

Piebaldism in Roatán Spiny-tailed Iguanas, *Ctenosaura oedirhina*

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ABSTRACT - Abnormal colouration has been reported in both wild and captive reptile populations. In wild populations, piebaldism is a type of leucism where individuals have normal-coloured eyes, but exhibit white patches, giving them a blotched or mottled appearance. It is a common form of abnormal colouration. Some species show an ontogenetic shift in this patchy colouration, while others start life with a piebald appearance. We studied colouration across populations of Roatán Spiny-tailed Iguanas (*Ctenosaura oedirhina*), a species endemic to the island of Roatán, Honduras. In general this lizard exhibits an ontogenetic shift in colouration from grey with green mottling in hatchlings to dark grey/black with white or cream patches in adults; however, there is substantial colour variation among both adults and populations. Based on an ordinal regression analysis there is a significant positive trend between size (snout-vent length) and percentage of white colouration, suggesting an ontogenetic shift toward increasing piebaldism. Additional data, specifically recapture and genetic information, are needed to elucidate the mechanisms responsible for these patterns.

INTRODUCTION

Piebaldism is a type of leucism, where an individual has normally coloured eyes but lacks pigment on some parts of the body, giving a spotted or blotched pattern, which often changes over time. Leucism is caused by anomalous pigment cells in the skin that lack colour. In most cases the specific causes of leucism have not been identified, but it may result from dominant Mendelian inheritance, ontogenetic expression (vitiligo), or post-inflammatory leukoderma (Dyrkacz, 1981; Bechtel, 1995).

Albinism is a complete lack of pigment and is known to occur in many species of reptiles (see Bechtel, 1995 for a review), though it is thought to be rare (Krečsák, 2008). Piebaldism, however, appears to be one of the more common forms of abnormal coloration across reptiles (Bechtel, 1995), but generally occurs as an atypical coloration. For example, in *Tarentola boettgeri bischoffi*, a lizard endemic to several islands in the Selvagens archipelago in Portugal, piebaldism has only been documented once (Rocha & Rebelo, 2010). In contrast, in taxa such as *Sauromalus varius*, all adult males show conspicuous reticulated or banded piebald coloration (Hollingsworth, 1998).

Reptiles with albinism or piebaldism are usually rare in nature for many reasons, most notably it is presumed that they are more visible to predators (Krečsák, 2008). They may also have health impacts, directly via associated detrimental genetic mutations, or indirectly via compromised thermoregulation (Bechtel, 1978; Krečsák, 2008). Abnormal coloration can also be caused by nutritional or environmental factors altering pigmentation (Hayley-McCardle, 2012). *Conolophus marthae* has a distinctive pink coloration, which may be indicative of piebaldism. Gentile & Snell (2009) showed that when a

pink scale was removed, the resulting blood loss caused it to lose its pink colour, appearing white. This may indicate that the pink coloration is an effect of blood circulating to achromatic scales. They also found that these iguanas have lower levels of vitamin D and seem to avoid the sun more than other similar species (G. Gentile, pers. comm.), which could have negative health consequences.

Ontogeny may also play a role in lizard coloration, reflecting a change in diet, habitat, predation risk, or sexual maturity (Booth, 1990). For example, Piebald Chuckwallas (*S. varius*), found on two islands in the Gulf of California, exhibit an ontogenetic change from brown as hatchlings to yellow/orange with mottled black as they grow (Hollingsworth, 1998). This unusual coloration may have been the result of a bottleneck in the island population (McAliley et al., 2006). The Acklins Rock Iguanas (*Cyclura rileyi nuchalis*) also exhibits a colour shift from

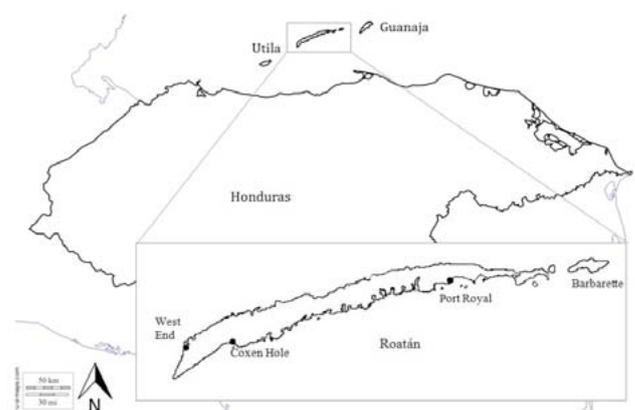


Figure 1 Map of Honduras. Inset shows close-up of the island of Roatán, with some larger towns identified. Adapted from Goode et al., 2016

hatchling to adult, which can vary over time. A colour shift within the adults seems to relate to size, with larger individuals tending to be lighter in colour (Iverson et al., 2016).

Though both ontogenetic colour change and piebaldism have been observed in iguanas, the combination of ontogenetic piebaldism has not yet been described. Delayed onset piebaldism has, however, been seen in wild-type corn snakes (*Pantherophis guttata*) bred in captivity, and in Burmese Pythons (*Python molurus bivittatus*) (Bechtel, 1995).

Roatán Spiny-tailed Iguanas, *Ctenosaura oedirhina*, are endemic to the island of Roatán, off the northern coast of Honduras (Fig. 1). They are listed as Endangered by the IUCN and are on Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (Pasachnik et al., 2010; Pasachnik & Ariano, 2010). In general, hatchlings are grey with green mottling. Most adult individuals are black and grey with “cream to white blotches arranged in bands” (de Queiroz, 1987). There is, however, great variation in the proportion of white observed in adults, ranging from the extremes of almost completely white to completely black (Figs. 2–4).

Differences in coloration among populations of the same species could indicate differences in genetics, environment or a combination of the two. Identifying and understanding these differences would be useful for future conservation efforts involving captive breeding or translocation of individuals. To understand these observations in the context of ontogeny, piebaldism, and sexual dimorphism, we quantified the relationship between colour and size, sex, and location. We also discuss potential mechanisms of colour change realizing that additional data are needed to fully elucidate these mechanisms.

METHODS

We collected colour data on 561 *C. oedirhina* over the course of four years from 2010-2014. Upon capture we measured snout-vent length (SVL) to the nearest millimeter using a tape measure and mass to the nearest gram using Pesola hanging scales. We sexed each individual by cloacal probing and an evaluation of external sex characteristics, including femoral pores, head shape, and dorsal crest spines. We photographed the left side of each individual before releasing it at the point of capture. Total processing took no longer than 20 minutes. Using the photos, one of us (SAP) later ranked each individual for percentage of white on a scale of 1-6 (1 = 0 % white, 2 = 1–25 %, 3 = 26–50 %, 4 = 51–75 %, 5 = 76–99 %, and 6 = 100 %) (see Figs. 2–4 for examples). We considered only the portion of the body from posterior of the front legs to anterior of the back legs on the left side, as this was typically the area with greatest variation across individuals. All photos were scored at the same time and each photo was scored independently at least two times. Once both scores were recorded we selected the photos with a discrepancy between scores and scored them once again, taking the majority rule as the final score. This was the case for 18% of the photos. Six photos were scored an additional, fourth time, due to discrepancy between the



Figure 2. A predominantly dark individual *C. oedirhina* (122 mm SVL, sex unknown as individual is a juvenile) from Roatán, Honduras. Colour ranking 1. Photograph by Stesha A. Pasachnik.



Figure 3. An intermediately coloured individual *C. oedirhina* (222 mm SVL, female) from Roatán, Honduras. Colour ranking 3. Photograph by Stesha A. Pasachnik.



Figure 4. A predominantly white individual *C. oedirhina* (270 mm SVL, male) from Roatán, Honduras. Colour ranking 6. Photograph by Stesha A. Pasachnik.



Figure 5. An individual *C. oedirhina* from Roatán, Honduras, that presumably suffered from an incomplete tail wound. The “second” tail appears to have regenerated with no pigment. Photograph by Stesha A. Pasachnik.

first three scores, and once again majority rule was used for the final score decision. We also noted all instances in which an individual had an injury resulting in a lack of pigmentation in the area of regrowth, such as regenerated tails (Fig. 5), combat wounds, and injured digits.

We used an ANOVA to determine if there was a significant difference in SVL across populations and then the Tukey's HSD post hoc test to determine differences among the populations. We performed an ordinal regression of SVL, sex, and location on colour ranking, using the six locations that had ≥ 10 individuals ($n = 546$) and six levels of coloration (see above). We also ran the same analysis with four levels of coloration (1 = 0–25 % white, 2 = 26–50 %, 3 = 51–75 %, and 4 = 76–100 %) to compare the effect of equal bin size. In the analysis, we compared five study locations to a sixth, reference population to evaluate the effect of location condition (e.g. disturbance, hunting pressure). The reference population was completely protected from illegal hunting, had little to no habitat alteration, and no supplemental food was available to the iguanas, thus it was likely to be the closest to the natural and historical population structure of this species. Fifty-six individuals were recaptured during the study. Nearly all of the recaptured individuals were larger adults (SVL mean = 223 mm, range = 150–283 mm). Individuals were considered to be mature if they had a SVL greater than 150 mm and to be hatchlings if they had a SVL < 60 mm (Pasachnik, 2013). We used qualitative statistics to determine any difference in growth, recapture intervals, or colour change because of the small sample size of recaptured individuals. All analyses were conducted in R Console and run with packages: foreign, ggplot2, MASS, Hmisc, and reshape (R Development Core Team, 2013).

The locations of our study sites are not identified by name due to the endangered status of the species, but can be obtained from the authors upon legitimate request.

RESULTS

Whether using four or six categories of coloration, the same variables were found to be statistically significant with only slightly different P values and regression coefficients (Table 1). We thus report the results using six bins, as we feel that those more accurately reflect the natural colour categories of the individuals. When compared to the reference population, we observed a significant positive trend in which larger (SVL) individuals had a higher percentage of white coloration ($P < 0.001$; Table 1, Fig. 6). Colour did not vary significantly by sex ($P = 0.06$; Table 1). However, colour did vary significantly across Locations 1, 2, and 3 ($P < 0.001$; Table 1, Fig. 7). In these three locations, regression coefficients were significant. These coefficients indicate the approximate coloration of the population (the more positive the coefficient, the more white the individual). Location 3 had the highest occurrence of white individuals and Location 2 had the highest occurrence of dark individuals (Table 1, Figs. 6 and 7). Size of individuals varied significantly between locations ($P = 0.0001$, Fig. 8). There was a significant SVL*Location interaction identified for Locations 1, 2, and 5 ($P < 0.01$ for all three locations; Table 1). This interaction term had a small, negative coefficient for all three locations.

Of the 56 recaptured individuals, four had more white coloration than at initial capture. The average change in SVL between recaptures for these four individuals was 37.7 mm (range = 3–86 mm). The average time between

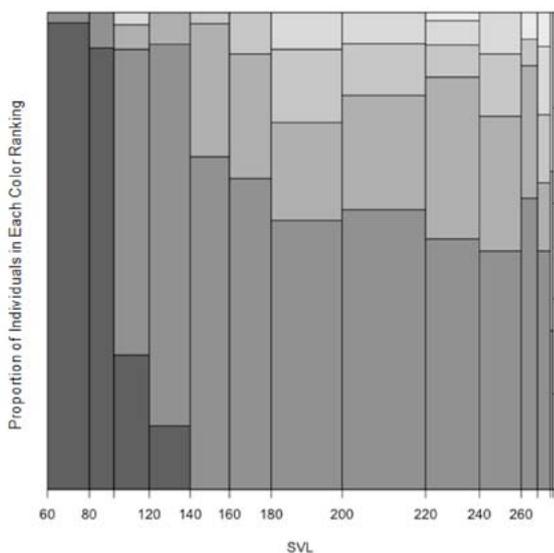


Figure 6. Ordinal regression of snout-vent length (SVL) to colour category in *C. oedirhina* from Roatán, Honduras. SVL sizes were binned by 20 mm increments and the width of bar is relative to number of individuals in that bin. Heights indicate the proportion of individuals in that size class that are in each colour category (percentage of white on a scale of 1-6, see Methods) with the darkest gray indicating individuals ranked 1 (0% white) and the lightest grey indicating individuals ranked 6 (100% white).

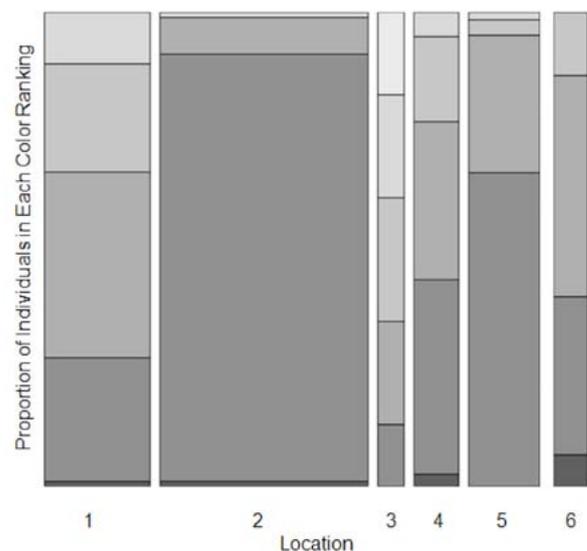


Figure 7. Ordinal regression of colour category by location in *C. oedirhina* from Roatán, Honduras. Height of bar segments indicates proportion of individuals in that size class that are in each colour category (percentage of white on a scale of 1-6, see Methods) with the darkest gray indicating individuals ranked 1 (0% white) and the lightest grey indicating individuals ranked 6 (100% white). Location 6 is an untouched population in pristine habitat, and was used as a reference population for comparison.

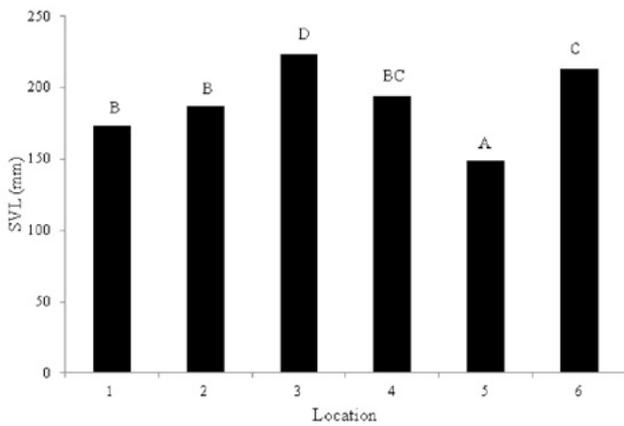


Figure 8. Average SVL of *C. oedirhina* at Study Locations in Roatán, Honduras. Letters indicate Turkey's HSD post hoc analysis results.



Figure 9. This *C. oedirhina* from Roatán, Honduras grew 31 mm over 160 days, a growth rate of 0.19 mm/day, nearly four times the average. A.) Initial capture on 28 February 2011 at a SVL of 134 mm. B.) Recaptured on 4 August 2011 at a SVL of 164 mm. Photographs by Stesha A. Pasachnik.

Table 1. Significance of Regression Variables in Four Level and Six Level Analysis. (*Indicates significance at $\alpha < 0.05$)

Variable	Four Level Analysis		Six Level Analysis	
	Coefficient	P value	Coefficient	P value
SVL	0.0227	< 0.001 *	0.0248	< 0.001 *
SEX	0.325	0.18	0.439	0.0611
Location - 1	1.648	< 0.001 *	1.923	< 0.001 *
Location - 2	-2.683	< 0.001 *	-2.253	< 0.001 *
Location - 3	2.301	< 0.001 *	2.744	< 0.001 *
Location - 4	0.407	0.384	0.576	0.227
Location - 5	-0.322	0.475	-0.0168	0.971
Location - 6	0.348	0.18	0.462	0.0611
SVL*Loc - 1	-0.0015	0.773	-0.0154	0.0056*
SVL*Loc - 2	-0.0017	0.807	-0.02	< 0.001*
SVL*Loc - 3	0.011	0.17	0.003	0.712
SVL*Loc - 4	-0.006	0.462	-0.013	0.149
SVL*Loc - 5	-0.009	0.173	-0.253	< 0.001*
SVL*Loc - 6	0.008	0.18	0.0189	0.0611

their recaptures was 681 days (range = 160–1043 days). Their average growth rate was 0.0568 mm/day. The other 52 recaptures received the same colour ranking in their first (n = 43), second (n = 8), and third (n = 1) captures. Their average change in SVL was 21.9 mm (range = 1–110 mm), average time between these recaptures was 386.3 days (range = 6–1315 days), and average growth rate was 0.0554 mm/day. The recaptured individuals with the increased white coloration had SVL and time between recaptures within the range of the other group and a very similar growth rate. One individual exhibited rapid colour change over a short period (Fig. 9A & B). We noted observations of injuries or regenerated tails that were abnormally white (n = 3, Fig. 5) in three captured individuals.

DISCUSSION

Though piebaldism has been observed in many reptile taxa, the patterns of occurrence and underlying mechanisms have not been rigorously assessed. Further, delayed onset piebaldism is something rarely observed, even in captive populations (Booth, 1990; Bechtel, 1995). The positive relationship between SVL and colour category found in *C. oedirhina*, elucidates a pattern of larger individuals exhibiting a higher degree of piebaldism. Hatchlings are exclusively dark in colour and usually have some green pigmentation, while individuals observed to be completely white are exclusively large adults. In addition, limited recapture data show a very slow increase in piebaldism over time. Given these patterns, we suggest an ontogenetic increase in the frequency of piebaldism in this species, although some individuals remain dark their entire lives. This pattern seems relatively clear, however understanding the underlying mechanisms is much more complex.

In Piebald Chuckwallas (*Sauromalus varius*) an ontogenetic shift in coloration has been reported to be the result of a genetic bottleneck in their island populations (Hollingsworth, 1998). In the present study, the effects of location, population structure, and genetics may all be influencing the observed colour patterns. Population structure varies by location. This is supported by the significant interaction term associated with three of the locations. Some locations also have human-supplemented food available, which may cause faster growth and in turn may skew the occurrence of piebaldism because of the increased number of larger individuals. While all of the study locations offer protection from hunting, some are better protected, and some have been protected longer and or more consistently. These factors could lead to populations with predominately older or larger individuals. Locations 1–5 have the added factor of hunting pressure outside of the site boundaries, limiting migration and reducing gene flow (Goode et al., 2016 ; Pasachnik & Hudman, 2016).

In order to better understand these various factors we used Location 6 as a reference population as it appears to be large, is not supplementally fed, and is less likely affected by inbreeding than many of the other populations (Pasachnik & Hudman, 2016). The physical isolation of this location and the presence of security eliminate hunting and introduced predators as a threat. Thus, the demographics

of this population are likely more natural than the other locations. As predicted, the degree of piebaldism is relatively evenly distributed within this population (Fig. 7).

This species occurs primarily in isolated populations across the main island of Roatán (Pasachnik, 2013; Goode et al., 2016). There is little migration among populations, resulting in low genetic diversity and inbreeding depression (Pasachnik & Hudman, 2016). This population genetic structure may account for at least some of the differences seen in the occurrence of piebaldism in *C. oedirhina*. In a randomly mating, wide-ranging species, one may expect to find equal variation in traits, such as colour, across all populations. However, this is not what we observed when comparing Locations 1–5. Location 4 had a varied colour distribution but a large percentage of extremely piebald individuals (Fig. 7), and was by far the smallest of our study populations. Location 2 on the other hand, had mostly darker individuals, and is one of the locations most affected by inbreeding. The isolation, size, differing predation histories, and inbreeding in these populations may have resulted in certain colour patterns becoming more prominent in these populations.

When considering protection, Location 4 had the longest and most consistent protection and also provides supplemental food. This is one of the smallest sites, hatchlings were rarely seen in this area, and the average SVL is on the larger side (Fig. 8). Thus, this population may exhibit more piebald individuals due to the increased number of larger or older individuals. Location 2 also provided supplemental food and was afforded protection, but to a lesser degree than Location 4. Many hatchlings and juveniles were seen at Location 2, but adult recapture rate was very low (Campbell & Pasachnik, pers. obs.). It is thus possible that this population was younger and fast-growing, which may account for the lack of piebald individuals. There were large adults in this population; however, since we were unable to age the adults in this study it may be that older, and not necessarily just larger individuals, present a higher degree of piebaldism. Similar to Location 2, Location 5 was made up of mostly darker individuals (Fig. 7) and has the smallest average SVL (Fig. 8). This location was afforded some level of protection, and hatchlings and large adults were present, but supplemental food was not provided. Following the previous logic one would then expect this population to have more darker individuals because the older individuals would be smaller. Given that this was not what was found, it might be the case that genetics is playing a larger role in fixing this population for a darker colour morph.

Some individuals exhibited postinflammatory leukoderma, a condition where injured tissue heals without pigment. An example of this can be seen in Figure 5, where an individual that had lost a portion of their tail had healed with completely white epidermis. Only a few of the captured individuals exhibited this characteristic; many were also caught with previous injuries that healed with normal pigmentation. This could be another indication that the increased loss of pigmentation in certain populations

is primarily genetically driven, as the individuals with the postinflammatory leukoderma were of varying sizes.

C. oedirhina adults exhibit size-related piebaldism, but is likely that demographic, anthropogenic, and genetic factors play a role in its expression across locations. The evolution of this species on an island with few natural predators could account for the persistence of the piebaldism in the population. Additional studies should focus on understanding the genetic basis for piebaldism in this species, as well as accumulating more recaptures to determine colour change over time.

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