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NATURAL SELECTION AND AGE-RELATED VARIATION IN MORPHOLOGY OF A
COLONIAL BIRD

by

Mary Bomberger Brown

A DISSERTATION

Presented to the Faculty of
The Graduate College at the University of Nebraska
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(Applied Ecology)

Under the Supervision of Professor Larkin Powell

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NATURAL SELECTION AND AGE-RELATED VARIATION IN MORPHOLOGY OF A COLONIAL BIRD

Mary Bomberger Brown, Ph.D.

University of Nebraska, 2011

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In May 1996, inclement weather led to the deaths of thousands of Cliff Swallows (*Petrochelidon pyrrhonota*) in Nebraska. Survivors had larger skeletons, shorter wings and tails, and less wing asymmetry than non-survivors. This population was followed for 10 years to study 1) whether natural selection events result in permanent microevolutionary changes, 2) if variation in climate affects the development of morphological traits, and 3) if morphological traits vary systematically with age.

Patterns in morphology exhibited by swallows following the selection event were studied by measuring yearling birds. Wing and middle tail lengths decreased, beak length and width increased, tarsus length was unchanged, and the amount of wing asymmetry increased. The cumulative directional change in wing, tail, and beak length was greater after the selection event than during the event. This variation was not explained by phenotypic plasticity resulting from better environmental conditions, because conditions were not significantly different before and after the event. There was no evidence opposing selection restored skeletal size or wing or tail length to that before the selection event. This continued change in morphology may represent the population shifting to a different fitness peak in the adaptive landscape.

The way variation in climatic conditions (and food resources) affects the morphological development of juvenile swallows was studied. In cooler years birds allocated less growth to wings and tails than they did in warmer years, while maintaining normal levels of skeletal growth and body mass. Changes in juvenile feather growth in response to rearing conditions persisted into the first breeding season.

The extent morphological traits vary with age across a bird's lifetime was examined. Juveniles had shorter wings and tails, lower body mass, smaller skeletal size and lower levels of fluctuating asymmetry than adults. Among adult age classes, wing and tail length increased with age and wing and tail fluctuating asymmetry decreased with age. There was no evidence for

degenerative senescence in swallows, as the decline in fluctuating asymmetry suggests the oldest birds maintain high levels of phenotypic performance. This age-related variation in morphology suggests that age should be considered in future analyses of morphological variation in passerines.

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To Charles R. Brown, it has been a wonderful life. Thank you.

Cliff Swallows

Is it some turn of wind
that funnels them all down at once, or
is it their own voices netting
to bring them in – the roll and churr
of hundreds searing through river light
and cliff dust, each to its precise
mud nest on the face
none of our own isolate
groping, wishing need could be sent
so unerringly to solace. But
this silk-skein flashing is like heaven
brought down: not to meet ground
or water – to enter
the riven earth and disappear.

--Debra Nystrom

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INTRODUCTION

“Natural selection tends only to make each organic being as perfect as, or slightly more perfect than, the other inhabitants of the same country with which it comes into competition... Natural selection will not produce absolute perfection, nor do we always meet, as far as we can judge, with this high standard under nature.”

Charles Darwin – The Origin of Species (1859)

Natural selection events that affect wild populations vary substantially across time and the resulting long-term pattern in the observed phenotypic effects cannot be predicted. These phenotypic effects can be morphological or behavioral in nature and the patterns may be unidirectional, oscillating, episodic or gradual in form (Grant and Grant 2001). Hence, long-term research programs, such as the Nebraska Cliff Swallow study, a small part of which is described here, are needed to detect and interpret the consequences of natural selection events and other forms of ‘non-uniform evolutionary change’ (Grant and Grant 2002) in natural populations. The results, analysis and interpretations presented in the following three chapters represent one of the first broad-scale, long-term examinations of natural selection in a wild population that incorporates a measure of developmental stability (fluctuating asymmetry, a repeated formation index; see Appendix) with morphological measures (Brown and Brown 1998, 2000, 2002a, b, 2003, 2011).

The following study is divided into three chapters. Chapter 1 addresses whether episodic selection events result in permanent microevolutionary changes in natural populations or if they are reversed by opposing selection pressures. Specifically, 1) whether the directional selection on morphology documented after a selection event has been reversed, 2) whether there is evidence of continuing directional selection on any of the morphological traits, 3) whether the morphological changes over time are consistent with possible responses to global climate

change, and 4) whether the microevolutionary changes documented in the selection event resulted in a relatively permanent shift in body size in this population of Cliff Swallows. Chapter 2 addresses the question of how juvenile Cliff Swallows allocate growth among different morphological traits in response to climatically-based annual variation in food availability. Chapter 3 addresses questions of age-related changes in individual Cliff Swallows. Specifically, 1) if morphological traits increase in size early in life or among older age classes and 2) if there is evidence for senescence-related decreases in body size and/or fluctuating bilateral asymmetry among the oldest individuals.

Study Background

Study Animal – Cliff Swallows (Hirundinidae: *Petrochelidon pyrrhonota*: Vieillot, 1817) are highly colonial, 20 – 28 gram Neotropical migrant passerine birds. They breed throughout much of North America and winter in South America (reported anecdotally to be Paraguay, Uruguay, and Argentina) (Brown and Brown 1995). Cliff Swallows exhibit no obvious sexual dimorphism and are sexually monochromatic, with males and females essentially identical in all plumages (Brown and Brown 1995); within pairs the male may have a larger dark blue patch on the lower throat than the female (Brown and Brown 1995; MBB, pers. obs.). They are diurnal, aerial insectivores, feeding exclusively on flying insects and can only forage when weather conditions allow flying insects to be active. Cliff Swallows begin arriving in the study area (southwestern Nebraska) in late April (earliest arrival date is 13 April) and continue to arrive throughout the month of May. They build enclosed, gourd-shaped mud nests that are approximately 15 – 20 cm in diameter and have entrances that are 5 – 7 cm wide. Individual nests in Cliff Swallow colonies are often densely packed and nesting is highly synchronous within colonies, but less so between colonies. Nest entrances are, on average, approximately 25 –

30 cm apart and nests often share walls. Most nests contain clutches ranging in size from 1 – 6 eggs; clutches with more than 4 eggs likely are cases of intraspecific brood parasitism or egg transfer; extra pair fertilization occurs frequently. Eggs are incubated for approximately 2 weeks. Brood sizes generally range from 1 – 4 nestlings per nest (7 is the largest brood reported in the study area to date) and most nestlings fledge 23 – 26 days after hatching. After they leave the nest, fledglings congregate in crèches where they are fed and escorted by their parents for 4 – 5 days. After that time the fledglings are independent of their parents but do remain in the study area for several weeks before migration. During this time they range widely, visiting colonies that still have active nests. Nesting is largely complete by the end of July and nesting success declines as the season advances. The study animal is described in greater detail in Brown (1998) and Brown and Brown (1995, 1996).

Cliff Swallows have been studied near the University of Nebraska School of Biological Sciences' Cedar Point Biological Station (41° 13' N, 101° 39' W) continuously since 1982; data collection for the current study took place between 1996 and 2006. The 150 x 50 kilometer study area lies along the North and South Platte rivers in southwestern Nebraska and includes parts of Keith, Deuel, Garden, and Lincoln counties. Historically, Cliff Swallows built their mud nests on the sides of cliffs, but with the advent of human-created structures such as road bridges, box-shaped road culverts, and the eaves of buildings, most birds in the study area are now placing their nests on these sorts of structures. In the study area, this change in nest placement habits began when Kingsley Dam was closed in 1940. The construction of the dam and the associated irrigation structures and canals provided the birds with an abundance of stable, protected "cliffs". There are approximately 170 structures in the study area that have supported nesting colonies during the course of the Cliff Swallow study. In any given year, about a third of these structures

will not be used by nesting birds. Each colony site tends to be separated from the next nearest by 1 – 10 kilometers but in a few cases colonies are separated by more than 20 kilometers. The size of nesting colonies varies widely within and between years; the mean colony size is 405 nests (SE = ± 14 , N = 2,209 colonies, 29 years), with some birds nesting solitarily. The study area is described in greater detail by Brown and Brown (1996).

Capture and Banding – Cliff Swallows were captured using mist nets. Mist nets were placed across the entrances of culverts or along the sides of bridges; at some bridge sites mist nets were dropped down from above, catching birds when they flushed from their nests. During the years of this study, Cliff Swallows were mist-netted at 27 – 40 colony sites each year and each colony site was mist-netted on 1 – 37 days during each year. The number of netting days at a colony site was determined by the number of birds at the colony, the phenology of the colony, and a variety of other factors that affected the practicality of netting at a colony site. All birds caught in nets were banded with individually numbered, aluminum USGS leg bands upon first capture, sexed by the presence of a cloacal protuberance or brood patch, aged on the basis of facial plumage patterning, and weighed to the nearest 0.5 gram with a Pesola® scale by placing the bird in a soft cloth bag. The netting and banding protocols are described in greater detail by Brown and Brown (1996) and Brown (1998).

Colony and Population Size Estimates – The entire study area was surveyed for colony site usage and colony size in late June and early July during every year of the study. Colony size is defined as the maximum number of active nests at a particular location during one breeding season. The use of a single colony size estimate for each colony site for each year is a useful metric for comparing different colonies. The protocol for estimating colony size and usage is described in greater detail by Brown and Brown (1996).

Morphometric Measurements – All measurements in this study were conducted by one individual (MBB), consequently no corrections to the data for multiple measurers was required (e.g., Price and Grant 1984; Jones 1987; Bryant and Jones 1995; Grant and Grant 1995). This same individual (MBB) measured all birds in other Cliff Swallow morphometric studies (e.g., Brown and Brown 1998, 2002a, b, 2003, 2011), so the measurements reported here are comparable with those in earlier work. For all birds the following measurements were taken: the length of the flattened, closed wing (from the anterior most part of the wrist (carpal) joint to the tip of the outermost primary feather or rectrice) with a stoppered wing ruler; the length of the middle and two outermost tail feathers or remiges (from their emergence from the follicle at the surface of the skin to the distal tip) with a ruler; the length of each tarsus (from the proximate end of the tarso-metatarsus to the base of the hallux) with a calipers, and the length and width of the exposed culmen (length from the proximate end of the exposed culmen to the beak tip along the upper mandible and width of the exposed mandibles at the nares) with calipers. Wing and tail feathers that were broken or damaged were not measured for any birds. Likewise, no tarsi or culmen were measured if they appeared damaged or malformed in any way. Wing and tail lengths were measured to the nearest whole millimeter and tarsus and culmen lengths were measured to the nearest 0.1 millimeter. These measurement limits were chosen because taking more precise measures on living Cliff Swallows in the field was impractical.

1996 Mortality Event – Since Cliff Swallows are obligate aerial insectivores, they are particularly affected when cold, rainy weather reduces the availability of aerial insects. Mortality due to starvation may result if these conditions last for several (usually four or more) days (Anderson 1965; DuBowey and More 1985; Littrell 1992; Brown and Brown 1996, 1998, 2011). One such event occurred in May 1996. A six day period of unusually cold and wet

weather began on 24 May 1996. During this period, most Cliff Swallows in the study area were building nests or incubating eggs. According to National Weather Service records, during this period, the average maximum temperature was 11.1° C (123-year average = 24.0° C) and the average minimum temperature was 6.4° C (123-year average = 9.0° C). There was measurable precipitation on three of the six days, and the average amount of precipitation per day was 1.7 cm. The six day period of cold rainy weather caused considerable Cliff Swallow mortality, the total number of active nests in the study area fell from 29,490 in 1995 to 13,827 in 1996.

The geographic scope of this mortality event was extensive. There were reliable reports of Cliff Swallow mortality ranging from north-central Iowa westward to eastern Wyoming. Based on an analysis of weather records, it appears that the mortality event extended north into South Dakota and south into northern Kansas (Brown and Brown 1998). The population of Cliff Swallows was reduced by at least 53% (Brown and Brown 1998) and more likely by as much as 73% (Price et al. 2000). According to records maintained by the United States National Weather Service (North Platte, NE reporting station), there has been only one other weather event comparable to this one (in 1967) since records began being kept in 1875 (Brown and Brown 1998).

The Cliff Swallows that survived this mortality event tended to have shorter wings, longer tarsi, longer and wider beaks (culmen), and lower asymmetry than those that did not survive. These differences led to a substantial shift toward larger body size and greater symmetry in bilateral traits in this population of Cliff Swallows (Brown and Brown 1998, 2011). There was also selection for later arrival in the spring (Brown and Brown 2000).

The Cliff Swallow population that survived this mortality (and selection) event was followed for 10 years (1997 – 2006). The current study is based on the measurements of this

population of Cliff Swallows. The population that survived the event has been used in studies of weather-mediated natural selection on arrival time (Brown and Brown 2000) and effects of ectoparasites on asymmetry (Brown and Brown 2002a); the population that did not survive the event has been used in studies of the effects of coloniality on spleen and testis size (Brown and Brown 2002b, 2003).

Statistical Analyses –Repeatabilities (r_i) of the morphological measurements were estimated using intraclass correlation (Zar 1974; Kuehl 2000; Soper 2009) from a sample of 1525 birds that were measured twice while alive during the same breeding season (1997 – 2006) (1997 = 142 , 1998 = 108, 1999 = 216 , 2000 = 118, 2001 = 95, 2002 = 99, 2003 = 215, 2004 = 280, 2005 = 148, 2006 = 104). These samples of birds were chosen haphazardly by virtue of the repeated measures being done haphazardly (at the time of measurement, the birds' identities were not referenced by the measurer). Repeatabilities were high and statistically significant ($P < 0.0001$). Wing asymmetries were all significantly higher than expected based on measurement error. Measures of wing, tail, and tarsus asymmetry all have a relatively high degree of precision in this study and do not represent noise in the measurement process. Repeatabilities of the morphometric measurements made by this measurer (MBB) in an earlier study (Brown and Brown 1998) are given provided below.

Repeatability of morphological measurements of Cliff Swallows; data taken from 52 birds measured twice while alive during the same breeding season (1996) and 111 dead birds measured twice as specimens are shown below. Missing repeatabilities are due to those measurements not being taken on live birds in 1996. All repeatabilities were highly significant ($P < 0.0001$, sequential Bonferroni corrections).

Trait	Alive	Dead
Right wing	0.921 ± 0.056	0.955 ± 0.029
Left wing	0.873 ± 0.085	0.968 ± 0.024
Middle tail	0.724 ± 0.095	0.943 ± 0.032
Right outer tail		0.988 ± 0.015
Left outer tail		0.965 ± 0.025
Right tarsus		0.910 ± 0.039
Left tarsus	0.755 ± 0.093	0.938 ± 0.033
Culmen length	0.657 ± 0.107	0.921 ± 0.038
Culmen width	0.615 ± 0.111	0.899 ± 0.043
Wing fluctuating asymmetry	0.771 ± 0.058	0.891 ± 0.020
Tail fluctuating asymmetry		0.851 ± 0.004
Tarsus fluctuating asymmetry		0.682 ± 0.051

In this study, as in Brown and Brown (1998), there was evidence for directional asymmetry in wing length measurements. Asymmetry in wing, tail, and tarsus is expressed as the unsigned or absolute R – L values (right minus left) of each measurement. Mean signed asymmetry for wing length differed significantly from 0 (one-sample *t*-test, $P < 0.001$). Mean signed asymmetry for all other measurements did not differ significantly from 0 ($P > 0.10$). This directional asymmetry most likely reflects a handedness bias in this particular measurement by the measurer (MBB; Brown and Brown 1998). It is not uncommon to find this bias with wing measurements taken of living birds (Helm and Albrecht 2000). As suggested by Palmer (1994), this directional asymmetry was corrected by calculating the ([mean signed asymmetry] / 2) for wing and subtracting these values from the longer average side and adding these values to the shorter average side for each observation. The corrected signed and unsigned asymmetry values are both presented and analyzed in this study.

Fumigation – The number and variety of ectoparasites associated with Cliff Swallows is substantial and includes fleas (Siphonaptera: Ceratophyllidae-*Ceratophyllus celsus celsus*),

chewing lice (Mallophaga; Amblycera: Menoponidae-*Machaerilaemus malleus* and Ischnocera: Philopteridae-*Brueelia longa*), plumicolus feather mites (Acari: Astigmata, Avenzoariidae-*Pteronyssoides obscurus*), swallow bugs (Hemiptera: Cimicidae-*Oeciacus vicarius*), and soft-bodied ticks (Acarina: Argasidae-*Ornithodoros concanensis*) (Brown and Brown 1996; Brown et al. 2006). Of these parasites, the hematophagous swallow bug appears to have the greatest impact on the general health, survival and reproductive success of the Cliff Swallow (Brown and Brown 1986, 1992, 1996, 2006); infestations can reach up to 2600 swallow bugs per nest. In order to study the impact of swallow bugs on Cliff Swallows, nests were fumigated in selected colonies (every year since 1984) by regularly spraying them with a dilute solution of Dibrom® (also known as 'naled'; Chevron Chemical Corporation). This acaricide is highly effective in killing swallow bugs (Brown and Brown 1986, 1996). The nest fumigation protocol is described in greater detail by Brown and Brown (1996).

Number of Cliff Swallows measured during the course of this study.

Year	Male	Female	Total Adults	Juveniles
1997	1081	924	2005	
1998	1160	1106	2266	
1999	1731	1414	3145	
2000	1932	1432	3364	558
2001	1621	1249	2870	505
2002	1856	1428	3284	551
2003	2394	2044	4438	1017
2004	3141	2369	5510	607
2005	2029	1473	3502	1124
2006	1725	1325	3050	351

Total males = 18,670

Total females = 14,764

Total adults = 33,434

Total Juveniles = 4,713

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CHAPTER 1. Intense natural selection on morphology of Cliff Swallows a decade later: did the population move between adaptive peaks? ¹

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Abstract: Unusual climatic events often lead to intense natural selection on organisms. Whether episodic selection events result in permanent microevolutionary changes or are reversed by opposing selection pressures at a later time is rarely known, because most studies do not last long enough to witness rare events and document their aftermath. In 1996, unusually cold and wet weather in southwestern Nebraska led to the deaths of thousands of Cliff Swallows (*Petrochelidon pyrrhonota*) over a 6-day period. Survivors were skeletally larger, with shorter wings and tails, and had less asymmetry in wing length than those that died. We determined trajectories of morphological traits in the decade following this event by measuring yearling birds each year from 1997 to 2006. Wing and middle tail-feather lengths continued to decrease, bill length and width continued to increase, tarsus length was unchanged, and levels of asymmetry in wing length increased. Cumulative directional change in wing, tail, and bill length was greater in the decade after selection than during the selection event itself. Morphological variation could not be explained by phenotypic plasticity resulting from better environmental conditions during growth, because weather variables (that influence food supply and ectoparasitism) were not significantly different before and after selection. There was no evidence that opposing selection restored skeletal size or wing or tail length to that before the selection event. The reasons for continued change in morphology in this population are unclear but may represent a population shift to a different fitness peak in the adaptive landscape as a consequence of the intense selection in 1996.

Key words: body size, Cliff Swallow, episodic selection, fluctuating asymmetry, morphological evolution, *Petrochelidon pyrrhonota*.

Intense episodic selection events often provide opportunities for observing significant evolutionary change over short time frames (Bumpus 1899, Endler 1986, Grant and Grant 2002, Siepielski et al. et al. 2009). Most often, unusual climatic conditions are the drivers for rapid selection, but other events, such as epidemics, drastic habitat modification, introduction of new predators, or colonization of vacant island habitats, may also cause short-term microevolutionary shifts in particular traits (e.g., Hairston and Walton 1986, Grant and Grant 1993, Benkman and Miller 1996, Clegg et al. 2008, Mathys and Lockwood 2009, Wilcoxon et al. 2010; reviewed in Carroll et al. 2007). However, the extent to which rare selective episodes result in relatively permanent microevolutionary change is less well documented. Although many studies have measured the intensity of natural selection in the wild (Siepielski et al. 2009), most field studies are not conducted over long enough periods to both witness rare selection events and determine the long-term trajectories of any trait changes. A notable exception is Grant and Grant's (2002) 30-year study of evolution in body size and bill dimensions of two species of Darwin's finches of the Galápagos. They documented several periods of intense selection on morphology brought about by rare climatic events, and as they followed these populations after selection, unpredictable patterns of change were revealed (Grant and Grant 2002). In most cases, intense selection during unusual climatic conditions tended to be reversed in the years immediately after these events by opposing selection pressures, yet both species showed long-term changes in morphology apparently brought about by recurring bouts of directional selection in response to environmental changes (Grant and Grant 1989, 1993, 1995, 2002; Price et al. 1984). The Darwin's finch study suggests that rare selection events can serve as catalysts to the evolution of some traits, yet the generality of this conclusion is difficult to evaluate because so few comparable long-term studies of natural populations exist.

In late May 1996, unusually cold and wet weather extending over a 6-day period resulted in the deaths of thousands of Cliff Swallows (*Petrochelidon pyrrhonota*) in southwestern Nebraska, reducing the breeding population by at least half (Brown and Brown 1998). The cold and wet weather depressed the abundance of the flying insects that Cliff Swallows feed on to the extent that a portion of the population starved to death. The comparison of morphological measurements of those that died and birds that survived the event revealed strong selection on tarsus, wing and bill length, and bill width; in addition, those with lower levels of fluctuating asymmetry in wing length tended to survive. We hypothesized that the skeletally larger birds were favored because they had been able to store more fat before the inclement weather commenced and that low levels of wing asymmetry may have promoted more efficient foraging in cold weather (Brown and Brown 1998). These morphological characteristics were retained in the first-generation offspring of the survivors.

Here, we report how morphology of Cliff Swallows in the Nebraska study area changed in the 10 years subsequent to the 1996 selection event. Measuring the same traits of birds known to be born in the study area and taking the measurements at identical ages (1 year old) in each year over a decade allowed us to investigate long-term changes in morphology. Specifically, we asked whether the directional selection on morphology documented in 1996 had been reversed by opposing selection pressures in response to potential environmental change in subsequent years; whether there is evidence of continuing directional selection on any of the traits, perhaps as a result of the population crossing to a different adaptive peak (Feare and Price 1998, Arnold et al. 2001); whether the morphological changes over time are consistent with possible responses to global climate change (e.g., Van Buskirk et al. 2010, Yom-Tov 2001); and whether the microevolutionary changes documented in the episodic selection event resulted in a relatively

permanent shift in body size in this population of Cliff Swallows.

METHODS

Study site. Cliff Swallows have been studied since 1982 near the Cedar Point Biological Station (41°13'N, 101°39'W) in Keith County, southwestern Nebraska, along the North Platte and South Platte rivers; the study area also includes portions of Deuel, Garden, and Lincoln counties. Cliff Swallows construct gourd-shaped mud nests, often in dense, synchronously breeding colonies. In our study area, the birds nest mostly on the sides of bridges, in box-shaped road culverts, or underneath overhangs on the sides of cliffs. The study area contains about 170 colony sites, of which about a third are not used in a given year. Colony size varies widely; in our study area it ranges from 2 to 6,000 nests (mean \pm SE = 393 \pm 15, n = 1,812 colonies), with some birds nesting solitarily. The study site is described in detail in Brown and Brown (1996).

Measurements. Beginning in 1997 and continuing through 2006, birds were measured during the course of a long-term mark–recapture project in which we rotated among 25–40 colonies in the study area on a regular basis each year, mist netting birds for studies of survival and movement (Brown and Brown 2004, 2009; Brown et al. 2007, 2008a, b). At smaller colonies, we measured most birds captured on a given occasion; at larger sites, processing time allowed us to measure only a subset of those netted. At the latter sites, we generally tried to include as many previously banded birds as possible, because more information on those individuals was potentially available (e.g., age, prior colony-site use). In the field at the time of measurement, however, we knew only a previously marked bird's band prefix and thus the approximate year it was banded. Birds were sexed by the presence of a cloacal protuberance, brood patch, or both.

M.B.B. measured all birds in all years of the present study and in the 1996 weather event

(Brown and Brown 1998), and thus no corrections to the data for multiple measurers were necessary. For all birds, the length of each unflattened, closed wing (from the anterior-most part of the wrist joint to the tip of the outermost primary) was measured to the nearest 1 mm with a stoppered wing ruler; the length of the middle tail feather (from its emergence from the skin to the distal-most point) was measured to the nearest 1 mm with a ruler; the length of each tarsus (from the proximate end of the tarso-metatarsus to the hallux) was measured to the nearest 0.1 mm with calipers; and the length and width of the exposed culmen (length from the proximate end of the exposed culmen to the bill tip along the upper mandible and width of the exposed mandibles at the nostrils) were measured to the nearest 0.1 mm with calipers. Asymmetry in wing length was expressed by the unsigned difference between the right and left wing measurements (Palmer 1994).

Repeatabilities of morphometric measurements by M.B.B. are given in Brown and Brown (1998); repeatabilities, in general, were high and statistically significant. We also found that wing asymmetry was higher than expected given the measuring error, which means that asymmetry could be distinguished from random noise (Brown and Brown 1998). We found evidence for directional asymmetry in wing measurements, as indicated by signed asymmetry values that differed significantly from zero (Brown and Brown 2002). This asymmetry likely reflected a handedness bias by the measurer (Brown and Brown 1998, 2002). To correct for directional asymmetry (Palmer 1994), we divided the mean signed asymmetry by 2 and subtracted the result from the wing measurement of the larger average side and added the latter value to the measurement of the smaller average side for each observation. The corrected unsigned asymmetry values are those presented and analyzed here. Statistical analyses were performed with SAS (SAS Institute 2004).

Individuals included in analyses. For the period 1997–2006, our analyses used only birds known by banding to have been born in the Nebraska study area the previous summer and that were thus 1 year old at the time of measurement. These birds had been banded as nestlings or recently fledged juveniles; those measured in 1998 and 1999 included some that were part of a cross-fostering experiment the previous year (Brown and Brown 2000) in which 5-day-old nestlings were moved between nearby colonies and reared in colonies of different sizes. Although many individuals were measured in multiple years, for the present study we used a bird's measurements only from the season in which it was 1 year old; thus, all measurements were from birds of identical life stage, and data from each year were statistically independent. The number of 1-year-old birds measured varied between 113 and 314 per season, reflecting differences in the number of nestlings and juveniles banded, recapture effort, and population size in a given year. If a 1-year-old bird was measured multiple times that season, average within-year values for its respective measurements were used.

For comparison, we used measurement data from the birds that did or did not survive the 1996 event, as described in Brown and Brown (1998). The principal difference between the data reported in the earlier publication and in these analyses is that here we separated the survivors and nonsurvivors from 1996 by sex. Sex had not been determined for the initial analyses (Brown and Brown 1998), but subsequently all nonsurviving birds were prepared as skins and dissected, so the sexes were known. Briefly, nonsurvivors were dead birds collected on the ground below nests or found inside nests on the day after the cold and rainy weather ended, and survivors were live birds (at least 1 year old) measured in 1996 in the immediate aftermath of the event. See Brown and Brown (1998) for additional details. The trait distributions prior to the 1996 selection event were unknown (Price et al. 2000).

Weather data.—To evaluate potential weather changes in the study area that could have influenced growth and development of nestlings before and after the selection event, we compared total June rainfall and average June high temperature in the 10 years prior to the selection event (1986–1995), during which time most of the birds that experienced the event hatched, with the same measures for the 10 years after the selection event (1996–2005), during which time the descendants of the survivors would have hatched. June was used because that is when most brood-rearing by Cliff Swallows occurs in the study area and their reproductive traits depend heavily on climatic conditions such as temperature and rainfall (Brown and Brown 1999a, b). Climatological data were taken from a long-term monitoring site in Arthur County, Nebraska, about 48 km directly north of the study area (Brown and Brown 1996). This site, part of the University of Nebraska’s Automated Weather Data Network, recorded daily high and low temperatures and total precipitation.

Quantitative changes in traits during selection. As a measure of relative changes in trait means during the 1996 selection event versus in the subsequent 10 years, we computed the directional change among trait means expressed as a fraction of the before-selection standard deviation (SD) using the following formula: $(\text{trait mean after selection} - \text{trait mean before selection}) / \text{trait SD before selection}$.

For the 1996 event, we used survivors and nonsurvivors combined as the before-selection population and survivors as the after-selection population. For the decade following selection, we used the 1996 survivors as the before-selection population and those measured in 2006 as the after-selection population.

RESULTS

Body-size variation over time. For skeletal measures of body size, the strong differences

documented between survivors and nonsurvivors of the 1996 selection event were maintained in the 10 years afterward (Fig. 1). Tarsus length remained essentially unchanged in later years, with mean values for both sexes mostly within the 95% confidence interval of the 1996 survivors' tarsus length (Fig. 1A). Mean tarsus length per year did not vary over time in the 1996–2006 interval for either males ($r_s = -0.16$, $P = 0.64$, $n = 11$ years) or females ($r_s = -0.21$, $P = 0.53$). By contrast, both bill length (Fig. 1B) and width (Fig. 1C) continued to increase in the years after the kill. Bill length of both sexes by 2003 was well outside the 95% confidence interval for that of the 1996 survivors (Fig. 1B), and bill width in males also tended to track to larger values than in 1996 (Fig. 1C). Bill length in both males ($r_s = 0.61$, $P = 0.046$) and females ($r_s = 0.68$, $P = 0.022$) increased significantly across the 11 years from 1996 to 2006, as did bill width ($r_s = 0.72$, $P = 0.012$ for both sexes). There was no evidence that any of the skeletal measures drifted back toward those of the nonsurvivors in 1996 (Fig. 1).

Wing length showed a continuing decline in the years following the 1996 kill, with yearly means for both sexes generally outside the 95% confidence interval for the survivors (Fig. 2A). Male mean wing length per year declined significantly after 1996 ($r_s = -0.87$, $P = 0.005$, $n = 11$ years), with females showing the same trend, though it was not significant ($r_s = -0.55$, $P = 0.078$). Wing length did not exhibit a directional increase in size toward that of the non-survivors from 1996 (Fig. 2A). Length of middle tail feathers showed a less consistent pattern, with some yearly values near the 95% confidence interval of the 1996 survivors, but the trend was for smaller middle tail feathers and generally away from that of the nonsurvivors (Fig. 2B). Neither males ($r_s = -0.46$, $P = 0.15$, $n = 11$) nor females ($r_s = -0.38$, $P = 0.25$) showed a significant change in middle tail feather length over the 11-year interval.

Average wing asymmetry increased in the years after the 1996 event, although by 2006

wing asymmetry was still below that of the nonsurvivors (Fig. 3). Both males ($r_s = 0.82$, $P = 0.002$, $n = 11$ years) and females ($r_s = 0.66$, $P = 0.026$) showed significant yearly increases in wing asymmetry with time over the 11-year period.

Quantitative changes in traits. Wing, middle tail, and bill lengths exhibited greater change (and in the same direction) in the subsequent 10 years than during the selection event (Table 1). Bill width showed about the same level of change, and tarsus length showed much less change in the subsequent period, compared with the selection event (Table 1). Wing asymmetry showed opposite patterns of change during the two periods (Table 1).

Weather before and after selection. Total June rainfall in the years prior to the selection event (mean = 6.8 cm year⁻¹; range: 3.0–13.9 cm) did not differ significantly from that in the years after the selection event (mean = 6.3; range: 2.3–9.9 cm; Wilcoxon test, $Z = 0.00$, $P = 0.98$). Average June high temperature in the years prior to the selection event (mean = 26.3°C year⁻¹; range: 23.1–30.2°C) did not differ significantly from that in the years after the selection event (mean 25.5°C year⁻¹; range: 22.9–29.5°C; Wilcoxon test, $Z = 0.49$, $P = 0.62$). In 2004, however, cold and wet weather in late June resulted in the mean high temperature for the second half of the month being at least 6°C colder than that for the comparable period in any other year either before or after the selection event. Many nestling Cliff Swallows in the study area, and some adults, starved to death during the period 17–20 June 2004, although overall population size was unaffected.

DISCUSSION

The most striking result of our study is that none of the measures of Cliff Swallow body size showed evidence of opposing selection in the years after the unusual climatic event, and the patterns of directional selection that occurred during that event have been maintained for most

traits. The trend toward evolution of birds of larger skeletal size (especially bill size) with shorter wings and tail has continued even in the apparent absence of similar selection events in subsequent years. Hypotheses to explain these results include (1) relaxation of opposing selection pressure for smaller body size due to recent environmental changes; (2) undetected continuing episodic selection events similar to that of 1996; and (3) as a consequence of the intense selection in 1996, the population may have crossed to another fitness peak on the adaptive landscape, leading to further directional selection on body size. In contrast to what we observed for body size, levels of fluctuating asymmetry in wing length moved in the opposite direction in the subsequent decade, likely indicating relaxed selection for high levels of symmetry in the apparent absence of extreme climatic events. All patterns seemed roughly similar between the sexes, with no sex-specific differences in any of the results.

Body size in Bank Swallows (*Riparia riparia*) in Great Britain in the 1980s showed evidence of oscillating selection, believed to be related in part to climatic conditions. Populations crashed in 1983–1984, with smaller birds being favored, presumably because of unusual drought conditions on the African wintering grounds (Jones 1987, Bryant and Jones 1995). Larger birds have reproductive advantages on the breeding grounds, however, and by 1990 countervailing selection for large size had restored the population to a body-size morphology largely characteristic of that before the drought (Bryant and Jones 1995). Similar reversals of selection were documented for Darwin's finches in response to short-term changes in rainfall that affected seed availability (Price and Grant 1984; Gibbs and Grant 1987; Grant and Grant 1989, 1995). Because we observed little shift in any morphological trait (except wing asymmetry; see below) back toward that before the severe weather event, opposing selection based on advantages associated with smaller skeletal size and longer wings and tail did not apparently occur in the

decade following selection in this Cliff Swallow population. We have not identified any obvious ecological or social benefit of small body size per se during the course of our long-term research on Cliff Swallow coloniality. Furthermore, the fact that the Nebraska population has retained its morphological characteristics in the aftermath of the selection event suggests that there have been no environmental changes or episodic disturbances (e.g., drought on the wintering range; Bryant and Jones 1995) that favored smaller body size or longer wings and tail during other times or stages of the annual life cycle, at least during the past decade. Not finding evidence of directional selection for smaller size is not surprising, given the lack of empirical evidence for advantages of being small in animals generally (Blanckenhorn 2000).

Long-term change in body morphology can occur through recurring bouts of directional selection, as documented in Darwin's finches (Price et al. 1984, Grant and Grant 2002). This requires environmental conditions that favor continued directional changes. Climatological records (Brown and Brown 1998) show that the 1996 weather event in our study area was one of only two such events of this magnitude in southwestern Nebraska since 1875. Cold and rainy weather of shorter duration occurs more frequently, which can result in limited Cliff Swallow mortality. These events also lead to viability selection for birds of larger skeletal size, lower asymmetry, and shorter wings (Brown and Brown 1998). One such selection event occurred in June 2004. The extent of mortality that resulted from the severe weather of 2004 was limited, but it was second only to the major mortality event of 1996 in severity of mortality. The 2004 event was also the latest in the summer that weather-related mortality is known to have occurred in our study area. We detected apparent selection for lower wing asymmetry in 2004, with a corresponding drop in levels of wing asymmetry among yearlings the following summer (Fig. 3). Other morphological traits seemed unaffected by the 2004 event. Although another drop in wing

asymmetry in 2001 (Fig. 3) might suggest another selection event of some kind in 2000, if so, it was not cold-weather-related and we did not detect it. The summer of 2000 was unusually dry, perhaps exerting selection on asymmetric individuals if drought affected foraging conditions. Although drought in 2000 and the cold weather event in 2004 may have produced patterns of selection similar to that of 1996, on balance it seems unlikely that directional selection in response to unusual environmental events has recurred frequently enough or has been intensive enough since the 1996 selection event to account, in general, for the morphological trajectories that we observed (Figs. 1 and 2).

The continued evolution of Cliff Swallow morphology in the same direction as that following the 1996 selection event suggests that this population may have crossed to another fitness peak in the adaptive landscape (Wright 1931, Arnold et al. 2001, Benkman et al. 2005). With the virtual elimination of the smaller and longer-winged birds in 1996 (Brown and Brown 1998, Price et al. 2000), any ecological or social niche occupied by individuals of this phenotype would have been permanently vacated. In addition, and in the absence of appreciable immigration from populations not exposed to the 1996 selection event, presumably most of the additive genetic variation for small skeletal size and longer wings and tail would have been eliminated from the study population, given that avian morphology is highly heritable (Alatalo and Lundberg 1986, van Noordwijk et al. 1988, Wiggins 1989, Grant and Grant 1995). Consequently, the population may now experience a different fitness function in which larger birds with shorter wings continue to be favored. In support of this, we found some evidence that at least the distribution of tarsus length may have been bimodal before the selection event (Price et al. 2000). With the smaller mode now eliminated, the population as a whole cannot cross the adaptive “valley” of intermediate size because larger birds are continually favored over ones that

drift back toward the deleterious intermediate size on the population's way to becoming smaller. The adaptive landscape model has been criticized, and few empirical studies support it (Coyne et al. 1997), but it seems consistent with the patterns reported here. Linking size to fitness measures such as annual survival and nesting success would be necessary to better determine the applicability of the adaptive landscape concept to Cliff Swallow morphological evolution.

Why might birds with larger bills and shorter wings continue to be favored in the aftermath of the 1996 climatic event and in the absence of continuing episodic selection of this nature? One possibility is that avian morphology is responding to global climate change. Studies on multiple species from both North America and Europe have documented directional changes in morphological measurements over the past 50 years that are interpreted as responses to warming global climate (Przybylo et al. 2000, Yom-Tov 2001, Kanuscak et al. 2004, Guillemain et al. 2005, Yom-Tov et al. 2006, Monahan 2008, Van Buskirk et al. 2010). For many of these species, the trend is for shorter wings, as we documented for Cliff Swallows. The rationale offered is that warming climate favors smaller body size, based on Bergmann's biogeographic rule that animals in colder, more northerly latitudes tend to be larger (Ashton 2002).

However, for several reasons the climate-change hypothesis seems an unlikely explanation for directional change in morphology of Cliff Swallows in particular, and probably of birds in general. Although the trend in wing length for Cliff Swallows matched the pattern seen in other species, our skeletal measures showed that the Nebraska population increased in bill size and did not change in tarsus length (an index of body size), despite exhibiting the decline in wing length. Thus, studies that use only wing length (or mass, which in Cliff Swallows, at least, varies enormously across a day, a season, and with colony size; Brown and Brown 1996) as a surrogate of body size may not reflect a true reduction in skeletal size in some

species. In addition, global climate change may lead to an increased frequency of unusually severe weather events such as we saw in 1996 (Easterling et al. 2000, Greenough et al. 2001, Rosenzweig et al. 2001), and if so, larger (not smaller) body size should be favored to allow animals to better endure these unpredictable events (e. g., by retaining more body fat; Ashton 2002). Finally, there is little evidence for most species that recent changes in morphology (that are putatively linked to climate) reflect microevolutionary genetic change, and they more likely represent phenotypic plasticity (Gienapp et al. 2008, Teplitsky et al. 2008).

Developmental plasticity (Cooch et al. 1991, Larsson et al. 1998) could account for these patterns (Figs. 1 and 2) if conditions during brood rearing were routinely better in the decade after the selection event (1996–2005) than in the decade before (1986–1995). With more food available or smaller populations of ectoparasitic swallow bugs (*Oeciacus vicarius*) in the nests, both of which may be influenced by summer rainfall and temperature (Brown and Brown 1999a, b), birds may attain larger skeletal size simply as a result of favorable conditions for nestling growth. This might also be brought about by higher-quality parents being overrepresented in the population after the selection event (see below), which could lead to greater nestling-provisioning rates. However, we found no evidence that seasonal weather patterns in the study area were different before and after the selection event. Thus, phenotypic plasticity during growth, at least that attributable to weather-driven environmental conditions, can probably be ruled out.

The gradual increase in wing asymmetry (presumably a deleterious trait) in Cliff Swallows following the selection event (Fig. 3) supports studies on other organisms that show that fluctuating asymmetry has low heritability (e.g., Palmer and Strobeck 1997, Bryden and Heath 2000, Campo et al. 2005); otherwise, we should have seen a trajectory of little change in

the population following the intense selection event. The elimination of highly asymmetric individuals in this population in 1996 may have reflected simply a culling of low-quality individuals, with fluctuating asymmetry being a reliable index of phenotypic quality. With the relaxation of the episodic selection event in subsequent years (except in 2004; see above) and the population returning by 2001 to a size equal to that before the selection event, individuals of lower (non-genetically-based) phenotypic quality presumably increased and led to higher average levels of observed fluctuating asymmetry (Fig. 3).

Morphometric traits in many bird populations clearly are not static and often show directional change over time (e.g., Larsson et al. 1998; Nowakowski 2000, 2002; Acquarone et al. 2004; Guillemain et al. 2005; Yom-Tov et al. 2006; Monahan 2008; Van Buskirk et al. 2010). There may be no single general explanation for these patterns (e.g., climate change), and understanding each species' ecology may be necessary to interpret why morphology is so temporally variable. However, even in a population as well studied as the Cliff Swallows of southwestern Nebraska, the causes of the continuing directional changes in bill size and wing length remain unknown. Possibilities (at least for bill size) could include an increase in competition for the best nesting sites within colonies, increased competition to settle in colonies of particular sizes or to exclude other individuals from settling, better nest defense against conspecifics, increased competition (among males) for access to extrapair matings or (for females) to place as many parasitic eggs in other nests as possible, or habitat modifications (e.g., conversion of native prairie to crops) that have changed the size distribution of the birds' insect prey. All of these could potentially favor birds with larger bills, but there is no direct evidence that these factors have intensified in the study area in the past decade to the extent that we would expect an evolutionary response. The 1996 selection event and the subsequent directional

changes in morphology may be two relatively independent phenomena; lack of similar data prior to 1996 prevents us from knowing this with certainty. Nevertheless, the directional selection that occurred in the extreme climatic event has clearly not been reversed in the subsequent decade, leading apparently to a relatively permanent microevolutionary change in the morphology of this species. Whether opposing selection occurs in discrete bouts at even longer intervals (≥ 10 years) and results in directional change in the other direction can be determined only by continued long-term study of this population.

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Table 1-1. Intensity of selection as indicated by change in trait mean, expressed as the fraction of the before-selection standard deviation (ΔSD), for morphology of Cliff Swallows during an intense selection event in 1996 (Brown and Brown 1998), compared with that during the period from 1996 to 2006. ΔSD for the 1996 event was calculated using combined survivors and nonsurvivors (before selection) and survivors (after selection), and ΔSD for 1996–2006 used 1996 survivors (before selection) and birds measured in 2006 (after selection). Negative values indicate that trait mean decreased in size during the respective selection event; positive values indicate that the trait mean increased in size.

Trait	<u>1996 selection event</u>	<u>1996:2006</u>
	Δ SD	Δ SD
Male mean wing length	-0.18	-0.82
Female mean wing length	-0.14	-0.46
Male middle-tail-feather length	-0.16	-0.44
Female middle-tail-feather length	-0.16	-0.29
Male mean tarsus length	+0.95	-0.12
Female mean tarsus length	+1.29	-0.04
Male bill length	+0.72	+1.58
Female bill length	+0.98	+2.72
Male bill width	+0.78	+0.64
Female bill width	+1.12	+1.08
Male wing asymmetry	-0.20	+0.61
Female wing asymmetry	-0.50	+0.46

Figure 1-1. Average (A) mean tarsus length, (B) bill length, and (C) bill width for male and female Cliff Swallows each year following a 1996 selection event in southwestern Nebraska. Birds in 1997–2006 were 1 year old and born in the study area the previous summer. Sample sizes (number of birds measured) are shown next to symbols; error bars indicate SE. For comparison, survivors and nonsurvivors of the 1996 event (Brown and Brown 1998) are also shown, and the dotted line indicates 95% confidence interval for the 1996 survivors.

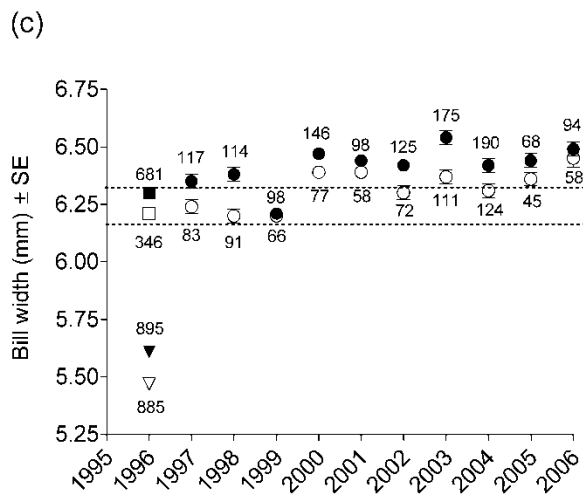
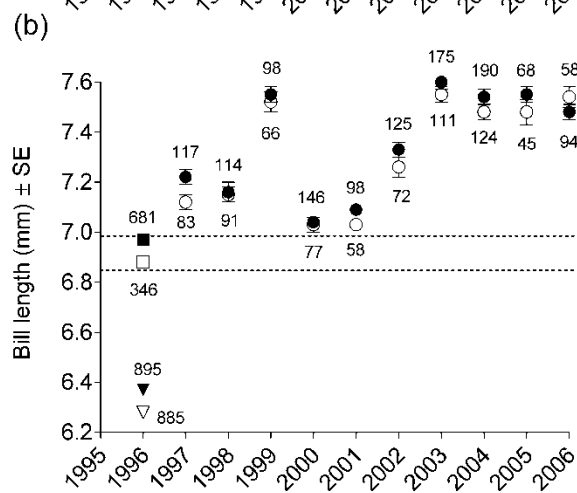
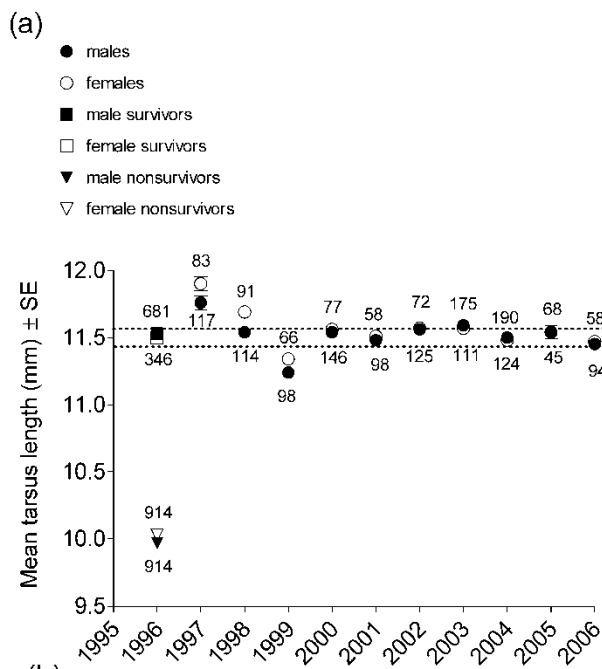
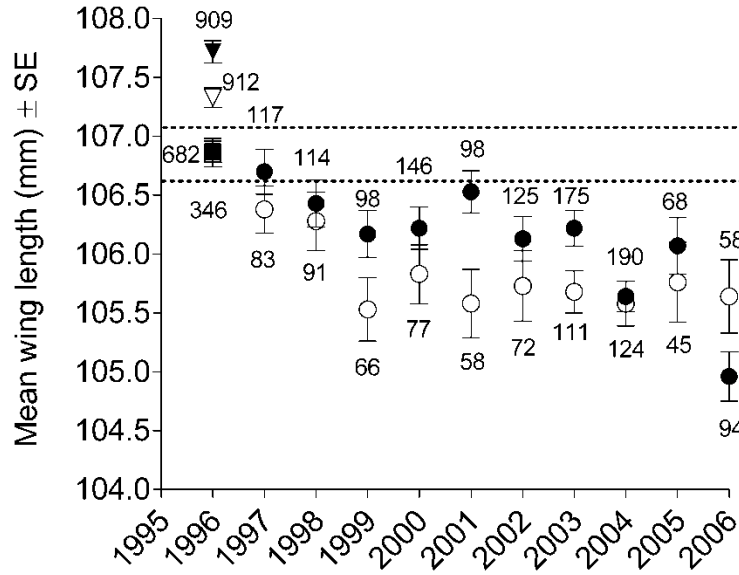


Figure 1-2. Average (A) mean wing length and (B) middle-tail-feather length for male and female Cliff Swallows each year following a 1996 selection event in southwestern Nebraska. Birds in 1997–2006 were 1 year old and born in the study area the previous summer. Sample sizes (number of birds measured) are shown next to symbols; error bars indicate SE. For comparison, survivors and nonsurvivors of the 1996 event (Brown and Brown 1998) are also shown, and the dotted line indicates 95% confidence interval for the 1996 survivors.

(a)

- males
- females
- male survivors
- female survivors
- ▼ male nonsurvivors
- ▽ female nonsurvivors



(b)

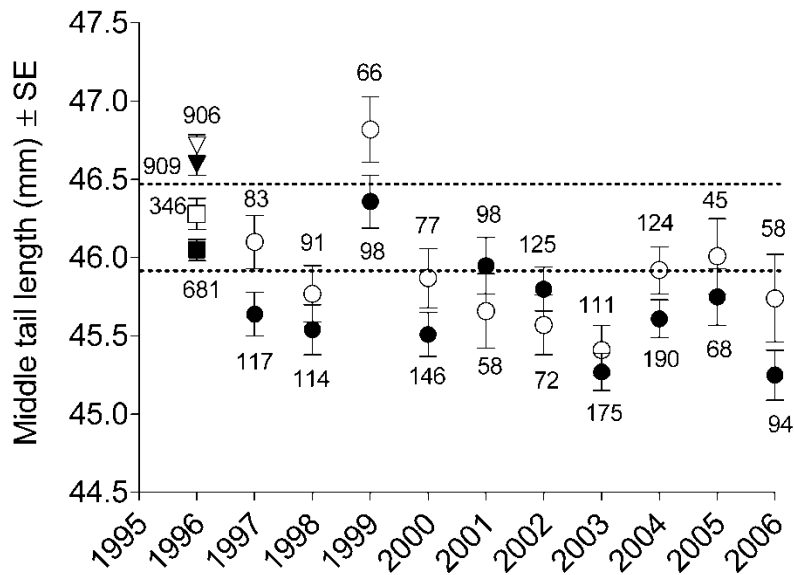
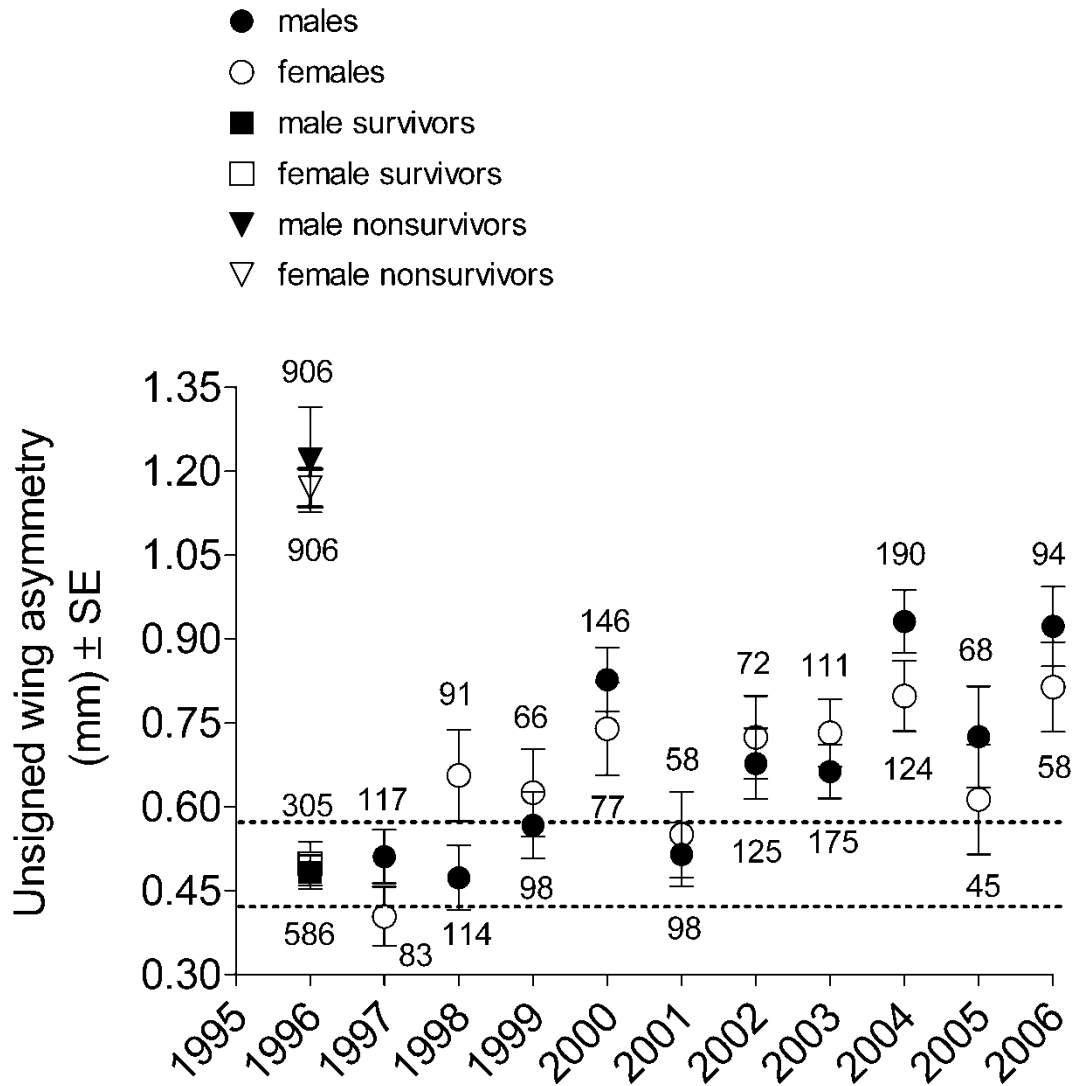


Figure 1-3. Average unsigned fluctuating asymmetry in wing length for male and female Cliff Swallows each year following a 1996 selection event in southwestern Nebraska. Birds in 1997–2006 were 1 year old and born in the study area the previous summer. Sample sizes (number of birds measured) are shown next to symbols; error bars indicate SE. For comparison, survivors and nonsurvivors of the 1996 event (Brown and Brown 1998) are also shown, and the dotted line indicates 95% confidence interval for the 1996 survivors.



CHAPTER 2. Effects of climatic variables on feather growth, skeletal traits, and body mass of juvenile Cliff Swallows (*Petrochelidon pyrrhonota*)¹

¹To be submitted to Climate Research. Coauthor: Charles R. Brown

Abstract: We investigated how seasonal variation in climatic variables (summer maximum temperature and total precipitation) that influence food availability in turn affect growth of wing, tail, and skeletal traits, and body mass in Cliff Swallows (*Petrochelidon pyrrhonota*), colonial, insectivorous birds that regularly experience brief periods of food deprivation during the nesting season. Birds were caught as juveniles soon after fledging and morphometric measurements taken; only birds occupying large colonies where the confounding effects of ectoparasites had been removed were studied. Average wing and tail length increased significantly with June maximum air temperature only. No skeletal traits, nor body mass, varied with May maximum temperature, and no traits showed a relationship with May or June precipitation. We conclude that only feather growth in Cliff Swallows is sensitive to climatically based natural variation in food availability during the summer, with birds in cooler years allocating less growth to wing and tail feathers while maintaining normal levels of body mass and skeletal growth. Because adult and juvenile feather lengths were positively correlated, any changes in juvenile feather growth in response to rearing conditions apparently persist into a bird's first breeding season.

Many birds in temperate latitudes experience periods of food deprivation during the breeding season, often due to transient periods of inclement weather which reduce food abundance or curtail foraging activities. Reductions in food availability occur regularly for aerial insectivorous species that depend exclusively on flying insects that are not active in cold or rainy weather (e.g., Löhrl 1971; Murphy 1983, 1985; Blancher and Robertson 1987; Brown and Brown 1996, 1998; McCarty and Winkler 1999; Bize et al. 2006). In response to periods of food deprivation, nestling birds might be expected to allocate energy for growth differently among skeletal traits, wing and tail feathers, and fat deposition (e.g., Congdon 1990; Ashton and Armstrong 2002; Dahdul and Horn 2003; Bize et al. 2006). The nature of these growth tradeoffs among morphological traits seems to vary across species, presumably reflecting differences in ecology and life histories (Dow and Gill 1984; Boag 1987; van Heezik 1990; Negro et al. 1994; Lepczyk and Karasov 2000; Ashton and Armstrong 2002; Benowitz-Fredericks et al. 2006; Sears and Hatch 2008).

Swallows are aerial insectivores that exhibit relatively long nestling periods and are particularly sensitive to periodic disruptions in food availability caused by weather conditions (e.g., Stewart 1972a, b; Bryant 1975; Brown 1976, 1997; Hoogland and Sherman 1976; DuBowy and Moore 1985; Littrell 1992; Brown and Brown 1996, 1998). Unusually cold or rainy weather leads to widespread mortality of both adult and nestling birds, but less dramatic climatic variation can also influence many aspects of these birds' reproductive biology, likely through daily fluctuations in flying insect abundance (Bryant 1975, 1978; Turner 1983; Brown and Brown 1999a, b; McCarty and Winkler 1999). In this study, we examine how juvenile Cliff Swallows (*Petrochelidon pyrrhonota*) potentially allocate growth among different morphological traits in response to climatically-based annual variation in food availability. These traits in

adults were previously shown to be targets of intense natural selection during periods of food deprivation (Brown and Brown 1998, 2011). By understanding the relative priority of skeletal and feather growth and body mass deposition during periods of relatively high versus low resource (food) availability, we may gain insight into the selective pressures potentially promoting long-term change in these trait trajectories (Brown and Brown 2011).

We make the explicit assumption in this study that annual variation in temperature and precipitation affects the flying insect prey upon which Cliff Swallows rely. Numerous studies have shown that flying insect activity varies directly with temperature and inversely with precipitation (e.g., Williams 1961; Taylor 1963; Johnson 1969). We previously demonstrated strong relationships between these annual climatic variables and time of laying, clutch size, and fledging success, and in all cases the relationships were ones predicted if food availability varied systematically with seasonal weather conditions (Brown and Brown 1999a,b). Summers with lower mean temperatures and higher precipitation reflect more days with conditions marginal for Cliff Swallow foraging, and thus in those years we assumed less food was available during brood rearing. The design of our study was to examine skeletal variables, feather growth, and body mass for juveniles in six different years and relate that to broad measures of climate during each season. We removed the confounding effects on nestling growth of (i) ectoparasites (Brown and Brown 1986, 1996) by using only fumigated, parasite-free nests; (ii) colony size (Brown and Brown 1996) by using only large colonies; and (iii) annual differences in ambient temperatures by studying a species living in an enclosed, mud nest where outside temperature extremes are buffered by the insulating properties of the mud nest (Mayhew 1958; Withers 1977). We use temperature and precipitation values for May to evaluate whether the foraging conditions female swallows experience during egg provisioning and laying affects their investment in egg quality

that is subsequently reflected in juvenile morphology. We also use these same variables to evaluate whether the foraging conditions the birds experience in June during brood rearing is reflected in juvenile morphology.

METHODS

Study species. Cliff Swallows (*Petrochelidon pyrrhonota*) are highly colonial Neotropical migrant passerine birds. They breed throughout much of North America and winter in southern South America (Brown and Brown 1995). They are diurnal, aerial insectivores and only forage when flying insects are active (Brown and Brown 1996, 1998). Juvenile Cliff Swallows have distinctive, mottled head and throat plumage, making them easy to distinguish from adults (Stoddard and Beecher 1983; Brown and Brown 1995). Upon fledging, many juvenile Cliff Swallows spend several days near their natal colony site, often entering other nests in the colony in apparent attempts to steal food from parents of smaller nestlings (Brown and Brown 1996). During this time, they can be easily caught in mist nets placed across the entrances of highway culverts or along the side of bridges. After they become independent of their parents, they remain in the study area for several weeks before migration, foraging and sometimes visiting colonies that still have active nests.

Study site. Cliff Swallows have been studied near the University of Nebraska School of Biological Sciences' Cedar Point Biological Station (41° 13' N, 101° 39' W) since 1982; data collection for this study occurred in June and July, 2001–2006. The study area lies along the North and South Platte rivers in southwestern Nebraska and includes parts of Keith, Deuel, Garden, and Lincoln counties. Historically, Cliff Swallows built their nests on the sides of cliffs, but most birds in the study area now place their nests underneath road bridges or inside box-shaped highway or railroad culverts. The size of nesting colonies varies within and between

years; the mean colony size is 405 nests (SE = ± 14 , N = 2209 colonies, 29 years), with some colonies as large as 6000 nests and other birds nesting solitarily. The study area is described in greater detail by Brown and Brown (1996).

Capture and banding. Juvenile Cliff Swallows were captured and banded as part of a long term mark-recapture project in which we rotated among different colony sites throughout the nesting season (Brown and Brown 1996, 2004, 2009). Mist nets were placed across the entrances of culverts or along the sides of bridges to catch birds as they flew through the culverts or underneath the bridges. Juveniles were birds capable of sustained flight that had hatched earlier that summer, known by the plumage pattern of the head and throat and their overall much duller coloration compared to adults. We restricted all juveniles in this study to those whose wing lengths were greater than 100 mm; this was done to ensure that only individuals that were independent of their parents and whose feathers had ceased growing were included in the analyses (MBB, CRB, pers. obs.). All juveniles caught in nets were banded with numbered USGS leg bands and weighed to the nearest 0.5 gram. Birds used in this study were caught primarily at two main study colonies that were fumigated each season to remove ectoparasites (Brown and Brown 1986, 1996); these sites varied from 955 to 1810 active nests in size during 2001–2006, which for each year at each site constituted a relatively large Cliff Swallow colony. Some comparisons in this study also used birds banded as nestlings or juveniles in the study area and re-caught and measured as breeding adults during their first nesting season the next summer; data collection for these birds is described fully in Brown and Brown (2011).

Morphometric measurements – All measurements of wing, tail, and skeletal traits in this study were conducted by one individual (MBB), and consequently no corrections to the data for multiple measurers was required (e.g., Price and Grant 1984; Bryant and Jones 1995; Grant and

Grant 1995). The following measurements were taken: the length of the flattened, closed wing; the length of the middle and two outermost tail feathers; the length of each tarsus; and the length and width of the exposed culmen. Wing and tail feathers that were damaged were not measured; likewise, no tarsi or culmen were measured if they appeared damaged or malformed. Wing and tail lengths were measured to the nearest whole millimeter with a stoppered wing ruler and tarsus and culmen lengths were measured to the nearest 0.1 millimeter with calipers (see Brown and Brown 1998, 2011). We measured the entire length of the birds' wing and tail feathers, because we were interested in a cumulative measure of how foraging conditions varied over the entire nestling period.

Fumigation. To remove the deleterious effects of blood-feeding swallow bugs (Hemiptera: Cimicidae: *Oeciacus vicarius*) on Cliff Swallow growth and development, nests in the colonies used in this study were fumigated by regularly spraying them with a dilute solution of Dibrom® (naled; Chevron Chemical Corporation). The nest fumigation protocol is described in greater detail by Brown and Brown (1986, 1996).

Weather. Weather data (May and June maximum daily air temperature and May and June daily precipitation) was acquired from the University of Nebraska High Plains Regional Climate Center's Kingsley Dam station located near the center of the study area (<http://www.hprcc.unl.edu>, ID: 254455; 41°21' N, 101°67' W, elevation 1011 meters). Using the daily data, we calculated the mean maximum air temperature (\pm 1SE) and the total precipitation for the months of May and June during each year of the study. We chose maximum air temperature and precipitation because previous studies have suggested that these weather variables have the greatest effect on the availability of the flying insects that Cliff Swallows depend on (Williams 1961; Taylor 1963; Johnson 1969) and that consequently are most likely to

affect the growth and development of nestling birds and the nutritional condition of females when they are provisioning and laying eggs (Bryant 1975, 1978, 1979; Turner 1983; Dow and Gill 1984; Murphy 1985; Blancher and Robertson 1987; Grubb et al. 1991; McCarty 2001). The months of May and June were used in our index of seasonal conditions because most Cliff Swallow eggs are laid in May and most brood rearing occurs during June (Brown and Brown 1996).

Statistical analyses. Repeatabilities (r_i) of the morphological measurements were estimated using intraclass correlation (Zar 1974; Kuehl 2000; Soper 2009) from a sample of 1525 birds that were measured twice while alive during the same breeding season. Repeatabilities for all traits were high and statistically significant ($P < 0.0001$; Brown and Brown 1998).

Fluctuating asymmetry in wing and tail was defined as the difference in measurements between the right and left sides, and asymmetry in both traits was significantly higher than expected based on measurement error. In this study, as in Brown and Brown (1998, 2011), there was evidence for directional asymmetry in wing length measurements. Mean signed asymmetry for wing length differed significantly from 0 (one-sample t -test, $P < 0.001$). This directional asymmetry likely reflects a handedness bias by the measurer (Brown and Brown 1998). We corrected for directional asymmetry by calculating ($[\text{mean signed asymmetry}] / 2$) for wing and subtracting these values from the longer average side and adding these values to the shorter average side for each observation (Palmer 1994). The corrected unsigned asymmetry values are presented in this study.

Spearman correlations were used to evaluate the relationship between temperature, precipitation, and morphological measurements and between juvenile and adult morphological

measurements. We used mean annual values for the morphological measurements in the analyses. Statistical analyses were performed with SAS (SAS Institute 2004), GraphPad Prism (GraphPad 2000), and Soper Calculators (Soper 2009).

RESULTS

During the six years of the study the mean May maximum air temperature varied between 21.7°C and 25.9°C and the mean June maximum air temperature varied between 25.5°C and 33.1°C (Fig. 1). The total precipitation for May varied between 2.6 cm and 5.4 cm (2001, 5.0 cm; 2002, 2.6 cm; 2003, 5.4 cm; 2004, 4.8 cm; 2005, 4.1 cm; 2006, 2.7 cm. The total precipitation for June varied between 2.1 cm and 10.4 cm (2001, 2.3 cm; 2002, 6.7 cm; 2003, 5.0 cm; 2004, 6.7 cm; 2005, 10.4 cm; 2006, 9.0 cm. There was no correlation between mean maximum air temperature and total precipitation (May $r_s = 0.4857$, $P = 0.3556$, $N = 6$; June $r_s = 0.2571$, $P = 0.6583$, $N = 6$); thus, warm years were not drier or wetter than cool years.

Of the nine morphological traits measured in this study, only wing length ($r_s = 0.9429$, $P = 0.0048$, $N = 6$) and outer tail length ($r_s = 0.8286$, $P = 0.0416$, $N = 6$) of juvenile Cliff Swallows were correlated with the June maximum air temperature (Table 1a); both traits increased in size with air temperature (Fig. 1a, b). None of the nine morphological traits were correlated with May maximum air temperature (Table 1b). For wing, there was about a 2-mm average difference between the coldest and warmest summers; the magnitude of the average change in tail length was small (< 1 mm; Fig. 1a, b). There was no association between mean maximum air temperature and any measure of skeletal traits, body mass, or fluctuating asymmetry (Table 1a). There was no association between total May or June precipitation and any measure of feather length, skeletal traits, body mass, or fluctuating asymmetry (Table 1b). Using the standard error of the May and June mean maximum temperature as our descriptor of seasonal temperature

variability, there was no association between temperature variation and any measure of feather length, skeletal traits, body mass, or fluctuating asymmetry (Table 1c).

Because of the significant relationships between wing and tail length and June temperature, we examined for these variables the likelihood that juvenile-based variation in these feather lengths was maintained for birds during their first breeding season. For 417 birds measured at both life stages, there was a significant positive relationship between both wing and tail length as juveniles and those traits as breeding adults the next summer (Fig. 2a, b).

DISCUSSION

Our results suggest that only feather growth in nestling Cliff Swallows is sensitive to climatically based natural variation in food availability during a summer, with birds in cooler years allocating less growth to wing and tail feathers while maintaining relatively normal levels of body mass and skeletal growth that were apparently unaffected by changes in resource availability. Reductions in juvenile feather growth in response to rearing conditions appeared to persist into a bird's first breeding season, despite an intervening molt that occurs on the wintering grounds (November – January; Brown and Brown 1995), because adult and juvenile feather lengths were positively correlated. While rainfall can have drastic effects on insectivorous birds such as Cliff Swallows by curtailing or entirely preventing foraging during rainy days (and also by altering prey abundance), we found no indication that juvenile morphology was related in any way to the extent of rainfall during the six summers of this study. However, this may have partly reflected the fact that these years overlapped extensively with a multi-year drought in the western Great Plains, and there may not have been enough natural variation in rainfall during this time to detect rainfall-related effects.

While some bird species exhibit reductions in skeletal traits such as tarsus or culmen during food deprivation (e.g., Rodway 1997; Bize et al. 2006; Sears and Hatch 2008), in most species skeletal measures seem the least sensitive to nutritional deficits (e.g., Dow and Gill 1984; Boag 1987; van Heezik 1990; Negro et al. 1994; Lepczyk and Karasov 2000; Dahdul and Horn 2003). Cliff Swallows conformed to this pattern, with no indication that skeletal growth in any way varied with seasonal climatic variables. Body mass also showed no relationship with presumed measures of food availability, in contrast to some species including other swallows (Boag 1987; Quinney et al. 1986; Ashton and Armstrong 2002; Benowitz-Fredericks et al. 2006). It appears that nestlings can prioritize body mass gain at the potential expense of growth of other traits during times of nutritional deficits, perhaps in part by exhibiting accelerated mass gain immediately following the food deprivation (McCarty and Winkler 1999; Lepczyk and Karasov 2000; Bize et al. 2006). Allocation to fat deposition might be especially important in aerial insectivores such as Cliff Swallows that experience frequent short-term fluctuations in food availability. Even in a relatively unusual summer such as 2004 in which June temperatures were well below seasonal norms (Brown and Brown 2011), juvenile body mass was unaffected.

Cliff Swallows are similar to a variety of other species in which wing growth in particular is compromised under stressful rearing conditions (e.g., Murphy 1985; Quinney et al. 1986; Grubb 1989; Johnston 1993; Negro et al. 1994; McCarty and Winkler 1999; Lepczyk and Karasov 2000; Benowitz-Fredericks 2006; Bize et al. 2006). Juvenile wings decreased by as much as 2 mm, on average, between relatively warm and cool summers, which was a reduction equivalent to or greater than that seen in intense episodic survival selection on wing length in adult Cliff Swallows (Brown and Brown 1998, 2011). The long-term consequences of shorter wings for juveniles (that are apparently maintained into adulthood) are unclear; analyses are

currently underway to determine whether juvenile wing length is associated with annual survival. Developmental strategies that sacrifice wing growth in favor of skeletal traits and/or body mass may reflect, in general, the phenotypic plasticity of feather growth and may also result from reduced amounts of protein, antioxidants, calcium, and other nutrients in the diet (Perrins 1976; Johnston 1993; Saino et al. 2002a, b; de Ayala et al. 2006; Gill 2007) during stressful conditions.

Greater phenotypic plasticity of wing and tail length in birds such as Cliff Swallows may be because most skeletal development takes place inside the egg during incubation; after hatching the skeleton increases in size and later ossifies, but all the components are present at hatching. Final skeletal size may depend more upon the female's nutritional state at the time the egg is provisioned and laid (Hamburger and Hamilton 1951; Deeming and Ferguson 1991; Stark 1998; Gill 2007) than upon foraging conditions after hatching. Nearly all feather development occurs outside the egg after hatching in altricial birds (Hamburger and Hamilton 1951; Deeming and Ferguson 1991; Stark 1998; Gill 2007). The epidermal and dermal tissues that form the feather follicles are present but not active until after hatching, so feather growth might be expected to be more sensitive to environmental or nutritional conditions.

That we did not see a relationship between foraging conditions during egg-laying and subsequent juvenile morphology suggests that foraging conditions during brood rearing are more important determinants of juvenile morphology than is egg provisioning.

Another possibility to explain the observed reduction in wing and tail lengths of juvenile Cliff Swallows during periods of low food availability is viability selection against birds with longer wings, perhaps occurring after fledging. Adults with smaller wings and tails were favored during intense selection during periods of food deprivation brought about by unusually cold weather in late spring (Brown and Brown 1998), and the same trajectory toward smaller wings

and tail has occurred in the generations since selection (Brown and Brown 2011). In what way birds with shorter wings and tails may survive better during periods of resource scarcity is unclear, but may involve advantages associated with maneuverability that increase the potential prey-size breadth. The survival advantages associated with larger skeletal size for adult Cliff Swallows in times of food shortage (Brown and Brown 1998) were not apparent for juveniles, however, and the trend in all cases was in the direction opposite that we would predict based on the patterns of morphological selection in adults (Table 2a). For this reason, viability selection on juveniles after fledging is probably less likely to explain the reduction in wing and tail lengths than is redirection of energy during nestling growth in colder summers. Nevertheless, this study adds to the general conclusion that wing and tail length in Cliff Swallows is subject to rapid changes in response to climatically related events (Brown and Brown 1998, 2011). An analysis, comparable to this one, of adult feather length and the relationship between temperature and precipitation the birds experienced the previous summer would help address this issue.

This study is unique in that we were able to control for several of the variables that can affect the growth and development of nestling birds, including temperature inside the nests, presence of blood-feeding ectoparasites in the nests, and foraging efficiency of parent birds due to social foraging opportunities. For example, the enclosed, mud nests of Cliff Swallows buffer ambient temperature extremes (Mayhew 1958; Withers 1977) and reduce fluctuations in microclimate inside the nest. In addition, nests in swallow colonies are often closely packed together, which may also help moderate temperatures inside the nest by providing greater insulation. Thus, effects of ambient temperature specifically on feather follicle development (Zuberbier and Grubb 1992) or generally on the energetic demands of chicks, which might be

severe especially in cases of altricial birds nesting in open, exposed nests, were ameliorated for Cliff Swallows and unlikely to have affected our results.

In cooler years, the amount of energy expended by adult swallows foraging for themselves and their nest-bound chicks might be greater than in warmer years and expressed in reduced chick provisioning and juvenile morphology and survival. For example, in the unusually cool summer of 1996 in which substantial adult mortality occurred in May (Brown and Brown 1998), juvenile survival was markedly lower than in other years. This suggests adult swallows that were food- and temperature-stressed early in the season did not invest as much in their offspring, resulting in the juveniles' lower survival (Brown and Brown 2004). During the course of this study (2001 – 2006), the birds did not experience such extreme conditions early in the season, so the energetic effects on adults during egg provisioning, egg laying, and brood rearing was minimized. We used strictly fumigated colonies in this study to remove the confounding effects of hematophagous swallow bugs, the principal Cliff Swallow nest ectoparasite. When infestations are severe, these insects can reduce nestling body mass, lead to lower blood hemoglobin, hematocrit and erythrocytes, and cause increased nestling mortality (Brown and Brown 1986, 1996, 2002; Chapman and George 1991). The juveniles at our fumigated colonies were not exposed to the physiological stress caused by the bugs. Previous work on juvenile Cliff Swallows showed that ectoparasitism by swallow bugs led to the birds' exhibiting higher levels of feather asymmetry (Brown and Brown 2002); developmentally, this effect is likely mediated by stress hormones (corticosterone; Raouf et al. 2006). Because we saw no effect of either temperature or precipitation on juvenile asymmetry in this study (Table 2a, b), it appears that in the absence of bugs, even normal fluctuations in food availability are not sufficiently stressful to lead to elevated levels of fluctuating asymmetry during nestling growth.

Colonial bird species often exhibit wide variation in the size of a colony that forms at a given site (Brown and Brown 2001). In Cliff Swallows, colony size can both positively and negatively affect nestling growth: opportunities to transfer information about food locations may enhance nestling growth (e.g., body mass) in medium to large colonies, competition for food can increase the likelihood of nestling starvation in larger broods at the largest colonies, and colonies of different sizes may be settled by birds of different phenotypic characteristics (Brown and Brown 1996). These potential effects on nestling growth and survival were controlled in this study by restricting our comparison to juveniles that were all captured at colonies of roughly similar size. Since these birds were raised in large colonies without ectoparasites, they were subject to less physiological stress (as measured by corticosterone levels; Raouf et al. 2006). Possibly, growth tradeoffs in response to changes in resource availability would be different for birds raised under more stressful conditions that included ectoparasitism or variation in colony size (with its associated variation in parental foraging success; Brown and Brown 1996).

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Table 2-1a-c. Spearman correlation coefficients and associated *P* values for nine morphological traits of juvenile Cliff Swallows in relation to three indices of the climatic conditions (mean daily maximum temperature, total monthly precipitation and variance in maximum temperature) the birds experienced during egg laying and pre- and early post-fledging development (N = 6 years).

(a)

Trait	Mean May Maximum Temperature	Total May Precipitation
Wing Length	-0.3143, $P = 0.5639$	-0.5429, $P = 0.2972$
Tail Length	-0.0857, $P = 0.9194$	-0.4286, $P = 0.4194$
Middle Tail Length	0.1429, $P = 0.8028$	-0.3143, $P = 0.5639$
Tarsus Length	-0.6429, $P = 0.1670$	0.4286, $P = 0.4194$
Culmen Length	0.0286, $P = 0.9999$	-0.2000, $P = 0.7139$
Culmen Width	-0.1429, $P = 0.8028$	0.3143, $P = 0.5639$
Wing FA	-0.2561, $P = 0.6583$	0.0857, $P = 0.9194$
Tail FA	-0.3714, $P = 0.4972$	-0.1429, $P = 0.8028$
Tarsus FA	0.7143, $P = 0.1361$	-0.5429, $P = 0.2972$
Body Mass	0.1429, $P = 0.8028$	0.2000, $P = 0.7139$

(b)

Trait	Mean June Maximum Temperature	Total June Precipitation
Wing Length	0.9429, $P = 0.0048^{**}$	0.0286, $P = 0.9572$
Tail Length	0.8286, $P = 0.0167^{*}$	0.1429, $P = 0.7872$
Middle Tail Length	0.6000, $P = 0.2080$	-0.2000, $P = 0.7040$
Tarsus Length	0.2571, $P = 0.6228$	-0.6000, $P = 0.2080$
Culmen Length	0.1429, $P = 0.7872$	0.5429, $P = 0.2657$
Culmen Width	0.0857, $P = 0.8717$	-0.1429, $P = 0.7872$
Wing FA	0.2000, $P = 0.7040$	0.2571, $P = 0.6228$
Tail FA	0.5429, $P = 0.2657$	0.2857, $P = 0.9572$
Tarsus FA	-0.0857, $P = 0.9194$	0.7143, $P = 0.1361$
Body Mass	-0.2571, $P = 0.6583$	0.3143, $P = 0.5639$

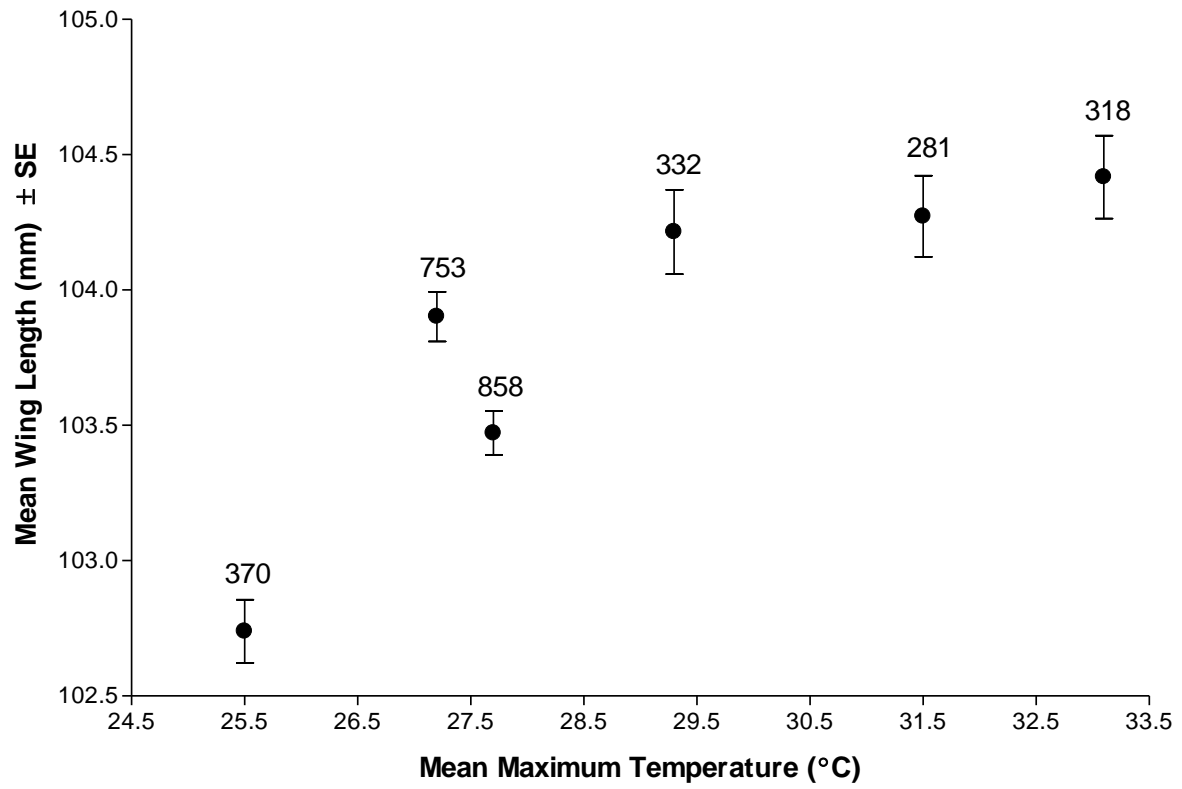
(c)

Trait	May Maximum Temperature Standard Error	June Maximum Temperature Standard Error
Wing Length	0.3714, $P = 0.4972$	-0.3714, $P = 0.4972$
Tail Length	0.6000, $P = 0.2417$	0.0857, $P = 0.9194$
Middle Tail Length	0.1429, $P = 0.8082$	-0.5429, $P = 0.2972$
Tarsus Length	-0.0286, $P = 0.9999$	-0.0857, $P = 0.9194$
Culmen Length	0.4286, $P = 0.4194$	-0.7143, $P = 0.1361$
Culmen Width	0.3143, $P = 0.5639$	0.4857, $P = 0.3556$
Wing FA	0.2571, $P = 0.6583$	-0.4286, $P = 0.6228$
Tail FA	0.4857, $P = 0.3556$	-0.6571, $P = 0.1750$
Tarsus FA	0.3143, $P = 0.5639$	0.3143, $P = 0.5639$
Body Mass	0.2000, $P = 0.7139$	-0.3143, $P = 0.5639$

Figure 2-1. Mean wing length (a) and mean tail length (b) of juvenile Cliff Swallows each year in relation to the mean maximum air temperature during the month of June in the study area each year, 2001 – 2006. Sample sizes are shown next to symbols and error bars indicate ± 1 SE.

Spearman correlation coefficients are given in Table 1.

(a)



(b)

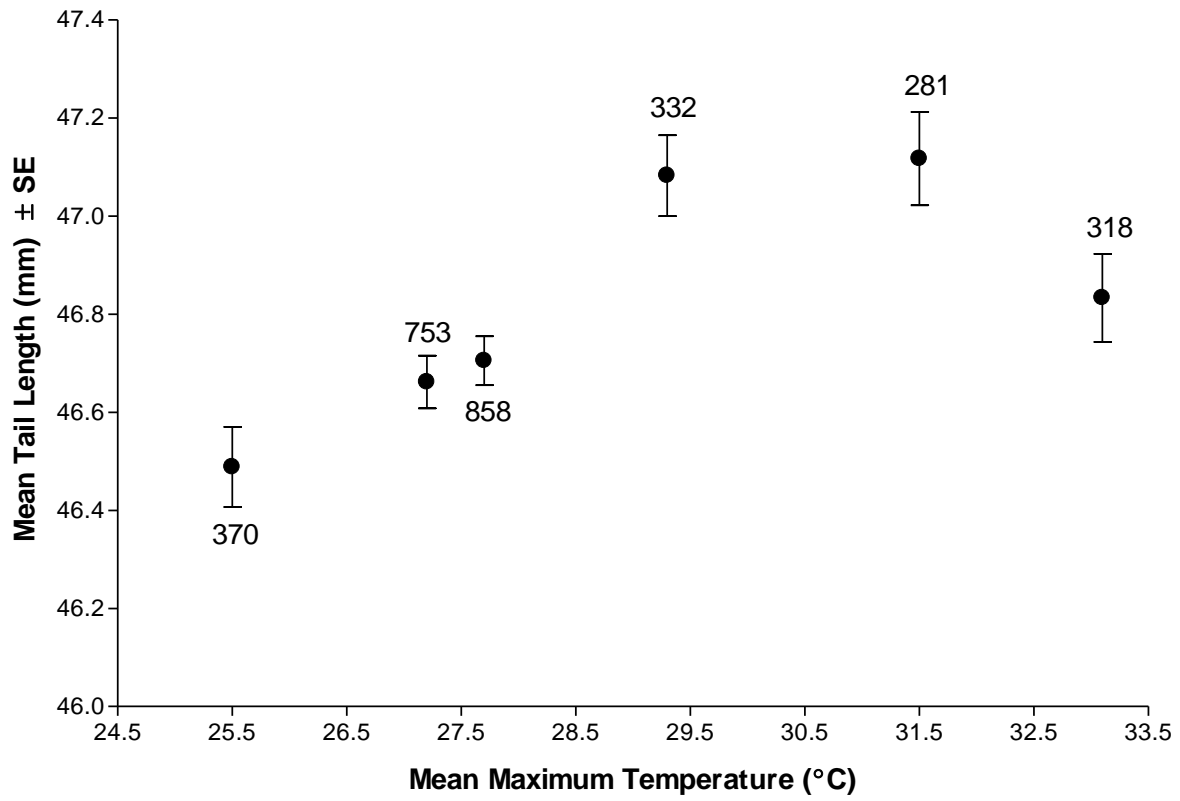
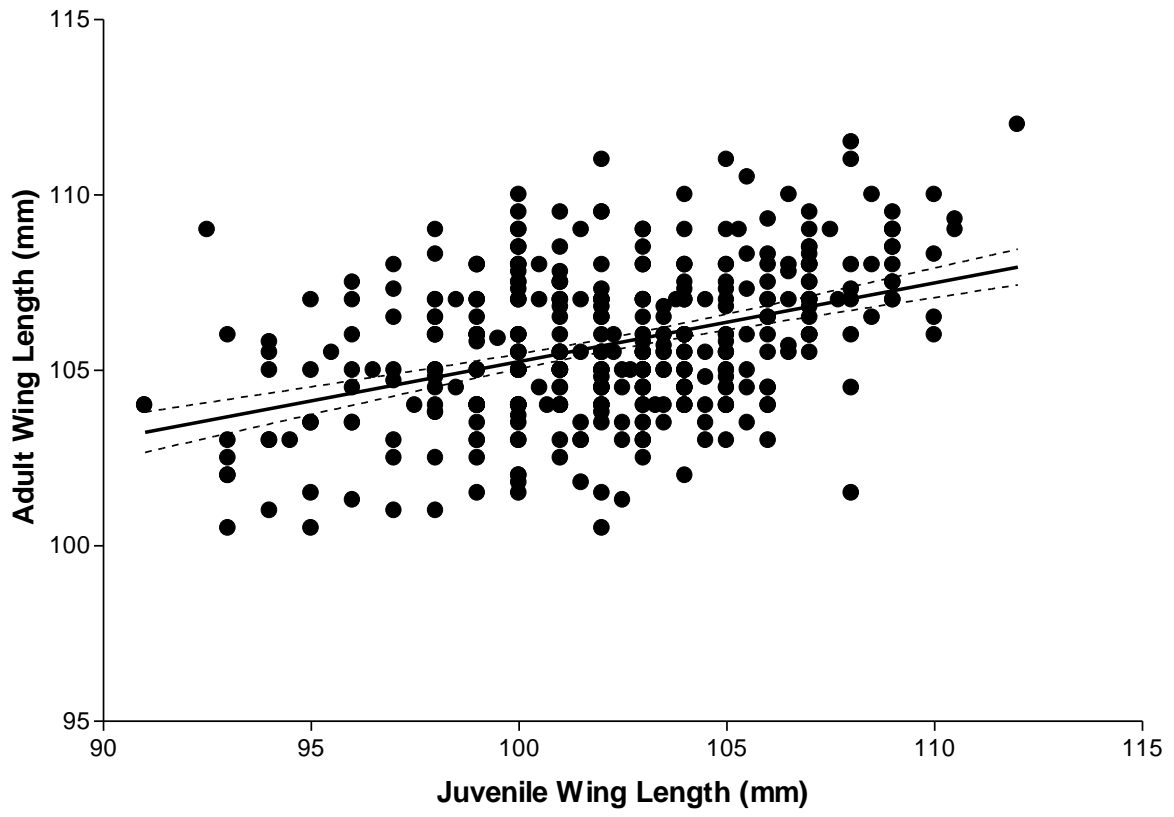
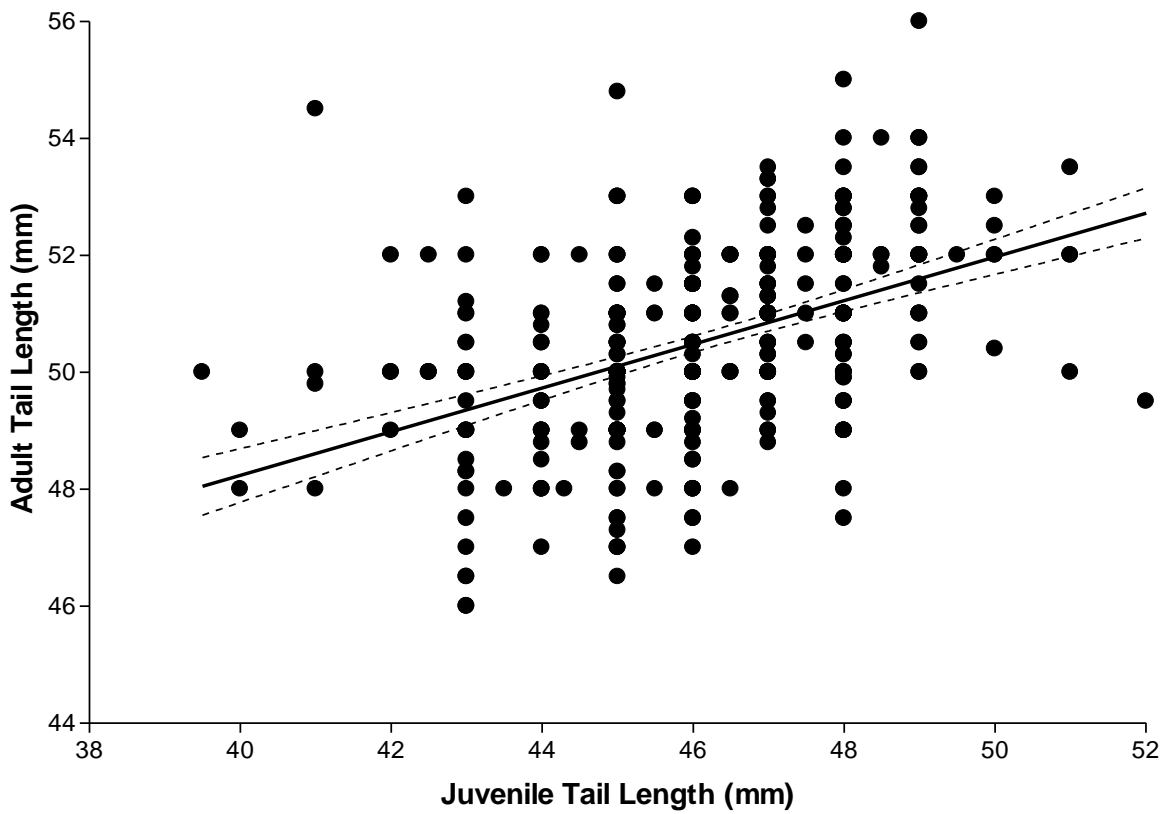


Figure 2-2. Adult wing length (a) and adult tail length (b) in relation to the same measurement for the same bird as a juvenile for 417 Cliff Swallows. Solid lines indicate the best fitting curve and dotted lines the 95% confidence limits. Both wing length ($r_s = 0.3545$, $P < 0.0001$) and tail length ($r_s = 0.4854$, $P < 0.0001$) as a juvenile was positively correlated with the same measurement as an adult.

(a)



b)



Chapter 3. Lifetime changes in morphology of Cliff Swallows (*Petrochelidon pyrrhonota*): wings and tails get longer but no evidence of senescence

Abstract: The extent to which morphological traits in birds vary systematically with an individual's age has been addressed in a variety of field studies, but relatively little information exists on changes in morphology occurring across birds' lifetimes. We used data on over 4000 Cliff Swallows (*Petrochelidon pyrrhonota*) of known ages measured in western Nebraska to examine age-related variation in wing and tail length, skeletal measures of body size, and extent of fluctuating asymmetry. Juvenile birds measured in the days immediately after fledging had shorter wings and tails, and smaller body size as measured by skeletal traits, than adults; juveniles also had lower levels of fluctuating asymmetry. Morphological differences among adult age classes were slight, but wing and tail length increased significantly with age and levels of fluctuating asymmetry in wing and tail tended to decline among adults. Nutritional constraints on juveniles and younger adults may lead to the age-related variation in feather growth, and skeletal size in this species clearly is not fully developed until well after fledging. The results suggest no evidence for senescence through age 7 in Cliff Swallows, as the decline in levels of fluctuating asymmetry with age suggest that the oldest adults maintain high phenotypic performance. Age-related variation in morphology of Cliff Swallows suggests that bird age should be taken into account in analyses of morphological variation in small passerines.

Morphological traits are commonly measured in field studies of birds, and widely used to infer, for example, patterns of geographic variation (Hamilton 1961; James 1970), intensity of sexual selection (Searcy 1979; Andersson 1994), habitat selection (Ulfstrand et al. 1981), parentage (Alatalo et al. 1984a, 1989), diet and energetics (Feinsinger et al. 1979; Yom-Tov and Yom-Tov 2006; González-Gómez and Estades 2009), migratory propensity (Pérez-Tris and Tellería 2001), and, most recently, potential effects of climate change (Przybylo et al. 2000a; Yom-Tov 2001; Kanuscak et al. 2004, Guillemain et al. 2005; van Buskirk et al. 2010). In addition, beak dimensions and other aspects of body size have often been studied as targets of natural selection (Bryant and Jones 1995; Barbraud 2000; Przybylo et al. 2000b; Covas et al. 2002; Hall et al. 2004), in some cases allowing demonstration of selection in contemporary time (Bumpus 1899; Schluter and Smith 1986; Grant and Grant 1989, 1993, 1995, 2002; Brown and Brown 1998, 2011).

The implicit assumption in many studies is that a bird's morphology is largely fixed over its lifetime, and thus measurements of an individual at any one time in its life accurately reflect its size or shape at other times and that any fitness correlate does not vary temporally. However, increasing evidence indicates that individual morphology is malleable, and proper interpretation of morphological variation requires knowing how skeletal and feather measurements may potentially vary with ecology or phenotype. For example, feather wear may lead to a decrease in measured wing length from one season to another (Dhondt et al. 1979; Yosef and Meissner 2006) and wing shape (including length) may vary among individuals within a population depending on the extent to which they are migratory or resident (Pérez-Tris and Tellería 2001; Ellrich et al. 2010; Jakubas and Wojczulanis-Jakubas 2010; Kovács et al. 2010).

A bird's age may have perhaps the greatest potential effect on its size or shape. In a number of species, yearlings have shorter wings than older adults (Löhr 1954; van Balen 1967; Thorne 1975; Dhondt et al. 1979; Ábu 1983; Alatalo et al. 1984b; Arcese 1984; Hogstad 1985; Smith et al. 1986; Ewald and Rohwer 1980; Wojciechowski 1992; Merom et al. 1999; Nowakowski 2000, 2002; Wysocki and Kiriaka 2007; Arizaga et al. 2009; Jakubas and Wojczulanis-Jakubas 2010), with wing length presumably reflecting either nutritional constraints on feather growth in young birds (Lucas and Stettenheim 1972; Jacober and Stauber 1980; Slagsvold 1982; Merom et al. 1999) and/or increased aerodynamic maneuverability conferred by shorter wings in younger birds (Alatalo et al. 1984b; Merom et al. 1999). Wing length is not generally thought to vary with age among birds older than 1 year, although some evidence indicates that both wing and tail length may decline among the oldest individuals in Barn Swallows (*Hirundo rustica*), perhaps reflecting senescence (Møller and de Lope 1999). Age-related changes in skeletal size (tarsus and beak) have also been reported in a few species although without a consistent pattern (Slagsvold 1982; Price and Grant 1984; Alatalo and Lundberg 1986; Smith et al. 1986).

In this study we use repeated morphometric measurements of wings, tail, tarsus, and beak to investigate age-related changes in individual Cliff Swallows (*Petrochelidon pyrrhonota*) as part of a long-term study on how a rare climatic event influenced the evolution of morphology (Brown and Brown 1998, 2011). Using a data set of over 4000 known-age birds measured at more than one age during their lifetimes, this study is among the largest to investigate effects of age on morphometrics in birds. Our objective is to determine if morphological traits increase in size for individuals early in life, as reported in other species, or among older age classes, and

then to examine the evidence for potential senescence-related decreases in body size among the oldest individuals.

In addition to studying body size metrics, we also investigate age-related variation in the extent of fluctuating bilateral asymmetry. With asymmetry widely regarded as an index of individual quality (reviewed in Møller and Swaddle 1997), systematic changes in the level of fluctuating asymmetry may reflect either enhanced performance due to experience or deterioration of condition due to senescence (Møller and de Lope 1999).

METHODS

Study animal

Cliff Swallows (*Petrochelidon pyrrhonota*) are highly colonial Neotropical migrant passerine birds. Breeding throughout much of North America and wintering in southern South America (Brown and Brown 1995), they are diurnal, aerial insectivores and can only forage when weather conditions allow insects to be active (Brown and Brown 1996, 1998). Based on 29 years of band re-sightings, Cliff Swallows in the study area can live as long as 13 years, but relatively few birds eight years of age or older have been found (MBB, CRB, pers. obs.). Juvenile Cliff Swallows after fledging can be identified by the mottled plumage of their heads and throats and by their overall duller coloration (Stoddard and Beecher 1983). Juveniles molt into adult plumage and adults replace their plumage while on the wintering grounds from approximately November to January (Brown and Brown 1995). The study animal is described in greater detail in Brown and Brown (1995, 1996).

Study site

Cliff Swallows were studied near the University of Nebraska School of Biological Sciences' Cedar Point Biological Station (41° 13' N, 101° 39' W); data collection for this study took place

over 11 years from 1996 to 2006. The study area lies along the North and South Platte rivers and includes parts of Keith, Deuel, Garden, and Lincoln counties. Historically, Cliff Swallows built their gourd-shaped mud nests on the sides of cliffs, but with the advent of human-created structures such as road bridges, highway culverts, and eaves of buildings, nearly all birds in the study area now place their nests on artificial structures. The number of and size of nesting colonies varies widely within and between years; the mean colony size is 405 nests (SE = ± 14 , N = 2,209 colonies, 29 years), with some birds nesting solitarily. The study area is described in greater detail by Brown and Brown (1996).

Capture and banding

Cliff Swallows were captured and banded as part of a long-term capture-mark-recapture project in which we rotated among 27 – 40 colony sites throughout each nesting season (Brown and Brown 1996, 2004, 2009, 2011). Mist nets were placed across the entrances of road culverts or along the sides of bridges to capture birds as they flew through the culverts or beneath the bridges. The number of netting days at a colony site was determined by the number of birds at the colony, the phenology of the colony, and the practicality of netting at the site. All adult birds in this study were captured in mist nets, banded with individually numbered, aluminum USGS leg bands and weighed to the nearest 0.5 gram. All adults were captured during May, June and July.

Juvenile Cliff Swallows were birds capable of sustained flight that had hatched earlier in the summer. Some of the juveniles were first banded as nestlings as part of other study protocols, while others were banded as free-flying birds that had been captured in mist nets along with adult swallows; all were marked with numbered USGS leg bands. We restricted all juveniles in this study to those whose wing lengths were greater than 100 mm; this was done so that only

individuals that were independent of their parents and whose feathers had ceased growing were included in the analyses (MBB, CRB, pers. obs.). Juveniles were captured during June and July. The capture and banding protocols are described in greater detail by Brown and Brown (1996, 2004).

Morphometric measurements

All measurements in this study and others were conducted by one individual (MBB; e.g., Brown and Brown 1996, 1998, 2002a, b, 2003, 2011); consequently no corrections to the data for multiple measurers were required (e.g., Price and Grant 1984; Bryant and Jones 1995; Grant and Grant 1995). The following measurements were taken of both adult and juvenile swallows: the length of the unflattened, closed wing chord, the length of the middle and two outermost tail feathers, the length of each tarsus, and the length and width of the exposed culmen. Wing and tail feathers that were damaged were not measured; likewise, no tarsi or culmen were measured if they were damaged or malformed. Wing and tail lengths were measured to the nearest whole millimeter with a stoppered wing ruler, and tarsus and culmen lengths were measured to the nearest 0.1 millimeter with calipers (see Brown and Brown 1998, 2011). Birds were also weighed to the nearest 0.5 g using a Pesola scale. Fluctuating asymmetry in wing, tail, and tarsus was calculated as the unsigned right side minus the unsigned left side value of each bilateral measurement.

At smaller colonies, we measured most of the birds caught on each capture occasion; at larger sites, we measured a subset of those captured. In selecting birds to measure, we included as many birds banded in earlier years as possible, because more information on those individuals was available (e.g., age, previous year measurements, colony site usage). At the time of measurement, the measurer knew only a previously marked bird's band prefix and thus the

approximate year it was banded. Birds caught and measured more than once within the same summer were assigned the mean value of their respective measurements for that year.

Fumigation

To remove the deleterious effects of nest-based, blood-feeding ectoparasites (swallow bugs; Hemiptera: Cimicidae: *Oeciacus vicarius*) on Cliff Swallow growth, development and maintenance, nests in some of the colonies used in this study were fumigated by regularly spraying them with a dilute solution of Dibrom® (naled; Chevron Chemical Corporation). The nest fumigation protocol is described in greater detail by Brown and Brown (1986, 1996).

Statistical analyses

Repeatabilities (r_I) of the morphological measurements were calculated using intraclass correlation (Zar 1996; Palmer 1994; Kuehl 2000; Soper 2009) from a sample of 1,525 birds that were measured twice during the same breeding season. Repeatabilities were high and statistically significant ($P < 0.0001$; MBB, unpubl. data). Repeatabilities of the morphometric measurements made by this measurer (MBB) in an earlier study are given in Brown and Brown (1998).

In this study, as in Brown and Brown (1998, 2011), there was evidence for directional asymmetry in wing length measurements. Mean signed asymmetry for wing length differed significantly from 0 (one-sample t -test, $P < 0.001$); mean signed asymmetry for all other measurements did not differ significantly from 0 ($P > 0.10$). The directional asymmetry in wing length most likely reflects a handedness bias by the measurer (Brown and Brown 1998). This directional asymmetry was corrected by calculating the ([mean signed asymmetry] / 2) for wing and subtracting these values from the longer average side and adding these values to the shorter average side for each observation (Palmer 1994). The corrected asymmetry values are presented and analyzed in this study.

Only birds that were first banded as nestlings or juveniles and thus of known age were included in this study. Birds measured as juveniles were assigned age 0; those measured in their first summer as an adult were assigned age 1; their second summer as an adult, age 2; etc. The measurements were analyzed using a linear mixed model-repeated measures technique in ASReml (Gilmour et al. 2009; <http://www.vsni.co.uk/software/asreml/>). This technique allowed us to use repeated-measures (birds measured at more than one age), and did not require complete measurement sets on each individual. The model structure included fixed effects for age, sex, colony fumigation class, colony size, and an interaction between age and sex. Random effects included in the model were year, colony identity nested within year, and age by colony identity nested within year. After comparing alternative covariance structures using Akaike's Information Criteria (AIC), we selected a first order antedependence covariance structure for the residual covariance matrix. The residual covariance matrix captures the fact that measurements on the same individual will be correlated. The antedependence structure allows for the correlation between observations on the same individual to decrease over time. Wald-type F statistics, with the Kenward and Roger adjustment (Kenward and Roger 1997), were used to test for the effect of age on morphology. Least square mean estimates (in relation to age) for the six morphological traits and the three measures of fluctuating asymmetry were adjusted for the effect of years, colony sizes, colony identities, and gender (Fig. 1, Table 1). The least square means estimated values allow us to make comparisons across age categories after adjusting for the other effects in the model. No birds older than 7 years were included in this analysis as the sample sizes in these categories were too small for rigorous analysis (age 8 = 24, age 9 = 13, age 10 = 6, age 11 = 1). . The sample sizes by age (Fig. 1) include all individuals of that age measured; the same individual may be included in more than one age category. Bonferroni Multiple Comparison tests

were used to determine if mean estimates varied systematically by age category and Spearman correlations were used to determine if mean estimates varied across age categories (GraphPad Prism 2000; SAS 2009; Soper 2009). Each bird was the unit of analysis and we controlled for correlated effects in the model structure.

RESULTS

All six morphological traits and the measures of wing and tail fluctuating asymmetry were significantly larger in birds during their adult years compared to their juvenile year (Fig. 1a - i); only tarsus fluctuating asymmetry did not increase after age 0 (Fig 1i). Also using each bird as the unit of analysis, we found no significant differences among the means for the different ages for any traits in adult birds of ages 1–7, including all three of the indices of fluctuating asymmetry (Fig. 1a - i).

In looking at a linear trend among age-specific trait means with time, however, we found that wing and tail length increased with age for adult birds aged 1–7, as measured by a Spearman correlation analysis (Table 1). Middle tail length, tarsus length, culmen length, and culmen width, as measured by mean values, did not increase or decrease over the birds' adult lives. Wing fluctuating asymmetry tended to decrease in size over ages 1–7 (although not significantly after Bonferroni correction), but tail and tarsus fluctuating asymmetry were not correlated with age (Table 1).

Of the 10 birds that were measured in five or more years during their lifetimes (seven measured in five years and three measured in six years), none showed consistently significant positive or negative correlation with age for any of the six measured traits or three measures of fluctuating asymmetry (Table 2). See Table 3 for least square mean estimates separated by colony fumigation class and gender.

DISCUSSION

Our results indicate that morphological characters in Cliff Swallows are not invariant over an individual's life, with these birds in general tending to exhibit slightly longer wing and tail feathers as they get older. While most of the changes were between juveniles versus adults (of all ages collectively), and the differences among birds aged 1–7 years were slight, nevertheless the results indicate that age does influence annual feather growth when potentially confounding variables are statistically controlled. We found no evidence of senescence in Cliff Swallows through age 7; levels of fluctuating asymmetry in wing and tail (an indicator of individual quality that is expressed each year during molt) tended to decline among the older age classes, indicating, if anything, that higher quality birds were those that reached old age. Two hypotheses can explain these results: (1) an individual grows longer wings and tails and expresses less feather asymmetry as it gets older, or (2) only longer-winged, longer-tailed, and possibly less asymmetric individuals survive to reach the older age classes.

Age-related changes in morphology

Cliff Swallows appear to exhibit the typical avian pattern in which younger birds have slightly shorter wings and tails (Löhrl 1954; van Balen 1967; Thorne 1975; Dhondt et al. 1979; Ewald and Rohwer 1980; Álbú 1983; Alatalo et al. 1984b; Arcese 1984; Hogstad 1985; Smith et al. 1986; Wojciechowski 1992; Merom et al. 1999; Nowakowski 2000, 2002). While finding this among juveniles is not surprising, and may reflect continued feather growth after fledging and even after young become independent of their parents, there was a non-significant trend for younger adults (1–2 years) to also have shorter wings and tails than the oldest birds. That shorter wings and tails in juveniles reflect developmental trajectories with age is suggested by similar patterns for skeletal traits in juveniles. Tarsus and culmen clearly continued to grow after

juveniles had fledged, calling into question the assumption that skeletal traits are fixed in size relatively early during the nestling period or that they can be used to predict parentage (e.g., Stoner 1945; Alatalo et al. 1984a, 1989; Smith et al. 1986; Møller 1987; Sherman and Morton 1988; Norris and Blakey 1989; Payne and Payne 1989; Dale et al. 2002; Nowakowski 2002). Growth of tarsus and culmen had stopped by the bird's first season as an adult, and these traits showed no change over the remainder of a bird's life, as might be expected for skeletal traits. That skeletal body size continues to grow for an undetermined time after birds fledge has rarely been reported for those few species in which skeletal traits were measured during that phase of the life cycle (e.g. Smith et al. 1986; Ng et al. 1997; Brown and Bhagabati 1998; Dale et al. 2002; Nowakowski 2002).

The hypothesis that shorter wing length in younger birds may reflect nutritional constraints on feather growth early in life (Lucas and Stettenheim 1972; Jacober and Stauber 1980; Slagsvold 1982; Merom et al. 1999) seems likely for Cliff Swallows. This species is exposed to large numbers of blood-feeding ectoparasitic swallow bugs, and the bugs reduce nestling body mass, retard feather growth, and lead to premature fledging (Brown and Brown 1986, 1996; Chapman and George 1991; Loye and Carroll 1991). In support of this, although our sample of juveniles caught and measured at non-fumigated colonies was relatively small, we found that these birds tended to have shorter wings and tails than juveniles at fumigated sites: fumigated, wing = 102.4 ± 0.27 ; fumigated tail = 46.1 ± 0.03 ; non-fumigated wing = 101.3 ± 0.37 ; non-fumigated tail = 45.5 ± 0.09 ; these differences were not statistically significant, as indicated by fumigation status not being significant in the model. The alternative hypothesis that shorter wings in younger birds are adaptive responses to increase aerodynamic maneuverability during their first year (Alatalo et al. 1984b; Merom et al. 1999) is difficult to evaluate because

we lack information on age-specific foraging behavior and/or diet of Cliff Swallows during their first year of life. Only if younger birds adopt different foraging strategies or select different prey types than do older birds might this hypothesis hold.

Senescence

If we define senescence as accelerating phenotypic deterioration of individuals with advancing age that leads to increased mortality (degenerative senescence; Fisher 1930; McDonald et al. 1996; von Hardenberg et al. 2004), then our population of Cliff Swallows does not exhibit senescence. If senescence did occur, one would expect to find evidence of it before the birds reached age 7. The strongest evidence against senescence is that measures of fluctuating asymmetry in wing (and, to a non-significant extent, tail) declined in the older age classes. Because Cliff Swallows molt and re-grow their wing and tail feathers each winter, their ability to symmetrically develop these bilateral traits should reflect relative to an individual's condition and its ability to sequester the energetic resources necessary for molt each season. Older birds exhibited no decline in phenotypic performance with age, and if anything, increased in measures of symmetry. Furthermore, unlike in Barn Swallows (*Hirundo rustica*; Møller and de Lope 1999), wing and tail length did not decline among the oldest birds.

Some work has suggested that increased glucocorticoid (stress) hormone levels in birds can cause osteoporosis and lead to skeletal shrinkage in birds as they age (Siegel 1980). Previous studies on this population of Cliff Swallows (Brown et al. 2005a, b; Raouf et al. 2006) have shown that corticosterone levels increase in birds living in larger sized colonies and in colonies with large ectoparasite infestations. One might expect these increased corticosterone levels to cause osteoporosis-related skeletal size shrinkage in Cliff Swallows as they get older. However, this is not apparent in this study; none of the skeletal traits changed in size in relation to age. If

phenotypically higher quality and more experienced birds survive to the older age classes, they must be able to counteract any degenerative effects of osteoporosis.

In contrast to our results, a study of senescence and morphology in the related European Barn Swallow found the length of the outer tail feathers decreased among older individuals and that wing and tail fluctuating asymmetry increased among older individuals (Møller and de Lope 1999). Saino et al. (2002) found that measures of individual and offspring quality decreased with age in Barn Swallows. Why Cliff Swallows presumably differ so much from Barn Swallows is unclear, but the difference could be due in part to a recent (1996) intense viability selection event that favored phenotypically higher-quality individuals (as measured by levels of fluctuating asymmetry) and presumably eliminated many of the inferior birds (Brown and Brown 1998, 2011). This may have masked our ability to detect senescence-related changes in morphological characters in the immediate aftermath of the selection event (when the present study was done); with time, age-related decreases in performance might become more apparent as the population accumulates individuals of lesser phenotypic quality.

Interestingly, we found that measures of fluctuating asymmetry in wing and tail length were significantly lower in juveniles than in adult Cliff Swallows. This likely reflected the fact that the majority of our sample of juveniles measured came from fumigated colonies, where the effects of ectoparasites were removed during the nestling period when these birds were growing their feathers, and in such situations, juvenile asymmetry is typically low (Brown and Brown 2002a). Adults, on the other hand, molt on the wintering grounds, and asymmetry in adult characters is unaffected by whether they occupied fumigated or non-fumigated breeding colonies the previous summer (Brown and Brown 2002a) and thus presumably more likely reflects conditions in South America.

Age-related selection on morphology?

Our data at present do not allow us to determine whether any of the age-related changes in morphology among adult age classes in Cliff Swallows are due to physiological-based growth processes that change with age, or whether there is viability selection on birds with certain morphological characteristics that result in, for example, only relatively long-winged and long-tailed individuals surviving to the older age classes. Because we had relatively few individuals measured at all ages throughout their lives (only 10 birds), we cannot conclusively determine the extent and direction of individual-specific variation over time, although some of these birds did exhibit increases in trait sizes as they got older (Table 2). That viability selection on morphology with age might be primarily responsible for these results is suggested by the fact that these morphological traits demonstrably respond to intense selection in Cliff Swallows (Brown and Brown 1998, 2002a, 2011).

On the other hand, the trend for an increase in wing and tail length with age is opposite the direction of the prevailing selection on these traits measured in the 1996 rare climatic event and in the decade subsequent to this catastrophe (Brown and Brown 1998, 2011). Wing and tail become shorter during the selection event and continued on the same trajectory afterwards, suggesting the population is under selection for smaller wings and tails for unknown reasons. This result seems difficult to reconcile with the age-related changes reported here if survival selection alone is responsible for increased wing and tail lengths among older birds. Formal viability selection analyses with marked birds, investigating to what extent morphological measurements are targets of selection in Cliff Swallows, are presently underway (CRB, MBB, unpubl.).

In summary, Cliff Swallows exhibit slight but predictable changes in wing and tail length with age as adults, and even measures of skeletal morphology increased between the time that birds fledged and the following summer. However, no evidence of senescence was apparent in this population. The results of this study suggest the need to consider bird age in any evolutionary, ecological, or behavioral analysis that relies on morphometric measurements taken at single points in time for small passerines such as Cliff Swallows.

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Table 3-1. Spearman correlation coefficients and associated *P* values for nine morphological traits in relation to an individual's age in Cliff Swallows. Values in the Juvenile and Adult column include the juvenile year (0) and adult years (1 – 7) categories; values in the adult column include only adult values (years 1 – 7). After Bonferroni correction, culmen width and wing fluctuating asymmetry were not significantly correlated with age.

Trait	Juvenile and Adult (N = 8)	Adult (N = 7)
Wing length	0.9286, $P = 0.0009^{**}$	0.8930, $P = 0.0068^{**}$
Tail length	0.9286, $P = 0.0009^{**}$	0.8929, $P = 0.0068^{**}$
Middle tail length	0.6667, $P = 0.0710$	0.5000, $P = 0.2532$
Tarsus length	-0.0476, $P = 0.9108$	-0.5714, $P = 0.1803$
Culmen length	0.5000, $P = 0.2070$	0.2500, $P = 0.5887$
Culmen width	0.7381, $P = 0.0366^*$	0.6071, $P = 0.1482$
Wing fluctuating asymmetry	-0.1667, $P = 0.6932$	-0.7500, $P = 0.0522^*$
Tail fluctuating asymmetry	0.1429, $P = 0.7358$	0.2857, $P = 0.5345$
Tarsus fluctuating asymmetry	-0.3571, $P = 0.3851$	-0.2500, $P = 0.5687$

Table 3-2. Direction of Spearman correlation coefficients for nine morphological traits of 10 Cliff Swallows measured five or more times during their lives. None of the correlations was statistically significant.

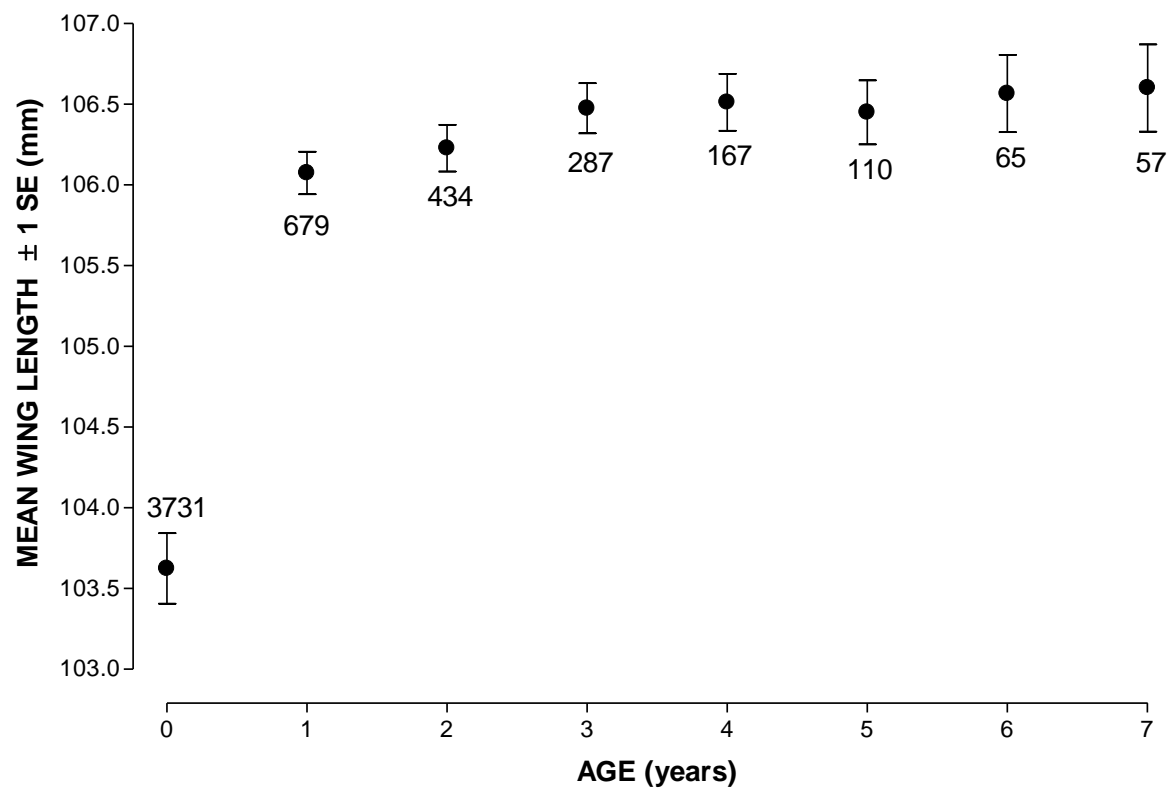
Trait	Positive r_s	Negative r_s
Wing length	7	3
Tail length	7	3
Middle tail length	4	6
Tarsus length	6	4
Culmen length	5	5
Culmen width	6	4
Wing fluctuating asymmetry	5	5
Tail fluctuating asymmetry	5	5
Tarsus fluctuating asymmetry	4	6

Table 3-3. Least square mean estimates of nine morphological traits separated by colony fumigation status and gender (mean \pm 1 SE).

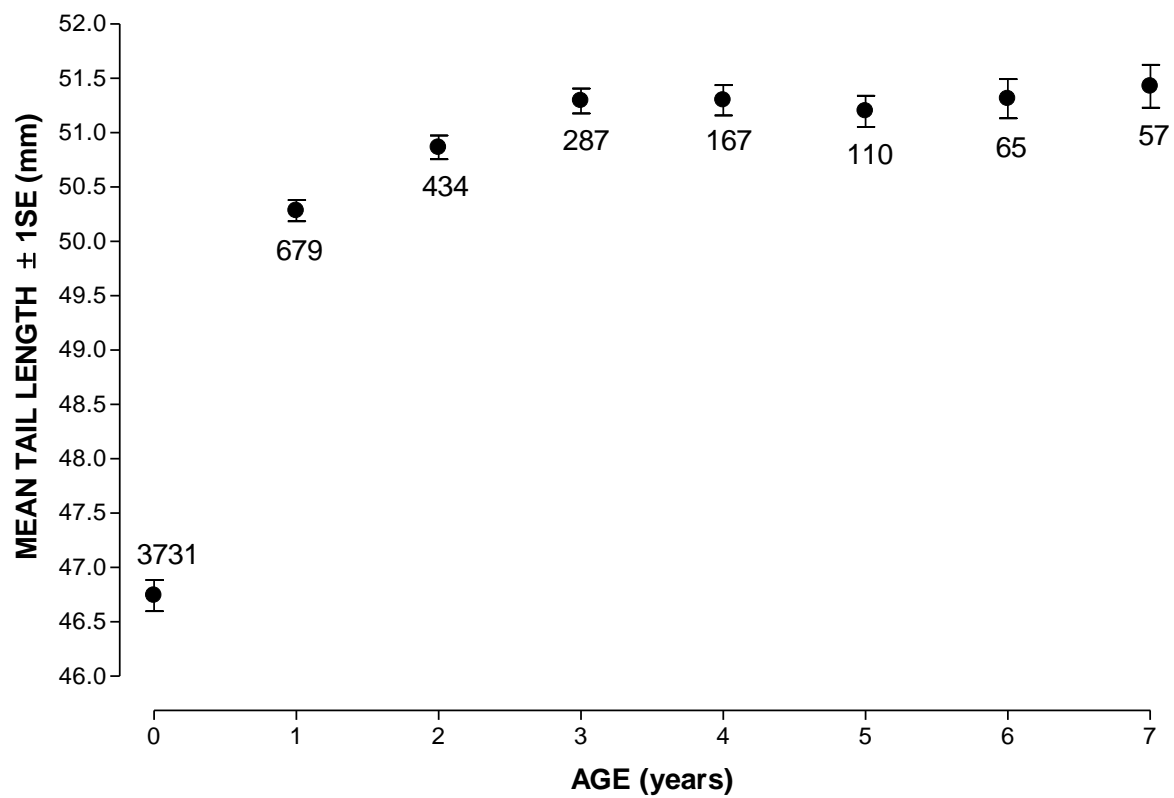
	Fumigated	Non-fumigated	Male	Female
Wing	106.0181 ± 0.0999	106.1145 ± 0.1255	106.2723 ± 0.1136	105.8604 ± 0.1370
Tail	50.5013 ± 0.0752	50.6031 ± 0.0975	50.4563 ± 0.0858	50.6481 ± 0.1038
Middle Tail	45.8319 ± 0.0791	45.9337 ± 0.1102	45.8145 ± 0.0903	45.9511 ± 0.1087
Tarsus	11.5285 ± 0.0162	11.5263 ± 0.0209	11.5247 ± 0.0175	11.5301 ± 0.0203
Culmen Length	7.3277 ± 0.0223	7.3346 ± 0.0264	7.3430 ± 0.0235	7.3193 ± 0.0259
Culmen Width	6.3785 ± 0.0138	6.3624 ± 0.0180	6.4209 ± 0.0147	6.3200 ± 0.0169
Wing Asymmetry	0.5741 ± 0.0312	0.6609 ± 0.0491	0.6202 ± 0.0319	0.6148 ± 0.0371
Tail Asymmetry	0.4162 ± 0.0251	0.4389 ± 0.0410	0.3812 ± 0.0289	0.4739 ± 0.0359
Tarsus Asymmetry	0.1359 ± 0.006	0.1494 ± 0.0091	0.1413 ± 0.0066	0.1440 ± 0.0076

Figure 3-1. Least square mean estimates of (a) wing length, (b) tail length, (c) middle tail length, (d) tarsus length, (e) culmen length, (f) culmen width, (g) wing fluctuating asymmetry, (h) tail fluctuating asymmetry and (i) tarsus fluctuating asymmetry. Sample sizes (number of birds in each age category used in the estimations) are shown next to symbols; error bars indicate ± 1 SE. Wald F statistics for each least square mean estimate are: wing ($F = 21.38$, $df = 7$, 38.6 , $P < 0.001$), tail ($F = 118.99$, $df = 7$, 47.3 , $P < 0.001$), middle tail ($F = 60.55$, $df = 7$, 53.8 , $P < 0.001$), tarsus ($F = 4.47$, $df = 7$, 42.1 , $P < 0.001$), culmen length ($F = 4.85$, $df = 7$, 47.4 , $P < 0.001$), culmen width ($F = 16.94$, $df = 7$, 46.6 , $P < 0.001$), wing fluctuating asymmetry ($F = 2.89$, $df = 7$, 46.6 , $P = 0.014$), tail fluctuating asymmetry ($F = 30.57$, $df = 7$, 288.1 , $P < 0.001$) and tarsus fluctuating asymmetry ($F = 0.36$, $df = 7$, 76.3 , $P = 0.923$).

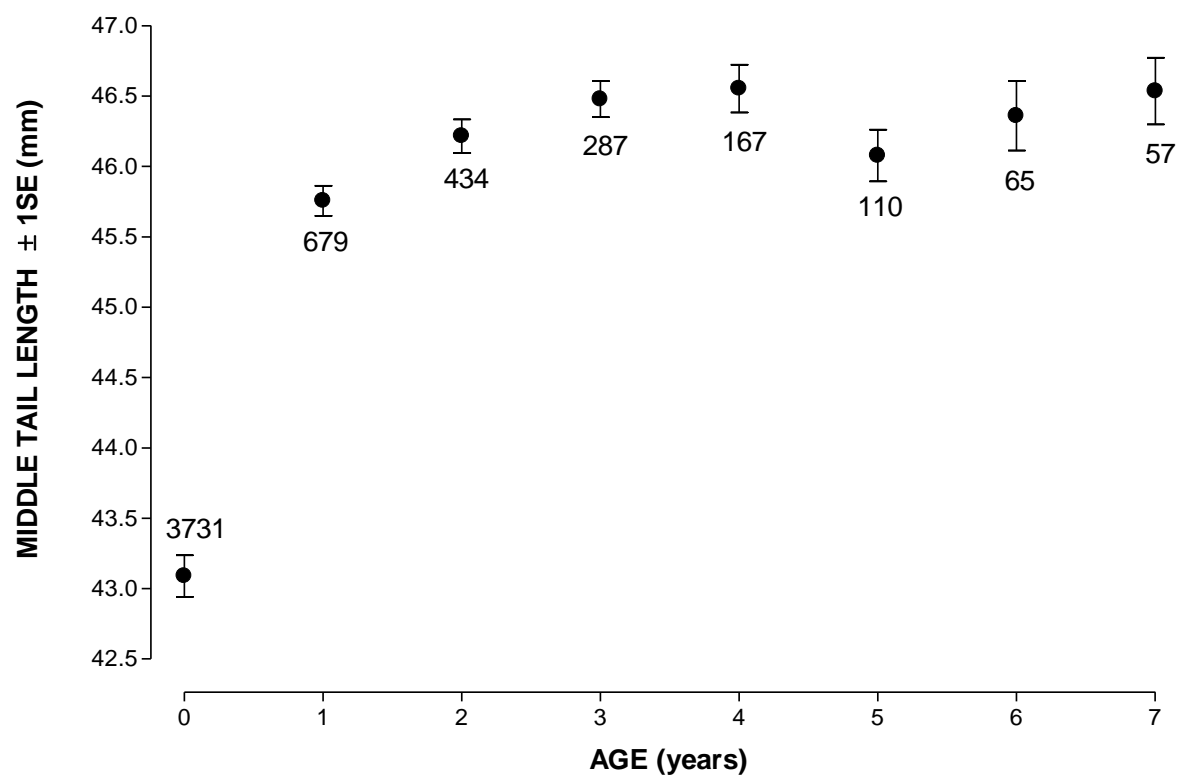
(a)



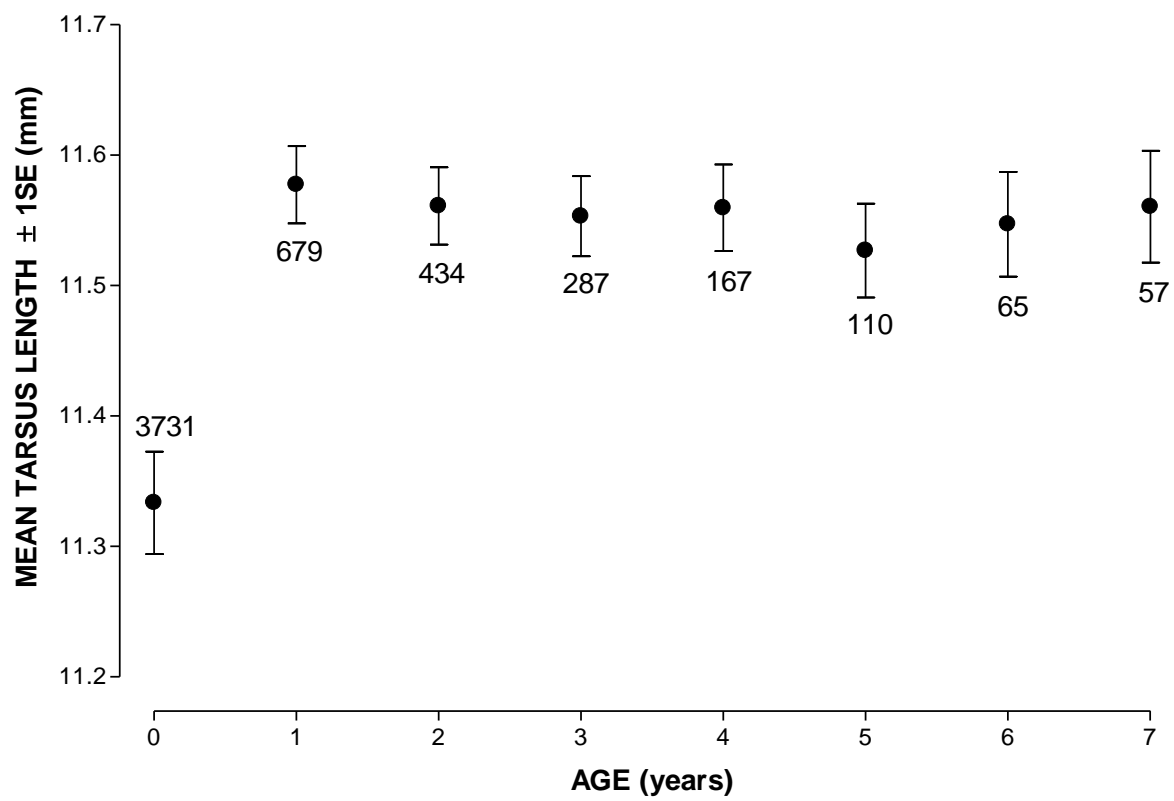
(b)



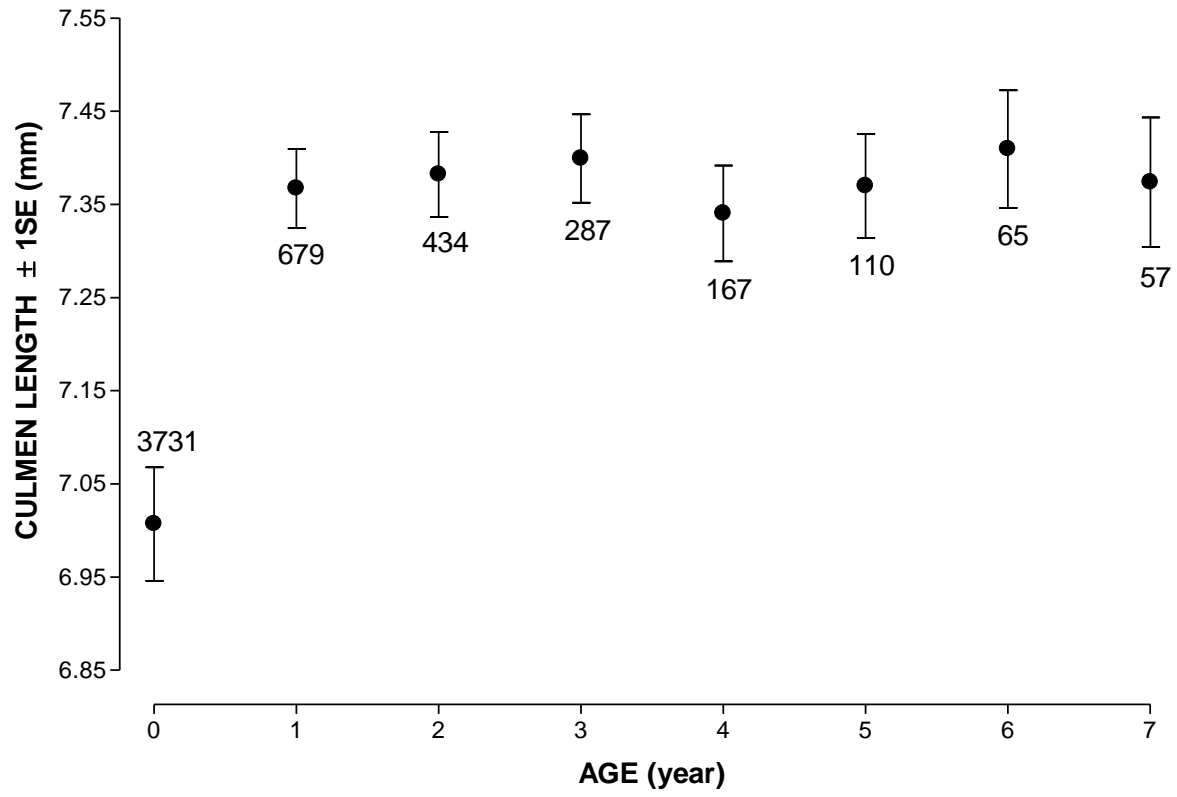
(c)



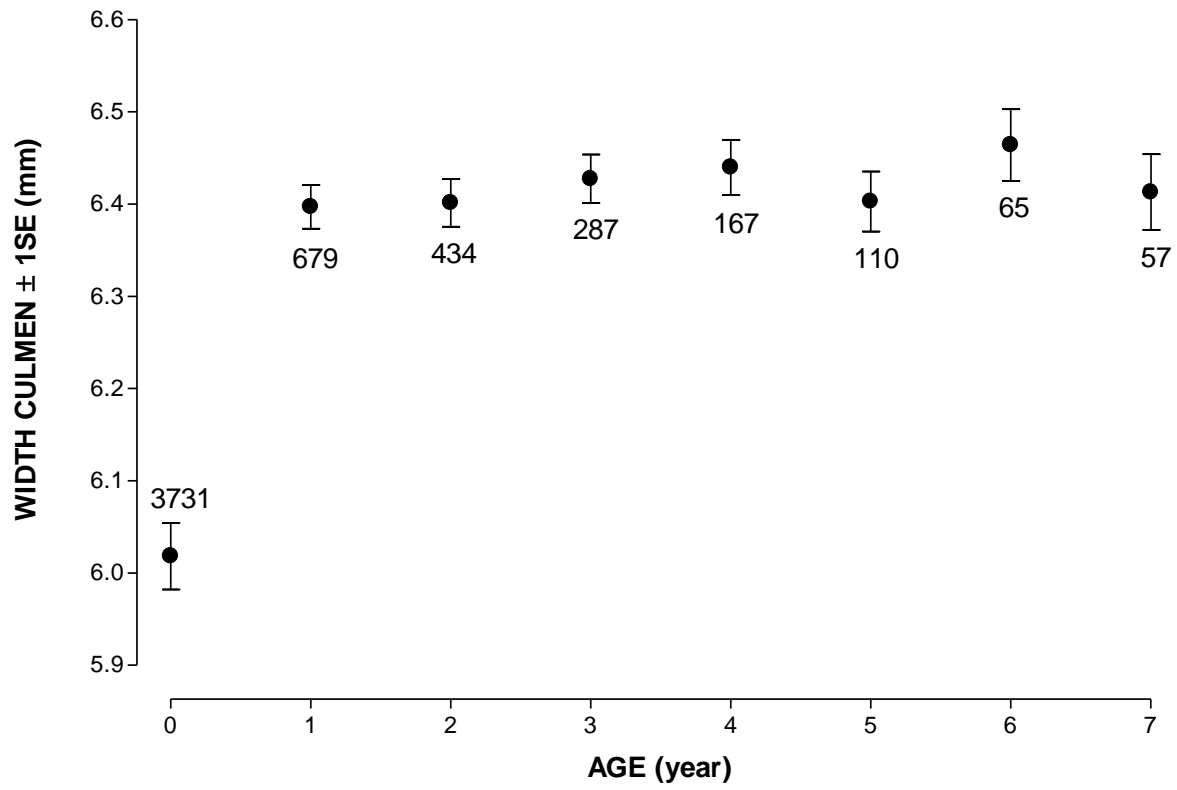
(d)



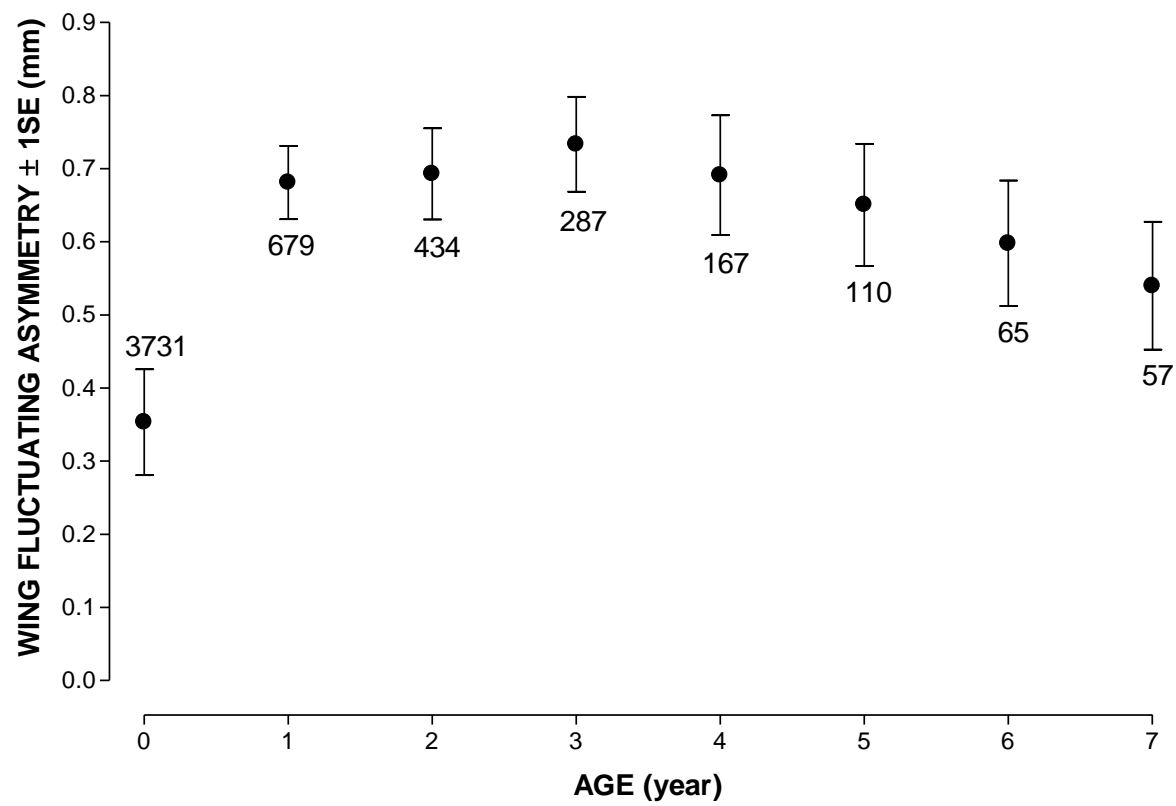
(e)



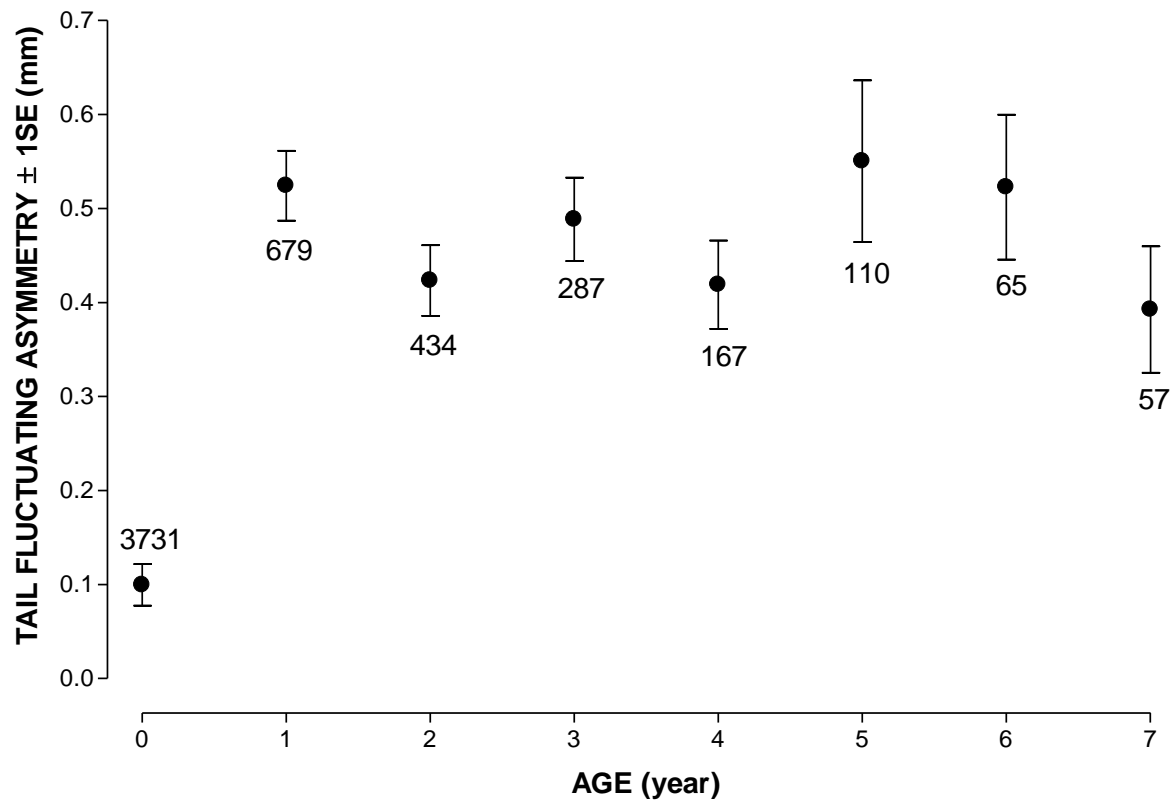
(f)



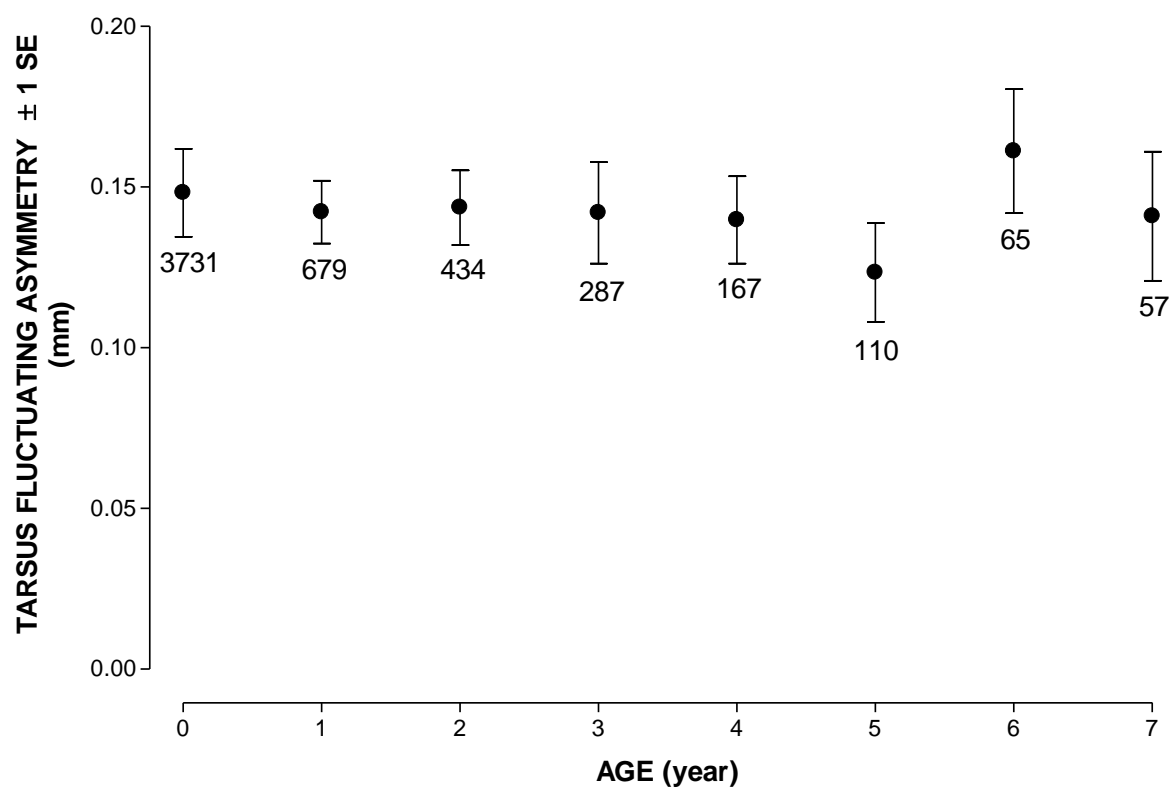
(g)



(h)



(i)



SUMMARY

In May 1996, a 6-day period of unusually cold and wet weather in southwestern Nebraska led to the starvation deaths of thousands of Cliff Swallows. The birds that survived had larger skeletons, shorter wing and tail feathers, and less asymmetry in wing feather length than those that did not survive. By following this population of swallows for 10 years, this incident provided us with the opportunity to study 1) whether natural selection events result in permanent microevolutionary changes or if they are reversed by opposing selection pressures, 2) if seasonal variation in climate (temperature and precipitation) affect the growth and development of morphological traits, and 3) if morphological traits vary systematically in size with an individual bird's age.

In the first section of this study, we examined patterns in morphological traits exhibited by Cliff Swallows following this selection event by measuring yearling birds born in the study area from 1997 to 2006. Wing and middle tail-feather lengths continued to decrease, bill length and width continued to increase, tarsus length was unchanged, and the amount of asymmetry in wing length increased over the 10-year period. The cumulative directional change in wing, tail, and bill length was greater in the years after the selection event than it was during the event. The variation in morphology we observed cannot be explained by phenotypic plasticity resulting from better environmental conditions during growth, because climatic conditions (temperature and precipitation) were not significantly different before and after the selection event. In this study, there was no evidence that opposing selection restored skeletal size or wing or tail length in this population of birds to that before the selection event. The reasons for the continued change in morphology in this population of Cliff Swallows are not clear but may represent the population shifting to a different fitness peak in the adaptive landscape.

In the second section of this study, we examined how variation in the climatic conditions that influence the availability of the insects Cliff Swallows feed on affect the growth of their wing and tail feather lengths, size of skeletal traits and body mass. Juvenile Cliff Swallows were captured and morphometric measurements taken; only birds from large colonies where the effects of ectoparasites had been removed by fumigation were included in the study. It appears that feather growth in juvenile Cliff Swallows is sensitive to climatically based variation in food availability. None of the skeletal traits we measured, or body mass, varied with May temperature, and none of the traits showed a relationship with the amount of precipitation that fell in May or June. The average length of the birds' wing and tail feathers increased significantly with June temperature. In cooler years birds appear to allocate less growth to wing and tail feathers than they do in warmer years, while maintaining normal levels of skeletal growth and body mass. Because adult and juvenile feather lengths are positively correlated, it appears that any changes in a bird's juvenile feather growth in response to rearing conditions persist into its first breeding season.

In the third section of this study, we examined the extent to which morphological traits in Cliff Swallows vary systematically with age across an individual bird's lifetime. The juvenile birds that we measured immediately after fledging had shorter wing and tail feathers, lower body mass, smaller skeletal size and lower levels of fluctuating asymmetry than adult birds. Among adult age classes, morphological differences were slight, but wing and tail feather length increased significantly with age and levels of wing and tail fluctuating asymmetry tended to decline. Nutritional constraints experienced by juvenile and younger adult swallows may lead to the age-related variation in feather growth that we observed; it appears that skeletal size in this species is not fully developed until well after fledging. These results provide no evidence for

degenerative senescence occurring in Cliff Swallows, as the decline in levels of fluctuating asymmetry with age suggest that the oldest birds in the population maintain high levels of phenotypic performance. The age-related variation in morphology we observed in Cliff Swallows suggests that bird age should be considered in future analyses of morphological variation in passerines.

Future analyses of this long-term data set will involve evaluations of the effects of morphology (and fluctuating asymmetry) on the survival of Cliff Swallows and of the effects of colony size on the morphology of Cliff Swallows.

APPENDIX

Developmental stability analyses based on morphological measurements or counts of repeated structures are used to make inferences about the fitness of individuals and populations (van Valen 1962; Møller and Swaddle 1997; Polak 2003). These analyses are based on the assumption that deviations from the ideal phenotype provide information about the precision of the development of individuals in the population; lower developmental precision reflects the disruptive effects of genetic quality and environmental stressors or both (van Valen 1962; Endler 1986; Leary and Allendorf 1989; Parsons 1990; van Dongen et al. 1999; Kellner and Alford 2003; van Dongen 2006). Unfortunately, the underlying principles of canalization, homeostasis, genetic architecture, heterogeneity, and compensatory growth which underlie developmental stability are imperfectly understood (Møller and Swaddle 1997; Kellner and Alford 2003; Polak 2003; van Dongen et al. 1999; van Dongen 2006). Recognizing these limitations, micro- and macro-evolutionary studies utilizing the techniques and analytical methodology of developmental stability (morphology) are still possible (Kellner and Alford 2003; Polak 2003; van Dongen 2006).

The developmental stability of individuals and populations are assessed and measured in two general ways, with frequency indices and repeated-formation indices. Frequency indices monitor the frequency of deviant phenotypes (phenodeviant) or measure the morphological variation in trait size among and within populations; they do not control for genetic differences between individuals or for the effects of environmental conditions on development. Examples of the use of frequency indices include studies of jaw deformities of fish in polluted and unpolluted lakes (Graham et al. 1993) or wing-vein abnormalities in *Drosophila* (Waddington 1953; Bateman 1959). While useful, these techniques are limited in that they do not allow *a priori*

predictions of the true frequency of phenodeviants or variations within and among populations—the optimal phenotype in the population is not known. For frequency indices to be effective in providing accurate assessments of developmental stability, populations have to be exhaustively sampled (Møller and Swaddle 1997). Repeated-formation indices are derived from comparisons of measurements of morphological traits repeatedly formed on the same individual. This is the technique utilized in the present study. They control for genetic differences between individuals and for the effects of environmental conditions on development, which is their great advantage. Examples of traits useful for repeated-formation indices include scales in fish (Shakell and Doyle 1991), microchaetae in *Drosophila* and molar cusps in *Peromyscus* (van Valen 1962). These techniques allow *a priori* predictions of the true frequency of phenodeviants or variation within and among populations -- the optimal phenotype [symmetry] is known. They can be used to track populations to changing environmental conditions. Repeated-formation indices are typically presented in terms of the symmetry or asymmetry of the measured individuals (Møller and Swaddle 1997).

There are three types of asymmetry considered in studies of developmental stability: directional asymmetry (DA), antisymmetry asymmetry (AA), and fluctuating asymmetry (FA). The first two are developmentally controlled and normally are adaptive as asymmetries (van Valen 1962; Graham et al. 1993; Pratt and McLain 2002). The third is reduced by natural selection and may not be an adaptive asymmetry (Beardmore 1960; Thoday 1958; van Valen 1962; Graham et al. 1993; Simmons et al. 1999; Polak 2003; van Dongen 2006). FA is commonly used to estimate the effects of developmental accidents in individuals and to serve as a measure of individual phenotypic quality (van Valen 1962; Emlen et al. 1993; Watson and Thornhill 1994; Gangestad and Thornhill 1999; Møller and Swaddle 1997; van Dongen et al.

1999; Simmons et al. 1999; van Dongen 2006); hence it is used in this study. Fluctuating asymmetry is referred to by Waddington (1957) as the being the result of ‘developmental noise’.

Directional is the form of asymmetry where one side of a trait develops more than the other and where it is possible to predict which side of the trait will be larger before the trait has begun to develop (handedness of the trait; van Valen 1962; Palmer and Strobeck 1986; Markow 1992; McManus 1992; Graham et al. 1993; Møller 1994a, b; Runyon and Hurley 2004).

Examples of DA include a variety of internal organs (i.e., lungs, heart, and testes), the beaks of Wry-billed Plovers (*Anarhynchus frontalis*) (Neville 1976) and the ears of owls (Norberg 1978).

The presence of DA can be detected by the mean values of a trait differing systematically between the two sides of an individual (van Valen 1962; van Dongen et al. 1999; Kellner and Alford 2003; van Dongen 2006).

Antisymmetry is the form of asymmetry where one side of a trait is larger than the other but it is not possible to predict which side of the trait will be larger before the trait has begun to develop (no handedness of the trait: Timofeeff-Ressovsky 1934). Examples of AA include the signaling claws of fiddler crabs (*Uca musica*) (Neville 1976; Pratt and McLain 2002) and the beaks of Red Crossbills (*Loxia curvirostra*) (Neville 1976; Benkman and Lindholm 1991). The presence of AA can be detected by a bimodal distribution of the signed differences between the two sides of an individual or by a tendency toward platykurtosis rather than a normal distribution (van Valen 1962; van Dongen et al. 1999; van Dongen 2006).

Fluctuating asymmetry, first described by Ludwig (1932, 1936), results from the inability of organisms to develop along precisely determined paths (van Valen 1962; Palmer and Strobeck 1986; van Dongen et al. 1999; Kellner and Alford 2003; van Dongen 2006). This inability results in randomly occurring defects in the development and expression of traits. Since the defects are

random, their expression in an individual is symmetry; however, asymmetries may develop in a proportion of individuals in a population. While there is a substantial debate in the FA literature regarding the best method of analyzing FA data (Palmer and Strobeck 1986; van Dongen et al. 1999; Palmer 1994; Green 2001; Kellner and Alford 2003; van Dongen 2006), the presence of FA can be detected by an approximately normal distribution of signed asymmetry ($R - L$) scores around a mean of zero and an equal mean development of the trait on each side (van Valen 1962; Palmer and Strobeck 1986; Palmer 1994; Swaddle et al. 1994; van Dongen et al. 1999; Kellner and Alford 2003; van Dongen 2006). Palmer and Strobeck (1986) assessed a variety of methods (14) of calculating FA for individuals and populations. For populations, they recommend using indices based on the variance of asymmetry in the population ($\text{var}(R - L)$). However, when studying asymmetry at the level of the individual, as in the current study, they recommend using indices based on the unsigned ($R - L$) asymmetry values (Palmer and Strobeck 1986; Swaddle et al. 1994); this is the method employed in this study.

Since FA is characterized by a normal distribution of unsigned $R - L$ around a mean of zero, it can be difficult to distinguish FA from measurement error, which will have a similar distribution (Lundström 1960; Greene 1984; Palmer and Strobeck 1986; Palmer 1994; Swaddle et al. 1994; Merilä and Bjorklund 1995; Fields et al. 1995). Typically, FA measurements are very small in relation to the trait being measured ($< 1\%$ of trait size), so the difficulty in discriminating between FA and measurement error is exacerbated (Møller and Pomiankowski 1993). To address this problem, repeated measurements of the same trait must be taken from the same individual to assess the relative effect of measurement error on asymmetry measurements (Lundström 1960; Palmer and Strobeck 1986; Palmer 1994). The inability to distinguish asymmetry from measurement error within a sample does not necessarily invalidate a

comparison between populations, especially if biologically significant differences are found (Swaddle et al. 1994, Møller 1997, Brown and Brown 1998, 2011), however, it does require caution in concluding there is no difference (Brown and Brown 1998, 2011).

Individual FA and population FA have proven to be useful indices in evolutionary, ecological and behavioral studies. For populations, FA can track evolutionary change over time (i.e., van Valen 1962; Møller and Pomiankowski 1993; Brown and Brown 1998, 2000, 2011, this study; Grant and Grant 2002) or to study population responses to changes in environmental conditions (i.e., Kirpichnikov 1981; Nilsson 1994; Brown and Brown 1998, 2000, 2011, this study; Leary and Allendorf 1989; Zakharov 1987, 1989; Leary et al. 1992; Swaddle and Witter 1994; Bustnes et al. 2002; Grant and Grant 2002; Bize et al. 2004). For individuals, FA can indicate individual condition (i.e., Møller 1990, 1992, 1993, 1994a, b; Swaddle and Witter 1994; Brown and Brown 1998, 2000, 2002, 2011, this study; Pratt and McLain 2002; Bize et al. 2004) or be used as signaling trait (i.e., Møller 1992, 1993; Swaddle and Cuthill 1994; Palmer 1996; Swaddle 1996, Bowyer et al. 2001, Pratt and McLain 2002).

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