Report

Current Biology

Industrial Melanism in the Seasnake *Emydocephalus annulatus*

Highlights

- Turtle-headed seasnakes are melanic (black) in polluted sites, but not in other areas
- Melanin binds trace elements that are excreted when the snake sloughs
- Trace-element concentrations are higher in polluted sites and in darker skin
- Melanism in seasnakes from urban sites may facilitate excretion of pollutants

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In Brief

Goiran et al. found that within a wide-ranging seasnake species, melanism (black color) was common only in polluted sites. Darker skin enables snakes to excrete trace-element pollutants by binding pollutants to melanin in the shed skin and by inducing more frequent sloughing.

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Industrial Melanism in the Seasnake *Emydocephalus annulatus*

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SUMMARY

Although classically associated with urban environments in invertebrates, melanism in terrestrial snakes is more often linked to occupancy of cool climates [1–3]. Thermal advantages to melanism do not apply in aquatic snakes [4], but although turtle-headed seasnakes (*Emydocephalus annulatus*) are banded or blotched across a wide geographic range [5], most individuals are melanic in polluted inshore bays of the Pacific island of New Caledonia [4]. Why has melanism evolved in these urban sites? Because trace elements bind to melanin, darker feathers enhance a bird's ability to shed pollutants [6]. Reptiles in polluted habitats also accumulate trace elements, which are expelled when the skin is sloughed [7–11]. Might melanism enable snakes to rid themselves of harmful pollutants? We measured trace elements in sloughed skins of seasnakes from urban-industrial versus other areas and in dark versus light skin. For the latter comparison, we used data from laticaudine seasnakes (sea kraits *Laticauda* spp.), in which each individual is dark and light banded, facilitating comparisons between dark and light skin. As predicted, concentrations of trace elements were higher in snakes from urban-industrial areas and higher in darker than paler skin (even within the same slough). The rate of excretion of trace elements is further enhanced by higher frequencies of sloughing in melanic than banded individuals, even within the same population, because of higher rates of algal settlement on darker skin. Thus, melanism of seasnakes in polluted sites may facilitate excretion of trace elements via sloughing.

RESULTS

We surveyed color morphs of turtle-headed seasnakes across their geographic range, using a combination of field observations and examination of museum specimens (Table S1). Melanism was common in *E. annulatus* from urban-industrial sites within New Caledonia and in a remote Barrier Reef atoll used as a bombing range in Australia (association between % melanism with the categories of urban-industrial, non-industrial, and river-mouth: $F(2,20) = 27.61$, $p < 0.001$; post hoc Tukey tests show that urban-industrial > non-industrial or river-mouth), whereas most snakes from less heavily polluted sites were banded or blotched (Figure 1 and Table S1).

Mean concentrations of the 13 trace elements in sloughed skins analyzed ranged from 0.14 $\mu g.g^{-1}$ for Cd to 1,385 $\mu g.g^{-1}$ for Fe, with a maximum concentration of 6,195 $\mu g.g^{-1}$ of Fe (Figures 2 and S1; Tables S2 and S3). There was no significant difference in trace-element concentrations between *E. annulatus* versus the laticaudine species (MANOVA, $F(1,13) = 4.74$, $p = 0.35$; Figure 3). For all 13 trace elements, mean concentrations were significantly higher in urban-industrial sites than in non-industrial sites (Figures 2 and S1; Table S2; for statistical results, see Table S3). Concentrations of five trace elements (Co, Mn, Ni, Pb, Zn) were significantly higher in darker bands than in lighter bands (for statistical results see Table S3).

Our mark-recapture data from a color-polymorphic population in New Caledonia [4] show that the proportions of snakes that exhibited heavy algal fouling and sloughing at the time of capture were higher in melanic snakes than in banded snakes (logistic regression, algae $\chi^2 = 20.86$, degrees of freedom [df] = 1, $p < 0.0001$; sloughing $\chi^2 = 9.12$, df = 1, $p < 0.003$), but with similar seasonal patterns of sloughing in both color morphs.

DISCUSSION

In the seasnake *Emydocephalus annulatus*, melanism is more frequent in urban-industrial sites than in less polluted locations. Melanic snakes slough more often than banded conspecifics, and sloughing eliminates more trace elements from darker skin than from lighter skin. In combination, these results suggest that industrial melanism enhances a seasnake's ability to dispose of trace elements.

Concentrations of trace elements in these sloughs were higher than most previous records for marine reptiles [12, 13], including seasnakes [14, 15] and fishes (including the eels consumed by sea kraits [16]), and higher than can cause health problems in mammals and birds [17]. Seasnakes in the

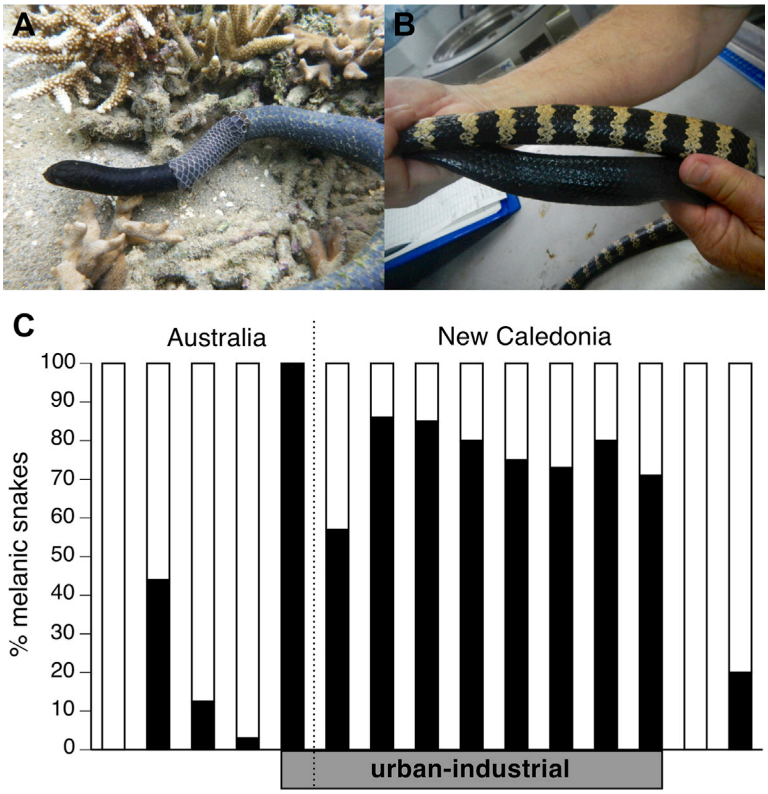

Figure 1. Geographic Variation in Coloration in Turtle-Headed Seasnakes

(A) *Emydocephalus annulatus* (melanic specimen sloughing).

(B) Melanic and banded *E. annulatus* from a peri-urban population near Noumea.

(C) Frequencies of melanism in snakes from urban-industrial sites versus other areas.

See also Table S1.

Noumea Lagoon are exposed to pollutants via run-off from terrestrial systems [16, 18]. New Caledonia's rich mineral deposits create high levels of trace-element contamination, further increased by mining activities [16]. High trace-element concentrations in sloughs of sea kraits from close to a river-mouth (but far from urban-industrial activity) suggest that melanism may benefit seasnakes in many areas. The primary uptake of trace elements presumably comes via ingestion of prey, with predatory snakes accumulating trace elements through time (i.e., bioaccumulation [15, 19]). These snakes also might take up trace elements directly from the water [20], given their high ratio of surface area to volume, and significant rates of gas exchange across the skin [21, 22]. However, radiotracer studies on other aquatic species suggest that feeding is the primary pathway for uptake of trace elements in invertebrates [23], fish [24], seabirds [25], and cetaceans [26].

Importantly, concentrations of trace elements were higher in darker than in lighter bands within the same slough (Table S3). As in birds, then, melanin-rich areas of a snake's outer surface accumulate trace elements, and, hence, sloughing reduces the trace-element load faster in melanic snakes than in paler conspecifics. That effect is amplified by the higher sloughing frequency of melanic *Emydocephalus* (Figure 1A), presumably because algal spores settle onto dark substrates, enhancing rates of algal fouling [27].

The melanic morph appears to be a derived trait in *E. annulatus*, but the number of independent evolutionary increases in the frequency of melanism is unclear. A single origin may have been involved, as in peppered moths [28], but occasional melanism is geographically widespread in *E. annulatus* (Table S1). The only sites where melanism in *E. annulatus* was common, but which were not urban-industrial sites, were Saumarez Reef, an isolated reef that is used as a bombing range, and Ashmore Reef (Table S1), a site where seasnake populations have plummeted in recent years, possibly due to pollution from fishing boats [29]. Future work should measure concentrations of trace elements at these reefs, to quantify the correlation between melanism and pollutant levels more robustly, and compare trace-element concentrations to snake coloration in populations of *E. annulatus* where the banded morph occurs at a high frequency.

What alternative hypotheses could explain the high frequency of melanism in seasnakes from urban-industrial habitats? Melanin plays diverse and important physiological roles. For example, enhanced immune function in melanin-rich individuals might be advantageous in polluted sites where the animals are subject to chemical stresses [2, 6], or melanism might protect snakes from high UV levels in clear shallow water [30]. Ecological advantages to melanism (such as local color matching to the habitat, to avoid visual predation) or reproductive advantages (mate choice) seem less likely: there are no clear differences in habitat use between banded and melanic snakes in our study populations, and a snake's color appears to play little role in mate recognition [31].

In summary, melanism has evolved under diverse selective advantages. Intriguingly, the seasnakes we studied in the Indo-Pacific exhibit the same correlation as seen in insects and pigeons in European cities: melanism is more common in urban-industrial environments. However, the selective advantages underlying that common pattern may involve antipredator camouflage and physiological benefits in insects versus trace-element excretion in pigeons and seasnakes.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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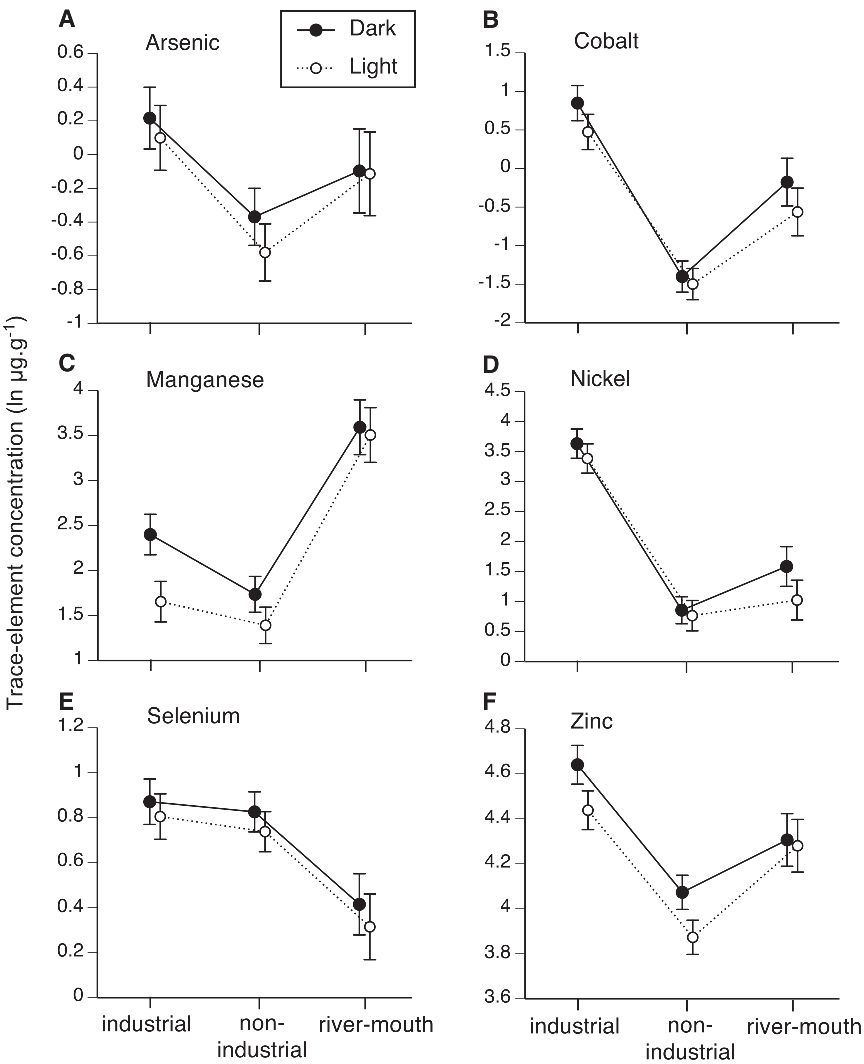

Figure 2. Concentrations of Trace Elements (ln $\mu g.g^{-1}$ Dry Weight) in Sloughed Skins of Sea Kraits (*Laticauda* spp.) from the Great Lagoon of New Caledonia

Some sites were close to urban-industrial areas, some were non-industrial, and one site was near the mouth of a major river that carried significant volumes of sediment. The panels show data (mean ± SE) for levels of trace elements in light- and dark-colored rings of sloughs from sea kraits from each type of site.

(A) Arsenic.
(B) Cobalt.
(C) Manganese.
(D) Nickel.
(E) Selenium.
(F) Zinc.
See also Figure S1 and Tables S2 and S3.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.06.073.

A video abstract is available at http://dx.doi.org/10.1016/j.cub.2017.06.073#mmc3.

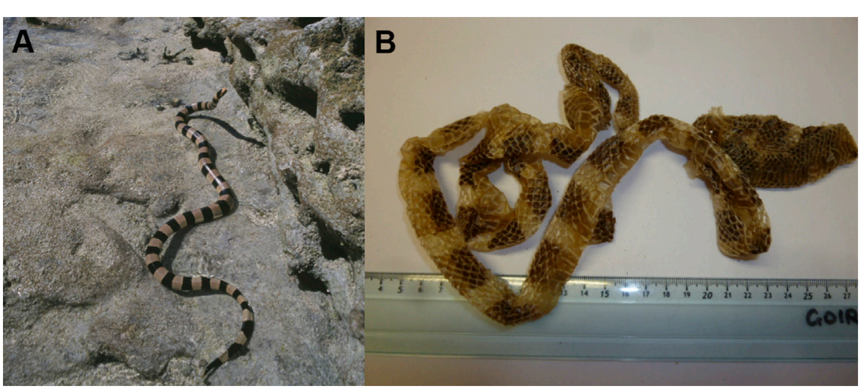

Figure 3. Amphibious Sea Krait and a Sloughed Skin

(A) A sea krait (*Laticauda saintgironsi*). Photo credit, Xavier Bonnet.

(B) A sloughed skin from a sea krait, showing dark and light rings. Photo credit, C.G.

AUTHOR CONTRIBUTIONS

C.G. conceived the study and gathered sloughs. P.B. analyzed trace elements. C.G. and R.S. conducted fieldwork. All authors contributed to writing the paper.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
JMP Pro v.11 statistical analysis software	SAS Institute	N/A
Other		
Trace element analysis	[32]	N/A

CONTACT FOR REAGENT RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Richard Shine (rick.shine@sydney.edu.au).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All procedures were approved by, and performed in compliance with, all guidelines for animal research in New Caledonia. Our analyses of trace elements in sloughs were based on sloughs found after they had been shed (and were collected under New Caledonian permits) in the case of sea kraits, and from shedding animals in the case of turtle-headed seasnakes. The subjects used in the mark-recapture study were 1,253 turtle-headed seasnakes (*Emydocephalus annulatus*) captured, marked and released (and in some cases, recaptured) in small bays beside the city of Noumea over the period 2004 to 2017. No animals were kept in captivity for this study.

METHOD DETAILS

Experimental design

In this paper, we describe the case of a seasnake species in which melanism is more frequent in urban-industrial populations than in those from less disturbed areas; and identify a possible selective advantage. We hypothesized that melanism might enhance the viability of urban-industrial snakes by enhancing their ability to eliminate trace-element pollutants, and thus, tested three predictions from the above hypothesis:

(1) melanism will be more common in urban-industrial populations of *E. annulatus* than in conspecifics from less disturbed areas;
(2) sloughed skins of seasnakes from urban-industrial areas will contain higher levels of trace elements than those of conspecifics from less disturbed areas; and
(3) concentrations of trace elements will be higher in dark skin than in pale skin. Because most individuals in our study populations are melanic, we tested this third prediction using data from sympatric laticaudine seasnakes that are dark-and-light banded and occur in both non-polluted and industrialized areas. That situation facilitates comparison of trace-element levels in dark versus light skin (from the same snake) and from areas with differing levels of pollution; and (because laticaudines slough on land) to obtain our samples from terrestrial situations where trace-element concentrations in sloughs are not rapidly leached out, as may occur in water.

Study species

The turtle-headed seasnake (*Emydocephalus annulatus*) is a small hydrophiine with a wide distribution in the Indo-Pacific. Most individuals are brightly banded, but some are melanic [5] (Figure 1). A snake retains its color pattern throughout life (R. Shine, *unpubl. data*). In contrast to this entirely aquatic species, brightly-banded sea kraits (*Laticauda saintgironsi* and *L. laticaudata*) forage in the ocean but return to land to slough [33]. Rings of color are clearly evident in their shed skins (Figure 3B).

Survey of snake coloration

We scored colors of *E. annulatus* in 15 populations (1,456 specimens) across the species' range (Table S1). Although some locations are close together, philopatry of snakes reduces gene flow [34]. The snakes were collected over a long period of time, but no significant temporal shifts in morph frequency were apparent in any sites.

Collection and analysis of sloughed skins

In 2015 and 2016, we collected recently-sloughed skins while processing *E. annulatus* during mark-recapture studies [35], and from terrestrial sites where *L. saintgironsi* and *L. laticaudata* aggregate [33]. Sampling locations for *Laticauda* spp. included urban-industrial zones (Baie des Citrons n = 1, Kuendu n = 10) and non-industrial locations (Mato Islet n = 6, Signal Islet n = 8), including close to the mouth of a large river (Bourail n = 6). We did not distinguish between sloughs of the two laticaudines. For *E. annulatus*, all sloughs were manually removed from shedding snakes, so that the skin was not exposed to water (urban-industrial: Anse Vata n = 1 snake, Baie des Citrons n = 14; non-industrial: Isle des Pins n = 1).

Rate of sloughing

During long-term fieldwork (2004–2017) in inshore bays beside Noumea [27] (2,377 total captures), we scored whether or not recently-captured *E. annulatus* were covered by algae or were sloughing).

Analysis of trace elements

In order to remove dirt and adsorbed trace elements, sloughs were rinsed with distilled water, dried, cut into pieces, cleaned twice in 2:1 chloroform:methanol solution in an ultrasound bath for 2 min, rinsed in ethanol between and after the cleanings and dried at 48°C for 24 h. Then they were ground with an agate mortar and pestle and sent to the LIENSs laboratory where they were analyzed for elements [32]. Briefly, aliquots of 50 to 150 mg were digested with a mixture of hydrochloric and nitric acids in a microwave. As, Cr, Cu, Fe, Mn, Ni, Se and Zn were analyzed by inductively coupled plasma optical emission spectrometry on a Varian Vista-Pro ICP-OES (Varian Inc., Palo Alto, CA, USA), and Ag, Cd, Co, Pb and V were analyzed by inductively coupled plasma mass spectrometry on an ICP-MS Series II (ThermoFisher Scientific, Waltham, MA, USA). The analytical performances for each trace element and method were checked using two certified reference materials (CRM): dogfish liver NRCC-DOLT-4 and lobster hepatopancreas NRCC-TORT-3. Quality control showed recoveries ranging from 69 to 108% according to the element. Trace-element concentrations are presented in micrograms per gram on a dry weight basis ($\mu g.g^{-1}$ dw).

QUANTIFICATION AND STATISTICAL ANALYSIS

To quantify the impact of skin color on trace-element concentrations, we took samples of both the black rings and the light rings from each banded slough. Using JMP Pro v11 we compared mean concentrations of each trace element in the two taxa (*E. annulatus* versus *Laticauda* spp.) using Multivariate Analysis of Variance (MANOVA), with species as the factor and concentrations of trace elements (ln-transformed to attain normality of distributions) as the dependent variables. To examine specific trace elements more closely within the sample of *Laticauda*, we used ANOVA on each trace element, with water quality (urban-industrial versus non-industrial versus river-mouth) and skin color (light versus dark rings) as factors, plus their interaction, and with snake ID# included as a random factor to account for the fact that both light and dark rings were analyzed from each slough.