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Sexual dichromatism in the marbled salamander, *Ambystoma opacum*

Brian D. Todd and Andrew K. Davis

Abstract: Reports of sexual dichromatism in salamanders are rare and have been generally restricted to a few species in the families Hynobiidae and Salamandridae. We used image analysis techniques to examine sexual dichromatism in the marbled salamander, *Ambystoma opacum* (Gravenhorst, 1807). We measured the average hue of white saddles on male and female marbled salamanders (n = 118), as well as the proportion of white dorsal surface area relative to the black dorsal surface area, to determine the extent of sexual dichromatism in this species. We also tested whether patterning and coloration were correlated with body size or relative body mass. Males had significantly whiter saddles and higher relative proportions of white coloration on their dorsal surfaces than did females. Furthermore, the relative proportion of white areas on the dorsum was positively correlated to body condition in both males and females. Body size was not correlated with hue or proportion of white area on the dorsum. To our knowledge, we report the first confirmation of sexually dimorphic coloration in the ambystomatid salamander family, extending the known distribution of sexual dichromatism in the order Caudata.

Résumé : On a rarement signalé le dichromatisme sexuel chez les salamandres et généralement seulement chez quelques espèces des familles Hynobiidae et Salamandridae. Nous avons utilisé des techniques d'analyse d'images pour étudier le dichromatisme sexuel chez la salamandre marbrée, *Ambystoma opacum* (Gravenhorst, 1807). Afin de déterminer l'importance du dichromatisme sexuel chez les salamandres marbrées, nous avons mesuré la teinte moyenne des selles blanches sur les mâles et femelles (n = 118), ainsi que la proportion de surface blanche par rapport à la surface noire sur le dos. Nous avons vérifié si les patrons et la coloration sont en corrélation avec la taille corporelle ou la masse relative du corps. Par rapport aux femelles, les mâles possèdent des selles significativement plus blanches et une proportion relativement plus élevée de coloration blanche sur leur surface dorsale. De plus, la proportion relative des surfaces blanches sur le dos est en corrélation positive avec la condition corporelle tant chez les mâles que les femelles. La taille corporelle n'est reliée ni à la teinte, ni à la proportion de surface blanche dorsale. À notre connaissance, c'est la première fois qu'on signale une coloration sexuellement dimorphique chez des salamandres de la famille des ambystomatidés, ce qui élargit la répartition du dichromatisme sexuel dans l'ordre des Caudata.

[Traduit par la Rédaction]

Introduction

Sexual dimorphism in body characters is a common feature of many animal species. Body size, ornamentation, and coloration are characters that are often exaggerated in one sex relative to the other. Traditional explanations for intersexual differences in observed characters are based largely on Darwin's theory of sexual selection, which holds that limited access to mates can lead to the evolution of conspicuous secondary sex characters (Darwin 1871; Andersson 1994). However, ecological factors can also lead to sexual dimorphism between the sexes (Slatkin 1984).

In the Amphibia, sexual dimorphism in body size and morphology has been explored, particularly with regard to intrasexual combat and sexual selection (e.g., Shine 1979; Woolbright 1983). Sexual dichromatism (i.e., sexually dimorphic coloration) has received less attention but has been reported in some species and families, mostly among anurans (frogs and toads). For example, males of many anurans develop prominent dark or colored patches on their vocal sacs during the breeding season (Conant and Collins 1998), and in other frog species, sexual dichromatism takes form in divergent body coloration between the sexes (Savage and Donnelly 1992; Hoffman and Blouin 2000). In some cases, sexual dichromatism in anurans has been attributed to sexual selection in the form of mate choice (Summers et al. 1999) and has been shown to affect offspring survival under predation risk (Sheldon et al. 2003).

Reports of sexual dichromatism in salamanders have been generally restricted to a few newts, including *Neurergus*

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B.D. Todd.[1] The University of Georgia, Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802, USA.
A.K. Davis. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602, USA.

[1]Corresponding author (e-mail: todd@srel.edu).

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strauchii (Steindachner, 1887) (Sparreboom et al. 2000) and some newts of the genus *Triturus* Rafinesque, 1815 (Duellman and Trueb 1994), and also to the clouded salamander, *Hynobius nebulosus* (Temminck and Schlegel, 1838) (Thorn 1967). In the aforementioned newts, males develop bright coloration on the tail, which is presumably used in courtship (Salthe 1967), whereas male *H. nebulosus* develop a white gular patch, which is also presumably used during courtship (Thorn 1967). The sexual dichromatism is condition-dependent and manifests during the mating season but typically diminishes outside of the breeding season. Sexual dichromatism in other salamanders is presumably nonexistent or has gone unreported.

The marbled salamander, *Ambystoma opacum* (Gravenhorst, 1807), is widely distributed in eastern North America, and its dorsal coloration has long puzzled herpetologists (Noble and Brady 1933). As aquatic larvae, animals are typically greenish-yellow to entirely black and emerge from natal ponds as solidly dark animals with minor white speckling. As juveniles age, they develop conspicuous, apparently random, dorsal patterns of white saddles, crossbands, or in some cases, longitudinal lines (Conant and Collins 1998). There does not appear to be any consistency in dorsal patterns within the sexes (Noble and Brady 1933). However, males of this species often appear noticeably whiter than females, although the degree of sexual dimorphism in the quality and quantity of white coloration remains unknown. Recent advances in computer-assisted measuring techniques now allow for the quantification of surface features of animals that contrast in color (Davis and Maerz 2007), as well as the measurement of color itself (Davis et al. 2007), and the measurement of traditional metrics such as body size (Davis and Grayson 2007), all from a single photograph. In this paper, we used image analyses to determine the extent of sexual dimorphism in the coloration of marbled salamanders. We also tested whether there was a relationship between salamander size or body condition and the color or extent of white coloration present on salamanders. Based on firsthand observations of the animals and anecdotal reports of this species, we predicted that males would have whiter coloration than females and that white patterns would cover relatively more of the dorsum in males than in females. Furthermore, because increased coloration is often correlated to increased body condition or body size in some animal taxa (Hill and Montgomerie 1994; Weiss 2006), we predicted that salamanders of greater relative body mass would have whiter coloration and more extensive white patterns than individuals of lower relative body mass.

Materials and methods

Animal capture and photography

Marbled salamanders undergo yearly reproductive migrations during rainy nights in autumn in the southeastern United States (Scott 2005). Our study animals were intercepted by a drift fence (Gibbons and Semlitsch 1982) as they immigrated to reproduce at Ginger's Bay, a dry pond basin located in Aiken County, South Carolina, on the US Department of Energy's Savannah River Site. A sample consisting of 118 animals (67 females and 51 males) was collected on 9 and 12 October 2006 and returned to the laboratory for photographing. Because temperature can affect coloration in some amphibians (e.g., King et al. 1994), we maintained all animals overnight in a darkened, temperature-controlled chamber at 8 °C and we removed them just prior to photographing them. The animals were not rinsed but were kept on damp paper towels moistened with aged well water. We used a Nikon D70 to photograph the animals and we photographed all specimens in the same session using identical camera settings and lighting. We placed all animals on the same white background with an embedded ruler while photographing them. We recorded the mass of each animal to the nearest milligram using an electronic balance at the time the photographs were taken. All animals were released back to their breeding wetland after the photographs were taken.

Fig. 1. Example of the image analysis procedure used to generate morphological data of marbled salamanders (*Ambystoma opacum*) in this study. A line was drawn down the back of the salamander in the initial image (A) and its length (mm) was measured to generate snout–hindlimb length (SHL). The head, torso, and tail were then selected (B) and the surface area (mm^2) of this entire selection was measured. We next selected all white areas from within the larger selection (C) and determined the average hue of all white colored pixels. The image was then inverted to absolute black and white (D) to easily measure the area of all white saddles (now represented in absolute black) for comparison against the entire dorsal surface area.

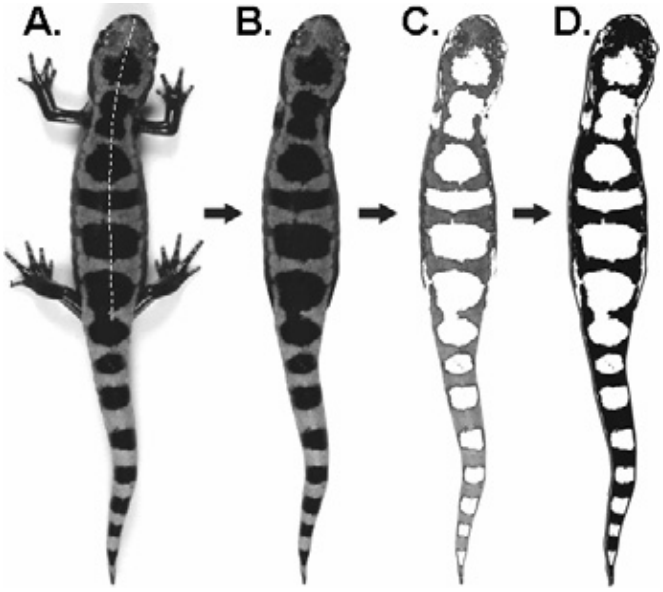

Image analysis

We imported all images into Adobe Photoshop with Fovea Pro plug-ins (Reindeer Graphics, Inc., Asheville, North Carolina) installed. We followed Davis and Maerz (2007) in the measurement of morphological characteristics of the salamanders. Briefly, we calibrated the software with the embedded ruler that appeared in each photograph so that all measurements could be output as millimetres. On each salamander image, we drew a line from the tip of the snout to the point of hind-limb attachment following the curvature of the salamander torso and recorded the length of this line as snout–hindlimb length, SHL (Fig. 1A); this technique

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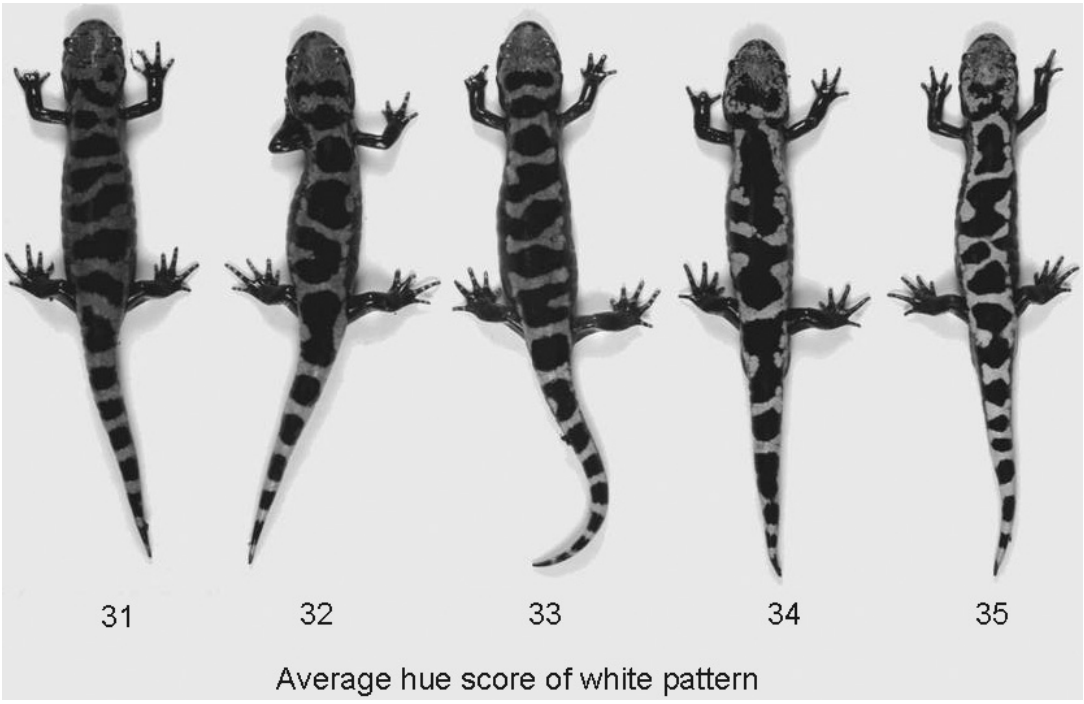
Fig. 2. Five marbled salamanders from our sample that demonstrate the gradient of dorsal hue values assigned by the image analysis procedures (for details see the Materials and methods). Salamanders with brighter white areas had higher hue scores than those with darker white areas.



slightly underestimates true snout–vent length (SVL) but is consistent within our study. Next, we traced the outline of the salamander head, torso, and tail and measured the total surface area of this selection (Fig. 1B). From within this selection, we picked all white areas (Fig. 1C) and measured the hue of this selection following Davis et al. (2004). In this case, we used the software to calculate the average hue of all white regions on the salamander. Finally, we inverted the image into an absolute black and white image and measured the collective surface area of all white areas (now represented as absolute black, Fig. 1D). Using this value and the surface area of the entire dorsum (obtained from Fig. 1B), we calculated the amount of dorsal surface area encompassed by the white patterns as a proportion of the entire surface area. We note here that this value probably underrepresents actual coverage of white areas because we were measuring two-dimensional photographs.

Visual assessment of the quantitative output from within Adobe Photoshop indicated that the hue scores of white areas increased as the white saddles became increasingly white (Fig. 2). With this scoring system, individuals that had duller coloration were assigned lower hue scores than those that had brighter coloration with more white, which were assigned higher scores. Thus, we computed two measures of white dorsal coloration for each salamander: the average hue of the white areas and the relative proportion of white areas on the dorsum.

Data analysis

We used analyses of variance to test whether the hue and the proportion of white area on the dorsum differed between the sexes. We used analyses of covariance to test whether the hue and the proportion of white area on the dorsum were correlated with size-specific body mass, using both sex and length (SHL) as covariates. We also used an analysis of covariance to test whether the hue and the proportion of white area on the dorsum were correlated with body size, using sex as covariates. Lastly, we used a linear regression to determine whether hue was positively correlated to the proportion of white area on the dorsum in the salamanders. We examined all data prior to analyses to ensure that statistical assumptions were met. We log-transformed SHL and mass, and arcsine square-root transformed the relative proportion of white area for all analyses. We performed all tests using the SAS® version 9 statistical package (SAS Institute Inc. 2003).

Results

Sexual dichromatism

On average, males had significantly more dorsal surface area covered in white relative to black than did females ($F_{[1,116]} = 65.7$, $p < 0.001$). Males ranged from 42% to 58% of the dorsum covered in white compared with 34% to 53% in females (Fig. 3A). Likewise, the average hue in males was significantly whiter than that of females ($F_{[1,116]} = 165.9$, $p < 0.001$; Fig. 3B). Salamanders with greater proportions of their dorsum covered in white also tended to have whiter saddles ($F_{[1,116]} = 70.67$, $p < 0.001$).

Effects of body condition and size on coloration

Salamanders that were heavier at a given length were not significantly whiter than their lower-mass counterparts of equal length as determined using the average hue of all white areas ($F_{[1,114]} = 0.19$, $p = 0.67$). However, there was a significant positive relationship between the body condition

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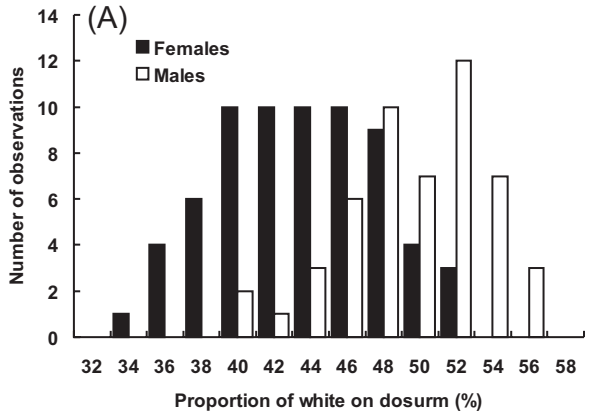
Fig. 3. Frequency histograms demonstrating the degree of sexual dimorphism between male and female marbled salamanders for (A) proportion of white surface area relative to black on the dorsum and (B) average hue of all white areas on the dorsum. Males had greater proportions of their dorsal areas covered in white compared with females; also, the average hue of white areas in males was greater than in females.

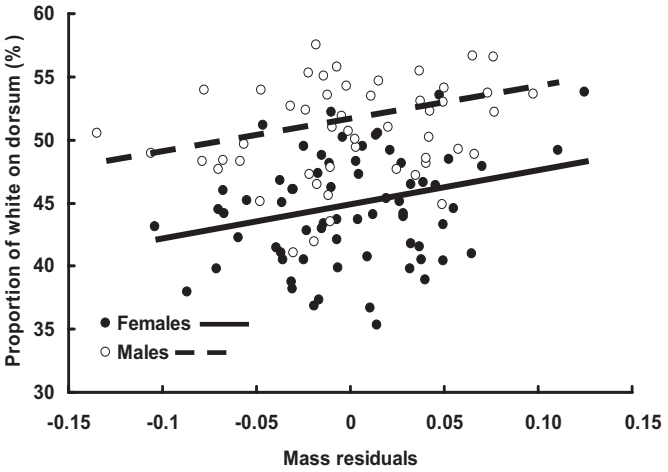


and the relative proportion of white areas in salamanders ($F_{[1,114]} = 8.11$, $p = 0.005$; Fig. 4). Neither hue nor proportion of white areas on the dorsum were correlated with body size in males or females (hue: $F_{[1,115]} = 0.19$, $p = 0.66$; proportion white area: $F_{[1,115]} = 0.72$, $p = 0.40$).

Discussion

Our study represents the first quantitative confirmation of sexual dichromatism in *A. opacum*. Generally, we confirmed anecdotal descriptions that males appear appreciably different from females of this species during the reproductive period (Noble and Brady 1933; Petranka 1998). It is unknown whether this dichromatism persists outside of the breeding season. Using image analysis techniques, we were also able to characterize both the quality of white coloration (hue) and the relative proportion of white coloration present, as well as demonstrate that males had higher scores than females for both measures. There was very little overlap in coloration between the sexes, although a few bright females and darker males did occur in our sample. To our knowledge, the sexual dichromatism that we report for *A. opacum* represents the first confirmation of sexually dimorphic coloration in the ambystomatid salamander family. It also extends the

Fig. 4. There was a significant positive relationship between the condition of the salamander body and the proportion of white area covering the dorsum ($F_{[1,114]} = 8.11$, $p = 0.005$). Mass residuals for the figure were calculated from linear regressions of log-transformed mass versus SVL for males and females separately.



previously scant phenomenon of sexual dichromatism in salamanders beyond the few currently documented species of the families Hynobiidae and Salamandridae.

Although we found evidence of sexual dichromatism in this species, we did not find evidence that coloration in males or females was correlated with their length, often used as a rough proxy for age in salamanders. In contrast, the relative proportion of white patterns on dorsal surfaces was positively correlated to body condition (size-specific body mass) in both males and females, a trait that has been tied to individual quality. Heavier salamanders generally have greater fat stores and, in the case of females, produce larger egg clutches (Scott and Fore 1995). The lack of a similar relationship between hue and body condition suggests that the quantity of white coloration on the dorsum rather than the quality of the white pattern (hue) may be a better indicator of an animal's fitness.

Studies of inheritance in hybrid crosses of salamander species demonstrate that pigment traits are largely heritable (Lipsett and Piatt 1936; Spurwell 1953; Twitty 1961). Nevertheless, factors such as prey availability or foraging success may additionally affect pattern development to ultimately produce the resulting phenotype. Juvenile *A. opacum* generally develop adult coloration in the months that follow metamorphosis and patterns tend to remain unchanged throughout their adult lives (Ravela and Gamble 2004). However, at least one study has demonstrated the important and pervasive effects that larval and juvenile fitness in *A. opacum* have on adult demographic traits such as age at first reproduction, body size, and clutch size (Scott 1994), suggesting that environmental control of pattern development in juveniles may signal an animal's fitness. This idea has also been raised in recent examinations of spot patterns in spotted salamanders, *Ambystoma maculatum* (Shaw, 1802), which also develop their dorsal pattern in the months following metamorphosis (Davis and Maerz 2007). Specifically, healthy juveniles exposed to adequate larval and post-metamorphic resources may develop more elaborate patterns and colors, a prediction that remains to be tested.

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We did not test in our study whether sexual selection plays a role in the sexual dichromatism that we observed in *A. opacum*. However, secondary evidence, as well as reports and observations of courtship and breeding patterns in *A. opacum*, do suggest a possible role for sexual selection in the sexual dichromatism of *A. opacum*. For example, sex ratios in breeding populations of *A. opacum* are nearly always male-biased, meaning that reproductive females are limiting (Petranka 1998; Scott 2005). Also, males arrive at reproductive sites weeks earlier than females (Krenz and Scott 1994). Thus, conspicuous traits that increase a male's chance of being noticed by later-arriving, limiting females should increase its reproductive success. Also, because these animals court and breed at night (Scott 2005), the development of maximally contrasting patterns of black and white may be more suitable than alternative colors more useful in daytime advertisement. The value of having noticeable white patches for use in intraspecific signaling at night has been demonstrated in other species that breed and display under low light (Penteriani et al. 2006, 2007). Additionally, female *A. opacum* arriving at dry pond basins to breed at night have been observed cueing in on groups of active, brightly patterned males to join them in breeding congresses (Scott 1998; D. Scott, personal communication). However, we caution that the precise cause(s) of sexual dichromatism in *A. opacum* and its correlation with body condition remain unknown, and the possibility that sexual selection and mate choice are responsible remains speculative. Only future studies can discern whether ecological factors, physiological factors, sexual selection, or a combination of these factors is responsible for sexual dichromatism in *A. opacum*.

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