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Natural Selection of Melanism in Costa Rican Jaguar and *Oncilla*: A Test of Gloger's Rule and the Temporal Segregation Hypothesis

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Abstract

The persistence of coat color polymorphisms—such as the coexistence of melanistic and “wild-type” coat color—is an ongoing evolutionary puzzle. We tested the predictions of Gloger's rule and the Temporal Segregation hypothesis that propose that melanistic individuals will (a) occur more frequently in closed tropical forest versus open habitat due to camouflage and thermoregulation advantages and (b) be more active during brighter times of the circadian and lunar cycle because black pigmentation is cryptic under bright illumination. Based on 10 years of camera trap records of jaguar and *oncilla* from dense tropical forest in Costa Rica, we compared activity and relative abundance of non-melanistic wild-type morphs (rosetted or spotted) versus melanistic morphs. Twenty-five percent of jaguar records in dense forest were melanistic compared with the global average of 10% in both open and closed habitats; 32% of *oncilla* records were melanistic compared with 18% overall in Brazil. Overlap analysis indicated that melanistic jaguars were more active during daylight hours compared with non-melanistic jaguars, which were more nocturnal and crepuscular. Likewise, melanistic *oncillas* were significantly more diurnal than non-melanistic *oncillas*; melanistic *oncillas* were also more active during full moon, while non-melanistic *oncillas* were less active. These results imply that melanistic jaguar and *oncilla* enjoy the adaptive benefits of superior camouflage when inhabiting dense forest and accrue a fitness advantage when hunting during conditions of brighter illumination. If true, natural selection would ensure that melanistic individuals persist when dense forest is retained but may be threatened by deforestation and accelerating human presence.

Keywords

melanistic, Felidae, coat color polymorphism, Gloger's rule, temporal segregation, jaguar, *oncilla*, Costa Rica

Of the 41 species of felids (Kitchener et al., 2017), 14 species exhibit a melanistic (black) coat morph that coexists with a wild-type “non-melanistic” coat pattern of visible spots, rosettes, or uniform color (Graipel et al., 2019; Schneider et al., 2012; Silva, 2017; Silva et al., 2016). The presence of alternative color morphs that coexist within a population, called coat color polymorphism, has long presented an evolutionary puzzle (Forsman et al., 2008; Graipel et al., 2014). The multiple independent evolutionary origins of the melanistic mutation in Felidae (at least eight) and the high frequency of color polymorphism in some felid species strongly suggest that melanism is favored by natural selection in wild populations (Eizirik et al., 2003; Schneider et al., 2012; Silva, 2017). Although it has been proposed that

melanistic coloration is adaptive under certain ecological conditions (Schneider et al., 2015; Silva, 2017), the functional advantages of melanism remain an enigma. The

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proposed functions of melanism include camouflage, thermoregulation, parasite resistance, and sexual selection (Graipel et al., 2019).

Of the six felid species that occupy Costa Rica, three are known to exhibit melanistic polymorphisms—jaguar (*Panthera onca*), jaguarundi (*Herpailurus yagouaroundi*), and northern tiger cat (*Leopardus tigrinus oncilla*); there are no records of melanistic puma (*Puma concolor*) or ocelot (*Leopardus pardalis*) in Costa Rica, and melanistic margay (*Leopardus wiedii*) have only recently been reported (González-Maya et al., 2018). It is reported that about 10% of jaguars globally are melanistic (Silva, 2017), and melanism in this species is inherited as a dominant trait caused by a 15-bp deletion in the MC1R gene that produces eumelanin in the background regions of the coat (Eizirik et al., 2003; Haag et al., 2010). As of this writing, there are only two published records of melanistic jaguar in Costa Rica in this century (Cartín & Carrillo, 2009; Sáenz-Bolaños et al., 2015). The northern tiger cat (*Leopardus tigrinus*) ranges from central Brazil to Central America and is locally known as oncilla (tigrillo in Spanish), which is the common name we will use hereafter. About 18% of Brazilian oncilla surveyed by Graipel et al. (2014) were melanistic. The genetic mechanism for melanism in oncilla is currently unknown, and nothing has previously been published regarding oncilla melanism in Central America.

Gloger's rule is named after C. W. L. Gloger, who noted the association between climatic variation (temperature and humidity) and animal coloration, observing that birds and mammals appear more pigmented in tropical regions (Rensch, 1929). A recent survey of the evidence for Gloger's rule confirmed the predicted effect of humidity, but not temperature, and concluded that humidity is the core climatic variable behind Gloger's rule (Delhey, 2019). The basic version of Gloger's rule predicts that endothermic animals will be darker in warm and wet tropical areas from increased deposition of melanin pigments, which may be favored by natural selection for camouflage or thermoregulatory advantages (Delhey, 2017, 2019; Jaroš, 2012; Silva et al., 2016). Darker individuals are hypothesized to be more cryptic and to thermoregulate more efficiently in shaded, closed habitats (Figure 1). Both mechanisms of Gloger's rule predict a higher frequency of melanism in tropical forests compared with open grasslands or savannah. In addition, melanistic individuals may be selected against in open areas where they would be more conspicuous, and where dark coats would absorb more solar radiation and heat up faster (Delhey, 2017; Silva, 2017), leading to the prediction that melanistic individuals should occur less frequently in open habitats.

It has been proposed that for coat color polymorphisms such as melanism to persist, conspecifics of different color morphs must be able to exploit different

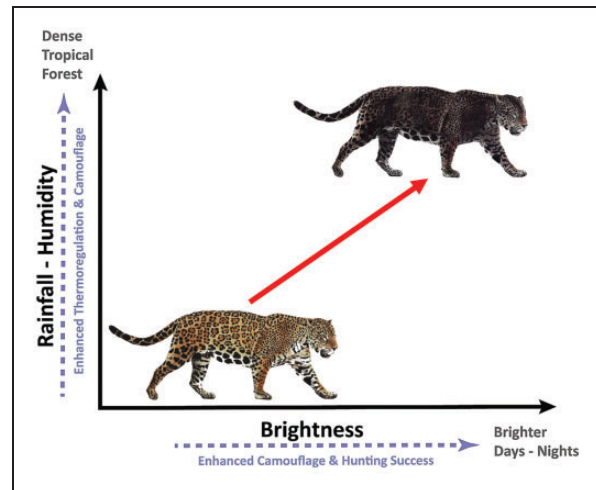


Figure 1. Graphical Representation of the Hypotheses to Explain the Adaptive Advantage of the Melanistic Color Morph Under Specific Ecological Conditions. Gloger's rule states that warm and humid conditions enhance the deposition of melanin pigments and that melanism is correlated with wetter and more humid conditions due to more efficient thermoregulation or camouflage of darker individuals, predicting a higher frequency of melanism in dense tropical forests than grassland and savannas. The Temporal Segregation hypothesis posits that melanistic individuals will be more camouflaged than wild-type color morphs (non-melanistic) in the brighter light of day or night and thus be more successful predators; we therefore predicted that melanistic individuals will shift their circadian activity to the day (or brighter hours of the night) and/or shift their nocturnal activity to brighter times of the lunar month (e.g., full moon). Artwork by Emma Mooring.

habitat niches and take advantage of a broader range of resources (Forsman et al., 2008; Graipel et al., 2014). The Temporal Segregation hypothesis proposes that dark morph melanistic individuals will have a cryptic advantage over wild-type non-melanistic individuals at times of brighter illumination (Figure 1). If melanistic coloration affords greater camouflage in a closed forest habitat during brighter times of the day or night, melanistic individuals could expand their feeding niche by being more active than non-melanistic individuals during periods of greater illumination. Graipel et al. (2014) proposed that cryptic melanistic oncilla should be more active during bright moonlit nights compared with non-melanistic (spotted) oncilla. Such temporal segregation could result from melanistic individuals choosing to be more active, non-melanistic individuals avoiding activity during bright nights, or both. Melanistic individuals hunting during bright lunar illumination would succeed in expanding their food niche and enhance their hunting success at a time when some prey animals may become "lunarphilic," increasing activity during full moon (Graipel et al., 2014; Prugh & Golden, 2014). By extension, the Temporal Segregation hypothesis could apply to brighter times

during the 24-hr day, with melanistic individuals being better camouflaged during the day versus night.

An additional factor is that many felids (including jaguar and oncolla) have white ear markings that function in intraspecific communication but are absent in melanistic individuals (Graipel et al., 2019). The combination of a dark coat and absence of white ear markings would confer melanistic felids with superior camouflage against visual predators and prey compared with more conspicuous non-melanistic conspecifics (Graipel et al., 2019). It has recently been proposed that the benefit of short-range communication and the cost of greater conspicuousness in non-melanistic individuals should be balanced by the adaptive advantage of superior camouflage in melanistic individuals, which might explain the evolutionary persistence of melanistic polymorphisms by balancing selection in which each morph gains a different benefit (Graipel et al., 2019).

The purpose of this study was to test the predictions for the selective advantage of melanism proposed by Gloger's rule and the Temporal Segregation hypothesis (Delhey, 2017; Graipel et al., 2014), particularly in regard to enhanced camouflage. Here, we present data from our long-term camera trap surveys in and around the Talamanca Cordillera of Costa Rica to compare the ecological and behavioral correlates of melanistic versus non-melanistic coat colors in jaguar and oncolla. Our hypotheses are that (a) melanistic felids have an adaptive advantage compared with non-melanistic individuals, (b) melanistic individuals will tend to be found in habitats in which the thermoregulatory and camouflage benefits of melanism can be most fully realized, and (c) melanistic felids will be active at times of the day or month when the camouflage advantages of melanism can garner greater resource exploitation in the form of superior hunting success.

The following were the predications that we explicitly tested:

- The percentage of melanism in tropical forest habitat will be higher than the global average (10%) for jaguar in open and closed habitats and the Brazilian percentage (18%) for oncolla.
- Melanistic jaguar and oncolla will be more active during the day than non-melanistic jaguar or oncolla.
- Melanistic jaguar and oncolla will be more active during bright lunar phases than the non-melanistic morphs.

Finally, we suggest that our results raise troubling concerns about the survival of the melanistic polymorphism given the current threats to populations of jaguar and other felids from fragmentation, habitat loss, and increasing human presence (De la Torre et al., 2018; Srbek-Araujo et al., 2018).

Methods

Study Area

We conducted camera trap surveys at 12 sites in the mountains of the Talamanca Cordillera or lowland forest in the Pacific lowlands (Figure 2). Across sites, rainfall varied from 300 to 800 cm per year, and temperature varied from 10°C to 20°C in the highlands and 24°C to 32°C in the lowlands (Costarica.com, 2019). All sites were characterized by two distinct seasons, with the dry (or summer) season from December to April and the wet (or winter) season from May to November. Rainfall is largely dependent on location within the country, leading to a range of 300 to 800 cm of average precipitation throughout our sites. The average temperature in the highlands varies between 10°C to 20°C depending on altitude and location; lowland temperatures average 24°C to 32°C (Santa Ana Conservation Center, 2019; Costarica.com, 2019).

We worked in collaboration with national park officials and local community volunteers in national parks, private reserves, and biological corridors from June 2010 through August 2019. These areas range from primary forest in protected areas to a mosaic of primary and secondary forest fragments in biological corridors. The lowland sites (Alexander Skutch Biological Corridor, Cabo Blanco National Park, Proyecto Campanario, La Marta National Wildlife Refuge, Carara National Park, and La Cangreja National Park) are located from 28 m to 1,000 m above sea level. The highland sites (Chirripo National Park, La Amistad International Park, Los Quetzales National Park, Tapantí Macizo de la Muerte National Park, and the Savegre Lodge Reserve in the upper Rio Savegre Valley) range from 1506 m to 3,464 m in elevation. Bosque de Agua Biological Corridor and El Copal Reserve are located within the mid-elevational range of 1,000 to 1500 m. No major changes to protection status or environmental impacts occurred during the study.

Camera Traps

Cameras were deployed at each of 12 survey sites from June 2010 to August 2019 (Table 1). The six highland sites were in dense tropical montane forest, and the six lowland sites were in lowland rainforest. Bushnell Trophy Cam™ units were deployed either as single units or as paired camera stations to image both sides of the animal. The Bushnell Trophy Cam™ has a passive infrared sensor that detects the surface temperature of objects in the detection zone and triggers the camera when a rapid change in temperature is detected due to motion and body temperature of a passing mammal (Welbourne et al., 2016). The Trophy Cam™ is distinguished by a very quick detection trigger and recovery

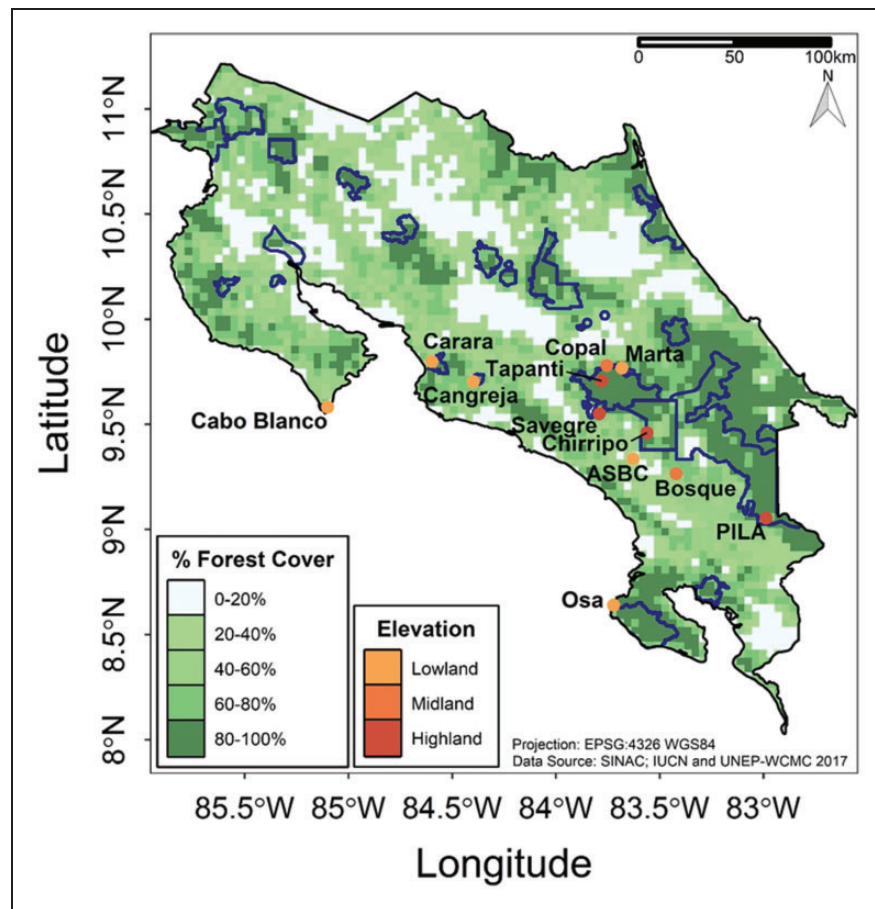


Figure 2. Map of Camera Trap Survey Sites in Costa Rica With Reference to Forest Cover and Elevation. Outlines in blue are the boundaries of the SINAC national park protected areas; forest cover is in shades of green; elevation is by color of the survey site points; and short names of the 12 survey sites are in black (see Table 1 for details).

SINAC = National System of Conservation Areas; IUCN = International Union for Conservation of Nature; UNEP = United Nations Environment Programme; WCMC = World Conservation Monitoring Centre.

speed, a 75° detection angle and 42° field of view, and long battery life (Bushnell Corporation, Lenexa, KS, USA). We avoided using cameras with incandescent white flash because studies have shown that the white flash can produce a startle effect that contributes to avoidance behavior (Gibeau & McTavish, 2009; Meek et al., 2014, 2016; Sequin et al., 2003). The cameras were equipped with a 2- to 8-GB SanDisk SD memory card (Western Digital Technologies, Milpitas, CA, USA) and Ultimate lithium AA batteries (Energizer Holdings, St. Louis, MO, USA), often with silica desiccant capsules (DRICAP capsule dehydrators; Ted Pella Inc., Redding, CA, USA) to minimize humidity inside the camera. Camera units were attached to trees approximately 1 m from the ground with Python 3/8" cable locks (Master Lock Company, Oak Creek, WI, USA) within steel security boxes to deter vandalism (CAMLOCKbox, Green Bay, WI, USA). Units were positioned at 1 to 2 km intervals, primarily along established trail systems

and access roads within protected areas. Although species vary in trail use, felids and large mammals in general tend to walk along trails (Harmsen et al., 2010). We assume that activity patterns recorded on trails reflects activity patterns off trails. This assumption is supported for large felids (e.g., jaguar, puma, ocelot) that prefer to travel along trails (Harmsen et al., 2010) but may be less accurate for small felids such as oncilla that may use trails less often. The cameras were oriented to face down the trail (vs. perpendicular across the trail) to maximize the time that animals were within the camera's field of view, thus maximizing the number of photos taken. Garmin eTrex GPS units (Garmin Ltd., Olathe, KS, USA) were used to record the coordinates and elevation of each camera station. Cameras were operated continuously, and all photos were stamped with the date and time of occurrence; camera monitoring involved switching out SD memory cards and checking battery charge.

Table 1. Camera Trap Surveys With Number of Cameras, Camera Trap Days, Survey Period, Coordinates, Elevation, and Elevation Type.

Study site (abbreviation)	Number of cameras ^a	Camera days	Start date	End date	Latitude	Longitude	Elevation (m)	Elevation type ^b
Alexander Skutch Biological Corridor (CoBAS)	4–18	3,472	7/3/2012	3/30/2015	9.5633	–83.7839	338–888	Lowland
Bosque de Agua Biological Corridor (CoBBA)	8	597	6/21/2016	2/19/2017	9.2650	–83.4210	851–1,672	Low/Mid/High
Cabo Blanco National Park (PNCB)	6	2,034	7/17/2015	8/25/2018	9.5820	–85.1010	63–321	Lowland
Carara National Park (PNC)	3	653	9/26/2014	4/17/2019	9.7984	–84.5979	28–64	Lowland
Proyecto Campanario Biological Station (PC)	10	1,533	7/16/2016	8/27/2017	8.6397	–83.7226	62–158	Lowland
Chirripo National Park (PNCH)	2–22	6,710	6/26/2012	7/8/2019	9.4599	–83.5619	2,308–3,464	Highland
El Copal Private Reserve (ECR)	4	634	1/8/2013	9/17/2016	9.7804	–83.7546	1,158–1,225	Midland
La Amistad International Park (PILA)	5	7,613	5/30/2017	6/25/2019	9.0539	–82.9876	2,086–2,308	Highland
La Canguja National Park (PNLC)	5	3,972	10/24/2014	5/30/2019	9.7001	–84.39206	338–584	Lowland
La Marta National Wildlife Refuge (LMR)	2–4	720	7/20/2013	4/26/2015	9.7685	–83.6823	747–1,000	Lowland
Tapantí Macizo de la Muerte National Park (PNTMM)	4–14	10,354	6/18/2012	9/17/2018	9.7068	–83.7793	1,506–2,803	Highland
Savagrey Valley /Los Quetzales National Park (PNLQ)	5–15	19,988	6/29/2010	8/16/2019	9.5502	–83.7911	2,112–3,118	Highland
Total = 12 sites	58–114	58,280	6/29/2010	8/16/2019				

^aNumber of cameras is the range of total cameras operating during one sampling period in a given survey. For example, in the summer of 2012, there were 15 cameras operating in the Savagrey Valley Private Reserve but only 5 operating in the fall of 2013.

^bElevational categories: Lowland <1,000 m, Midland 1,000 to 1,500 m, and Highland >1,500 m.

Scent Stations

From 2012 on, all camera stations had scent stations within the field of view to stimulate passing animals to slow down and investigate the novel scent. Because less light is produced by infrared flash compared with the white flash, the scent stations were beneficial in maximizing photo quality by minimizing blurriness of night photos. Scent stations are widely used to increase photo quality for studies aimed at species and individual identification (Barea-Azcón et al., 2007; Conner et al., 1983; Randel & Peace, 2010; Thorn et al., 2009; Travaini et al., 1996; Weaver et al., 2005). In an experimental study, Mills et al. (2019) concluded that attractants are an effective tool for increasing the detection of elusive carnivores, although responses are species-specific. Each station consisted of a free-standing polyvinyl chloride pipe (sometimes a strap attached to a tree) with a piece of sponge scented with Calvin Klein's "Obsession for Men" cologne (Calvin Klein Inc., New York, NY, USA) inside a clear plastic tube. The scent is only detected at short range; thus, scent stations do not attract animals that are not already on the trail. Studies have found that scent stations do not affect temporal activity, distance traveled, or total photo capture (Brackzkowski et al., 2016). The Obsession was recharged every 1 to 2 months with 4 to 5 sprays; investigators used surgical rubber gloves to avoid leaving human scent.

Database Creation

Once the SD memory cards were collected from cameras, photographs were manually sorted into folders by date, species, and site, and then loaded into Camera Base 1.7 data management software (Tobler, 2015). Photographic records were considered independent if photos of a given species at a particular camera station were at least 30 min apart, which is a commonly used standard (Anile & Devillard, 2016; Ridout & Linkie, 2009; Si et al., 2014; Sollmann, 2018; Van Berkel, 2014). Photographs of multiple individuals of the same species were classified as a single observation, as were photographs from paired cameras. Only independent records were included in the data analysis. Data were exported from Camera Base into an Excel .CSV file for analysis. The relative abundance indices (RAI) were calculated for each species from all survey sites using the equation: $RAI = (\text{number independent records}) / (\text{days camera was active}) \times 1,000$.

Circadian and Lunar Activity Patterns

To characterize activity patterns, records between sunrise and sunset were categorized as daytime, while records between sunset and sunrise were regarded as nighttime. In the tropics, the "clock time" of sunrise/

sunset changes slightly over the course of the year depending on distance from the equator and time of year. In our dataset, the average time of sunrise/sunset was 05:27/17:34 for jaguar, and 05:29/17:37 for oncilla—the actual sunrise/sunset clock time was as much as 30 min before or after the average time, a significant change in illumination for wildlife. For this reason, it is important to use “sun time” rather than clock time to analyze day/night activity patterns (Nouvellet et al., 2012). Species were classified as diurnal (<10% of records at night), nocturnal ($\geq 90\%$ of records at night), mostly diurnal (10% to 29% of records at night), mostly nocturnal (70% to 89% of records at night), or cathemeral (30% to 69% of records at night) according to Azevedo et al. (2018, and references therein). To account for the successive changes of the sun’s position throughout the year (Nouvellet et al., 2012), we used the “sunTime” function of “Overlap” version 0.3.2 as detailed later (Meredith & Ridout, 2018a, 2018b). “Overlap” works with time in radians, normally mapping 06:00 to $\pi/2$ and 18:00 to $3\pi/2$. The “sunTime” function remaps these times, so that $\pi/2$ corresponds to sunrise and $3\pi/2$ to sunset, using the dates and locations of the observations (Meredith & Ridout, 2018a). Lunar activity was characterized using the “getMoonIllumination” function from the package “suncalc” version 0.5.0 (Thieurmel & Elmarhraoui, 2019) to obtain the moon phase for each observation based on its date. Moon phase is a value from 0 to 1, with the designations 0 as New Moon, 0.25 as First Quarter, 0.5 as Full Moon, and 0.75 as Last Quarter. These values were linearly scaled for each complete lunar cycle in radians by multiplying each value by 2π .

Activity Patterns and Coefficient of Overlapping (Δ)

All analysis was done in the R programming language (R Core Team, 2019). To determine the overlap of activity budgets between melanistic and non-melanistic felids, the package “Overlap” version 0.3.2 was used (Meredith & Ridout, 2018a, 2018b) following the two-step method developed by Ridout and Linkie (2009). First, the circadian or lunar phase activity patterns were determined using either a kernel density with a standard bandwidth of 1 and a bandwidth adjustment of 0.8 for large sample sizes ($n > 75$), or with a nonnegative trigonometric sum distribution for small sample sizes ($20 \leq n \leq 75$; Ridout & Linkie, 2009). The minimum sample size was set at 20 independent observations to minimize overestimation of Δ (discussed later; Lynam et al., 2013; Rowcliffe et al., 2014; Tan et al., 2018). This calculation used the standard assumptions that (a) observations were random samples from a continuous distribution and (b) captures were equally likely to occur at any time during the period when active (Ridout & Linkie, 2009). Next, the

coefficient of overlapping (Δ) was calculated either using the $\Delta 1$ measure for small sample sizes or $\Delta 4$ for large sample sizes (Ridout & Linkie, 2009). The Δ values fall between 0 (no overlap) to 1 (complete overlap) and represent the shared area under two density curves.

Statistical Measures

For this study, Δ was used to assess possible divergence of temporal activity patterns between melanistic and non-melanistic conspecifics. Currently, the field of statistics associated with this type of cyclical data (“circular statistics” or “directional statistics”) is relatively undeveloped, and thus, there are few statistical tests available. In light of this, we chose to calculate and report three statistical measures to assess potential divergence of temporal activity patterns between melanistic and non-melanistic conspecifics, as no single method is currently accepted as the standard. The first statistic we report is Watson’s two-sample U^2 test with ties (Zar, 1999, section 27.6). A p value for this statistic was calculated through the chi-square approximation (Tiku, 1965). The second statistic we report is W_r , a uniform scores statistic (Fisher, 1993, section 5.3.6). A p value for this statistic was calculated using the chi-square distribution according to the recommendations of Fisher (1993). The third statistical method we used was Fisher’s exact test (Upton, 1992). For this test, observations of each species were binned by time into 12 bins corresponding to intervals of 2 hr each. The p values comparing activity overlap were then calculated using the Fisher’s exact test with a Monte Carlo simulation of 1,000 replicates. (The chi-squared test could not be used because Cochran’s rule was violated for most of our data: not all bins were >0 , or $>20\%$ of bins were <5 .) We determined that 1,000 replicates were necessary to reduce the variation inherent with the use of a Monte Carlo simulation. To test whether any one of the color morphs (jaguar melanistic, jaguar non-melanistic, oncilla melanistic, and oncilla non-melanistic) deviated significantly from uniformity by having periods of higher activity during the 24 hr period, we ran Rao’s Spacing Test because it has the ability to detect multimodality (Rao, 1969; Mutwiri et al., 2013). Rao’s test was implemented via the `rao.spacing.test` in the “circular” package version 0.4–93 (Agostinelli & Lund, 2017). All of the R code used in this analysis is available online at GitHub (<https://github.com/rbotts/Melanism>).

Results

Melanistic Jaguar

Collectively, we analyzed photographic records from a total of 58,280 camera trap days (Table 1). We observed both melanistic and non-melanistic jaguar in the camera

trap surveys (Figure 3). From 2010 to 2019, we amassed a total of 104 independent records of jaguar, of which 25% were melanistic (melanistic: $n = 26$; non-melanistic: $n = 78$). Likewise, the Relative Abundance Index (RAI) of melanistic jaguar represented 25% of the RAI of all morphs of jaguar (Melanistic RAI = 0.480; Non-melanistic RAI = 1.440). Due to single cameras and some blurred photos, we were unable to individually identify all jaguar records. However, we estimate that the number of individuals represented in our surveys was a minimum of 15 non-melanistic and 3 to 4 melanistic individuals (17% to 21%).

As predicted, melanistic individuals were more active during daylight hours compared with non-melanistic jaguars, which were more nocturnal (Figure 4). The activity budget of non-melanistic jaguar tended to be more crepuscular and evenly divided between day and night (51% diurnal; 49% nocturnal) compared with the more diurnal activity of melanistic jaguar (58% diurnal, 42% nocturnal; Figure 4). The circadian activity pattern of melanistic jaguar (Figure 5) was significantly different from uniformity by Rao's Spacing Test ($n = 26$, $U = 166.1$, $p < 0.05$), whereas the circadian activity of non-melanistic jaguar (Figure 5) was not significantly non-uniform by Rao's test ($n = 78$, $U = 134.3$, $p > 0.10$). Melanistic jaguar had an activity peak during the day from about 9:00 a.m. to 3:00 p.m., while non-melanistic jaguars were most active in the early morning (3:00 to

6:00 a.m.) and early night (7:00 to 9:00 p.m.; Figure 5). The coefficient of overlapping Δ between non-melanistic and melanistic jaguar for circadian activity was 0.77 (95% CI [0.63, 0.90]), which was significantly different

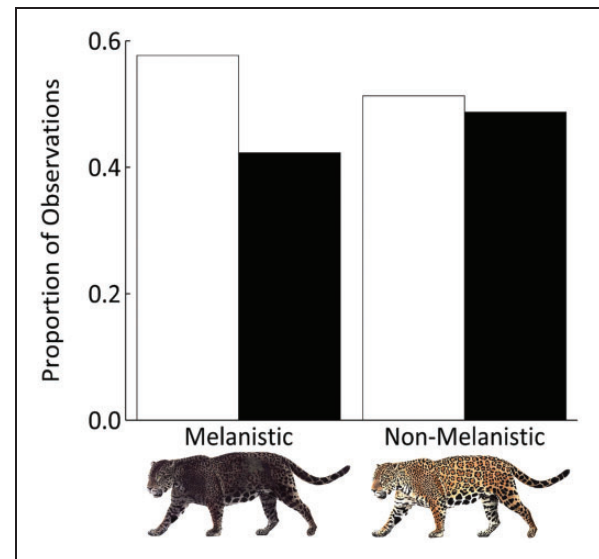


Figure 4. Proportion of Photographic Observations During the Day (White Bars) Versus Night (Black Bars) for Melanistic ($n = 26$) and Non-melanistic ($n = 78$) Jaguar. Melanistic jaguars were more active during the day (58% of records) compared with non-melanistic jaguars (51%), based on 10 years of camera trap records in Costa Rica. Artwork by Emma Mooring.



Figure 3. Camera Trap Photos of Non-melanistic (Top) and Melanistic (Bottom) Jaguars. Photographs from the camera trap survey.

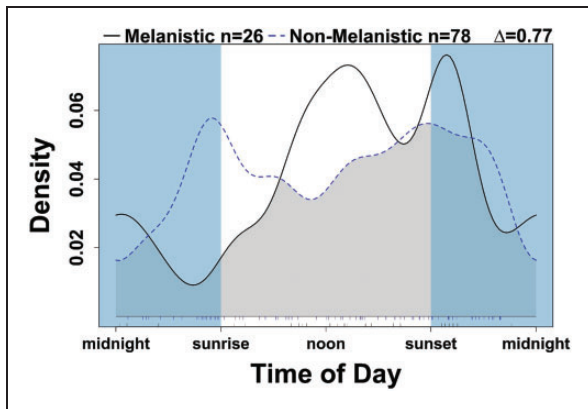


Figure 5. Circadian Activity Overlap Between Non-melanistic (Dotted Line) and Melanistic (Solid Line) Jaguars; Gray Is the Area of Overlap. Melanistic jaguars were more active at midday compared with the more crepuscular activity pattern of non-melanistic jaguars. The coefficient of overlapping Δ between non-melanistic and melanistic jaguar was 0.77 and significantly different by Fisher's exact test ($p = .01$).

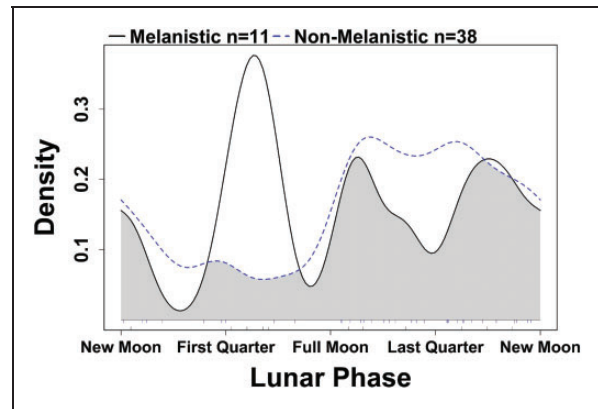


Figure 6. Lunar Phase Activity Overlap Between Non-melanistic (Dotted Line) and Melanistic (Solid Line) Jaguars; Gray Is the Area of Overlap. It appears that melanistic jaguars were more active during the first quarter of the moon compared with non-melanistic jaguars, although the sample size of nocturnal records for melanistic jaguar was small ($n = 11$).

by Fisher's exact test ($p = .01$) but not by the Watson's two-sample U^2 test or the W_r uniform scores test (Watson's $U^2 = 0.118$, $p = .20$; $W_r = 2.912$, $p = .23$). Although it appeared that melanistic jaguars were more active during the first quarter of the moon compared with non-melanistic jaguars, this may be the result of a small sample size of nocturnal records (Figure 6). In fact, the sample size of nocturnal records was too small to calculate overlap and run the associated statistical tests (melanistic $n = 11$, non-melanistic $n = 38$).

Melanistic Oncilla

Both melanistic and non-melanistic oncillas were observed in the surveys (Figure 7). From 2010 to 2019, we recorded a total of 203 independent photos of oncilla, of which 32% were melanistic (melanistic: $n = 65$; non-melanistic: $n = 138$). Likewise, based on the RAI, melanistic oncilla represented 29% of the RAI of all morphs of oncilla (Melanistic RAI = 1.200; Non-melanistic RAI = 2.918). Because oncillas are small and many photos were blurred because the animal was moving, we can estimate the number of oncilla individuals in the survey assuming a maximum of one melanistic and one non-melanistic individual potentially detected per camera station (typically 1 to 2 km apart), which yielded 55 non-melanistic and 30 melanistic oncillas (35% melanistic).

We tested the prediction that melanistic oncilla would be more active during brighter times of the circadian or lunar cycle because they are better camouflaged in bright light compared with non-melanistic individuals. Activity analysis showed that melanistic oncillas were much more

active during the day compared with non-melanistic oncillas, which were more nocturnal (Figure 8). The activity budget of non-melanistic oncilla was primarily nocturnal (85% nocturnal; 15% diurnal) compared with the more diurnal activity of melanistic oncilla (66% nocturnal, 34% diurnal; Figure 8), as we predicted. The circadian activity pattern of melanistic oncilla (Figure 9) was not significantly different from uniformity by Rao's Spacing Test ($n = 65$, $U = 139.2$, $p > 0.10$); however, the circadian activity of non-melanistic oncilla was significantly non-uniform ($n = 138$, $U = 159.1$, $p < 0.001$). The coefficient of overlapping Δ between non-melanistic and melanistic oncilla for circadian activity was 0.77 (95% CI [0.67, 0.87]), which was significantly different by all three statistical tests (Watson's $U^2 = 0.231$, $p = .02$; $W_r = 6.694$, $p = .04$; Fisher's exact test: $p = .05$; Figure 9). Analysis of activity overlap of oncilla during the lunar phases (Figure 10) gave a coefficient of overlapping $\Delta = 0.87$ (95% CI [0.75, 0.97]). The lunar phase analysis indicated that melanistic oncillas were more active during the full moon and last quarter compared with non-melanistic oncillas that were more active during the first quarter (Figure 10), but this was not significantly different (Watson's $U^2 = 0.088$, $p = .35$; $W_r = 2.332$, $p = .31$; Fisher's exact test: $p = .70$; Figure 10).

Discussion

Based on 10 years of survey data, we tested the hypothesis that melanism gives a selective advantage to jaguar and oncilla in dense tropical forest and specifically that the dark morph provides a cryptic advantage during

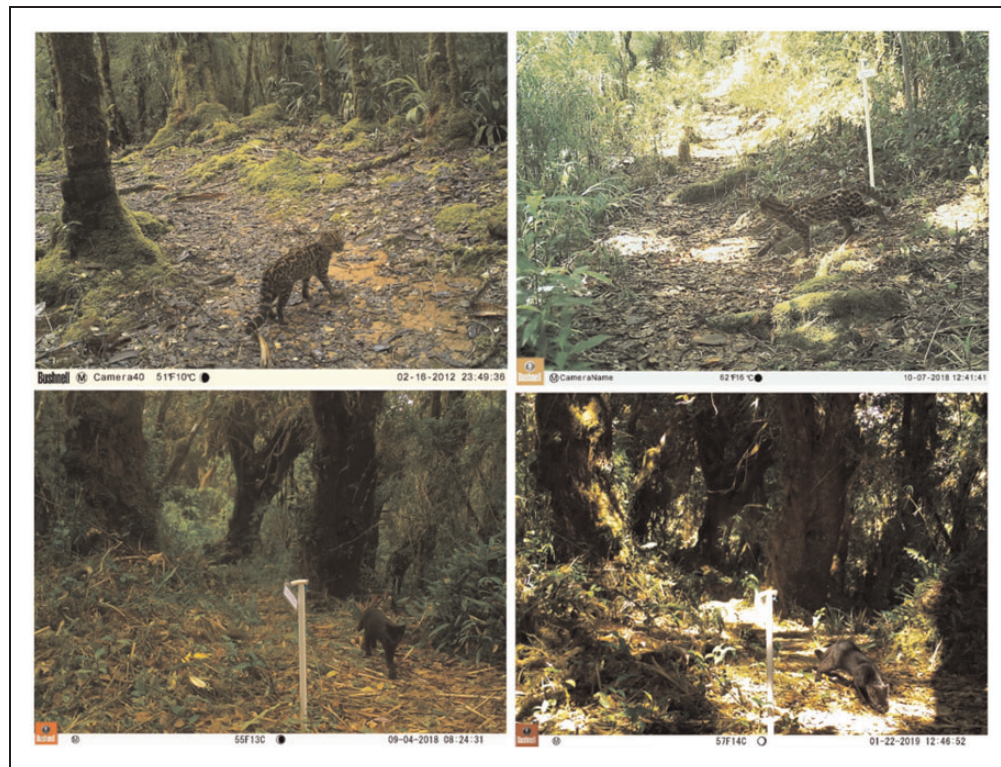


Figure 7. Camera Trap Photos of Non-melanistic (Top) and Melanistic (Bottom) *Oncilla*. Photographs from the camera trap survey.

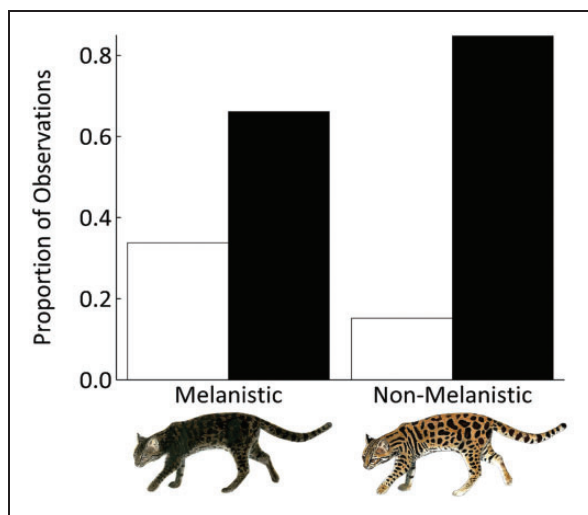


Figure 8. Proportion of Photographic Observations During the Day (White Bars) Versus Night (Black Bars) for Melanistic ($n = 65$) and Non-melanistic ($n = 138$) *Oncilla*. Melanistic *oncillas* were more active during the day (34% of records) compared with non-melanistic *oncillas* (15%); conversely, non-melanistic *oncillas* were more nocturnal (85% of records) compared with melanistic *oncillas* (66% of records). Artwork by Emma Mooring.

brighter times of the circadian and lunar cycle. Gloger's rule predicts that coat color polymorphisms are driven by climate variables (e.g., rainfall and moisture), with increased fitness of melanistic individuals resulting

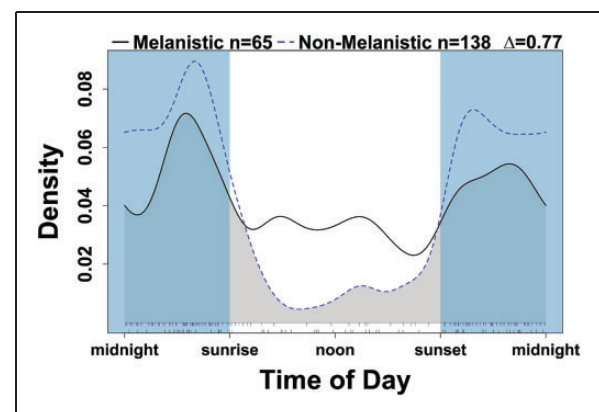


Figure 9. Circadian Activity Overlap Between Non-melanistic (Dotted Line) and Melanistic (Solid Line) *Oncilla*; Gray Is the Area of Overlap. The coefficient of overlapping Δ between non-melanistic and melanistic *oncilla* was 0.77 and significantly different by all three statistical tests ($p < .05$).

from either enhanced thermoregulation or because tropical forest cover enhances the camouflage of darker individuals that thereby enjoy greater hunting success (Silva et al., 2016). The Temporal Segregation hypothesis predicts that the melanistic morph will accrue greater fitness benefits when inhabiting tropical forest because they are better camouflaged during times of brighter illumination

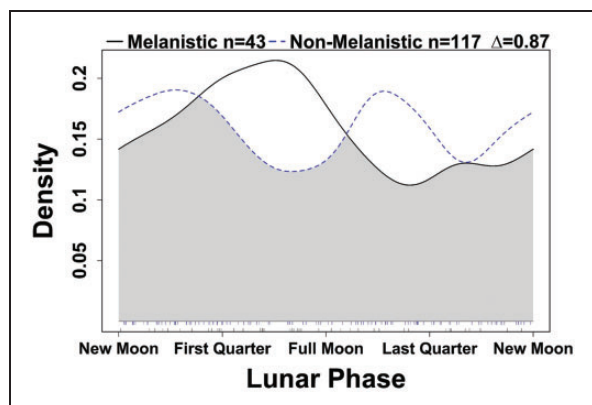


Figure 10. Lunar Phase Activity Overlap Between Non-melanistic (Dotted Line) and Melanistic (Solid Line) Oncilla; Gray Is the Area of Overlap. Melanistic oncilla tended to be more active during the full moon compared with non-melanistic oncilla. The coefficient of overlapping Δ between non-melanistic and melanistic oncilla was 0.87 and not significantly different ($p > .30$).

compared with non-melanistic individuals (Graipel et al., 2014, 2019) and would also benefit from enhanced hunting success at those times of the day or night. We tested the prediction that the percentage of melanistic jaguar in our surveys would be higher than the global average because all of our cameras are situated in primary or secondary forests. As predicted, 25% of independent camera trap records of jaguar involved melanistic individuals, over twice the global average of 10% based on both open and closed habitat (Silva, 2017). Similar to the jaguar results, 32% of independent records of oncilla were melanistic, higher than the percentage for Brazilian oncilla of 18% (Graipel et al., 2014). Estimates of the minimum number of individuals represented in our surveys indicated a similarly high percent of melanistic jaguar and oncilla that concur with the results based on unidentified capture records. Thus, the high percentage of melanistic records supports the hypothesis that melanistic color polymorphisms in these species are adaptive in dense forest habitat, most likely due to camouflage or thermoregulatory benefits.

In support of our results, Silva (2017) analyzed 37 environmental factors in relation to the geographical distribution of melanism in jaguars throughout their range. Based on 980 records of jaguar, he found that melanism was present in most forest habitats but was completely absent in grassland and savannah habitat. Silva (2017) concluded that (a) the spatial distribution of melanism was nonrandom, (b) the occurrence of melanism was related to humidity, and (c) the frequency of melanism may be regulated by climate. Interestingly, the models that Silva (2017) generated to explain melanism showed high suitability for melanism occurrence in Belize and Costa Rica.

Support for Gloger's rule has also been reported for other felids that exhibit melanistic and non-melanistic polymorphisms. Although leopards (*Panthera pardus*) are found in more than 100 ecoregions, melanistic leopards are most common in tropical and subtropical moist forests (Silva, 2017). However, the frequency of melanism in leopards is much lower in drier and more open habitats, such as deserts, shrublands, grasslands, savannas, and broadleaf forests (Silva, 2017). Jaguarundi exhibits a distinct biogeographic pattern insofar as the derived gray/dark color morph (which evolved more recently) is associated with moist and dense forests, while the ancestral reddish morph is associated with dry and open areas such as deserts and xeric landscapes (Silva et al., 2016). Melanistic Geoffroy's cat (*Leopardus geoffroyi*)—a small spotted cat similar to oncilla—followed Gloger's rule by occurring more frequently in ecoregions with a humid environment (Silva et al., 2017). These results suggest a selective advantage for dark morph individuals in dense tropical forests versus open habitats. Although Costa Rican oncillas are almost entirely found in high-elevation mountains (montane oak forests), throughout its broad range in South America, the species can inhabit a variety of habitats, including rainforests, dry deciduous forest, savannas, semiarid thorny scrub, and disturbed areas (Payan & de Oliveira, 2016). It would be interesting to see whether the melanistic morph of oncilla is less common or entirely absent from more open habitats, just as melanistic jaguars are less common or missing from grassland and savannah habitats (Silva, 2017).

The prediction that melanistic jaguar and oncilla would be better camouflaged in brighter light compared with non-melanistic individuals was supported, with melanistic jaguar more active during the day and melanistic oncilla significantly more diurnal compared with non-melanistic oncilla. Melanistic oncillas were much more active during the day compared with non-melanistic conspecifics, and the overlap analysis revealed a significant difference. The greater frequency of diurnal records among melanistic jaguar also supported the temporal segregation prediction. As a confirmation of our results, Graipel et al. (2014) found that melanistic oncillas were more active during bright nights compared with non-melanistic oncillas, and melanistic oncillas were also more active on bright nights compared with other felids (margay, ocelot, puma) or small mammalian prey (mostly rodents). They concluded that melanistic oncilla would be better camouflaged on bright nights than non-melanistic oncilla and would thereby benefit from both increased hunting success and decreased competition from non-melanistic oncilla or other felids (Graipel et al., 2014).

We also tested the prediction that melanistic color morphs would be more active during the brighter

phases of the moon. The prediction was not supported for melanistic jaguar, with no clear pattern in the lunar phase activity patterns of melanistic versus non-melanistic jaguar detected. In contrast, the lunar activity prediction was supported for melanistic oncilla because there was an increase in activity by melanistic oncilla during the first quarter and full moon (brightest phase) and a decrease in activity during this time by non-melanistic individuals. Oncillas were generally twice as nocturnal as jaguar, which suggests that evolutionary mechanisms acting to facilitate shifts in nocturnal behavior may provide more “return on investment” for oncilla than for jaguar. These trends should be tested with other felid species from a wide geographic range and across different habitats.

Our results raise the fascinating question of how melanistic individuals can shift their activity cycle to take advantage of their more cryptic coloration. We suggest that it is most likely that predators adapt their activity budgets to better hunt their prey, in accordance with optimal foraging theory. A study in Brazil showed that oncillas are primarily nocturnal in the absence of other felids, but in the presence of potential felid competitors (margay, ocelot, puma), they become more diurnal (Oliveira-Santos et al., 2012). The presence of prey or intraguild competitors was associated with behavioral shifts in activity patterns, presumably as the result of learning reinforced by improved hunting success. Felids are constantly assessing the scent and marks of competitors and potential mates, which facilitates the maintenance of complex social structures (Wooldridge et al., 2019). Scent marking is an integral part of felid behavior and regulates social hierarchies and mate choice, thereby providing a means by which melanistic felids could monitor their environment for prey and competitors and subsequently adjust their hours of activity in appropriate, fitness-enhancing ways.

Implications for Conservation

Many subpopulations of jaguar face accelerating threats from deforestation, fragmentation, road building, and illegal hunting (De la Torre et al., 2018; Espinosa et al., 2018; Srbek-Araujo et al., 2018). According to the International Union for Conservation of Nature (IUCN) Red List, jaguar is listed as Near Threatened with declining populations (Quigley et al., 2018). The geographical range of jaguar has contracted by 55% in the past century, and 33 of 34 subpopulations of jaguar are critically endangered or endangered according to IUCN criteria, and all are experiencing accelerating decline (De La Torre et al., 2018). The jaguar population of the Talamanca Cordillera of Costa Rica has been estimated at between 25 and 69 individuals (De La Torre et al., 2018). However, as recently as 2006, experts

ranked the Talamanca population to be at 200 to 500 individuals and stable or increasing, with a medium-to-high probability of long-term survival (Sanderson et al., 2002; Zeller, 2007). With many small jaguar subpopulations in decline due to habitat fragmentation, loss of genetic diversity is inevitable, followed by genetic subdivision from loss of connectivity among populations (Soto Fournier, 2014; Srbek-Araujo et al., 2018; Wultsch, Caragiulo, et al., 2016; Wultsch, Waits, et al., 2016).

Similar threats face the oncilla, with habitat loss from agricultural development considered the greatest threat to their survival (Payan & de Oliveira, 2016). Oncillas are listed as Vulnerable with declining populations according to the IUCN Red List (Payan & de Oliveira, 2016). Oncilla appears to be rare throughout their range, and it has been called the most obscure of all Neotropical felids due to often inexplicably low densities (Giordano, 2012; Tortato & de Oliveira, 2005). The taxonomy of the *Leopardus* “tigrina” species complex is currently under revision, and molecular and morphometric studies indicate a confusing picture of ancient and current hybridization, introgression, genetic isolation, and divergence among closely related and geographically overlapping species (Li et al., 2016; Nascimento & Feijó, 2017; Trigo et al., 2013). There is evidence that the subspecies found in Costa Rica and Panama (*L. t. oncilla*) is geographically disjunct and genetically distinct from the subspecies found in South America (*L. t. tigrinus*) and probably qualifies as a separate species (Johnson et al., 1999; Kitchener et al., 2017; Payan & de Oliveira, 2016). Taxonomic revision of *L. tigrinus* into a northern and southern species would result in smaller geographic ranges and population sizes that would likely increase the extinction risk of Costa Rican oncilla (Nascimento & Feijó, 2017).

Our results support the hypothesis that melanism provides adaptive benefits favored by natural selection. The results of activity overlap analysis suggest that melanistic oncilla and jaguar can persist in the face of conspecific competition by non-melanistic conspecifics because of their superior camouflage under brighter daytime or lunar light. This perspective not only contributes to a better understanding of the evolution, biogeography, and behavior of melanistic color polymorphisms (Payan & de Oliveira, 2016; Quigley et al., 2018), but it can also provide conservation policy makers, resource agencies, and communities with tools to better manage wild felid populations. One implication of these findings is that melanistic jaguar (and perhaps oncilla) requires dense tropical forests for their continuing existence (Delhey, 2017; Silva, 2017); thus, future land-use changes could have a more devastating effect on melanistic individuals compared with non-melanistic individuals. Absence of tropical forest would remove the

selective advantage of melanism indicated by our study, and we propose that continuing forest loss threatens the persistence of the melanistic coat color polymorphism in jaguar populations. Furthermore, felids are known to avoid human beings, and anecdotal evidence suggests that increased human presence can result in avoidance of areas that are intensely visited by people (Tortato & de Oliveira, 2005). This presents the troubling possibility that increasing human disturbance from farmers, hunters, or even ecotourists could have the unintended consequence of reducing the ability of melanistic individuals to hunt during daylight hours and thereby compromise their prey capture success. Melanism may be a “double-edged sword” that provides adaptive advantages in the absence of human disturbance but is a competitive disadvantage in the face of strong human impacts.

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