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CAN A TIGER CHANGE ITS SPOTS? A TEST OF THE STABILITY OF SPOT PATTERNS FOR IDENTIFICATION OF INDIVIDUAL TIGER SALAMANDERS (*AMBYSTOMA TIGRINUM*)

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Abstract.—**There is increasing interest in the use of unique spot patterns as a way to "mark" individual amphibians as an alternative to invasive techniques for studies of free-ranging populations. However, studies testing the efficacy of the pattern recognition technique have largely drawn their conclusions from the ability to recognize recaptured individuals that were identified solely by spot pattern. Individuals whose color pattern changed significantly would therefore not be identified upon recapture. For this study, Tiger Salamanders (*Ambystoma tigrinum*) were captured in west-central Minnesota and maintained in captivity for one year, and their dorsal and ventral surfaces photographed approximately every six weeks. The stability of the spot patterns and their utility as individual identifiers were examined through comparison tests that required the matching of photographs taken 12 months apart. Each of the 23 volunteers who took the test was given a sample photograph and asked to choose the corresponding photograph from four others or to choose "no match". On average, volunteers were able to correctly match the photographs only 67% of the time. Four of the salamanders (36%) could be identified by all volunteers, whereas another 36% were matched at a rate that was no better than guessing. Two of these salamanders changed dramatically in appearance, from the spotted *A. tigrinum* pattern to the blotched *A. mavortium melanostictum* pattern. The high frequency of misidentifications and the dramatic changes in coloration have implications not only for studies that involve identification of recaptured individuals, but potentially for efforts to classify the different subspecies of Tiger Salamanders.**

Key Words.—Ambystoma tigrinum; mark-recapture; pattern identification; Tiger Salamander

INTRODUCTION

Studies of free-ranging populations of animals can require the identification of specific individuals to determine movement or life history characteristics of recaptured individuals over time. Individual identification can be achieved by "marking" individuals, either by applying an artificial mark or by using a feature of the animal itself. Artificial marks used in the study of amphibians include passive integrated transponder (PIT) tags, toe-clipping, external tags, branding, and other similarly invasive (and often expensive) techniques (reviewed by Donnelly et al. 1994 and Ferner 2010).

A potential alternative is the use of unique color patterns or markings as a way to identify individual amphibians. The patterns can be sketched or photographed (Ferner 2010), depending on their complexity and the number of individuals involved in the study. Pattern matching is non-invasive, and usually less expensive than the more invasive methods (although see Arntzen et al. 2004 for a cost-benefit comparison of the use of PIT tags and pattern mapping). However, like all methods for marking individuals, the use of patterns must be validated for the species of interest to ensure that a marked animal will be recognized upon recapture (Ferner 2010). Studies testing the validity of this technique for various species of amphibians have largely derived their conclusions from the ability to recognize recaptured individuals that were identified solely by spot pattern (e.g., Loafman 1991; Grant and Nanjappa 2006; Church et al. 2007; Gamble et al. 2008; Jonas et al. 2011) or that were recaptured over a relatively short period of time (e.g., Bailey 2004; Bradfield 2004; Grant and Nanjappa 2006; Del Lama et al. 2011). Individuals with significant changes in pattern would therefore not be identified upon recapture, skewing estimates of population size or individual movement patterns.

I study habitat use of Tiger Salamanders in the prairie potholes of west-central Minnesota, and wanted to determine whether spot patterns can be used for individual identification over multiple years for spotted *Ambystoma* species. Tiger salamanders are found in many types of aquatic habitats across most of North America (Stebbins 1985; Conant and Collins 1998; Petranka 1998). Once identified as various races or subspecies of *Ambystoma tigrinum*, the various geographical groups are now split between the western *A. mavortium* and the eastern *A. tigrinum* (Crother et al. 2008; Collins and Taggart 2009). As described by Vörös et al. (2007), morphological features can be used to identify an individual to species or subspecies (originally described for Tiger Salamanders by Dunn 1940). Therefore, it is important to establish the stability of an individual's color pattern over a significant period of time, not only for long-term population studies but for species identification.

Materials and Methods

We collected a total of eleven Tiger Salamanders during fall movement events in October 2011, from one site approximately 5 km north of Alberta, Minnesota, USA and from another site approximately 10 km east of the first location, in Morris, Minnesota. Salamanders were housed individually at the University of Minnesota, Morris in 37.8 L (10 gallon) aquaria with a combination of soil and moss and a water bowl filled with dechlorinated water. Lighting (10L:14D) was provided by a 20 watt helical fluorescent bulb placed over one end of each aquarium and by sunlight ambient to the room.

I photographed each salamander every four to six weeks using a Panasonic Lumix DMC-TS2 14 megapixel camera. I took photographs under ambient indoor lighting without a flash, with the salamander placed on a 1x1 cm grid printed on white paper to record snout-vent length (SVL). Beginning in November 2011, I also photographed the ventral surface of each salamander by placing it in a glass dish and taking the photograph from the underside. The background of each photograph was therefore the same for each salamander every time (white paper for dorsal photographs, white ceiling tile for ventral photographs).

As a measure of the amount of change in spot pattern demonstrated by the salamanders over a 12 month period, and to test the relative utility of dorsal patterns versus ventral patterns, I recruited 23 volunteers to match photographs from November 2011 to those from October 2012. These volunteers had no prior experience with the use of pattern matching to identify individual animals and did not receive any training. I cropped the dorsal and ventral photographs of each salamander taken in November 2011 and October 2012 and lightened them if necessary to enhance contrast. A colleague wrote a custom computer program specifically for this task in a language called Processing (processing.org) to present the photographs to the volunteers and to record their responses (source code available upon request). We designed the program to produce 20 trials (10 dorsal and 10 ventral) for each volunteer, each trial contrasting one photograph from November 2011 against four from October 2012. These four included the matching photograph plus three chosen randomly from the 11 possibilities. Prior to presentation the program randomly selected two of the dorsal and two of the ventral trials to exclude the October 2012 match to the salamander being presented.

In each trial, I presented the volunteers with five options: the four photographs from October 2012 and "no match." Therefore, for any given trial, guessing would result in a correct choice with a probability of 1/5, and for any given volunteer taking the test, guessing would result in an expected accuracy rate of 20%. I summarized test results to determine the number of correct trials for each volunteer, and the number of correct matches for each salamander. I compared the number of correct trials (total, dorsal only, and ventral only) to each other by calculating 95% confidence limits for each mean. Statistically significant deviations from 100% (perfect matching) and from 20% were determined by calculating binomial 95% confidence limits for the proportion of correct matches for each salamander (Zar 1984).

Results

Average SVL in November 2011 was 9.8 cm (8.5–12 cm); whereas, the average in October 2012 was 10.9 cm (10–12.5 cm). A qualitative visual comparison of the photographs concluded that of the 11 salamanders, #1, 5, 7, 9, and 10

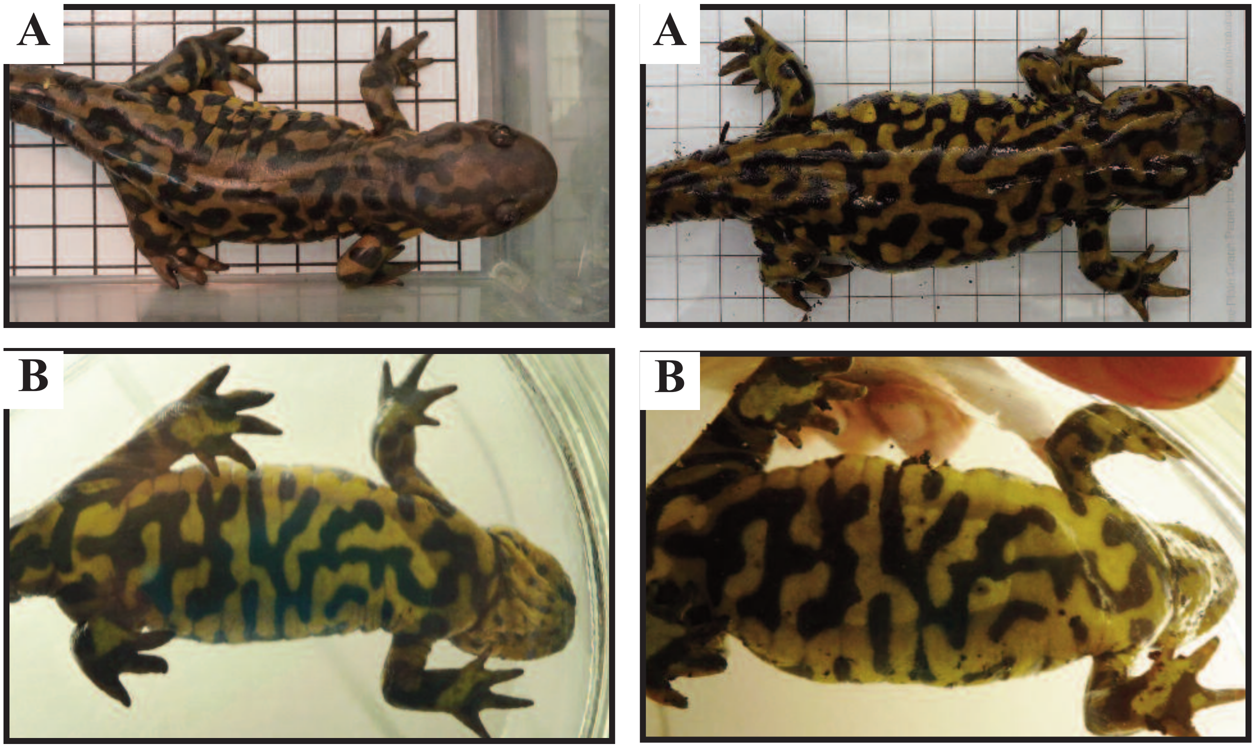

Figure 1. Dorsal (a) and ventral (b) views of salamander #1 from November 2011 (left) and October 2012 (right), demonstrating little change in spot pattern or degree of pigmentation. Photographs were lightened and cropped. (Photographed by Heather Waye)

(Fig. 1a) showed very little change in the spot pattern on the dorsal surface, and #1, 2, 6, 8, 9, and 10 (Fig. 1b) showed very little change on the ventral surface. The rest had noticeable changes in pattern and degree of coloration on the dorsal and/or ventral surfaces. The dorsal pattern changed more than the ventral for four individuals (#2, 4, 6, and 8), whereas the ventral pattern changed more than the dorsal for two others (#5 and 7). Some salamanders showed an increase in the area of lighter yellow or olive coloration (#3, 5, 9, and 11; Fig. 2) whereas others showed an increase in the amount of darker black coloration (#7 and 8). One maintained a fairly constant degree of pigmentation but the dorsal pattern of spots changed (#4; Fig. 3).

The results of the pattern matching trials generally supported the qualitative comparison. The average correct matches for the spot pattern

Figure 2. Dorsal views of salamander #3 from November 2011 (left) and October 2012 (right), demonstrating a reduction in degree of pigmentation over time. Photographs were lightened and cropped. (Photographed by Heather Waye)

Figure 3. Dorsal views of salamander #4 from November 2011 (left) and October 2012 (right), demonstrating a change in spot pattern but no significant change in degree of pigmentation. Photographs were lightened and cropped. (Photographed by Heather Waye)

matching tests was 13.3 (± 2.12 SD) out of 20 (67%), and whereas the average number of ventral matches (7.0 ± 1.18 out of 10) was higher than dorsal matches (6.3 ± 1.40 out of 10), they were not significantly different from each other (Fig. 4). Considering matching scores for individual salamanders (expressed as percent correct), four of the dorsal matches were not significantly different from 100% (salamanders #1, 10, 6, and 7); whereas, three were not significantly different from 20% (Fig. 5). The three that could not be matched were salamanders #3, 4, and 11. Of the ventral matches, four were not significantly different from 100% (#1, 10, 6, and 9), and four were not significantly different from 20%. The four that could not be matched were #3, 5, 7, and 11.

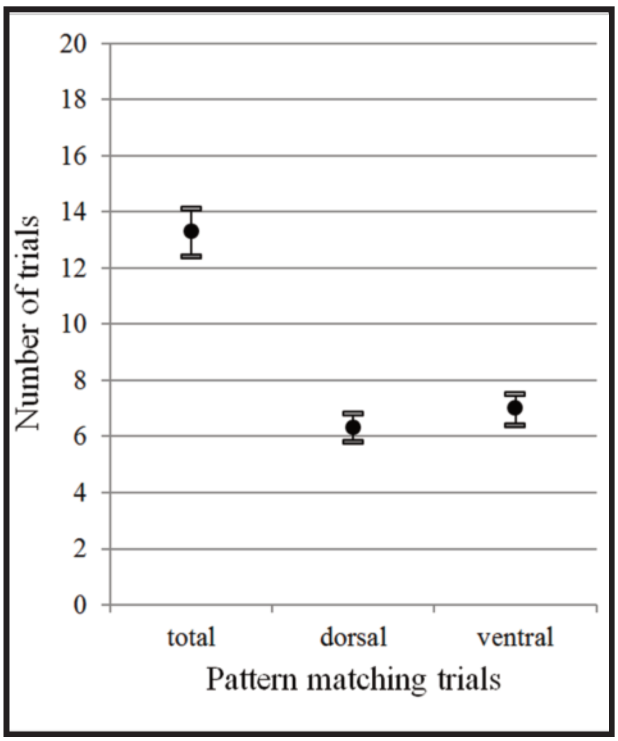

Figure 4. Average percent correct matches (and 95% CL) for Tiger Salamander (*Ambystoma tigrinum*) spot pattern matching trials. The category "total" is out of 20 trials, while "dorsal" and "ventral" are each out of 10 trials.

Discussion

A major assumption when using color patterns as unique identifiers of individual animals is that the pattern is stable enough to be recognizable when the animal is recaptured. In this case, the overall accuracy rate for 23 volunteers matching 11 salamanders was 67%, less than ideal for most purposes. The accuracy rate could improve with training, but contributing to this less-than-perfect rate is the fact that some of the salamanders used in this study exhibited pattern changes over time. At least half of the salamanders had a 50% or less matching rate using dorsal spot patterns, and three were essentially unrecognizable (the "correct" matches for these salamanders were largely the result of volunteers choosing "no match" when the matching photograph was not presented).

The dramatic change in spot pattern in the salamanders in our sample is interesting and worrisome. There have been claims that Tiger Salamander spots can change (e.g., Rose and Armentrout 1976; Stebbins 1985; Reaser 1995) or, conversely, that they change little after maturity (Church et al. 2007), but there is little published evidence supporting either position or

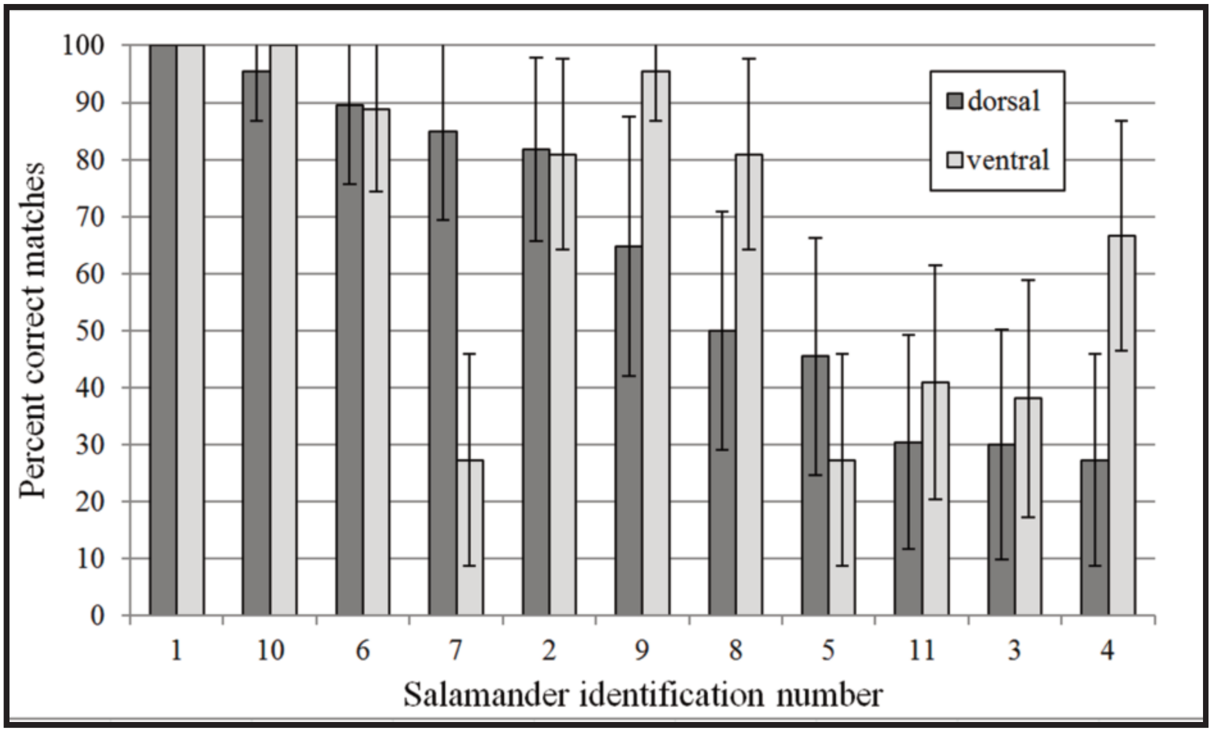

Figure 5. The proportion of correct matches (and 95% CL) for each salamander, separated into dorsal and ventral matches and arranged by dorsal scores. A score of 100% indicates perfect matching; for example, every volunteer presented with the November 2011 dorsal photograph for salamander #1 was able to choose the October 2012 photograph out of all of the offered choices. A score of 20% is no better than random.

documenting change in spot pattern. Clearly the patterns of some individuals do remain stable over longer periods of time (Church et al. 2007) but the lack of a second marking technique in many of the studies cited above means that dramatic changes in spot patterns will not be detected. In addition, the results of the current study demonstrate that significant remodeling of pigment patterns can occur in less than a year, even though these changes may go unnoticed over the course of several months. Kenyon et al. (2009) also found significant changes in the dorsal pattern (using toe clip patterns to verify) in one out of three recaptured *Litoria genimaculata* frogs over a two-month interval.

Why do spot patterns change? The spot pattern of an adult Tiger Salamander can differ from the pattern it had as a larva as part of the normal process of development, which may allow for adaptive modifications as the individual transitions between life history stages (Parichy 1998). Therefore, the environment that the individual inhabits may dictate whether its color pattern will change, and in what way (lighter or darker). On the other hand, Stebbins (1985) states that Tiger Salamanders of all subspecies may darken with age, suggesting that age, not environment, is a key factor in color change. In the current study, the changes to spot patterns occurred while the salamanders were in captivity and not in their natural habitat. However, the extent and especially the variety of changes in a relatively uniform and stable environment make it difficult to identify any particular factor directing those changes. Some changes may be the result of final pattern remodeling after metamorphosis, as nine of the 11 salamanders were between 8.5 and 10.5 cm SVL when captured, small enough to have metamorphosed earlier that year or to at least not have reached maturity (Semlitsch 1983; Lannoo and Phillips 2005). However, five of the 11 salamanders grew no more than 1 cm and still had noticeable pattern changes on either the dorsal or ventral surface. In addition, salamanders #8 and 10 grew the most over the course of the study (2 cm); whereas, salamanders #1, 2, and 5 grew the least (less than 0.5 cm), but there appears to be little relationship between the amount of growth and degree of pattern change (Fig. 5).

The spot patterns of most of the salamanders in this study were recognizable to some degree one year later; however, two salamanders (#3 and 11) changed dramatically in appearance. When initially captured, these salamanders had the spotted pattern typical of the Eastern Tiger Salamander, *A. tigrinum*, but after one year their color pattern was like that of the Blotched Tiger Salamander, *A. mavortium melanostictum* (Fig. 2). I have not observed any salamanders with this blotched pattern at my study site in west-central Minnesota, but they have been reported at other locations in the area (Carol Hall, pers. comm.). This dramatic change, from the color pattern typical of one species to the coloration of another species, has implications not only for Tiger Salamander studies that involve identification of recaptured individuals, but potentially for efforts to classify the different groups of Tiger Salamanders on the basis of appearance. The flexibility of color patterns of at least some Tiger Salamanders should be kept in mind when designing studies that rely on these patterns for individual or species identification.

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