Testis size increases with colony size in cliff swallows

Charles R. Brown and Mary Bomberger Brown
Department of Biological Sciences, University of Tulsa, Tulsa, OK 74104, USA

By using a sample of over 800 male cliff swallows (*Petrochelidon pyrrhonota*) that died during a rare climatic event in our Nebraska study area in 1996, we investigated how testis size was related to body size, age, parasite load, a bird's past colony-size history, and spleen size. Testis volume increased with body size. After correcting for body size, testis volume was lowest for birds age 1 and 2 years but did not vary with age for males 3 years old or more. Birds occupying parasite-free (fumigated) colonies had significantly larger testes than did birds at nonfumigated sites. Testis volume increased significantly with the size of the breeding colonies a bird had used in the past. Testis volume had no relationship with spleen volume after correcting for body size. The results show within a species that larger testes are favored in more social environments, probably reflecting a response to increased rates of extrapair copulation (and thus sperm competition) among cliff swallows in large colonies. The presence of ectoparasites, by inflating levels of plasma corticosterone, may in turn reduce testis mass. These data provide no support for the hypothesis that large testes, perhaps by producing more testosterone, are immunosuppressive and thus costly for that reason. *Key words:* coloniality, immunocompetence, life history, *Petrochelidon pyrrhonota*, social behavior. *[Behav Ecol 14:569–575 (2003)]*

One way that males can increase their probability of fertilizing a female's eggs is by producing large quantities of sperm and copulating frequently with a given female. The ability to produce frequent ejaculates with a high volume of sperm is determined in large part by testis size (Møller, 1988, 1989; Stockley et al., 1997). Large testes are beneficial both in permitting males to copulate more frequently with their own mate(s) as a defense against extrapair mating and in allowing males to copulate with more extrapair females. Because for some species the incidence of multiple mating by females through extrapair copulation increases in large groups such as breeding colonies (Birkhead et al., 1992; Brown and Brown, 1996; Hatchwell, 1988; Møller, 1985; Ramo, 1993), males with larger testes should be favored in those social environments. Cross-species comparisons have shown that testis size tends to be larger in more highly social taxa, presumably as a direct result of heightened within-pair and extrapair sperm competition (Gage, 1994; Harcourt et al., 1995; Møller, 1991; Stockley et al., 1997). The same selective pressures on testis size might also apply within a species when individuals routinely experience different degrees of sociality. However, few studies have looked at intraspecific variation in testis size, and no previous work has examined whether testis size is correlated with group size (and thus possibly with the expected intensity of sperm competition) within a species.

In this study we examine variation in testis size in cliff swallows (*Petrochelidon pyrrhonota*), asking specifically whether testis size varies with social environment (colony size). If male cliff swallows that traditionally occupy large colonies are subject to greater sperm competition, we would expect testis size to vary directly with colony size. Because there is heritable variation in these birds' choice of colony size (Brown and Brown, 2000), testis size could be under directional selection in large, but not necessarily in small, groups. We also investigate the relationship between testis size and spleen size. Larger testes potentially produce more testosterone, and

testosterone is thought to be immunosuppressive (Folstad and Karter, 1992; Wedekind and Folstad, 1994). Spleen size is an index of exposure to parasites and pathogens in general (Møller and Erritzøe, 1996, 1998; Møller, 1998; Møller et al., 2001) and for cliff swallows in particular (Brown and Brown, 2002), and a larger spleen (splenomegaly) is associated with disease in a variety of taxa (Ali and Behnke, 1985; John, 1994, 1995; Molyneux et al., 1983; Vincent and Ash, 1978; Watkins et al., 1991). Consequently, we might expect birds with larger testes to be more sickly, and thus, their spleens should also be larger. Finally, we took advantage of the fact that ectoparasites had been removed from some of the colonies in our study area, and we examined whether ectoparasitism might have directly affected testis size, as reported in some species (Dunlap and Schall, 1995).

Our analyses use a unique dataset in which we measured testis and spleen sizes for over 800 male cliff swallows. These were birds that died during a rare climatic event in our Nebraska study area in 1996 (Brown and Brown, 1998). Because they all died at once, our results are not confounded by seasonal variation in size of either the testis or spleen (Fänge and Silverin, 1985; Johnston, 1956; Oakeson, 1953, 1956; Silverin, 1981; Wright and Wright, 1944).

METHODS

Study animal

Cliff swallows are highly colonial passerines that breed throughout most of western North America (Brown and Brown, 1995). They build gourd-shaped mud nests and attach them to the vertical faces of cliff walls, rock outcrops, or artificial sites such as the eaves of buildings or bridges. Their nests tend to be stacked closely together, often sharing walls. Cliff swallows are migratory, wintering in southern South America, and have a relatively short breeding season in North America. They begin to arrive at our study site in late April or early May and depart by late July. They generally raise only one brood. Cliff swallows are associated with a variety of ectoparasites, endoparasites, and viruses throughout their range (Brown and Brown, 1995; Brown et al., 2001; Monath et al., 1980; Scott et al., 1984). The ectoparasites, in particular the hematophagous swallow bug (Hemiptera: Cimicidae:

Oeciacus vicarius), are responsible for much of the nestling mortality and nest failures that occur in our study area (Brown and Brown, 1986, 1996).

Study site

Our study site was centered 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 included portions of Deuel, Garden, and Lincoln counties, southwestern Nebraska. We have studied cliff swallows there since 1982. There were approximately 160 cliff swallow colony sites in our 150 × 50-km study area, with about one third of these not used in a given year. Colony size at a site varied widely; in our study area, it ranged from two to 3700 nests, with some birds nesting solitarily. Over a 20-year period, mean (±SE) colony size ($n = 1363$) was 363 (±16) nests. Each colony site tended to be separated from the next nearest by 1–10 km, but in a few cases by 20 km or more. In our study area, the birds nested on both natural cliff faces and artificial structures such as bridges, buildings, and highway culverts. The study site is described in detail by Brown and Brown (1996).

Fumigation and designating colony sizes

We removed swallow bugs and other ectoparasites by applying a dilute solution of Dibrom as a light mist to the outsides of nests. This insecticide was highly effective against most arthropods, and we used it extensively in our past work (see Brown and Brown, 1996). Nests were lightly sprayed once a week to remove any bugs immigrating into the colony on arriving birds. The two fumigated colonies used in this study were fumigated in their entirety, and we began fumigation in early May each year as the first cliff swallows were arriving. Fumigation had no detectable effects on the cliff swallows themselves (Brown and Brown, 1996). The same sites, and other sites in some years, had been fumigated in similar ways in earlier years of our study.

Cliff swallow colony size is defined as the maximum number of nests to have housed one or more eggs. Active nests were counted at some sites by periodically checking the nest contents with a dental mirror and flashlight, whereas the colony size at other sites was estimated by counting the number of nests in active sections of the colony. Full details on these methods of determining colony sizes are given in Brown and Brown (1996). For some analyses, we used colony sizes in 1996 at sites before the bad-weather event (below), which was a period before all birds had laid eggs. These colony-size estimates were derived from observations of the number of cliff swallows present at the colonies in the week preceding the bad weather, primarily at dusk when the birds returned to the colony site to roost (a time when accurate estimates of colony size can be made; Brown, 1998; Brown and Brown, 1996). Past breeding-colony size histories were calculated for all birds in the sample that had been banded in our study area before 1996; we did this by averaging the sizes of all breeding colonies a given individual had been known to occupy during its lifetime. This varied from one to seven colonies per bird, depending in part on how long an individual had lived, when we first caught it, and the extent to which we had caught it in each previous year (some individuals were missed in some years).

Weather event and collection and measurement of birds

The cliff swallows for which testis and spleen measurements were taken were ones found dead during a rare climatic event in the study area in 1996 (Brown and Brown, 1998). This period of unusually cold and wet weather occurred 24–29 May after most cliff swallows had arrived and settled in colonies. Mortality was extensive, with the population reduced by at least 53% during this 6-day period (Brown and Brown, 1998). We salvaged 1856 specimens. These were birds dead underneath nests at colony sites and, in a few cases, inside nests. We visited most colony sites in the area immediately after the bad weather and collected all specimens we could find. External morphology of specimens was measured (Brown and Brown, 1998), all were prepared as skins, carcasses were saved, and later all testes (if specimen condition allowed; $n = 844$) and spleens ($n = 1723$) were measured by a single person (M.B.B.). The length and width of each testis and spleen were taken in millimeters with calipers. Testes and spleens were ellipsoid in shape, and the volume (in mm^3), V, was calculated by using the formula

$$V = (\pi \times \text{length} \times \text{width}^2)/6. \tag{1}$$

Volume served as our measure of size for both organs. We added the volumes for the right and left testis on each bird to get a combined testes volume for each individual. Because spleen volume was determined in part by exposure to ectoparasites and because birds occupying fumigated colonies in 1996 had significantly smaller spleens than those in nonfumigated colonies (Brown and Brown, 2002), we separated all analyses involving the spleen into ones based on birds from fumigated and nonfumigated colonies.

We used a multivariate index of overall body size that combined measurements of the mean wing length (the unflattened closed wing from the most anterior part of the wrist joint to the tip of the outermost primary), the mean tail length and the middle tail length (the length of the outermost tail feather on each side and the middle tail feather, respectively), the mean tarsus length (from the proximate end of the tarso-metatarsus to the hallux for each leg), and the length and width of the exposed bill (length from the proximate end of the exposed culmen to the tip of the bill along the upper mandible and width of the exposed mandibles at the nostrils). All measurements were taken by one person only (M.B.B.). Overall body size was calculated as the mean of the log-transformed, m, original variables, $[\Sigma\ln(X_i)/m]$, where X_i was the value of the ith variable for a given observation, and in our case $m = 6$ (Brown and Brown 1998; Via and Shaw 1996). See Brown and Brown (1998) for additional details and measurement repeatabilities.

Testis and spleen volumes and body size were log$_{10}$-transformed before analysis because these variables were not normally distributed. Because we found that testis volume increased significantly with body size (linear regression: $\beta = 5.901$, $p < .0001$, $n = 828$), we present our data as residuals from a linear regression of combined right and left testes volume on body size. Use of residuals corrects for any effect of body size (see Gage, 1994; Møller, 1988, 1989). However, significance tests based on residuals can be biased in both conservative and liberal ways (Darlington and Smulders, 2001; Freckleton, 2002). A recommended alternative is to perform significance testing on the raw data (nonresiduals) by using GLM with any confounding variable as a covariate in the regression model (Darlington and Smulders, 2001). Thus, our statistical analyses used GLM with the log$_{10}$-transformed raw data and always with body size as a covariate; we report the significance (F test, p) for the independent variable of interest from each of these tests. Although spleen volume did not vary significantly with body size (linear regression: $\beta = 0.541$, $p = .59$, $n = 1685$), for consistency we also used residuals from a linear regression of spleen volume on body size in presenting testis volume in relation to spleen volume. Statistical analyses were done with SAS (SAS Institute, 1990).
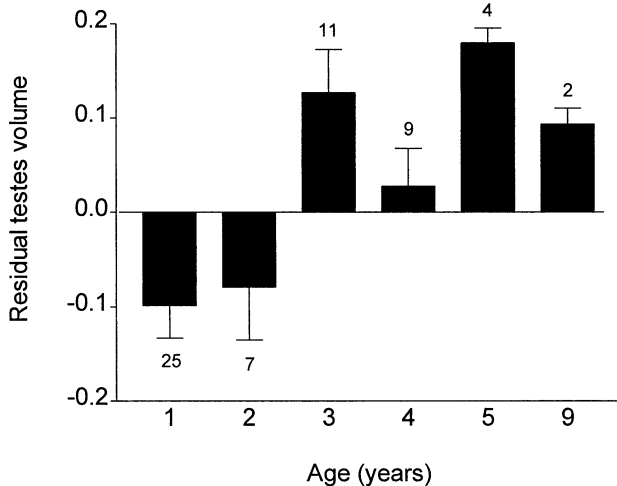
Figure 1
Mean ($\pm$SE) residual testis volume in relation to age in cliff swallows. Testis volume varied significantly with age (GLM: $F_{5,51} = 4.81$, $p = .001$). Sample sizes (number of birds) are shown above the SE bars.

RESULTS

Effect of age

We had 59 males in our sample that had been banded as nestlings or juveniles in an earlier year and thus for whom we knew exact ages. Testis volume varied significantly with age (Figure 1). This was brought about primarily by the lower testis volumes for birds 1 and 2 years of age; testis volume did not vary significantly among the age classes of 3 years or more (GLM: $F_{2,20} = 2.37$, $p = .12$).

Effects of nest fumigation

Because earlier we had found a strong effect of ectoparasite removal on spleen size (Brown and Brown, 2002), we examined whether testis volume differed between males occupying fumigated versus nonfumigated colonies. Testis volume for males at parasite-free colony sites in 1996 (mean residual = 0.049, SE = 0.014, $n = 134$) was significantly larger than that for males at naturally infested sites in 1996 (mean residual = –0.009, SE = 0.007, $n = 695$; GLM: $F_{1,826} = 12.21$, $p < .001$). In case this result might have been caused by different age distributions among birds in fumigated versus nonfumigated colonies and the effect of age on testis volume (above), we considered only the subset of birds for whom we knew colony-size histories before 1996 ($n = 243$ males). This ensured that all birds in the analysis were at least 2 years old (each having occupied at least one breeding colony before 1996). Among these birds, we found that testis volume for males at parasite-free colony sites in 1996 (mean residual = 0.097, SE = 0.017, $n = 68$) again was larger (although not quite significantly at the $p = .05$ level) than that for males at naturally infested sites in 1996 (mean residual = 0.065, SE = 0.010, $n = 175$; GLM, $F_{1,241} = 3.37$, $p = .067$).

We also examined whether past use (in years before 1996) of fumigated versus nonfumigated colonies affected testis size. For this analysis, each individual for whom we knew at least one of its breeding colonies before 1996 was scored as having a fumigated history if it had used at least one fumigated colony in the past, or a nonfumigated history if it was not known to have ever used a fumigated site, irrespective of past colony size. For birds using nonfumigated colonies in 1996, testis volume did not differ significantly between those that
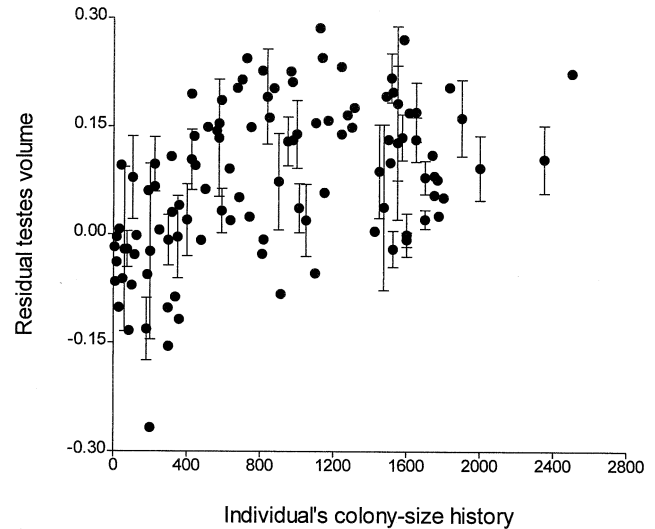

Figure 2
Residual testis volume in relation to a male cliff swallow's past history of colony-size use, defined as the mean breeding-colony size over all years before 1996 for which we knew the individual's history. Each circle represents a single individual, unless SE bars are shown, in which case the mean $\pm$ SE for that colony-size history is plotted; each colony-size history counted equally in the statistical analysis. Testis volume increased significantly with colony-size history (GLM: $\beta = 0.125$, SE = 0.017, $F_{1,112} = 56.7$, $p < .0001$, $n = 114$ size histories).

had (mean residual = 0.052, SE = 0.017, $n = 59$) versus had not (mean residual = 0.073, SE = 0.012, $n = 116$) used fumigated sites in the past (GLM: $F_{1,173}$, $p = .39$). For birds using fumigated colonies in 1996, testis volume did not differ significantly between those that had (mean residual = 0.100, SE = 0.018, $n = 59$) versus had not (mean residual = 0.078, SE = 0.069, $n = 9$) used fumigated sites in the past (GLM: $F_{1,65} = 0.07$, $p = .79$). Thus, we disregarded fumigation status of past colony use in further analyses (below).

Effects of colony size

Because of the effect of age on testis volume (Figure 1) and because most of the birds in our data set were of unknown exact ages, we restricted our analysis of colony size to the birds for whom we knew colony-size histories before 1996 and thus were at least 2 years old in 1996 ($n = 243$ males). By removing all yearlings, we minimized confounding covariation between male age, testis size, and colony size.

Testis volume increased significantly with the average breeding-colony size an individual had occupied (Figure 2). Males traditionally residing in the larger colonies had testes almost three times larger, corrected for body size, than did males living in the smaller colonies (Figure 2). This analysis combined birds from nonfumigated and fumigated colonies in 1996 because we had a relatively small range of past histories for fumigated birds; however, we got the same result if the analysis was restricted just to those using nonfumigated colonies in 1996 (GLM: $\beta = 0.123$, SE = 0.016, $F_{1,84} = 56.0$, $p < .0001$, $n = 86$ colony histories).

Correlations between testis size and spleen size

By using birds from all colonies in 1996 and with body size controlled for as a covariate, we found no significant relationship between spleen volume and testis volume for birds in either nonfumigated (Figure 3a) or fumigated
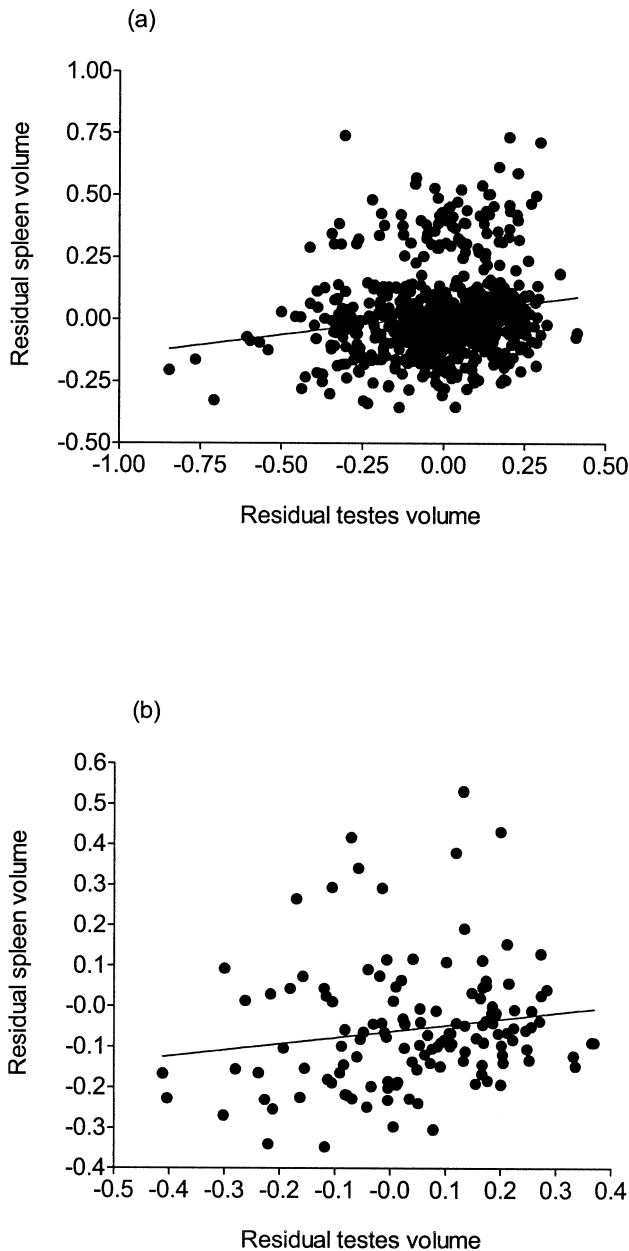
(a)

Table 1

Results of GLM tests on the relationship between testis volume and spleen volume, controlling for body size as a covariate, for birds at 15 cliff swallow colonies in 1996

Approximate colony size (nests) in 1996	$\beta \pm SE$	p^a	n
10	0.997 ± 1.021	.38	8
200	-0.281 ± 0.156	.09	20
300	-0.139 ± 0.313	.66	13
300	1.834 ± 0.628	.04	8
450	-0.110 ± 0.148	.46	35
500	-0.701 ± 0.453	.17	9
500	0.176 ± 0.201	.39	44
500	0.078 ± 0.152	.61	61
500	0.085 ± 0.161	.60	49
600	-0.032 ± 0.100	.75	137
750	0.835 ± 0.259	.02	8
750	0.027 ± 0.266	.92	48
800	0.084 ± 0.083	.72	95
1000	0.004 ± 0.077	.96	59
1500	0.131 ± 0.113	.25	127

Regression tests were based on $\log_{10}$-transformed raw data; p values are from F test.

[a] None significant at the $p \leq .05$ level after correcting for multiple comparisons (Rice, 1989).

(b)

Figure 3
Residual spleen volume in relation to a male cliff swallow's residual testis volume for birds occupying nonfumigated (a) and fumigated (b) sites in 1996. Each circle represents a single bird. Spleen volume did not vary significantly with testis volume for birds from either nonfumigated colonies (GLM: $\beta = 0.068$, SE = 0.041, $F_{1,650} = 2.7$, $p = .10$) or fumigated colonies (GLM: $\beta = 0.132$, SE = 0.112, $F_{1,127} = 1.4$, $p = .24$). Line in each indicates best-fit linear least-squares regression.

regression coefficient was positive in 10 cases and negative in five. None was statistically significant after correcting for multiple comparisons (Table 1). There was thus no evidence that pooling colonies (Figure 3) affected our conclusions or that 1996 colony size itself obviously influenced the overall testis/spleen relationship (Table 1). The separate colony-by-colony analyses were consistent with the combined analysis in suggesting that testis size and spleen size tended not to vary together in any predictable way.

DISCUSSION

The strong association between an individual's colony-size history and its testis size is the first such relationship reported within a species to our knowledge. The results may indicate that there is greater sperm competition among males that traditionally occupy the larger colonies. Larger testes allow the production of larger quantities of sperm and thereby are presumably advantageous when male-male competition occurs via sperm competition.

Because our data were taken from individuals that had succumbed in a rare climatic event (Brown and Brown, 1998), it is probable that our sample of birds was not a truly random one. We know that the birds that died were smaller and had greater levels of asymmetry in wing and tail than those that survived. Thus, our reported testis volumes may not be representative for the population as a whole. However, our conclusions should be unaffected by this because all of our comparisons (e.g., the effect of colony size) were relative ones done among birds that all died at the same time and thus presumably had similar attributes at least with respect to their ability to survive inclement weather.

Testis size and colony size

Three aspects of cliff swallow ecology suggest that selection should favor increased testis size as an adaptation to living in large colonies. First, female cliff swallows often engage in extrapair copulation attempts, and this activity occurs at a higher frequency in larger colonies (Brown and Brown,

(Figure 3b) sites. There was considerable variation in both data sets, although both regression coefficients were positive (Figure 3). We had no way to correct for the effect of age on testis volume in these analyses; however, spleen volume itself was found not to vary significantly with age in earlier analyses based on both sexes (Brown and Brown, 2002).

In case combining colonies might have obscured a different pattern, we also examined the relationship between testis volume and spleen volume for each colony in 1996 (where $n \geq 8$ birds) separately (Table 1). For these 15 colonies, the

1996). Extrapair copulation happens often at mud-gathering sites while the birds are nest-building; females seem not to resist them in many cases. Males that are not residents of a colony come to mud-gathering sites and attempt extrapair copulations with nest-building females (Brown and Brown, 1996). Other extrapair copulation attempts occur at nests among resident birds who are close neighbors. Second, male cliff swallows do not mate-guard, instead guarding the nest whenever the female leaves during nest-building and egg-laying. This presumably is a defense against nest usurpation, loss of nesting material, egg tossing by conspecifics, and intraspecific brood parasitism (Brown and Brown, 1996). In lieu of mate-guarding, males copulate frequently with their mate, usually after she returns from each trip to collect mud or to forage, a pattern seen in other highly social species (Birkhead, 1998; Møller and Birkhead, 1993), suggesting that the ability to make large volumes of sperm is important for these birds. Third, cliff swallows show a heritable basis to their choice of group size (Brown and Brown, 2000), meaning that individuals tend to occupy colonies of similar size throughout their lives. Thus, directional selection can favor larger testes in those males perennially using larger colonies, while at the same time not necessarily favoring larger testes (especially if they are costly) in individuals residing in small colonies. With the birds' behavioral preferences for choosing colonies of particular sizes, testis size has apparently become a morphological specialization (perhaps in response to the level of sperm competition) linked to colony choice.

Other possibilities for the increase in testis size with colony size are unlikely. One is that the effect of age on testis size might have accounted for the results if age distributions of males varied with colony size. This is unlikely both because our analysis specifically excluded all yearlings and because, if anything, younger birds tend to be overrepresented in the larger colonies (Brown and Brown, 1996). Thus, age should have biased us toward finding smaller testis sizes in larger colonies, opposite of what we observed. Arrival time, and thus stage in the breeding cycle, is also unlikely to have confounded our results. Spring arrival times do not differ significantly among cliff swallows age 2 years or more in our population (Brown and Brown, 1996). That fact, plus the date when our birds were sampled (29 May), suggests that all were in breeding condition when their testes were measured.

Although comparative studies across species in various taxa have shown positive correlations between testis size and the extent of multiple mating by females (Gage, 1994; Harcourt et al., 1995; Møller, 1991; Stockley et al., 1997), the same relationship has rarely, if ever, been found within a species. The most closely similar results to ours come from fishes, in which gonad mass in several species varies depending on whether males are territorial or engage in satellite ("sneaking") behavior (Taborsky, 1994). But these gonadal differences are part of a radical divergence among males in overall morphology and behavior. An effect of social environment per se (and resulting levels of sperm competition) on testis size within a species in birds and mammals may not have been reported simply because no one has looked; measuring testis sizes requires killing animals, and to do it within a species requires killing lots of the same kind of animal. We were able to take advantage of a rare mortality event in which thousands of cliff swallows died naturally.

The increase in testis size in larger groups could come about through either directional selection on the heritable component of testis size or through facultative adjustment by males who invest more in testis mass when in large colonies. We know of no examples of the latter in other species, and this scenario seems unlikely for cliff swallows. Males collected in our study area on arrival in the spring—before colony sites

are chosen and colony sizes are established—tend to have well-developed testes (Brown C and Brown M, unpublished data), meaning that testis development often occurs before individuals have actually settled anywhere. It seems more likely that the genetic basis to the cliff swallow's choice of colony size (Brown and Brown, 2000) means that this species is primed for directional selection on testis size in relation to social environment. Cliff swallows occupying colonies of different sizes experience a variety of different selective pressures and show differences in behavior (e.g., foraging strategies) as a result (Brown and Brown, 1996). A greater intensity of both within-pair and extrapair sperm competition in bigger groups is probably another of these selective pressures and, in this case, has apparently brought about a morphological difference related to colony size.

In a comparative analysis across bird species, Westneat and Sherman (1997) found no effect of breeding dispersion on rates of extrapair fertilizations. This was in contrast to other comparative studies and some on single species (Birkhead and Møller, 1992; Brown and Brown, 1996; Gladstone, 1979; Møller and Birkhead, 1993), in which rates of extrapair copulations were greater among colonial species or populations. One explanation for this discrepancy is suggested by our data and the cross-species studies of testis size (see Møller, 1988, 1991). If individuals in larger groups have larger testes, males may be better able to defend against extrapair fertilizations by copulating frequently with their mates. The result could be to lower the rate of extrapair fertilizations among highly social species, even though the frequency of extrapair copulations remains high. This does not mean that sperm competition is not more intense in colonial birds, only that larger testes may be one effective counter-defense. It may be that selection for larger testes in male cliff swallows (and other species) occupying large colonies may be caused largely by the advantages of being able to make frequent ejaculates for within-pair copulations and perhaps less so by the greater opportunities to copulate with extrapair females.

Parasites and testis size

We had not expected to find an apparent effect of ectoparasitism on testis size. However, the increased testis volume for cliff swallows occupying fumigated colonies is consistent with Dunlap and Schall's (1995) hypothesis that elevated levels of plasma corticosterone, induced by exposure to parasites, can alter the adrenal response to stress, resulting in both a suppression of testosterone and reduced testis mass. Lizards infected with malarial parasites had smaller testes than those of uninfected ones (Dunlap and Schall, 1995). This hypothesis is further supported in cliff swallows by (1) the fact that the fumigation status of only the current colony size (and thus the current exposure to ectoparasites), not whether a bird used a fumigated or nonfumigated colony in the past, was related to testis size; and (2) our finding in a separate study of higher levels of plasma corticosterone in birds residing in large nonfumigated colonies than in those inhabiting large fumigated colonies (Brown C, Brown M, Raouf S, Smith L, Wingfield J, unpublished data). To the degree that any reduction in potential testis volume is disadvantageous to cliff swallows in sperm competition, this represents yet another cost of ectoparasitism in this species.

Costs of large testes

That higher levels of testosterone may be costly through their suppressive effects on the immune system has been a widely discussed hypothesis in recent years (see Braude et al., 1999; Folstad and Karter, 1992; Hillgarth and Wingfield, 1997;

Hillgarth et al., 1997; Wedekind and Folstad, 1994). Some studies have found empirical support (see Saino and Møller, 1994; Verhulst et al. 1999; Zuk et al., 1995), others have not (see Dunlap and Schall, 1995; Hasselquist et al., 1999; Ros et al., 1997), and some have found evidence both for and against in the same species (depending, e.g., on whether individuals were captive or free-living; Duckworth et al., 2001; Peters, 2000). Because the testes produce testosterone, males with larger testes have the potential to produce higher levels of circulating testosterone (although the relationship between testis size and circulating testosterone levels has apparently rarely been studied). If males with larger testes have on average higher circulating levels of testosterone, we would predict that male cliff swallows with larger testes should have more suppressed immune systems.

In an earlier study, we found that cliff swallows more exposed to ectoparasites and their associated pathogens had larger spleens (Brown and Brown, 2002). Males in non-fumigated colonies had spleens about 20% larger than those of males in parasite-free sites. If we assume that more sickly birds have larger spleens, as has been demonstrated in various taxa (see Ali and Behnke, 1985; John, 1994, 1995; Molyneux et al., 1983; Morand and Poulin, 2000; Vincent and Ash, 1978; Watkins et al., 1991), the immunosuppression hypothesis would predict a positive relationship between testis size and spleen size in cliff swallows. However, we found little evidence that testis size and spleen size were correlated in cliff swallows, with neither the analyses based on all data nor any of those for each colony separately being statistically significant. Therefore it would be difficult to construe these results as support for the immunosuppression hypothesis. Moreover, some of the variation in spleen size is probably unrelated to levels of parasitism or immunological challenge and thus cannot reflect an effect of immunosuppression or testis size.

Could there be other costs of large testes? That males in smaller colonies have not undergone directional selection for increased testis size suggests that any advantages of large testes in small groups do not outweigh the costs. Potential costs could include the metabolic expense of seasonal testis enlargement and mass-related costs of carrying them around. We have no data to evaluate either of these possibilities, but birds in the largest colonies have total testis masses of approximately 2% of the average early-season body mass. This compares to 0.25% for males in the smallest colonies. If cliff swallow mass is optimized by flight considerations (see Freed, 1981; Norberg, 1981; Veasey et al., 1998), males in larger colonies with bigger testes may be forced to carry slightly smaller fat stores in order to not be too heavy for efficient flight. This, in turn, could be a severe disadvantage in periods of bad weather when food is scarce, such as the mortality event of 1996 (Brown and Brown, 1998).

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