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The Prevalence and Manifestation of Wing De-melanization in the Eastern Carpenter Bee (Hymenoptera, Apidae *Xylocopa virginica)* Associated with Urban Areas

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ABSTRACT: Animals that live in urban environments face unique stressors due to increased temperatures associated with these novel ecosystems. Large bees in the family Apidae thermoregulate in a variety of fashions including wing muscle vibrations, basking, and collecting heat with the wings. Thus, the coloration of the wing will impact the degree of heat absorption. The eastern carpenter bee, *Xylocopa virginica* Linnaeus, is a widespread, univoltine species found throughout eastern North America. Typically, *X. virginica* has solid dark brown to black wings. We report on a newly discovered wing phenotype in which the wing has lost significant amounts of coloration, making them nearly transparent. Using specimens from St. Louis, MO, we found that the de-melanization is usually restricted to the distal portions of the wing, yet the coverage differs between individuals. The proportion of individuals exhibiting pigment loss is similar between males and females. We examined observations on the iNaturalist platform and found that the phenotype is found in urban areas throughout the species range. We hypothesize that de-melanization could be an adaptation to heat stress, however more research is needed.

KEYWORDS: Wing color, iNaturalist, urban heat island

The modern urban environment represents a unique set of challenges to almost all organisms that are contained within it (Lambert *et al.,* 2020; Des Roche *et al.,* 2021). Patterns of abiotic conditions including light, water, sound, and chemical composition are likely very different from the natural and historical conditions that urban-dwelling species originally encountered (Morse *et al.,* 2014). Furthermore, increased warming due to climate change (Parmesan and Yohe, 2003) coupled with the urban heat island effect exacerbates environmental stressors. The combination of all these factors is putting tremendous selection pressures on the species that have made cities their homes (Diamond and Martin, 2021).

Many species in the bee family Apidae (e.g., *Bombus* and *Xylocopa*) tend to use their wings for thermoregulation, especially during cool mornings, conveying them an ecological advantage over other flower visiting insects (Heinrich, 1975; Baird, 1986). They can increase their internal body temperatures by passively collecting heat from solar radiation with the melanized portions of the wing and transmitting that heat to their flight muscles (Baird, 1986; Heinrich and Esch, 1994). Conversely, translucent portions of the wing absorb less heat and in other insect orders it has been posited that less-melanized wings may be an adaptation against overheating (Clusella-Trullas and Nielsen, 2020).

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The eastern carpenter bee, *Xylocopa virginica* Linnaeus (Fig. 1) is a common species native to the eastern half of the United States and Canada (Skandalis *et al.,* 2011). This bee often nests in human-associated wood such as fence posts, eaves, and wood piles (Balduf, 1962). *Xylocopa virginica* is one of the earliest emerging species in the spring (Gerling and Hermann, 1978) and is active below 16 °C —a temperature at which many other bee species are incapable of flight (Baird, 1986; Skandalis *et al.,* 2011).

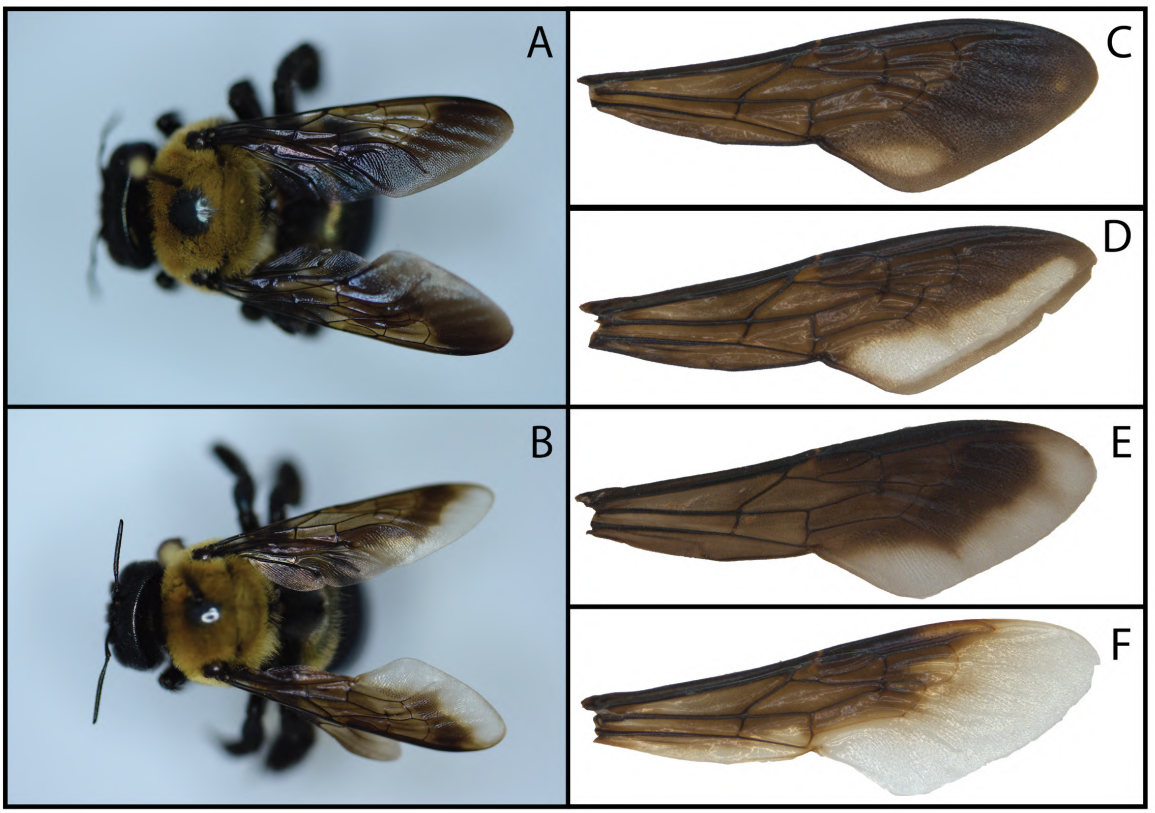

Figure 1: Eastern carpenter bee, *Xylocopa virginica*, showing loss of wing pigmentation. A) Individual with normal wing pigmentation; B) Individual with de-melanized wings; C-F) Variability among degree of de-melanization.

During the summer of 2014 we noticed *X. virginica* individuals with partly translucent wings in St. Louis, MO (Fig. 1B). Later, inspection of local insect collections yielded additional specimens dating as far back as 1995. Individuals with this phenotype were not found in several of the large entomological collections in the center of the continental US (Illinois Natural History Museum, University of Texas-Austin, Purdue University, University of Missouri-Columbia, University of Missouri-St. Louis, and Southern Illinois University-Carbondale) nor the east (Cornell University and American Museum of Natural History). Given its widespread prevalence within St. Louis, we hypothesized that this phenotype is found in other regions of the species' range but was undetected, possibly due to the rural-bias in large entomology collections. Therefore, we sought to utilize the citizen science platform iNaturalist (www.inaturalist.org) to broaden our collection pool since observations on iNaturalist have an urban bias (Di Cecco *et*

al., 2021). Furthermore, *X. virginica* is the second most common North American native bee on the platform, making iNaturalist an excellent source to investigate the prevalence of wing de-melanization.

Given the putative novelty of this phenotype we set out to ask some fundamental questions: is the loss of pigmentation on the wing distributed at random? If not, what is the pattern? Is the prevalence similar between males and females? Our objective is to provide foundational information on the morph by (1) quantitatively describing the pattern of wing de-melanization and (2) investigating the prevalence of the morph in the St. Louis population as well as across the species' geographical range.

MATERIALS AND METHODS

De-melanization Pattern

To quantify the pattern of de-melanization, we removed the right forewing (Fig. 1 C-F) of every bee exhibiting the morph in our collection (specimens identified by PAM, GRC, and NSF). All bees were caught in St. Louis City and St. Louis County, MO. The specimens are deposited in the insect collection at Saint Louis University. The bees were collected from 2005 through 2020 as part of other projects, with a majority being from 2015 and 2020. Each wing was photographed using a Leica S6D digital stereomicroscope. For wings that were tattered, we manually filled in the wing edge with the appropriate pixel color using the color picker and brush tools in GIMP (version 2.10). We omitted wings that were severely tattered from our analysis due to the difficulty of assessing the shape and color of the missing portions. The variation among 62 de-melanized wings was assessed using the R package 'patternize' (version 0.0.2, Van Belleghaem *et al.,* 2018, Supp. S1). This provided a visual summary of the translucent portion of the wing, wherein lighter pixels indicate a higher proportion of de-melanization.

Population Prevalence

As part of a separate project on bee diversity in the city of St. Louis, we sampled a range of restored native vegetation sites (n = 4), community gardens (n = 9), and urban farms (n = 5) across the city during the growing seasons of 2013 through 2016 (Camilo *et al.,* 2017). Sampling was conducted via aerial netting between 10:00 and 15:00 on sunny days at a rate of one person-hour per 0.25 ha. Given that the sampling was standardized and the coverage was equally distributed among sites, the *X. virginica* individuals represent a randomly drawn sample. We took advantage of that fact to determine the prevalence of the wing de-melanization phenotype in the population (Supp. Table S1). We also used those data to compare the frequency of wing de-melanization between the sexes. The comparison was done with a Chi-square test in the R computational environment (R Core Team 2021).

Geographical Distribution

To assess the morph's prevalence throughout its range, we searched the citizen science platform iNaturalist. All observations that were classified as 'Research Grade' by September 30th, 2021 (N = 35,696) were examined for the presence of de-melanization. Most of the iNaturalist observations were from the last decade but some predated 2011. We excluded observations where it appeared that the individual had malformed wings resulting from a genetic defect or virus. We did not tabulate the number of observations in which the wings were not in suffi-

cient focus to assess the presence of de-melanization. Therefore, we cannot make any conclusions about relative abundance across the range. All observations can be found in the iNaturalist project titled "*Xylocopa virginica* Wing Morph."

Occurrence data obtained from iNaturalist were mapped using ArcPro 10.3 (ESRI, 2020). Urban areas within the United States were designated at the census block level using 2010 census data (ESRI, 2021). For Canada, metropolitan areas were based on 2016 boundary files (StatCan, 2019).

RESULTS

The earliest known records of the de-melanized phenotype are specimens from 1995 and 1998 captured at a single site in St. Louis, MO. An additional specimen was collected in 2004, 60km from the first location (found in the R. Clinebell collection at the Missouri Botanical Garden). We suspect that the morph has been present in St. Louis predating 1995. However, there was little routine bee sampling prior to that time.

De-melanization Pattern

Color pattern analysis showed the morph's presence was highly associated with the non-vascularized distal regions of the forewing (Fig 2). Individual bees had left and right wings with similar patterns, but among individuals the de-melanized portion varied from a small spot to covering the entirety of the non-vascularized area. Often the de-melanization went all the way to the edge, but sometimes there was a rim of color (e.g., Fig 1D). Vascularized portions (basal to the second recurrent vein) located anteriorly and proximally on the wing rarely exhibited de-melanization. A visual inspection of the image analysis output from ten normally pigmented wings yielded no observable variation in coloration pattern.

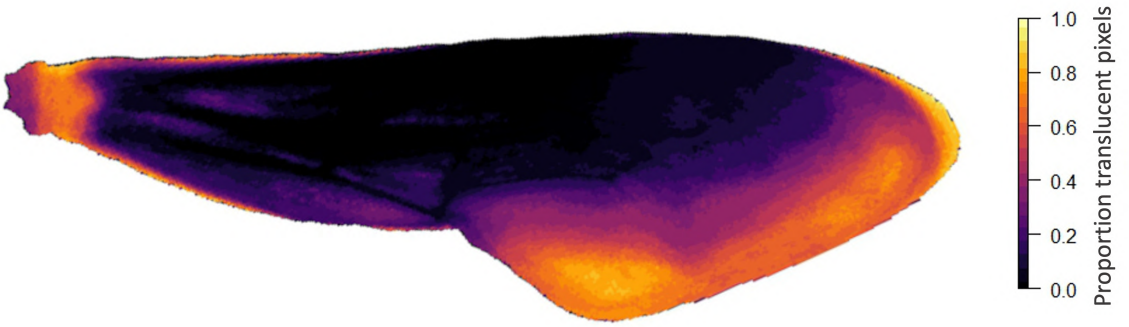

Figure 2: Color analysis summary of the location and proportion of forewing pigmentation from 62 *Xylocopa virginica* exhibiting de-melanization. The lighter the color, the higher proportion of translucent pixels (de-melanization). The light-colored portion at the proximal tip of the wing and along the proximal edges is due to slight differences in inherent wing shape and from cutting the wings off the specimens.

Population Prevalence

A total of 484 *X. virginica* individuals were collected between 2013 and 2016 across 18 sites in St. Louis. Of those, 41 females and six males exhibited wing de-melanization, whereas 365 females and 71 males had all dark wings. A contingency analysis of frequencies in the population showed that there was not a statistically significant difference in the frequency of the de-melanized wings between females (10%) and males (7.8%; Chi-square = 0.391, p = 0.5313). The overall population frequency was 9.7%.

Geographic Distribution

On iNaturalist, a total of 533 individuals exhibiting de-melanization were found in 30 of the 35 states and territories that make up the species' range (Fig 3). Three of the five states without evidence of wing de-melanization are the northernmost edge of the range—Vermont, New Hampshire, and Maine. Another northern state, Wisconsin, had a single observation. The metro areas of St. Louis, MO, Houston, TX, and Dallas, TX represented 54.6% of the morphs found. Among the 442 bees that could be sexed from photos, 314 were female and 128 were male.

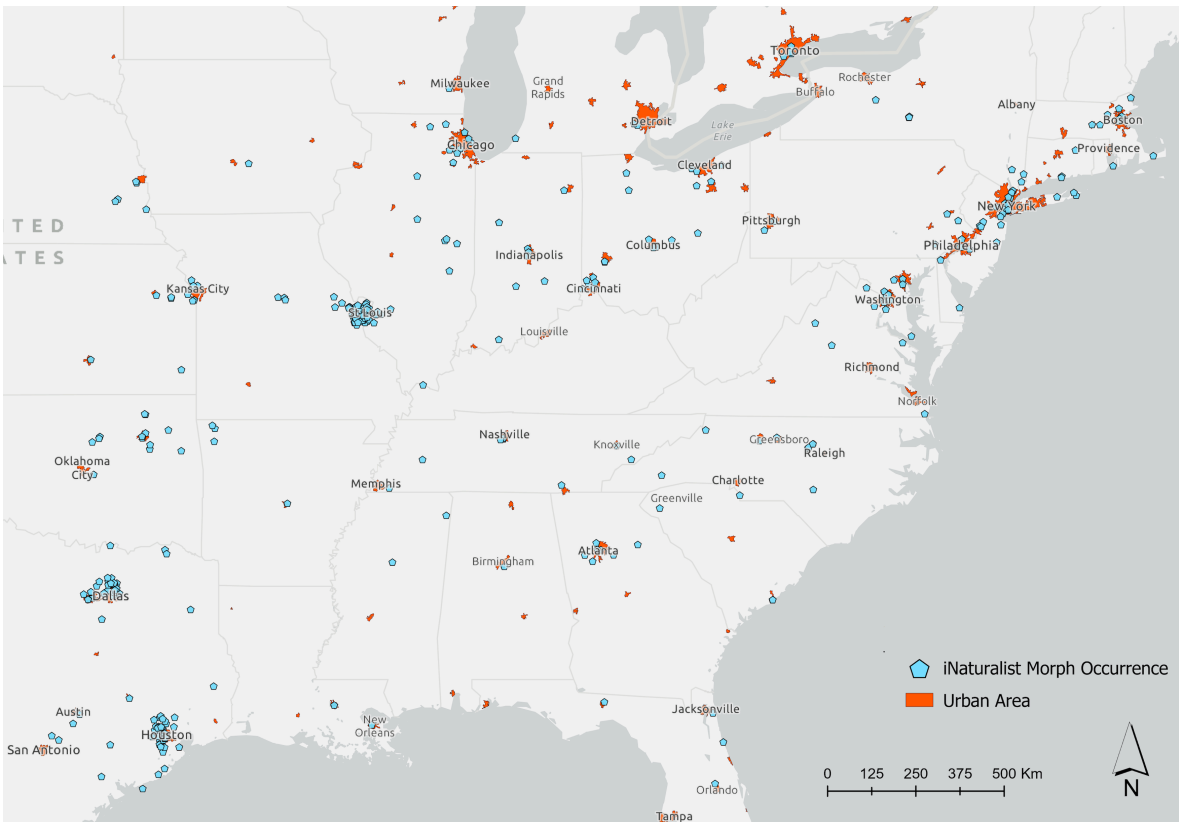

Figure 3: Locations of *Xylocopa virginica* exhibiting wing de-melanization from iNaturalist that were Research Grade by September 30th, 2021. Across the species range, the phenotype was found in 29 states and the province of Ontario.

DISCUSSION

Loss of pigmentation in wings due to a warming climate has been documented in various insect groups, including Odonata (Lis *et al.,* 2020), Lepidoptera (Kingslover and Buckley 2018), and Orthoptera (Smith *et al.,* 2021). The specific mechanisms identified in the various groups ranged from classical adaptation to developmental plasticity. Yet de-melanization can be disadvantageous. Under cooler conditions it can lead to decreased activity, limiting individual's ability to forage, find mates, and defend territory (Moore *et al.,* 2019).

The loss of melanin in the wings of *X. virginica* followed a distinct pattern, with most of the pigment loss occurring in the distal portions (Fig. 2). We hypothesize that de-melanization may be an adaptation or a developmental plasticity to combat overheating in urban areas. iNaturalist observations are more prevalent in urban areas (Di Cecco *et al.,* 2021), yet lack of de-melanized individuals in large entomology collection provides credence to the urban-bias of this phenotype.

The prevalence of the phenotype among the St. Louis specimens was surprisingly high at 7.5%, with no difference between males and females, suggesting that loss of melanin is not sex-specific, as has been reported for some other insects (e.g., Lis *et al.,* 2020). There is no evidence that de-melanization is an artifact of aging. Although we cannot age the specimens, bee's wings tend to tatter over time. We observed de-melanization on both pristine and highly worn wings.

Wing de-melanization would allow the bee to be active at the hottest part of the day or in locations with increased temperatures due to climate change and urban heat island effect. A loss of pigmentation should result in less efficient heat collection, which could be consequential in early spring and during cooler days. Determining if individuals with this phenotype have a disadvantage when temperatures are cooler remains to be investigated. This can be done by measuring the heat absorption between normally pigmented and de-melanized wings.

We were unable to examine the relationship between time of year and the amount of pigment loss along with the prevalence of loss within populations due to the seasonal bias in our collecting efforts. Future research into pigmentation loss throughout the year in this species would be insightful. Ultimately, much still needs to be done to determine the underlying mechanism of the de-melanization. To do this, future research must address if mechanism is due to phenotypic plasticity or if there is a genetic component. Rearing bees at different temperatures may provide insights. Additionally, a detailed analysis of behavior within populations over time could indicate if there are fitness advantages to presence and/or degree of wing de-melanization.

As locations around the globe become increasingly urbanized, human's impact on the fitness of wildlife is of increasing concern and importance. With the information from this study, progress can be made to understand the impact urbanization is having on pollinators, including how they could be adapting to anthropogenic changes.

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Supplemental Table and Materials

The prevalence and manifestation of wing de-melanization in the eastern carpenter bee (Hymenoptera, Apidae *Xylocopa virginica*) associated with urban areas

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