Latitudinal variation in testis size in six species of North American songbirds

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Abstract: Six songbird species were studied to determine how testis size varies in relation to latitude, using data from breeding areas at latitudes 7°N to 69°N. Three of the species (*Carpodacus mexicanus*, *Vireo olivaceus*, *Geothylpis trichas*) exhibited a significant positive relationship, one (*Passerculus sandwichensis*) a significant negative relationship, and two (*Agelaius phoeniceus*, *Spizella passerina*) no significant association between relative testis mass and latitude. These results suggest that sperm competition may also vary latitudinally, likely in response to geographic variation in breeding season length, extrapair mating intensity, and social mating system.

Résumé : Six espèces d'oiseaux chanteurs ont été étudiées dans des territoires de reproduction entre les latitudes 7°N et 69°N pour déterminer la relation entre la taille des testicules et la latitude. Chez trois des espèces (*Carpodacus mexicanus*, *Vireo olivaceus*, *Geothylpis trichas*), il y a une relation positive significative entre la masse relative des testicules et la latitude, chez une autre espèce (*Passerculus sandwichensis*), la relation est négative et significative et chez deux autres (*Agelaius phoeniceus*, *Spizella passerina*), il n'y a pas de relation entre les deux variables. Ces résultats semblent indiquer que la compétition entre les spermatozoïdes peut également varier selon la latitude, probablement en réaction à la variation géographique de la durée de la saison de reproduction, de l'intensité des appariements hors couple et du système social d'appariement.

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Introduction

Avian mating system theory has undergone a revolution in the last decade because of an increased focus on the role of extrapair fertilizations and the advent of DNA fingerprinting. Increasingly, a great deal of attention has been focused on the way in which sperm competition affects behaviour, ecology, and morphology (Birkhead 1988; Westneat et al. 1990; Wagner 1992; Birkhead et al. 1993). Testis size and its relation to sperm competition (Short 1979; Harcourt et al. 1981; Birkhead and Møller 1992; Hosken 1997), mating system (Kenagy and Trombulak 1986; Kusano et al. 1991; Briskie 1992, 1993; Rising 1996; Kappeler 1997), extrapair paternity (Mulder and Cockburn 1993; Moller and Briskie 1995), asymmetry (Møller 1994; Kimball et al. 1997), and body size (Cartar 1985; Kenagy and Trombulak 1986; Olsen 1991; Dahl et al. 1993; Rising 1996) have been thoroughly examined. However, little is known about geographic variation in testis size (see Rising 1987). Some authors predict intraspecific geographic variation in testis size (Stutchbury and Morton 1995; Morton 1996), while others make the assumption that there is no geographic variation in testis size (Møller and Briskie 1995). Our primary objective is to describe major geographical patterns in testis sizes in six species of songbirds collected over a broad latitudinal range.

Latitude is hypothesized to have an effect on mating systems because of the dramatic difference in breeding-season length between the temperate and tropical zones (Murton and Westwood 1977; Weatherhead 1979; Morton 1996). Temperate-zone populations of songbirds are constrained to breed in a relatively short period of time, whereas tropical populations generally have an extremely long breeding season. For example, the breeding season for Great Tits (*Parus major*) lasts approximately 7 months at latitude 8°N, but only 2 weeks at 50°N (Murton and Westwood 1977). Compression of breeding activities into a short period results in modifications of adult mating behaviour (Carey 1986) and morphological adaptations such as unusually large testis (Briskie 1992; Stutchbury and Morton 1995). A short breeding season at extreme northern latitudes generally permits the raising of only one brood per year (Weatherhead 1979; Briskie 1992). This suggests that at northern latitudes, competition between males for paternity (i.e., sperm competition) is likely very intense, to avoid having to provide significant amounts of parental care at their one and only nest per year to another male's offspring (i.e., extrapair young) (Briskie 1992; Stutchbury and Morton 1995). Testis mass is strongly correlated with the intensity of sperm competition (Birkhead and Møller 1992; Møller and Briskie 1995), so it is expected that testis size will vary geographically.

Methods

Testis sizes, body masses, and localities of songbirds were obtained from museum specimen tags and data bases from 10 museum collections in Canada and the United States: Royal Ontario Museum (Toronto), Canadian Museum of Nature (Ottawa), Smithsonian Institution (Washington, D.C.), Field Museum of Natural History (Chicago), Cornell University Museum (Ithaca), Slater Museum of Natural History (Tacoma), The Burke Museum (Seattle), Carnegie Museum of Natural History (Pittsburgh), University of Kansas Museum of Natural History (Lawrence), and the Centennial Museum of Natural History (El Paso). Testis sizes of adult males were collected for six species, the House Finch (*Carpodacus mexicanus*), Common

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Yellowthroat (*Geothylpis trichas*), Red-eyed Vireo (*Vireo olivaceus*), Savannah Sparrow (*Passerculus sandwichensis*), Chipping Sparrow (*Spizella passerina*), and Red-winged Blackbird (*Agelaius phoeniceus*). The species were chosen on the basis of their broad geographical breeding range and the availability of testis sizes from a large number of specimens from a diversity of latitudes.

From each specimen examined we recorded testis length and width (to the nearest 0.1 mm), body mass (to the nearest 0.1 g), latitude (to the nearest degree), date of collection, and any comments regarding breeding condition. Because of extreme seasonal variation in testis size (Murton and Westwood 1977; Wingfield 1984), this was recorded for (*i*) adult specimens whose breeding status was indicated on the specimen tag and (*ii*) specimens collected during the period from May to July, which is generally the core of the breeding season for the species of interest (see Peck and James 1983; Brauning 1992; Wheelright and Rising 1993; Yasukawa and Searcy 1995; Hill 1993; Middleton 1998), in order to exclude data collected in the nonbreeding season. Testis length and width data were converted to testis mass using the corrected formula from Møller (1991). To control for potential confounding effects of body size (Cartar 1985; Kenagy and Trombulak 1986), relative testis mass (RTM), defined as testis mass divided by body mass, was used for all analyses (see Bercovitch and Rodriguez 1993; Hill 1994; Stutchbury and Morton 1995).

To assess geographic variation in testis size, Spearman's rank correlation was used, correlating mean RTM and latitude (Sokal and Rohlf 1995). We examined an average of approximately 2500 specimens of each species, but because of incomplete information, fewer than 10% of these were useful to us. The most frequent grounds for inadmissibility of data were (*i*) lack of gonad measurements and (*ii*) lack of body mass information. The necessary data were available for an average of 212 specimens per species (range 71–593). Samples were often large for a given latitude ($\bar{x} = 16$ specimens per degree of latitude; range 3–61), and often represented individuals from the same population. For example, some populations of Savannah Sparrows that represented a single degree of latitude had upwards of 60 specimens. All specimens from a particular degree of latitude were grouped and the mean RTM for each latitude was used in the correlation analysis. The sample sizes in the correlations are thus the number of degrees of latitude for which the mean RTM could be calculated. For most degrees of latitude there were more than 10 specimens, but the southern latitudes (<15°) were underrepresented, owing to a lack of specimens collected from these latitudes. The standard error of the mean (± SE) was calculated for all results.

Results

For 144 House Finches examined over a broad latitudinal range, all mean RTM values fell within the range 0.0009–0.0086. The relationship between RTM and latitude was significantly positive ($r_s = 0.72$, $P = 0.04$, $n = 8$; Fig. 1*a*). Similar results were found for the Common Yellowthroat ($r_s = 0.70$, $P = 0.03$, $n = 8$; Fig. 1*b*) and Red-eyed Vireo ($r_s = 0.45$, $P = 0.05$, $n = 19$; Fig. 1*c*).

A significant negative association between testis size and latitude was found for the Savannah Sparrow ($r_s = -0.56$, $P = 0.004$, $n = 24$; Fig. 1*d*). No significant association was found between mean RTM and latitude for the Chipping Sparrow ($r_s = 0.19$, $P = 0.65$, $n = 8$; Fig. 1*e*) or Red-winged Blackbird ($r_s = 0.001$, $P = 0.94$, $n = 10$; Fig. 1*f*).

There was extensive variation in testis size at the same latitude, with standard errors ranging from 0.0009 to 0.007 (Fig. 1). The variation may have been due to fluctuations in testis size during the breeding season (Murton and Westwood 1977), error in measuring testis size, and differences in museum preparation protocols. Age is an unlikely contributor to the variation, as only adult specimens were examined. However, because it was impossible to differentiate between second year (SY) and after second year (ASY) specimens, we assumed that there were no differences between the groups (see Hill 1994). This would not likely have contributed to latitudinal variation in testis size, as it is extremely unlikely that there is any bias in the numbers of SY versus ASY individuals collected at different latitudes.

Discussion

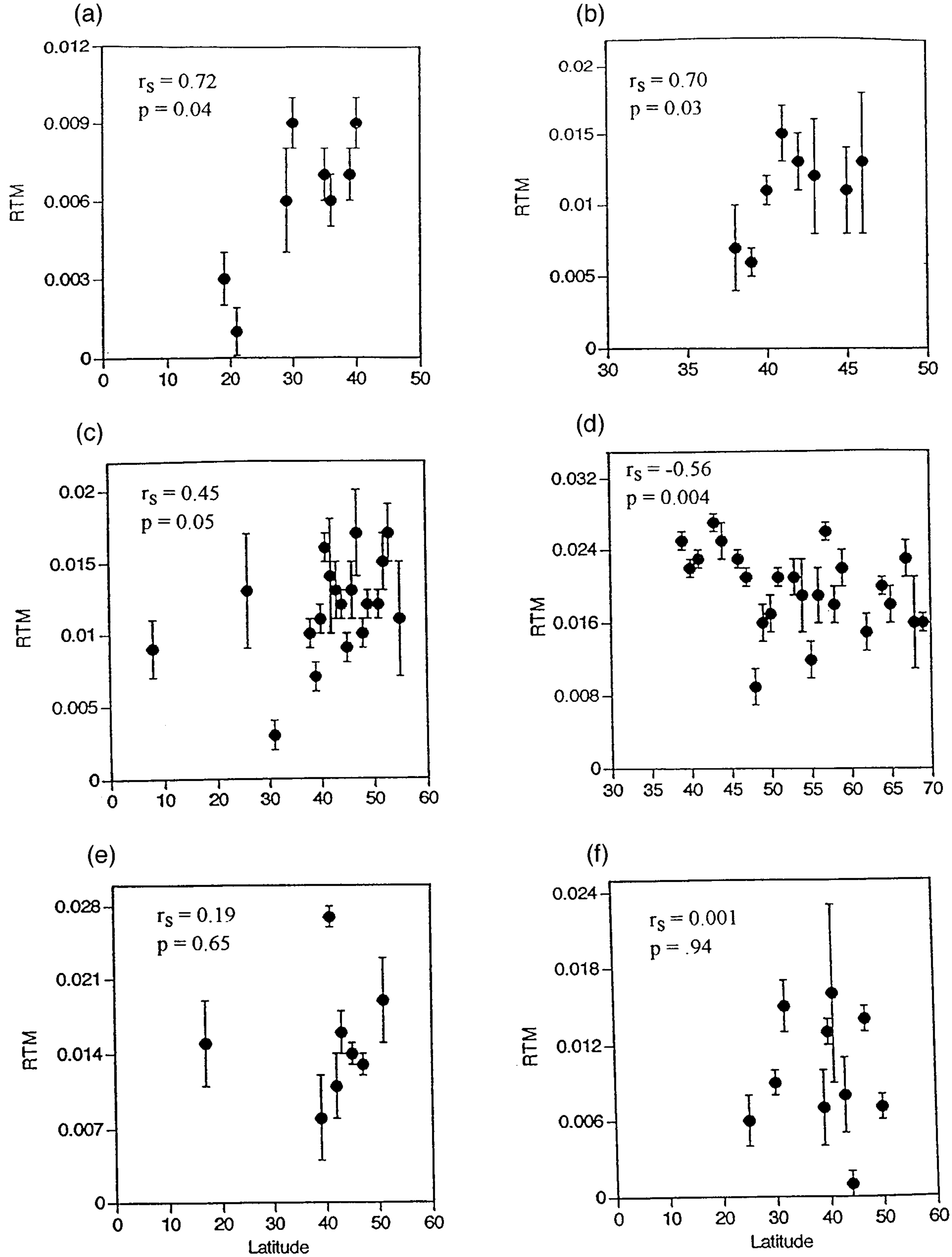
Songbirds demonstrate significant intraspecific geographic variation in testis size (Fig. 1), which suggests that sperm competition may also vary latitudinally. Among the six species examined, representing a wide range of ecological niches and life histories, there is considerable variation in the effects of latitude on testis size.

Three of the six species examined showed a significant positive relationship between testis size and latitude (Figs. 1*a*–1*c*). The increases in testis size at northern latitudes may be a direct adaptation to more intense sperm competition resulting from a short breeding season. More intense sperm competition during short breeding seasons is likely due to the increase in extrapair fertilizations associated with synchronous breeding (Stutchbury and Morton 1995; Stutchbury and Neudorf 1997). Larger testes are capable of producing more sperm in order that a bird may engage in the intense sperm competition expected in extrapair mating systems (Møller 1988; Birkhead and Møller 1992). Extrapair males can increase their own reproductive success via frequent extrapair copulations, requiring larger testes to meet the increased demand for sperm (Møller and Briskie 1995). Extrapair mating systems may also select for larger testes via an increase in the frequency of within-pair matings as a type of paternity guard (Birkhead et al. 1987). A complete test of this hypothesis requires DNA fingerprinting of populations from a diversity of latitudes.

Savannah Sparrows exhibited a significant negative relationship between testis size and latitude (Fig. 1*d*). This finding is similar to that reported by Rising (1987), who found that latitude was a better predictor of testis size per se than a variety of climatic measures at the collection sites. Geographic patterns in sperm competition are hard to predict because social mating systems vary among populations. Savannah Sparrows are known to be socially monogamous or polygynous depending on their geographic location, but to what extent is still in question (see Weatherhead 1979; Rising 1987, 1989; Weatherhead and Shutler 1989; Wheelwright and Rising 1993). Furthermore, extrapair mating systems occur in at least some, and probably most, populations of Savannah Sparrows (Freeman-Gallant 1996), so genetic mating systems may also affect geographic variation in testis size. Socially monogamous Savannah Sparrows have significantly higher extrapair fertilization rates than polygynous individuals (Freeman-Gallant 1997). The negative correlation between testis size and latitude in the Savannah Sparrow is likely due to the fact that the social mating system varies geographically, in contrast to the House Finch and Red-eyed Vireo, whose social mating system is uniform.

We did not find any relationship between latitude and testis size in Red-winged Blackbird and Chipping Sparrow populations,

Fig. 1. Relationship between latitude and relative testis mass (RTM) (mean ± SE) for six species of songbirds. Sample sizes (in parentheses) are the number of data points used in the correlation analysis followed by the number of specimens with appropriate data. (*a*) House finch, *Carpodacus mexicanus* ($n = 8$, 144). (*b*) Common Yellowthroat, *Geothylpis trichas* ($n = 8$, 155). (*c*) Red-eyed Vireo, *Vireo olivaceus* ($n = 19$, 212). (*d*) Savannah Sparrow, *Passerculus sandwichensis* ($n = 24$, 593). (*e*) Chipping Sparrow, *Spizella passerina* ($n = 8$, 71). (*f*) Red-winged Blackbird, *Agelaius phoeniceus* ($n = 10$, 94).



which suggests that many other ecological (i.e., longitude, altitude) and mating-system factors (i.e., double-broodedness) undoubtedly play important roles in determining optimal testis size. The mean size of Red-winged Blackbird harems varies among geographically distinct populations (Searcy and Yasukawa 1995), so this may be a case of sperm competition in a social mating system masking any relationship due to the genetic mating system. Although extrapair paternity appears similar in eastern and western populations, social mating is significantly more polygynous in western populations (Gray 1996; Beletsky 1996). Scrutinizing the relationship between longitude and testis size in Red-winged Blackbirds may reveal other interesting patterns and hypotheses.

Alternative hypothesis

Although five of the six trends shown in Fig. 1 appear to be positive (three significantly, two nonsignificantly), as would be expected if sperm competition was more prevalent at higher latitudes, this result could potentially be expected from the nature of the sampling we performed. At higher latitudes, where the breeding season is short, at any given time the number of birds with atrophying testes following reproduction will be small. In contrast, populations with a longer breeding season (at southern latitudes) will consist of a more heterogeneous group of birds, some of which are breeding (with large testes) and some have not yet begun to invest in large testes or have finished breeding for the season. Therefore, it is possible that as latitude decreases, so will the number of nonbreeding individuals in the sample, reducing the mean testis size. However, this alternative hypothesis is unlikely to account for our data set, for numerous reasons. First of all, testis-size sampling was limited to those specimens known to be in breeding condition at the time of collection (via specimen-tag descriptions, especially common on Savannah Sparrows and Red-winged Blackbirds) and those collected within the appropriate dates during the breeding season. Secondly, to test the alternative hypothesis we examined the coefficients of variation, which are used extensively when comparing the variation among different populations for the same character (i.e., testis size) (see Sokal and Rohlf 1995). There was no relationship between the coefficients of variation of testis mass and latitude for any of the six species examined (Spearman's rank correlation, $P > 0.05$), contrary to what is predicted by the alternative hypothesis.

Conclusion

It is essential that geographic variation be considered when examining the evolution of testis size, sperm competition, and extrapair mating systems. This study demonstrates that there is a significant amount of intraspecific geographic variation in testis size and, hence, sperm competition. An "arms race" has likely evolved in numerous species, which has led to larger testes in birds living at northern latitudes, where breeding seasons are extremely short and the levels of extrapair activity are consistently high (Stutchbury and Morton 1995). Within-species variation in sperm competition as a result of latitudinal varriation could therefore be an important tool for understanding the evolution of avian sperm competition and extrapair mating systems.

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