

Androgens, interspecific competition and species replacement in hybridizing warblers[†]

Noah T. Owen-Ashley^{1*} and Luke K. Butler^{1,2}

¹Department of Biology, 24 Kincaid Hall, University of Washington, Seattle, WA 98195-1800, USA

²Burke Museum of Natural History, University of Washington, Seattle, WA 98105, USA

*Author for correspondence (nowenash@u.washington.edu).

Recd 17.03.04; Acptd 05.07.04; Published online 02.11.04

The steroid hormone testosterone regulates aggressive behaviour in many vertebrates and is important for territorial defence among males of the same species. However, its role in mediating interspecific competition, and ultimately species distributions, is unknown. We show that testosterone may influence the geographical replacement of one species by another. Townsend's warblers (*Dendroica townsendi*) have replaced hermit warblers (*D. occidentalis*) over a vast portion of their historical range, partly because Townsend's males are more aggressive than hermit males and outcompete them for territories in areas of sympatry. We report differences in plasma androgen levels that parallel these aggressive asymmetries and the historical pattern of species replacement between Townsend's and hermits. Using hybrids, we provide evidence that these hormonal differences are partially genetically based and thus may have evolved through sexual selection during Pleistocene glacial maxima. Hormone-behaviour mechanisms can therefore have important effects on species distributions and can even influence the pathways underlying extinction.

Keywords: extinction; hybrid zone; hybridization; interspecific competition; testosterone; warbler

1. INTRODUCTION

The steroid hormone testosterone influences aggressive behaviour in males of many vertebrates, especially in relation to territoriality (Wingfield 1994). For many seasonally breeding birds, a rise in androgen levels during the territorial phase of the breeding period facilitates the persistence of aggression during territorial fights (Wingfield *et al.* 1987). Despite the role of testosterone in regulating territory density and population dynamics within species (e.g. Mougeot *et al.* 2003), its contribution to interspecific competition, and ultimately to species distributions, remains unexplored.

[†] Both authors contributed in equal part to this work.

In this study, we examined the role of androgens (testosterone and dihydrotestosterone) in mediating the geographical replacement of hermit warblers (*Dendroica occidentalis*) by Townsend's warblers (*D. townsendi*) in western North America. Over the past 5000 years, Townsend's warblers have replaced hermit warblers by genetic introgression in a series of southward-moving hybrid zones (Rohwer *et al.* 2001). An important factor influencing this replacement is the aggressive superiority of male Townsend's warblers over male hermit warblers (Pearson & Rohwer 2000). Townsend's males that take over hermit territories mate with hermit females and produce reproductively viable hybrid offspring.

Because elevated androgens are associated with territorial aggression in temperate-breeding birds, we tested the hypothesis that differences in plasma androgen levels parallel the asymmetries in aggression between Townsend's and hermit warblers. We predicted that Townsend's males would have higher androgen levels than hermit males, with hybrids producing intermediate levels. To test for a genetic contribution, we compared androgen levels across a range of hybrid phenotypes.

2. METHODS

In 2001 and 2002, we studied male hermit, Townsend's and hybrid warblers as they established territories on their breeding grounds in Washington State, USA. Parental species were captured outside the hybrid zones in two phenotypically pure breeding areas, and hybrids were sampled at the midpoint of the southern Cascades hybrid zone (Rohwer & Wood 1998).

Males were captured on their territories using mist-nets, song playback and a conspecific taxidermic mount (electronic Appendix A). Only the most aggressive birds of each phenotype were netted, but this bias favoured the less aggressive species (electronic Appendix A). We captured males only before females arrived in the area or before individual males were socially mated (electronic Appendix A). Males were captured between 1 and 34 min after the start of playback, and immediately removed from the mist-net. A 100–200 µl blood sample from the alar vein was taken within 10 min of capture. The blood was placed on ice and then centrifuged to separate the plasma within 6 h. Plasma was stored at –20 °C until transport to the University of Washington. Capture of warblers was performed between 07.00 and 13.00 Pacific Daylight Time. Most birds were collected to assess hybrid score accurately (see next paragraph) and donated to the Burke Museum of Natural History for another study.

Each collected warbler was scored for seven plumage characteristics following the method of Rohwer & Wood (1998). We used these seven scores to calculate a single 'hybrid score', which reflected the degree of backcrossing for each individual. Hybrid scores vary reliably from 0 to 0.24 for hermits, 0.25 to 0.75 for hybrids and 0.76 to 1.0 for Townsend's (Rohwer & Wood 1998).

Androgen levels were measured by radioimmunoassay (RIA) according to procedures by Wingfield & Farner (1975) except that column chromatography was not used. We did not separate testosterone from dihydrotestosterone. Although dihydrotestosterone had a 60% cross-reactivity with our T antiserum, it closely tracks testosterone secretion at much lower concentrations (less than 1 ng ml⁻¹) during the breeding season (Wingfield & Farner 1978). We used distilled dichloromethane for extractions. Intra-assay and inter-assay variations were 10.8% and 2.4%, respectively. The assay detection limit was ca. 0.1 ng ml⁻¹.

All RIAs were performed by N.T.O., all hybrid scoring was conducted by L.K.B., and data were combined without prior knowledge of the other results. Comparisons of plasma androgen levels among parental and hybrid warblers at different localities were performed using a two-way analysis of variance (ANOVA) with warbler type (hermit, Townsend's, or hybrid) and sampling year as factors. Relationships between plasma androgen level and hybrid score were assessed with analysis of covariance (ANCOVA) with sampling year as the factor and hybrid score as the covariate. Subsequent pooling of years led to simple linear regression with hybrid score as the independent variable and plasma androgen level as the dependent variable.

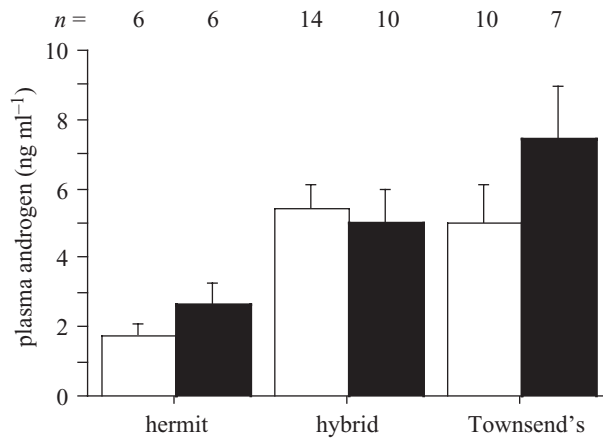


Figure 1. Plasma androgen levels (mean \pm s.e.) of male hermit, Townsend's and hybrid warblers (2001, open bars; 2002, filled bars). Sample sizes are shown above each bar.

Hormone data were logarithmically transformed (\log_{10}) to meet the requirements of normality and homogeneity of variances.

3. RESULTS

Time exposed to playback and mount before capture did not differ among the three warbler phenotypes (ANCOVA: $F_{2,44} = 0.45$, $p = 0.64$) nor covary with androgen levels (time exposed as the covariate: $F_{1,44} = 0.15$, $p = 0.70$; time exposed \times warbler type: $F_{2,44} = 0.71$, $p = 0.50$). Time of day had no effect on androgen levels ($p > 0.3$). Thus, we cannot attribute potential differences in androgen levels to our sampling technique.

Plasma androgen levels differed significantly among hermit, Townsend's and hybrid warblers but not between years (two-way ANOVA, warbler type: $F_{2,47} = 5.70$, $p = 0.006$; year effect: $F_{1,47} = 1.53$, $p = 0.22$; warbler type \times year interaction: $F_{2,47} = 1.33$, $p = 0.28$; figure 1). Comparisons among the three warbler types revealed that androgen levels were significantly higher in male Townsend's warblers than in male hermit warblers (Fisher's protected least significant difference test: $p = 0.0006$; figure 1). Hybrid males had significantly higher androgen levels than hermit males ($p = 0.002$), but hybrid androgen levels were not significantly lower than Townsend's levels ($p = 0.41$; figure 1). However, we cannot rule out the possibility that species differences in androgen levels were driven by environmental or social differences between localities.

To test the hypothesis that differences in androgen levels had a genetic and not solely an environmental basis, we compared androgen levels across a phenotypic range of hybrids from one locality in the hybrid zone, thus controlling for environmental differences between sampling localities. The degree of backcrossing (hybrid score) varied significantly with androgen levels (ANCOVA: $F_{1,18} = 15.40$, $p = 0.001$), suggesting that differences in androgen levels are inherited. Because neither the main effect (year: $F_{1,18} = 1.31$, $p = 0.28$) nor the interaction term (year \times hybrid score: $F_{1,18} = 0.57$, $p = 0.46$) was significant, we pooled data from both years and conducted a simple linear regression. There was a significant positive relationship between androgen level and hybrid score ($r^2 = 0.34$, $p = 0.002$; figure 2).

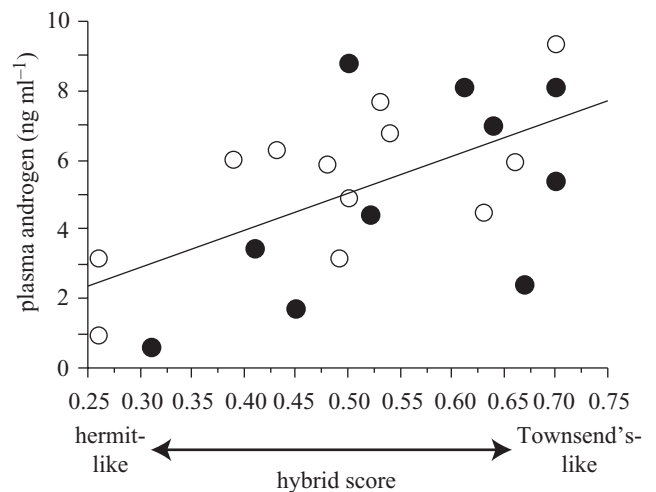


Figure 2. Plasma androgen varies with the degree of backcrossing in male hybrid warblers ($n = 22$) captured within the Washington Cascades hybrid zone (2001, open circles; 2002, filled circles).

4. DISCUSSION

As in most field endocrinology studies, we could not distinguish between baseline and socially elevated levels of androgen because it was impossible to ascertain whether territorial males were challenged by other males before capture. Androgen levels did not increase in relation to the amount of time males were experimentally challenged, indicating that levels were not confounded by our sampling method. Some birds may have been previously challenged, or male warblers may require more than 30 min of territorial intrusion to elevate androgen levels.

Plasma androgen levels of male Townsend's were higher than those of hermit males, corresponding to higher aggression in male Townsend's than male hermits (Pearson & Rohwer 2000). Just as hybrids are intermediate in aggressive behaviour compared to parentals (Pearson & Rohwer 2000), androgen levels of hybrids were significantly higher than hermit androgen levels, as predicted, and hybrid levels were lower, but not significantly lower, than Townsend's levels. However, these comparisons did not account for possible differences in density-dependent competition among the sampling localities.

To control for possible locality effects, we sampled a range of hybrids from one area in the hybrid zone. Heritability estimates of plasma testosterone levels are high ($h^2 > 0.40$) in human twin studies (Meikle *et al.* 1987; Harris *et al.* 1998), implying that genetic factors can contribute substantially to the variation in androgen profiles. Similarly, in this study, androgen level varied significantly with the degree of backcrossing in hybrids, suggesting that differences in androgen levels between hermit and Townsend's warblers may be partially genetically based. Alternatively, the relationship between hybrid score and androgen levels within the hybrid zone could be caused solely by social effects. According to this hypothesis, Townsend's-like hybrids (hybrid score greater than 0.5) would initiate more territorial encounters, or experience higher intrusion rates, than hermit-like hybrids, leading to elevated androgen levels. However, this conflicts with the findings that, within the Washington Cascades hybrid zone, (i) males do not respond more aggressively to pure

Townsend's mounts than to pure hermit mounts and (ii) hybrid score is not a strong overall predictor of individual male aggression (Pearson & Rohwer 2000). The latter finding is thought to be the result of males sorting into 'neighbourhoods' inside the hybrid zone.

We found that androgen levels were related to hybrid score. However, the lack of a relationship between hybrid score and aggression (Pearson & Rohwer 2000) might appear to contradict our suggestion that hormonal differences may underlie species differences in aggression. Importantly, the relationship between androgen levels and aggression within individuals is sufficiently labile that non-androgenic effects on aggression could easily obscure hormone-behaviour associations (Wingfield 1994). Androgens and social effects (e.g. intrusion rates), and their interactions, contribute to the expression of territorial aggression and the outcome of male-male competition (Wingfield 1994). Although high levels of circulating androgen can increase the likelihood that an aggressive behaviour will be initiated and maintained under the appropriate social stimuli, androgens do not cause aggression to occur *per se* (Wingfield *et al.* 1987). Thus, these complex interrelationships may explain why androgens, but not aggression (Pearson & Rohwer 2000), varied with the degree of backcrossing in hybrids.

This was a correlational study. Thus, experiments using testosterone implants, prolonged social challenges and plumage manipulations are required to understand the precise relationship between androgens and aggression in these species. In addition, relationships between androgen levels and individual genotypes would substantiate our conclusion that interspecific differences in androgen levels have a genetic component. Finally, female mate choice for Townsend's males is also an important factor for movement of the hybrid zone (Pearson 2000), and further study is warranted to examine whether females are choosing males according to androgen-dependent traits.

What are the historical selective pressures mediating these genetically based differences in androgen levels between species? It is suggested that during Pleistocene glacial maxima Townsend's warblers were sequestered in small isolated refugia in the Rocky Mountains, while hermit warblers inhabited larger refugia on the Pacific coast (Rohwer *et al.* 2001). Assuming similar effective population sizes between species during this time, competition for breeding habitat was greater for Townsend's than for hermits. Thus, in the Townsend's refugia, extreme competition for territories may have favoured the most aggressive males. If the glacial refugia hypothesis is correct, our results suggest that selection for aggression in Townsend's warblers may have acted, in part, on genetic determinants of androgen levels. Unfortunately, because the hormonal profile of the common ancestor of hermit and Townsend's warblers is unknown, we cannot determine directly whether, after divergence, androgens were selected to increase in Townsend's, decrease in hermits or both.

These findings, to our knowledge, describe the first hormone-behaviour mechanism that could contribute to a historical shift in species distributions. The relatively high androgen levels and aggression in male Townsend's promote the displacement of male, but not female, hermits, facilitating hybridization and potentially leading to the extinction of hermits. Thus, sexually selected traits, such as hormone-mediated aggressive behaviour, may be among the more important factors determining the outcome of interspecific competition after secondary contact.

Acknowledgements

The authors thank S. Rohwer and J. Wingfield for providing logistical support, and J. Wingfield funded the hormone assays (NSF IBN no. 9905679). They thank D. Froehlich, Q. Hays, S. Rohwer and B. Zelano for assistance in the field and are indebted to L. Erckmann for help with radioimmunoassays, C. Wood for help with scoring specimens, and S. Pearson for locality information. The authors acknowledge S. Bensch, C. Filardi, D. Hasselquist, R. Huey, P. Loesche, S. Naeem, S. Rohwer, C. Smith, J. Wingfield and five anonymous reviewers for comments on the manuscript. The NSF, the Society of Integrative and Comparative Biology, and the Garrett Eddy Ornithological Endowment at the Burke Museum funded this research. This study was conducted under licences from the US Fish and Wildlife Department and the University of Washington Animal Care Committee (IACUC protocol no. 2614-08).

- Harris, J. A., Vernon, P. A. & Boomsma, D. I. 1998 The heritability of testosterone: a study of Dutch adolescent twins and their parents. *Behav. Genet.* **28**, 165–171.
- Meikle, A. W., Bishop, D. T., Stringham, J. D. & West, D. W. 1987 Quantitating genetic and nongenetic factors that determine plasma sex steroid variation in normal male twins. *Metabolism* **35**, 1090–1095.
- Mougeot, F., Redpath, S. M., Leckie, F. & Hudson, P. J. 2003 The effect of aggressiveness on the population dynamics of a territorial bird. *Nature* **421**, 737–739.
- Pearson, S. F. 2000 Behavioral asymmetries in a moving hybrid zone. *Behav. Ecol.* **11**, 84–92.
- Pearson, S. F. & Rohwer, S. 2000 Asymmetries in male aggression across an avian hybrid zone. *Behav. Ecol.* **11**, 93–101.
- Rohwer, S. & Wood, C. 1998 Three hybrid zones between hermit and Townsend's warblers in Washington and Oregon. *Auk* **115**, 284–310.
- Rohwer, S., Bermingham, E. & Wood, C. 2001 Plumage and mtDNA haplotype variation across a moving hybrid zone. *Evolution* **55**, 405–422.
- Wingfield, J. C. 1994 Communication in vertebrate aggression and reproduction: the role of hormones. In *The physiology of reproduction* (ed. E. Knobil & J. D. Neill), pp. 303–342. New York: Raven Press.
- Wingfield, J. C. & Farner, D. S. 1975 The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. *Steroids* **26**, 311–327.
- Wingfield, J. C. & Farner, D. S. 1978 The annual cycle of plasma irLH and steroid hormones in feral populations of the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Biol. Reprod.* **19**, 1046–1056.
- Wingfield, J. C., Ball, G. F., Dufty, A. M. J., Hegner, R. E. & Ramenofsky, M. 1987 Testosterone and aggression in birds. *Am. Sci.* **75**, 602–608.

Visit www.journals.royalsoc.ac.uk and navigate to this article through Biology Letters to see the accompanying electronic appendix.