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Changes in Tree Frog (*Hyla savignyi*) Coloration in Unstable Habitats at the Southern Border of Its Distribution

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Abstract

This study examined the relationship between tree frog (*Hyla savignyi*) coloring and its different seasonal habitats at the southern border of its distribution. The results show that tree frog color is affected by the dominant colors in its habitat, which vary seasonally, especially between winter and summer. Tree frog colors were various shades of green, white, brown, and black. No genetic marker was found to characterize the color. The ability of a small frog to infer its own time with the help of color changes occurring in the habitat on the southern border of its distribution, which are relatively broad, gives this species an advantage.

Keywords

Tree Frog, *Hyla savignyi*, Colors, Genetic Marker, Winter, Summer, Habitats

1. Introduction

The systematics of the tree frog (*Hyla savignyi*) at the southern border of its distribution has been extensively described in a number of studies [1] [2] [3] [4] [5]. Gvoždík [6] used molecular methods to describe *H. savignyi* found in Israel, Syria, Lebanon and Jordan. In Israel, an additional species, *Hyla heinzsteinitzi*, was also described [2], found at 730 - 895 m above sea level (ASL). Genetic diversity, life cycle, and adaptation to unstable water bodies, among other aspects, have been studied in this species in Israel. The life cycle of the tree frog in Israel was described by Degani [7]. Interactions between tree frog tadpoles and other amphibian species in Israel were intensively investigated [5] [8] [9] [10] [11] and their genetic variability among the country's different habitats has also been stu-

died [12].

Several studies have been performed on changes in the coloration of the European species *Hyla arborea*, with the dominant colors being green, yellow, gray and black, and the colors between these phenotypes [13] [14] [15]. The color variations in *H. savignyi*'s dorsal patterns have been described in a Cypriote population [15] and in Israel [5] [14]. This study is to find out the connection between the habitat and the different colors of the *Hyla arborea* in the habitat about which little is known.

The aim of the present study was to examine the influence of the semiarid habitat at the southernmost border of the tree frog's distribution, where large changes in climate are found between winter and summer, on tree frog colors.

2. Materials and Methods

Study Area

The study was conducted in the Golan Heights, Galilee and Hula Valley around water bodies where tree frog tadpoles have been previously found [10]. The tree frogs were photographed. When there was water in the ponds, the tree frog tadpoles were trapped by net and their length was measured using millimeter paper as described previously [7]. The locations surveyed are listed in **Table 1** (n = 10).

Sample collection and DNA extraction were as described previously [12]. Samples were cut up and placed in 1.5-ml Eppendorf tubes containing 95% ethanol and stored at −20˚C. Extraction was carried out using the QIAamp DNA Mini Kit (Qiagen, Germany) after proteinase K digestion. One mitochondrial

Table 1. Some of the habitats around the breeding sites of *H. savignyi* larvae in the various water bodies examined in northern Israel.

Longitudes	Latitudes	Altitude (m ASL)	
157,022	236,119	446	Fara Pond
157,022	236,119	665	Matityahu Pond
192,873	274,808	663	Navoraya Spring
197,970	267,307	110	Jauda Spring
186,972	270,855	810	Sasa Pond
174,280	249,160	300	Leshem Pond
184,200	252,800	195	Dir-Hanna Pond
157,022	236,119	446	Balad Spring
161,200	224,200	140	Gahar Stream
286,429	222,677	1060	Elrom Pond

Sample Collection and DNA Extraction (Degani, 2014) [16].

gene and one nuclear gene were analyzed by PCR amplification from genomic DNA and direct sequencing. The mitochondrial gene was cytochrome b (*Cytb*). Primers used in the current study for *Cytb* were Cytkoi 1 (*FW*) GTTCAGAATAGGAATTGGG [16] [17].

Analysis of Population Variation

The DNA sequences were analyzed as described in detail [12] [18]. Multiple sequence alignment and phylogenetic cluster analysis were carried out using the MegAlign computer program (Windows32 MegAlign6.1, DNASTAR Inc.). Phylogenetic trees were generated by the neighbor-joining method from distance matrices, which were based on the sequence differences found in the multiple sequence alignment [12].

3. Results

The tree frog body colors were found to differ in the different habitats and in different seasons, depending on the colors in the habitats. Examples of the color changes in a tree frog habitat are shown in **Figure 1**.

In winter and early spring, different shades of green are found in and around the water bodies. Colors gradually change from light green to brown and even black. The tree frogs exhibited matching colors throughout the year.

During the breeding season, when the tree frogs move to the ponds, the winter pond area is covered with green vegetation and the tree frog colors match these green shades. When the winter ponds dry up, the tree frog colors change to brown and black to match the habitat colors (**Figure 2**).

The breeding season of tree frogs in northern Israel starts at the end of February/beginning of March and ends sometime in April. The tree frogs breed in unpredictable habitats, where the winter tadpoles can grow and complete metamorphosis (**Figure 3**).

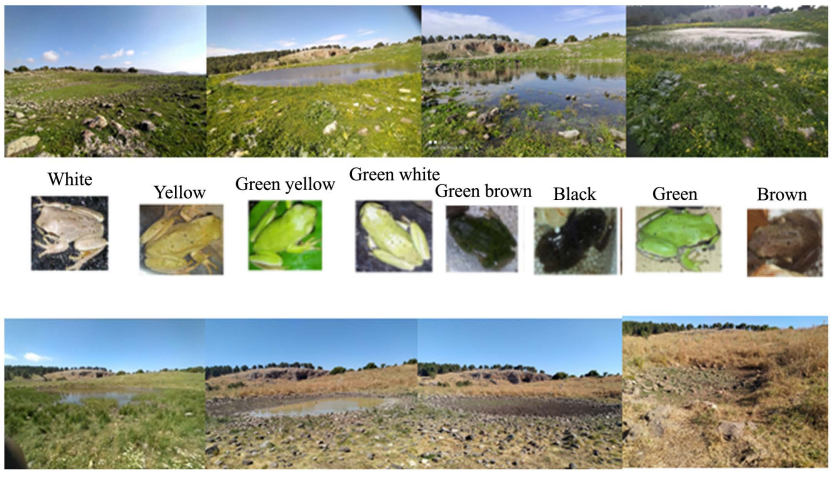
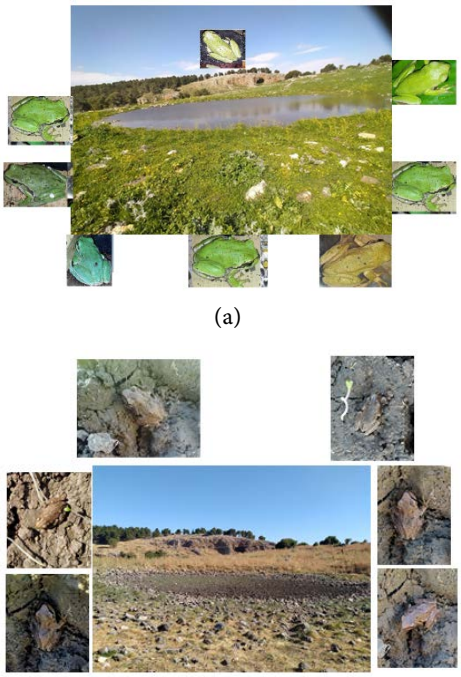


Figure 1. Changes in a winter pond (Nahalit Pool—35˚27'48"E 33˚04'56"N, 665 m ASL) and the area around the pond, and the various colors of the tree frogs found there, from winter to summer.



(a)

(b)

Figure 2. The winter pond area is covered with green on 22 February (a) and the dried pond on 24 May 2021. Brown and black tree frogs were found in the dry pond, between and underneath the stones (b).

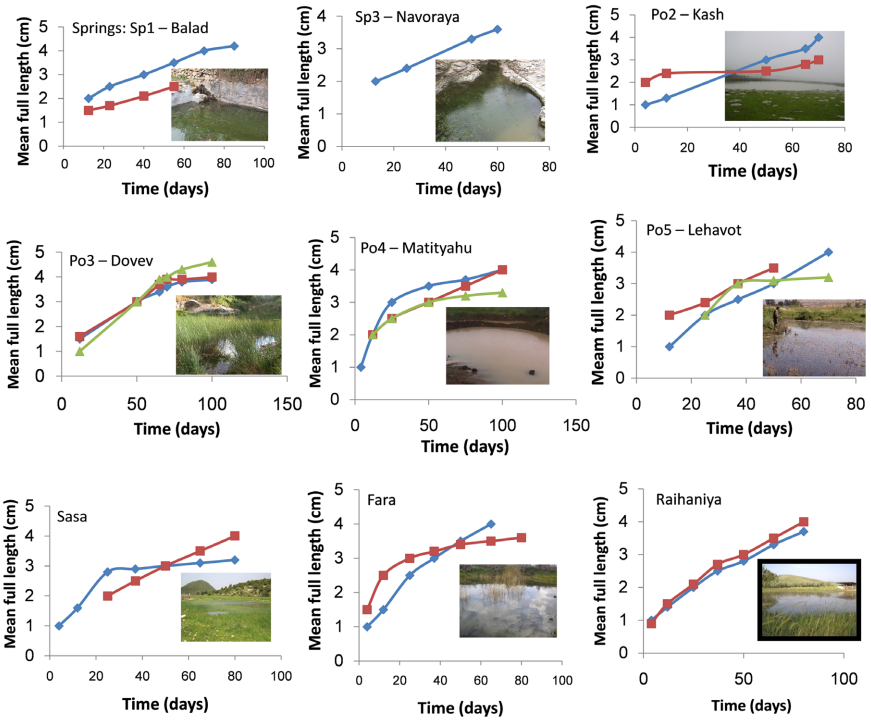


Figure 3. Winter ponds and springs in which the tree frog tadpoles grow during the winter. The curves in different colors represent tadpole growth in different years. During this period, the reproducing mature frogs are in different shades of green. Three main seasons. From winter to spring to summer.

The tree frogs move to the breeding places in February/March when green colors dominate. Sometimes the frog reaches a pond area to breed and the pond and plants have dried up. The area colors are brown and black, and the tree frogs change color from green to brown (Figures 1-4). The results of the present study show that the tree frogs move to their breeding sites when water may no longer be available consistently every year for breeding (Figures 1-3). In ponds like these, all of the tree frogs are brown, matching the colors of the dry breeding site.

No single color was recognized as typical of a particular habitat. All color patterns were found in many habitats according to the color of the subtract the frog are linked. A study of different molecular markers did not reveal any genetic markers that were strongly correlated with color (Figure 5).

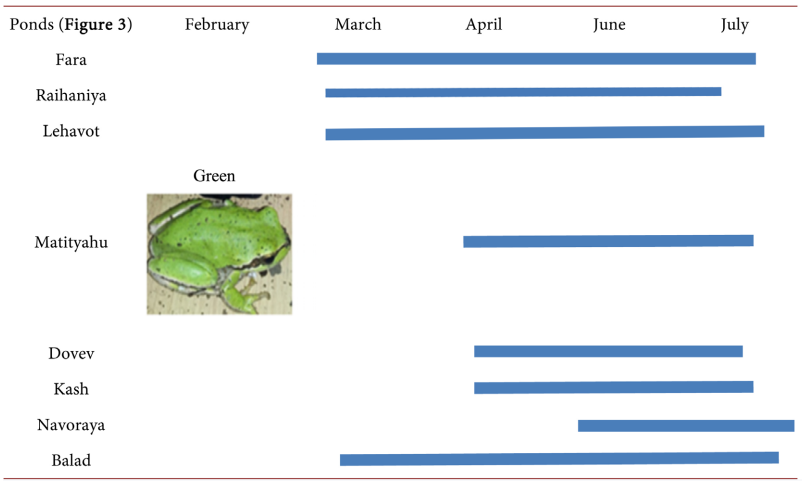


Figure 4. The period when water is found in the various winter ponds and springs and the tree frog tadpoles grow and complete metamorphosis.

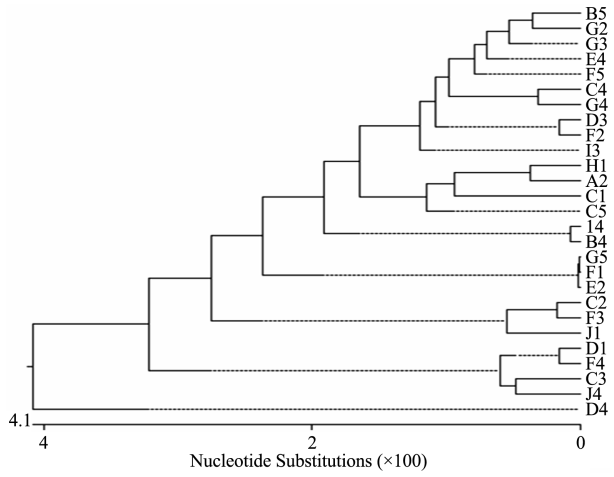


Figure 5. Based on the partial Cytb nucleotide sequences of *H. savignyi* from 10 habitats. The phylogenetic tree was constructed using the MegAlign program (DNASTAR) and CLUSTALW method. A—Elrom, B—Fara, C—Matityahu Pond, D—Navoraya Spring, E—Jauda Spring, F—Sasa Pond, G—Leshem Pond, H—Dir-Hanna Pond, I—Balad Spring, J—Gahar.

4. Discussion

Many variables of tree frog (*H. savignyi*) adaptation to its northern border habitat have been investigated, the most important being life cycle [7], physiological adaptation [19], genetic variability [5] [6] [12] [20], environmental behavior and its different colors [14] [15]. The unique contribution of this study is that for the first time, it shows the tree frog's ability to adapt its color to that of the changing habitat between winter and summer at the southern border of its distribution. This ability to change color is especially important in this region because the habitat shows extreme variations from summer to winter (**Figure 1**). During the breeding season, the frogs migrate to the winter pond which, along with its surroundings, is green due to the vegetation described in this and other studies [7] [21]. When the tree frog tadpoles complete their metamorphosis in the spring or summer, the whole area has changed color from green to brown, yellow and black. The tree frog's ability to change its color accordingly is a great advantage in terms of camouflage. In some cases, the trees frogs reach the pond to reproduce in the spring, but it has already dried up and the area has shifted from green to earth colors. The mature tree frogs do not reproduce but adapt their colors to the earth colors (**Figure 2**), in support of experiments examining the behavior of tree frogs on substrates with colors matching those found in nature [14]: there, tree frogs that chose a green substrate changed their color to green, while others that preferred yellow and black-brown substrates changed their colors to black [14]. In studies of the genetic variability [5] [6] [12] [20] of tree frogs in their different habitats, no genetic marker was found correlated to color, supporting results obtained in this work (**Figure 5**). All of the habitats had different color variants of tree frogs, and no genetic marker to color was found. Rather, the colors were correlated to the substrate.

In summary, the ability of a small frog to infer its own time with the help of color changes occurring in the habitat on the southern border of its distribution, which are relatively broad, gives this species an advantage [5].

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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