2008, Revista Eletrônica Lato Sensu 3: 1-14; De Toni, D.C., and P.R.P. Hofmann 1995, Rev. Brasil. Biol. 55 (3): 347-350; De Toni, D.C., M. S. Gottschalk, J. Cordeiro, P.R.P. Hofmann, and V.L.S. Valente 2007, Neotrop. Entomol. 36: 356-375; Döge, J.S., V.L.S. Valente, and P.R.P. Hofmann 2008, Rev. Bras. Entomol. 52: 615-624; Ehrman, L., and J.R. Powell 1982, In: *The Genetics and Biology of* Drosophila, vol. 3b. (Ashburner, M., H.L. Carson, and J.N. Thompson, Jr., eds.). Academic Press, New York, pp. 345–384; Fundação SOS Mata Atlântica and Instituto Nacional de Pesquisas Espaciais 2010, Atlas dos Remanescentes Florestais da Mata Atlântica – Período 2005-2008– Relatório Final, Fundação SOS Mata Atlântica e Instituto Nacional de Pesquisas Espaciais, São Paulo; Fundação SOS Mata Atlântica 2013, Atlas dos remanescentes florestais da Mata Atlântica período 2011-2012. Relatório Técnico, São Paulo; Garcia, A.C.L., V.H. Valiati, M.S. Gottschalk, C. Rohde, and V.L.S. Valente 2008, Iheringia, Sér. Zool. 98: 329-338; Garcia, A.C.L., D.M.I.O. Silva, A.F. Monteiro, Amanda G.F. Oliveira, M.A. Montes, and C. Rohde 2014, Ann. Entomol. Soc. Am. 107(5): 975-982; Garcia, C.F., C.J.C. Hochmüller, V.L.S. Valente, and H. J. Schmitz 2012, Neotrop. Entomol. 41: 1-12; Gottschalk, M.S., L. Bizzo, J.S. Döge, M.S. Profes, P.R.P. Hofmann, and V.L.S. Valente 2009, Iheringia Ser. Zool. 99(4): 442-448; Gottschalk, M.S., P.R.P. Hofmann, and V.L.S. Valente 2008, Check List 4: 485-518; Klein, R.M., 1978, Mapa fitogeográfico do estado de Santa Catarina, Herbário Barbosa Rodrigues, Itajaí; Machado, I.C., A.V. Lopes, and K.C. Pôrto 1998, Reserva Ecológica de Dois Irmãos: Estudos em áreas de remanescentes de Mata Atlântica em áreas urbanas. Secretaria de Ciência, Tecnologia e Meio Ambiente, Editora Universitária-UFPE, Recife; Malogolowkin, C., 1952, Bras. J. Biol. 12(4): 455-457; Mata, R.A., M.A. Mcgeoch, and R. Tidon 2008, Biodivers. Conserv. 17: 2899–2916; Poppe, J.L., H.J. Schmitz, and V.L.S. Valente 2013, Neotrop. Entomol. 42: 269–277; Powell, J.R., 1997, *Progress and Prospects in Evolutionary Biology*, *The* Drosophila *Model* (Powell, J.R., ed.), New York, Oxford University Press; Roque, F., R.A. Mata, and R. Tidon 2013, Biodivers. Conserv. 22: 657-672; Tabarelli, M., L.P. Pinto, J.M.C. Silva, M. Hirota, and L. Bedê 2005, Conserv. Biol. 19 (3): 695–700; Tidon, R., and F.M. Sene 1988, Dros. Inf. Serv. 67: 89; Valadão, H., J.D.V. Hay, and R. Tidon 2010, International Journal of Ecology 2010: 1-7; Wheeler, M.R., and M.P. Kambysellis 1966, Univ. Texas Public. 6615: 533-565.

Lack of evidence for directional selection on *Sex combs reduced* gene in *Drosophila* species differing in sex comb morphology.

Mittleman, Briana E., Amanda B. Abrams, and Mohamed A.F. Noor. Biology Department, Duke University, Durham, NC, USA; bem29@duke.edu.

Introduction

Sex combs are secondary sex characteristics located on the first and second tarsals segments in males of some *Drosophila* species. They are characterized by a set of one or two modulated transverse bristle rows which point distally, and are oriented along the proximo-distal axis of the prothoracic legs. Sex combs can influence male mating success (*e.g.*, Ng and Kopp, 2008). While sex combs may have originally evolved for pushing female wings apart prior to copulation, the combs are now used for varying purposes across *Drosophila* subspecies (Spieth, 1952). For example, in the *obscura* group, sex combs aid in the abdominal attachment of the male to the female. When sex combs have been reduced, copulation rates have consequently dropped (Spieth, 1952).

Dramatic phenotypic variations exist in sex comb morphology between species of *Drosophila*, but the genetic underpinnings of this variation are still poorly understood (Graze *et al.*, 2007). In this study, we focus on three species in the *obscura* group of *Drosophila* differing in the number of sex comb rows per foreleg and number of teeth per comb. Figure 1 illustrates the variation in sex combs of the species examined here. *D. subobscura* males have large sex combs on both the first and second tarsal segments, with the combs lying perpendicular to the tarsus (Beckenbach and Prevosti, 1986). *D. pseudoobscura* have smaller sex combs on both segments than *D. subobscura* (Beckenbach and Prevosti, 1986). Finally, *D. azteca* has a still smaller sex

comb, bearing only 4-5 teeth (Sturtevant and Dobzhansky, 1936), with only a single comb on the tarsal section. Sizes of sex combs vary within species as well.

The *Sex combs reduced* (*Scr*) gene influences the development of sex combs (Kopp, 2011). Here, we examine DNA sequences of *Scr* to infer types of selection operating on the coding region using the ratio of nonsynonymous to synonymous differences within *vs*. between species. Our ultimate goal is to assess the genetic and evolutionary contribution *Scr* may have had on sex comb morphology in *D. subobscura*, *D. pseudoobscura*, and *D. azteca.*

Figure 1. The male sex combs of *D. azteca* (top), *D. pseudoobscura* (lower left), and *D. subobscura* (lower right).

Methods

Stocks:

D. azteca strains used were MSH4 and MSH7, collected from Mt. St. Helena (MSH), California in 2013 by A. Hish. *D. subobscura* strains used were MSH12, also collected from Mt. St. Helena, California (MSH) in 2013, and Seattle, Washington 6, collected in 2011. *Scr* sequences of 11 *D. pseudoobscura* strains were extracted from the genome sequences in McGaugh *et al.* (2012) and are available online in Pseudobase (http://pseudobase.biology.duke.edu/): refer to website for original stock/ collection details.

DNA isolation:

We isolated genomic DNA from *D. subobscura* and *D. azteca* using a standard fly squish protocol (Gloor and Engel, 1992). The *Scr* gene was amplified via PCR using primers (5' - CCTGCTATCCGCAGCAGATGAATC - 3') and (5' - CCAGGACTGTGCATCGGGAC – 3') in 25µl reaction, amplifying a region of approximately 850 bases from the largest exon. The sizes of the PCR products were confirmed on a 1% TBE agarose gel. Samples were purified using ExoSAP-IT (Affymetrix) reactions. The samples were sent to Eton Bioscience for sequencing and submitted to GenBank as accessions KM596822-KM596825.

Data Analysis:

Sample DNA sequences were aligned with the *Scr* sequences from *D. pseudosoobscura* from McGaugh *et al*. (2012) using the ClustalW program in BioEdit (Thompson *et al*., 1994). Analysis was performed using DNAsp (Rozas and Rozas, 1999). A McDonald Kreitman (1991) test and Ka/Ks calculation (Nei and Gojobori, 1986) were done to compare sequences of each pair of the three species and test for evidence of natural selection.

Results

Results of the McDonald Kreitman test are presented in Figure 2. No within-species nonsynonymous variation was detected in the region of *Scr* we examined, and only 2-3 nonsynonmous variants differentiated the species studied. No significant difference was observed in the nonsynonymous to synonymous ratio within

vs. between species. Similarly, Ka and the Ka/Ks ratio were very low (Table 1), indicating strong purifying selection but not showing any clear signal of positive selection on the coding regions of *Scr*.

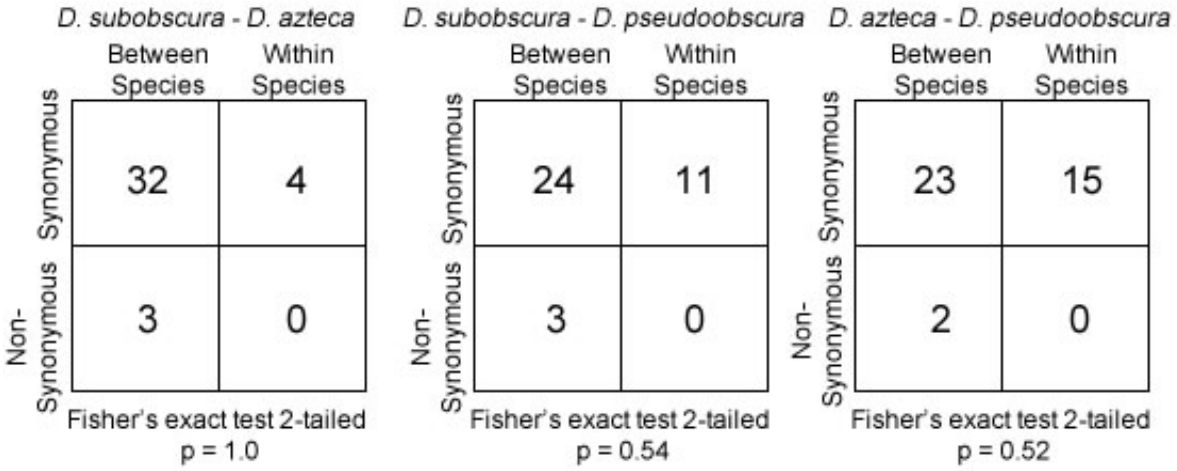

Figure 2. McDonald Kreitman test results.

Table 1. Estimates of Ka, Ks, and Ka/Ks in interspecies comparisons.

	D. subobscura – D. azteca	*D. subobscura – D. pseudoobscura*	*D. azteca – D. pseudoobscura*
Ka	0.0079	0.0087	0.0087
Ks	0.237	0.221	0.225
Ka/Ks	0.028	0.034	0.018

Discussion

While the three *Drosophila* species studied vary dramatically in sex comb morphology, our analysis indicates strong selective constraint but no statistically significant signal for positive selection acting on coding sequence variation in the first exon of the *Sex combs reduced* gene. Despite this result, our analyses cannot completely rule out three means by which *Scr* variation may still affect sex comb variation in these species: 1) via the small amount of nonsynonymous variation observed in our sequences, 2) via differences in the other (much smaller) exon of this gene, or 3) via noncoding regulatory differences among these species. Other genes have also been implicated in sex comb diversity either via correlations in expression (Kopp, 2011) or genetic mapping (Graze *et al.*, 2007). Future research can focus on these amongst other possibilities.

Acknowledgments: We thank B. Manzano-Winkler and J. Gredler for technical assistance. This research was conducted as part of undergraduate independent study projects of the first two authors.

References: Beckenbach, A.T., and A. Prevosti 1986, Am. Midl. Nat. 115: 10-18; Gloor, G.B., and W.R. Engels 1992, Dros. Inf. Serv. 71: 148-149; Graze, R.M., O. Barmina, D. Tufts, E. Naderi, K.L. Harmon, M. Persianinova, and S.V. Nuzhdin 2007, Genetics 176: 2561-2576; Kopp, A., 2011, Evol. Dev. 13: 504-522; McDonald, J.M., and M. Kreitman 1991, Nature 351: 652-654; McGaugh, S.E., C.S.S. Heil, B. Manzano-Winkler, L. Loewe, S. Goldstein, T.L. Himmel, and M.A.F Noor 2012, PLoS Biol. 10: e1001423; Nei, M., and T. Gojobori 1986, Mol. Biol. Evol. 3: 418-426; Ng, C.S., and A. Kopp 2008, Behav. Genet. 38: 195-201; Rozas, J., and R. Rozas 1999, Bioinform. 15: 174-175; Spieth, H.T., 1952, Bull. Am. Mus. Nat. Hist. 99: 394-474; Sturtevant, A.H., and T.H. Dobzhansky 1936, Am. Nat. 70: 574-584; Thompson, J.D., D.G. Higgins, and T.J. Gibson 1994, Nucleic Acid Res. 22: 4673-4680.