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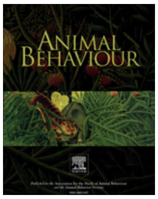

Spawning coloration, female choice and sperm competition in the redside dace, *Clinostomus elongatus*

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Understanding pre- and postcopulatory mechanisms of sexual selection (male–male competition and female choice) can provide insight into the evolution of male ornamentation. In this study, we use an integrative approach to study sexual selection by incorporating measures of both mechanisms using the redside dace, *Clinostomus elongatus*, a freshwater, externally fertilizing fish found in streams of eastern North America. We measured the success of individual male redside dace in both pre- and postspawning sexual selection and related the success of males to a range of traits, including spectral properties and the size of their red spawning coloration, body size metrics and sperm quality. We found significant sexual dimorphism in the relative amount and spectral properties (saturation and brightness) of the red spawning coloration, with males being more ornamented than females. Using dichotomous mate choice trials, we found that females did not favour males with relatively larger red spawning coloration area and more saturated or brighter red spawning coloration. Males with relatively more red spawning area would be favoured in male–male postspawning sperm competition because they possess sperm with higher velocity, and sperm velocity was determined to be the key predictor of sperm competition success as assessed by paternity analyses related to in vitro sperm competition trials. These results suggest that prespawning selection via female choice is not driving the evolution of red spawning coloration and that postspawning selection via male–male competition is primarily determined by sperm velocity, which was correlated with the relative amount of red spawning coloration.

A central aim of evolutionary biology is to understand how selection acts on traits in natural populations. For example, sexual selection acts on secondary sexual traits when there is variation in reproductive success associated with these traits, which arises from two mechanisms: intrasexual selection (male–male and female–female competition) and intersexual selection (e.g. female and male mate choice) (reviewed in Andersson 1994; Andersson & Simmons 2006). Although the importance of both of these mechanisms for the evolution of secondary sexual characters in males has been acknowledged, most researchers focus their attention on one or the other mechanism within the context of their study system. This can be problematic, however, because even though both mechanisms may operate at the same time, they do not necessarily act in the same direction (reviewed by Hunt et al. 2009). For example, the two mechanisms can reinforce each other, as in the western rainbow fish, *Melanotaenia australis*, and hissing cockroaches, *Gromphadorhina portentosa*, where male–male competition and female choice both act to favour large body size in males (Clark & Moore 1995a, b, c; Young et al. 2010). Alternatively, if sexual conflict is pronounced, one mechanism may oppose the other (reviewed in Arnqvist & Rowe 2005). In water striders, *Aquarius remigis*, for example, male–male competition appears to select for larger body size, whereas female choice appears to select against larger body size (Sih et al. 2002). As such, when differences in the relative strengths of the two mechanisms exist, the outcome for sexual selection will depend on an interaction between the magnitude and direction of each selective force (see Hunt et al. 2009). Differentiating between the effects of female choice and male–male competition within a mating system is therefore critical in order to better understand the relative contribution of the two mechanisms in the evolution of sexually selected traits.

A recent review of 83 studies (of 51 species) that investigated both male–male competition and female mate choice acting on an apparent sexually selected trait, male body size, found that in most cases selection was linear and that both mechanisms were more likely to be reinforcing rather than opposing (Hunt et al. 2009). However, the generality of these results are questionable because

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although body size is commonly thought to be important in sexual selection, it is also a metric that is crucial in many other contexts (e.g. different life history stages) and is likely to be influenced by natural selection as well. As such, Hunt et al. (2009) suggest that there is a need for more empirical studies of both mechanisms that examine traits that are limited in expression to the adult stage and that have little relevance outside of courtship and mating, such as colourful male secondary sexual characters (e.g. Setchell 2005; Tarof et al. 2005). In addition to considering sexual traits other than body size, there is also a need to examine how both pre- and postcopulatory processes might affect the evolution of male secondary sexual characters. For example, if there is a relationship between male secondary sexual characters and sperm quality (Blount et al. 2001; Peters et al. 2004), postcopulatory sperm competition success by more ornate males might reinforce or oppose precopulatory female choice on male sexual traits. Predicting the directionality of the relationship between sperm quality metrics and sexual ornamentation is difficult because two different hypotheses make opposite predictions. The phenotype-linked fertility hypothesis (Sheldon 1994) suggests that male sperm quality and ornament elaboration will positively covary, whereas, the sperm competition hypothesis (Parker 1998) predicts a negative association between investment in sperm quality and ornamentation related to mate acquisition. For example, in guppies, *Poecilia reticulata*, males with relatively more orange body coloration (a trait subject to precopulatory female choice: Magurran 1995; Pitcher et al. 2003) have more and faster sperm (Pitcher & Evans 2001; Locatello et al. 2006; Pitcher et al. 2007) and a paternity advantage following artificial insemination of females with equal numbers of sperm from two competing males (Evans et al. 2003). Whereas, in Arctic charr, *Salvelinus alpinus*, the most brightly coloured males (i.e. males with more intense red sexual ornamentation) have the lowest fertilization probability following artificial fertilization of female's eggs using equal numbers of sperm from two competing males (Liljedal et al. 2008).

In this study we used mate choice trials, spectral and sperm analysis, competitive in vitro fertilization and paternity assignment to examine factors affecting the evolution of a male secondary sexual character. We did this by quantifying prespawning female mate choice and postspawning male–male competition in the redside dace, *Clinostomus elongatus*. The redside dace is a small, externally fertilizing cyprinid native to headwater streams in the basins of the Mississippi River and Lakes Michigan, Huron, Erie and Ontario (Parker et al. 1988). Sexual selection has not been explored in this species, but the sexual dichromatism exhibited in sexually mature adults during the spawning season suggests that it may be present in this species' mating system (Andersson 1994). Throughout the year, adult redside dace have pink coloration on their body just behind their head, but during the breeding season both sexes develop intense red spawning coloration. Based on a chemical test that identifies the presence of carotenoid pigments in animal tissues (McGraw et al. 2005) and preliminary high performance liquid chromatography analyses, the red spawning coloration is carotenoid based (T. E. Pitcher & K. McGraw, unpublished data). The red spawning coloration manifests itself as an intense red stripe extending from the opercula to below the dorsal fin or beyond (Fig. 1). Males appear to possess a larger area of more intensely red spawning coloration than females, although this has yet to be quantified. Throughout their range, redside dace, which become sexually mature at the age of 3 years (they can live to be 4 years old), spawn between mid-May and early June (Scott & Crossman 1998; Werner 2004). Like some other small minnows, redside dace typically parasitize gravel nests built by larger minnows or suckers such as creek chub, *Semotilus atromaculatus*. These nests are found just upstream or downstream of fast riffles in coarse sand or fine gravel. Redside dace leave the eggs to be guarded by the larger species and provide no parental care to their offspring. During spawning, males typically congregate in dense schools downstream from creek chub nests, with the females positioned on the outer regions of the school. Spawning typically begins when a female moves upstream towards a creek chub nest and is followed by up to six males (Koster 1939; T. E. Pitcher, unpublished data). During spawning, multiple males crowd around the female and release their sperm as she deposits her eggs into a gravel nest. Females carry between 400 and 1500 eggs, depending on their body size (Scott & Crossman 1998; Werner 2004). Spawning of eggs by females and sperm from multiple males can be repeated every few seconds for about 1 min, with several minutes often elapsing between each spawning bout. Unlike other minnows that have a lek-like mating system (e.g. European minnow, *Phoxinus phoxinus*, Jacob et al. 2009), behavioural observations of wild redside dace prior to spawning suggest that prespawning male–male competition plays a limited role in this mating system because males do not appear to defend territories or have consistent spawning hierarchies (T. E. Pitcher, personal observation). However, it is likely that postspawning intrasexual competition in the form of sperm competition, the competition between sperm of two or more males for the fertilization of ova (Parker 1970), is an important selective factor in this mating system. Recently, Pitcher et al. (2009a) documented significant intermale variance in sperm size and shape metrics (total sperm length, sperm head length, flagellum length and sperm head length to width ratio) for redside dace and also found positive relationships between these morphometric indices and sperm velocity. Because sperm velocity correlates with sperm competition success in many fish species (e.g. Gage et al. 2004), these findings suggest that male redside dace vary in their sperm competitive ability.

Figure 1. Male redside dace showing typical sex-specific spawning coloration. The circle indicates the location where reflectance spectrometry measurements were collected. Note that the bluish colour on the dorsal surface is not structural coloration but a reflection from excess water, which causes specular glare by reflecting incident light. Prior to taking spectral readings, we removed excess water by wiping the body surface with a lint-free delicate task wiper.

We examined the success of individual male redside dace in both pre- and postspawning sexual selection and related the success of males to a range of traits, including body size, spectral properties and size of the red spawning coloration, and sperm quality. Our first objective was to quantify sexual dimorphism in this species and to test whether females show preferences for sexually dimorphic male traits. Using mate choice trials in which females were allowed to choose between two males, we predicted that the degree of female preference for a particular male would increase as a function of the difference in phenotype between the two males; that is, females should be more responsive to more colourful (i.e. more ornamented) males. Our second objective was to examine postspawning male–male competition by using in vitro sperm competition trials to determine which sperm traits are important for competitive fertilization success and whether males that are more colourful possess these sperm traits. To accomplish this objective, we investigated the importance of sperm velocity, density and longevity with respect to competitive fertilization success by conducting in vitro fertilization trials using sperm from pairs of males and eggs from individual females. We then used