

Ectoparasites cause increased bilateral asymmetry of naturally selected traits in a colonial bird

CHARLES R. BROWN & MARY BOMBERGER BROWN

Department of Biological Sciences, University of Tulsa, Tulsa, OK, USA

Keywords:

bilateral symmetry;
cliff swallow;
coloniality;
natural selection;
Oeciocercus vicarius;
parasitism;
Petrochelidon pyrrhonota.

Abstract

Parasitism has been shown to correlate with levels of bilateral symmetry in some organisms, with more asymmetric individuals often having more parasites. However, few studies have shown experimentally that parasitism directly causes increased asymmetry. By fumigating some cliff swallow (*Petrochelidon pyrrhonota*) colonies and leaving others untreated, we investigated experimentally whether ectoparasitism by the cimicid swallow bug led to higher levels of asymmetry in length of wings, outer tail feathers, and tarsus among juvenile and adult birds. Juveniles from fumigated colonies measured soon after fledging had significantly less asymmetry in wing and outer tail length than juveniles from nonfumigated colonies; asymmetry in tarsus length was unaffected by parasitism. Adults that had undergone one or more post-juvinal molts on the wintering grounds showed no differences in asymmetry between those reared in fumigated vs. nonfumigated colonies. These results show that ectoparasitism directly leads to increased feather asymmetry in cliff swallows, probably through parasite-induced nutritional stress. Because wing and tail asymmetry impair flight performance and reduce foraging efficiency, the increased asymmetry caused by parasites represents a fitness cost to cliff swallows. This is among the few experimental studies to show an effect of parasites on asymmetry of naturally selected characters.

Introduction

The subtle, random deviations from perfect symmetry seen in most bilateral organisms have stimulated much research in recent years. The extent of asymmetry in certain traits has been interpreted as a reflection of conditions individuals experience during growth and development, with the prevailing view that environmental, physiological, or developmental stress of various kinds leads to higher levels of fluctuating asymmetry (Van Valen, 1962; Palmer & Strobeck, 1986; Parsons, 1990; Leung & Forbes, 1996; Møller & Swaddle, 1997). Some have also argued that animals may use the degree of fluctuating asymmetry in choosing mates (reviews in Møller & Swaddle, 1997; Møller & Thornhill, 1998).

Correspondence: Charles R. Brown, Department of Biological Sciences, University of Tulsa, Tulsa, OK 74104, USA.
Tel.: 918-631-3943; fax: 918-631-2762;
e-mail: charles-brown@utulsa.edu

Despite warnings that the biological significance of small differences in asymmetry have been overstated (Palmer, 1996, 1999; Houle, 1997, 1998; Clarke, 1998) and little understanding of the genetic and developmental basis to fluctuating asymmetry in particular (Bjorksten *et al.*, 2000a), many researchers are now using asymmetry as an index of individual quality or condition.

Although much of the early emphasis was on patterns of asymmetry in sexually selected traits and how these affect mating decisions, more recently many other studies have focused on asymmetry in naturally selected traits and how asymmetry is associated with environmental stress (e.g. Møller, 1995a; Rettig *et al.*, 1997; Carbonell & Telleria, 1998; Siikamaki & Lammi, 1998; Imasheva *et al.*, 1999; Roy & Stanton, 1999; Reimchen & Nosil, 2001). For example, fish from polluted or otherwise altered waters exhibit higher levels of asymmetry in gill rakers and fin rays than those inhabiting nonpolluted or natural habitats (Østbye *et al.*, 1997; Campbell *et al.*,

1998), logging and consequent habitat alteration leads to increased mandibular asymmetry in shrews (Badyaev *et al.*, 2000), and food deprivation in the laboratory is associated with higher levels of asymmetry in feather growth in some birds (Swaddle & Witter, 1994). Another potential source of environmental stress is parasitism by macroparasites and pathogens. Several studies have demonstrated a positive correlation between levels of parasitism and extent of asymmetry in various traits (Møller, 1996), and these studies are consistent with the widespread assumption drawn from other work that parasitism can be detrimental to host individuals.

Relatively little is known, however, about the causative nature of the relationship, if any, between parasitism and a host's developmental instability as reflected in bilateral asymmetry (Polak, 1997a). Positive correlations between extent of parasitism and degree of asymmetry could result because a higher level of developmental instability itself (perhaps through its effects on the immune system) causes greater susceptibility to parasites. In addition, the environmental conditions (e.g. poor nutrition, pollutants) that promote developmental instability may also promote parasitism of hosts by creating greater exposure to parasites (e.g. Reimchen & Nosil, 2001), perhaps through positive effects on local parasite abundance. These possibilities have not been ruled out for most of the studies that have shown a relationship between parasitism and asymmetry (Møller, 1996; Møller & Swaddle, 1997). Almost all of this work has been correlative in nature, and only a few studies have investigated the effect of parasites experimentally (Møller, 1992; Folstad *et al.*, 1996; Agnew & Koella, 1997; Polak, 1997a; Shykoff & Kaltz, 1998). Some have proposed that if parasites affect developmental stability, traits under strong sexual selection should be those most likely to be affected (Møller & Pomiankowski, 1993; Møller & Swaddle, 1997; Polak, 1997a,b; cf. Bjorksten *et al.*, 2000a). Although most workers have assumed that we should see relatively low levels of fluctuating asymmetry in naturally selected traits that are under strong stabilizing selection, we know relatively little about how developmental stress brought about specifically by parasites or pathogens directly affects traits under either sexual or natural selection.

In this study, we investigate how ectoparasites affect asymmetry of three naturally selected traits (wing length, outer tail length and tarsus length) in the colonially nesting cliff swallow (*Petrochelidon pyrrhonota*). We have previously shown strong survival advantages associated with low levels of wing and tail asymmetry in this species (Brown & Brown, 1998), and thus any environmental stress likely to cause increased asymmetry in these characters will have a substantive effect on fitness. Here we use an experimental approach in which we removed parasites from cliff swallow nests and measured levels of bilateral asymmetry among (1) juveniles raised in parasitized and parasite-free nests and (2) adults raised in

those nests after their subsequent molt(s) on the wintering grounds. This study is among the few experimental investigations of how parasitism affects levels of bilateral symmetry of hosts under natural conditions.

Methods

Study animals and study site

Cliff swallows are highly colonial passerines that breed throughout most of western North America (Brown & Brown, 1995). They build gourd-shaped mud nests and attach them to the vertical face of cliff walls, rock outcroppings, or artificial sites such as the eaves of buildings or bridges. Nests tend to be stacked closely, often sharing walls, which facilitates the movement of ectoparasites between nests (by crawling). Cliff swallows are migratory, wintering in southern South America, and have a relatively short breeding season in North America. At our study site, they arrive beginning in late April or early May, and most depart by late July. They generally raise only one brood.

The cliff swallows in our study area are associated with several ectoparasites, but the most important one is the swallow bug (Hemiptera: Cimicidae: *Oeciacus vicarius*; Brown & Brown, 1986, 1992, 1996; Brown *et al.*, 2001). This wingless bug parasitizes primarily cliff swallows and is found throughout the bird's wide geographical range (Brown & Brown, 1995). Swallow bugs are nest-based ectoparasites that overwinter in the cliff swallow nests or in cracks and crevices of the nesting substrate near the nests. They are exclusively haematophagous, feeding on the birds mostly at night, and they travel on the adult birds relatively rarely (George, 1987; Brown & Brown, 1996). Infestations can reach 2600 bugs per nest, and the bugs have substantive effects on cliff swallow reproductive success and social behaviour (Brown & Brown, 1986, 1992, 1996; Chapman & George, 1991; Loye & Carroll, 1991).

Our study site is centred at the Cedar Point Biological Station (41°13'N, 101°39'W) near Ogallala, in Keith County, along the North and South Platte Rivers, and also includes portions of Deuel, Garden and Lincoln counties, south-western Nebraska. We have studied cliff swallows there since 1982. There are approximately 160 cliff swallow colony sites in our 150 × 50 km study area, with about a third of these not used in a given year. Colony size at a site varies widely; in our study area, it ranges from 2 to 3700 nests, with some birds nesting solitarily. Over a 20-year period, mean (\pm SE) colony size ($n = 1363$) was 363 (± 16) nests. Each colony site tends to be separated from the next nearest by 1–10 km but in a few cases by ≥ 20 km. In our study area, the birds nest on both natural cliff faces and on artificial structures such as bridges, buildings and highway culverts. The study site is described in detail by Brown & Brown (1996).

Field methods

We removed swallow bugs from nests by applying a dilute solution of Dibrom (naled) as a light mist to the outsides of nests. This insecticide is highly effective against swallow bugs and we have used it extensively in our past work (Brown & Brown, 1986, 1996). Nests are lightly sprayed once a week to remove any bugs immigrating into the colony on arriving birds. All nests used in this study were from colonies that were fumigated in their entirety. Fumigation has no detectable effects on the cliff swallows themselves (Brown & Brown, 1996).

Cliff swallows were captured in mist nets at colony sites, usually by placing a net across the mouth of a culvert or the side of a bridge, and the birds were caught as they exited the colony (Brown & Brown, 1996, 2000). Both adults (birds at least 1-year old) and juveniles (birds hatched that season that had fledged generally within 1–10 days of capture) were caught in nets and measured. We assumed that any juvenile caught at a colony was from that colony site and thus from a colony of that fumigation status. We know this assumption was violated for some individuals, as juveniles do move around and sometimes visit non-natal sites (Brown & Brown, 1996). However, this served to make any test for differences between fumigated and nonfumigated classes of birds conservative, and thus tended to bias us against detecting any real differences if they existed. All measurements on juveniles were taken during the 2000 breeding season from three fumigated colonies that were 88, 825 and 1500 nests in size and from six nonfumigated colonies that were 24, 415, 500, 575, 700 and 1150 nests in size. Analyses that treated each individual juvenile bird as the unit of analysis and that used colony-wide means for the sites where juveniles were measured were performed. Measurement data on adults spanned the period 1997–2000. Most adults were caught and measured in multiple years, and in these cases we used average trait values for the analyses. Only adults that had been caught as juveniles at fumigated or nonfumigated colonies and later recaptured when ≥ 1 -year old were included in the analyses presented here. Because adults were caught at many different colonies (often the same individual at different sites in different years), we did not analyse the effect of colony size or site *per se* for adults.

All morphometric measurements were taken by one person only (MBB), and thus no corrections to the data for multiple measurers were necessary. The same person measured birds in this study as in Brown & Brown (1998), so the measurements reported here are comparable with those in the earlier work. For all birds, we measured the length of each unflattened, closed wing (from the anterior most part of the wrist joint to the tip of the outermost primary) to the nearest 1 mm with a stoppered wing ruler; the length of the middle and two outermost tail feathers (from their emergence from the skin to the distal most point) to the nearest 1 mm with a

ruler; and the length of each tarsus (from the proximate end of the tarso-metatarsus to the hallux) to the nearest 0.1 mm with calipers.

Statistical analyses

Asymmetry in wing, tail and tarsus was expressed in most analyses as the unsigned R–L values. Repeatability of these morphometric measurements by this measurer (MBB) are given in Brown & Brown (1998); repeatabilities, in general, were high and statistically significant. We also found that wing asymmetry was significantly higher than expected based on measurement error but that tail and tarsus asymmetry were not (Brown & Brown, 1998). The inability to distinguish asymmetry from measurement error for tail and tarsus within a sample does not invalidate a comparison between samples (e.g. fumigated vs. nonfumigated), especially if a significant difference is found (Swaddle *et al.*, 1994; Møller, 1997), but it does require caution in concluding that there is no difference between the samples.

We found evidence for directional asymmetry in some measurements (Brown & Brown, 1998); in this study, mean signed asymmetry for wing in both adults and juveniles in both fumigated and nonfumigated samples differed significantly from 0 (one-sample *t*-test, $P < 0.001$ for each). Adults also showed directional asymmetry in tarsus ($P < 0.001$). Mean signed asymmetry for all other samples did not differ significantly from 0 ($P > 0.10$). This directional asymmetry likely reflected a handedness bias by the measurer (Brown & Brown, 1998); this is common particularly with measurements of wings in living birds (Helm & Albrecht, 2000). Thus, to correct for directional asymmetry (Palmer, 1994), we calculated the [mean signed asymmetry]/2 for wing by age and fumigation class and for adult tarsus by fumigation class, and subtracted these values from the larger average side and added these values to the smaller average side for each observation in each respective data set. The corrected signed and unsigned asymmetry values are those presented and analysed here (see Results). We applied sequential Bonferroni corrections (Rice, 1989) in analyses that involved multiple, related univariate statistical tests. Statistical analyses were performed with SAS (SAS Institute, 1990).

Results

Statistical descriptors of asymmetry variation

Asymmetry variation in cliff swallows exhibited properties of both fluctuating asymmetry and antisymmetry (Table 1; also see Brown & Brown, 1998) but were more consistent with fluctuating asymmetry. In fluctuating asymmetry, the mean of the signed R–L values should be zero, and the values should be distributed normally. We found that mean signed asymmetry did not differ

Table 1 Statistical descriptors of bilateral asymmetry among the classes of cliff swallows studied.

Trait	Signed R-L asymmetry*					<i>n</i>	<i>r_s</i> †§	<i>P</i> (<i>r_s</i>)
	Mean (±SE)	<i>P</i> (<i>t</i>)†	Skewness	Kurtosis	<i>P</i> (<i>w</i>)‡			
Juveniles, fumigated								
Wing	0.00 ± 0.04	0.89	1.10	3.17	<0.001	372	0.08	0.11
Tail	0.03 ± 0.02	0.20	0.08	5.96	<0.001	372	-0.08	0.13
Tarsus	-0.02 ± 0.01	0.10	0.19	-0.30	<0.001	372	-0.03	0.59
Juveniles, nonfumigated								
Wing	0.00 ± 0.10	0.99	-0.01	-0.20	<0.001	182	-0.06	0.40
Tail	-0.09 ± 0.06	0.13	0.58	-0.20	<0.001	182	-0.23	0.002¶
Tarsus	0.00 ± 0.01	0.94	-0.09	8.21	0.001	182	-0.04	0.57
Adults, fumigated								
Wing	0.00 ± 0.03	0.86	0.20	2.77	<0.001	1148	0.01	0.79
Tail	-0.05 ± 0.03	0.14	3.02	58.61	<0.001	1089	-0.01	0.74
Tarsus	-0.01 ± 0.01	<0.001¶	2.22	8.39	<0.001	1148	0.03	0.27
Adults, nonfumigated								
Wing	0.00 ± 0.06	0.96	1.47	2.34	<0.001	215	0.06	0.40
Tail	0.04 ± 0.07	0.60	1.66	10.49	<0.001	198	-0.07	0.31
Tarsus	-0.01 ± 0.01	<0.001¶	1.85	5.15	<0.001	216	-0.02	0.82

*Statistics computed after corrections for directional asymmetry (see Results).

†Probability that mean signed asymmetry equals zero (one-sample *t*-test).

‡Probability that signed asymmetry is normally distributed (*W*-test; SAS Institute, 1990).

§Correlation between unsigned asymmetry and trait size.

¶Significant at *P* < 0.05 after sequential Bonferroni correction.

significantly from zero for all traits except tarsus in adults after correcting for the handedness bias of the measurer (Table 1; see Methods). However, the distribution of the signed asymmetry values for all traits departed significantly from a normal distribution (Table 1). Rather than indicating true antisymmetry or some other pattern, this was most likely a statistical artefact of our rather large sample size in all cases; with large sample sizes, even slight deviations from a theoretical normal distribution can generate statistical significance. In addition, for all traits and all classes of birds, the lower bound (fifth percentile) of each half of the unsigned asymmetry distribution equalled zero (see Rowe *et al.*, 1997). This result is more consistent with fluctuating asymmetry than antisymmetry. That the asymmetry we observed was more likely fluctuating asymmetry was also suggested by the strong leptokurtosis seen in most classes of birds (Table 1).

There was little indication that unsigned asymmetry was related to trait size for the age and fumigation classes we studied (Table 1). Correlations between average length of wing, average length of outermost tail feathers, or average length of tarsus and unsigned asymmetry were very small and statistically nonsignificant in all cases except for tail among juveniles from nonfumigated nests. We found a similar result when correlations between trait size and level of asymmetry were performed for the fumigated and nonfumigated classes of birds combined; correlation coefficients (*r_s*) ranged from -0.13 to 0.08. We, therefore, did not correct unsigned asymmetry values by trait size in subsequent analyses.

The fact that there was, in general, no (or only a weak) relationship between trait size and levels of asymmetry (Table 1) also meant that we could rule out trait size as a potential effect on asymmetry in later analyses (below).

We found no significant correlations between unsigned asymmetry of different traits within individuals. For both adults and juveniles separated by nonfumigated and fumigated colonies, pair-wise rank correlations (*r_s*) between wing and tail asymmetry, tail and tarsus asymmetry, and wing and tarsus asymmetry ranged from -0.11 to 0.08 (*P* > 0.05 on all). We thus treated asymmetry of each trait as independent in our analyses.

Asymmetry in relation to parasitism and age class

Using our combined data set with all juveniles treated as independent units of analysis, unsigned asymmetry in length of both wing and outermost tail feathers was significantly lower in juveniles from fumigated colonies than in juveniles from nonfumigated colonies (Fig. 1, Table 2). For both wing and tail, average asymmetry was over twice as great for birds from nonfumigated colonies. In contrast, unsigned asymmetry in tarsus length did not differ significantly between juveniles from fumigated and nonfumigated colonies (Fig. 1, Table 2). The differences in wing and tail asymmetry could not be explained by date of measurement (which might have been related to age) between fumigated and nonfumigated juveniles (Table 2).

The effect of fumigation on levels of wing and tail asymmetry was unaffected by colony size of the sites

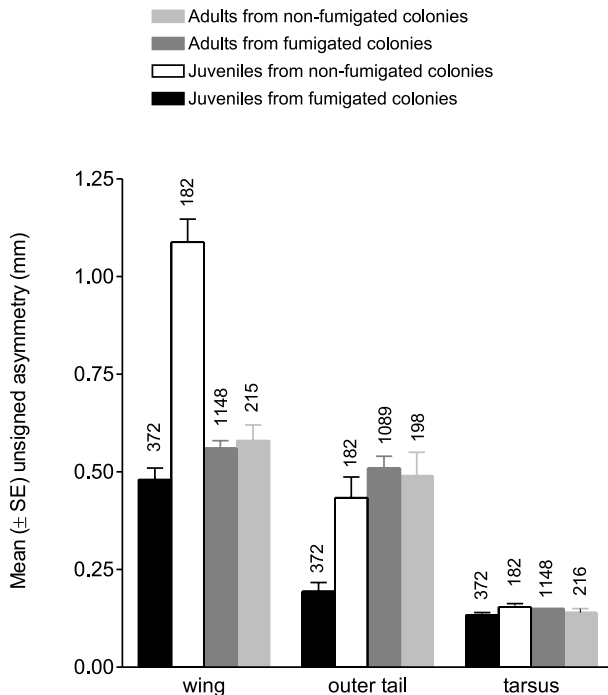


Fig. 1 Mean (\pm SE) unsigned asymmetry in length of wing, outer tail feathers and tarsus for juvenile and adult cliff swallows from fumigated and nonfumigated natal colonies. Among juveniles, wing and tail asymmetry each was significantly greater in birds from nonfumigated colonies than in those from fumigated colonies; tarsus asymmetry did not differ between the two classes of birds (Table 2). Among adults, asymmetry in none of the traits differed significantly between birds from nonfumigated and fumigated colonies (Table 2). Sample sizes (number of birds) shown above error bars.

studied. We found no significant effect of colony size when considering each juvenile as an independent unit of analysis (Table 2). We also examined the effect of colony site, independent of colony size, using an ANOVA with the effect of site nested within fumigation treatment

(Table 2). There was no significant effect of colony site on asymmetry levels of either wing or tarsus asymmetry; however, tail asymmetry did vary significantly (for unknown reasons) with colony site independent of fumigation treatment (Table 2). Thus, both fumigation and colony site itself seemed to affect levels of tail asymmetry, but the strong effects of parasite removal on wing asymmetry were not confounded by site-specific variation in wing asymmetry levels.

To further separate any effect of colony site when using individual birds as the unit of analysis, we analysed only the mean values of asymmetry for the three fumigated and six nonfumigated colonies where juveniles were measured. Using colony mean as the unit of analysis, we found a similar pattern: mean (\pm SE) wing asymmetry per colony for fumigated sites (0.47 ± 0.01 mm) was significantly less than that for nonfumigated sites (1.06 ± 0.15 mm; Wilcoxon test, $P = 0.03$); mean tail asymmetry per colony for fumigated sites (0.13 ± 0.07 mm) was significantly less than that for nonfumigated sites (0.71 ± 0.22 mm; Wilcoxon test, $P = 0.03$); but mean tarsus asymmetry per colony for fumigated sites (0.14 ± 0.01 mm) did not differ significantly from that for nonfumigated sites (0.13 ± 0.03 mm; Wilcoxon test, $P = 0.80$).

In contrast, asymmetry in adults was unaffected by whether they came from a fumigated or nonfumigated natal colony (Fig. 1, Table 2). In most cases, average asymmetry values were very similar for adults irrespective of fumigation status (Fig. 1). Sex was unrelated to asymmetry (Table 2). For adults, we found only a significant effect of year of birth (on tail asymmetry; Table 2).

In comparing birds measured as juveniles with those measured as adults within each fumigation class, juveniles had significantly smaller wing and tail asymmetry than adults among fumigated birds (Wilcoxon test, $P < 0.0001$), but tarsus asymmetry did not differ significantly between the two age classes ($P = 0.09$). Among nonfumigated birds, juveniles had significantly greater

Table 2 ANOVA results for comparing measures of bilateral asymmetry in juvenile and adult cliff swallows. For juveniles, the effect of colony site was nested within fumigation treatment.

	Wing			Tail			Tarsus		
	Mean square	F^*	P	Mean square	F^\dagger	P	Mean square	F^*	P
Juveniles									
Fumigation treatment	4.071	11.00	<0.001	1.340	4.53	0.03	0.002	0.19	0.66
Natal colony size	0.378	1.01	0.31	0.034	0.12	0.73	0.001	0.09	0.76
Date measured	0.191	0.51	0.91	0.362	1.23	0.26	0.012	0.92	0.52
Natal colony site	0.649	1.75	0.09	0.780	2.64	0.01	0.012	0.95	0.46
Adults‡									
Fumigation treatment	0.001	0.00	0.95	0.257	0.67	0.41	0.010	0.31	0.58
Natal year	0.272	0.76	0.62	1.801	4.72	<0.0001	0.050	1.53	0.16
Sex	0.349	0.98	0.32	0.001	0.00	0.95	0.010	0.31	0.58

*d.f. = 21(1,1,12,7 for the main effects, respectively), 532 for juveniles; 9(1,7,1 for the main effects, respectively), 397 for adults.

†d.f. = 21(1,1,12,7 for the main effects, respectively), 532 for juveniles; 9(1,7,1 for the main effects, respectively), 312 for adults.

‡Because only adults of known age (natal year) were included, sample size for adults was less than in Table 1 and Fig. 1.

wing asymmetry than adults (Wilcoxon test, $P < 0.0001$), but tail and tarsus asymmetry did not differ significantly between the two age classes ($P > 0.05$ for each).

Discussion

Our results indicate that removal of ectoparasites leads to markedly lower levels of asymmetry in wing and outer tail feathers of juvenile cliff swallows. Average asymmetry among birds from fumigated colonies was less than half that seen among birds exposed to natural levels of parasites. Ectoparasites apparently affect only feather growth, however, as there was no obvious difference in tarsus asymmetry among fumigated and nonfumigated birds (although the fact that tarsus asymmetry could not be distinguished from measurement error tempers any conclusion relating to tarsus). The asymmetry differences between juveniles had largely disappeared by the time birds had become adults (≥ 1 -year old), with all birds showing essentially the same level of feather asymmetry. This suggests that the increased asymmetry in wing and tail among juveniles in nonfumigated colonies is a direct result of the heavy exposure to ectoparasites they experience in the nest (Brown & Brown, 1986, 1996) while these wing and tail feathers are growing.

Asymmetry in wing and tail feathers is known to be disadvantageous to cliff swallows. Birds with higher levels of asymmetry were more likely to die during spells of cold weather in late spring in our study area (Brown & Brown, 1998), presumably because they were less efficient in foraging during marginal and stressful conditions. Other studies on birds have shown a reduction in flight manoeuvrability and lift as wing and tail asymmetry increases (Møller, 1991; Balmford *et al.*, 1993; Thomas, 1993), and increased wing asymmetry in European starlings (*Sturnus vulgaris*) negatively affected flight speed and angle of takeoff (Swaddle, 1997). These sorts of performance impairments illustrate potential foraging-related costs of being asymmetrical. In cliff swallows, the reduction in average wing and tail asymmetry between the juvenile and adult age classes for nonfumigated birds may reflect some survival selection against juveniles with higher asymmetry prior to the post-juvinal molt or at other times prior to their return to the breeding grounds as adults.

These results indicate that parasites impose a substantial fitness cost on cliff swallows through increased feather asymmetry at the juvenile stage. This is another in a suite of parasite-related costs of coloniality in this species (Brown & Brown, 1986, 1996). Given the strong survival selection against asymmetry during periodic climate-driven selection episodes (Brown & Brown, 1998), it had been unclear how the higher observed levels of asymmetry were maintained in between these selection events. This study provides one answer: the ectoparasites to which cliff swallows are continually exposed generate feather asymmetry at the population

level. It is thus probable that the periodic climate-driven survival selection, in part, favours birds that have been less exposed to ectoparasites in the past and thus that have lower feather asymmetry.

The experimental approach used in this study establishes parasitism as a direct cause of increased feather asymmetry in juvenile cliff swallows. That asymmetry was unrelated to natal colony size, body size and (for wing asymmetry) colony site suggests that these factors did not vary among fumigated or nonfumigated colonies in a way that could account for the results. Although colony size does reflect phenotypic sorting in this species (Brown & Brown, 1996, 2000, 2001), the lack of any colony-size effects in particular indicated that the patterns of observed juvenile asymmetry did not reflect preferential settlement of birds in colonies of different sizes with respect to overall developmental stability (cf. Møller, 1995b). Furthermore, that there were no effects of body size on levels of asymmetry suggests that morphological sorting among sites (even had it occurred) could not have explained our results.

Parasitism as a cause of increased asymmetry is further suggested by the differences between juveniles and adults. Juveniles grow wing and tail feathers (that we measured soon after fledging) while in the nest, during which time they are continually fed upon by the nest-based swallow bugs (and other ectoparasites such as fleas). In contrast, adults molt and grow wing and tail feathers largely during the winter while in South America. During this period, cliff swallows are nomadic (Brown & Brown, 1995) and not associated with nests or swallow bugs. Once not directly exposed to the stress of swallow bug parasitism during growth, adult asymmetry levels were unaffected by whether a bird was from a fumigated or nonfumigated colony. For birds under nonexperimental conditions, feather asymmetry was much higher for juveniles and then declined once birds underwent their first post-juvinal molt, a result consistent with a parasite effect during the nestling stage. These results tend to confirm recent predictions (Aparicio, 2001) that fluctuating asymmetry should be more pronounced, and better reflect developmental stability, in growing traits than in fully grown ones.

Although there have been several studies showing a positive correlation between levels of parasitism and asymmetry, only a few have tested experimentally (by adding or removing parasites) whether parasitism was directly linked to asymmetry. Experimental infection of a weed by an anther-smut pathogen led to higher petal asymmetry in infected plants (Shykoff & Kaltz, 1998). Female mosquitoes that were experimentally infected by a microsporidian showed greater wing asymmetry (Agnew & Koella, 1997). In *Drosophila*, experimental infections of females with mites resulted in their sons having higher asymmetry in thoracic bristles (Polak, 1997b). Removal of endoparasites in reindeer resulted in greater antler symmetry among females 6 months later

(Folstad *et al.*, 1996). In a study more similar to ours, Møller (1992) manipulated ectoparasite loads of barn swallows (*Hirundo rustica*), finding that asymmetry of the outer tail feathers of adults with heavier parasite loads was greater following their next molt. However, these results differ from ours in that the barn swallows were not directly exposed to parasites during the time the asymmetrical feathers were actually growing (and thus whether this represented a true manipulation is debatable), and we found no differences in asymmetry among adult birds growing feathers on the wintering grounds. Three of these studies report a delayed effect of parasites on asymmetry, in contrast to ours in which asymmetry of feather growth was closely associated with the period of actual parasitism.

The studies by Møller (1992), Folstad *et al.* (1996) and Polak (1997b) also found increased asymmetry expressed in ornamental traits presumably subject to intense sexual selection, and they found no effect of parasitism on nonornamental characters under stabilizing natural selection. This again differs from our results: the traits for which we found higher asymmetry in relation to parasitism in cliff swallows – wing length and outer tail length – are naturally selected ones not subject to strong sexual selection. Unlike in barn swallows, outer tail feathers in cliff swallows are not elongated into streamers. The rounded tail shape in cliff swallows presumably reflects aerodynamic advantages (principally reduction of drag; Rowe *et al.*, 2001) associated with their high-altitude aerial foraging on swarming insects (Brown & Brown, 1996) and the high cost of streamer breakage in their enclosed nests. Wing length itself has been shown to be subject to strong viability selection in cliff swallows, particularly during unfavourable weather conditions (Brown & Brown, 1998).

The mechanisms by which parasites cause increased character asymmetry in general and for cliff swallows in particular are unknown. Most likely, parasite-induced nutritional deprivation of various forms destabilizes host development and elevates levels of fluctuating asymmetry (Polak, 1993, 1997a). Parasitism can limit host nutrient availability by reducing host food intake, digestion, absorption and nutrient assimilation (Whitfield, 1979; Thompson, 1983; Polak, 1997a). Some experimental studies have shown that nutritional stress directly leads to elevated asymmetry (Sciulli *et al.*, 1979; Swaddle & Witter, 1994; Imasheva *et al.*, 1999), although others have shown no such effect (Hovorka & Robertson, 2000; Bjorksten *et al.*, 2000b). In addition, Polak's (1997b) experiments on *Drosophila* suggest that degraded maternal condition, possibly via nutritional stress, can lead to impaired developmental stability *in utero*, resulting in increased asymmetry among offspring. For cliff swallows, given the high ectoparasite loads experienced in many colonies and the serious nutritional stress on nestlings that results (Brown & Brown, 1986, 1996), it seems likely that parasitism causes nutritional

deprivation which in turn causes increased fluctuating asymmetry among birds raised in infested nests. Maternal effects are less likely in cliff swallows, because females produce and lay eggs relatively early in the nesting season before parasite numbers become large. In the related but solitarily nesting tree swallow (*Tachycineta bicolor*), experimental food deprivation of nestlings for 3 h each day was found not to cause greater asymmetry in wing, tail, or tarsus (Hovorka & Robertson, 2000); however, birds in that study were not subjected to the heavy ectoparasite loads characteristic of cliff swallow colonies.

Interestingly, we found a detectable effect of parasites on feather asymmetry but not on skeletal (i.e. tarsus) asymmetry, suggesting that feather growth is more susceptible to parasite-induced nutritional stress than bone growth. This could result because tarsus growth in cliff swallows occurs during a relatively short period of time immediately after hatching, with the tarsus reaching its full development by day 12 or 13 (Stoner, 1945). In contrast, wing and tail feather growth occurs over a longer time period, including the entire ~25-day nestling period and for up to a week or more after fledging (Stoner, 1945; C. Brown & M. Brown, personal observations). Thus, young birds growing feathers are exposed to the effects of parasites for a much longer time period than when growing the tarsus. Furthermore, during the second half of the nestling period when feather development is at its maximum, young birds are larger and may have greater total nutritional demands that make them more sensitive to parasite-induced stress. Feather development continues to occur once birds fledge (when we measured them), which is likely to be a nutritionally stressful period in general as juveniles are learning to feed themselves. Birds from parasite-infested nests fledge at lower body mass than those from fumigated nests, contributing to the potential food deprivation that parasitized juveniles suffer during the time they are becoming independent.

In conclusion, this study provides experimental evidence that parasites can lead directly to increased levels of fluctuating asymmetry. Blood-feeding ectoparasites are likely to be a significant component of environmental stress, and their effects on levels of feather asymmetry probably affect substantively survival and fitness of colonially nesting cliff swallows exposed to them. Our results add to those of others suggesting that asymmetry in naturally selected characters believed to be under strong stabilizing selection for symmetry may prove to be a useful index of ecological conditions.

Acknowledgments

We thank the 54 research assistants who have worked on our cliff swallow project since its inception and in particular Scott Aldridge, Kimberly Cornett and Jennifer Klaus who assisted in the 2000 season. We are grateful to the University of Nebraska-Lincoln for allowing us to use

the facilities of the Cedar Point Biological Station. Financial support was provided most recently by the National Science Foundation (DEB-9613638, IBN-9974733, DEB-0075199). We thank two anonymous reviewers for helpful comments on the manuscript.

References

- Agnew, P. & Koella, J.C. 1997. Virulence, parasite mode of transmission, and host fluctuating asymmetry. *Proc. R. Soc. Lond. B* **264**: 9–15.
- Aparicio, J.M. 2001. Patterns of growth and fluctuating asymmetry: the effects of asymmetrical investment in traits with determinate growth. *Behav. Ecol. Sociobiol.* **49**: 273–282.
- Badyaev, A.V., Foresman, K.R. & Fernandes, M.V. 2000. Stress and developmental stability: vegetation removal causes increased fluctuating asymmetry in shrews. *Ecology* **81**: 336–345.
- Balmford, A., Jones, I.L. & Thomas, A.L.R. 1993. On avian asymmetry: evidence of natural selection for symmetrical tails and wings in birds. *Proc. R. Soc. Lond. B* **252**: 245–251.
- Bjorksten, T.A., Fowler, K. & Pomiankowski, A. 2000a. What does sexual trait FA tell us about stress? *Trends Ecol. Evol.* **15**: 163–166.
- Bjorksten, T., David, P., Pomiankowski, A. & Fowler, K. 2000b. Fluctuating asymmetry of sexual and nonsexual traits in stalk-eyed flies: a poor indicator of developmental stress and genetic quality. *J. Evol. Biol.* **13**: 89–97.
- Brown, C.R. & Brown, M.B. 1986. Ectoparasitism as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). *Ecology* **67**: 1206–1218.
- Brown, C.R. & Brown, M.B. 1992. Ectoparasitism as a cause of natal dispersal in cliff swallows. *Ecology* **73**: 1718–1723.
- Brown, C.R. & Brown, M.B. 1995. Cliff swallow (*Hirundo pyrrhonota*). In: *The Birds of North America* (A. Poole & F. Gill, eds), no. 149. Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, DC.
- Brown, C.R. & Brown, M.B. 1996. *Coloniality in the Cliff Swallow: the Effect of Group Size on Social Behavior*. University of Chicago Press, Chicago, IL.
- Brown, C.R. & Brown, M.B. 1998. Intense natural selection on body size and wing and tail asymmetry in cliff swallows during severe weather. *Evolution* **52**: 1461–1475.
- Brown, C.R. & Brown, M.B. 2000. Heritable basis for choice of group size in a colonial bird. *Proc. Natl. Acad. Sci. USA* **97**: 14825–14830.
- Brown, C.R. & Brown, M.B. 2001. Avian coloniality: progress and problems. *Curr. Ornithol.* **16**: 1–82.
- Brown, C.R., Komar, N., Quick, S.B., Sethi, R.A., Panella, N.A., Brown, M.B. & Pfeffer, M. 2001. Arbovirus infection increases with group size. *Proc. R. Soc. Lond. B* **268**: 1833–1840.
- Campbell, W.B., Emlen, J.M. & Hershberger, W.K. 1998. Thermally induced chronic developmental stress in coho salmon: integrating measures of mortality, early growth, and developmental instability. *Oikos* **81**: 398–410.
- Carbonell, R. & Telleria, J.L. 1998. Increased asymmetry of tarsus-length in three populations of blackcaps *Sylvia atricapilla* as related to proximity to range boundary. *Ibis* **140**: 331–333.
- Chapman, B.R. & George, J.E. 1991. The effects of ectoparasites on cliff swallow growth and survival. In: *Bird-Parasite Interactions: Ecology, Evolution and Behaviour* (J. E. Loye & M. Zuk, eds), pp. 69–92. Oxford University Press, Oxford.
- Clarke, G.M. 1998. Developmental stability and fitness: the evidence is not quite so clear. *Am. Nat.* **152**: 762–766.
- Folstad, I., Arneberg, P. & Karter, A.J. 1996. Antlers and parasites. *Oecologia* **105**: 556–558.
- George, J.E. 1987. Field observations on the life cycle of *Ixodes baergi* and some seasonal and daily activity cycles of *Oeciacus vicarius* (Hemiptera: Cimicidae), *Argas cooleyi* (Acari: Argasidae), and *Ixodes baergi* (Acari: Ixodidae). *J. Med. Entomol.* **24**: 683–688.
- Helm, B. & Albrecht, H. 2000. Human handedness causes directional asymmetry in avian wing length measurements. *Anim. Behav.* **60**: 899–902.
- Houle, D. 1997. Comment on 'a meta-analysis of the heritability of developmental stability' by Møller and Thornhill. *J. Evol. Biol.* **10**: 17–20.
- Houle, D. 1998. High enthusiasm and low R-squared. *Evolution* **52**: 1872–1876.
- Hovorka, M.D. & Robertson, R.J. 2000. Food stress, nestling growth, and fluctuating asymmetry. *Can. J. Zool.* **78**: 28–35.
- Imasheva, A.G., Bosenko, D.V. & Bubli, O.A. 1999. Variation in morphological traits of *Drosophila melanogaster* (fruit fly) under nutritional stress. *Heredity* **82**: 187–192.
- Leung, B. & Forbes, M.R. 1996. Fluctuating asymmetry in relation to stress and fitness: effects of trait type as revealed by meta-analysis. *Ecoscience* **3**: 400–413.
- Loye, J.E. & Carroll, S.P. 1991. Nest ectoparasite abundance and cliff swallow colony site selection, nestling development, and departure time. In: *Bird-Parasite Interactions: Ecology, Evolution and Behaviour* (J. E. Loye & M. Zuk, eds), pp. 222–241. Oxford University Press, Oxford.
- Møller, A.P. 1991. Sexual ornament size and the cost of fluctuating asymmetry. *Proc. R. Soc. Lond. B* **243**: 59–62.
- Møller, A.P. 1992. Parasites differentially increase the degree of fluctuating asymmetry in secondary sexual characters. *J. Evol. Biol.* **5**: 691–699.
- Møller, A.P. 1995a. Leaf-mining insects and fluctuating asymmetry in elm *Ulmus glabra* leaves. *J. Anim. Ecol.* **64**: 697–707.
- Møller, A.P. 1995b. Developmental stability and ideal despotic distribution of blackbirds in a patchy environment. *Oikos* **72**: 228–234.
- Møller, A.P. 1996. Parasitism and developmental instability of hosts: a review. *Oikos* **77**: 189–196.
- Møller, A.P. 1997. Developmental stability and fitness: a review. *Am. Nat.* **149**: 916–932.
- Møller, A.P. & Pomiankowski, A. 1993. Fluctuating asymmetry and sexual selection. *Genetica* **89**: 267–279.
- Møller, A.P. & Swaddle, J.P. 1997. *Asymmetry, Developmental Stability, and Evolution*. Oxford University Press, Oxford.
- Møller, A.P. & Thornhill, R. 1998. Bilateral symmetry and sexual selection: a meta-analysis. *Am. Nat.* **151**: 174–192.
- Østbye, K., Øxnevad, S.A. & Vøllestad, L.A. 1997. Developmental stability in perch (*Perca fluviatilis*) in acidic aluminum-rich lakes. *Can. J. Zool.* **75**: 919–928.
- Palmer, A.R. 1994. Fluctuating asymmetry analyses: a primer. In: *Developmental Instability: its Origins and Evolutionary Implications* (T. A. Markow, ed.), pp. 335–364. Kluwer, Dordrecht, The Netherlands.
- Palmer, A.R. 1996. Waltzing with asymmetry. *Bioscience* **46**: 518–532.
- Palmer, A.R. 1999. Detecting publication bias in meta-analyses: a case study of fluctuating asymmetry and sexual selection. *Am. Nat.* **154**: 220–233.

- Palmer, A.R. & Strobeck, C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Ann. Rev. Ecol. Syst.* **17**: 391–421.
- Parsons, P.A. 1990. Fluctuating asymmetry: an epigenetic measure of stress. *Biol. Rev.* **65**: 131–145.
- Polak, M. 1993. Parasites increase fluctuating asymmetry of male *Drosophila nigrospiracula*: implications for sexual selection. *Genetica* **89**: 255–265.
- Polak, M. 1997a. Parasites, fluctuating asymmetry, and sexual selection. In: *Parasites and Pathogens: Effects on Host Hormones and Behavior* (N. E. Beckage, ed.), pp. 246–276. Chapman & Hall, New York.
- Polak, M. 1997b. Ectoparasitism in mothers causes higher positional fluctuating asymmetry in their sons: implications for sexual selection. *Am. Nat.* **149**: 955–974.
- Reimchen, T.E. & Nosil, P. 2001. Lateral plate asymmetry, diet and parasitism in threespine stickleback. *J. Evol. Biol.* **14**: 632–645.
- Rettig, J.E., Fuller, R.C., Corbett, A.L. & Getty, T. 1997. Fluctuating asymmetry indicates levels of competition in an even-aged poplar clone. *Oikos* **80**: 123–127.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- Rowe, L., Repasky, R.R. & Palmer, A.R. 1997. Size-dependent asymmetry: fluctuating asymmetry versus antisymmetry and its relevance to condition-dependent signaling. *Evolution* **51**: 1401–1408.
- Rowe, L.V., Evans, M.R. & Buchanan, K.L. 2001. The function and evolution of the tail streamer in hirundines. *Behav. Ecol.* **12**: 157–163.
- Roy, B.A. & Stanton, M.L. 1999. Asymmetry of wild mustard, *Sinapis arvensis* (Brassicaceae), in response to severe physiological stresses. *J. Evol. Biol.* **12**: 440–449.
- SAS Institute 1990. *SAS/STAT User's Guide*, Version 6. SAS Institute, Cary, NC.
- Sciulli, P.W., Doyle, W.J., Kelley, C., Siegel, P. & Siegel, M.I. 1979. The interaction of stressors in the induction of increased levels of fluctuating asymmetry in the laboratory rat. *Am. J. Phys. Anthropol.* **50**: 279–284.
- Shykoff, J.A. & Kaltz, O. 1998. Phenotypic changes in host plants diseased by *Microbotryum violaceum*: parasite manipulation, side effects, and trade-offs. *Int. J. Plant Sci.* **159**: 236–243.
- Siikamaki, P. & Lammi, A. 1998. Fluctuating asymmetry in central and marginal populations of *Lychnis viscaria* in relation to genetic and environmental factors. *Evolution* **52**: 1285–1292.
- Stoner, D. 1945. Temperature and growth studies of the northern cliff swallow. *Auk* **62**: 207–216.
- Swaddle, J.P. 1997. Within-individual changes in developmental stability affect flight performance. *Behav. Ecol.* **8**: 601–604.
- Swaddle, J.P. & Witter, M.S. 1994. Food, feathers and fluctuating asymmetries. *Proc. R. Soc. Lond. B* **255**: 147–152.
- Swaddle, J.P., Witter, M.S. & Cuthill, I.C. 1994. The analysis of fluctuating asymmetry. *Anim. Behav.* **48**: 986–989.
- Thomas, A.L.R. 1993. The aerodynamic costs of asymmetry in the wings and tail of birds: asymmetric birds can't fly round tight corners. *Proc. R. Soc. Lond. B* **254**: 181–189.
- Thompson, S.N. 1983. Biochemical and physiological effects of metazoan endoparasites on their host species. *Comp. Biochem. Physiol.* **74B**: 183–211.
- Van Valen, L. 1962. A study of fluctuating asymmetry. *Evolution* **16**: 125–142.
- Whitfield, P.J. 1979. *The Biology of Parasitism: an Introduction to the Study of Associating Organisms*. University Park Press, Baltimore, MD.

Received 15 March 2002; revised 10 April 2002; accepted 16 May 2002