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## Interactions between sexual signaling and wing size drive ecology and evolution of wing colors in Odonata

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Insect coloration has evolved in response to multiple pressures, and in Odonata (dragonflies and damselflies) a body of work supports a role of wing color in a variety of visual signals and potentially in thermoregulation. Previous efforts have focused primarily on melanistic coloration even though wings are often multicolored, and there has yet to be comprehensive comparative analyses of wing color across broad geographic regions and phylogenetic groups. Percher vs. flier flight-style, a trait with thermoregulatory and signaling consequences, has not yet been studied with regard to color. We used a new color clustering approach to quantify color across a dataset of over 8,000 odonate wing images representing 343 Nearctic species. We then utilized phylogenetically informed Bayesian zero-inflated mixture models to test how color varies with mean ambient temperature, body size, sex and flight-style. We found that wing coloration clustered into two groups across all specimens - light brown-yellow and black-dark brown - with black-dark brown being a much more cohesive grouping. Male perchers have a greater proportion of black-dark brown color on their wings as do species with longer wings. In colder climates, odonates were more likely to have black-dark brown color present, but we found no relationship between the proportion of black and temperature. Light brown-yellow showed similar scaling with wing length, but no relationship with temperature. Our results suggest that black-dark brown coloration may have a limited role in thermoregulation, while light brown-yellow does not have such a role. We also find that the odonate sexes are divergent in wing color in percher species only, suggesting a strong role for color in signaling in more territorial males. Our research contributes to an understanding of complex interactions driving ecological and evolutionary dynamics of color in animals.

**Keywords** Insect color, Odonata, Ecological traits, Thermal biology, Sexual signaling, Sexual dimorphism, Color quantification

Insect coloration is diverse in appearance and function, and the cosmopolitan order Odonata containing both dragonflies and damselflies displays a wide range of wing colors in addition to a sophisticated vision apparatus<sup>1–3</sup>. For odonates and many other insect groups, colors serve multiple functions including visual signaling such as intra- and inter-species signals, warnings to predators and camouflage, or in some cases as a means to support thermoregulation or desiccation tolerance in more adverse climatic conditions<sup>4–7</sup>. Previous work focusing on odonate wing color has documented complex ecological and evolutionary forces and interacting drivers that may be governing wing coloration<sup>8–10</sup>.

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Of particular note are key color differences between sexes<sup>8,10</sup> related to their differing life history strategies. Male odonates often fly to secure and defend suitable territory for foraging and mating while females can be more cryptic, focusing on avoiding predators and seeking to oviposit eggs. Males with darker<sup>8</sup> or more vibrant wings<sup>9,11,12</sup> have been linked to more successful territoriality displays. These males have also been observed to be more successful in attracting females, demonstrating that wing color can also be a sexual ornament for males that either distinguishes the sexes or signals quality or age<sup>10–14</sup>. In summary, the diverging wing color patterns of males and females appear to fit their divergent needs related to their life histories. Beyond key differences across sexes, odonates species also differ in other aspects of flight behavior of males<sup>15</sup>. “Percher” dragonfly species spend most of their time stationary, making short but energetic flights, while “flier” species move continuously during active times. Percher flight style is associated with territoriality – male odonates choose perches in high-quality territories and aggressively defend this territory against intruding males<sup>16</sup>. Thus, percher males may show increased wing coloration as a way to signal broadly to conspecific or heterospecifics. While a few species may be “intermediate” between percher and flier<sup>15</sup>, we predict that different species-specific flight behaviors will impose selection on wing color, with the strongest forces operating on male wings.

So far we have focused on behavior and life-history characteristics, but structural constraints may also determine coloration. Organisms that are larger have a smaller surface area to volume ratio, providing an insulating effect that slows the rate at which they gain and lose heat<sup>17</sup>. However, odonate wings are relatively thin, and assuming wing area and thickness is isometric with body size, larger wings may actually lose heat-per-unit-area at a similar or faster rate to smaller wings. The veins and muscles that large wings rely on for motion and rigidity may also take longer to heat than in smaller wings<sup>18</sup>. For these reasons, we might expect differences in proportion of coloration in wings depending on size if pigments have a role in mitigating heat loss or supporting heat gain. However, it remains uncertain whether using wing color to warm the small volume of hemolymph circulating through the wings has thermoregulatory value either for capturing or retaining heat<sup>19,20</sup>. The only clear evidence of heat transfer between body and wings in Odonata comes from the libellulid *Zenithoptera lanei*, which has a complex multifunctional structure of the wing membrane not yet found in any other odonates. In this group, the wings are filled with tracheae and tracheoles that turn the wings into thermal windows, besides melanized cuticle, structural coloration, wax coverage and nanospheres<sup>21</sup>. However, even if there is no direct thermoregulatory function for wing color, dark coloration may still relate to climate, for example via resistance to other stressful factors such as increased ultraviolet radiation. As well, it may be that larger odonates have both competitive advantages in cooler climate and proportionally more wing coloration for success against competitors. In this scenario, the thermoregulatory effect is indirect via body size but leads to relationships among the three. For all these reasons, there may still be strong interactions between darker coloration, body size and climate.

Despite significant efforts to untangle the complex factors that are driving coloration of odonate wings, no studies have quantified the ecological and evolutionary forces driving wing coloration in a broad, comprehensive framework. Previous work<sup>8,22</sup> has focused on the darkest color on wings, although more recent work has called into question making simplistic assessments of function using one color alone<sup>23</sup>. It seems probable that other colors common on wings (e.g. orange, yellow, brown, or white color) might perform different functions or be evolving in a different way compared to more melanistic colorations. We therefore developed and utilized a novel color clustering approach that takes a data-driven approach to infer color groupings and identify what kinds of colors are present across odonates. We used this approach to test the predictions that percher species and males in general (across flight categories) have more dark wing pigment, with percher males having the most pigment. We also tested whether there is a relationship between body size, wing color and climate. As a means for comparison, we performed these same tests on lighter wing colors discovered during color clustering. Finally, we tested if our main delimited color groupings—one darker and one lighter—are phylogenetically correlated, e.g., appearing together across the Nearctic odonate phylogenetic tree. Our approach explicitly allows capturing presence and absence of coloration, as well as measures of proportion of wing containing a particular color, and provides a means to test key predictions while also accounting for spatial and phylogenetic autocorrelation. This work provides a framework that extends our ability to understand the forces shaping insect color and can be extended broadly to other taxa and body regions.

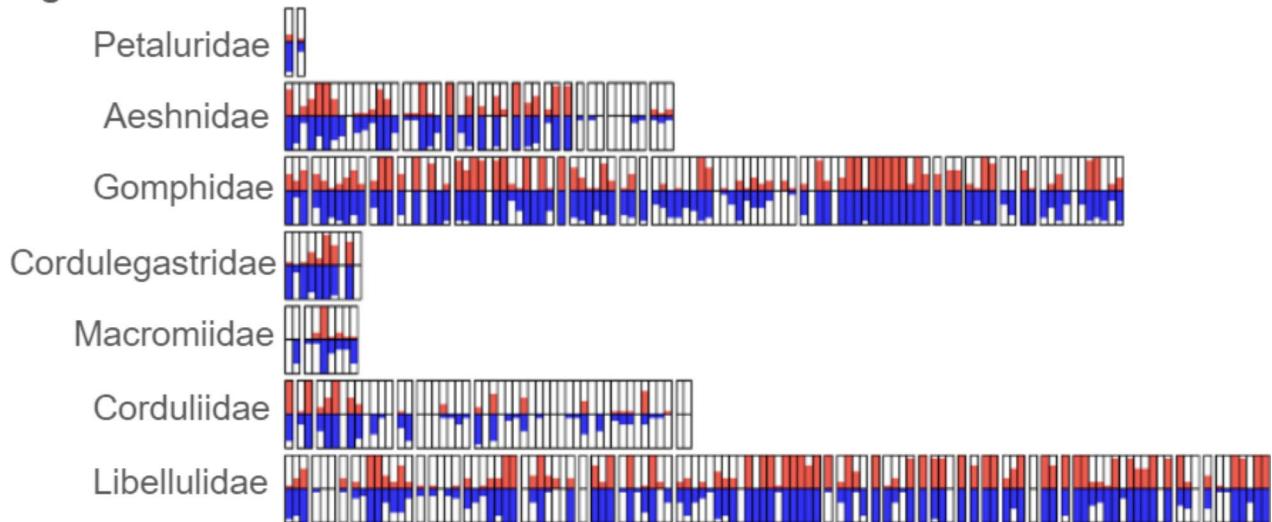
## Methods

### Specimen imaging and the Targeted Odonata Wing Digitization dataset

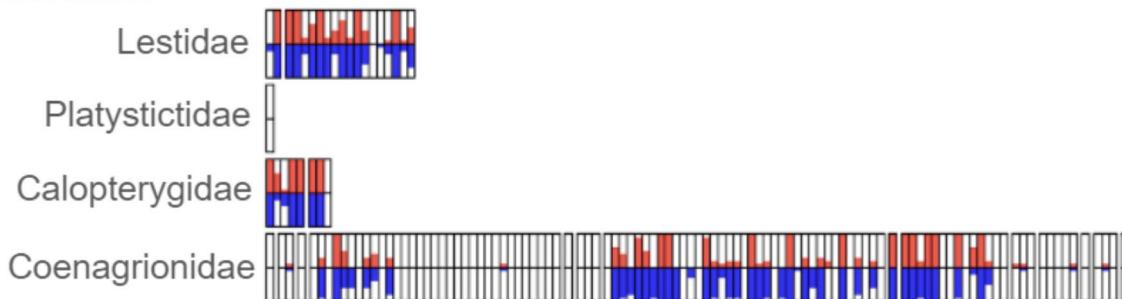
We used the fore and hind wings of 4,091 individual odonate specimens, and thus over 8,000 wings, imaged on a commercially-available Epson desktop flatbed scanner and color-calibrated using a color-checker, comprising the Targeted Odonata Wing Digitization dataset (TOWD; <https://digitizingdragonflies.org/>). The imaged odonates are all from the Nearctic, and represent 343 species. The average number of males and females per species is 8 and 15 respectively, thus strong intraspecific sampling for both sexes in most species. However 23% of species had either 0 males or females. Figure 1 provides sampling rates across known species of Nearctic Odonates, showing extensive overall coverage and relatively strong sampling across families.

In this dataset, 47% of images come from the Alabama Museum of Natural History (ALMNH), 19% from the PhD thesis collection of W.R.K. (now housed at the American Museum of Natural History, AMNH), 19% from the collection of the late Michael L. May, and 13% from J.W.’s collection at the AMNH. The TOWD dataset is well curated and includes needed specimen metadata, including date of collection, locality, sex of the specimen, etc. Each image from that dataset includes the dorsal views of a fore and hind wing, which were excised from the body of an individual odonate, a Tiffen Q-13 Color Separation Guide for standardizing the image for color, and the specimen label card containing collection locality information. We used a thresholding approach to segment the wings from their white backgrounds. As part of TOWD, wing length (measured from wing articulation to wing tip) had already been measured for each specimen. The TOWD dataset does not include precise geolocation

## Dragonflies



## Damselflies



**Fig. 1.** A visual representation of our sampling coverage across all 466 North American odonate species. Each bar shows the proportion (out of 10 individuals) of females (red) and males (blue) of a species represented in the wing image dataset. Clusters of bars indicate genera, where both genera and species are arranged alphabetically, and genera are listed by suborder and family. Many species are missing data for males, females, or both sexes. Species where a sex is represented by more than 10 individuals in the dataset are not indicated here.

where each specimen was captured, but does include the county of capture and provincial, state or country information for most individuals. We retrieved the 10 km resolution BioClim annual mean temperature closest to the center of each county and used this value for the 89% of specimens having a county<sup>24</sup>. This approximation captures a reasonable estimate of climate context given the continental scale of the analysis.

### Assembling a key trait: percher versus flier

We classified all species with suitable image data as perchers or fliers using the literature and personal observations. Following the criteria developed by Corbet and May<sup>15</sup>, we classified a species as a “percher” if its members (1) typically spend less than 60% of their time in flight and spend the rest of their time perched on vegetation or the ground, and (2) that regulate body temperature with movement or habitat choice. Those that usually spend more than 60% of their time actively or passively flying were classified as “fliers,” while “intermediates” *sensu* Corbet and May<sup>15</sup> was used for those that spend much of their time on the wing, but do not neatly fit into the dichotomous bins of percher/flier. Since only two species (*Tachopteryx thoreyi* and *Tanypteryx hageni*) were coded as intermediate based on consensus scoring by J.A. and J.W., we opted to filter these from further consideration.

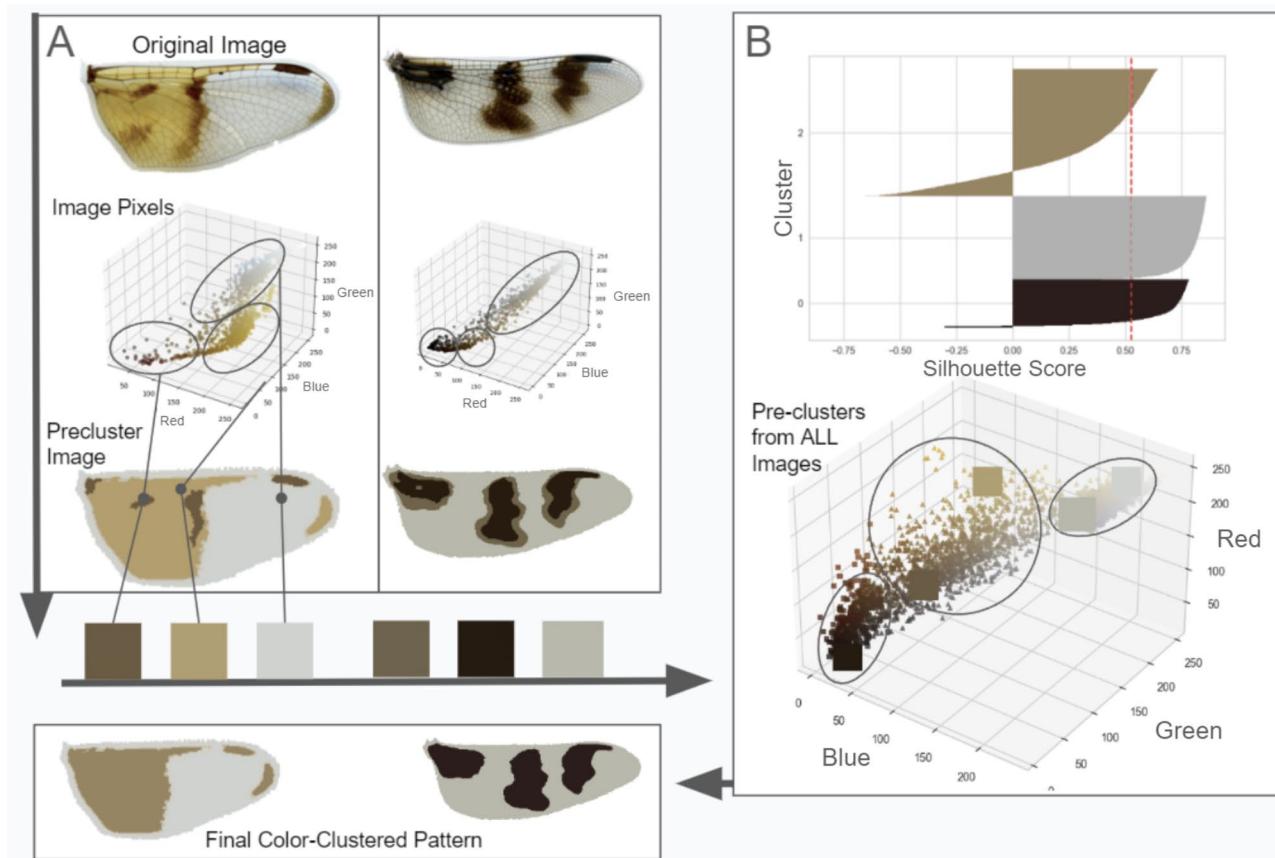
### Image processing and color clustering

Our goal in image processing and color clustering was to identify similar categories of wing color across the wing images. Color clustering identifies a finite set of “bins” that optimally captures the diversity of pixel colors across all these wings. Pixels placed into a bin should be similar to other pixels in that bin and as many pixels as possible should fit neatly into one bin rather than existing in an uncertain “gray area” between bins. If two expectations were met: (1) that different kinds of colors exist across odonate wings and (2) that they are defined with sufficient separation in 3-channel color space, we expected that clustering would be able to find them.

Using the R package *recolorize*<sup>25</sup> we first conducted a “pre-clustering” step where the pixels of each image were clustered separately in RGB space, creating a pattern from each image that contained color patches with each patch having a unique RGB value. We applied a bilateral blur (pixel kernel size = 6) to each image to blur out the wing veins, then hierarchically clustered the pixels to turn the image into a finite series of color patches. This pre-clustering step was intended to reduce noise in the colors we were clustering and also reduce the complexity of the main clustering step by reducing the number of colors needing to be clustered. In the main clustering step, we clustered all pre-clustered color patches together using a custom Python script, where each color patch from each image is a data point to cluster (Fig. 2A). The data points representing the continuum of color patches of all our odonate wings were grouped to a finite set of self-similar bins using Gaussian-mixture clustering. Gaussian-mixture clustering, which models each datapoint as the weighted sum of cluster centroids that take the shape of Gaussian curves, is more effective at identifying ellipsoid-shaped clusters than k-means or other traditional clustering methods.

In many clustering algorithms including Gaussian-mixture, it is necessary to choose how many clusters to fit data points to in the model; here that is equivalent to identifying how many color clusters are represented among odonate wings. We calculated Calinski-Harabasz scores, Davies-Bouldin scores, silhouette scores (Fig. 2B), and AIC for clustering results using one to nine clusters to identify which number of clusters resulted in the least dispersion within clusters and the least overlap with other clusters. Because of the strong peak in the Calinski-Harabasz score along with only small decreases in the Davies-Bouldin and silhouette scores relative to the alternative of two clusters, we chose to use three clusters. The wing patterns resulting from this approach are available for download on Dryad. Our color clusters were pale white (corresponding in almost all cases to the transparent wing background), black-dark brown, and light brown-yellow (Fig. 2).

Color clustering is a statistical approach that helps with ordination and discovery of color groups. If a cluster contains wing colors with very different amounts of melanins, ommochromes, or pterins, colors in that cluster will have different physiological outcomes and optical or physicochemical behaviors and thus may not be directly comparable to one another in relation to structural basis or function. Even if the amounts of pigments are similar, differences in cuticle material and pigment deposition may influence comparability. However, it is reasonable to assume that tightly-packed dark brown and black colors are at least partially the result of melanins. Finally,



**Fig. 2.** Schematic diagram of the process of clustering wing images to find related color clusters across images. Pixel pre-clustering (Panel A) is applied to each image separately using the histogram-binning method provided by the R package *recolorize*. Group reclustering (Panel B, bottom) clusters all the pixel clusters from all images taken together using Gaussian-mixture clustering in Python. Silhouette plot (Panel B, top) visualizes the quality of clusters by showing how close each data point in one cluster is to data points in neighboring clusters. The dotted red line shows the average silhouette score of all points (0.52).

we argue that using a data-discovery oriented approach to group colors and examining their cohesiveness and dispersion is complementary to techniques for large-scale color quantification relying on human scoring of colors, based on prior belief and often subjective assessment<sup>25,26</sup>.

## Modeling wing coloration as a function of morphometrics, environment, and traits

We modeled the presence and amounts of black-dark brown and light brown-yellow wing coloration in a hierarchical Bayesian framework using a zero-inflated beta distribution using the R package *brms*<sup>27</sup>. While many odonates (> 80%) have some light brown-yellow color on their wings (unlike brown-black, which is more rare), there are enough individuals missing these lighter colors to justify utilizing this approach. The zero part of the model estimated the effects of wing length, local mean annual temperature, flight type, and sex on the probability of having no wing coloration. A nested random intercept was included for this part of the model that estimated an intercept for each wing type (either fore or hind wing) nested within each species. The non-zero part of the model estimated the effects of wing length, temperature, flight type (percher vs. flier), sex, and the interactions between temperature and flight type and sex and flight type. Once again, we estimated an intercept for each wing type nested within each species.

Phylogenetic relatedness among species in trait-environment analyses can lead to unreliable parameter estimates<sup>28</sup>. Thus, a phylogenetic estimate of Odonata was performed using data gathered from NCBI in October 2022. These data included ~ 1,800 taxa and ~ 10 loci and included extensive North American species. The topology, although less resolved and well supported, reflected our current understanding of odonate phylogeny (Bybee et al., 2021). This phylogeny was then trimmed to only species in the wings dataset and used to generate a distance matrix of phylogenetic relatedness among species as a random intercept term in our model to address potentially problematic phylogenetic autocorrelation. To deal with potentially problematic spatial autocorrelation, we included a spline based smooth term of the spatial coordinates. We set weakly informative priors for all parameters. At the end of model runs (see below), we estimated the precision parameter of the beta distribution as a function of temperature and the random intercept of wing type nested within species.

Models were independently fit for both color clusters in Stan using the R package *brms*. For both models, *Rhat* was < 1.01 for all parameters, the number of effective samples was > 400 for all parameters, and no models had divergent transitions. We ran these models for 4,000 iterations with a 2,000 iteration warm up. Graphical posterior predictive checks were used to ensure that the model generated data similar to that used to fit the model<sup>29</sup>. Data simulated from the posterior predictive distribution were similar to the observed data (SI Fig. 1). We examined the residuals of these models for spatial and phylogenetic autocorrelation and did not find evidence for either (SI Fig. 2). We also tested whether presence or amount of black-dark brown and light brown-yellow are phylogenetically correlated, that is, that lineages containing black-dark brown are also likely to have light brown-yellow, using phylogenetic generalized least squares. We used the *gls* function from the package *nlme* with a Brownian phylogenetic correlation structure generated using the *corBrownian* function in *phytools*. For all models, wing length and temperature were scaled using z-score transformation.

## Results

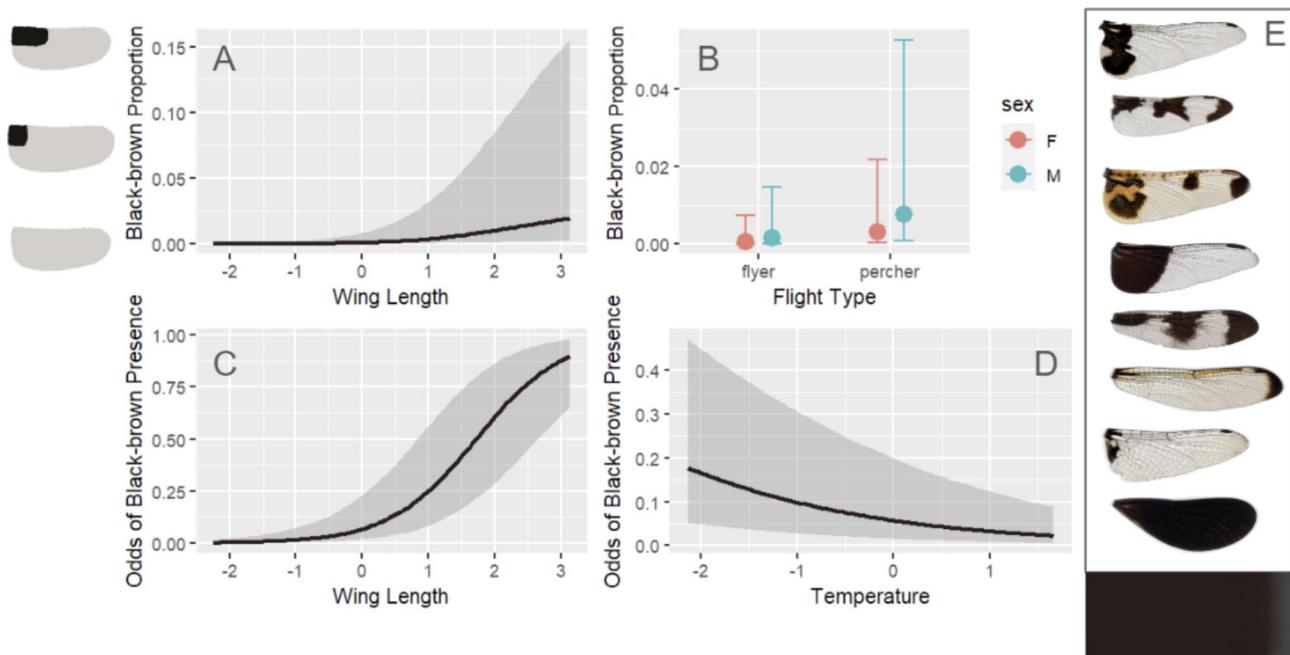
### Color clustering

The three color clusters were as follows: (1) a pale white representing the unpigmented wing background which is seen through a clear, colorless wing, (2) a cluster containing dark brown and black which appears strongly melanized, and (3) a cluster of a range of lighter browns and yellows which includes orange. Cluster 2 corresponds to color patches such as the dark central stripe of *Platylthemis lydia* and the basal black patch of *Tramea lacerata* (Fig. 3E) and will be referred to as black-dark brown throughout the remainder of the paper. Cluster 3 captured a wide range of colors from yellow to light brown (referred to as light brown-yellow going forward), all not nearly as dark and achromatic as the black-dark brown type and likely including a much wider variety of melanins and ommochromes in combination (Fig. 4D).

A silhouette score measures the similarity of a data point (wing color patch) to its own cluster compared to other clusters. Therefore, a silhouette plot containing ordered silhouette scores for each cluster visualizes the cohesion and separation between clusters. Our silhouette plot (Fig. 2B) shows that the clusters for black-dark brown and unpigmented are cohesive and well-separated, with almost every color patch observation having a silhouette score of at least 0.5 and a majority having a score of about 0.7. However the cluster for light brown-yellow partially overlaps with the clusters for black and unpigmented in addition to being much more dispersed and less cohesive across RGB space. It is a more disparate color bin - a weaker grouping - that captures a range of colors. While we do not know the underlying pigments for either grouping, we assume that the black-dark brown grouping likely does capture melanin, known to be responsible for such coloration. We know much less about underlying pigments for the broader light brown-yellow cluster.

### Rarity of black-dark brown and light brown-yellow and phylogenetic correlation between them

The presence of even small patches of the black-dark brown color cluster is rare in Nearctic odonates - just 7.5% of the 343 species in the dataset had > 5% or more of their wing covered by these black or nearly black patches and ~ 20% had any darker coloration at all. These colors are most common in the 95 Libellulidae species examined with 20% of these species with at least 5% coverage. The light brown-yellow color cluster was much more common, with 29% of species having > 5% coverage and > 80% having some light brown-yellow coloration. We also found no strong phylogenetically structured correlations between presences ( $r=0.005, p=0.93$ ) or amounts ( $r=0.06, p=0.31$ ) of black-dark brown and light brown-yellow. This phylogenetic independence suggests treating the two color clusters separately in models is warranted.



**Fig. 3.** Wing length (Panel A, C), percher/flyer flight type (Panel B), and temperature (Panel D) influence the coverage proportion or presence of the black-dark brown color cluster in odonate wings. Wing length and temperature are scaled using z-score transformation. The black-dark brown color cluster was identified in the wings using two clustering steps. Panel E: examples of wings that contained the black-dark brown color cluster, with the mean color of the cluster at the bottom.

### Black-dark brown modeling results

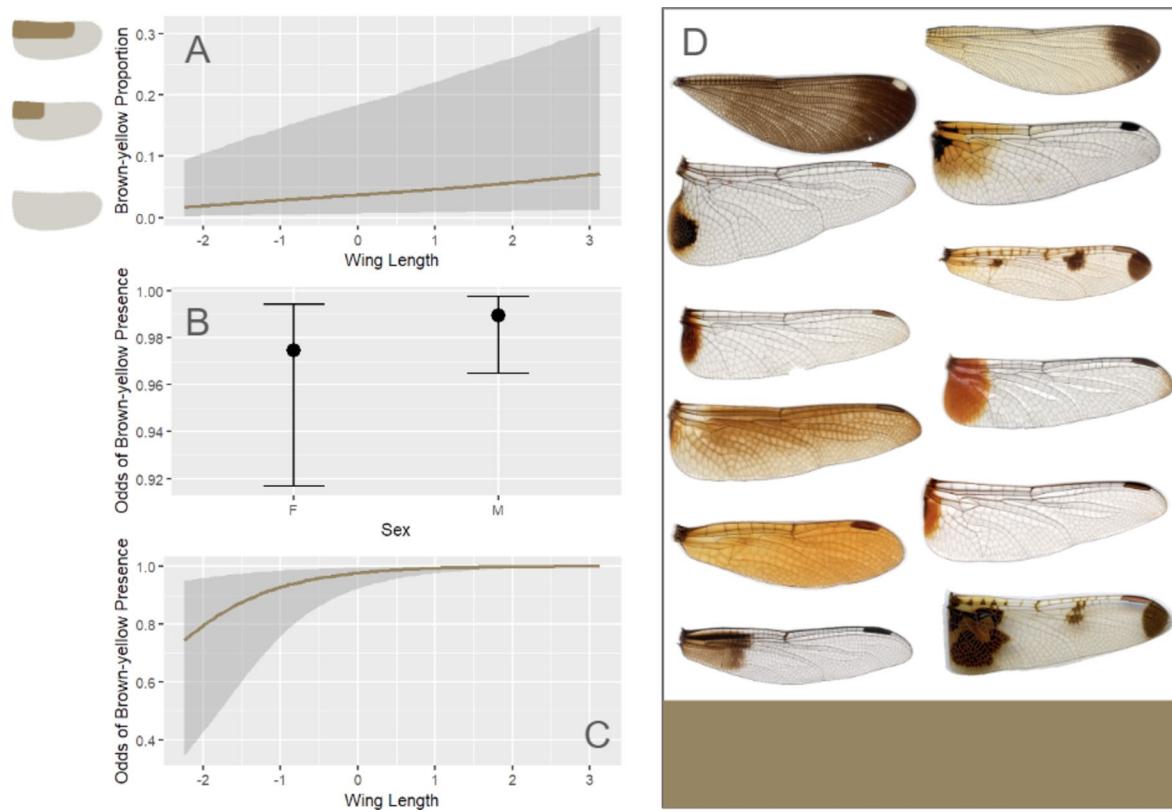
When estimating the percent of wing area covered by black-dark brown, the zero inflation model results indicate that individuals with longer wings are more likely to have black-dark brown color (population-level effect estimate = 1.53 [95% credible interval 1.09–1.97]) and individuals found in warmer areas are more likely to lack black color (population-level effect estimate = -0.60 [95% CI -0.78 - -0.42] (Fig. 3A, C,D). Additionally, males were more likely than females to have black color (population-level effect estimate = 1.03 [95% CI 0.82–1.24]). The non-zero part of the model indicates that more black color is associated with having longer wings (population-level effect estimate = 0.22 [95% CI 0.11–0.33]). An important interaction between flight type and sex was also shown in the non-zero part of the model, with percher males having significantly ( $\alpha <= 0.05$ ) more wing area covered by black than other groups (Fig. 3B).

### Light brown-yellow modeling results

The results of the model estimating the percent of wing area covered by yellow was similar to the percent black model with a few notable differences. In the zero inflated part of the model, longer wing lengths are associated with higher likelihood of light brown-yellow colors, similar to the black-dark brown cluster above (population-level effect estimate = 1.19 [95% CI 0.80–1.60] and males were more likely to have color (Fig. 4B, C). However, the temperature at which an individual was collected did not influence the probability of having zero yellow color (population-level effect estimate = -0.12 [95% CI -0.34–0.11]. For the non-zero part of the model, the only predictor variable with 95% CI estimates that did not overlap with zero was wing length, with longer wings being associated with more percent of wing area covered by yellow (population-level effect estimate = 0.21 [95% CI 0.13–0.29] (Fig. 4A). We note there is no indication of more light brown-yellow in percher males compared to other groups as with the black-dark brown color cluster.

### Discussion

Using a dataset of over 8,000 museum-specimen odonate wings from over 4,000 individual specimens, we tested hypotheses that expanded the scope of our understanding of odonate thermoregulatory and signaling interactions. We are the first to incorporate species-level percher-flier codings to examine how these dramatically different life strategies influence color evolution. Our color clustering approach was able to inductively and reproducibly identify what consistent color categories are present across the entire odonate clade. Using these color clusters, our phylogenetically informed Bayesian mixture models allow us to interpret how color varies both within and between species, and model both the presence-absence and amount of color present in relation to set of well-established hypothesis related to territoriality, signaling, and potentially thermoregulation.



**Fig. 4.** Results from model fitting (see Methods) for significant determinants for presence and amount of light brown-yellow coloring on wings. Panel A shows that the proportion of light brown-yellow increases with wing length. Panel B and C shows that the odds of light brown-yellow increase with increase in males compared to females and when wing lengths are longer. Panel D: examples of wings containing light brown-yellow coloring (note that some of these also show black-dark brown regions as well), with the mean color of the cluster at the bottom.

### The value of color clustering

Our inductive clustering is a novel way to categorize colors from images of animals, a process essential for understanding what functions different colors serve. While the premise of clustering colors is not new, studies thus far typically use it to match up pre-conceived color categories based on expert opinion. This is different from our approach of identifying color groupings based on the cohesion and dispersion of clusters. We argue that inductive color clustering is a more reproducible and agnostic approach compared to thresholding for black, picking template colors using human observers<sup>30</sup>, or scoring for the presence or absence of a color trait using human observers<sup>8,22</sup>. It allows us to empirically define color grouping, determine how broad those groupings are in the full space of colors, and identify colors in different individuals and species that belong to a given group. A particular advantage compared to looking at mean color lightness directly comes in the situation where a certain color type (for example a black melanin) is truly important for a particular function e.g. thermoregulation while another dark pigment is not (for example a dark brown ommochrome). If mean lightness alone is examined, a color with no bearing on a key function may be counted.

Here the key color groups supported by empirical analyses roughly break down into black-dark brown, light brown-yellow and white (representing the background). Darker “black” colors have often been the focus of wing color studies using human coding or thresholding, and our approach captures this and provides a clear view of the variation in “black” (Fig. 2). Given that this cluster is relatively narrow compared to the light brown-yellow color cluster (Fig. 2), we expect it may be the result of similar amounts of melanins or ommochromes in wing color patches belonging to it. By contrast, the light brown-yellow cluster covers a much larger part of the color space suggesting a wider range of pigment and structural components comprise this cluster. Relatively simple experiments selectively bleaching pigments from specimens could help confirm expectations of a wider set of pigments comprising light brown-yellow clusters than black-dark brown<sup>31</sup>.

### Wing pigments and relationship to climatic factors and wing size

Our predictions that dragonfly wing pigmentation is related to wing length, percher vs. flier flight style, and climate were generally supported, but exceptions paint a complex picture. First and based on a body of research spanning dragonflies<sup>8,22</sup> and other insects<sup>32</sup>, we predicted that both presence and amount of dark coloration would be found in colder environments. Alternatively, some have argued that dark pigmentation may not serve a strong thermoregulatory function in odonates<sup>23</sup>. Our results present mixed-evidence but generally support that

darker coloration *does not* have a strong thermoregulatory function. The lack of an area relationship is consistent with the idea that muscle warming through hemolymph (which should increase with pigment coverage) is likely not the function of wing pigment. It is possible that black-dark brown presence in key veins along the wing could have more of an impact on flight performance than full black coverage, supported by our data showing a presence-absence effect but not an effect related to proportion of dark colors.

By contrast, the increase of dark coloration with wing length was among the strongest in our study. We had anticipated that retaining heat in the larger veins and muscles supporting larger wings may be more difficult than in smaller wings, such that darker colors might allometrically increase in larger wings especially in colder climates. However, and critically, we found no interaction with temperature; regardless of the thermal environment that larger winged odonates live in, they consistently have more pigments. If there is a thermoregulatory function for black-dark brown pigment in wings, it is not related to climate context, and instead may provide a benefit across a range of climates. As well, both black-dark brown and light brown-yellow colors increase in area with wing length - a contrast from our expectation that only black-dark brown is important for thermoregulation. Rather than thermoregulation, it may be size and color are related in the context of signaling. Outomuro et al.<sup>33</sup> found that in the genus *Trithemis* hindwings with more coloration were wider at the base, especially in males, consistent with colored and wide hindwings being a sexual ornament or wings that convey gliding ability being associated with colorful displays. Work focusing on relationships between color and wing shape would help illuminate potential drivers and constraints of these complex color-size-shape relationships.

### Wing pigments and signaling: male vs. female and percher vs. flier flight style

Our final prediction that male percher odonates would have more pigments compared to any other group (percher females or male fliers) was strongly supported (Fig. 3B). This result emphasizes the importance of the specific needs of individuals of different sexes and flight styles in wing pigment evolution. It also suggests that there may be complex relationships between wing and body pigmentation. Relative to fliers, species of the percher flight style require an external source of heat from sunlight or the ambient climate to warm themselves for flight<sup>15,34</sup>. Meanwhile males, which both make more energetic and aggressive flights and often need to signal their territorial dominance to other males, should have more wing and body pigments for both of these reasons<sup>8</sup> - and this could be doubly so in perchers given studies suggesting more territoriality in general<sup>16,35</sup>. While we have not measured body pigmentation here, coupling wing and body pigmentation in order to better understand aspects of pigmentation related to signaling, such as wing color and aspects related to thermoregulation, particularly in terms of body coloration, is an exciting next step. Further, our data suggests that flight style and sex strongly interact with one another to determine darker coloration on wings, but we didn't find this same interaction for lighter coloration, again potentially marking those light brown-yellow colors as potentially functionally different. In sum, our work strongly suggests that dark wing pigments play a key role in sexual signaling and found only weak evidence for a direct role in thermoregulation.

### Shortcomings of color clustering and future directions

Our inductive color clustering approach to identify color groupings still has several shortcomings which we acknowledge here. Clustering gives little weight to color categories that are rare across the images, causing these to often get grouped alongside more densely sampled colors. Saturated red and orange colors were too rare in our dataset to comprise their own cluster and were thus grouped into light brown-yellow despite the strong possibility that these colors have different physical basis and function. As well, while structural colors that differ depending on perspective are not present in our wing data, color information from 2-dimensional images may fail to capture their variation accurately should we expand this approach to structural color. White pruinosity is known to be present on the wings of some species, it was not visibly present in our wing images perhaps due to it rubbing off during specimen handling, fading over time, and/or being hard to see against the white background. The very small number of specimens in which white pruinosity was visible were grouped with the white background cluster. Finally, our images do not contain UV color information, a limitation given that UV color is present in the wings of several species<sup>36</sup> and was found to be a signal for sex recognition in a damselfly<sup>12</sup>. However, UV colors discovered thus far have mostly arisen from either pruinosity or iridescence, components of color that we do not expect are included in our black-dark brown color cluster.

The accuracy of clustering may also be improved through advances in the approach itself. One avenue we are pursuing is clustering separated color patches by their mean colors instead of clustering individual pixels by their RGBs. This should help prevent rare, distinctive color patches like the reds in our dataset from being washed out, given that color patches are likely more biologically realistic color units than individual pixels. In this study we also do not establish that the colors belonging to each cluster have a similar physiological basis in terms of the pigments comprising them. If presences or amounts of these pigments are similar across the colors assigned to a cluster group, this would help establish a connection between our color groupings, their functions as supported by our models, and their physiological basis and associated constraints.

We recognize there are unaccounted sources of variation in our analyses, and other potential drivers of wing color, but as we discuss below, we doubt these affected our results. A key source of variation is that pigmentation is expected to generally increase with the age of an individual, especially when it reaches sexual maturity<sup>37</sup>. We carefully surveyed our best-sampled species and observed a range of ages but no tenerals (recently emerged individuals), which would have the most extreme reductions in color. Given the absence or near-absence of tenerals and the lack of systemic bias in age across species, we do not expect age to be impacting our results. Dark wing color has also been found to increase over the course of a flight season, possibly due to sexual signaling needs varying across the season<sup>38</sup> but again these biases are unlikely to be systematic within or across species. While unlikely to be impacting our results, we note that these issues highlight the complexity of wing pigmentation and suggest that future work may be able to pull forward more interactions between seasonal and spatial factors.

These unexplained sources of variation and the scaling between variability expressed at the intraspecific versus interspecific level are areas where there should be further exploration with increased within-species sample sizes.

We also recognize that much remains to be studied regarding factors shaping wing color and our work here is merely a start at accounting for key factor. Still, our work contributes to a growing body of research suggesting complex interactions between style of flight, sex and climatic context. We are the first to expand the scope to hundreds of species across the Nearctic, and clarify how these interactions play out according to key hypotheses in the literature. The results of our large-scale study are indirect, not causative, and this work still leaves much unanswered about the importance of thermoregulatory and territorial signaling functions of dark color in percher males. Given that body color is much more likely to aid in thermoregulation than wing color, a key next step is analyzing wing and body color in tandem with regard to thermoregulation. We do note that the relationship between presence but not amount of black-dark brown pigment and temperature was significant in our models, suggesting simple linear relationships between proportion of dark wing coloration and thermoregulatory function are almost certainly incorrect. Our findings also suggest avenues for further physiological and behavioral research investigating precisely why percher males have such dark wings. Finally, by distinguishing black-dark brown from light brown-yellow colors, we find evidence that only dark colors have any relationship to temperature and interactions between sex and flight style, suggesting that light brown-yellow colors, while more prevalent, may have more limited or different functional roles.

## Data availability

The data input to our study (a series of ~8,000 PNG images of dragonfly wings) are available on Zenodo [<https://zenodo.org/records/10714418>]. The Python and R code for analysis - consisting of steps in clustering and modeling - is available on GitHub [<https://github.com/jidec/odomatic-wings-analysis>].

Received: 17 June 2024; Accepted: 19 September 2024

Published online: 23 October 2024

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## Acknowledgements

This work was supported by NSF grants 2002489, 2002432, 2002473, 2002457 to J. Abbott, S. Bybee, J. Ware and R. Guralnick for the GEODE project (Collaborative Research: Genealogy of Odonata (GEODE): Dispersal and color as drivers of 300 million years of global dragonfly evolution). Further support was also provided by NSF grants 1611642 and 1564386. GEODE and TOWD are enabled by the work of field biologists and museum staff who have collected and managed specimens, and we acknowledge their hard work and dedication to enabling new science.

## Author contributions

J.I and R.G. and S.B. and J.W. conceived of the study. J.W. and J.A. coded species as percher or flier. J.I and R.G. wrote the main manuscript text. S.B. and J.W. and J.A. and R.G.F. and W.K. and R.G. edited the manuscript. A.S. and M.K created the phylogeny. J.I. and L.E. created the color clustering methodology. J.I. and R.G. designed the modeling and hypothesis-testing framework. M.B. created the models. J.I. created the figures. All authors reviewed the manuscript.

## Declarations

### Competing interests

The authors declare no competing interests.

### Additional information

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-73612-4>.

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