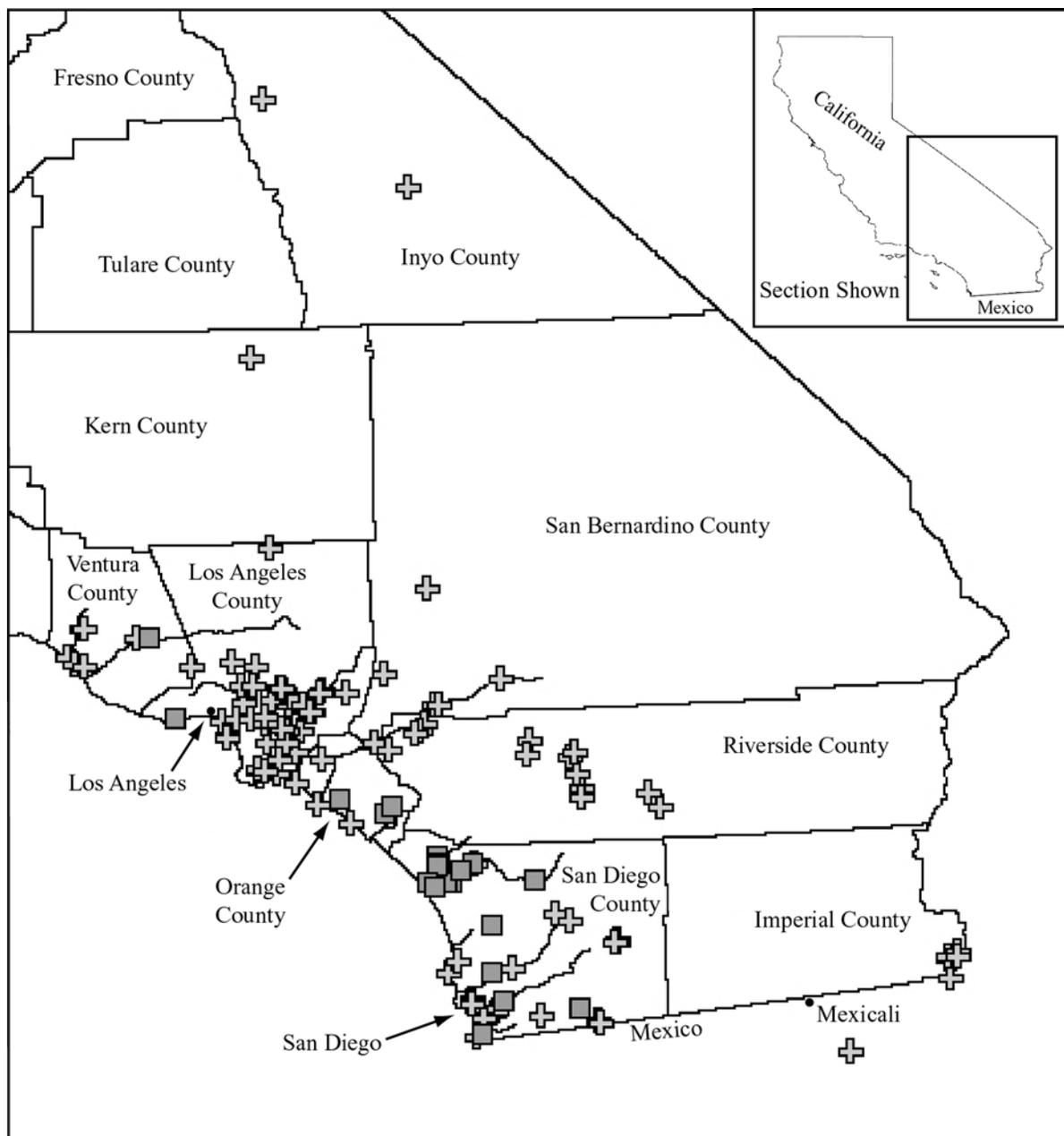


Figure 1. Locations of collection sites for live birds (squares) and museum skins (crosses) within southern California and northern Mexico.

To avoid the possibility of sampling migrating birds, all birds captured in nets, or skins residing in museums, were processed only if their collection date fell within each species' designated "Breeding season/Resident period" (Unitt 1984, Kus pers. comm.). The collection periods for each of the focal species were: Song Sparrow (16 February – 31 August), Common Yellowthroat (10 April – 5 September), Yellow-breasted Chat (28 April –

31 August), Least Bell's Vireo (26 March – 5 September), and Southwestern Willow Flycatcher (1 June – 31 July). To increase the sample size of historic Southwestern Willow Flycatchers, 12 museum skins collected outside of the 1 June – 31 July dates were measured. Therefore, the sampling period for Willow Flycatcher museum skins ranged from 4 May - 28 September.

Measurement of Fluctuating Asymmetry

Nine bilaterally symmetric characters were initially evaluated to assess their feasibility of measurement and repeatability (see Appendix A for a list of traits evaluated). After initial evaluation, three traits were selected for further scrutiny: length of tarsus, length of bill, and length of hallux. Skeletal characters were specifically chosen to avoid the possibility of measuring “false” asymmetries that could result from uneven wear of less durable materials such as feathers. A subsequent power analysis revealed that only the length of tarsus and the length of bill could be measured with enough precision to warrant their inclusion in this study (Appendix B).

Tarsus length was measured from the notch on the back of the intertarsal joint to the lower edge of the last complete scale before the toes diverge (Lens et al. 1999, Baldwin et al. 1931). Length of bill was measured from the anterior end of the nostril to the anterior end of the maxilla (Baldwin et al. 1931). To be able to statistically quantify measurement error, three repeated measurements of the right and left tarsus, and four repeated measurements of the right and left bill, were made on each specimen. Determination of the appropriate number of measurements to be taken on each trait was derived from results of a Pearson correlation analysis conducted on preliminary bill and tarsus data (Appendix C). Measurements were taken to the nearest 0.01 mm using digital slide calipers. To reduce bias, measurements were (1) alternated between traits on each side of a specimen (sequence left bill, left tarsus, left bill, left tarsus, left bill, left tarsus, left bill; right bill, right tarsus, right bill, right tarsus, right bill, right tarsus, right bill), (2) entered directly into a Handspring Visor Deluxe personal digital assistant (PDA) using Pendragon Forms 3.2 software (once entered, the measurement was stored and the user was prompted for the next measurement, making previous measurements unavailable for review), and (3) all taken by James Rourke. Damage to museum skins incurred during initial collection, and the escape of live birds,

prevented all measurements from being taken on all specimens. As a result, sample sizes vary among analyses.

Fluctuating Asymmetry Analyses

Fluctuating asymmetry scores for individuals or populations can be greatly influenced by measurement error (ME) if certain precautions are not taken prior to conducting statistical analyses (Palmer and Strobeck 2003). The principal reason for this is that the distribution of signed FA scores and ME display the same statistical properties (i.e. are normally distributed with a mean of zero) and are therefore indistinguishable (Palmer and Strobeck 1986, Palmer 1994, Swaddle et al. 1994). Consequently, the result of sloppily taken measurements is an unwarranted inflation in FA estimates. It is therefore essential to assess the relative influence of measurement error on asymmetry estimates prior to conducting analyses to determine if FA levels differ between individuals, populations, through time, etc. (Palmer and Strobeck 2003).

To ensure that extreme measurements, which could result from injury, wear, or calibration or data recording errors, would not unduly bias overall FA estimates of individual birds, differences between repeated measurements of a trait were computed (i.e. the difference between the first and second right tarsus measurements, the first and third measurements, etc.). Differences between repeated measurements were grouped by species, trait (bill or tarsus), and collection type (museum skin or live bird), and each point was tested against zero and the group mean to evaluate it as a possible outlier using Grubb's test (t_G) (Palmer 1994, Palmer and Strobeck 2003):

$$(1) \quad t_G = (X_i - \bar{X}) / SD$$

where X_i is the difference between two repeated measurements of a trait, \bar{X} is the mean of the sample group of repeated measurements, and SD is the standard deviation of \bar{X} . Alpha was set at 0.01 and a sequential Bonferroni correction for multiple tests was then applied (Palmer 1994). Specimens determined to contain statistically significant outliers for a particular trait were removed from further analyses for that trait.

The data were visually inspected for unusually large (or small) trait values, which could result from injury, wear, or calibration error, by computing and plotting the average left vs. right bill and tarsus size per specimen from the sets of repeated measurements. Any aberrant points were flagged as possible fluctuating asymmetry outliers. Individual fluctuating asymmetry scores per trait were then calculated by subtracting the averaged left trait size from the averaged right trait size for bill and tarsus. To identify more subtle, but statistically significant FA outliers, individual FA scores were grouped by species, trait, and collection type, and tested against the appropriate group mean FA using Grubb's test (Palmer and Strobeck 2003). Since it is possible for extreme outliers to unduly influence the results of FA analyses, outliers were removed at three significance levels to better evaluate their effects, creating three datasets. Subsequent FA analyses were run using each dataset and FA results were compared between all datasets. The significance levels at which outliers were removed were: $\alpha = 0.05$ (hereafter, 0.05 dataset), 0.01 (hereafter, 0.01 dataset), and 0.01 with a 20 group Bonferroni multiple comparisons correction. Because no specimens were considered outliers after the Bonferroni correction was applied, this dataset will be referred to as the "Uncorrected dataset". Specimens determined to be outliers were removed from further analyses for that trait within the appropriate dataset.

To be confident that observed asymmetries were a result of developmental instability and not an artifact of measurement error, mixed model two-way ANOVAs were applied to each dataset to determine if asymmetries due to FA were greater than asymmetries associated with ME (Palmer and Strobeck 2003, Swaddle et al. 1994). Within the ANOVA, Measurement Side (hereafter Side, i.e. left and right) was specified as a fixed factor, Individual Specimen (hereafter Individual) was designated a random factor, and the Individual Repeated Measurements were specified as the dependent variable. F-statistics for the mixed model ANOVAs were calculated according to Zar (1998). Statistical significance was set at $\alpha = 0.05$. Within the models, statistically significant p-values for Side are indicative of directional asymmetry, significant p-values for Individual are indicative of differences in trait size among individuals within a group, the interaction term Side*Individual tests whether asymmetries are greater than measurement error, and the Error term describes the variance in the repeatability of the repeated measurements. Because

measurement error could differ between traits, species, or collection types, separate analyses were performed for each species*trait*collection combination.

Fluctuating asymmetry has been shown to change in a predictable way with trait size in some species (Palmer and Strobeck 2003, Moller and Swaddle 1997). Typical changes include asymmetries positively scaling with trait size, resulting in large individuals possessing proportionally larger asymmetries than smaller individuals. Such instances may yield differences between groups that are an artifact of trait size alone, with significant results disappearing when asymmetry scores are corrected for the size of the trait. To investigate whether fluctuating asymmetries were size-dependent, scatterplots of trait asymmetry $|\text{left} - \text{right}|$ vs. trait size $[(\text{left} + \text{right})/2]$ for bill and tarsus were inspected for each dataset. Next, nonparametric Spearman correlation analyses were conducted for each species and dataset to ensure that there was no association between trait asymmetry and trait size. A nonparametric analysis was used because parametric tests can be influenced greatly by a few extreme observations, and are therefore more likely to yield erroneous results.

Fluctuating asymmetry is characterized as having deviations from symmetry normally distributed around a mean of zero. Departures from this distributional type include directional asymmetry, where the distribution is shifted away from zero, and antisymmetry, which is characterized by a bi-modal distribution. To test whether asymmetry data were centered around a mean of zero, one sample t-tests were run against zero for each trait by species and collection type, using all datasets. To ensure the data were normally distributed, left vs. right bill and tarsus length probability plots were inspected.

Contemporary vs. Historic Asymmetry

Levene's test for heterogeneity of variance was used to test for differences in measurement error between measurements taken on historically collected museum skins and contemporary live birds of the same species (Palmer and Strobeck 2003). Because measurement error was found to differ significantly between historic and contemporary samples of all species for both bill and tarsus length (all $p < 0.0001$), only statistical methods that corrected for ME were used.

For each trait, fluctuating asymmetry differences between contemporary and historic populations of the same species were analyzed using two methods that corrected for both measurement error and directional asymmetry to yield unbiased FA estimates. In the first

analysis, Palmer and Strobeck's (Palmer and Strobeck 2003) FA10 index was used to compute asymmetry variance terms that were corrected for ME and directional asymmetry (DA) for each population sampled (i.e. historic and contemporary Song Sparrows, historic and contemporary Common Yellowthroats, etc.). This analysis uses the mean square statistics generated in the above measurement error analyses to compute the adjusted FA variances:

$$(2) \quad \sigma_i^2 = (MS_{si} - MS_e)/M$$

where σ_i^2 is the population level fluctuating asymmetry corrected for ME and DA, MS_{si} is the sides*individual mean square term for that population, MS_e is the error mean square of the population, and M is the number of replicate measurements taken on a trait. F-statistics were then calculated by dividing the larger population variance by the smaller variance to test for FA differences between contemporary and historic populations of the same species (e.g. contemporary Willow Flycatcher bill length σ_i^2 / historic Willow Flycatcher bill length σ_i^2). Within the FA10 analyses, degrees of freedom for the corrected variance terms are computed using the following formula (Palmer and Strobeck 2003):

$$(3) \quad \text{Approximate degrees of freedom for } \sigma_i^2 = \frac{(MS_{sj} - MS_e)^2}{\left(\frac{(MS_{sj})^2}{(S-1)(J-1)} + \frac{(MS_e)^2}{SJ(M-1)} \right)}$$

where S is the number of sides measured per specimen, and J is the number of individuals in the group sampled. The computation of degrees of freedom using equation 3 results in a more conservative estimate of the significance of the main effects by adjusting the degrees of freedom downward as measurement error increases and approaches the variability associated with the fluctuating asymmetry of a sample (MS_{sj}).

In the second analysis, first principles (hereafter FPA analysis) were used to correct for measurement error and directional asymmetry when calculating population level FA variance terms that were then used to generate F-statistics to test for differences between

populations for each trait. First, the population level FA variance (σ_u^2), uncorrected for ME, was computed as follows for each trait:

$$(4) \quad \sigma_u^2 = \frac{\sum ((\bar{R}_i - \bar{L}_i) - \bar{Y})^2}{n}$$

where \bar{R}_i is the mean of the repeated measurements taken on the right side of specimen i , \bar{L}_i is the mean of the repeated measurements taken on the left side of specimen i , \bar{Y} is the grand mean computed from all $(\bar{R}_i - \bar{L}_i)$ in a population, and n is the number of individuals in the population. This equation corrects for directional asymmetry by using the grand mean instead of zero in calculating the variance between the right and left sides for a particular trait.

Second, the variability due to measurement error (σ_{me}^2) was calculated for a population using the following formula:

$$(5) \quad \sigma_{me}^2 = \frac{2}{M} \left(\frac{\sum (X_{i,j,k} - \bar{X}_{i,j})^2}{n} \right)$$

where $\frac{2}{M}$ is the number of sides measured on a specimen (i.e. left and right) over the number of repeated measurements (M) taken per side on a trait, $X_{i,j,k}$ designates an individual measurement (k) taken on side j of specimen i , $\bar{X}_{i,j}$ is the mean of M repeated measurements taken on side j of specimen i , and n is the number of specimens in the sample population.

The FA variance of a population, corrected for measurement error and directional asymmetry, was then computed by subtracting the variance associated with ME (σ_{me}^2) from the population FA variance corrected for DA (σ_u^2):

$$(6) \quad \text{Population variance corrected for ME and DA } (\sigma_{fa}^2) = \sigma_u^2 - \sigma_{me}^2$$

F-statistics were then calculated by dividing the larger population variance by the smaller variance as done in the FA10 analysis (e.g. corrected contemporary σ_{fa}^2 / corrected historic σ_{fa}^2). Degrees of freedom were calculated as the number of specimens in the population minus one ($n - 1$).

Species of Like Conservation Status

Levene's test for heterogeneity of variance was used to test for differences in ME between measurements taken on contemporary specimens of species of like conservation status (e.g. healthy, sensitive, endangered) prior to testing for differences in FA. Because measurement error differed significantly between the abundant species' (i.e. Common Yellowthroat and Song Sparrow) in two of the bill length datasets (0.01 and uncorrected datasets, $p < 0.05$) and all of the tarsus length datasets (all $p < 0.02$), the FA10 and FPA statistical techniques, which correct for ME and directional asymmetry, were used when testing for differences in FA as described above. To evaluate whether fluctuating asymmetry levels were similar in abundant species, contemporary FA levels of the Common Yellowthroat and Song Sparrow were compared. To test for differences in fluctuating asymmetry levels of endangered species, contemporary FA levels of the Least Bell's Vireo and Southwestern Willow Flycatcher were contrasted. Differences between sensitive species could not be evaluated because only a single sensitive species (Yellow-breasted Chat) was included in the study.

FA and Conservation Status

Because ME associated with the measurement of the contemporary birds differed between species (see above), analyses such as linear regression/correlation that are powerful in detecting associations between variables were not used because they do not correct for measurement error or the presence of DA. Consequently, the FA10 and FPA analyses were employed to evaluate the hypothesis that fluctuating asymmetry and conservation status are correlated, with endangered species possessing the highest FA levels, followed by species of special concern, and finally abundant species. Because the variability in measurement error made grouping species according to conservation status inappropriate, individual tests were conducted for pairs of all possible combinations of the five focal species to investigate differences in FA levels. The resulting P-values between comparisons were then examined

to determine if fluctuating asymmetry and conservation status were correlated in a predictable manner.

RESULTS

Two hundred and fifty live birds were measured during the 2002 and 2003 breeding seasons. Two hundred and ninety-four museum skins, collected within the designated year range and sampling periods for each species, were measured from 26 August 2002 to 4 April 2003. However, because some museum skins had suffered damage during collection and a few live birds possessed obvious physical deformities, the sample sizes across traits within species are not equal (Table 1).

Table 1. Sample sizes of live birds and museum skins measured by species and trait.

Collection Type	BEVI ^a		COYE ^b		SOSP ^c		SWFL ^d		YBCH ^e	
	Bill ^f	Tarsus ^g	Bill	Tarsus	Bill	Tarsus	Bill	Tarsus	Bill	Tarsus
Live	44	45	69	69	68	67	37	37	31	31
Museum	63	49	42	34	110	99	33	30	34	34

^aBEVI = Least Bell's Vireo

^bCOYE = Common Yellowthroat

^cSOSP = Song Sparrow

^dSWFL = Southwestern Willow Flycatcher

^eYBCH = Yellow-breasted Chat

^fBill = bill length (mm)

^gTarsus = tarsus length (mm)

Grubb's test identified outliers in the repeated measurements associated with five tarsi (all museum skins) and 11 bills (six museum skins and five live birds) of 15 specimens (Table 2). These birds were removed from further analyses for the appropriate traits.

Results from plotting average left bill and tarsus length vs. average right bill and tarsus length, respectively, indicated no overtly asymmetric individuals that could have resulted from trait damage, trait wear, calibration error, etc. (Figure 2). Grubb's test to identify statistically significant fluctuating asymmetry outliers that could unduly influence population level FA tests identified between 1 and 4 specimens (depending on the trait and collection type) as outliers when alpha was set at 0.01, and between 7 and 15 specimens when alpha was set at 0.05 (Table 3). None of the specimens were considered outliers after a Bonferroni correction for multiple comparisons was used ($n = 20$ groups). Because outliers were removed at different significance levels, sample sizes relevant to a specific species and

Table 2. Statistically significant results of Grubb's test on individual repeated measurement differences versus the mean trait difference and versus zero, applied to bill and tarsus length, to detect outliers. Tests were two-tailed. A sequential Bonferroni correction for multiple tests was applied ($N_{\text{Groups}} = 20$, 5 species x 2 collection types x 2 morphological traits).

Species ^a	Type ^b	Trait	Differences between repeated measurements			Outlier ^c (mm)	Test vs. mean		Test vs. zero	
			df	Mean	SD		t_G	p	t_G	p
BEVI	Museum	Bill	827	-0.003	0.076	0.34	4.51	< 0.001	4.47	< 0.001
BEVI	Museum	Bill	827	-0.003	0.076	-0.29	3.78	< 0.001	3.82	< 0.001
		Bill	827	-0.003	0.076	-0.36	4.70	< 0.001	4.74	< 0.001
		Bill	827	-0.003	0.076	-0.31	4.04	< 0.001	4.08	< 0.001
		Bill	827	-0.003	0.076	-0.31	4.04	< 0.001	4.08	< 0.001
BEVI	Museum	Tarsus	323	0.005	0.091	0.33	3.57	< 0.001	3.63	< 0.001
COYE	Live	Bill	827	-0.002	0.123	-0.49	3.97	< 0.001	3.98	< 0.001
COYE	Live	Bill	827	-0.002	0.123	0.44	3.59	< 0.001	3.58	< 0.001
COYE	Museum	Tarsus	317	0.008	0.073	0.42	5.64	< 0.001	5.75	< 0.001
		Tarsus	317	0.008	0.073	0.27	3.59	< 0.001	3.70	< 0.001
SOSP	Live	Bill	815	0.003	0.112	0.46	4.08	< 0.001	4.11	< 0.001
		Bill	815	0.003	0.112	-0.42	3.78	< 0.001	3.75	< 0.001
SOSP	Museum	Bill	1643	0.008	0.078	0.29	3.62	< 0.001	3.72	< 0.001
SOSP	Museum	Bill	1643	0.008	0.078	-0.27	3.56	< 0.001	3.46	< 0.05
SOSP	Museum	Bill	1643	0.008	0.078	0.38	4.77	< 0.001	4.87	< 0.001
		Bill	1643	0.008	0.078	0.28	3.49	< 0.001	3.59	< 0.001
SOSP	Museum	Tarsus	749	0.002	0.078	0.28	3.56	< 0.001	3.59	< 0.001
SOSP	Museum	Tarsus	749	0.002	0.078	0.30	3.82	< 0.001	3.85	< 0.001
SWFL	Live	Bill	443	-0.003	0.122	-0.54	4.40	< 0.001	4.43	< 0.001
		Bill	443	-0.003	0.122	-0.44	3.58	< 0.001	3.61	< 0.001
YBCH	Live	Bill	371	0.002	0.134	-0.53	3.97	< 0.001	3.96	< 0.001
YBCH	Museum	Bill	431	-0.004	0.086	-0.33	3.79	< 0.001	3.84	< 0.001
		Bill	431	-0.004	0.086	-0.36	4.14	< 0.001	4.19	< 0.001
		Tarsus	215	-0.003	0.070	-0.25	3.53	< 0.001	3.57	< 0.001

^a Species: BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat

^b Type: Museum = Museum Skin, Live = Live Bird

^c The difference in millimeters between two repeated measurements of a trait

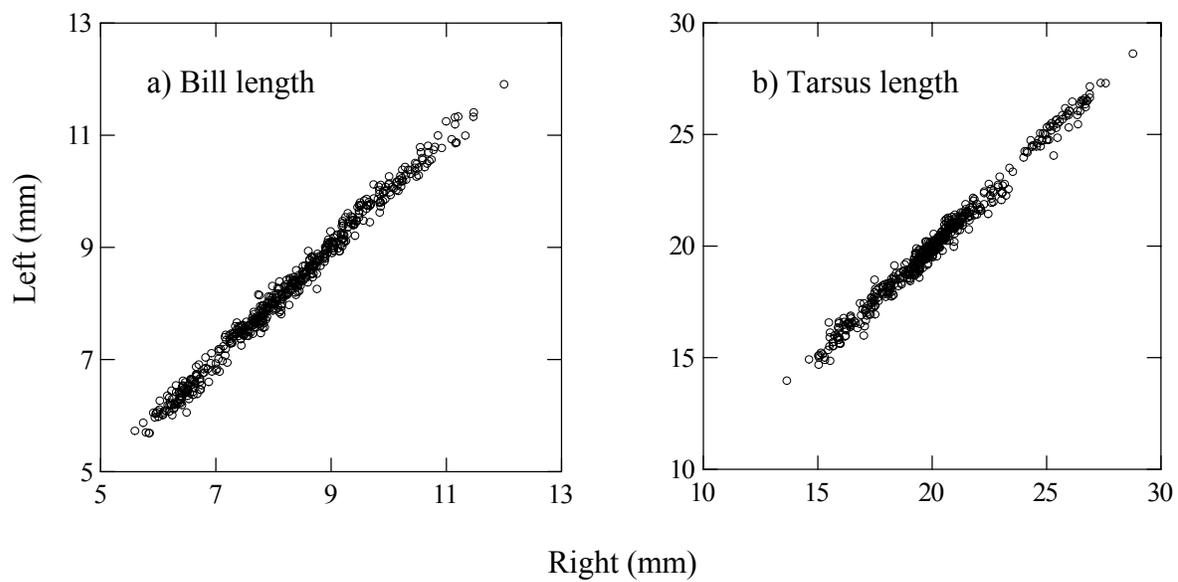


Figure 2. Average length of left side vs. right side of bill (a) and tarsus (b) for all species combined (Least Bell's Vireo, Common Yellowthroat, Song Sparrow, Southwestern Willow Flycatcher, and Yellow-breasted Chat).

Table 3. FA outliers identified by Grubb's test. Specimens determined to be outliers at $\alpha = 0.01$ and/or 0.05 are marked with an "X".

Trait	Species	Type	df	Mean	Std	FA ^a (mm)	t_G	p	0.01	0.05
Bill	BEVI	Live	43	-0.038	0.131	0.303	-2.024	0.049	-	X
Bill	BEVI	Museum	60	-0.071	0.128	0.205	2.168	0.034	-	X
Bill	BEVI	Museum	60	-0.071	0.128	0.213	2.227	0.030	-	X
Bill	BEVI	Museum	60	-0.071	0.128	0.463	-3.066	0.003	X	X
Bill	COYE	Live	66	-0.012	0.146	0.363	2.559	0.013	-	X
Bill	COYE	Live	66	-0.012	0.146	0.353	-2.325	0.023	-	X
Bill	COYE	Live	66	-0.012	0.146	0.522	-3.487	0.001	X	X
Bill	COYE	Museum	41	0.016	0.133	0.398	2.881	0.006	X	X
Bill	COYE	Museum	41	0.016	0.133	0.280	-2.231	0.031	-	X
Bill	SOSP	Live	66	-0.066	0.097	0.143	2.156	0.035	-	X
Bill	SOSP	Live	66	-0.066	0.097	0.133	2.053	0.044	-	X
Bill	SOSP	Museum	106	-0.014	0.126	0.335	-2.543	0.012	-	X
Bill	SOSP	Museum	106	-0.014	0.126	0.288	-2.166	0.033	-	X
Bill	SOSP	Museum	106	-0.014	0.126	0.305	2.535	0.013	-	X
Bill	SOSP	Museum	106	-0.014	0.126	0.260	2.178	0.032	-	X
Bill	SOSP	Museum	106	-0.014	0.126	0.278	-2.087	0.039	-	X
Bill	SOSP	Museum	106	-0.014	0.126	0.325	-2.464	0.015	-	X
Bill	SOSP	Museum	106	-0.014	0.126	0.302	2.515	0.013	-	X
Bill	SWFL	Live	35	0.065	0.148	0.248	-2.105	0.043	-	X
Bill	SWFL	Museum	32	0.071	0.106	0.292	2.094	0.044	-	X
Bill	SWFL	Museum	32	0.071	0.106	0.152	-2.107	0.043	-	X
Bill	YBCH	Live	29	-0.039	0.129	0.328	-2.238	0.033	-	X
Bill	YBCH	Live	29	-0.039	0.129	0.225	2.046	0.050	-	X
Bill	YBCH	Live	29	-0.039	0.129	0.225	2.046	0.050	-	X
Bill	YBCH	Museum	32	-0.046	0.132	0.330	-2.161	0.038	-	X
Tarsus	BEVI	Live	44	-0.203	0.361	0.717	2.546	0.014	-	X
Tarsus	BEVI	Live	44	-0.203	0.361	0.947	3.183	0.003	X	X
Tarsus	BEVI	Museum	47	-0.011	0.244	0.543	-2.179	0.034	-	X
Tarsus	BEVI	Museum	47	-0.011	0.244	0.493	2.063	0.045	-	X
Tarsus	BEVI	Museum	47	-0.011	0.244	0.563	-2.260	0.028	-	X
Tarsus	COYE	Live	68	-0.149	0.293	0.733	-1.998	0.050	-	X
Tarsus	COYE	Museum	33	-0.135	0.246	0.910	-3.149	0.003	X	X
Tarsus	SOSP	Museum	96	-0.040	0.191	0.340	1.993	0.049	-	X
Tarsus	SOSP	Museum	96	-0.040	0.191	0.397	2.291	0.024	-	X
Tarsus	SOSP	Museum	96	-0.040	0.191	0.560	-2.728	0.008	X	X
Tarsus	SOSP	Museum	96	-0.040	0.191	0.353	2.063	0.042	-	X
Tarsus	SOSP	Museum	96	-0.040	0.191	0.517	2.920	0.004	X	X
Tarsus	SOSP	Museum	96	-0.040	0.191	0.353	2.063	0.042	-	X
Tarsus	SOSP	Museum	96	-0.040	0.191	0.450	-2.151	0.034	-	X
Tarsus	SOSP	Museum	96	-0.040	0.191	0.360	2.098	0.038	-	X
Tarsus	SWFL	Live	36	-0.012	0.400	1.027	2.597	0.014	-	X
Tarsus	SWFL	Live	36	-0.012	0.400	1.083	-2.681	0.011	-	X
Tarsus	SWFL	Museum	29	-0.006	0.306	0.743	-2.408	0.023	-	X
Tarsus	YBCH	Live	30	-0.257	0.332	0.977	-2.165	0.038	-	X
Tarsus	YBCH	Live	30	-0.257	0.332	1.320	-3.198	0.003	X	X
Tarsus	YBCH	Museum	32	-0.012	0.213	0.687	-3.168	0.003	X	X

^a The difference in millimeters between the mean left and mean right trait size

trait varied between the three datasets (Table 4) (i.e. 0.01, 0.05, and uncorrected Bonferroni datasets).

Table 4. Sample sizes by species, trait, and collection type for the 0.05, 0.01, and uncorrected datasets.

Dataset	Type	BEVI ^a		COYE ^b		SOSP ^c		SWFL ^d		YBCH ^e	
		Bill	Tarsus								
0.05	Live	43	43	64	68	65	67	35	35	27	29
0.05	Museum	58	45	40	33	100	89	31	29	32	32
0.01	Live	44	44	66	69	67	67	36	37	30	30
0.01	Museum	60	48	41	33	107	95	33	30	33	32
Uncorrected	Live	44	45	67	69	67	67	36	37	30	31
Uncorrected	Museum	61	48	42	34	107	97	33	30	33	33

^a BEVI = Least Bell's Vireo

^b COYE = Common Yellowthroat

^c SOSP = Song Sparrow

^d SWFL = Southwestern Willow Flycatcher

^e YBCH = Yellow-breasted Chat

Signed FA (MS_{sj}) was several orders of magnitude larger than ME (MS_e) for all groups tested, indicating fluctuating asymmetry could be separated from measurement error (Tables 5-7 and Appendix B).

A Spearman correlation analysis failed to show evidence of scaling between fluctuating asymmetry levels and trait size for either bill or tarsus length, within any species or collection type (all $p > 0.05$) (Figure 3). Therefore, a log transformation to correct for size-dependence was not applied to the asymmetry data.

Directional asymmetry was detected in the bill length of contemporary specimens of Southwestern Willow Flycatcher and Song Sparrow, and in historic populations of Least Bell's Vireo and Southwestern Willow Flycatcher (all $p < 0.015$). Directional asymmetry in tarsus length was found in contemporary populations of all species (all $p < 0.001$), except Southwestern Willow Flycatcher, and in historic samples of Common Yellowthroat ($p < 0.005$) and Song Sparrow ($p < 0.05$). All groups were normally distributed.

Contemporary vs. Historic Asymmetry

Preliminary results, prior to correcting for measurement error, indicated that tarsus asymmetry in contemporary populations of Least Bell's Vireos, Song Sparrows, and Yellow-breasted Chats (all $p < 0.007$) (Figure 4), and bill asymmetry in contemporary samples of

Table 5. Summary results from two-way mixed model ANOVAs to test whether fluctuating asymmetry could be separated from measurement error within the 0.05 dataset for museum skins and live birds.

Trait	Species ^a	Type	df _e ^b	MS _e ^c	df _{si} ^d	MS _{si} ^e	F _{si} ^f	p _{si} ^g
Bill	BEVI	Museum	348	0.00268	57	0.02334	8.71	< 0.0001
Bill	BEVI	Live	258	0.00740	42	0.03161	4.27	< 0.0001
Bill	COYE	Museum	240	0.00276	39	0.02497	9.05	< 0.0001
Bill	COYE	Live	384	0.00697	63	0.02839	4.07	< 0.0001
Bill	SOSP	Museum	600	0.00271	99	0.02147	7.92	< 0.0001
Bill	SOSP	Live	390	0.00601	64	0.01668	2.78	< 0.0001
Bill	SWFL	Museum	186	0.00353	30	0.01733	4.91	< 0.0001
Bill	SWFL	Live	210	0.00689	34	0.03940	5.72	< 0.0001
Bill	YBCH	Museum	192	0.00297	31	0.03034	10.22	< 0.0001
Bill	YBCH	Live	162	0.00857	26	0.01982	2.31	< 0.001
Tarsus	BEVI	Museum	180	0.00350	44	0.06673	19.07	< 0.0001
Tarsus	BEVI	Live	172	0.03525	42	0.12398	3.52	< 0.0001
Tarsus	COYE	Museum	132	0.00227	32	0.06469	28.50	< 0.0001
Tarsus	COYE	Live	272	0.03587	67	0.12249	3.41	< 0.0001
Tarsus	SOSP	Museum	356	0.00281	88	0.03248	11.56	< 0.0001
Tarsus	SOSP	Live	268	0.04538	66	0.25438	5.61	< 0.0001
Tarsus	SWFL	Museum	116	0.00145	28	0.11560	79.72	< 0.0001
Tarsus	SWFL	Live	140	0.03950	34	0.15562	3.94	< 0.0001
Tarsus	YBCH	Museum	128	0.00226	31	0.04747	21.00	< 0.0001
Tarsus	YBCH	Live	116	0.04034	28	0.08340	2.07	< 0.005

^a Species: BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat

^b df_e = Error degrees of freedom

^c MS_e = Error mean square [variation in ME]

^d df_{si} = Side*Individual degrees of freedom

^e MS_{si} = Side*Individual mean square [variation in signed FA]

^f F_{si} = Side*Individual F-statistic

^g P_{si} = significance level of F_{si}

Table 6. Summary results from two-way mixed model ANOVAs to test whether fluctuating asymmetry could be separated from measurement error within the 0.01 dataset for museum skins and live birds.

Trait	Species ^a	Type	df _e ^b	MS _e ^c	df _{si} ^d	MS _{si} ^e	F _{si} ^f	p _{si} ^g
Bill	BEVI	Museum	360	0.00263	59	0.02781	10.57	< 0.0001
Bill	BEVI	Live	264	0.00743	43	0.03420	4.60	< 0.0001
Bill	COYE	Museum	246	0.00276	40	0.02854	10.34	< 0.0001
Bill	COYE	Live	396	0.00707	65	0.03538	5.00	< 0.0001
Bill	SOSP	Museum	642	0.00269	106	0.03177	11.81	< 0.0001
Bill	SOSP	Live	402	0.00600	66	0.01877	3.13	< 0.0001
Bill	SWFL	Museum	198	0.00342	32	0.02243	6.56	< 0.0001
Bill	SWFL	Live	216	0.00685	35	0.04400	6.42	< 0.0001
Bill	YBCH	Museum	198	0.00320	32	0.03460	10.81	< 0.0001
Bill	YBCH	Live	180	0.00815	29	0.03326	4.08	< 0.0001
Tarsus	BEVI	Museum	192	0.00392	47	0.08961	22.86	< 0.0001
Tarsus	BEVI	Live	176	0.03471	43	0.15300	4.41	< 0.0001
Tarsus	COYE	Museum	132	0.00227	32	0.06469	28.50	< 0.0001
Tarsus	COYE	Live	276	0.03588	68	0.12834	3.58	< 0.0001
Tarsus	SOSP	Museum	380	0.00288	94	0.04640	16.11	< 0.0001
Tarsus	SOSP	Live	268	0.04538	66	0.25438	5.61	< 0.0001
Tarsus	SWFL	Museum	120	0.00142	29	0.14072	99.10	< 0.0001
Tarsus	SWFL	Live	148	0.03910	36	0.23976	6.13	< 0.0001
Tarsus	YBCH	Museum	128	0.00226	31	0.04747	21.00	< 0.0001
Tarsus	YBCH	Live	120	0.03957	29	0.11103	2.81	< 0.0001

^a Species: BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat

^b df_e = Error degrees of freedom

^c MS_e = Error mean square [variation in ME]

^d df_{si} = Side*Individual degrees of freedom

^e MS_{si} = Side*Individual mean square [variation in signed FA]

^f F_{si} = Side*Individual F-statistic

^g P_{si} = significance level of F_{si}

Table 7. Summary results from two-way mixed model ANOVAs to test whether fluctuating asymmetry could be separated from measurement error within the uncorrected dataset for museum skins and live birds.

Trait	Species ^a	Type	df _e ^b	MS _e ^c	df _{si} ^d	MS _{si} ^e	F _{si} ^f	p _{si} ^g
Bill	BEVI	Museum	366	0.00264	60	0.03252	12.32	< 0.0001
Bill	BEVI	Live	264	0.00743	43	0.03420	4.60	< 0.0001
Bill	COYE	Museum	252	0.00274	41	0.03513	12.82	< 0.0001
Bill	COYE	Live	402	0.00701	66	0.04286	6.11	< 0.0001
Bill	SOSP	Museum	642	0.00269	106	0.03177	11.81	< 0.0001
Bill	SOSP	Live	402	0.00600	66	0.01877	3.13	< 0.0001
Bill	SWFL	Museum	198	0.00342	32	0.02243	6.56	< 0.0001
Bill	SWFL	Live	216	0.00685	35	0.04400	6.42	< 0.0001
Bill	YBCH	Museum	198	0.00320	32	0.03460	10.81	< 0.0001
Bill	YBCH	Live	180	0.00815	29	0.03326	4.08	< 0.0001
Tarsus	BEVI	Museum	192	0.00392	47	0.08961	22.86	< 0.0001
Tarsus	BEVI	Live	180	0.03507	44	0.19558	5.58	< 0.0001
Tarsus	COYE	Museum	136	0.00225	33	0.09086	40.38	< 0.0001
Tarsus	COYE	Live	276	0.03588	68	0.12834	3.58	< 0.0001
Tarsus	SOSP	Museum	388	0.00291	96	0.05450	18.73	< 0.0001
Tarsus	SOSP	Live	268	0.04538	66	0.25438	5.61	< 0.0001
Tarsus	SWFL	Museum	120	0.00142	29	0.14072	99.10	< 0.0001
Tarsus	SWFL	Live	148	0.03910	36	0.23976	6.13	< 0.0001
Tarsus	YBCH	Museum	132	0.00222	32	0.06796	30.61	< 0.0001
Tarsus	YBCH	Live	124	0.03842	30	0.16570	4.31	< 0.0001

^a Species: BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat

^b df_e = Error degrees of freedom

^c MS_e = Error mean square [variation in ME]

^d df_{si} = Side*Individual degrees of freedom

^e MS_{si} = Side*Individual mean square [variation in signed FA]

^f F_{si} = Side*Individual F-statistic

^g P_{si} = significance level of F_{si}

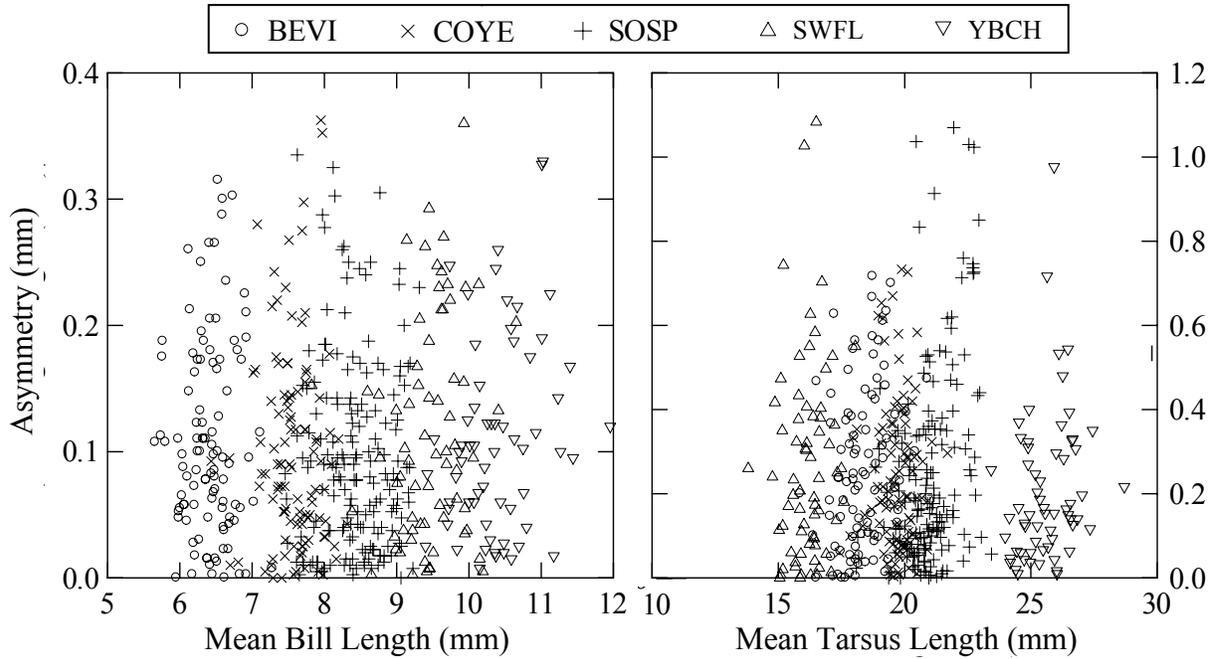


Figure 3. Bill and tarsus asymmetry ([Left - Right]) as a function of mean trait size $[(\text{Left} + \text{Right})/2]$ for five avian species, after FA outliers were removed at $\alpha < 0.01$. (BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat).

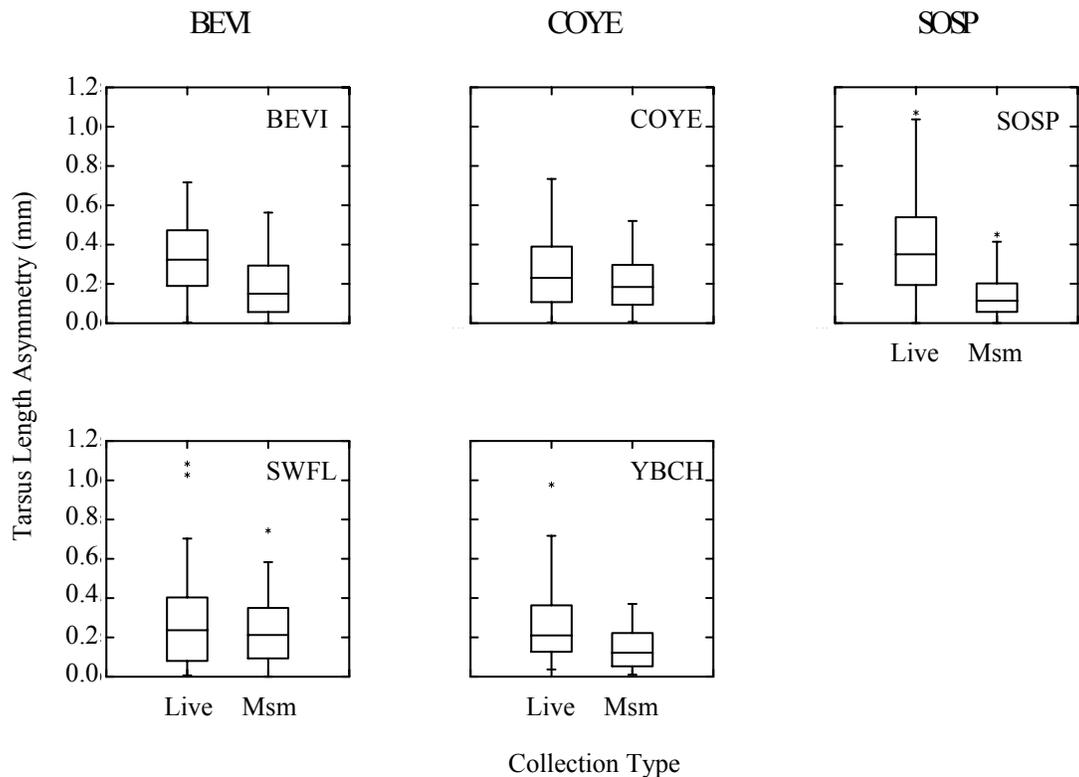


Figure 4. Contemporary (Live) and historic (Msm) levels of unsigned tarsus length asymmetry (|Left - Right|) in five species of southern Californian riparian birds (BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat) using the 0.01 dataset. Figures display asymmetry data that have not been corrected for measurement error.

Southwestern Willow Flycatchers ($p = 0.05$) (Figure 5) were significantly greater than in historic populations. However, the variability associated with measuring contemporary live birds was greater than that for museum skins (Figure 6), indicating a need to correct for measurement error. Once corrected, using the FA10 index and FPA analysis, Least Bell's Vireo and Yellow-breast Chat tarsus FA were no longer statistically different from historic FA levels, in two of the three datasets (Tables 8, 9 and Figure 7a). Additionally, bill asymmetry in Song Sparrows became significant, but in the opposite direction to that predicted, with contemporary populations possessing more symmetric bills than historic populations within all datasets (Tables 8, 9 and Figure 7b).

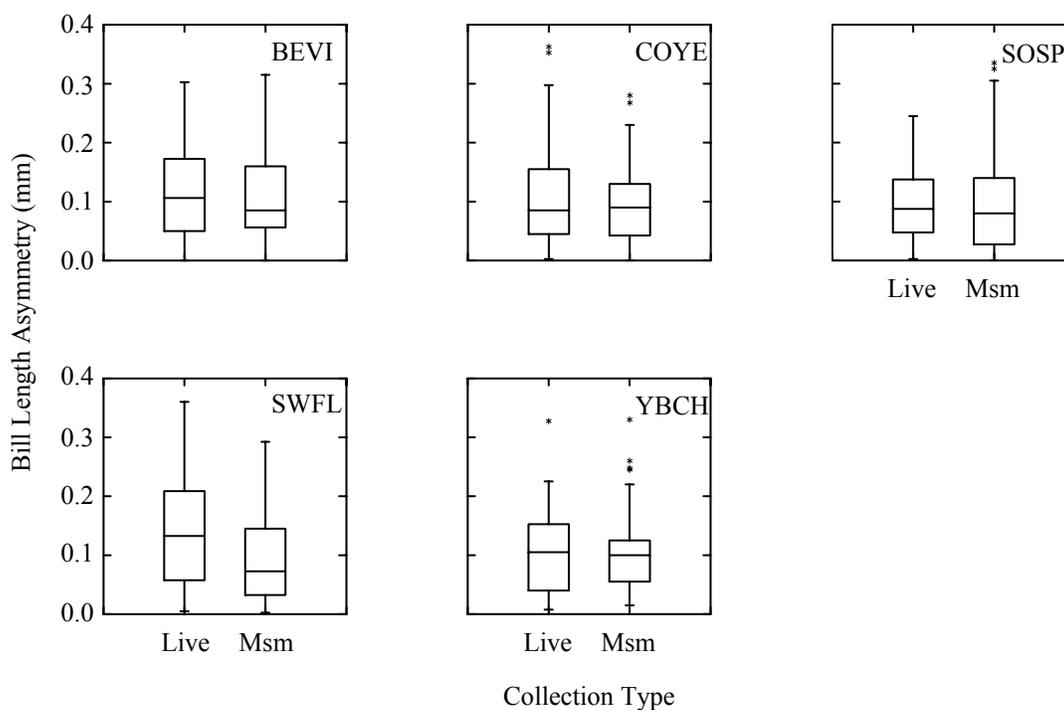


Figure 5. Contemporary (Live) and historic (Msm) levels of unsigned bill length asymmetry ($|\text{Left} - \text{Right}|$) in five species of southern Californian riparian birds (BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat) using the 0.01 dataset. Figures display asymmetry data that have not been corrected for measurement error.

Other tests yielded statistically significant differences between contemporary and historic populations, but results differed by analytic technique and dataset (Tables 8 and 9).

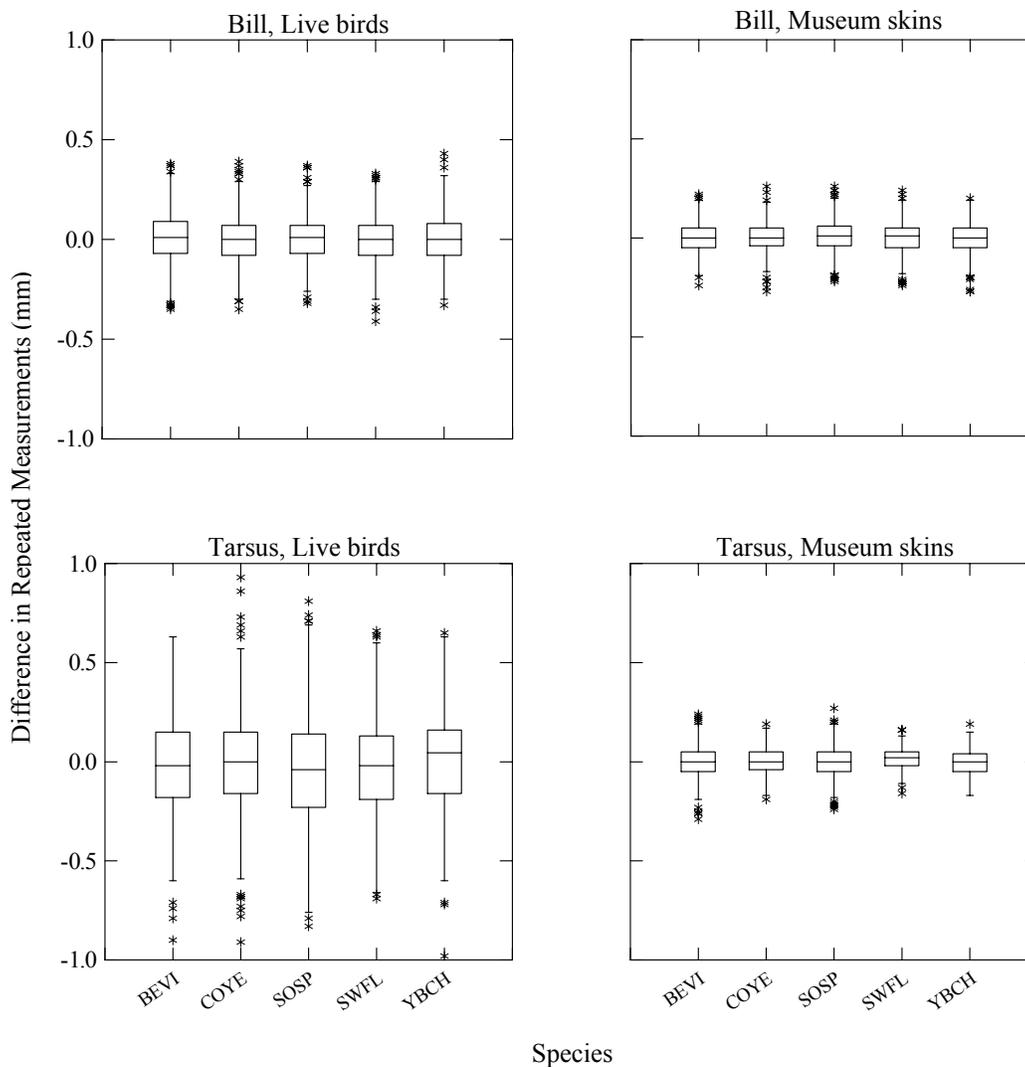


Figure 6. Distribution of differences between repeated measurements of bill and tarsus length taken on live specimens and museum skins of five species of birds (BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat).

Table 8. Results from the FA10 analyses (data corrected for ME) testing for differences in bill and tarsus length fluctuating asymmetry between contemporary and historic populations in five species of birds (BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat). (Num df = numerator degrees of freedom, Dem df = denominator degrees of freedom).

Species	Trait	Dataset	Num df	Dem df	<i>F</i>	<i>p</i>
BEVI	Bill	0.05	24.4	44.6	1.17	0.317
BEVI	Bill	0.01	26.1	48.3	1.06	0.416
BEVI	Bill	Uncorrected	50.6	26.1	1.12	0.389
BEVI	Tarsus	0.05	21.1	39.5	1.40	0.176
BEVI	Tarsus	0.01	25.4	43.0	1.38	0.175
BEVI	Tarsus	Uncorrected	29.4	43.0	1.87	0.031^a
COYE	Bill	0.05	30.8	35.5	1.04	0.456
COYE	Bill	0.01	41.3	32.6	1.10	0.396
COYE	Bill	Uncorrected	46.0	34.8	1.11	0.383
COYE	Tarsus	0.05	32.8	29.8	1.39	0.188
COYE	Tarsus	0.01	34.6	29.8	1.48	0.142
COYE	Tarsus	Uncorrected	34.6	31.4	1.04	0.454
SOSP	Bill	0.05	75.4	25.6	1.76	0.057^b
SOSP	Bill	0.01	88.7	30.0	2.28	0.006^b
SOSP	Bill	Uncorrected	88.7	30.0	2.28	0.006^b
SOSP	Tarsus	0.05	44.2	73.3	7.04	< 0.001^a
SOSP	Tarsus	0.01	44.2	82.6	4.80	< 0.001^a
SOSP	Tarsus	Uncorrected	44.2	86.0	4.05	< 0.001^a
YBCH	Bill	0.05	25.2	8.1	2.43	0.097
YBCH	Bill	0.01	26.3	16.4	1.25	0.326
YBCH	Bill	Uncorrected	26.3	16.4	1.25	0.326
YBCH	Tarsus	0.05	28.1	7.2	1.05	0.516
YBCH	Tarsus	0.01	11.7	28.1	1.58	0.159
YBCH	Tarsus	Uncorrected	17.5	29.9	1.94	0.057^a
SWFL	Bill	0.05	23.0	18.9	2.36	0.034^a
SWFL	Bill	0.01	24.9	22.9	1.95	0.059^a
SWFL	Bill	Uncorrected	24.9	22.9	1.95	0.059^a
SWFL	Tarsus	0.05	18.6	27.3	1.02	0.473
SWFL	Tarsus	0.01	25.1	28.4	1.44	0.174
SWFL	Tarsus	Uncorrected	25.1	28.4	1.44	0.174

^a Contemporary population more asymmetric than historic population.

^b Contemporary population more symmetric than historic population.

Table 9. Results from the First Principles Analyses (FPA) (data corrected for ME) testing for differences in bill and tarsus length fluctuating asymmetry between contemporary and historic populations in five species of birds (BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat). (Num df = numerator degrees of freedom, Dem df = denominator degrees of freedom).

Species	Trait	Dataset	Num df	Dem df	<i>F</i>	<i>p</i>
BEVI	Bill	0.05	42	57	1.22	0.239
BEVI	Bill	0.01	43	59	1.11	0.353
BEVI	Bill	Uncorrected	43	59	1.11	0.353
BEVI	Tarsus	0.05	42	44	1.56	0.074
BEVI	Tarsus	0.01	43	47	1.49	0.090
BEVI	Tarsus	Uncorrected	44	47	1.98	0.011^a
COYE	Bill	0.05	39	63	0.99	0.507
COYE	Bill	0.01	65	40	1.14	0.336
COYE	Bill	Uncorrected	66	41	1.14	0.334
COYE	Tarsus	0.05	67	32	1.56	0.084
COYE	Tarsus	0.01	68	32	1.65	0.060^a
COYE	Tarsus	Uncorrected	68	33	1.17	0.317
SOSP	Bill	0.05	99	64	1.60	0.023^b
SOSP	Bill	0.01	106	66	2.09	0.001^b
SOSP	Bill	Uncorrected	106	66	2.09	0.001^b
SOSP	Tarsus	0.05	66	88	7.32	< 0.001^a
SOSP	Tarsus	0.01	66	94	5.04	< 0.001^a
SOSP	Tarsus	Uncorrected	66	96	4.26	< 0.001^a
YBCH	Bill	0.05	31	26	2.10	0.029^b
YBCH	Bill	0.01	31	29	1.19	0.323
YBCH	Bill	Uncorrected	32	29	1.19	0.323
YBCH	Tarsus	0.05	28	31	1.23	0.287
YBCH	Tarsus	0.01	29	31	1.84	0.049^a
YBCH	Tarsus	Uncorrected	30	32	2.11	0.020^a
SWFL	Bill	0.05	34	30	2.33	0.010^a
SWFL	Bill	0.01	35	32	1.96	0.029^a
SWFL	Bill	Uncorrected	35	32	1.96	0.029^a
SWFL	Tarsus	0.05	34	28	1.13	0.375
SWFL	Tarsus	0.01	36	29	1.53	0.121
SWFL	Tarsus	Uncorrected	36	29	1.53	0.121

^a Contemporary population more asymmetric than historic population.

^b Contemporary population more symmetric than historic population.

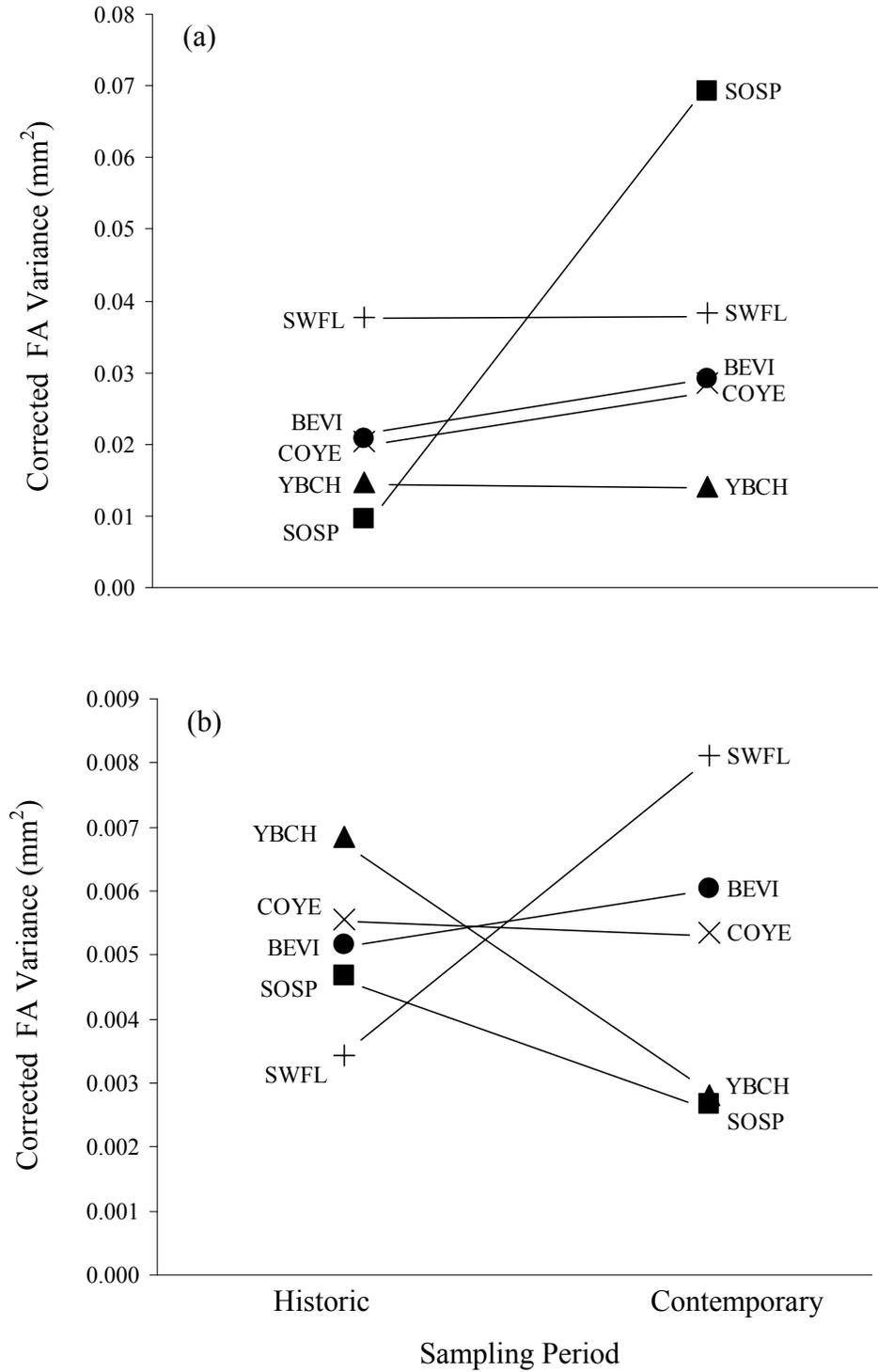


Figure 7. FA10 results comparing changes in tarsus (a) and bill (b) length fluctuating asymmetry between historic and contemporary populations of five species of bird, using the 0.05 dataset. (BEVI = Least Bell’s Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat).

For example, Least Bell's Vireo and Yellow-breasted Chat tarsus length were more asymmetric in contemporary populations than in historic samples within the uncorrected dataset using the FA10 analyses. The First Principles Analysis yielded similar results; however tarsus length was also significantly more asymmetric within the 0.01 dataset for chats. Contemporary Common Yellowthroat populations were more asymmetric than historic samples according to the FPA analysis using the 0.01 dataset, but were not significantly different within the 0.05 or uncorrected datasets. These differing results appear to be the product of a few deviant FA scores that caused the significance level to change depending on their inclusion or exclusion. This phenomenon can be seen when the FPA results using the uncorrected and 0.01 datasets for the tarsus trait are contrasted (Figure 8, Table 9). In this instance, the removal of a single Common Yellowthroat specimen resulted in the P-value changing from 0.32 to 0.06, while the removal of one Least Bell's Vireo had less impact, but still caused the P-value to change from "significance" (0.01) to "non-significance" (0.09).

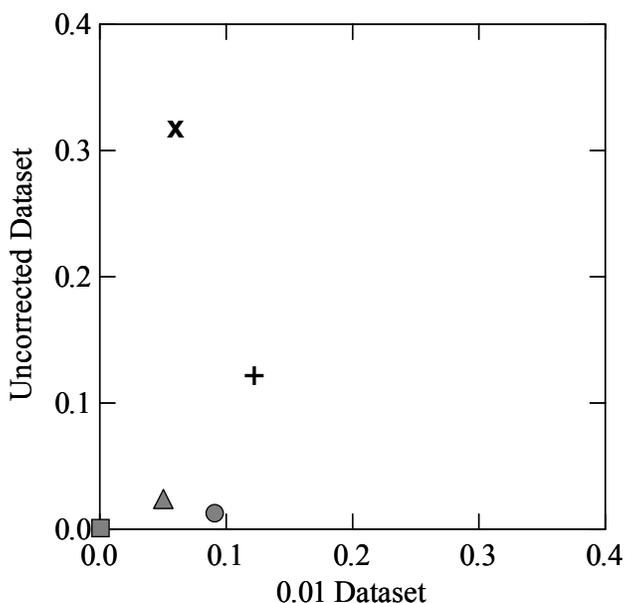


Figure 8. Contrast of P-values generated from the uncorrected and 0.01 datasets investigating whether contemporary tarsus length fluctuating asymmetry levels in five avian species differed from their historic counterparts. P-values were generated using the First Principles Analyses (FPA). (Square = Song Sparrow, triangle = Yellow-breasted Chat, circle = Least Bell's Vireo, plus = Southwestern Willow Flycatcher, and X = Common Yellowthroat).

Species of Like Conservation Status

FA10 and FPA analyses to test the hypothesis that species of like conservation status would possess similar levels of FA yielded mixed results. Bill and tarsus asymmetry did not differ between the endangered species (Least Bell's Vireo and Southwestern Willow Flycatcher) (all $p > 0.05$), supporting to the hypothesis (Figure 9). However, levels of bill and tarsus asymmetry between the common species (Song Sparrow and Common Yellowthroat) differed significantly in all tests, regardless of which dataset or analytic method was used. Common Yellowthroats possessed more asymmetric bills (all $p < 0.04$) while Song Sparrows had more asymmetric tarsi (all $p < 0.01$).

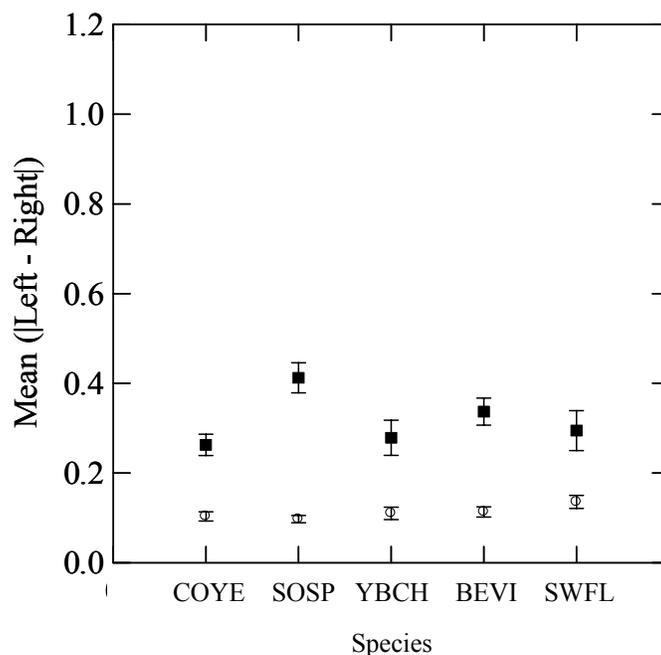


Figure 9. Mean (mm) unsigned tarsus (filled squares) and bill (open circles) length fluctuating asymmetry scores of contemporary populations of five species of southern California riparian birds. Error bars represent one SEM. Data displayed in figure has not been corrected for measurement error. FA outliers were excluded at $\alpha = 0.01$. (BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat).

FA and Conservation Status

Results from the analysis to further investigate a possible association between FA and conservation status did not support the hypothesis that fluctuating asymmetry and conservation status are correlated in a predictable manner for either the bill or tarsus character (Figure 10). Results from the FA10 and FPA analyses were similar. Contrary to my prediction, FA levels of species did not tend to group by conservation status. Consequently, species often possessed FA levels that were more similar to species of a different conservation designation than to species of the same status regardless of the dataset or statistical method used. As a result, statistically significant differences in fluctuating asymmetry levels between species displayed no visible correlation with a species' State and/or Federal conservation designation.

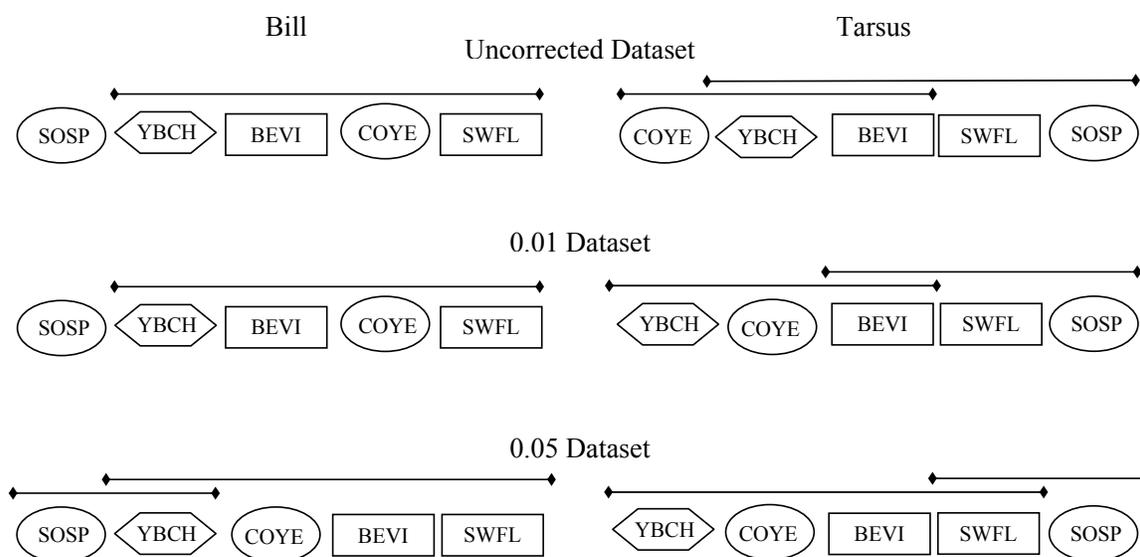


Figure 10. FA10 results comparing contemporary bill and tarsus length fluctuating asymmetry levels of the five focal bird species, across three datasets. The most symmetric species are positioned to the left side of each figure, with asymmetry increasing to the right. Geometric shapes indicate conservation status (oval = abundant/healthy, hexagon = species of concern, rectangle = endangered). Species under the same line were not statistically different from each other. BEVI = Least Bell's Vireo, COYE = Common Yellowthroat, SOSP = Song Sparrow, SWFL = Southwestern Willow Flycatcher, YBCH = Yellow-breasted Chat.

DISCUSSION

If fluctuating asymmetry is to be widely used as a conservation tool to identify imperiled populations, it must be expressed in a predictable manner. However, results from this study indicate that the relationship between FA level/expression, stress, and conservation status may not be straight forward. Furthermore, because it was found that statistical significance can be influenced greatly by the presence of one or two deviant points in a sample, only results that are consistent across all datasets and analyses will be used to evaluate fluctuating asymmetry's applicability to wildlife management.

Contemporary vs. Historic Asymmetry

One of the major assumptions in this study was that environmental and/or genetic factors that could negatively impact species are greater now than they were 60 to 100 years ago. This added “stress” on individuals was predicted to translate into elevated fluctuating asymmetry in contemporary populations compared to their historic counterparts. Under this hypothesis, threatened/endangered species were expected to display elevated fluctuating asymmetry levels as an indicator of the stressor(s) that acted as a proximate factor in their decline. Similarly, it would not be unusual to observe an increase in FA of the abundant species, as such an increase could be considered confirmation of the greater “stress” presumably experienced by contemporary populations in general.

However, out of the five species examined in this study, elevated FA levels were only detected consistently in the tarsus of contemporary Song Sparrows, an abundant species, and the bill of Southwestern Willow Flycatchers, an endangered species. The lack of a detectable increase in the fluctuating asymmetry levels of the second abundant species (Common Yellowthroat) or even in the sole sensitive species in this study (Yellow-breasted Chat) does not necessarily run counter to the above hypothesis. However, not detecting an increase in either tarsus or bill FA of the second endangered species, the Least Bell's Vireo, questions the validity of the hypothesis that contemporary populations of endangered species should display elevated FA levels compared to their historic populations, and therefore warrants further discussion. Furthermore, the lack of correspondence in FA expression through time

between the bill and tarsus traits is troublesome from a conservation perspective and will be discussed later in this document.

There are several possible reasons why the sample of contemporary Least Bell's Vireos in this study did not possess elevated fluctuating asymmetry levels relative to their historic population as hypothesized. Some explanations are consistent with the existing hypothesis while others question its validity. The first possible reason is one of sampling error. That is, that the sample in this study was comprised of extremely fit individuals and was not representative of the true vireo population. Although this is a possibility, it is unlikely, as vireos were sampled across a broad range of habitat at fourteen different locations throughout the study area.

The second possibility is that the stressor(s) that caused the vireo to decline have been removed through management actions since the species was federally listed as endangered in 1986. This possibility has merit as the vireo population in southern California has increased over the past 25 years from approximately 300 territories in 1980 (USF&WS unpublished data) to approximately 1600 territories in 2003 (USGS unpublished data). The early implementation of successful management actions may have allowed enough time for any deleterious alleles that were expressed as a result of inbreeding during the period of limited population size to be removed from the population through selection. The removal of harmful alleles could have lowered the level of stress vireos were under by restoring their genetic balance and thereby causing a reduction in FA levels. Therefore, if it had been possible to sample birds just before or during the vireo's decline, 20-40 years ago when environmental and/or genetic stressors were still present, elevated levels of FA might have been detected. Under such a scenario not finding elevated fluctuating asymmetry levels in contemporary populations of an endangered species does not run contrary to the stated hypothesis.

A third possibility is that what caused vireo populations to decline was not the type of "stress" that would have manifested itself in elevated levels of fluctuating asymmetry. This seems reasonable when one of the major causes of the vireo's decline, Brown-headed Cowbird (*Molothrus ater*) nest parasitism (U.S. Fish and Wildlife Service 1986, Franzreb 1989), is considered. Brood parasitism is characterized by the parasite species, in this case the Brown-headed Cowbird, laying its egg in the nest of the host (i.e. the Least Bell's Vireo)

(Ortega 1998). The host species is then left to incubate and care for the parasite's young, often to the detriment of its own offspring. Typically, the host's reproductive success is reduced by the parasitism event through the removal/destruction of its own eggs or young, or through competition between the parasite and host offspring for food (Ortega 1998). Least Bell's Vireo productivity is particularly impacted by cowbird parasitism because it is not unusual for parasitized nests to be abandoned or fledge no vireo young (Kus 1999, Kus unpublished data). Under such extreme conditions, where nests either avoid parasitism and fledge "unstressed" young, or are parasitized and fledge no host offspring, the impacted species may decline without manifesting an increase in fluctuating asymmetry.

Consequently, for fluctuating asymmetry to be useful as a management tool, the "stress" acting on populations cannot be so severe that it kills the individuals on which it is acting, as the case may be with Brown-headed Cowbirds and Least Bell's Vireos. Other instances in which populations/species may decline without manifesting an increase in fluctuating asymmetry levels include: declines due to high mortality resulting from increased predation pressure, exposure to lethal doses of chemical pollutants, or situations where large scale habitat loss prevented surviving offspring from breeding and contributing to the next generation. Under such circumstances, contemporary populations of a species would possess fluctuating asymmetry levels similar to their historic counterparts, and as a result FA techniques would be ineffective in identifying them as being at risk. Therefore, it seems likely that the applicability of fluctuating asymmetry as a conservation tool in monitoring population stress may be case-specific.

As mentioned earlier, there was a lack of correspondence in FA expression through time between the bill and tarsus traits for the two species that displayed a change in asymmetry. Results from this study indicate that the bill of the Southwestern Willow Flycatcher in southern California has become more asymmetric over time, while the symmetry level of its tarsus has not changed. Within the Song Sparrow, contemporary populations possess more asymmetric tarsi, but more symmetric bills, relative to their historic counterparts. The Southwestern Willow Flycatcher findings are not surprising as a similar lack of FA concordance between traits has been observed in other species (Moller 1993, Aparicio and Bonal 2002, Bonada and Dudley Williams 2002, Franco et al. 2002, Jentzsch et al. 2003). However, the situation observed in contemporary Song Sparrows of fluctuating

asymmetry levels of different traits changing in opposite directions through time has not, to my knowledge been observed previously.

Within the fluctuating asymmetry literature, there are two hypotheses that have been put forth to explain the lack of correspondence in changes in FA between characters in an organism. The first hypothesis suggests that there is a window in the development of characters during which time they are vulnerable to the effects of environmental and/or genetic stressors. Consequently, only those traits whose windows are open while the stress is acting will be affected. Furthermore, since stressor(s) may vary in intensity (or presence) during an organism's potentially protracted development, traits will be affected differentially according to the amount of stress they receive (Palmer 1994, Clarke 1995, Moller and Swaddle 1997, Clarke 1998).

The second hypothesis concerns the canalization of traits that are important to an organism's fitness. Under this hypothesis, characters that are highly correlated with fitness and survival within a species are physiologically buffered against stress to a greater degree than other less essential traits (Clarke 1995, Moller and Swaddle 1997). For example, in studying aerodynamic efficiency in birds, Balmford et al. (1993) found that migratory species and species that spent much of their time in flight had greater wing symmetry than other birds. This hypothesis is also backed by experimental studies conducted on *D. melanogaster* to increase the canalization of specific characters against environmental stress (see Moller and Swaddle 1997 for review). Through canalizing selection, researchers were able to remove alleles from the *Drosophila* genome that caused susceptibility to environmental stress for a particular character, resulting in a reduction in FA expression when exposed to specific stressors.

Regardless of the reasons for these discrepancies, they are problematic from a conservation monitoring perspective because the conclusions drawn about a species' health could differ depending on the trait selected. For example, a manager using results from this study could draw differing conclusions regarding Southwestern Willow Flycatcher and Song Sparrow population health depending on the trait considered. In the case of the Song Sparrow, contemporary bill fluctuating asymmetry levels indicated that the species was under less stress than historic populations, while tarsus FA levels indicated the opposite.

FA and Conservation Status

The hypothesis that species of like conservation status should possess similar fluctuating asymmetry levels was not supported. While contemporary tarsus and bill FA levels of the two endangered species were not statistically different, asymmetry levels between the two abundant species, the Common Yellowthroat and Song Sparrow, differed significantly for both the tarsus and bill traits. In fact, as indicated by the subsequent analyses to determine if conservation status and FA were correlated, Song Sparrows possessed the largest tarsus fluctuating asymmetry of any species (Figure 10). Additional results also ran counter to this hypothesis as in no case did fluctuating asymmetry level segregate according to the level of management concern (Figure 10).

However, it is worth noting that FA levels of the endangered species were often greater than those of the other species in the study. Therefore, the lack of correspondence observed may be the result of the limited number of species involved in the analysis. As a result only continued research over a broad range of species of varying conservation status may be able to answer this question.

Recommendations Concerning FA Analyses

The presence of even a few fluctuating asymmetry outliers in a sample added to the difficulty of analyzing the data and interpreting results. On three instances, within the First Principles Analyses, the “significance” of results changed because of the addition or exclusion of a single specimen (Table 9). This phenomenon can easily be seen by contrasting the FPA results for the tarsus trait when data are analyzed using the uncorrected and 0.01 datasets (Figure 8). In this instance, the removal of a single Common Yellowthroat specimen resulted in the P-value changing from 0.32 to 0.06. The removal of one Least Bell's Vireo had less impact, but still caused the P-value to change from “significance” (0.01) to “non-significance” (0.09). These results are worrisome from a conservation standpoint, and stress the need for researchers to fully understand the distributional qualities of their data before conducting fluctuating asymmetry analyses.

A primary reason FA outliers have such a great influence on results stems from the fact that both the FA10 and FPA analyses calculate a variance for each sample, derived from

the squared difference of individual FA scores from the sample mean $(X_i - \bar{X})^2$, where X_i is the fluctuating asymmetry score of specimen i and \bar{X} is the sample mean. Because the deviations are squared, outlying observations become even more extreme and can have a large effect on the sample variance used in the calculation of F-statistics when samples are compared. A possible solution to this issue could have been to apply a procedure that used the mean of FA scores instead of the variance to make statistical comparisons between samples, since means are influenced less by outliers than variances. However, no procedure involving the mean is capable of correcting for measurement error and directional asymmetry, both of which were present to some degree in the data, and therefore were judged less likely to yield valid results than the chosen analyses.

An encouraging aspect of the analyses conducted was the concordance of results between the FA10 and FPA methodologies. Both analytical methods yielded strikingly similar F-statistics across all datasets, species, and traits, regardless of the question under investigation (Figure 11a in Appendix E). Where the analyses diverged was in the subsequent generation of P-values (Figure 11b in Appendix E), with the FA10 methodologies typically producing larger P-values than the First Principles Analyses. This result stems from FA10's use of a corrected degrees of freedom (equation 3) that adjusts the df downward as measurement error increases and approaches the variability associated with the fluctuating asymmetry of a sample. As can be seen, this procedure results in a more conservative estimate of the significance of the main effects.

Conclusions and Implications for Conservation

The peer reviewed literature demonstrating the expression of fluctuating asymmetries in organisms when they are exposed to ample environmental and/or genetic stress is copious. Traditionally, biologists studied fluctuating asymmetries in hopes of elucidating the physiological mechanisms that determined the developmental stability of organisms. However, over the past two decades, there has been a growing interest in using FA as a tool to monitor species/population health in natural systems. Although other researchers have documented changes in fluctuating asymmetry levels of specific species over time, or under varying environmental or genetic conditions, results from this study raised questions

concerning FA's usefulness as a conservation tool in monitoring the status of avian species on a broad scale.

Results from this study indicated no consistent relationship between the expression of fluctuating asymmetry over time and conservation status, FA and conservation status of contemporary populations, or fluctuating asymmetry expression among different traits within a species. These results do not imply that FA is an unreliable technique in assessing population stress, but speak to the difficulty in selecting traits that are not highly canalized, and are also under development when the stressor(s) affecting the population is being applied. Fluctuating asymmetry levels of the morphological traits examined here reflect the stressors affecting individuals from the time of conception until they fledged from the nest, fully developed, approximately 30-40 days later, and are not indicative of any stress inflicted on that individual after that point. Therefore, the usefulness of fluctuating asymmetry as a conservation tool is dependent upon the identification of such traits, and should be limited to cases where the agent causing the stress or reduction in population numbers has the opportunity of affecting a species' physiology during development of the trait under study so it can be manifested in the organism's morphology. As stated earlier, severe stressors that cause mortality of organisms will not be reflected in the population level FA of the remaining individuals. Therefore, under such situations, using fluctuating asymmetry as a monitoring technique is inappropriate.

However, under the right circumstances, fluctuating asymmetry monitoring techniques may prove to be very reliable. If FA techniques are to be used in the future to monitor the condition of avian populations, it is recommended that care be taken when selecting traits to ensure characters with ample phenotypic plasticity are chosen. To that end, it is recommended that a number of traits be initially evaluated. Two osteological characters were selected for this study to avoid the possibility of measuring false asymmetries in museum skins associated with possible feather wear. However, additional traits that have been used to study avian fluctuating asymmetry are numerous and include primary and tail feather lengths, and maximal width and height of eye rings, cheek patches, wing bars, and pre-ocular spots.

This study demonstrates the influence outliers can have on the significance of the results and ultimately on the conclusions drawn. Therefore, it is strongly recommended that

researchers thoroughly understand the distributional properties of their data prior to conducting FA analyses. As part of this, it is also recommended that analyses be conducted over multiple datasets that have a subsequently larger percentage of “outliers” removed. This methodology leads to robust conclusions by ensuring that significance between samples is not a result of one or two aberrant points.

Finally, since signed FA and measurement error display the same properties and are often indistinguishable (Palmer and Strobeck 1986, Palmer 1994, Swaddle et al. 1994), it is strongly recommended that fluctuating asymmetry analyses be conducted using an analytic technique that corrects for ME, such as FA10 or the First Principles Analysis. If measurement error is not taken into account prior to testing for differences between samples, there is an increased probability that spurious significant results will be found, based solely on the presence of ME.

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APPENDIX A

**TRAITS EVALUATED FOR INCLUSION IN STUDY AND
REASON(S) FOR THEIR EXCLUSION.**

**TRAITS EVALUATED FOR INCLUSION IN STUDY AND
REASON(S) FOR THEIR EXCLUSION.**

Trait	Reason for Exclusion
Length of hallux	Initially included, but excluded from study once the power analysis indicated that it would take extremely large sample sizes to detect differences between populations with the effect size desired.
Length of bill from gape	Gape often obscured by feathers, making measurement difficult. Also, there was a high likelihood that this measurement was correlated with the “length of bill from nostrils” measurement, which was deemed more feasible.
Length of exposed ramus	Ramus often obscured by feathers, making measurement difficult. Also, there was a high likelihood that this measurement was correlated with the “length of bill from nostrils” measurement, which was deemed more feasible.
Length of tibia	Proximal end of tibia obscured by muscle tissue making accurate measurement difficult.
Length of middle toe	Since the middle toe contains multiple bones and is able to flex, accurate repeated measurement was deemed problematic.
Length of tail feathers	Asymmetry within tail feathers of the migratory species considered in this study would reflect conditions of their wintering grounds (i.e. Central and South America) and would not be comparable to levels of resident species. Therefore, they were excluded from measurement in this study.
Length of 8th primary	Asymmetry within primary feathers of the migratory species considered in this study would reflect conditions of their wintering grounds (i.e. Central and South America) and would not be comparable to levels of resident species. Therefore, they were excluded from measurement in this study.

APPENDIX B

**POWER ANALYSIS AND SAMPLE
SIZE DETERMINATION**

POWER ANALYSIS AND SAMPLE SIZE DETERMINATION

Two-sample t-test power analyses were performed on tarsus, bill, and hallux measurements to investigate the sample sizes needed to achieve a power of 80 percent with alpha equal to 0.05. Sample means and standard deviations were obtained from 12 Wrentit (*Chamaea fasciata*) study skins housed within San Diego State University's ornithological collections. The test statistic for each analysis was computed by taking the absolute value of the difference between the mean of three left trait measurements and three right trait measurements.

Fluctuating asymmetry levels expressed in organisms tend to be one percent or less of the trait being measured (Moller and Swaddle 1997). Therefore, sample sizes were determined that would allow the detection of FA levels equal to one, one-half, and one-quarter of a percent of each trait (Table 10). Results indicated that sample sizes in the low to mid-thirties would allow the detection of asymmetries in the 0.5 percent of trait size range for the tarsus and bill characters. However, the greater variability associated with measuring the hallux resulted in a required sample size of over 475 specimens to detect a similar FA level. As a result, the hallux trait was not measured during the 2002 and 2003 field seasons.

Table 10. Two-sample t-test power analysis results. Sample size required to be able detect fluctuating asymmetry differences of 1, 0.5, and 0.025 percent of the mean trait size. N = 12 Wrentit study skins.

Trait	Trait Size (mm)	Mean FA Difference (mm)	SD (mm)	Sample Size Difference Detectable		
				1 %	0.5 %	0.025 %
Tarsus	25.23	0.22	0.17	9	30	115
Bill	6.70	0.08	0.05	10	36	140
Hallux	7.25	0.25	0.20	121	477	1920

APPENDIX C**DETERMINATION OF THE NUMBER OF REPEATED
MEASUREMENTS PER SIDE IN RELATION
TO MEASUREMENT PRECISION**

**DETERMINATION OF THE NUMBER OF REPEATED
MEASUREMENTS PER SIDE IN RELATION
TO MEASUREMENT PRECISI**

To assess measurement precision, Pearson correlation analyses were performed on a series of six repeated measurements taken on the left and right tarsus and bill of 12 Wrentit study skins. Metrics for the analysis were computed by calculating a series of means from the repeated measurements for right and left traits and subtracting appropriate side averages to compute mean differences. For example, the first two repeated measurements of a trait for the right side of a study skin were averaged and subtracted from the mean of the first two left measurements. This procedure was replicated for all six repeated measurements. Pearson correlation analyses were performed using the mean differences, for each trait, to investigate how well averages of 2 to 5 measurements predicted the results from six measurements. It was assumed that the mean difference from six repeated measurements was an accurate predictor of the true difference between right and left sides. For both traits, the averaged mean difference of three repeated measurements correlated highly with the mean of six, indicating that three measurements per trait should yield high accuracy (Table 11).

Table 11. Pearson correlation statistics for the mean difference of two, three, four and five repeated measurements against the mean difference of six measures for tarsus and bill. $n = 12$ birds.

Trait	Number of Averaged Measurements (correlation statistic)				
	Two	Three	Four	Five	Six
Tarsus	(0.94)	(0.97)	(0.99)	(0.99)	(1.00)
Bill	(0.62)	(0.92)	(0.97)	(0.99)	(1.00)

To be confident that ME could be separated from FA with three repeated measurements, mixed model two-way ANOVAs were applied to data from each trait (Palmer and Strobeck 2003, Swaddle et al. 1994). Within the ANOVA, Measurement Side (i.e. left and right) was specified as a fixed factor, Individual Specimen was designated a random factor, and the Individual Repeated Measurements were specified as the dependent variable. F-statistics for the mixed model ANOVAs were calculated according to Zar (1998). Statistical significance was set at $\alpha = 0.05$. Results indicated that three repeated measurements were sufficient to distinguish asymmetry and measurement error for tarsus

length ($F = 9.75$, $P < 0.0001$). However, four repeated measurements were required to distinguish asymmetry from measurement error in bill length ($F = 2.80$, $P = 0.005$).

APPENDIX D**FLUCTUATING ASYMMETRY VS. MEASUREMENT
ERROR ANOVA TABLES**

**FLUCTUATING ASYMMETRY VS. MEASUREMENT
ERROR ANOVA TABLES**

Mixed model ANOVA tables showing the results of analyses to determine if fluctuating asymmetry can be separated from measurement error, using the dataset in which FA outliers were excluded at $\alpha = 0.05$.

Least Bell's Vireo, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.642	1	0.642	27.508	2.39E-06
Individual	43.716	57	0.767	286.172	1.58E-259
Side * Individual	1.331	57	0.023	8.709	3.21E-40
Error	0.932	348	0.003		

Least Bell's Vireo, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.086	1	0.086	2.731	1.06E-01
Individual	34.077	42	0.811	109.642	3.29E-141
Side * Individual	1.327	42	0.032	4.272	1.94E-13
Error	1.910	258	0.007		

Common Yellowthroat, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.015	1	0.015	0.584	4.49E-01
Individual	39.138	39	1.004	363.601	1.14E-191
Side * Individual	0.974	39	0.025	9.047	2.48E-29
Error	0.661	240	0.003		

Common Yellowthroat, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.003	1	0.003	0.098	7.56E-01
Individual	48.376	63	0.768	110.169	8.88E-210
Side * Individual	1.788	63	0.028	4.073	6.74E-18
Error	2.675	384	0.007		

Song Sparrow, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.028	1	0.028	1.325	2.53E-01
Individual	151.511	99	1.530	564.727	0.00E+00
Side * Individual	2.125	99	0.021	7.923	1.04E-61
Error	1.624	600	0.003		

Song Sparrow, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.685	1	0.685	41.097	1.99E-08
Individual	91.867	64	1.435	238.839	9.48E-276
Side * Individual	1.067	64	0.017	2.775	7.60E-10
Error	2.344	390	0.006		

Southwestern Willow Flycatcher, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.310	1	0.310	17.896	2.02E-04
Individual	37.882	30	1.263	357.717	3.17E-148
Side * Individual	0.520	30	0.017	4.909	4.49E-12
Error	0.657	186	0.004		

Southwestern Willow Flycatcher, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.379	1	0.379	9.616	3.86E-03
Individual	50.536	34	1.486	215.727	1.10E-144
Side * Individual	1.340	34	0.039	5.718	4.52E-16
Error	1.447	210	0.007		

Yellow-breasted Chat, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.087	1	0.087	2.880	9.97E-02
Individual	38.290	31	1.235	415.879	3.97E-159
Side * Individual	0.941	31	0.030	10.215	1.37E-26
Error	0.571	192	0.003		

Yellow-breasted Chat, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.123	1	0.123	6.195	1.95E-02
Individual	76.631	26	2.947	343.912	6.33E-128
Side * Individual	0.515	26	0.020	2.313	8.10E-04
Error	1.389	162	0.009		

Least Bell's Vireo, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.000	1	0.000	0.004	9.48E-01
Individual	125.173	44	2.845	812.809	1.95E-185
Side * Individual	2.936	44	0.067	19.066	7.21E-48
Error	0.630	180	0.004		

Least Bell's Vireo, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	4.054	1	4.054	32.697	1.01E-06
Individual	146.997	42	3.500	99.289	2.26E-100
Side * Individual	5.207	42	0.124	3.517	3.63E-09
Error	6.063	172	0.035		

Common Yellowthroat, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.614	1	0.614	9.498	4.21E-03
Individual	126.195	32	3.944	1737.273	4.11E-158
Side * Individual	2.070	32	0.065	28.498	5.89E-45
Error	0.300	132	0.002		

Common Yellowthroat, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	2.005	1	2.005	16.367	1.37E-04
Individual	124.200	67	1.854	51.679	9.85E-122
Side * Individual	8.207	67	0.122	3.415	6.41E-13
Error	9.756	272	0.036		

Song Sparrow, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.453	1	0.453	13.959	3.31E-04
Individual	333.260	88	3.787	1347.701	0.00E+00
Side * Individual	2.858	88	0.032	11.559	1.60E-64
Error	1.000	356	0.003		

Song Sparrow, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	7.698	1	7.698	30.263	6.60E-07
Individual	323.586	66	4.903	108.039	4.27E-160
Side * Individual	16.789	66	0.254	5.606	1.87E-24
Error	12.163	268	0.045		

Southwestern Willow Flycatcher, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.017	1	0.017	0.145	7.06E-01
Individual	81.963	28	2.927	2018.786	6.45E-143
Side * Individual	3.237	28	0.116	79.724	5.23E-63
Error	0.168	116	0.001		

Southwestern Willow Flycatcher, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.006	1	0.006	0.038	8.47E-01
Individual	74.182	34	2.182	55.236	2.38E-65
Side * Individual	5.291	34	0.156	3.940	5.24E-09
Error	5.530	140	0.040		

Yellow-breasted Chat, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.004	1	0.004	0.076	7.85E-01
Individual	150.300	31	4.848	2145.301	3.22E-159
Side * Individual	1.471	31	0.047	21.004	1.15E-36
Error	0.290	128	0.002		

Yellow-breasted Chat, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	1.665	1	1.665	19.962	1.19E-04
Individual	147.097	28	5.253	130.230	8.95E-75
Side * Individual	2.335	28	0.083	2.067	3.89E-03
Error	4.679	116	0.040		

Mixed model ANOVA tables showing the results of analyses to determine if fluctuating asymmetry can be separated from measurement error, using the dataset in which FA outliers were excluded at $\alpha = 0.01$.

Least Bell's Vireo, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.506	1	0.506	18.207	7.28E-05
Individual	44.357	59	0.752	285.863	2.44E-268
Side * Individual	1.641	59	0.028	10.574	6.82E-50
Error	0.946	360	0.003		

Least Bell's Vireo, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.126	1	0.126	3.685	6.16E-02
Individual	34.943	43	0.813	109.370	2.53E-144
Side * Individual	1.471	43	0.034	4.603	3.96E-15
Error	1.963	264	0.007		

Common Yellowthroat, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.003	1	0.003	0.116	7.36E-01
Individual	40.708	40	1.018	368.732	3.70E-197
Side * Individual	1.142	40	0.029	10.341	5.79E-34
Error	0.680	246	0.003		

Common Yellowthroat, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.003	1	0.003	0.071	7.91E-01
Individual	50.395	65	0.775	109.661	6.57E-216
Side * Individual	2.300	65	0.035	5.004	2.42E-24
Error	2.800	396	0.007		

Song Sparrow, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.045	1	0.045	1.414	2.37E-01
Individual	160.599	106	1.515	563.230	0.00E+00
Side * Individual	3.368	106	0.032	11.810	1.19E-96
Error	1.727	642	0.003		

Song Sparrow, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.590	1	0.590	31.422	4.40E-07
Individual	93.143	66	1.411	235.210	6.91E-283
Side * Individual	1.239	66	0.019	3.128	2.09E-12
Error	2.412	402	0.006		

Southwestern Willow Flycatcher, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.330	1	0.330	14.700	5.57E-04
Individual	55.770	32	1.743	509.599	1.17E-172
Side * Individual	0.718	32	0.022	6.558	9.63E-18
Error	0.677	198	0.003		

Southwestern Willow Flycatcher, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.301	1	0.301	6.840	1.31E-02
Individual	50.551	35	1.444	210.851	9.67E-148
Side * Individual	1.540	35	0.044	6.423	9.01E-19
Error	1.479	216	0.007		

Yellow-breasted Chat, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.139	1	0.139	4.007	5.38E-02
Individual	43.024	32	1.345	420.159	1.73E-164
Side * Individual	1.107	32	0.035	10.813	9.02E-29
Error	0.633	198	0.003		

Yellow-breasted Chat, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.090	1	0.090	2.720	1.10E-01
Individual	83.715	29	2.887	354.201	4.12E-143
Side * Individual	0.965	29	0.033	4.081	2.54E-09
Error	1.467	180	0.008		

Least Bell's Vireo, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.008	1	0.008	0.094	7.60E-01
Individual	126.010	47	2.681	683.946	1.55E-190
Side * Individual	4.212	47	0.090	22.860	5.55E-57
Error	0.753	192	0.004		

Least Bell's Vireo, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	3.452	1	3.452	22.565	2.28E-05
Individual	147.183	43	3.423	98.613	2.04E-102
Side * Individual	6.579	43	0.153	4.408	1.41E-12
Error	6.109	176	0.035		

Common Yellowthroat, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.614	1	0.614	9.498	4.21E-03
Individual	126.195	32	3.944	1737.273	4.11E-158
Side * Individual	2.070	32	0.065	28.498	5.89E-45
Error	0.300	132	0.002		

Common Yellowthroat, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	2.291	1	2.291	17.854	7.27E-05
Individual	124.212	68	1.827	50.910	1.11E-122
Side * Individual	8.727	68	0.128	3.577	5.09E-14
Error	9.903	276	0.036		

Song Sparrow, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.232	1	0.232	5.000	2.77E-02
Individual	352.836	94	3.754	1303.323	0.00E+00
Side * Individual	4.361	94	0.046	16.111	2.32E-88
Error	1.093	380	0.003		

Song Sparrow, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	7.698	1	7.698	30.263	6.60E-07
Individual	323.586	66	4.903	108.039	4.27E-160
Side * Individual	16.789	66	0.254	5.606	1.87E-24
Error	12.163	268	0.045		

Southwestern Willow Flycatcher, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.002	1	0.002	0.011	9.18E-01
Individual	83.355	29	2.874	2024.155	7.38E-148
Side * Individual	4.081	29	0.141	99.099	1.74E-70
Error	0.170	120	0.001		

Southwestern Willow Flycatcher, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.007	1	0.007	0.031	8.62E-01
Individual	75.685	36	2.102	53.769	3.39E-68
Side * Individual	8.631	36	0.240	6.132	1.01E-15
Error	5.787	148	0.039		

Yellow-breasted Chat, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.004	1	0.004	0.076	7.85E-01
Individual	150.300	31	4.848	2145.301	3.22E-159
Side * Individual	1.471	31	0.047	21.004	1.15E-36
Error	0.290	128	0.002		

Yellow-breasted Chat, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	2.211	1	2.211	19.915	1.13E-04
Individual	147.175	29	5.075	128.253	6.50E-77
Side * Individual	3.220	29	0.111	2.806	4.60E-05
Error	4.749	120	0.040		

Mixed model ANOVA tables showing the results of analyses to determine if fluctuating asymmetry can be separated from measurement error, using the uncorrected dataset (i.e. no FA outliers were removed).

Least Bell's Vireo, Bill, Museum skin					
Source	SS	df	MS	F	<i>p</i>
Side	0.623	1	0.623	19.166	4.87E-05
Individual	44.542	60	0.742	281.201	1.65E-271
Side * Individual	1.951	60	0.033	12.318	6.16E-58
Error	0.968	366	0.003		

Least Bell's Vireo, Bill, Live bird					
Source	SS	df	MS	F	<i>p</i>
Side	0.126	1	0.126	3.685	6.16E-02
Individual	34.943	43	0.813	109.370	2.53E-144
Side * Individual	1.471	43	0.034	4.603	3.96E-15
Error	1.963	264	0.007		

Common Yellowthroat, Bill, Museum skin					
Source	SS	df	MS	F	<i>p</i>
Side	0.021	1	0.021	0.586	4.48E-01
Individual	42.211	41	1.030	375.745	6.09E-203
Side * Individual	1.440	41	0.035	12.821	8.56E-42
Error	0.691	252	0.003		

Common Yellowthroat, Bill, Live bird					
Source	SS	df	MS	F	<i>p</i>
Side	0.020	1	0.020	0.457	5.01E-01
Individual	56.702	66	0.859	122.555	2.68E-228
Side * Individual	2.829	66	0.043	6.114	1.45E-31
Error	2.818	402	0.007		

Song Sparrow, Bill, Museum skin					
Source	SS	df	MS	F	<i>p</i>
Side	0.045	1	0.045	1.414	2.37E-01
Individual	160.599	106	1.515	563.230	0.00E+00
Side * Individual	3.368	106	0.032	11.810	1.19E-96
Error	1.727	642	0.003		

Song Sparrow, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.590	1	0.590	31.422	4.40E-07
Individual	93.143	66	1.411	235.210	6.91E-283
Side * Individual	1.239	66	0.019	3.128	2.09E-12
Error	2.412	402	0.006		

Southwestern Willow Flycatcher, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.330	1	0.330	14.700	5.57E-04
Individual	55.770	32	1.743	509.599	1.17E-172
Side * Individual	0.718	32	0.022	6.558	9.63E-18
Error	0.677	198	0.003		

Southwestern Willow Flycatcher, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.301	1	0.301	6.840	1.31E-02
Individual	50.551	35	1.444	210.851	9.67E-148
Side * Individual	1.540	35	0.044	6.423	9.01E-19
Error	1.479	216	0.007		

Yellow-breasted Chat, Bill, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.139	1	0.139	4.007	5.38E-02
Individual	43.024	32	1.345	420.159	1.73E-164
Side * Individual	1.107	32	0.035	10.813	9.02E-29
Error	0.633	198	0.003		

Yellow-breasted Chat, Bill, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.090	1	0.090	2.720	1.10E-01
Individual	83.715	29	2.887	354.201	4.12E-143
Side * Individual	0.965	29	0.033	4.081	2.54E-09
Error	1.467	180	0.008		

Least Bell's Vireo, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.008	1	0.008	0.094	7.60E-01
Individual	126.010	47	2.681	683.946	1.55E-190
Side * Individual	4.212	47	0.090	22.860	5.55E-57
Error	0.753	192	0.004		

Least Bell's Vireo, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	2.770	1	2.770	14.165	4.92E-04
Individual	152.246	44	3.460	98.664	1.00E-104
Side * Individual	8.605	44	0.196	5.577	6.49E-17
Error	6.312	180	0.035		

Common Yellowthroat, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.928	1	0.928	10.215	3.06E-03
Individual	127.086	33	3.851	1711.600	2.05E-162
Side * Individual	2.999	33	0.091	40.382	3.43E-55
Error	0.306	136	0.002		

Common Yellowthroat, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	2.291	1	2.291	17.854	7.27E-05
Individual	124.212	68	1.827	50.910	1.11E-122
Side * Individual	8.727	68	0.128	3.577	5.09E-14
Error	9.903	276	0.036		

Song Sparrow, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.232	1	0.232	4.264	4.16E-02
Individual	358.902	96	3.739	1284.729	0.00E+00
Side * Individual	5.232	96	0.055	18.729	6.81E-100
Error	1.129	388	0.003		

Song Sparrow, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	7.698	1	7.698	30.263	6.60E-07
Individual	323.586	66	4.903	108.039	4.27E-160
Side * Individual	16.789	66	0.254	5.606	1.87E-24
Error	12.163	268	0.045		

Southwestern Willow Flycatcher, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.002	1	0.002	0.011	9.18E-01
Individual	83.355	29	2.874	2024.155	7.38E-148
Side * Individual	4.081	29	0.141	99.099	1.74E-70
Error	0.170	120	0.001		

Southwestern Willow Flycatcher, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	0.007	1	0.007	0.031	8.62E-01
Individual	75.685	36	2.102	53.769	3.39E-68
Side * Individual	8.631	36	0.240	6.132	1.01E-15
Error	5.787	148	0.039		

Yellow-breasted Chat, Tarsus, Museum skin

Source	SS	df	MS	F	<i>p</i>
Side	0.008	1	0.008	0.112	7.40E-01
Individual	150.303	32	4.697	2115.752	9.55E-164
Side * Individual	2.175	32	0.068	30.613	1.06E-46
Error	0.293	132	0.002		

Yellow-breasted Chat, Tarsus, Live bird

Source	SS	df	MS	F	<i>p</i>
Side	3.074	1	3.074	18.549	1.63E-04
Individual	157.657	30	5.255	136.784	4.30E-81
Side * Individual	4.971	30	0.166	4.313	4.07E-09
Error	4.764	124	0.038		

APPENDIX E**COMPARISON OF F-STATISTICS AND P-VALUES
GENERATED BY THE FA10 AND FPA ANALYSES**

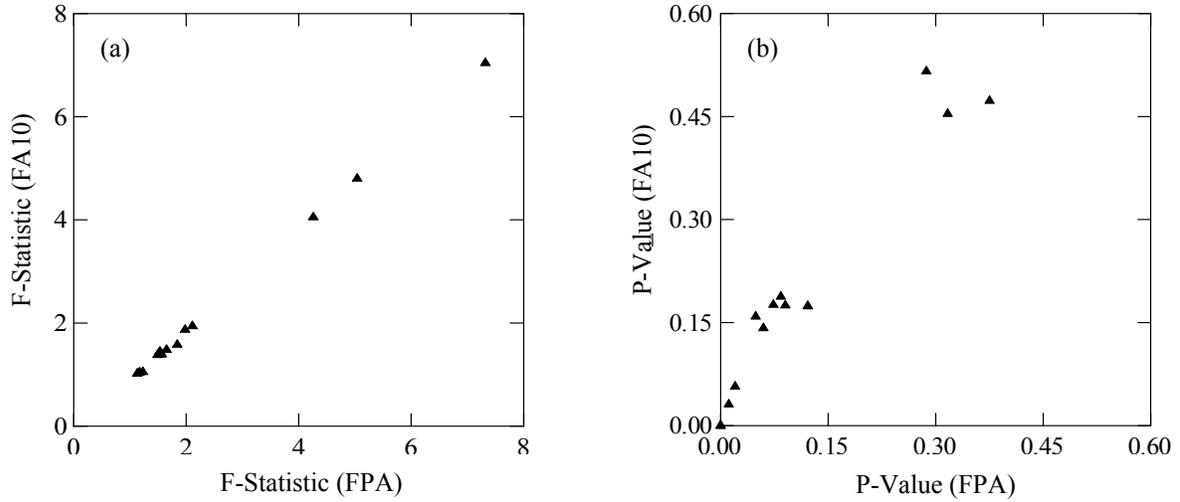


Figure 11. Comparison of F-statistics (a) and P-values (b) generated by the FA10 and FPA analyses for the tarsus trait of five avian species (Common Yellowthroat, Song Sparrow, Yellow-breasted Chat, Least Bell's Vireo, and Southwestern Willow Flycatcher) over three datasets (i.e. FA outliers removed at $\alpha = 0.05$, 0.01 , and no outliers removed - uncorrected). P-values were generated to test whether contemporary tarsus fluctuating asymmetry levels differed from their historic populations.

ABSTRACT

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Fluctuating asymmetry has been shown to increase in organisms exposed to environmental and/or genetic stress. I studied tarsus and bill length fluctuating asymmetry in five species of riparian obligate birds to investigate associations between a species level of endangerment and fluctuating asymmetry expression. Species ranged in Federal/State conservation status from endangered [Least Bell's Vireo (*Vireo bellii pusillus*) and Southwestern Willow Flycatcher (*Empidonax traillii extimus*)] and sensitive [Yellow-breasted Chat (*Icteria virens*)] to abundant [Song Sparrow (*Melospiza melodia cooperi*) and Common Yellowthroat (*Geothlypis trichas scirpicola*)]. Historic fluctuating asymmetry levels for each species were derived from museum skins collected between 1887 and 1938 in southern California. Contemporary asymmetry levels were derived from sampling live birds between 22 April 2002 and 14 July 2003 within the same region. Contemporary bill fluctuating asymmetry levels of the Southwestern Willow Flycatcher were significantly greater compared to historic levels. Comparing contemporary and historic Song Sparrow asymmetry indicated that Song Sparrow tarsus asymmetry increased, while bill asymmetry decreased. There was no significant change consistently detected in fluctuating asymmetry levels though time in either trait of the Least Bell's Vireo, Common Yellowthroat, or Yellow-breasted Chat, or in the tarsus of the Southwestern Willow Flycatcher. Contemporary fluctuating asymmetry levels of the two abundant species differed significantly for both the bill and tarsus traits. Results indicated no obvious correlation between a species' State/Federal conservation designation and its fluctuating asymmetry level.