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Body size is not correlated with the evolution of male coloration in darters (Percidae: Etheostomatinae)

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Coloration and body size are among the many morphological traits that vary among fish lineages. Elaborate coloration and body size covary in other animal groups, but relationships between these two morphological characteristics have not been rigorously examined in fishes. We formally test for correlations between coloration and body size in darters (Percidae: Etheostomatinae), a group of North American freshwater fishes that vary in the presence of male coloration and maximum body size. Although uncorrected analyses indicate a significant correlation between colour traits and body size in darters, phylogenetically corrected logistic regression models and ANOVAs revealed no significant correlations, suggesting body size does not act as a constraint on elaborate coloration or vice versa. These results are discussed in an ecological and behavioural context.

ADDITIONAL KEYWORDS: ecological selection – *Etheostoma* – *Percina* – phylogenetic comparative method – sexual dichromatism – total length.

INTRODUCTION

Body size is hypothesized to play a role in the evolution of elaborate animal coloration. In animals that utilize visual signals as predator deterrents, as in aposematism, size is associated with conspicuous coloration. In some aposematic lineages, elaborate coloration or visual conspicuousness is positively associated with body size across species, potentially to increase detectability (i.e. Hagman & Forsman, 2003; Hossie *et al.*, 2015; Forthman & Weirauch, 2018; Winebarger *et al.*, 2018). In other aposematic lineages, however, these same visual signals are negatively associated with body size, with smaller species possessing elaborate patterns potentially due to size-linked variation in physiology and behaviour, such as a higher metabolic cost for elaborate coloration in larger animals and/or an increased potential of predation due to a smaller body size (i.e. Cheney *et al.*, 2014; Winebarger *et al.*, 2018).

Less explored is whether body size and visual signals are correlated in animal groups for which elaborate coloration is not attributed to aposematism. In birds that use coloration in sexual displays, for example, the relationship between body size and elaborate coloration varies across taxa. In a broad comparative analysis of 18 avian orders, Galván *et al.* (2013) found that smaller species are more colourful, suggesting that large body size is a constraint on coloration in birds. For example, elaborate coloration might be more costly for larger species due to the higher total amounts of carotenoids required. Alternatively, conspicuousness might be more important for smaller species because they are more difficult for conspecifics to detect across long distances (Galván *et al.*, 2013). In contrast, Dale *et al.* (2015) and Carballo *et al.* (2020) found that larger species are more elaborately coloured in passerines and parrots, respectively, potentially due to lower predation risk and thus relaxed selection for crypsis. Similar patterns have yet to be explored in other vertebrates, including fishes.

Darters (Percidae: Etheostomatinae) comprise a species-rich group of freshwater fishes distributed throughout North America, primarily in basins draining to the Atlantic Ocean seaboard and the Gulf of Mexico. Males of many darter species exhibit

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elaborate nuptial coloration during spring and summer spawning seasons (Page & Burr, 2011). Most of the variation in male coloration in darters is derived from two main categories of colour, inferred from reflectance spectrophotometry as: (1) red or orange (herein referred to as 'red'), characterized by a single step-shaped curve in the visual spectrum, and (2) blue or green (herein referred to as 'blue'), characterized by a single peak in the short wavelength component of the visual spectrum (Gumm *et al.*, 2011; Zhou *et al.*, 2014). In darters, these colours can be present on the body, where they are constantly exposed to potential receivers. These colours can also be present on the fins, which can be raised to signal to intended receivers (i.e. conspecifics) and lowered to conceal from unintended receivers (i.e. predators). Male coloration likely affects both female mate choice and male-male competition in darters (Williams & Mendelson, 2011, 2013; Williams *et al.*, 2013; Martin & Mendelson, 2016). The presence and absence of red and blue coloration in darters exhibits a strong phylogenetic signal and is strongly and positively correlated across body regions and fins (Ciccotto & Mendelson, 2017).

Previous work suggests alternative predictions with respect to the relationship between body size and coloration in darters. For example, some studies have revealed correlations between ecological features and the presence of male coloration. Chromatic species with red or blue coloration (or a combination of both) tend to inhabit microhabitats where predation rates are likely reduced (Bossu & Near, 2015; Ciccotto & Mendelson, 2016); whereas, achromatic darters tend to be found in microhabitats with greater predation pressure, suggesting predator-mediated ecological selection against coloration in darters. If predation disproportionately affects smaller individuals or species, for example, due to gape limitations of predators (Hambright, 1991; Christensen, 1996), then the presence of colour in darters should be positively correlated with body size. Smaller species, which would be at a greater risk of predation, should thus tend to be achromatic. Alternatively, the presence of colour might be negatively correlated with body size if elaborate coloration is physiologically costly for larger species and/or if conspicuousness is more important for smaller species for detection by conspecifics (e.g. Galván *et al.*, 2013). Natural history observations suggest that body size is negatively correlated with coloration in darters, as species of the genus *Percina* tend to be larger than other darter species and generally lack elaborate male coloration compared to species of the genus *Etheostoma* (Page & Swofford, 1984; Page & Burr, 2011). The general absence of elaborate coloration in *Percina* might be due to predation by larger predators. Many larger species of *Percina* do not live directly on the benthos, as do other darter genera, and thus might be exposed to larger predators in the water column (Bossu & Near, 2015). The purpose of this study is to test whether correlations between colour traits and body size exist in darters using phylogenetic comparative methods.

MATERIAL AND METHODS

Body size data were gathered from Page & Burr (2011), which reports the maximum size in total length (in centimetres) of 197 darter species. Colour data for 99 species were gathered from Ciccotto & Mendelson (2016), in which the presence of red and blue colours was scored across three body regions (head, flank and caudal peduncle) and three fins (first and second dorsal fins and anal fin). Additional colour data were collected for the remaining 98 species using the same approach as Ciccotto & Mendelson (2016). In brief, descriptions and illustrations from Page & Burr (2011) were used to score the presence of red and blue on each of the aforementioned regions of the body and fins, which were then verified using digital images from Google Image searches, personal observations and other published sources (Kuehne & Barbour, 1983; Etnier & Starnes, 1993; Jenkins & Burkhead 1993; Mettee *et al.*, 1996; Thomas *et al.*, 2007; Ross, 2001; Rohde *et al.*, 2009; Robins *et al.*, 2018). The presence of red and blue were scored separately instead of in aggregate because of differences in the spectral properties of these colours (see above) and in the underlying physiological mechanisms that led to their production. These differences may lead to different selection pressures associated with size. For example, red pigment, which is derived from carotenoids from the diet, may be limited by food availability as size increases, while blue pigment may not be. Additionally, we previously showed that darter predators attack red significantly more than blue decoys (Ciccotto & Mendelson, 2016), suggesting red is more likely to be disadvantageous in larger darters where it could be more easily detected by predators.

For the present analyses, colour traits were grouped separately into body and fins by colour category, yielding four colour traits: red on the body, blue on the body, red on the fins and blue on the fins. This grouping allowed us to test for broad correlations between the exposed body and concealable fins. Grouping into these two main regions (body and fins) is warranted as the presence and absence of colour is strongly correlated across the three body regions and similarly across the three fin regions [for example, a species with red on the flank is highly likely to have red on the head and caudal peduncle; see Ciccotto & Mendelson (2017)].

Correlations between the presence and absence of colour traits and body size were tested with phylogenetic logistic regression models fitted in MATLAB (release 2021a, MathWorks, Inc., Natick, MA, USA) using the methods of Ives & Garland (2010) and the framework of Ciccotto & Mendelson (2016). The variance-covariance matrix required for the phylogenetic logistic regression models was created in the PDAP package v.1.16 (Midford *et al.*, 2010) in Mesquite (Maddison & Maddison, 2021) from the 50% majority rule consensus tree generated from a Bayesian analysis of AFLP data (Smith *et al.*, 2014) that was ultrametricized using the nonparametric rate smooth option and weighting rate differences at all nodes with the mean for all post burn-in trees in TreeEdit v.1.0a10 (Sanderson, 1997; Rambaut & Charleston, 2002). Body size data were standardized to have a mean equal to 0 and standard deviation equal to 1 prior to fitting regression models. Statistical significance of body size as a predictor of colour presence was assessed with 2000 nonparametric bootstrap replicates in each analysis. This analysis was repeated with the 50% majority rule consensus tree of Near *et al.* (2011) retrieved from TreeBASE (Piel *et al.*, 2009) pruned to have all 197 species from Page & Burr (2011). For brevity, only results from the taxonomically denser phylogeny of Near *et al.* (2011) are reported as results did not vary depending on the phylogeny used. *P*-values were corrected using the false discovery rate control for multiple comparisons, with *P*-values pooled from all eight (four standard and four controlling for phylogenetic relatedness) of the logistic regression models (Benjamini & Hochberg, 1995).

An implicit assumption of the phylogenetic regression models is that body size evolves prior to coloration; accordingly, body size in these analyses is the independent variable while colour traits are modelled as dependent variables. An alternative hypothesis is that colour evolves prior to body size, i.e. the body size of colourful species evolves due to selection for smaller or larger body sizes. We therefore conducted ANOVAs with log-transformed body size as the dependent variable, rather than the independent variable, in R 4.0.1 (R Core Team, 2021). Phylogenetic ANOVAs, which account for phylogenetic relatedness, were also conducted, using the R package phytools with 10 000 simulations (Revell, 2012). *P*-values were corrected using the methods described above.

RESULTS

A total of 197 [of the approximately estimated 250; see Near *et al.* (2011)] species of darters were included in analyses, ranging in size from 4.3 to 20.0 cm TL (mean ± SD = 8.4 ± 2.8 cm). In general, larger species of darters tended to lack red on the body and blue on the body and fins compared to smaller species (Fig. 1). Results of the standard logistic regression models

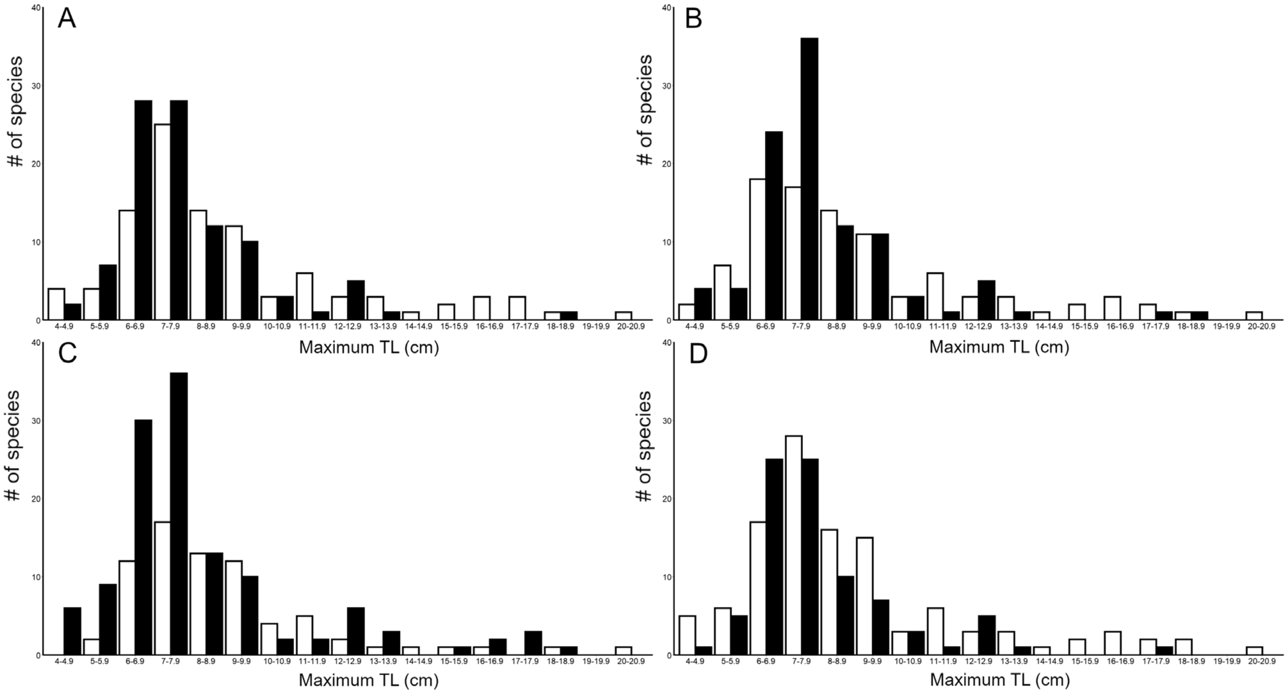

Figure 1. Number of darter species by total length (TL) size class. Black = present and white = absent for: (A) red on the body, (B) blue on the body, (C) red on the fins and (D) blue on the fins.

that indicated the presence of red on the body (mean bootstrap estimate = -0.57), blue on the body (mean bootstrap estimate = -0.44) and blue on the fins (mean bootstrap estimate = -0.45) were significant ($P < 0.05$) and negatively correlated with body size (Table 1). These results would suggest that larger darter species are less colourful; however, phylogenetic logistic regression revealed that this trend was not statistically significant ($P > 0.05$) when accounting for phylogenetic relatedness (Table 1).

Similarly, standard ANOVAs yielded significant associations between colour and size for every colour category: the presence of red on the body (F = 10.52, $P = 0.01$), blue on the body (F = 6.87, $P = 0.04$), red on the fins (F = 6.16, $P = 0.04$) and blue on the fins (F = 5.65, $P = 0.04$). However, phylogenetic ANOVAs revealed that these associations were not significant for the presence of red on the body (F = 10.52, $P = 0.25$), blue on the body (F = 6.87, $P = 0.34$), red on the fins (F = 6.16, $P = 0.33$) or blue on the fins (F = 5.65, $P = 0.45$). Results of both logistic regressions and ANOVAs therefore are consistent with the increased potential for Type I errors in comparative statistical analyses when studies fail to account for the non-independence of species data in a phylogenetic context (Martins & Garland, 1991; Martins *et al.*, 2002).

DISCUSSION

We tested for a relationship between body size and coloration in a clade of fishes known for their vivid nuptial colours. Although standard regression analyses and ANOVAs confirm impressions from the field that larger darter species are less colourful, this association appears to be driven by phylogenetic relatedness. Body size does not appear to act as a constraint on elaborate male coloration in darters, nor

Table 1. Parameter estimates for standard and phylogenetic logistic regression models comparing variation in male coloration with body size. SE = standard error, C.I. = confidence interval

Parameter	Estimate	SE	Bootstrap mean	Bootstrap 95% C.I.	Bootstrap *P*-value
Standard logistic regression					
Red on body					
B_0 (intercept)	-0.03	0.15	-0.03	(-0.32, 0.26)	0.84
B_1 (body size)	-0.55	0.17	-0.57	(-0.96, -0.23)	< 0.001
Blue on body					
B_0 (intercept)	0.09	0.15	0.09	(-0.20, 0.36)	0.55
B_1 (body size)	-0.43	0.16	-0.44	(-0.77, -0.15)	0.01
Red on fins					
B_0 (intercept)	0.54	0.15	0.55	(0.28, 0.84)	< 0.001
B_1 (body size)	-0.28	0.15	-0.28	(-0.61, 0.01)	0.11
Blue on fins					
B_0 (intercept)	-0.32	0.15	-0.32	(-0.63, -0.02)	0.034
B_1 (body size)	-0.43	0.17	-0.45	(-0.84, -0.14)	0.01
Phylogenetic logistic regression					
Red on body					
A	0.2	-	-0.03	(-1.10, 0.76)	0.001
B_0 (intercept)	-0.15	0.94	-0.12	(-1.58, 1.35)	0.92
B_1 (body size)	-0.07	0.14	-0.1	(-0.56, 0.26)	0.98
Blue on body					
A	-0.12	-	-0.34	(-1.20, 0.41)	0.002
B_0 (intercept)	0.03	0.71	0.02	(-1.18, 1.23)	0.98
B_1 (body size)	0.13	0.16	0.16	(-0.21, 0.57)	0.98
Red on fins					
A	0.13	-	-0.03	(-1.15, 0.84)	< 0.001
B_0 (intercept)	0.39	0.92	0.32	(-1.11, 1.69)	0.77
B_1 (body size)	-0.05	0.15	-0.07	(-0.48, 0.34)	0.98
Blue on fins					
A	0.34	-	0.18	(-1.11, 1.14)	0.001
B_0 (intercept)	-0.44	1.07	-0.42	(-2.01, 1.31)	0.77
B_1 (body size)	0.04	0.14	0.05	(-0.37, 0.49)	0.98

does elaborate coloration appear to act as a constraint on body size. Indeed, some of the smallest and largest species can possess striking male coloration on both the bodies and fins (e.g. the colourful *Etheostoma tippecanoe*, maximum TL = 4.3 cm and the colourful *Percina aurantiaca*, maximum TL = 18 cm).

One reason to predict that larger species would be less colourful is that larger species may have lower concentrations of carotenoids compared to smaller species (Galván *et al.*, 2013). In fishes, carotenoid-based coloration (i.e. orange and red) is derived from the diet (Grether *et al.*, 1999; Clotfelter *et al.*, 2007); larger species potentially need to consume higher concentrations of carotenoids than smaller species for these types of colorations. Diet may not be a limiting factor in explaining color variation in darters, however. Darter species generally share the same diet, primarily consuming chironomid larvae, although larger species have a greater dietary breadth. With a more diverse array and potential abundance of prey items available, larger darters may have more opportunities to consume carotenoids in their diet (Page & Swofford, 1984; Stauffer *et al.*, 1996; van Snik Gray *et al.*, 1997). Chironomids, which are high in carotenoid content (Czeczuga, 1970), and other invertebrates are frequently abundant in stream ecosystems, suggesting that large species are not carotenoid limited.

Second, larger species might be more visually conspicuous to potential predators compared to smaller species, such that elaborate coloration is maladaptive in larger species, or conversely larger body size is maladaptive in colourful species (Galván *et al.*, 2013). However, increased visual conspicuousness to predators due to larger body sizes is not likely to be a constraint in these colourful darter species. Ciccotto & Mendelson (2016) noted that chromatic darter species are more likely to occur in habitats that would support few predators, such as smaller streams and/or shallow riffles with coarse substrates. Achromatic species in contrast are more likely to inhabit larger streams and/or deeper pools with fine substrates that would support more predators. Thus, predation may drive the presence or absence of coloration, but this does not appear to be linked to body size. Large, colourful darters that inhabit smaller streams and/or shallow riffles with coarse substates likely encounter few predators and more opportunities to seek physical refuge. Additionally, large body size may act as a defence against predation due to gape limitation of predators. In darter species, small juveniles lack the vivid coloration of sexually mature adults. As darters grow into adulthood, predator-mediated selection against coloration may further weaken as adults of some species are too large to be captured or ingested (Hambright, 1991; Christensen, 1996). Thus, our finding that body size is not significantly correlated with coloration after controlling for phylogenetic relatedness is consistent with data suggesting that large species are not limited by carotenoid availability or predation risk.

Nuptial male coloration in darters appears to function in both female mate choice and male-male competition. Females in at least two species prefer the colour of conspecific over heterospecific males (Williams & Mendelson, 2011, 2013) and, in one species, females prefer certain conspecific colour variants (Williams *et al.*, 2013). Males in multiple species associate more and aggressively interact with similarly coloured males (Williams & Mendelson, 2013; Martin & Mendelson, 2016), suggesting male colour preference affects male-male interactions. Ciccotto & Mendelson (2016) tested colour preferences in females of 18 species of darters and found variation across species in the amount of time females spent associating with chromatic coloration. This variation in colour preference across species is not associated with body size (Ciccotto P, Mendelson T, unpubl. data). Thus, neither coloration nor preferences for coloration appear to be associated with body size in this clade.

The observation and documentation of the natural histories of organisms remains a critical endeavour in biology. A detailed understanding of natural history is necessary for the development of hypotheses regarding trait evolution that can help explain drivers of morphological and species diversification among different organismal groups. The ever-increasing number and availability of molecular phylogenetic data allows for the rigorous testing of such hypotheses, correcting for the non-independence associated with shared evolutionary history in statistical analyses. As demonstrated here, the incorporation of phylogenetic corrections into analyses of trait variation can challenge assumptions of co-evolution of traits. Darters, with their morphological, behavioural and ecological diversity and well-documented phylogenetic relationships, should continue to remain a useful study system for addressing similar assumptions and questions of the evolution of size, colour and other traits in fishes.

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DATA AVAILABILITY

Colour data are provided in the Supporting Information as a Microsoft Excel spreadsheet (Table S1).

REFERENCES

Benjamini Y, **Hochberg Y. 1995.** Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)* **27:** 289–300.

Bossu CM, **Near TJ. 2015.** Ecological constraint and the evolution of sexual dichromatism in darters. *Evolution* **69:** 1219–1231.

Carballo L, **Delhey K**, **Valcu M**, **Kempenaers B. 2020.** Body size and climate as predictors of plumage colouration and sexual dichromatism in parrots. *Journal of Evolutionary Biology* **33:** 1543–1557.

Cheney KL, **Cortesi F**, **How MJ**, **Wilson NG**, **Blomberg SP**, **Winters AE**, **Umanzör S**, **Marshall NJ. 2014.** Conspicuous visual signals do no coevolve with increased body size in marine sea slugs. *Journal of Evolutionary Biology* **27:** 676–687.

Christensen B. 1996. Predator foraging capabilities and prey antipredator behaviours: pre- versus postcapture constraints on size-dependent predator-prey interactions. *Oikos* **76:** 368–380.

Ciccotto PJ, **Mendelson TC. 2016.** The ecological drivers of nuptial color evolution in darters (Percidae: Etheostomatinae). *Evolution* **70:** 745–756.

Ciccotto PJ, **Mendelson TC. 2017.** The evolution of male nuptial colour in a sexually dimorphic group of fishes (Percidae: Etheostomatinae). *Journal of Fish Biology* **90:** 1768–1785.

Clotfelter ED, **Ardia DR**, **McGraw KJ. 2007.** Red fish, blue fish: trade-offs between pigmentation and immunity in *Betta splendens*. *Behavioral Ecology* **18:** 1139–1145.

Czeczuga B. 1970. Some carotenoids in *Chironomus annularius* Meig. larvae (Diptera: Chironomidae). *Hydrobiologia* **36:** 353–370.

Dale J, **Dey CJ**, **Delhey K**, **Kempenaers B**, **Valcu M. 2015.** The effects of life-history and social selection on male and female plumage coloration. *Nature* **527:** 367–370.

Etnier DA, **Starnes WC. 1993.** *The fishes of Tennessee*. Knoxville: Tennessee University Press.

Forthman M, **Weirauch C. 2018.** Phylogenetic comparative analysis supports aposematic colouration-body size association in millipede assassins (*Hemiptera*: *Reduviidae*: *Ectrichodiinae*). *Journal of Evolutionary Biology* **31:** 1071–1078.

Galván I, **Negro JJ**, **Rodríguez A**, **Carrascal LM. 2013.** On showy dwarfs and sober giants: body size as a constraint for the evolution of bird colouration. *Acta Ornithologica* **48:** 65–80.

Grether GF, **Hudon J**, **Millie DF. 1999.** Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proceedings of the Royal Society of London B* **266:** 1317–1322.

Gumm JM, **Feller KD**, **Mendelson TC. 2011.** Spectral characteristics of male nuptial coloration in darters (*Etheostoma*). *Copeia* **2011:** 319–326.

Hagman M, **Forsman A. 2003.** Correlated evolution of conspicuous coloration and body size in poison frogs (Dendrobatidae). *Evolution* **57:** 2904–2910.

Hambright KD. 1991. Experimental analysis of prey selection by largemouth bass: role of predator mouth width and prey body depth. *Transactions of the American Fisheries Society* **120:** 500–508.

Hossie TJ, **Skelhorn J**, **Breinholt JW**, **Kawahara AY**, **Sherratt TN. 2015.** Body size affects the evolution of eyespots in caterpillars. *Proceedings of the National Academy of Sciences of the United States of America* **21:** 6664–6669.

Ives AR, **Garland TD Jr. 2010.** Phylogenetic logistic regression for binary dependent variables. *Systematic Biology* **59:** 9–26.

Jenkins RE, **Burkhead NM. 1993.** *The freshwater fishes of Virginia*. Bethesda: American Fisheries Society.

Kuehne RA, **Barbour RW. 1983.** *The American darters*. Lexington: Kentucky University Press.

Maddison W, **Maddison DR. 2021.** *Mesquite: a modular system for evolutionary analysis. Version 3.90*. Available at: http://www.mesquiteproject.org/

Martins EP, **Diniz-Filho JAF**, **Housworth EA. 2002.** Adaptive constraints and the phylogenetic comparative method: a computer simulation test. *Evolution* **56:** 1–13.

Martins EP, **Garland TD Jr. 1991.** Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. *Evolution* **45:** 543–557.

Martin MD, **Mendelson TC. 2016.** Male behavior predicts trait divergence and the evolution of reproductive isolation in darters (Percidae: Etheostoma). *Animal Behaviour* **112:** 179–186.

Mettee MF, **O'Neil PE**, **Pierson JM. 1996.** *Fishes of Alabama and the Mobile Basin*. Birmingham: Oxmoor House Inc.

Midford PE, **Garland T Jr**, **Maddison WP. 2010.** *PDAP, PDTREE package for Mesquite, version 1.15*. Available at: http://www.mesquiteproject.org/pdap_mesquite/

Near TJ, **Bossu CM**, **Bradburd GS**, **Carlson RL**, **Harrington RC**, **Hollingsworth PR Jr**, **Keck BP**, **Etnier DA. 2011.** Phylogeny and temporal diversification of darters (Percidae: Etheostomainae). *Systematic Biology* **60:** 565–595.

Page LM, **Burr BM. 2011.** *A field guide to freshwater fishes of North America North of Mexico*, *2nd edn*. New York: Houghton Mifflin Company.

Page LM, **Swofford DL. 1984.** Morphological correlates of ecological specialization in darters. *Environmental Biology of Fishes* **11:** 139–159.

Piel WH, **Chan L**, **Dominus MJ**, **Ruan J**, **Vos RA**, **Tannen V. 2009.** TreeBASE v. 2: a database of phylogenetic knowledge. *e-Biosphere*. Available at: https://treebase.org/treebase-web/reference.html

R Core Team. 2021. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: https://R-project.org/

Rambaut A, **Charleston M. 2002.** *TreeEdit. Phylogenetic tree editor, version 1.0 alpha 10*. Available at: http://tree.bio.ed.ac.uk/software/treeedit/

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3:** 217–223.

Robins RH, **Page LM**, **Williams JD**, **Randall ZS**, **Sheehy GE. 2018.** *Fishes in the fresh waters of Florida: an identification guide and atlas*. Gainesville: University Press of Florida.

Rohde FC, **Arndt RG**, **Foltz JW**, **Quattro JM. 2009.** *Freshwater fishes of South Carolina*. Columbia: South Carolina University Press.

Ross ST. 2001. *The inland fishes of Mississippi*. Jackson: Mississippi University Press.

Sanderson MJK. 1997. A non-parametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution* **14:** 1218–1231.

Smith TA, **Ciccotto PJ**, **Mendelson TC**, **Page LM. 2014.** Dense taxon sampling using AFLPs leads to greater accuracy in phylogenetic estimation and classification of darters (Percidae: Etheostomainae). *Copeia* **2014:** 257–268.

van Snik Gray E, **Boltz JM**, **Kellogg KA**, **Stauffer JR Jr**. **1997.** Food resource partitioning by nine sympatric darter species. *Transactions of the American Fisheries Society* **126:** 822–840.

Stauffer JR Jr, **Boltz JM**, **Kellogg KA**, **van Snik ES. 1996.** Microhabitat partitioning in a diverse assemblage of darters in the Allegheny River System. *Environmental Biology of Fishes* **46:** 37–44.

Thomas C, **Bonner TH**, **Whiteside BG. 2007.** *Freshwater fishes of Texas: a field guide*. College Station: Texas A & M University Press.

Williams TH, **Gumm JM**, **Mendelson TC. 2013.** Sexual selection acting on a speciation trait in darters. *Behavioral Ecology* **24:** 1407–1414.

Williams TH, **Mendelson TC. 2011.** Female preference for male coloration may explain behavioral isolation in sympatric darters. *Animal Behaviour* **82:** 683–689.

Williams TH, **Mendelson TC. 2013.** Male and female responses to species-specific coloration in darters (Percidae: Etheostoma). *Animal Behaviour* **85:** 1251–1259.

Winebarger MM, **W orth Pugh M**, **Gangloff MM**, **Osbourn MS**, **Siefferman L. 2018.** Body size is positively correlated with conspicuous coloration in *Ambystoma* salamanders, but negatively correlated with conspicuous coloration in *Plethodon* salamanders. *Frontiers in Ecology and Evolution* **6:** 1–8.

Zhou M, **Johnson AM**, **Fuller RC. 2014.** Patterns of male breeding color variation differ across species, populations and body size in rainbow and orange-throat darters. *Copeia* **2014:** 297–308.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Darter colour data used for comparative analyses. See methods for description of data collection.