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TESTICULAR VOLUME AND ASYMMETRY ARE AGE-DEPENDENT IN BLACK-THROATED BLUE WARBLERS (*DENDROICA CAERULESCENS*)

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Abstract.—Passerine birds are favored models for studies of sperm competition and extrapair paternity, yet the intraspecific chronology of testicular maturation and its empirical and theoretical consequences in avian mating systems have been largely ignored. I analyzed age-dependent variation in testicular morphology in 25 breeding populations of the Black-throated Blue Warbler (*Dendroica caerulescens*) distributed throughout its geographic range in eastern North America. Yearlings (first breeding season) had significantly smaller testes than older males (≥2 years). Latitude, altitude, and Julian date had negligible effects on testicular morphology when effects of core body size were controlled. Preparator effects had significant influence on the estimation of testicular volume and asymmetry. Contrary to Møller's hypothesis that the smaller testis compensates for deficiencies in the larger, the volumes of the left and right testes were positively correlated in both yearlings and older males. Older males exhibited a higher degree of directional asymmetry because of the disproportionate enlargement of the left testis. These data suggest that testicular morphology and reproductive capacities of yearling passerines may not be equivalent to those of older males. In a broader context, these findings demonstrate that age class should be factored into quantitative models of sperm competition in birds. *Received 22 August 2003, accepted 16 January 2004.*

Resumen.—Las aves paserinas son frecuentemente empleadas como modelos en estudios sobre competencia espermática y paternidad extra-pareja, pero la cronología intraespecífica de la maduración testicular y sus consecuencias empíricas y teóricas sobre los sistemas de apareamiento de las aves han sido en buena parte ignoradas. En este estudio analicé la variación dependiente de la edad en la morfología testicular en 25 poblaciones reproductivas de *Dendroica caerulescens* distribuidas a través de su rango geográfico en el este de Norte América. Los machos añales (en su primera temporada reproductiva) presentaron testículos significativamente más pequeños que los machos más viejos (de 2 años). La latitud, altitud y fecha juliana tuvieron efectos despreciables sobre la morfología testicular una vez que los efectos del tamaño corporal fueron controlados. Los efectos del preparador tuvieron una influencia significativa sobre la estimación del volumen y la asimetría testicular. En contraste con la hipótesis de Møller de que el testículo más pequeño compensa las deficiencias del más grande, el volumen del testículo izquierdo estuvo positivamente correlacionado con el del derecho tanto en los machos añales como en los más viejos. Los machos más viejos mostraron un mayor grado de asimetría direccional debido al agrandamiento desproporcionado del testículo izquierdo. Estos datos sugieren que la morfología testicular y la capacidad reproductiva de los paserinos añales podrían no ser equivalentes a las de machos de mayor edad. En un contexto más amplio, estos hallazgos demuestran que la clase de edad debe ser tenida en cuenta en los modelos cuantitativos de competencia espermática en las aves.

Despite the likely importance of testicular morphology in avian mating systems (Birkhead 1987; Møller 1988, 1991, 1994a, b; Westneat et al. 1990; Birkhead and Møller 1992; Briskie 1993), with few notable exceptions (Manning 1985, Sheldon 1994) investigators have largely ignored the effects of age-dependent variation in testis volume in studies of sperm competition and extrapair paternity. Yearling passerines are widely believed to be reproductively mature in terms of testicular size and production of sperm during their first potential breeding season (Rohwer et al. 1980, Rohwer and Butcher 1988,

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Pitcher and Stutchbury 1998). That assumption is based on the observation that many yearlings breed successfully and on the rationale that abbreviated longevity (Klimkiewicz et al. 1983, Klimkiewicz and Futcher 1989) and high mortality rates (Lack 1967, Dobson 1990) in migratory passerines result in intense selection to breed as early as possible (Wittenberger 1979, Studd and Robertson 1985). However, the few examinations of age-related variation in testicular morphology conducted thus far on free-living individuals indicate that yearlings (first breeding season) have smaller testes than older males (second or later breeding season) in both monogamous (Davis 1958, Hill 1994, Merilä and Sheldon 1999) and polygynous species (Wright and Wright 1944, Selander and Hauser 1965, Payne 1969, Foster 1987). Conclusions drawn from those studies are somewhat weakened by the relatively small number of specimens examined during the peak of the breeding season. Nevertheless, because testes size in birds is thought to be positively correlated with ejaculate volume and number of sperm per ejaculate (Møller 1988, 1991, 1994a; Birkhead and Møller 1992; Briskie 1993), those data have important demographic implications for theories of sperm competition and extrapair paternity.

A second testicular metric that may correlate with reproductive potency and phenotypic condition is directional asymmetry. Right and left testes are both functional in passerines, although the left is larger in most species (Friedmann 1927, Lake 1981, Rising 1996). Left-side dominance begins early in embryological development and persists into adulthood (Lake 1981). Møller (1994a) hypothesized that the right testis increases in size to compensate for reduced function of the left and suggested further that the degree of directional asymmetry in testicular size reflects male quality and covaries with the expression of secondary sexual traits. Neither hypothesis was supported by an experimental test on semi-domestic Zebra Finches (*Taeniopyga guttata*) (Birkhead et al. 1998) nor by a correlative analysis of plumage color and incidence of parasitaemia in European Greenfinch (*Carduelis chloris*) populations (Merilä and Sheldon 1999).

Finally, females may choose older males by means of age-specific phenotypic or behavioral characters (phenotype-linked fertility hypothesis), in part, because such traits are correlated with ejaculate quality (Sheldon 1994, Birkhead and Fletcher 1995), which in turn may correlate with testicular volume. If age-dependent phenotypic characters signal functional fertility, the fact that age-specific plumages in passerine birds of breeding age are taxonomically widespread (Cramp and Perrins 1994, Pyle 1997, Shirihai et al. 2001) suggests that age-dependent variation in testes volume may be a common phenomenon. The female preference for older and more experienced males (Burley 1981, Buitron 1983, Manning 1985) has also been interpreted as selection for "good genes" carried by males that have proven their viability by surviving (Hansen and Price 1995, Wetton et al. 1995). It is possible that factors underlying both the "good genes" and "phenotype-linked fertility" hypotheses come into play when females choose mates.

This article focuses on the testicular morphology of the Black-throated Blue Warbler (*Dendroica caerulescens*), one of the most intensively studied passerines in the New World (Holmes et al. 1992, Rodenhouse and Holmes 1992, Holmes 1994, Chamberlain et al. 1997, Graves 1997b, Sillett et al. 2000, Webster et al. 2001, Graves et al. 2002, Rubenstein et al. 2002). The Black-throated Blue Warbler, a primarily monogamous insectivore, breeds in cool deciduous or mixed deciduous–coniferous forests in eastern North America and winters in the Caribbean basin (Holmes 1994, Price et al. 1995, Chamberlain et al. 1997, Rubenstein et al. 2002). Yearlings arrive on the breeding grounds later than older males (Hubbard 1965), are less likely to be polygynous (Holmes et al. 1992), and have lower annual reproductive outputs than older males ((Holmes et al. 1992). I analyzed effects of age class and a suite of morphological and spatio-temporal covariates on the testicular morphology of the Black-throated Blue Warbler in 25 breeding populations distributed from Georgia to New Brunswick (11° of latitude) and from New Brunswick to Michigan (22° of longitude). The geographic and temporal scope of this study made manipulative field experiments unfeasible. However, deficiencies inherent to correlative hypothesis testing were somewhat offset by the availability of relatively large sample sizes that are rarely achievable in experimental studies of free-living or captive birds. I addressed three questions of fundamental importance to studies of sperm competition in birds: (1) Do breeding males exhibit age-dependent differences in testicular volume and asymmetry? (2) Does the volume of the right testis increase to compensate for size deficiencies in the left, as hypothesized by Møller (1994a)? (3) Does testicular morphology exhibit "year" effects? Finally, I discuss the influence of preparator effects on the estimation of testicular size from length–width measurements.

Methods

The Black-throated Blue Warbler is an ideal candidate for ecological and behavioral studies because it is common to abundant in preferred habitat (Holmes 1994, Graves 1997b, Haney et al. 2001) and because yearlings (first alternate plumage; molt terminology follows Pyle 1997) can be distinguished from older males (definitive alternate plumage) in their second or later breeding seasons by subtle differences in appearance in the hand and under field conditions (Dwight 1900, Holmes 1994, Graves 1997a). Yearlings in the study were identified in the hand by the olive-green (rather than blue) edges of their alulae and primary coverts. Data used here were obtained from specimens collected under state and national licenses for a broader study of population genetics, isotope ecology (Chamberlain et al. 1997, Graves et al. 2002, Rubenstein et al. 2002), geographic variation of plumage and skeletal morphology, and avian malaria.

Territorial males were collected by the author over a 15-year period from populations distributed throughout the core breeding range in eastern North America during a narrow three-week period (9–29 June) that corresponds to the peak of the breeding season (Fig. 1; Appendix). Specimens were packaged in multiple layers of insulating tissue paper and aluminum foil, frozen in liquid nitrogen within 30 min of death, and transported to the National Museum of Natural History, Smithsonian Institution, where they were prepared as study skins and partial skeletons. A detailed description of the field sampling protocol appears in Graves (1997b) and Graves et al. (2002). The breeding distribution is strongly affected by altitude, especially south of 43°N. Most population samples from the Appalachian Mountains (Appendix: localities 1, 2, 5–10, 12–14, 16–20) were collected well above the species' lower elevational limit, which ranges from ~475 m above sea level in Pennsylvania to 750 m above sea level in Georgia. Other Appalachian populations (localities 3, 4, 11, 15) were sampled along transects that spanned the local elevational range of the warbler. Elevation of breeding territories was measured with an altimeter calibrated 2–3× daily.

Of particular interest, year effects in testicular morphology were analyzed in population samples collected during eight consecutive breeding seasons (9–23 June) in the Big Santeetlah Creek watershed (locality 4), a heavily forested basin (680–1,689 m above sea level) in the southern Appalachian Mountains (Graves et al. 2002). The collection period at that location coincided with the overlap of the fledging period for the first brood and nest building and egg laying for the second brood. Annual standardized censuses indicated that the removal of males had no demonstrable effect on the census population (G. R. Graves unpubl. data).

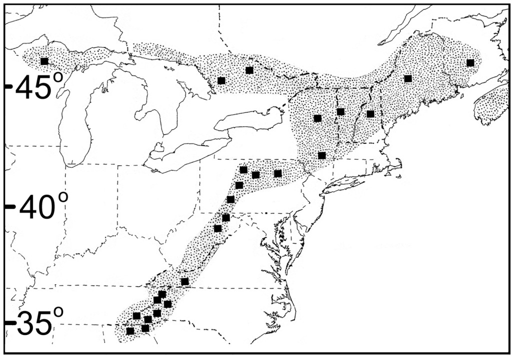

Fig. 1. Core breeding range of the Black-throated Blue Warbler (*Dendroica caerulescens*) in eastern North America. Population samples listed in the Appendix are represented by black squares.

Body mass, which is biased by gut contents and testicular mass, was measured to the nearest 0.1 g. I obtained unbiased estimates of core body size by calculating the geometric mean of keel length and sternum length from prepared skeletons (measured to the nearest 0.01 mm with digital calipers under a 10× stereo microscope). The sternum supports the principal flight muscles (pectoralis and supracoracoideus), which compose as much as 25% of lean body mass in wood warblers (Hartman 1961). Black-throated Blue Warblers apparently attain definitive skeletal dimensions within a few months of hatching because the core body size of yearling (x = 14.40 ± 0.47, n = 300) and older males (x = 14.42 ± 0.47, t = 0.51, P = 0.61, n = 542) was nearly identical.

Length and width of testes were measured to the nearest 0.5 mm. Data from testes punctured by shot were omitted. Measurements from left or right testis were available from 743 specimens (yearlings, n = 275; older males, n = 468). Complete bilateral testicular data were available for 588 specimens (yearlings, n = 225; older males, n = 377) prepared by a team of four skilled technicians (cumulative lifetime total of 10,000+ specimens) given the same preparation instructions. Multivariate analyses were limited to a subset of 521 specimens (complete testicular and skeletal data): J. Dean (n = 203 specimens), C. Dove (n = 122), B. Schmidt (n = 115), and C. Milensky (n = 81). I coded data by technician to test for preparator effects. Systematic bias in testis measurement can be introduced through rounding errors and observer differences in visual parallax.

I estimated testicular volume using the formula for a prolate spheroid (Hoyt 1979): testis volume = 0.51 $(\text{width})^2$ (length).

Although testis length is often used to estimate testis size in birds (e.g. Merilä and Sheldon 1999), length explained little more than half the variation in testicular volume in yearling (right testis: $r = 0.77$, $n = 252$; left testis: $r = 0.74$, $n = 248$) and older male (right testis: $r = 0.78$, $n = 416$; left testis: $r = 0.77$, $n = 429$) Black-throated Blue Warblers. Directional testicular asymmetry was defined as the volume of the left testis minus the volume of the right testis. Relative asymmetry (RA) was defined as

$$\text{RA} = \frac{\text{left-testis volume} - \text{right-testis volume}}{0.5\,(\text{left-testis volume} + \text{right-testis volume})}$$

I used analysis of covariance (ANCOVA) to investigate effects of categorical (age class, year, preparator) and continuous (latitude, altitude, Julian date, core body size) variables on testicular volume and asymmetry (GLM module of SYSTAT 1998). I used chi-square tests to assess differences in frequencies of yearling and older males with particular testicular asymmetries, *t*-tests to examine differences in mean character values, and Pearson correlation coefficients (r) to evaluate the correlation between pairs of variables.

Results

Testicular volume.—Yearling males had significantly smaller testes than older males (Table 1, Fig. 2). The percentage of individuals with relatively small testes (total volume <100 mm^3) was significantly higher in yearlings (14.2%) than in older males (6.6%). Nearly four-fifths (77.8%) of yearling warblers had a total testicular volume less than the median value (172.1 mm^3) observed in older males. Testis mass (mean specific density = 1.087 g cm^{-3}; Møller 1991) as a proportion of body mass was significantly lower in yearlings (0.017 ± 0.004, $n = 222$) than in older males (0.020 ± 0.006, $t = 7.94$, $P < 0.0001$, $n = 364$). Age class and preparator effects had significant influence on the variance in total testicular volume (Table 2). Core body size, latitude, altitude, and collection date (Julian date) had negligible effects on testicular volume.

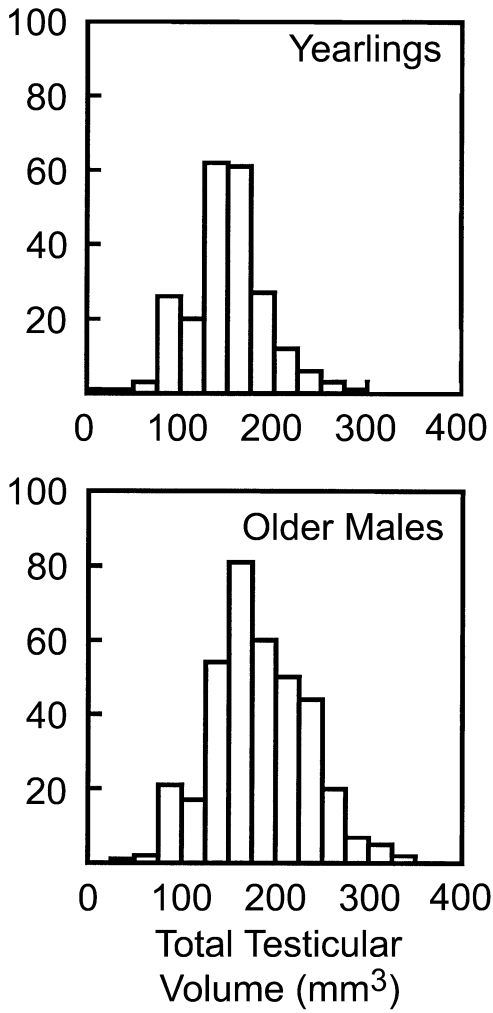

Fig. 2. Histograms of total testicular volume in yearling and older male (>2 years) Black-throated Blue Warblers. One outlier (423.9 mm^3) was omitted from the histogram for older males.

Table 1. Means and standard deviations (n = sample size) of testicular measurements of older male and yearling Black-throated Blue Warblers.

Characters	Older males	Yearlings	*t*-value	*P*
Volume, left testis	106.3 ± 38.4 (429)	82.1 ± 24.6 (248)	8.92	<0.0001
Volume, right testis	73.9 ± 26.1 (416)	65.5 ± 21.0 (252)	4.32	<0.0001
Total testicular volume	178.6 ± 51.5 (377)	146.8 ± 39.4 (225)	7.98	<0.0001
Directional asymmetry	33.9 ± 40.7 (377)	18.3 ± 24.2 (225)	5.23	<0.0001
Relative asymmetry	0.37 ± 0.40 (377)	0.25 ± 0.32 (225)	3.83	0.0001

Table 2. Independent analyses (ANCOVA) for total volume, directional asymmetry, and relative asymmetry of testes in 521 Black-throated Blue Warblers.

Dependent variable	Independent variables	df	MS	*F*-ratio	*P*
Total testicular volume	Age	1	119,201.49	64.58	<0.0001
(r^2 = 0.25)	Preparator	3	45,630.93	24.72	<0.0001
	Core body size	1	5,931.59	3.21	0.076
	Latitude (N°)	1	1,948.06	1.06	0.31
	Elevation (m) [a]	1	1,650.02	0.89	0.35
	Julian date	1	219.53	0.12	0.73
	Latitude × elevation	1	2,038.35	1.10	0.29
	Altitude × Julian date	1	1.76	0.00	0.98
	Error	510	1,846.69		
Directional asymmetry	Age	1	27,966.56	28.60	<0.001
(r^2 = 0.11)	Preparator	3	2069.04	2.12	0.097
	Core body size	1	5976.87	6.11	0.014
	Latitude (N°)	1	990.86	1.01	0.32
	Elevation (m) [a]	1	1,396.25	1.43	0.23
	Julian date	1	356.48	0.37	0.55
	Latitude × elevation	1	1993.04	2.04	0.15
	Altitude × Julian date	1	605.21	0.62	0.43
	Error	510	977.95		
Relative asymmetry	Age	1	1.65	12.89	<0.001
(r^2 = 0.08)	Preparator	3	0.36	2.82	0.038
	Core body size	1	0.60	4.71	0.030
	Latitude (N°)	1	0.11	0.84	0.36
	Elevation (m) [a]	1	0.11	0.83	0.36
	Julian date	1	0.12	0.96	0.33
	Latitude × elevation	1	0.21	1.64	0.20
	Altitude × Julian dates	1	0.12	0.93	0.34
	Error	510	0.13		

[a] Above sea level.

Testicular asymmetry.—The left testis was larger than the right in most yearlings ($L_{volume} > R_{volume}$, n = 180; $L_{volume} = R_{volume}$, n = 14; $R_{volume} > L_{volume}$, n = 29) and older males ($L_{volume} > R_{volume}$, n = 308; $L_{volume} = R_{volume}$, n = 18; $R_{volume} > L_{volume}$, n = 39). There was no difference in proportion of yearlings and older males with larger right testes (χ^2 = 1.32, df = 2, P > 0.50). Total testes volume averaged only slightly smaller when the right testis was larger in yearlings (142.9 vs. 147.2 mm^3) and older males (175.4 vs. 180.1 mm^3). Volumes of left and right testes were positively correlated in yearlings (r = 0.46, P < 0.0001, n = 225) and in older males (r = 0.25, P < 0.0001, n = 377). Older males exhibited a higher degree of directional asymmetry because of the disproportionate enlargement of the left testis (Table 1, Fig. 3). The volume of the left testis averaged 25.3% larger than the right in yearlings and 43.8% larger in older males. Age and core body size explained a small but significant amount of variance in directional asymmetry described by the general linear model (Table 2). In a bivariate analysis, core body size and directional asymmetry were uncorrelated in yearlings (r = 0.11, P = 0.13, n = 197) and marginally correlated in older males (r = 0.12, P = 0.027, n = 337). In other words, larger older males had more asymmetrical testes. Latitude, altitude, Julian date, and preparator effects had little effect on directional testicular asymmetry.

Relative testicular asymmetry was age dependent (Tables 1 and 2) and positively correlated with the volume of the left testis in yearlings (r = 0.43, P < 0.0001, n = 225) and in older males (r = 0.50, P < 0.0001, n = 377) (Fig. 4). However, relative testicular asymmetry was negatively correlated with the volume of the right testis in yearlings (r = –0.57, P < 0.0001, n = 225) and in

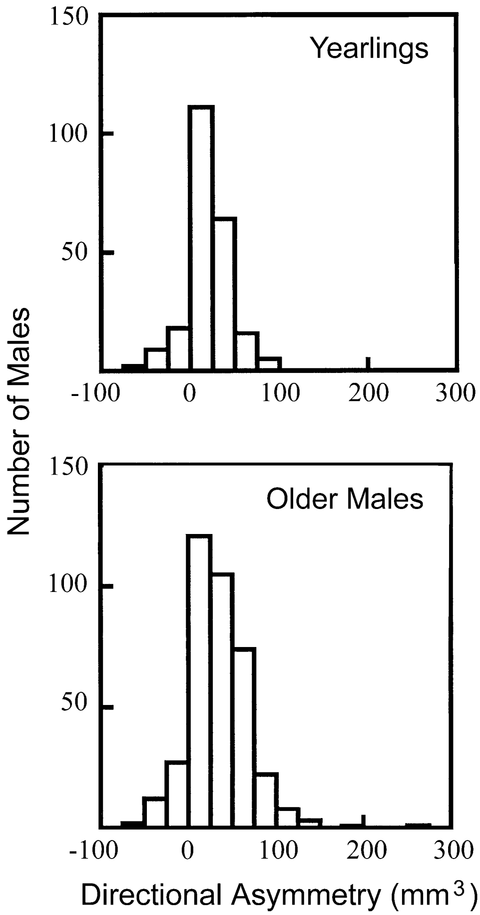

FIG. 3. Histograms of directional testicular asymmetry in yearling and older male (≥2 years) Black-throated Blue Warblers. One outlier (423.9 mm^3) was omitted from the histogram for older males.

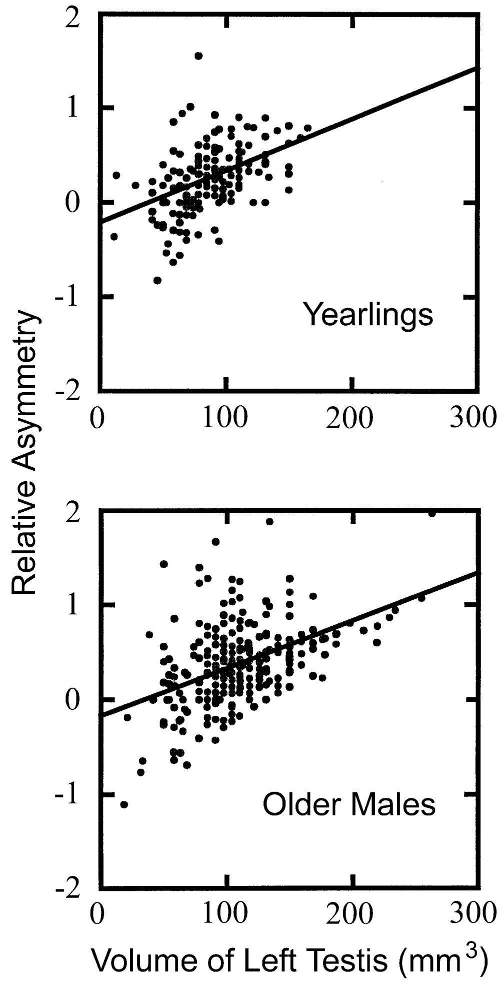

FIG. 4. Relationship of relative testicular asymmetry and volume of left testis in yearling and older male Black-throated Blue Warblers. Bivariate relationships are illustrated by least-square regression lines: yearlings (RA = –0.207 + 0.006 left volume) and older males (RA = –0.167 + 0.005 left volume).

older males ($r = -0.62$, $P < 0.0001$, $n = 377$) (Fig. 5). Core body size had marginal influence on relative asymmetry.

Year effects in testicular morphology.—Analysis of population samples from the Big Santeetlah Creek watershed (Appendix: locality 4; B. Schmidt, n = 108 specimens; C. Milensky, n = 72) revealed small but significant year effects in total testicular volume when the influence of other variables and interaction terms were controlled (Table 3). Relative testicular asymmetry showed marginal year effects.

DISCUSSION

The left testis averages larger than the right in North American passerines for which large numbers of specimens (≥50) have been examined (Wright and Wright 1944, Davis 1958, Selander and Hauser 1965, Rising 1987), although the opposite pattern (right > left) has been reported in populations of the European Greenfinch (Merilä and Sheldon 1999). In the Black-throated Blue Warbler, the left testis was larger than the right in 80.7% of yearlings and 84.4% of older males. The present study and two others (Davis 1958; Merilä and Sheldon 1999) indicated that older males exhibited a greater degree of testicular asymmetry than did

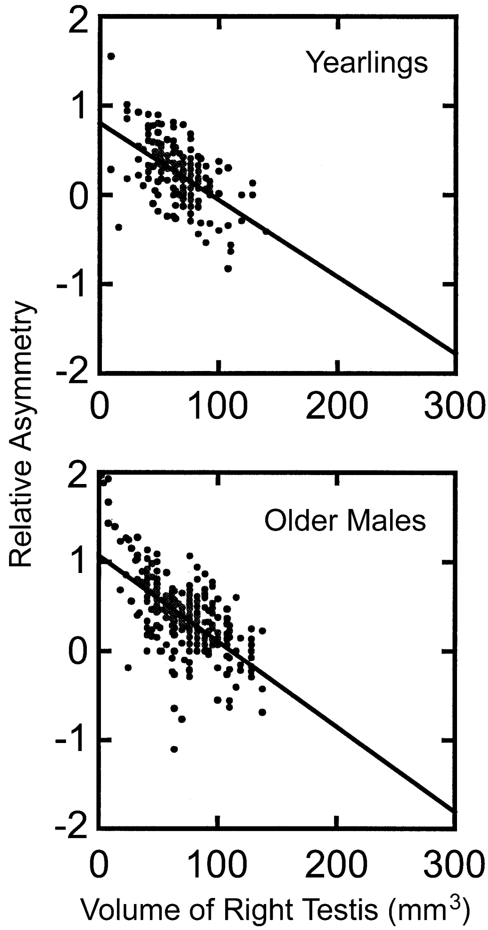

Fig. 5. Relationship of relative testicular asymmetry and volume of right testis in yearling and older male Black-throated Blue Warblers. Bivariate relationships are illustrated by least-square regression lines: yearlings (RA = 0.804 – 0.009 right volume) and older males (RA = 1.074 – 0.010 right volume).

yearlings. That pattern in the Black-throated Blue Warbler was due to the disproportionate enlargement of the left testis in older males. Møller (1994a) proposed that males in poor condition may be unable to develop large degrees of directional asymmetry and that deviations from asymmetry reflect developmental homeostasis. Møller (1994a) further hypothesized that the smaller testis increases in size to compensate for reduced function and size of the larger. Because both testes are very small and of similar size during the nonbreeding season in migratory songbirds, a consequence of that functional linkage is that the volumes of the left testis, in a fully-developed state, should exhibit an inverse relationship: if the left testis fails to enlarge, the right testis becomes larger. The testicular morphology of Black-throated Blue Warblers is inconsistent with the predictions of Møller's hypothesis. The volumes of the left and right testis exhibit a positive relationship in yearlings and older males. Moreover, relative testicular asymmetry was positively correlated with the volume of the left testis but negatively correlated with volume of the right testis. This indicates that the growth of the left testis usually outstrips that of the right and that the volume of the right testis was not a constant proportion of the volume of the left testis (cf. Birkhead et al. 1998). Finally, total testicular volume was smaller in yearlings and older males whose right testis was larger than the left.

Female choice in birds is almost certainly a multivariate cognitive process involving the discrimination of both phenotypic and behavioral cues (Trivers 1972, Burley 1981). In the Black-throated Blue Warbler, it can be inferred from field observations (Holmes et al. 1992, Holmes 1994) and genetic analyses (Webster et al. 2001) that at least some yearling males are capable of attracting females and siring young. However, if functional fertility is directly related to testicular volume (Møller 1988, 1991; Birkhead and Møller 1992; Birkhead et al. 1993; Sheldon 1994), yearlings may be less capable on average of effectuating frequent copulations (Birkhead et al. 1994) and perhaps less likely to fertilize eggs during extrapair copulations (Wetton et al. 1995). Several studies have shown that yearling passerines are more frequently cuckolded by older males than vice versa (Wetton et al. 1995, Richardson and Burke 1999, Møller and Ninni 1998). It follows then that the general female preference for older males observed in many avian species may partially reflect selection for large testes as signaled by the possession of definitive alternate plumage, a rephrasing of the phenotype-linked fertility hypothesis (see Sheldon 1994).

In the present study, nearly four-fifths (77.8%) of yearlings had testicular volumes smaller than the median value observed in older males. If large testis volume is advantageous, why do yearlings have smaller testes? Eight overlapping hypotheses appear most germane: (1) yearlings normally attain full reproductive maturity during the second breeding season;

TABLE 3. Independent analyses (ANCOVA) for total volume, directional asymmetry, and relative asymmetry of testes in 189 Black-throated Blue Warblers collected during eight consecutive breeding seasons in the Big Santeetlah Creek watershed (appendix, locality 4).

Dependent variable	Independent variables	df	MS	*F*-ratio	*P*
Total testicular volume	Age	1	36,976.03	28.60	<0.0001
(r^2 = 0.40)	Preparator	1	34,611.54	26.77	<0.0001
	Year	7	4,689.73	3.63	0.001
	Core body size	1	130.88	0.10	0.75
	Elevation (m) [a]	1	1,776.08	1.37	0.24
	Julian date	1	1,353.31	1.05	0.31
	Elevation × Julian date	1	1,953.69	1.51	0.22
	Preparator × year	7	3,073.48	2.38	0.024
	Error	168	1,292.91		
Directional asymmetry	Age	1	5,263.67	9.82	0.002
(r^2 = 0.22)	Preparator	1	440.35	0.82	0.37
	Year	7	1,125.20	2.10	0.046
	Core body size	1	27.15	0.05	0.82
	Elevation (m) [a]	1	494.96	0.92	0.34
	Julian date	1	651.81	1.22	0.27
	Elevation × Julian date	1	800.85	1.49	0.22
	Preparator × year	7	424.26	0.79	0.60
	Error	168	536.28		
Relative asymmetry	Age	1	0.40	5.65	0.019
(r^2 = 0.20)	Preparator	1	0.26	3.72	0.056
	Year	7	0.18	2.49	0.019
	Core body size	1	0.00	0.02	0.89
	Elevation (m) [a]	1	0.06	0.81	0.37
	Julian date	1	0.06	0.88	0.35
	Elevation × Julian date	1	0.09	1.24	0.27
	Preparator × year	7	0.09	1.31	0.25
	Error	168	0.07		

[a] Above sea level.

(2) testicular development of otherwise fit yearlings is directly suppressed by behavioral dominance of older males on the breeding grounds; (3) immunosuppressive effects of androgens secreted by testicular tissue select for small testis size in yearlings; (4) testis size is a true gauge of physiological health—smaller testes of yearlings reflect residual effects of poor-quality territories on their Caribbean wintering grounds; (5) yearling males with larger testes are more likely to survive long enough to become older males; (6) testicular morphology is affected by residual nutritional constraints experienced during the previous molt; (7) smaller testis size in yearlings is a correlative consequence of lower body mass; and (8) testis volume regresses earlier or increases later during the breeding season in yearlings than in older males.

The present study sheds light on the latter two hypotheses. First, testis volume was uncorrelated with core body size when other factors were statistically controlled. Moreover, testis mass as a proportion of body mass was significantly lower in yearlings than in older males. Consequently, the difference in testis size between yearlings and older males was not due to smaller body size of yearlings. Concerning the final hypothesis, there was no evidence that the testicular volume of yearlings or older males varied during the sampling period (9–29 June) or was influenced by the interactions between Julian date and altitude on a local scale or altitude and latitude on a regional scale. Thus, it seems unlikely that the observed differences in size and asymmetry were due to age-specific variation in the timing of testicular recrudescence or regression.

Two additional factors have been suggested to affect intraspecific variation in testis size. Rising (1987) found that polygynous populations of Savannah Sparrow (*Passerculus sandwichensis*) had slightly larger testes than those of monogamous populations, owing presumably to the greater demand for sperm in polygynous males. Although yearling Black-throated Blue Warblers are less likely to be polygynous than older males, at least in New Hampshire (Holmes et al. 1992), there seems to be no independent evidence to suggest that small testis size in yearlings is an adaptive consequence to reduced sperm demand.

Other investigators have suggested that the temporal compression of the breeding season at high latitudes promotes the evolution of large testis size because of the necessity to assure paternity in environments that permit the raising of but a single brood each year (Briskie 1992, Stutchbury and Morton 1995). Observation of latitudinal clines in testis size in several passerine species has been interpreted as evidence consistent with the temporal compression hypothesis (Pitcher and Stutchbury 1998). The Black-throated Blue Warbler appears to be double-brooded in the Appalachian Mountains northeast to Maine (Holmes et al. 1992, Holmes 1994, Graves 1997b, Harris and Reed 2002). Graves (1997b) suggested that populations breeding in regions with frost-free growing seasons less than ~95 days may be facultatively single-brooded. Growing seasons are frequently <100 days in several of the sampling locations (Appendix: localities 21, 22, 23, 25). Nonetheless, I found no evidence that testicular morphology varied geographically when the effects of age, core body size, and preparator were controlled. More generally, failure to discriminate yearlings from older males may prejudice studies of geographic variation in testis size, particularly in species that exhibit temporal (Sherry and Holmes 1991, Holmes et al. 1992) or geographic variation in age ratios (Rohwer et al. 1980, Graves 1997b, Rohwer 2004).

Year of collection (1995–2002) had a small but significant effect on total testicular volume in the Big Santeetlah Creek population. The cause of year-to-year fluctuations in testis size is unknown, but spring temperature and its influence on leafing phenology may subtly advance or retard the initiation of nesting (Marshall 1961, Crick et al. 1997, Winkel and Hudde 1997, McCleery and Perrins 1998, Brown et al. 1999, Dunn and Winkler 1999), resulting in concomitant changes in testicular volume.

Preparator effects are often overlooked in studies of testicular morphology. These analyses demonstrated that subtle differences in measurement techniques have a significant influence on the estimation of testicular volume and asymmetry. The influence of that factor in intraspecific (Pitcher and Stutchbury 1998) and interspecific analyses (Møller 1988, 1991; Briskie 1993; Stutchbury and Morton 1995; Dunn et al. 2001) has not been investigated, but my findings cast doubt on the widespread practice of pooling "length–width" data compiled by a number of specimen preparators. Preparator effect comes into play in specific situations, particularly in assaying patterns of intraspecific variation in testis size when data for population subsets are generated exclusively by different preparators. That bias will also affect interspecific studies that pool data from a number of species (each measured by a different observer), especially when testis size variation within the assemblage is modest. Ideally, testicular size should be computed directly from testicular mass (Silverin 1975; Hill 1994; Birkhead et al. 1997, 1998; Kimball et al. 1997) or from length–width measurements taken by a single preparator (Rising 1987, 1996).

Given the argued importance of testicular morphology in avian sperm competition, surprisingly little is known about the timing of testicular maturation in the 5,700+ described species of passerine birds. The pattern emerging from the analysis of Black-throated Blue Warblers and the handful of other applicable studies of species that breed at temperate latitudes (Wright and Wright 1944, Davis 1958, Selander and Hauser 1965, Payne 1969, Hill 1994, Merilä and Sheldon 1999) is that yearlings in their first potential breeding season have smaller testes than older males (≥2 years). The question of whether testis size continues to increase in size after the first year in resident tropical species (see Foster 1987) or in semi-domestic species, such as the Zebra Finch (Sossinka 1980), remains to be investigated. In any event, the aforementioned studies make it clear that sperm-producing capacities of yearling passerines may not be equivalent to those of older males. Correlative and experimental studies of sperm competition that fail to either directly measure testis volume or distinguish yearlings from older males by plumage characters, soft part colors, or other phenotypic criteria, may be systematically biased by age-dependent variation in testicular morphology.

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Appendix. Testicular data from breeding populations of Black-throated Blue Warblers.

State or province	Coordinates [a]		Collection dates	Older male (*n*)	Yearling (*n*)
(1) Georgia	34.75	84.03	10–11 June 1989	6	1
(2) South Carolina	35.00	83.10	10–12 June 1989	3	1
(3) North Carolina	35.13	83.70	14–17 June 1995	14	1
(4) North Carolina	35.33	84.02	18–23 June 1995	34	4
			13–20 June 1996	33	8
			11–19 June 1997	27	7
			10–18 June 1998	20	8
			10–16 June 1999	23	16
			10–18 June 2000	25	19
			9–17 June 2001	21	23
			11–18 June 2002	24	20
(5) North Carolina	35.40	82.66	17–18 June 1989	4	2
(6) North Carolina	35.71	82.20	25–27 June 1989	6	1
(7) Tennessee	36.13	82.33	19–20 June 1989	7	0
(8) Tennessee	36.20	82.13	22–23 June 1989	6	0
(9) Virginia	36.72	81.58	9–10 June 1988	4	0
(10) West Virginia	39.13	79.30	12–13 June 1993	14	9
(11) Maryland	39.85	79.18	14–15 June 1993	19	6
(12) Pennsylvania	40.23	78.67	16–17 June 1993	21	2
(13) Pennsylvania	40.83	78.13	13–18 June 1992/93	5	21
(14) Pennsylvania	41.32	76.33	9–12 June 1994	15	1
(15) Pennsylvania	41.37	77.57	13–14 June 1994	13	1
(16) Pennsylvania	41.75	77.75	15–17 June 1992	5	15
(17) New York	41.92	74.50	18–20 June 1992	11	9
(18) New York	43.70	74.72	23–25 June 1992	10	14
(19) Vermont	43.98	73.00	21–22 June 1992	8	16
(20) New Hampshire	44.00	71.32	27–28 June 1991	16	7
(21) Ontario	45.37	79.58	22–24 June 1990	21	14
(22) Maine	45.48	69.52	23–25 June 1991	7	14
(23) Ontario	45.67	77.50	24–29 June 1990	22	18
(24) New Brunswick	45.83	67.00	15–17 June 1991	13	7
(25) Michigan	46.37	88.93	12–14 June 1990	11	10

[a] Coordinates converted to decimals (i.e. 36°20′ = 36.33).